Carbohydrate Research
Elsevier Publishing Company, Amsterdam
Printed in Belgium

PHOTOCHEMICAL REACTIONS OF CARBOHYDRATES

II. THE PHOTOCHEMISTRY OF 6-DEOXY-6-IODO-1,2:3,4-DI-*O*-ISOPROPYLIDENE-α-D-GALACTOPYRANOSE

W. W. BINKLEY
The New York Sugar Trade Laboratory, 37 Warren Street, New York, N. Y. 10007 (U. S. A.)

AND R. W. BINKLEY
The Cleveland State University, Cleveland, Ohio 44115 (U. S. A.)

(Received January 30th, 1969)

ABSTRACT

Ultraviolet irradiation (Pyrex filter) of a methanol solution of 6-deoxy-6-iodo-1,2:3,4-di-*O*-isopropylidene-α-D-galactopyranose (**1**) in the presence of sodium hydroxide led to rapid, almost quantitative conversion of **1** into 6-deoxy-1,2:3,4-di-*O*-isopropylidene-α-D-galactopyranose (**2**). The yield of **2** was found to depend on the solvent and on the energy of the light used for irradiation. The direct irradiation of **1** in *tert*-butyl alcohol in the presence of sodium hydroxide gave **2** in only 36% yield, together with 6-deoxy-1,2:3,4-di-*O*-isopropylidene-L-*arabino*-hex-5-enopyranose (**4**) in 32% yield. A mechanism is proposed in which the initial step is the light-induced homolysis of the carbon–iodine bond in **1** to a radical species (**5**) and an iodine atom. The products formed from **5** depend on the relative ease of abstraction of hydrogen from the solvent.

INTRODUCTION

Although the naturally occurring carbohydrates are products of photochemical reactions, practical photochemical syntheses of carbohydrate substances in the laboratory are relatively rare[1–3]. In consequence, and also because of our interest in carbohydrates and in photochemistry, we have initiated a program for exploration of the possible utility of photochemical reactions in the study of sugars and sugar derivatives. The present paper describes, and discusses in detail[3], the photochemistry of 6-deoxy-6-iodo-1,2:3,4-di-*O*-isopropylidene-α-D-galactopyranose (**1**).

RESULTS

Direct ultraviolet irradiation (*i.e.*, without a filter; quartz lamp only) of **1** in methanolic sodium hydroxide under nitrogen for 2.0 h at 25° (see Expt. A, Table I) led to its complete disappearance, and, on removal of solvent, a milky white syrup

was produced. This syrup was divided into an ether-soluble and an ether-insoluble fraction. When the ether-insoluble fraction was dissolved in water and rendered neutral, addition of silver nitrate caused the formation of a precipitate having the pale-yellow color characteristic of silver iodide. Column chromatography, on Florisil, of the ether-soluble fraction afforded two photoproducts; the major product,

TABLE I

PHOTOCHEMISTRY OF 6-DEOXY-6-IODO-1,2:3,4-DI-*O*-ISOPROPYLIDENE-α-D-GALACTOPYRANOSE (1)

Expt.	*Solvent*	*Base added*	*Irradiation time, h*	*Yield of products, %*	
				2	4
A	Methanol	sodium hydroxide	2.0	83	none
B	Cyclohexane	sodium hydroxide	2.0	54	none
C	Cyclohexane	triethylamine	2.0	48	none
D	*tert*-Butyl alcohol	sodium hydroxide	3.0	36	32
E	Benzene	sodium hydroxide	6.0	none	none

isolated in 83% yield, was identified as 6-deoxy-1,2:3,4-di-*O*-isopropylidene-α-D-galactopyranose (**2**) by direct comparison with an independently synthesized sample[4]. The minor photoproduct (**3**), isolated in 11% yield, had an i.r. spectrum almost identical with that of **2**, but, in contrast to **2**, compound **3** was significantly less volatile and was much less mobile both on column and thin-layer chromatograms. Deacetalated **3** was markedly less mobile on paper than 6-deoxy-D-galactose (deacetalated **2**). Compound **3** has not yet been definitely identified; however, reasoning based on the available data allows a tentative assignment of structure to this compound (see DISCUSSION).

This photochemical conversion has also been achieved in cyclohexane in the presence of (*a*) sodium hydroxide (see Expt. B, Table I) and (*b*) trimethylamine (see Expt. C, Table I). In each case, however, the yield of product was less than from the irradiation in methanol. No reaction occurred on irradiation of a solution in benzene (see Expt. E, Table I).

A distinct change in the course of the reaction occurred when the irradiation of **1** was conducted in *tert*-butyl alcohol, a solvent that is a poor hydrogen-donor[5,6a]. In this case (see Expt. D, Table I), column chromatography of the irradiation products gave compound **2** (36%) and a second product (in 32% yield) which was found to be 6-deoxy-1,2:3,4-di-*O*-isopropylidene-L-*arabino*-hex-5-enopyranose[7] (**4**), identical by mixed m.p. and i.r. spectrum with an independently synthesized sample of **4**.

In Table II are given the yields of 6-deoxy-1,2:3,4-di-*O*-isopropylidene-α-D-galactopyranose (**2**) resulting from irradiation (of **1** in methanol) without a filter (quartz lamp only) and from those in which each of two different filters was placed between the light source and the reaction vessel.

TABLE II

DEPENDENCE OF YIELD OF PRODUCT ON EXCITATION WAVELENGTH EMPLOYED, FOR THE IRRADIATION OF 6-DEOXY-6-IODO-1,2:3,4-DI-*O*-ISOPROPYLIDENE-α-D-GALACTOPYRANOSE (**1**)

Expt.	*Irradiation time, h*	*Filter*	*Yield of* **2**, %
F	2.0	Vycor[a]	78
G	6.0	Pyrex[b]	97
H	2.0	quartz[c]	83

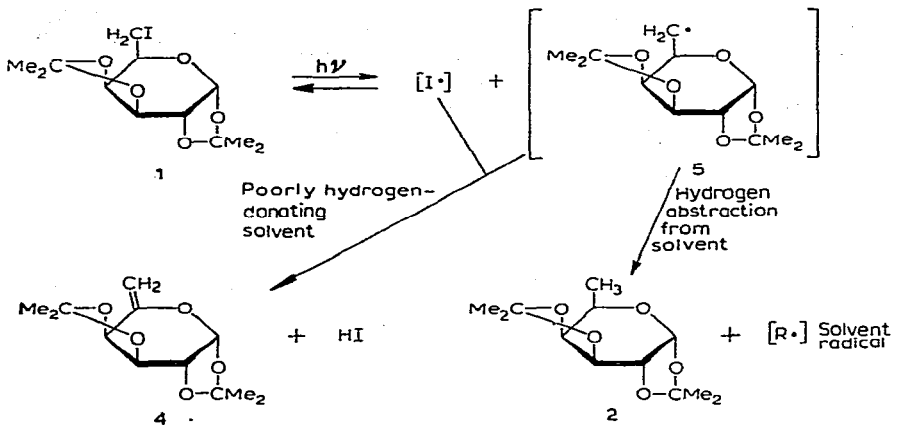
[a]Removes light of $\lambda < 205$ nm. [b]Removes light of $\lambda < 280$ nm. [c]Quartz is transparent above 200 nm (% transmittance = 73 at 200 nm).

DISCUSSION

The results presented in the previous section clearly show that the irradiation of 6-deoxy-6-iodo-1,2:3,4-di-*O*-isopropylidene-α-D-galactopyranose (**1**) under the proper conditions provides an excellent means for replacement of the iodine atom by a hydrogen atom. This photochemical reaction represents, as a synthetic process, a potentially attractive alternative to the well-known reductive procedure for accomplishing such a substitution[8], and may, therefore, be considered as one step in the general process for conversion of sugars into their deoxy derivatives. Although the possible synthetic utility of this reaction is clear, it is equally apparent from an inspection of Tables I and II that care must be taken in the selection both of the solvent and the energy of the light used in irradiation if the identity and yield of the photoproducts are to be controlled.

The data shown in Table I describe the dependence of the photochemistry of 6-deoxy-6-iodo-1,2:3,4-di-*O*-isopropylidene-α-D-galactopyranose (**1**) on the solvent used in the irradiation. The formation of 6-deoxy-1,2:3,4-di-*O*-isopropylidene-α-D-galactopyranose (**2**) is greatest in methanol, somewhat less in cyclohexane, and completely nonexistent in benzene. In *tert*-butyl alcohol, the formation of **2** is accompanied by a reaction that produces 6-deoxy-1,2:3,4-di-*O*-isopropylidene-L-*arabino*-hex-5-enopyranose (**4**). Although complete understanding of the effect of the solvent on the photochemistry of **1** has not been achieved some insight into the usual changes brought about by use of different solvents can be obtained by considering the most probable mechanism for this reaction and the effect of the solvent on molecules reacting *via* this proposed pathway.

In Scheme I is shown a proposed mechanism for the photochemical reactions observed on excitation of **1** in various solvents. The initial step in the process shown, regardless of the reaction solvent, is the light-induced homolysis of a carbon–iodine bond[9] to give the radical species **5**, as well as an iodine atom. Unlike the first step, the second step in the reaction sequence is critically dependent on the nature of the solvent. In methanol, an effective hydrogen-donor[6a], the free radical **5** abstracts a hydrogen atom from the solvent, to produce 6-deoxy-1,2:3,4-di-*O*-isopropylidene-α-D-galactopyranose (**2**), together with a hydroxymethyl radical. In *tert*-butyl alcohol,



where abstraction of a hydrogen atom is more difficult[5, 6a], hydrogen transfer from **5** to an iodine atom, to produce a molecule of 6-deoxy-1,2:3,4-di-*O*-isopropylidene-L-*arabino*-hex-5-enopyranose (**4**), is able to compete effectively with the reaction giving **2**. In benzene, which is an exceedingly poor hydrogen-donor, hydrogen abstraction leading to **2** is, reasonably, not observed. The apparent failure, in benzene, of the iodine atom to effect the abstraction of a hydrogen atom from radical **5** (to give **4**) is more difficult to rationalize; however, even though the explanation for this behavior still remains obscure, it is clear that any radicals formed by homolysis of a carbon–iodine bond in benzene tend to recombine. It seems unlikely that **1** is unreactive in benzene due merely to the failure of the carbon–iodine bond to undergo photochemical fragmentation.

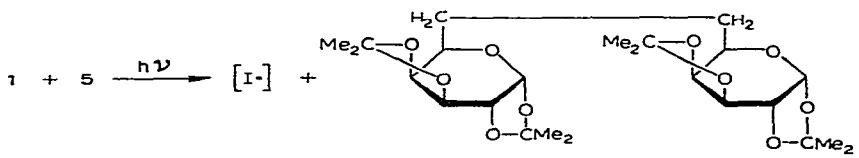
The energy of the light used for exciting 6-deoxy-6-iodo-1,2:3,4-di-*O*-isopropylidene-α-D-galactopyranose (**1**) also plays an important role in its photochemistry (see Table II). When the protons of higher energy are prevented from reaching the reaction mixture by the presence of a Pyrex filter, the yield of 6-deoxy-1,2:3,4-di-*O*-isopropylidene-α-D-galactopyranose (**2**) becomes almost quantitative. The exclusion of photons of very high energy also has the effect of terminating the photochemical side-reaction that results in the formation of compound **3**.

Although the structure of the minor product **3** from the irradiation of **1** has not yet been determined, the information available about this compound does allow some statement concerning its molecular framework. The i.r. spectrum of **3** is almost superposable upon that of 6-deoxy-1,2:3,4-di-*O*-isopropylidene-α-D-galactopyranose (**2**), indicating a very strong similarity in structure between **2** and **3**. Several additional observations are potential sources of further information about the structure of **3**: (a) **3** does not distil at 130° (bath temp.)/0.2 torr, whereas **2** readily distils at 59–61° under the same pressure; (b) on t.l.c. of **2** and **3** on silica gel, **2** has a much greater mobility than **3**; (c) the R_F values, on paper, of the deacetalated products from **2** and **3** also show the product from **3** to be less mobile than that from **2**.

In comparing two molecules, lower chromatographic mobility and higher b.p.

are usually associated either with an increased polarity within the molecule or an increased molecular weight, or both. The fact that the i.r. spectra of **2** and **3** are so similar favors the supposition that **3** is a species having a molecular weight higher than that of **2**, but suggests that **3** does not contain new, more-polar bonding, because increased polarity in the bonding in **3** (as compared to **2**) would produce corresponding changes in the infrared absorptions. Although, according to this reasoning, **3** should have a higher molecular weight than **2**, this increased molecular weight is clearly not a result of the incorporation of a molecule of the solvent into the starting material (or other reactive species) during photochemical reaction, as the same properties for the material designated **3** were found for the products formed in irradiations both in methanol and in cyclohexane.

On the basis of these considerations and the fact that free-radical species are known to attack alkyl halides with expulsion of a halogen atom[6b], we tentatively propose for the structure of compound **3** that shown in the following reaction depicting a probable process for the formation of **3**.



3

EXPERIMENTAL

General procedures. — In each reaction, a solution of compound **1** was irradiated at 25°, with constant stirring, with the light from a 100-watt, Hanovia, high-pressure, quartz, mercury-vapor lamp which had been lowered into a water-cooled, quartz immersion-well. Prepurified nitrogen was passed through the solution for 1 h prior to irradiation, and a slow stream of nitrogen was continued during photolysis. The solvent was removed from each irradiation mixture by distillation *in vacuo* below 30°, before column chromatography. T.l.c. was performed on silica gel (~100 μm thick) on polyester sheets (Eastman Kodak Co., Rochester, N.Y.) with 100:1 (v/v) benzene–*tert*-butyl alcohol as the developer and 1-naphthol–phosphoric acid as the indicator[10].

A. Direct irradiation in methanol in the presence of sodium hydroxide. — In a typical experiment, 1.000 g (2.78 mmoles) of compound **1** in 300 ml of methanol containing 390 mg of sodium hydroxide was irradiated for 2.0 h. No filter was used. T.l.c. of the crude reaction-mixture revealed, after treatment with 1-naphthol–phosphoric acid, the presence of two products. The more mobile of these (R_F 0.44) was salmon pink in color; the second product (R_F 0.22) was blue-gray.

After removal of solvent, 655 mg of ether-soluble, residual syrup was chromatographed on a column (2.5 × 80 cm) of Florisil, packed as a slurry in 1:9 ether–hexane. The column was developed with the following solvents: 200 ml of hexane, 200 ml

of 1:99 ether–hexane, 100 ml of 1:49 ether–hexane, 100 ml of 1:24 ether–hexane, 200 ml of 1:12 ether–hexane, and 600 ml of 1:6 ether–hexane. The effluent was collected in 100-ml fractions.

Fractions 4–9 gave 551 mg of a clear syrup, and fractions 11–12, 80 mg of another clear syrup. The major product had b.p. 59–61°/0.2 torr; $[\alpha]_D^{20}$ −62° (a supercooled melt; neat); n_D^{20} 1.45053; $\lambda_{max}^{CHCl_3}$ 3.35, 3.44, 6.93, 7.27, 9.24, and 10.01 μm; R_F 0.44 (1:100 *tert*-butyl alcohol–benzene). These constants are identical with those for a synthetic sample of 6-deoxy-1,2:3,4-di-*O*-isopropylidene-α-D-galactopyranose[4] (**2**).

Anal. Calc. for $C_{12}H_{20}O_5$: C, 59.00; H, 8.25. Found: C, 58.78; H, 8.21.

A portion (390 mg) of the major product was deacetalated by refluxing a solution in 1% aqueous sulfuric acid (16 g), followed by neutralization of the acid with barium carbonate, filtration, evaporation of the filtrate, and crystallization of the residue from ethyl alcohol (95%), to yield 147 mg of impure crystals. Recrystallization from the same solvent afforded elongated plates, m.p. 139–142°, $[\alpha]_D^{20}$ +76.6° (*c* 1, water), identical by mixed m.p. and paper chromatography with an authentic sample of 6-deoxy-α-D-galactopyranose[4].

Hence, the major product from the irradiation of 6-deoxy-6-iodo-1,2:3,4-di-*O*-isopropylidene-α-D-galactopyranose (**1**) is 6-deoxy-1,2:3,4-di-*O*-isopropylidene-α-D-galactopyranose (**2**), formed in 83% yield.

The minor product from the irradiation of **1** did not distil at 130° (bath)/0.2 torr, and had an i.r. spectrum essentially identical with that of **2**. Deacetalation with aqueous sulfuric acid (1%) yielded a syrup of R_F 0.16 on paper (with 2:2:1 butyl alcohol–ethyl alcohol–water as developer, and detection with *p*-anisidine hydrochloride to give a dark-green spot); 6-deoxy-α-D-galactose had R_F 0.56 and gave a green-brown spot. On the basis of these data and the mechanistic consideration described in the DISCUSSION, the structure **3** is tentatively assigned to the minor photoproduct from the irradiation of **1**.

B. Direct irradiation in cyclohexane in the presence of sodium hydroxide. — Irradiation of a solution of 360 mg (1.00 mmole) of **1** in 300 ml of cyclohexane containing 190 mg of sodium hydroxide for 2.0 h, followed by evaporation, gave a milky syrup. The ether-soluble portion of the reaction mixture was chromatographed on a column (2.5 × 80 cm) of Florisil, packed as a slurry in 1:9 ether–hexane. The column was developed with the following solvents: 100 ml of hexane, 100 ml of 1:99 ether–hexane, 100 ml of 1:49 ether–hexane, 100 ml of 1:24 ether–hexane, 100 ml of 1:12 ether–hexane, and 500 ml of 1:6 ether–hexane. The effluent was collected in 100-ml fractions.

Fractions 4–8 gave 128 mg (54%) of a clear syrup, identical in i.r. spectrum and t.l.c. mobility with 6-deoxy-1,2:3,4-di-*O*-isopropylidene-α-D-galactopyranose (**2**). Fraction 9 afforded 17 mg of a clear syrup identical in i.r. spectrum and t.l.c. mobility with the minor product, from the irradiation in methanol, that had tentatively been identified as **3**.

C. Direct irradiation in cyclohexane in the presence of triethylamine. — Irradi-

ation of a solution of 360 mg (1.00 mmole) of **1** in 300 ml of cyclohexane containing 1.0 ml of triethylamine for 2.0 h, followed by evaporation, gave a yellow syrup. Column chromatography of the ether-soluble portion of the reaction mixture was effected on Florisil, exactly as described in section C.

Fractions 4–8 afforded 120 mg (48%) of a clear syrup identical in i.r. spectrum and t.l.c. mobility with **2**.

A yellow solid that was isolated was identical (i.r. spectrum and mixed m.p.) with an authentic sample of triethylammonium iodide.

D. Direct irradiation in tert-*butyl alcohol in the presence of sodium hydroxide.* — A solution of compound **1** (360 mg, 1.00 mmole) and sodium hydroxide (190 mg, 2.3 mmoles) in 300 ml of *tert*-butyl alcohol was irradiated for 3.0 h as described under General Procedures. T.l.c. of the crude reaction mixture, followed by spraying with 1-naphthol–phosphoric acid, indicated the presence of four compounds. The most mobile component had R_F 0.79 and was pink, suggesting that it was unreacted **1**. A bright-blue spot (R_F 0.62) followed the pink one. The next spot was salmon pink and had R_F 0.44, indicating that this compound was 6-deoxy-1,2:3,4-di-*O*-isopropylidene-α-D-galactopyranose (**2**). Finally, the chromatogram showed a blue-gray spot near the origin.

After removal of solvent, 204 mg of the ether-soluble, residual syrup was chromatographed on a column (2.5 × 80 cm) of Florisil, packed as a slurry in 1:9 ether–hexane. The column was developed with the following solvents: 400 ml of hexane, 400 ml of 1:99 ether–hexane, 400 ml of 1:49 ether–hexane, 400 ml of 1:24 ether–hexane, 400 ml of 1:12 ether–hexane, 800 ml of 1:6 ether–hexane, and 400 ml of 1:3 ether–hexane. The effluent was collected in 20-ml fractions.

Fractions 99–106 gave 35 mg of unreacted **1**, identified by its i.r. spectrum and t.l.c. analysis. Fractions 130–156 afforded 75 mg (36%) of 6-deoxy-1,2:3,4-di-*O*-isopropylidene-α-D-galactopyranose (**2**), identified by i.r. spectrum and t.l.c. analysis. Fractions 114–124 gave 72 mg (32%) of colorless crystals, m.p. 76–81°. Recrystallization from ethyl alcohol (95%) yielded pure compound, m.p. 85–86°, $\lambda_{max}^{CHCl_3}$ 3.33, 6.04, and 7.26 μm, identical (mixed m.p., t.l.c., and i.r. spectrum) with a synthetic sample of 6-deoxy-1,2:3,4-di-*O*-isopropylidene-β-L-*arabino*-hex-5-enopyranose[7] (**4**).

E. Direct irradiation in benzene in the presence of sodium hydroxide. — Irradiation for 6.0 h of a solution of 360 mg (1.00 mmole) of **1** in benzene containing 190 mg (2.3 mmoles) of sodium hydroxide, as described under General Procedures, gave, on removal of solvent, a colorless solid. The ether-soluble portion of this material was identical, in i.r. spectrum, m.p., and mixed m.p., with the starting material (**1**).

F. Irradiation (Vycor filter) in methanol in the presence of sodium hydroxide. — The irradiation and isolation procedures were the same as those used in section A, except that a Vycor filter was placed between the light source and the reaction mixture.

Fractions 4–8 gave 510 mg (78%) of a clear syrup identical in i.r. spectrum and t.l.c. mobility with 6-deoxy-1,2:3,4-di-*O*-isopropylidene-α-D-galactopyranose (**2**).

Fraction 9 afforded 18 mg of a clear syrup identical in i.r. spectrum and t.l.c. mobility with the minor product (**3**) from the direct irradiation of a solution in methanol (section A).

G. Irradiation (Pyrex filter) in methanol in the presence of sodium hydroxide. — The irradiation and isolation procedures were the same as those used in section A, except that a Pyrex filter was placed between the light source and the reaction mixture.

Fractions 4–9 gave 635 mg (97%) of a clear syrup identical in i.r. spectrum and t.l.c. mobility with 6-deoxy-1,2:3,4-di-*O*-isopropylidene-α-D-galactopyranose (**2**). No other products were isolated.

Test of the stability of 6-deoxy-6-iodo-1,2:3,4-di-O-isopropylidene-α-D-galactopyranose (**1**) *under the conditions of reaction and isolation.* — In order to test the stability of the starting material under the conditions of reaction and isolation, compound **1** was treated exactly as described for direct irradiation in methanol in the presence of sodium hydroxide (section A), except that the light was not turned on. The isolation procedure was identical with that described in section A. Unreacted starting-material was isolated in quantitative yield.

ACKNOWLEDGMENT

R. W. B. thanks the Research Corporation for partial support of this work.

REFERENCES

1 D. HORTON AND W. N. TURNER, *Chem. Ind.* (London), (1964) 76; *Carbohyd. Res.*, 1 (1966) 444; D. HORTON AND J. S. JEWELL, *J. Org. Chem.*, 31 (1966) 509; R. H. BELL, D. HORTON, AND D. M. WILLIAMS, *Chem. Commun.*, (1969) 323.
2 P. M. COLLINS, *Chem. Commun.*, (1968) 403.
3 For a preliminary report of this work, see W. W. BINKLEY AND R. W. BINKLEY, *Carbohyd. Res.*, 8 (1968) 370.
4 K. FREUDENBERG AND K. RASCHIG, *Ber.*, 60 (1927) 1633.
5 S. G. COHEN AND H. M. CHAO, *J. Amer. Chem. Soc.*, 90 (1968) 165.
6 W. A. PRYOR, *Free Radicals*, McGraw–Hill Book Company, Inc., New York, 1966, (a) p. 219, (b) p. 145.
7 K. FREUDENBERG AND K. RASCHIG, *Ber.*, 62 (1929) 373.
8 O. T. SCHMIDT, *Methods Carbohyd. Chem.*, 1 (1962) 193.
9 J. G. CALVERT AND J. N. PITTS, JR., *Photochemistry*, Wiley, New York, 1967, p. 524.
10 W. W. BINKLEY, *Intern. Sugar J.*, 68 (1966) 10.

Carbohydrate Research
Elsevier Publishing Company, Amsterdam
Printed in Belgium

A FACILE SYNTHESIS OF 1,6-ANHYDRO DERIVATIVES OF 2-AZIDO-2-DEOXY-β-D-ALTROPYRANOSE AND 3-AZIDO-3-DEOXY-β-D-ALTROPYRANOSE FROM THE CORRESPONDING METHYL α-D-ALTROPYRANOSIDE DERIVATIVES

HIROYOSHI KUZUHARA, HIROSHI OHRUI, AND SAKAE EMOTO
The Institute of Physical and Chemical Research, Yamato-machi, Saitama-ken (Japan)
(Received January 24th, 1969)

ABSTRACT

The 2-*p*-toluenesulfonate (**6**) and the 2-benzoate (**7**) of 1,6-anhydro-3-azido-3-deoxy-β-D-altropyranose were prepared from the corresponding *p*-toluenesulfonate (**4**) and benzoate (**5**) of methyl 3-azido-3-deoxy-α-D-altropyranoside by treatment with *p*-toluenesulfonic acid in hot benzene. Alkaline hydrolysis of **7** gave crystalline 1,6-anhydro-3-azido-3-deoxy-β-D-altropyranose (**9**). In the same way, 1,6-anhydro-2-azido-2-deoxy-3-*O*-*p*-tolylsulfonyl-β-D-altropyranose (**15**) and 1,6-anhydro-2-azido-2-deoxy-3-*O*-methyl-β-D-altropyranose (**16**) were prepared from the corresponding methyl 2-azido-2-deoxy-α-D-altroside derivatives **13** and **14**. No polymerized products of these 1,6-anhydro compounds were detected in spite of the acidic condition of this reaction.

INTRODUCTION

A lack of reactivity in nucleophilic displacements was recently noted with *p*-toluenesulfonates[1] and chlorosulfates[2] of 1,6-anhydrohexopyranoses. This is probably one of the reasons why nitrogenous groups have never been introduced directly into 1,6-anhydrohexopyranoses by displacement reactions with such nucleophiles as ammonia, hydrazine, or the azide ion. Therefore various procedures that require a series of reactions have been developed to introduce nitrogenous groups into 1,6-anhydro sugars. The following procedures might be cited with 1,6-anhydro sugars: cleavage of an epoxide ring with ammonia[3], reduction of an oxime derived from the corresponding ketose[4], and oxidation with sodium periodate, followed by recyclization with nitromethane[5].

However, there have been a few examples where the nitrogenous group has been introduced into the sugar molecule before the formation of the 1,6-anhydro ring. One such rare example is 2-amino-1,6-anhydro-2-deoxy-β-D-glucopyranose hydrochloride, which was isolated[6] (together with 2-amino-2-deoxy-β-D-gulose hydrochloride) from the acidic hydrolyzate of the antibiotics streptothricin and streptolin B.

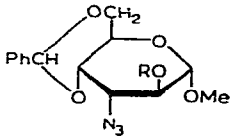
In this paper, we present another example, describing a facile synthesis of

1,6-anhydro-β-D-altropyranose derivatives that have an azido group at C-2 or C-3. Altrose is known as one of the sugars that easily form 1,6-anhydro rings under acidic conditions by way of the favorable *1C* (D) conformation. Thus, acid hydrolysis of methyl α-D-altropyranoside was reported[7] to give a mixture of 1,6-anhydro-β-D-altropyranose and D-altrose in the ratio of 57:43. Furthermore, methyl α-D-altropyranoside derivatives possessing an azido group at C-2 or C-3 are easily accessible[8]. For these reasons, the altrose derivatives were chosen for the preparation of a 1,6-anhydro sugar containing an azido group, which was required in the course of our study on the synthesis of optically active, polyamino compounds.

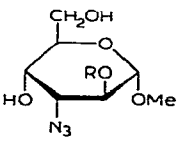
RESULTS AND DISCUSSION

Guthrie and Murphy[8] reported the preparation of methyl 3-azido-4,6-*O*-benzylidene-3-deoxy-α-D-altropyranoside (**1**) and methyl 2-azido-4,6-*O*-benzylidene-2-deoxy-α-D-altropyranoside (**10**) by cleavage of the epoxides of methyl 2,3-anhydro-4,6-*O*-benzylidene-α-D-mannopyranoside and of methyl 2,3-anhydro-4,6-*O*-benzylidene-α-D-allopyranoside with sodium azide. Both epoxide compounds are easily prepared from D-glucose.

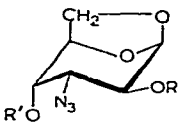
Compound **1** was *p*-toluenesulfonylated[8] and benzoylated in the usual manner to give the 2-*p*-toluenesulfonate (**2**) and 2-benzoate (**3**); these substances were then hydrolyzed under mildly acidic conditions to give crystalline methyl 3-azido-3-deoxy-2-*O*-*p*-tolylsulfonyl-α-D-altropyranoside (**4**) and methyl 3-azido-2-*O*-benzoyl-3-deoxy-α-D-altropyranoside (**5**). In the same way, compound **10** was *p*-toluenesulfonylated[8] to the 3-*p*-toluenesulfonate (**11**), which was debenzylidenated to give methyl 2-azido-2-deoxy-3-*O*-*p*-tolylsulfonyl-α-D-altropyranoside (**13**).



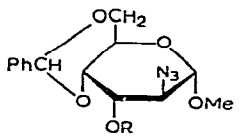
1 R=H
2 R=Ts
3 R=Bz



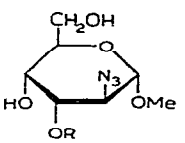
4 R=Ts
5 R=Bz



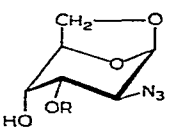
6 R=Ts, R'=H
7 R=Bz, R'=H
8 R=Bz, R'=Ms
9 R=R'=H



10 R=H
11 R=Ts
12 R=Me



13 R=Ts
14 R=Me



15 R=Ts
16 R=Me

The *p*-toluenesulfonylation and benzoylation had been carried out with an expectation of increasing the solubility of **4**, **5**, and **13** in solvents such as chloroform and benzene that were to be used in the subsequent cyclization reaction.

Aqueous *p*-dioxane containing a trace of sulfuric acid was used at 55° for the

removal of benzylidene groups from **2**, **3**, and **11**. These mild conditions seem superior to the usual hydrolysis with aqueous acetic acid, which sometimes causes acetylation of the hydroxyl group[9].

Various conditions were examined for formation of the 1,6-anhydro ring with **4**, **5**, and **13**. Compound **4** was recovered unchanged when its solution in *p*-dioxane was heated with Amberlite IR-120 (H^+) resin with stirring. The treatment of **4** with boron trifluoride etherate in hot chloroform gave a mixture of **4**, the 1,6-anhydro compound **6**, and an insoluble product (probably polymerized **6**). Treatment in hot benzene saturated with *p*-toluenesulfonic acid appeared to be most effective. Under the latter conditions, t.l.c. showed that 3 to 4 h was sufficient time to change **4**, **5**, and **13** into 1,6-anhydro-3-azido-3-deoxy-2-*O*-*p*-tolylsulfonyl-β-D-altropyranose (**6**), 1,6-anhydro-3-azido-2-*O*-benzoyl-3-deoxy-β-D-altropyranose (**7**), and 1,6-anhydro-2-azido-2-deoxy-3-*p*-tolylsulfonyl-β-D-altropyranose (**15**), respectively.

It is noteworthy that these reaction conditions give neither the polymerized product of the 1,6-anhydro compound nor the free sugar; these are always obtained together with the 1,6-anhydro compound by the usual hydrolysis procedure with aqueous acid.

Although compound **6** crystallized readily after the evaporation of the solvent, compound **7** resisted crystallization at first, and it was converted into the crystalline 4-methanesulfonate (**8**). However **7** also crystallized after purification by column chromatography. Further, compound **7** was converted into crystalline 1,6-anhydro-3-azido-3-deoxy-β-D-altropyranose (**9**) by alkaline hydrolysis.

Structural assignments of these 1,6-anhydro sugars (**6**, **7**, and **15**) were achieved as follows: dramatic rotational changes towards a negative value during the cyclization reaction suggested that inversions at the anomeric carbon atom had occurred. Elemental analyses of the products showed the loss of CH_3OH from the molecules of compounds **4**, **5**, and **13**. The azido, *p*-tolylsulfonyl, and benzoyl groups were found by i.r. spectra to be unchanged. Finally, the conformations of the products were determined from their n.m.r. spectra. The spectrum of **6** is shown in Fig. 1 and interpreted in terms of first-order vicinal couplings. The doublet centered at τ 4.48 having a splitting of 1.5 Hz was assigned to the anomeric proton on the basis of its chemical shift and its incomplete splitting, in which it resembles the case of 2,3,4-tri-*O*-acetyl-1,6-anhydro-β-D-talopyranose reported by Horton and Jewell[10]. In the region of τ 5.3–5.6, there are two multiplets that overlap each other in part. One of them, a typical quartet centered at τ 5.48, was assigned to H-2 coupled with H-1 ($J_{1,2}$ = 1.5 Hz) and with H-3 ($J_{2,3}$ = 9.0 Hz). The other multiplet of the overlapping signals was determined to be the H-5 signal after all other protons had been assigned. The C-4 proton gives a broad signal at τ 6.02 that separated into a sharp quartet ($J_{3,4}$ = 4.2, $J_{4,5}$ = 2.4 Hz) on the addition of D_2O, with simultaneous disappearance of the broad signal at τ 7.31 attributable to the hydroxyl proton. Both exo and endo protons at C-6 are observed in the region of τ 6.1–6.3 as the AB part of an ABX type of pattern. A quartet centered at τ 6.39 having splittings of 9 and 4.2 Hz was assigned to H-3.

The coupling constant of 9.0 Hz between H-2 and H-3 suggests that these protons are in the *trans* diaxial position. The n.m.r. data for **6** are fully consistent with the *1C* (D) conformation of the pyranose ring and cannot be rationalized in terms of a furanoid form.

The n.m.r. spectrum of **7** is essentially similar to that of **6**, suggesting that **7** has the same conformation as **6**. The H-2 and H-5 signals are well resolved in this case although the H-3, H-4, and H-6 signals overlap.

Compound **15** also gave a well-resolved pattern in its n.m.r. spectrum, as shown in Fig. 2, indicating that **15** exists in the *1C* (D) conformation.

In the series of 2-azido derivatives, the preparation of the 1,6-anhydro-3-benzoate was not attempted because of the possibility of acyl migration from C-3 to C-4 and further to C-6, but the preparation of the 1,6-anhydro-3-*O*-methyl compound was carried out. Compound **10** was methylated with a mixture of barium oxide, barium hydroxide, and methyl iodide in *N,N*-dimethylformamide, to afford crystalline methyl 2-azido-4,6-*O*-benzylidene-2-deoxy-3-*O*-methyl-α-D-altropyranoside (**12**), which was debenzylidenated to give syrupy methyl 2-azido-2-deoxy-3-*O*-methyl-α-D-altropyranoside (**14**). Purified **14** was treated in the same way as **4**, **5**, and **13**, giving syrupy 1,6-anhydro-2-azido-2-deoxy-3-*O*-methyl-β-D-altropyranose (**16**), which showed a large negative rotation, thus resembling compounds **6–9** and **15**. The conformation of **16** could not be determined conclusively from its n.m.r. spectrum because the chemical shifts of H-2 and H-3 were so close. Consequently the H-1 signal is not split as in **6**, **7**, and **15**, but appears as a somewhat broad singlet at τ 4.64 because of a virtual coupling with H-3. Nevertheless, the structural assignment of **16** appears not unreasonable on the basis of the satisfactory elemental analysis and the related examples shown in the formation of **6**, **7**, and **15**.

In order to examine the applicability of the reaction of 1,6-anhydro-ring formation to another sugar, methyl 2,3-di-*O*-*p*-tolylsulfonyl-α-D-glucopyranoside[11] [a derivative of the sugar that is the most unstable in the *1C*(D) conformation] was treated in a similar manner. This attempt, however, gave no 1,6-anhydro compound, and the starting material was recovered.

EXPERIMENTAL

General methods. — All melting points are uncorrected. Specific rotations were measured with a Perkin–Elmer 141 Polarimeter and a 1-dm tube. The i.r. spectra were recorded with a Shimadzu IRS-27 Model D i.r. spectrometer. The n.m.r. spectra were recorded at 60 MHz with a Japan Electron Optics C-60 Spectrometer with solutions in chloroform-*d* and tetramethylsilane as the internal standard. The t.l.c. was performed with Kieselgel G (E. Merck, Darmstadt) and the solvent system specified.

*Methyl 3-azido-2-*O*-benzoyl-4,6-*O*-benzylidene-3-deoxy-α-D-altropyranoside* (**3**). — Benzoyl chloride (8 g) was added to a solution of **1** (11.5 g) in pyridine (40 ml) with cooling (ice bath). The mixture was kept overnight at room temperature and then poured into ice–water (*ca.* 400 ml). The separated syrup was agitated until it

solidified. After filtration, the crude **3** was washed with water, dried, and recrystallized from isopropyl ether (*ca.* 300 ml), giving pure **3** (11.7 g). From the mother liquor an additional 2.0 g of **3** was obtained; total yield 13.7 g (88.9%), m.p. 136–137°, $[\alpha]_D^{24}$ −54.8° (*c* 3.80, chloroform); λ_{max}^{KBr} 2100 (N_3), 1730 cm^{-1} (>C=O).

Anal. Calc. for $C_{21}H_{21}N_3O_6$: C, 61.31; H, 5.15; N, 10.21. Found: C, 61.01; H, 5.11, N, 10.25.

*Methyl 3-azido-3-deoxy-2-*O*-p-tolylsulfonyl-α-*D*-altropyranoside* (**4**). — Aqueous sulfuric acid (1.2% w/w, 10 ml) was added dropwise to a solution of **2** (1.5 g) in *p*-dioxane (24 ml). The resulting transparent solution was kept for 24 h at 55° and then diluted with water (20 ml). After neutralization with a large excess of barium carbonate, the filtered solution was evaporated *in vacuo*. During the evaporation, water was added to the solution two or three times for the complete removal of benzaldehyde. The resulting crystals showed on t.l.c. (ethyl acetate–*n*-hexane, 2:1 v/v) one main product accompanied by traces of two other compounds. Recrystallization from water gave pure **4**; yield 600 mg (49.4%), m.p. 132–134°, $[\alpha]_D^{22}$ +52.8° (*c* 2.40, ethyl alcohol); λ_{max}^{KBr} 3380, 3240 (OH), 2100 (N_3), 1175, 1360 cm^{-1} (>SO_2).

Anal. Calc. for $C_{14}H_{19}N_3O_7S$: C, 45.04; H, 5.13; N, 11.26; S, 8.58. Found: C, 45.08; H, 5.02; N, 11.25; S, 8.47.

*Methyl 3-azido-2-*O*-benzoyl-3-deoxy-α-*D*-altropyranoside* (**5**). — Compound **3** (1.5 g) was treated in *p*-dioxane (25 ml) containing aqueous sulfuric acid (1.3% w/w, 10 ml), as described above for **4**, to give syrupy **5** which was chromotagraphed on silica gel (90 g) with ethyl acetate–benzene (3:4 v/v) as eluent. The purified syrup was suspended in a little water and allowed to crystallize overnight. Recrystallization from isopropyl ether gave pure **5**; yield 720 mg (61.5%), m.p. 91–92°, $[\alpha]_D^{23}$ −2.6° (*c* 2.77, methanol); λ_{max}^{KBr} 3540, 3400 (OH), 2100 (N_3), 1715 cm^{-1} (>C=O).

Anal. Calc. for $C_{14}H_{17}N_3O_6$: C, 52.01; H, 5.30; N, 13.00. Found: C, 52.01; H, 5.19; N, 12.99.

*1,6-Anhydro-3-azido-3-deoxy-2-*O*-p-tolylsulfonyl-β-*D*-altropyranose* (**6**). — Compound **4** (1 g) was added to a solution of *p*-toluenesulfonic acid (1 g) in hot benzene (150 ml). The mixture was stirred for 4 h under reflux. After cooling, the reaction mixture was washed with water and dried over anhydrous sodium sulfate, and the solvent was removed *in vacuo* to give crystalline **6**; yield 680 mg (74.4%), which showed one spot on t.l.c. with chloroform–methanol (94:6 v/v) as solvent. It was recrystallized from a mixture of ethyl acetate and *n*-hexane; m.p. 107–109°, $[\alpha]_D^{24}$ −75.9° (*c* 2.58, chloroform); λ_{max}^{KBr} 3500 (OH), 2100 (N_3), 1370, 1180 cm^{-1} (>SO_2); n.m.r. data (see also Fig. 1): τ 4.48 (1-proton, incomplete doublet, $J_{1,2}$ 1.5 Hz, H-1), τ 5.48 (1-proton quartet, $J_{1,2}$ 1.5 Hz, $J_{2,3}$ 9.0 Hz, H-2), τ 6.02 (1-proton, broad singlet which separated into a quartet on addition of D_2O, $J_{3,4}$ 4.2 Hz, $J_{4,5}$ 2.4 Hz, H-4), τ 6.1–6.3 (2-proton, multiplet, exo and endo H-6), τ 6.39 (1-proton, quartet, $J_{2,3}$ 9.0 Hz, $J_{3,4}$ 4.2 Hz, H-3), τ 7.31 (broad singlet which disappeared on addition of D_2O, OH).

Anal. Calc. for $C_{13}H_{15}N_3O_6S$: C, 45.75; H, 4.43; N, 12.31; S, 9.38. Found: C, 45.52; H, 4.61; N, 12.26; S, 9.41.

1,6-Anhydro-3-azido-2-O-benzoyl-3-deoxy-β-D-altropyranose (7). — Compound 5 (4.3 g) was added to a solution of *p*-toluenesulfonic acid (5 g) in hot benzene (600 ml). The mixture was heated for 2.5 h under reflux. After the cooled solution had been washed with water, it was dried over anhydrous sodium sulfate and evaporated *in vacuo* to afford a yellow syrup (2.8 g) which showed one major spot accompanied by one faint spot on t.l.c. (ethyl acetate–cyclohexane, 1:1 v/v). The syrupy product (2 g) was chromatographed on silica gel (100 g), with the same solvent system as that used for t.l.c., to afford a glass, which was dissolved in a little ether. Rubbing the mixture gave crystalline 7; yield 800 mg (28.9% from 5), m.p. 116–118°, $[\alpha]_D^{24}$ −273° (*c* 2.82, chloroform); λ_{max}^{KBr} 3450 (OH), 2100 (N_3), 1725 cm^{-1} (>C=O); n.m.r. data: τ 4.35 (1-proton, incomplete doublet, $J_{1,2}$ 1.2 Hz, H-1), τ 4.74 (1-proton, quartet, $J_{1,2}$ 1.2 Hz, $J_{2,3}$ 9.0 Hz, H-2), τ 5.28 (1-proton, multiplet, H-5), τ 5.77–6.25 (4-proton, multiplet, H-3, H-4, exo and endo H-6), τ 7.14 (1-proton, broad doublet, OH).

Anal. Calc. for $C_{13}H_{13}N_3O_5$: C, 53.61; H, 4.50; N, 14.43. Found: C, 53.51; H, 4.69; N, 14.45.

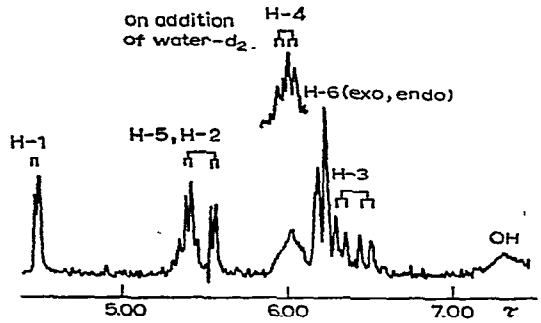


Fig. 1. The n.m.r. spectrum of 1,6-anhydro-3-azido-3-deoxy-2-*O*-*p*-tolylsulfonyl-β-D-altropyranose (6) in chloroform-*d* at 60 MHz.

1,6-Anhydro-3-azido-2-O-benzoyl-3-deoxy-4-O-(methylsulfonyl)-β-D-altropyranose (8). — Methanesulfonyl chloride (1 g) was added to a solution of crude syrupy 7 (2.1 g) in pyridine under cooling in an ice bath. The mixture was kept overnight at room temperature. After decomposition of the excess of methylsulfonyl chloride with a trace of water, the mixture was poured into ice–water. The resulting solid product was filtered off, washed with water, dried, and recrystallized from methanol to give pure 8; yield 2.5 g (93.9%), m.p. 181–182° (dec.), $[\alpha]_D^{23}$ −229° (*c* 2.0, chloroform); λ_{max}^{KBr} 2100 (N_3), 1740 (>C=O), 1360, 1180 cm^{-1} (>SO_2).

Anal. Calc. for $C_{14}H_{15}N_3O_7S$: C, 45.53; H, 4.09; N, 11.38; S, 8.67. Found: C, 45.31, H, 4.25; N, 11.27; S, 8.61.

1,6-Anhydro-3-azido-3-deoxy-β-D-altropyranose (9). — Aqueous sodium hydroxide (1% w/v, 9 ml) was added to a solution of 7 (600 mg) in ethyl alcohol (20 ml). After the mixture had been refluxed for 1 h, it was stirred with Amberlite IR-120 resin (H^+, 5 ml) and filtered. The filtrate was evaporated to dryness, and the resulting residue was dissolved in water (10 ml), which was extracted with ethyl ether in order

to remove benzoic acid. The water solution was evaporated *in vacuo* to give crude **9** which was chromatographed on silica gel (25 g), with chloroform–methanol (94:6 v/v) as eluent. The chromatographed syrup was kept at 5° for several days to give crystalline **9**; yield 250 mg (64.9%), m.p. 94–96°, $[\alpha]_D^{23}$ −209° (*c* 1.88, methanol); λ_{max}^{KBr} 3500, 3300–3200 (OH), 2100 cm^{-1} (N_3).

Anal. Calc. for $C_6H_9N_3O_4$: C, 38.50; H, 4.85; N, 22.45. Found: C, 38.68; H, 4.83; N, 21.88.

Methyl 2-azido-4,6-O-benzylidene-2-deoxy-3-O-methyl-α-D-altropyranoside (**12**). — To a solution of **10** (2 g) in *N*,*N*-dimethylformamide (20 ml) was added methyl iodide (5 ml), barium oxide (2.5 g), and barium hydroxide octahydrate (1 g). The mixture was stirred overnight under a reflux condenser at room temperature and then poured into water. After the mixture had been extracted with ethyl ether (*ca.* 200 ml), the extract was dried over anhydrous sodium sulfate and evaporated *in vacuo*, giving syrupy **12** (1.95 g, 93%), which solidified after a few days and was recrystallized from isopropyl ether; m.p. 93–95°, $[\alpha]_D^{21}$ +55.8° (*c* 1.08, chloroform); λ_{max}^{KBr} 2100 cm^{-1} (N_3).

Anal. Calc. for $C_{15}H_{19}N_3O_5$: C, 56.06; H, 5.96; N, 13.08. Found: C, 56.41; H, 5.51; N, 13.20.

Methyl 2-azido-2-deoxy-3-O-p-tolylsulfonyl-α-D-altropyranoside (**13**). — Compound **11** (1.5 g) was treated in *p*-dioxane (40 ml) containing aqueous sulfuric acid (3%, 10 ml) as described for the preparation of **4**, giving crude crystalline **13** which was recrystallized from water; yield 950 mg (78.5%), m.p. 134–136°, $[\alpha]_D^{23}$ +63.9° (*c* 2.69, ethyl alcohol); λ_{max}^{KBr} 3400–3300 (OH), 2100 (N_3), 1365, 1175 cm^{-1} (>SO_2).

Anal. Calc. for $C_{14}H_{19}N_3O_7S$: C, 45.04; H, 5.13; N, 11.26; S, 8.58. Found: C, 45.07; H, 5.09; N, 11.32; S, 8.59.

Methyl 2-azido-2-deoxy-3-O-methyl-α-D-altropyranoside (**14**). — Aqueous sulfuric acid (1.3% w/w, 9 ml) was added to a solution of **12** (800 mg), and the mixture was kept overnight at 55–60°. After neutralization with an excess of barium carbonate, the solution was evaporated *in vacuo* to afford syrupy **14**, which was chromatographed on silica gel (*ca.* 70 g), with cyclohexane–ethyl acetate (1:1 and then 2:3 v/v) as eluent; yield 400 mg (69%), $[\alpha]_D^{25}$ +67.9° (*c* 4.74, methanol); λ_{max} (film) 3450–3350 (OH), 2100 cm^{-1} (N_3).

Anal. Calc. for $C_8H_{15}N_3O_5$: C, 41.20; H, 6.48; N, 18.02. Found: C, 41.30; H, 6.30; N, 17.90.

1,6-Anhydro-2-azido-2-deoxy-3-O-p-tolylsulfonyl-β-D-altropyranose (**15**). — Compound **13** (1.5 g) was treated in hot benzene (240 ml) containing *p*-toluenesulfonic acid (1.5 g) as described for the preparation of **6**. Evaporation of the benzene solution afforded a yellow syrup that gave one spot on t.l.c. (chloroform–methanol, 94:6 v/v) and crystallized after several days. It was recrystallized from a mixture of ethyl acetate and *n*-hexane; yield 700 mg (52%), m.p. 86–87°, $[\alpha]_D^{26}$ −119° (*c* 2.74, chloroform); λ_{max}^{KBr} 3500 (OH), 2100 (N_3), 1360, 1180 cm^{-1} (>SO_2); n.m.r. data (see also Fig. 2): τ 4.59 (1-proton, incomplete doublet, $J_{1,2}$ 1.5 Hz, H-1), τ 5.46 (1-proton quartet, $J_{2,3}$ 9.6 Hz, $J_{3,4}$ 3.9 Hz, H-3), τ 5.65–5.90 (1-proton multiplet,

which collapsed to a quartet on addition of D_2O, $J_{3,4}$ 3.9 Hz, $J_{4,5}$ 2.7 Hz, H-4), τ 6.1–6.3 (2-proton, doublet, exo and endo H-6), τ 6.45 (1-proton quartet, $J_{1,2}$ 1.5 Hz, $J_{2,3}$ 9.6 Hz, H-2), τ 6.83 (1-proton doublet, which disappeared on addition of D_2O, $J_{4,OH}$ 4.5 Hz, OH).

Anal. Calc. for $C_{13}H_{15}N_3O_6S$: C, 45.75; H, 4.43; N, 12.31; S, 9.38. Found: C, 45.92; H, 4.36; N, 12.40; S, 9.33.

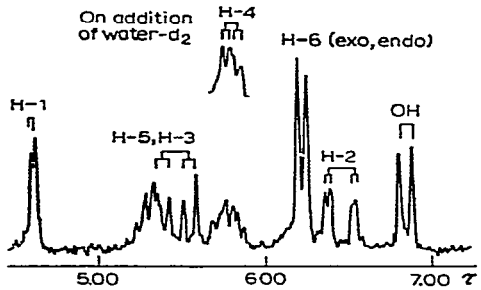


Fig. 2. The n.m.r. spectrum of 1,6-anhydro-2-azido-2-deoxy-3-*O*-*p*-tolylsulfonyl-β-D-altropyranose (**15**) in chloroform-*d* at 60 MHz.

1,6-Anhydro-2-azido-2-deoxy-3-O-methyl-β-D-altropyranose (**16**).—A suspension of **14** (2 g) and *p*-toluenesulfonic acid (2 g) in benzene (300 ml) was heated with stirring for 3 h at 80–90°. The mixture was washed with water (300 ml) after it had been cooled. The benzene solution was dried over anhydrous sodium sulfate and evaporated *in vacuo* to afford syrupy **16** (400 mg). The washing (water solution) was neutralized with an excess of barium carbonate and concentrated to dryness after filtration. The residue was extracted with acetone (120 ml) and evaporated *in vacuo*, giving additional **16** (700 mg); total yield 1.1 g (64%). The syrupy **16** was chromatographed on silica gel (80 g) with cyclohexane–ethyl acetate (3:2 v/v) as eluent; λ_{max} (solution in CCl_4) 3500 (OH), 2100 cm^{-1} (N_3); n.m.r. data: τ 4.64 (1-proton, singlet, H-1), τ 5.87–6.10 (1-proton, multiplet which collapsed to a quartet on addition of D_2O, $J_{3,4}$ = 4.5 Hz, $J_{4,5}$ = 2.4 Hz, H-4), τ 6.48 (3-proton, singlet, MeO), τ 7.19 (1-proton, doublet with 2.4 Hz splitting which disappeared on addition of D_2O, OH).

Anal. Calc. for $C_7H_{11}N_3O_4$: C, 41.79; H, 5.51; N, 20.89. Found: C, 41.60; H, 5.61; N, 20.88.

REFERENCES

1 A. K. Chatterjee, D. Horton, and J. S. Jewell, *Carbohyd. Res.*, 7 (1968) 212.
2 A. G. Cottrell, E. Buncel, and J. K. N. Jones, *Can. J. Chem.*, 44 (1966) 1483.
3 S. P. James, F. Smith, M. Stacey, and L. F. Wiggins, *J. Chem. Soc.*, (1946) 625.
4 A. K. Chatterjee, D. Horton, J. S. Jewell, and K. D. Philips, *Carbohyd. Res.*, 7 (1968) 173.
5 F. W. Lichtenthaler and T. Nakagawa, *Chem. Ber.*, 101 (1968) 1846.
6 E. E. van Tamelen, J. R. Dyer, H. E. Carter, J. V. Pierce, and E. E. Daniels, *J. Amer. Chem. Soc.*, 78 (1956) 4817.
7 N. K. Richtmyer and C. S. Hudson, *J. Amer. Chem. Soc.*, 57 (1935) 1716; 62 (1940) 961.
8 R. D. Guthrie and D. Murphy, *J. Chem. Soc.*, (1963) 5288.
9 J. Hill, L. Hough, and A. C. Richardson, *Carbohyd. Res.*, 8 (1968) 7.
10 D. Horton and J. S. Jewell, *Carbohyd. Res.*, 5 (1967) 149.
11 D. S. Mathers and G. J. Robertson, *J. Chem. Soc.*, (1933) 1076.

Carbohydrate Research
Elsevier Publishing Company, Amsterdam
Printed in Belgium

OXIDATIVE ALKALINE DEGRADATION OF CELLOBIOSE*

R. M. ROWELL**, P. J. SOMERS, S. A. BARKER, AND M. STACEY
Department of Chemistry, The University, Birmingham 15 (Great Britain)
(Received July 27th, 1968; in revised form, January 6th, 1969)

ABSTRACT

The nature and concentrations of products arising from the alkaline degradation of cellobiose were determined by varying the base, base concentration, sugar concentration, temperature, and atmosphere within the reaction vessel. In nitrogen, the oxidation reactions could effectively be eliminated. By introducing oxygen at various concentrations, oxidation reactions to aldonic acids predominated. The major acids found from the reducing end were 3,4-dihydroxybutyric acid and isosaccharinic acid, whilst D-arabinonic and glyceric acids were formed from the nonreducing end. In the stopping reaction, the major bound acids were 3-*O*-β-D-glycopyranosyl-D-arabinonic acid, 2-*O*-β-D-glucopyranosyl-D-erythronic acid, and 4-*O*-β-D-glucopyranosyl-D-mannonic acid. The yields of isosaccharinic and aldonic acids varied as the base and sugar concentrations were changed, and this clearly illustrated that the conditions of alkaline degradation determined the proportions of products obtained.

INTRODUCTION

Numerous papers have been concerned with the effects of alkali on carbohydrates, and several reviews have dealt with epimerizations to other sugars[1] or with rearrangements to isomeric saccharinic acids[2–4]. When cellobiose is treated with alkali in an inert atmosphere, α- and β-isosaccharinic acids[5–7] (3-deoxy-2-*C*-hydroxymethyl-D-*erythro*-pentonic acid and 3-deoxy-2-*C*-hydroxymethyl-D-*threo*-pentonic acid, respectively) are produced by a "peeling process". Thus, cellobiose is split into two fragments, isosaccharinic acids from the reducing end and D-glucose (hereafter referred to as liberated D-glucose) from the nonreducing end. Simultaneously, a "stopping reaction" occurs that involves the formation of a 3-deoxy-1,2-diketo intermediate (3-deoxyglucosone[4]) still connected by a β-(1→4)-linkage to D-glucose. The latter product is transformed by a benzilic acid type rearrangement

*Presented in part at the 156th National Meeting of the American Chemical Society, Atlantic City, Sept. 1968.
**Honorary Research Fellow, on study leave from Forest Products Laboratory, Madison, Wisconsin, U. S. A.

into α- and β-metasaccharinic acids[7-9] (3-deoxy-D-*ribo*-hexonic acid and 3-deoxy-D-*arabino*-hexonic acid, respectively) which render the molecule stable to alkali, thereby stopping the peeling process. In this case, D-glucose (hereafter referred to as bound D-glucose) remains as the nonreducing end. The acids which are linked to D-glucose after degradation are referred to as bound acids.

In oxygen, however, there occur quite different reactions[10]. When simple monosaccharides are treated with alkali in the presence of an oxidant, aldonic acids containing one carbon atom less than the starting sugars are obtained in good yield [9-16]. For example, D-glucose gives D-arabinonic acid, and D-ribose gives D-erythronic acid. Likewise, when disaccharides are treated in oxygen–alkali, aldonic acids bound to the nonreducing moiety were isolated [9,15-18]. For example, maltose lactose, and cellobiose all give bound D-arabinonic acid. Polysaccharides have also been reported to undergo this reaction [9,18], and cellulose was found to contain end units of D-arabinonic acid after treatment with polysulfide or sodium hydroxide in the presence of oxygen. Thus, two distinct types of reaction occur in alkali; rearrangements to isomeric saccharinic acids, and oxidations to aldonic acids. These two reactions were studied with cellobiose when the base, base concentration, sugar concentration, temperature, and atmosphere were varied.

EXPERIMENTAL

Reaction conditions. — To determine the effect of different cations on the alkaline degradation, cellobiose solutions (0.2 mg/ml) were treated in air-tight bottles in barium hydroxide (0.04N) or sodium hydroxide (0.04N) for 48 h at 25° and for 6 h at 50°. At the end of these times, the reaction mixture was neutralized with Dowex-50W x8 (H^+) resin and frozen. The samples were assayed manually with glucose oxidase[19] for liberated D-glucose and with 2-thiobarbituric acid[20,21] for peeled isosaccharinic acid. Other products were determined automatically as described later. The results are shown in Table I.

Degradations in which the atmosphere was varied were carried out in a 50-ml bubbling tower equipped with a reflux condenser and drying tube. The reactions under oxygen were done by bubbling oxygen into the reaction vessel throughout the degradation. Degradations in air were done in a closed vessel with no external gases added or removed. The degradations under nitrogen were performed by bubbling oxygen-free nitrogen (made by passing commercial nitrogen through a hot column of reduced copper) into the vessel during degradation. All of these degradations were in 0.04N barium hydroxide with a sugar concentration of 0.2 mg/ml. The time and temperature of reactions were as reported previously. The results are shown in Table II. Degradations at various concentrations of sugar and alkali are given in Table III.

Determination of products from alkaline degradation. — *(1) Ion-exchange separations. (A) Ammonium acetate.* Aliquots (5 ml) of the solution after degradation and neutralization were pumped (0.6 ml/min) by a peristaltic pump onto a column (0.6 × 30 cm) of Dowex-AG1 x8 (200–400 mesh) resin in the acetate form. The

TABLE I

ALKALINE DEGRADATION OF CELLOBIOSE IN AIR[a]

Temperature (degrees)	*Time (h)*	*Base*	*Liberated* D-*glucose*	*Bound* D-*glucose*	*Isosaccharinic acid*	D-*Arabinonic acid*	*3,4-Dihydroxybutyric acid*	*Glyceric acid*
25	48	$Ba(OH)_2$	14.4	4.2	15.6	10.2	11.9	3.5
50	6	$Ba(OH)_2$	8.1	1.7	23.4	15.2	12.5	12.4
25	48	NaOH	9.9	1.1	3.4	7.7	8.9	1.6
50	6	NaOH	0.5	0.9	8.5	14.9	25.8	12.2

[a]Percent yields given relative to cellobiose. Base concentration, 0.04N. Sugar concentration, 0.2 mg/ml.

TABLE II

DEGRADATION OF CELLOBIOSE BY BARIUM HYDROXIDE[a]

Atmosphere	*Temperature (degrees)*	*Time (h)*	*Liberated* D-*glucose*	*Bound* D-*glucose*	*Isosaccharinic acid*	D-*Arabinonic acid*	*3,4-Dihydroxybutyric acid*	*Glyceric acid*
N_2	25	48	9.9	trace	41.5	4.9	2.8	1.1
Air	25	48	14.4	4.2	15.6	10.2	11.9	3.5
O_2	25	48	13.1	17.8	13.1	21.4	15.5	3.2
N_2	50	6	5.0	0.0	39.5	1.6	1.1	1.9
Air	50	6	8.1	1.7	23.4	15.2	12.5	12.4
O_2	50	6	10.3	5.0	18.8	21.7	12.7	12.6

[a]Percent yields given relative to cellobiose. Base concentration, 0.04N. Sugar concentration, 0.2 mg/ml.

TABLE III

DEGRADATION OF CELLOBIOSE IN AIR WITH BARIUM HYDROXIDE[a]

Temperature (degrees)	*Time (h)*	*Base conc. (N)*	*Sugar conc. (mg/ml)*	*Liberated* D-*glucose*	*Bound* D-*glucose*	*Isosaccharinic acid*	D-*Arabinonic*	*3,4-Dihydroxybutyric acid*	*Glyceric acid*
25	48	0.04	0.2	14.4	4.2	15.6	10.2	11.9	3.5
50	6	0.04	0.2	8.1	1.7	23.4	15.2	12.5	12.4
25	48	0.04	2	7.4	4.7	7.0	9.8	5.4	1.5
50	6	0.04	2	0.9	11.5	30.4	3.2	9.6	3.4
25	48	0.4	0.2	8.1	33.4	0.9	9.0	4.1	1.5
50	6	0.4	0.2	9.0	15.6	21.6	5.0	5.6	1.4

[a]Percent yields given relative to cellobiose.

column was eluted with water (10 ml) followed by ammonium acetate (0.1M) to separate and elute the acids (see Table IV).

TABLE IV

RETENTION TIMES OF COMPOUNDS ON DOWEX AG-1 x8 (200–400 MESH)[a]

Compound	*Time to peak centre (min)*		*Response in*	*Periodate–pentane-2,4-dione assay*
	Standard	*Cellobiose*	*Cysteine–H_2SO_4 assay*	
Neutral sugars	30	+	+	+
Cellobionic acid	80	–	+	+
D-Glucose acids (no separation)	–	80	+	+
α,β-Isosaccharinic acids	95	+	–	+
D-Arabinonic acid	120	+	–	+
3,4-Dihydroxybutyric acid	122	+	–	+
Glyceric acid	150	+	–	+

[a]Acetate form, elution with ammonium acetate (0.1M).

(*B*) *Acetic acid*[22]. Aliquots (10 ml) of the solution after degradation and neutralization were concentrated to 0.5 ml and applied to a water-jacketed (30°) column (0.5 × 122 cm) of Dowex-AG1 x8 (200–400 mesh) resin in the acetate form.

TABLE V

RETENTION TIME OF COMPOUNDS ON DOWEX-AG1 x8 (200–400 MESH) RESIN[a]

Compound	*Time to peak centre (min)*			
	Standard	*Cellobiose*	*Peeled fraction*	*Bound fraction*
Neutral sugars	40	+	–	–
2-Deoxy-D-*erythro*-pentonic acid	135	–	–	trace
3,4-Dihydroxybutyric acid	165	+	+	–
Bound D-glucose-acid no. 1 (-D-mannonic acid)	...	245	–	–
α-Isosaccharinic acid	260	+	+	–
α-Metasaccharinic acid	290	–	–	–
Bound D-glucose-acid no. 2 (-D-arabinonic acid)	...	360	–	–
β-Metasaccharinic acid	400	–	–	–
D-Ribonic acid	455	–	–	–
Bound D-glucose-acid no. 3 (-D-erythronic acid)	...	525	–	–
D-Arabinonic acid	660	+	+	+
β-Isosaccharinic acid	660	+	+	–
D-Mannonic acid	810	–	–	+
D-Erythronic acid	820	+	–	+
D-Threonic acid	840	–	–	–
Glyceric acid	870	+	+	–

[a]Acetate form at 30°, elution with acetic acid (0.5M).

By using a positive displacement pump, the acids were separated and eluted with deaerated acetic acid (0.5N) at a flow rate of 0.8 ml/min (see Table V).

(*2*) *Automated detection of products.* The eluate from the bottom of the columns described above was split into three equal fractions. One part went to a fraction collector, and another was analysed by the automated periodate–pentane-2,4-dione assay[23] for released formaldehyde employing the Technicon Autoanalyser. The third part was analysed by the automated cysteine–sulphuric acid assay[24] for glucose. The colour developed in the two assays was simultaneously recorded at 520 nm. The amount of each compound was determined by comparison of areas under peaks relative to standard runs. For determination of the concentration of bound acids, a standard of cellobionic acid was used. All of the results given in Tables I, II, and III are reported in weight percentages based on the starting cellobiose.

(*3*) *G.l.c. analysis.* The collected, bound-acid fractions were hydrolyzed with hydrochloric acid (N, 2 ml) in a sealed tube for 18 h at 100°. Sodium hydroxide (N) was added until alkaline to ensure complete conversion into free acids. The solution was then pumped through a column (0.6 × 10 cm) of Dowex AG-1 x8 (200–400 mesh) resin in the acetate form. The column was washed with distilled water (10 ml) to remove neutral sugars, and then with ammonium acetate (M, 15 ml) to elute the acids. This solution was then passed through a column of Dowex-50W x8 (H^+) resin to remove the ammonium acetate, and hydrochloric acid (0.1N, 2 drops) was added to the effluent to ensure a low pH for complete conversion into lactones. After 6 h, the solution was concentrated to dryness, water (1 ml) was added, and the solution was again concentrated. This procedure was repeated four times, followed by a final concentration with ethanol–benzene (4/1 v/v). Trimethylsilyl ethers[25] of the lactones[26,27] were analysed by g.l.c. with a column (5 ft × 5 mm) of silicon ester (SE 30) (10%) on Celite (100–200 mesh) at 150°.

All other fractions were combined and passed through a column of Dowex 50-W x8 (H^+) resin, hydrochloric acid was added, and the mixture was worked up as given above. The results are given in Table VI.

DISCUSSION

The products observed from the alkaline degradation of cellobiose are consistent with the β-alkoxycarbonyl mechanism proposed by Isbell[28] (see Fig. 1). It has been shown that the 4-deoxy-2,3-hexodiulose (**5**) may be oxidized to 3,4-dihydroxybutyric acid and glycolic acid[29,30] and in addition undergoes the benzilic acid type rearrangement to isosaccharinic acid (**6**)[29–31]. Likewise, the 3-deoxy-D-*erythro*-hexosulose (**2**) has been rearranged to metasaccharinic acid (**3**)[32,33] and oxidized to 2-deoxy-D-*erythro*-pentonic acid[33], and this may explain the trace of bound 2-deoxy-D-*erythro*-pentonic acid found in the present degradation of cellobiose. Only trace amounts of bound acids were detected in the degradations performed under nitrogen, as compared with bound aldonic acids in the presence of oxygen, which illustrates the fact that the stopping reaction involving aldonic acids produces a greater percentage

TABLE VI

SEPARATION OF TRIMETHYLSILATED DERIVATIVES BY G.L.C.[a]

Compound	Time to peak centre (min)		
	Standard	Peeled fraction	Bound fraction
Glyceric acid	5.5	+	–
3,4-Dihydroxybutyro-1,4-lactone	3.0	+	–
3,4-Dihydroxybutyric acid	9.5	+	–
D-Erythrono-1,4-lactone	9.0	–	+
D-Erythronic acid	18.0	–	+
2-Deoxy-D-*erythro*-pentono-1,4-lactone	14.0	–	trace
D-Arabino-1,4-lactone	24.0	+	+
Isosaccharino-1,4-lactone			
alpha	31.0	+	–
beta	34.0	+	–
Metasaccharino-1,4-lactone	50.0	–	–
D-Mannono-1,4-lactone	112	–	+

[a]Column (5 ft × 4 mm) of SE30 (10%) on Celite (100–200 mesh) at 150°.

of bound materials. The bound aldonic acids were found to be stable under the conditions of degradation and once formed did not undergo further decomposition. The major bound acid is 3-*O*-*β*-D-glucopyranosyl-D-arabinonic acid with lesser amounts of 2-*O*-*β*-D-glucopyranosyl-D-erythronic acid and 4-*O*-*β*-D-glucopyranosyl-D-mannonic acid.

Several researchers have shown[34–36] that gluconic acid is not epimerized to mannonic acid in alkali at temperatures below 100°, so that the bound mannonic acid did not come from this source. D-Glucosone in alkali, however, gives good yields[37] of D-mannonic and D-arabinonic acids, and these two bound acids probably arise from this common intermediate. Thus, intermediate **1** can be converted into 4-*O*-*β*-D-glucopyranosyl-D-*arabino*-hexosulose which is then either rearranged to bound D-mannonic acid or oxidized to bound D-arabinonic acid. Part of the bound D-arabinonic acid may come directly from the oxidation of intermediate **1**. D-Erythronic acid was a product of the alkaline degradation of D-glucose[16, 18, 38] under conditions where arabinonic acid was stable, showing that erythronic acid does not arise from arabinonic acid. D-Erythronic acid was also found as a major product from the degradation of D-glucosone[37] in sodium hydroxide, and this may account for most of its production as a bound acid. It may also be formed, in part, from a bound pentose (D-arabinose) or from direct, oxidative cleavage of intermediate **4**.

Table I shows that percentages of the various products change with the type of base used. Divalent cations catalyse the benzilic acid type rearrangement[29,37,39], whereas monovalent cations favour fragmentation of the carbon skeleton. The yields of isosaccharinic acid are found to be higher in barium hydroxide than in sodium hydroxide, whereas the percentage of 3,4-dihydroxybutyric acid is higher in sodium hydroxide, especially at the higher temperature.

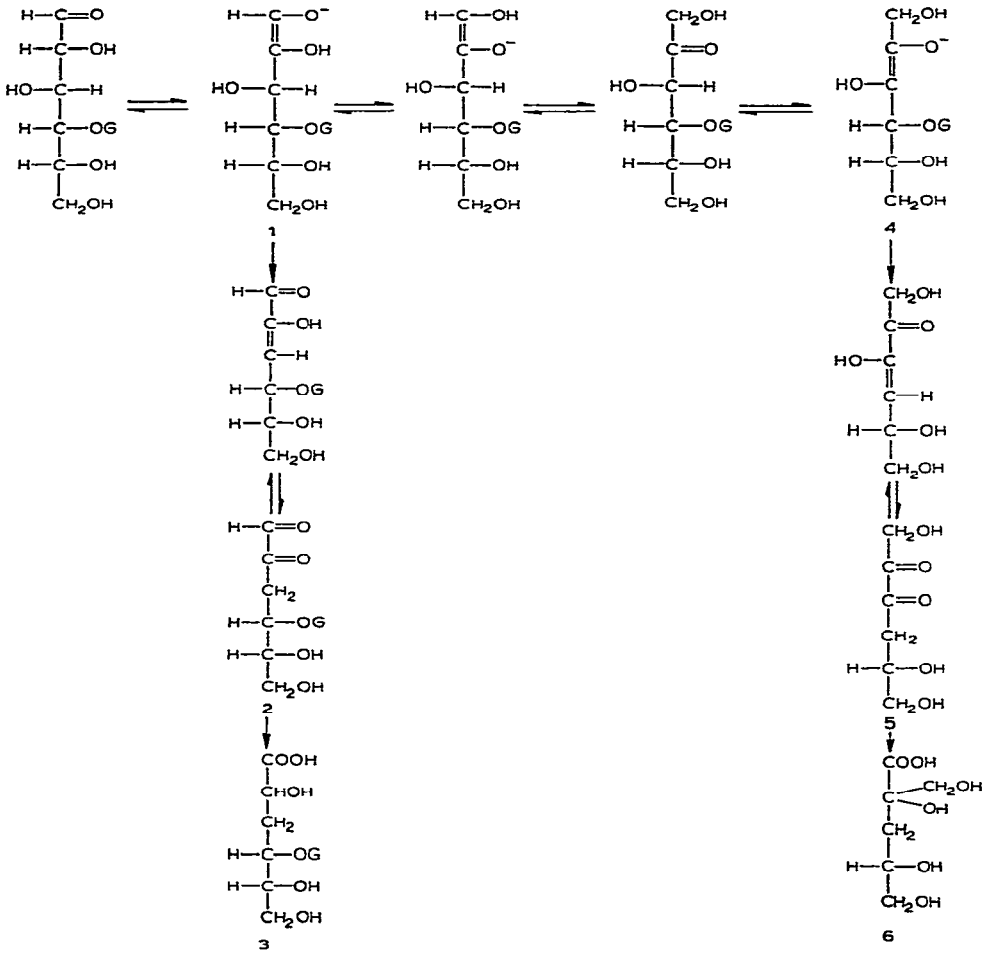


Fig. 1. Mechanism of the alkaline degradation of cellobiose.

Table II shows that the most-important, single factor in controlling the type of products in alkaline degradation is the oxygen concentration within the reaction vessel. The yields of isosaccharinic acid decrease as the oxygen content rises, whilst the yields of bound acids, arabinonic acid, and 3,4-dihydroxybutyric acid increase. It is only under nitrogen that the oxidation reactions can effectively be eliminated.

Finally, Table III shows how important the concentration of sugar and alkali are in the degradations. Isosaccharinic acid can almost be eliminated as a product of degradation by a tenfold increase in the alkali concentration. Of equal interest is the much greater yield of bound acids that are formed in preference to isosaccharinic acid under these conditions. This may be due to increased amounts of bound mannonic acid, which also arises by the benzilic acid type rearrangement[37].

ACKNOWLEDGMENT

The authors acknowledge the United States Forest Service, Forest Products Laboratory, for the Study authorisation granted under Public Law 85-507 which supported this research.

REFERENCES

1 J. C. SPECK, JR., *Advan. Carbohyd. Chem.*, 13 (1958) 63.
2 J. C. SOWDEN, *Advan. Carbohyd. Chem.*, 12 (1957) 35.
3 R. L. WHISTLER AND J. N. BEMILLER, *Advan. Carbohyd. Chem.*, 13 (1958) 289.
4 E. F. L. J. ANET, *Advan. Carbohyd. Chem.*, 19 (1964) 181.
5 S. V. HINTIKKA, *Ann. Acad. Sci. Fennicae*, 9 (1922) 3; *Chem. Zentr.*, I (1923) 296.
6 W. M. CORBETT AND J. KENNER, *J. Chem. Soc.*, (1955) 1431.
7 J. W. GREEN, *J. Amer. Chem. Soc.*, 78 (1956) 1894.
8 B. LINDBERG, O. THEANDER, AND J. E. UDDEGARD, *Svensk Papperstid.*, 69 (1966) 360.
9 P. AHLGREN, A. ISHIZU, I. SZABO, AND O. THEANDER, *Svensk Papperstid.*, in Press.
10 J. U. NEF, *Ann.*, 357 (1907) 214; 376 (1910) 1; 403 (1914) 204.
11 J. U. NEF, O. F. HEDENBURG, AND J. W. E. GLATTFELD, *J. Amer. Chem. Soc.*, 39 (1917) 1638.
12 O. SPENGLER AND A. PFANNENSTIEL, *Z. Wirtschaftsgruppe Zuckerind.*, 85 (1935) 546.
13 H. S. ISBELL, *J. Res. Natl. Bur. Stand.*, 29 (1942) 227.
14 E. HARDEGGER, K. KREIS, AND H. EL KHADEM, *Helv. Chim. Acta*, 34 (1951) 2343.
15 E. HARDEGGER, K. KREIS, AND H. EL KHADEM, *Helv. Chim. Acta*, 35 (1952) 618.
16 O. SAMUELSON AND L. THEDE, *Acta Chem. Scand.*, in press.
17 J. W. E. GLATTFELD AND M. T. HANKE, *J. Amer. Chem. Soc.*, 40 (1918) 973.
18 O. SAMUELSON AND L. THEDE, Tappi Second Dissolving Pulps Conference, New Orleans, June, 1968.
19 A. DAHLQVIST, *Biochem. J.*, 80 (1961) 547.
20 L. WARREN, *J. Biol. Chem.*, 234 (1959) 1971.
21 S. A. BARKER, A. R. LAW, P. J. SOMERS, AND M. STACEY, *Carbohyd. Res.*, 3 (1967) 435.
22 O. SAMUELSON AND L. THEDE, *J. Chromatog.*, 30 (1967) 556.
23 O. SAMUELSON AND H. STRÖMBERG, *Carbohyd. Res.*, 3 (1966) 89.
24 S. A. BARKER, R. G. JONES, A. R. LAW, AND P. J. SOMERS, *Carbohyd. Res.*, in press.
25 C. C. SWEELEY, R. BENTLEY, M. MAKITA, AND W. W. WELLS, *J. Amer. Chem. Soc.*, 85 (1963) 2497.
26 A. A. J. FEAST, B. LINDBERG, AND O. THEANDER, *Acta Chem. Scand.*, 19 (1965) 1127.
27 G. PETERSSON, H. RIEDL, AND O. SAMUELSON, *Svensk Papperstid.*, 70 (1967) 371.
28 H. S. ISBELL, *J. Res. Natl. Bur. Std.*, 32 (1944) 45.
29 M. J. BLEARS, G. MACHELL, AND G. N. RICHARDS, *Chem. Ind.* (London), (1957) 1150.
30 G. MACHELL AND G. N. RICHARDS, *J. Chem. Soc.*, (1960) 1932.
31 R. L. WHISTLER AND J. N. BEMILLER, *J. Amer. Chem. Soc.*, 82 (1960) 3705.
32 E. F. L. J. ANET, *J. Amer. Chem. Soc.*, 82 (1960) 1502; *Australian J. Chem.*, 14 (1961) 295.
33 G. MACHELL AND G. N. RICHARDS, *J. Chem. Soc.*, (1960) 1938.
34 B. ALFREDSSON, W. CZERWINSKY, AND O. SAMUELSON, *Svensk Papperstid.*, 64 (1961) 812.
35 P. H. ABENIUS, A. ISHIZU, B. LINDBERG, AND O. THEANDER, *Svensk Papperstid.*, 70 (1967) 612.
36 S. PETTERSSON AND O. SAMUELSON, *Svensk Papperstid.*, in press.
37 B. LINDBERG AND O. THEANDER, *Acta Chem. Scand.*, in press.
38 J. DUBOURG AND P. NAFFA, *Bull. Soc. Chim. France*, (1959) 1353.
39 G. MACHELL AND G. N. RICHARDS, *J. Chem. Soc.*, (1960) 1924.

Carbohydrate Research
Elsevier Publishing Company, Amsterdam
Printed in Belgium

INDUCED EFFECTS UPON THE SULPHATE METABOLISM OF ACIDIC MUCOPOLYSACCHARIDES OF HUMAN SKIN

S. A. BARKER AND J. F. KENNEDY

Department of Chemistry, The University of Birmingham, Birmingham 15 (Great Britain)

(Received June 12th, 1968; in revised form, January 3rd, 1969)

ABSTRACT

Factors affecting the incorporation of sulphate-^{35}S into the acidic mucopolysaccharides of human skin have been investigated, and conditions for maximal incorporation defined. Using these conditions, the effect of ascorbic acid, methyl sulphoxide, hydrocortisone, triparanol, and vitamin A upon the sulphate-^{35}S incorporation into each individual polysaccharide has been investigated, and the mode of action of these drugs is discussed.

INTRODUCTION

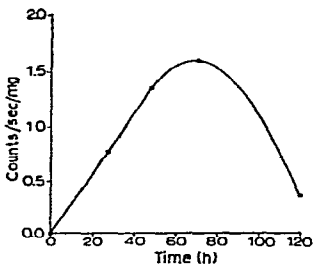
Characterisation of the acidic mucopolysaccharides[1] of human skin and the advent of radioactive incorporation methods for their identification on a nanogram scale[2] have made possible the investigation of induced effects upon their metabolism. The effects of ascorbic acid (vitamin C), methyl sulphoxide, hydrocortisone, triparanol {2-*p*-chlorophenyl-1-*p*-[2-(*N*,*N*-diethylamino)ethoxy]phenyl-1-*p*-tolylethanol}, and vitamin A are reported.

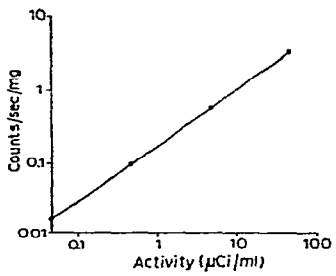
METHODS AND RESULTS

Determination of conditions for optimal incorporation. — Pieces (50 mg wet weight) of human skin from one sample were cultured in duplicate on balanced salt solution/horse serum media containing sodium sulphate-^{35}S as previously described[2]. In one series, the culture time was varied over the range 0–120 h, using normal, balanced salt solution and a medium having a specific activity of 50 μCi/ml. In the second, the specific activity of the medium was varied over the range 0–50 μCi/ml, using 24-h cultures and normal, balanced salt solution. In the third, the total concentration of sulphate was varied over the range 0–250 μg/ml, using 24-h cultures and a medium having a specific activity of 50 μCi/ml.

After treatment with liquid nitrogen for 10 min, the cultured tissue was dialysed against two changes of 0.1M sodium sulphate for 20 h at 20° to remove non-incorporated sulphate-^{35}S, washed in water, and air dried at 20° to constant weight. It was demonstrated that, for a particular sample of skin, the ratio of wet weight to

dry weight was constant, indicating the reliability of dry-weight measurement. Sulphate, liberated by hydrolysis conditions (10N hydrochloric acid for 16 h at 100°) which dissolved the whole tissue, was precipitated as barium sulphate-^{35}S in the presence of carrier barium sulphate, and the radioactivity was determined by automatic, planchet counting under conditions of infinite thickness (Figs. 1–3).





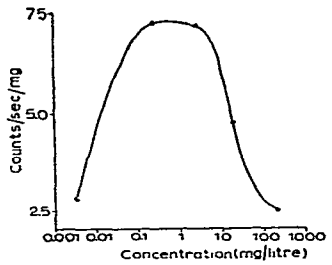


Fig. 1. Variation of incorporation with culture time.

Fig. 2. Variation of incorporation with specific activity of culture medium.

Fig. 3. Variation of incorporation with sulphate concentration of culture medium.

When culture time was varied, the viability of the tissue was estimated as described[2]. (The estimated degree of viability was 100% for the first 48 h but diminished as follows: 72 h, 90%; 96 h, 50%; 120 h, 10%.)

Investigation of induced effects. — Human skin was cultured for 24 h upon media having a total sulphate concentration of 22.26 μg/ml (normal, balanced salt solution) and a specific activity of 50 μCi/ml, to which had been added one of the drugs. The final concentrations were: ascorbic acid, 187; methyl sulphoxide, 1.178; hydrocortisone, 10; triparanol, 100; and vitamin A, 10 μM. The last three compounds were added as ethanolic solutions, the final concentration of ethanol being 0.1%; viability was not affected by this concentration of ethanol. Control cultures with tissue from the same samples were carried out in each case, and after establishment of viability, the epidermis and dermis were separated.

The dried, homogenised, defatted tissue was subjected to proteolysis, and the extraction procedure was completed by mild treatment with alkali, protein denaturation, and dialysis[1]. The polysaccharide solution was fractionated on De-Acidite FF resin, and the incorporation into each polysaccharide was determined by scintillation counting of the dialysed fractions. The results for both layers are expressed as a percentage of the incorporation into the control for each polysaccharide (Table I). Examples of the fractionation patterns are shown in Fig. 4. The following abbreviations have been used: chondroitin 4-sulphate, CS-4; chondroitin 6-sulphate, CS-6; dermatan sulphate, DS; heparin, HEP; keratan sulphate, KS; adenosine 3-phosphate 5-phosphosulphate, PAPS.

Approximately 1% of the available isotope was incorporated when 200 mg of tissue were cultured on 1 ml of medium under the conditions previously described. The extent of incorporation into the epidermis was approximately 22 nCi/mg and into the dermis was approximately 6 nCi/mg dry weight.

TABLE I

INDUCED EFFECTS

Drug	*Tissue layer*	*Incorporation (percentage of control)*						
		Total	*CS-4*	*CS-6*	*DS*	*HEP*	*KS*	*PAPS*
Ascorbic acid	epidermis	22	27	9	25	62	72	152
	dermis	24	32	7	13	36	—	121
Methyl sulphoxide	epidermis	10	9	7	19	9	7	20
	dermis	72	59	84	122	102	—	99
Hydrocortisone	epidermis	23	24	34	10	6	6	74
	dermis	87	70	53	428	95	—	102
Triparanol	epidermis	85	83	85	52	86	60	25
	dermis	140	104	184	244	105	—	136
Vitamin A	epidermis	139	129	185	109	170	150	85
	dermis	143	209	102	191	100	—	98

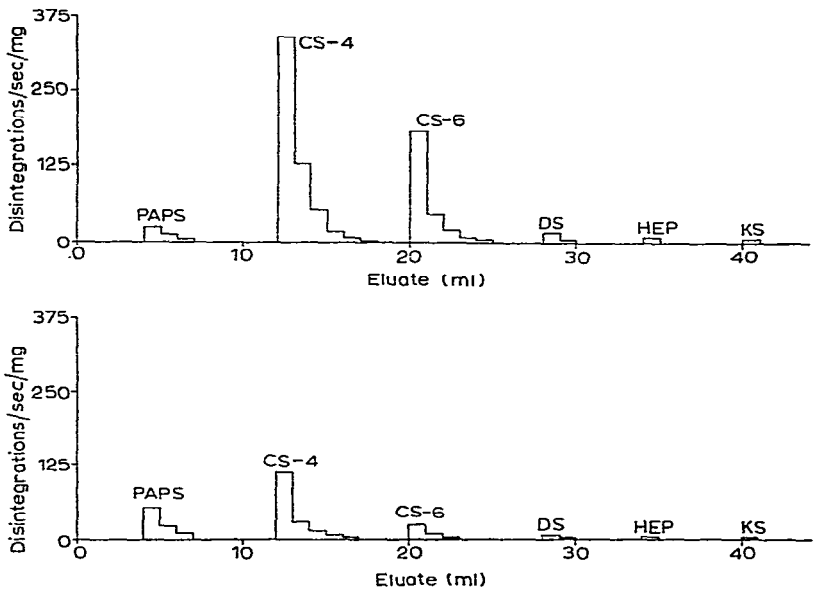


Fig. 4. Effect of ascorbic acid on incorporation of sulphate-^{35}S into the epidermal polysaccharides.

DISCUSSION

The reproducible results demonstrated that, whilst maximal incorporation was reached after 72 h, the tissue did not remain completely viable after 48 h. The

subsequent decrease in incorporation is explained by the further decrease in viability. Variation of the specific activity of the medium revealed that the incorporation was proportional to this factor. Incorporation could also be increased by a factor of 1.5 when a total sulphate concentration lower than that of normal, balanced salt solution was used. The culture conditions finally selected (medium: normal, balanced salt solution, 50 μCi of ^{35}S/ml; culture time, 24 h) were consistent with complete viability, normal growth of tissue, shorter time of culture, and maximal incorporation. The linearity of the relationships for incorporation/culture time and incorporation/specific activity of medium is advantageous, since, by correction, it allows comparison of results of different effects by using media differing slightly in these respects.

Ascorbic acid gave decreased incorporation in both epidermis and dermis, whereas incorporation into adenosine 3-phosphate 5-phosphosulphate was increased. This is consistent with decreased formation of polysaccharide chain and concomitant accumulation of the activated form of sulphate.

Of all the drugs investigated, methyl sulphoxide gave the most-striking result, in that it markedly inhibited incorporation into the epidermal polysaccharides. In both layers, the effect upon adenosine 3-phosphate 5-phosphosulphate was similar to the carbohydrate effect. It would appear that the drying, cracking, and scaling of skin resulting from the topical application of methyl sulphoxide[3] is associated with its effect upon polysaccharide metabolism, since the polysaccharides are concerned with tissue structure.

As found for other tissue[4], hydrocortisone generally decreased incorporation into the polysaccharides. There was less effect upon incorporation into the activated form of sulphate. Although hydrocortisone penetrates some parts of the epidermis slowly, it penetrates the dermis quite easily[5], and this may result in the increased incorporation into dermal dermatan sulphate, the polysaccharide associated with the fibrous lattice.

Triparanol has been used to relieve hypercholesterolaemia but has been withdrawn from clinical use, since its side effects included dry skin, cataract, and hair abnormalities. The present results indicate that the drug is at least responsible for a change in mucopolysaccharide metabolism. Such a change would be expected to be associated with abnormal tissue growth.

Vitamin A increased polysaccharide incorporation in both layers, consistent with the increased incorporation[4] and polysaccharide formation[6] found elsewhere. The activated form of sulphate maintained its normal level under this condition.

This work illustrates the value of the method for investigation of induced effects upon mucopolysaccharide metabolism and its applicability to the pre-clinical trial of drugs.

ACKNOWLEDGMENTS

The authors thank Professor M. Stacey C. B. E., F. R. S., for his interest, and Dr. C. N. D. Cruickshank for advice. This work was supported by a Medical Research Council research scholarship to J. F. K.

REFERENCES

1 S. A. BARKER, J. F. KENNEDY, AND P. J. SOMERS, *Carbohyd. Res.*, 10 (1969) 57.
2 S. A. BARKER, J. F. KENNEDY, AND C. N. D. CRUICKSHANK, *Carbohyd. Res.*, 10 (1969) 65.
3 D. M. BACKMAN, D. R. BOTTOMLY, AND Y. SHIMAZAKI, *Arthritis Rheumat.*, 7 (1964) 725.
4 S. A. BARKER, C. N. D. CRUICKSHANK, AND T. WEBB, *Exptl. Cell Res.*, 35 (1964) 255.
5 R. J. FELDMAN AND H. I. MAIBACH, *Arch. Derm.*, 91 (1965) 661.
6 S. R. PELC AND H. B. FELL, *Exptl. Cell Res.*, 19 (1960) 99.

Carbohydrate Research
Elsevier Publishing Company, Amsterdam
Printed in Belgium

DÉTERMINATION PAR RÉSONANCE MAGNÉTIQUE NUCLÉAIRE DU DEGRÉ DE SUBSTITUTION DANS LES MÉTHYL D-GLUCOPYRANOSIDES PARTIELLEMENT MÉTHYLÉS

DIDIER GAGNAIRE ET LÉON ODIER

(*Laboratoire de Chimie Organique Physique du Centre d'Études Nucléaires de Grenoble, et Centre de Recherches sur les Macromolécules Végétales (C. N. R. S.), CEDEX 53, 38-Grenoble-Gare (France)*

(Reçu le 27 janvier 1969)

ABSTRACT

In order to establish a method of determination, by n.m.r., of the degree of methylation at each position in mixtures of partially methylated methyl glycopyranosides, the chemical shift for each of the ten signals due to the methyl groups of methyl 2,3,4,6-tetra-*O*-methyl-α- and β-D-glucopyranoside has been determined. The chemical shift for each methyl group in both anomers was obtained by selectively deuterating the other four methyl groups. Five peaks corresponding to the five methyl groups were observed for each anomer in the solvent mixture benzene–chloroform (6:1, v/v).

The method was tested on the product of hydrolysis of a tri-*O*-methylcellulose, and the peaks observed corresponded to those for methyl groups at O-2, O-3, and O-6. Examination of the product of partial methylation of methyl β-D-glucopyranoside indicated that the degree of methylation at positions 2, 3, 4, and 6 was 86%, 40%. 28%, and 64%, respectively.

SOMMAIRE

Dans le but de mettre au point une méthode de dosage par r.m.n. du degré de substitution des groupes méthyles sur chaque position, dans des mélanges de méthyl glucopyranosides partiellement méthylés, nous avons déterminé, en r.m.n., les déplacements chimiques des dix signaux des groupes méthyles des méthyl 2,3,4,6-tetra-*O*-méthyl-α- et β-D-glucopyranosides. Cette détermination a été faite pour chaque anomère et pour chacune des cinq positions, en deutériant sélectivement les quatre autres. Le solvant utilisé, permettant d'observer en r.m.n. cinq pics méthyles pour chaque anomère, est un mélange de benzène et de chloroforme deutériés (6:1 en volume).

Un contrôle effectué à l'aide des produits d'hydrolyse d'une tri-*O*-méthylcellulose permet de vérifier que les pics observés correspondent bien aux groupes méthyles en positions 2, 3 et 6. Un dosage effectué sur le méthyl β-D-glucopyranoside partiellement méthylé sur les autres positions, donne les pourcentages de méthylation suivants: 88%, 40%, 28%, 64% pour les positions respectives 2, 3, 4 et 6.

INTRODUCTION

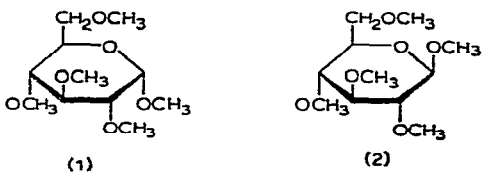
Le dosage des différents sucres obtenus par hydrolyse de polysaccharides partiellement méthylés se fait par la méthode classique d'isolement par chromatographie quantitative des différentes espèces obtenues et leur dosage par voie chimique[1].

Le but de ce travail est la mise au point d'une méthode de dosage par r.m.n. du degré de substitution des groupes méthyles, sur chaque position, dans des mélanges de méthyl D-glucopyranosides partiellement méthylés, sans séparation de ceux-ci. Il s'agit de produits de méthylation partielle des méthyl α- et β-D-glucopyranosides.

Le principe de l'analyse est simple: tout groupe hydroxyle non méthylé est remplacé par un groupe OCD_3. Le dosage de l'intensité des pics des groupes méthyles, après séparation éventuelle des deux isomères, donne alors le pourcentage de substitution sur chaque position avant substitution du OCD_3. Une méthode analogue a été appliquée par Goodlett[2] dans le cas de l'acétate de cellulose par acétylation sélective et totale de chaque position au chlorure d'acétyle deutérié.

Cette méthode exige, pour s'appliquer aux deux cas précédents:

1) La recherche d'un solvant r.m.n. pour lequel les cinq pics méthyles sont distincts dans les spectres de r.m.n. des méthyl 2,3,4,6-tétra-*O*-méthyl-α-D- (**1**) et β-D-glucopyranosides (**2**).



(1) (2)

2) L'identification non ambiguë des cinq pics dans les spectres de r.m.n. (Figs. 1 et 2) de ces deux substances (**1**) et (**2**).

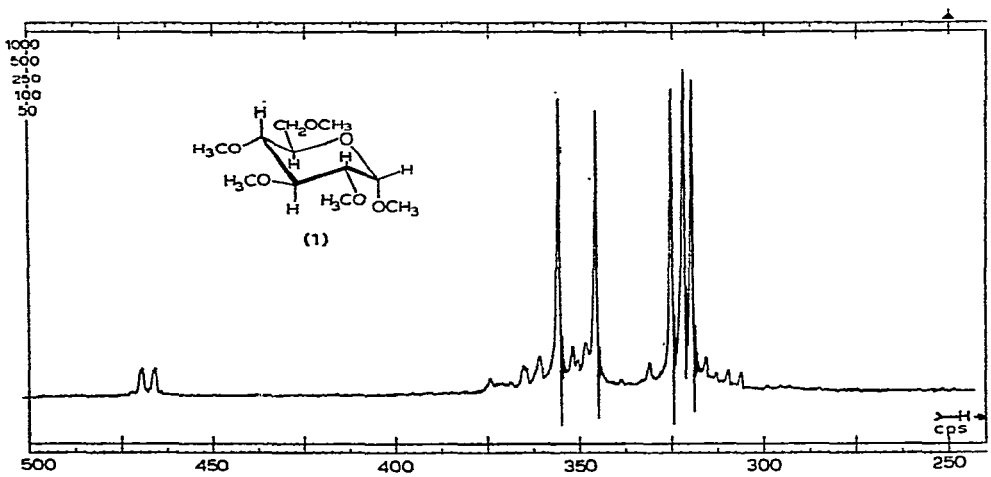


Fig. 1. Spectre de r.m.n. réalisé à 100 MHz dans le mélange benzène–chloroforme-*d* (6:1) du méthyl 2,3,4,6-tétra-*O*-méthyl-α-D-glucopyranoside.

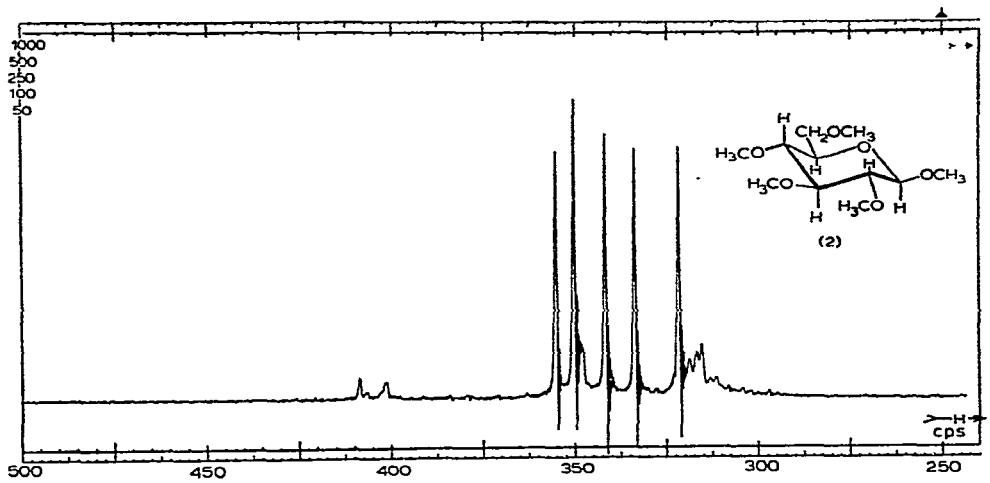


Fig. 2. Spectre de r.m.n. réalisé à 100 MHz, dans le mélange benzène–chloroforme-*d* (6:1) du méthyl 2,3,4,6-tétra-*O*-méthyl-β-D-glucopyranoside.

3) Une méthode rapide et totale de méthylation des méthyl α- et β-D-glucopyranosides partiellement méthylés.

La méthylation totale peut se réaliser par la méthode de Kuhn *et al.*[3] en utilisant ICD_3 (commercial) comme agent méthylant. Aucun solvant courant ne répond à la première condition, mais le mélange de benzène et de chloroforme deutériés (dans le rapport en volume 6:1) permet d'observer cinq pics bien séparés dans les spectres de r.m.n. réalisés à 100 MHz des méthyl 2,3,4,6-tétra-*O*-méthyl-α- et β-D-glucopyranosides (**1**) et (**2**) (Fig. 1 et 2).

Enfin l'identification des cinq pics de chaque anomère a été effectuée par synthèse de huit dérivés sélectivement deutériés, certains de ces composés ont été préparés par Kochetkov et collaborateurs[4] pour l'interprétation des spectres de masse des glycosides.

Les pics des groupes méthyles en position 1 sont caractérisés par méthylation avec l'iodure de méthyle deutérié (ICD_3) des méthyl α- et β-D-glucopyranosides: on obtient ainsi les deux méthyl tétraméthyl-d_3-α- et β-D-glucopyranosides.

L'identification du groupe méthyle en position 6 a été faite en perméthylant, par l'iodure de méthyle deutérié (ICD_3), le 6-*O*-méthyl-D-glucose obtenu par la méthode de Hough, Jones et Magson[5], conduisant ainsi aux deux méthyl-d_3 6-*O*-méthyl-2,3,4-tri-*O*-méthyl-d_3-α- et β-D-glucopyranosides.

L'attribution des signaux du groupe méthyle en position 3 a été faite d'une façon analogue en perméthylant par l'iodure de méthyle deutérié (ICD_3) le 3-*O*-méthyl-D-glucose préparé par la méthode classique[6], conduisant ainsi aux deux méthyl-d_3 3-*O*-méthyl-2,4,6-tri-*O*-méthyl-d_3-α- et β-D-glucopyranosides.

Enfin, le 2,3-diméthyl-D-glucose commercial, perméthylé par l'iodure de méthyle deutérié (ICD_3) nous a permis d'identifier les pics des méthyles en position 2. On obtient ainsi les deux méthyl-d_3 2,3-di-*O*-méthyl-4,6-di-*O*-méthyl-d_3-α- et β-D-glucopyranosides.

Dans tous les cas, les mélanges d'anomères obtenus ont été séparés par chromatographie préparative sur couche mince. L'attribution des signaux du groupe méthyle en position 4 résulte des identifications précédentes. Tous les groupes méthyles ont donc été identifiés de façon non ambiguë.

On peut alors dresser la liste des déplacements chimiques, comptés à partir du tétraméthylsilane, des signaux des groupes méthyles dans les spectres de r.m.n., réalisés dans le mélange benzène et chloroforme deutériés (6:1 en volume), des méthyl 2,3,4,6-tétra-*O*-méthyl-α- et β-D-glucopyranosides* (**1**) et (**2**) (Tableau I).

TABLEAU I

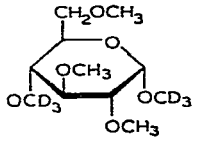
DÉPLACEMENTS CHIMIQUES DANS LES SPECTRES DE R.M.N. DES SIGNAUX DES GROUPES MÉTHYLES DES MÉTHYL 2,3,4,6-TÉTRA-O-MÉTHYL-α- ET β-D-GLUCOPYRANOSIDES[a]

Anomère	*Groupes méthyles en position*				
	1	*2*	*3*	*4*	*6*
α	3,20	3,26	3,56	3,46	3,22
β	3,34	3,50	3,56	3,42	3,21

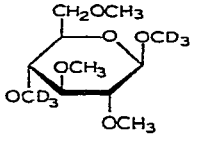
[a]Valeurs (δ) mesurées en p.p.m. à partir du tétraméthylsilane comme standard dans le mélange benzène–chloroforme (6:1).

Ces résultats sont bien illustrés par le spectre du produit d'épimérisation du méthyl 2,3,4,6-tétra-*O*-méthyl-α-D-glucoside (Fig. 3).

Un contrôle a été effectué à partir des 1,4-di-*O*-acétyl-2,3,6-tri-*O*-méthyl-α- et β-D-glucopyranoses obtenus par acétolyse d'une tri-*O*-méthylcellulose préparée dans ce laboratoire. Après perméthylation par le sulfate de méthyle deutérié $(CD_3)_2SO_4$, on obtient les deux méthyl-d_3 2,3,6-tri-*O*-méthyl-4-*O*-méthyl-d_3-α et β-D-glucopyranosides (**3**) et (**4**) qui ont été isolés.



(3)



(4)

Sur leurs spectres de r.m.n. (Fig. 4) les pics ont été désignés en fonction des numéros des carbones du cycle glucosidique. On voit sur ces figures que des spectres réalisés à 60 MHz donnent (quoique moins bien résolus qu'à 100 mHz) des pics distincts pour les groupes méthyles en position 2, 3 et 6 pour chaque anomère.

*Pour l'anomère α, Casu et collaborateurs[7] ont donné une identification identique pour les pics des groupes méthyles obtenus dans $CDCl_3$ et le Me_2SO, mais les pics des groupes méthyles en *O*-1 et 6 sont alors confondus.

La méthode de dosage recherchée résulte immédiatement des résultats précédents. Si l'on a un mélange de méthyl α- ou β-D-glucosides partiellement méthylés en position 2, 3, 4 et 6, le pourcentage de méthylation sur chaque position s'obtient ainsi :

méthylation totale par ICD_3 (voir partie expérimentale);

réalisation du spectre de r.m.n. du mélange anomérique obtenu dans le mélange de benzène et de chloroforme précédemment défini (de préférence à 100 MHz) et intégration des neuf pics méthyles.

L'intensité des pics correspondant aux groupes méthyles en position 1 donne le pourcentage des anomères α et β, l'intensité des autres pics par rapport à ces deux pics donne le pourcentage de méthylation de chacune des positions 2, 3, 4, et 6 dans le mélange initial. Seuls les pics du groupe méthyle en position 3 sont confondus pour les deux anomères (voir Fig. 3) et l'on ne pourra déterminer que le pourcentage

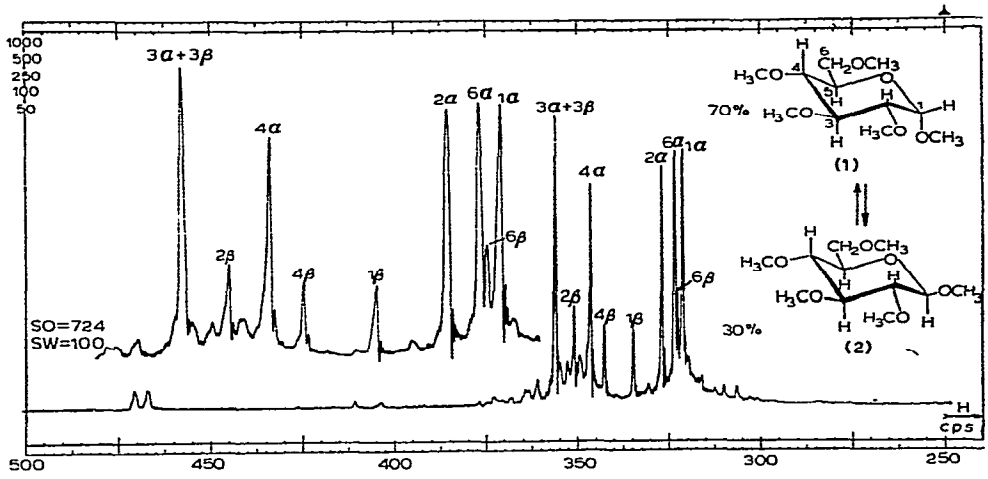


Fig. 3. Spectre de r.m.n. réalisé à 100 MHz, du mélange obtenu par épimérisation du méthyl 2,3,4,6-tétra-*O*-méthyl-α-D-glucopyranoside.

relatif à l'ensemble des deux anomères pour la position 3, sauf si l'on sépare les deux anomères. Par contre si l'on isole chaque forme anomérique, on pourra obtenir le pourcentage de méthylation sur chaque position pour chaque anomère. Certains pics des groupes méthyles se trouvant dans la même zone que ceux des protons des cycles, une correction peut être effectuée à l'aide des spectres des méthyl 2,3,4,6-tétra-*O*-méthyl-d_3-α- et β-D-glucopyranosides.

Un exemple d'application est donné ci-dessous : le méthyl β-D-glucopyranoside a été partiellement méthylé par la méthode de Purdie[8] qui utilise l'iodure de méthyle en présence d'oxyde d'argent dans le méthanol à 45°. Le mélange obtenu (16 isomères au maximum) est totalement méthylé par ICD_3 selon la méthode de Kuhn et Trischmann[3] dans la *N*,*N*-diméthylformamide en présence d'oxyde d'argent à température ambiante. On obtient ainsi du méthyl 2,3,4,6-tétra-*O*-méthyl-D-glucopyranoside partiellement deutérié. Son spectre de r.m.n. réalisé dans le solvant précédemment défini (Fig. 5) permet de doser, par l'intensité des pics méthyles, le pourcentage de

substitution sur chaque position du produit initial. Le pic du groupe méthyle en position 1 qui correspond à une position entièrement substituée sert de référence.

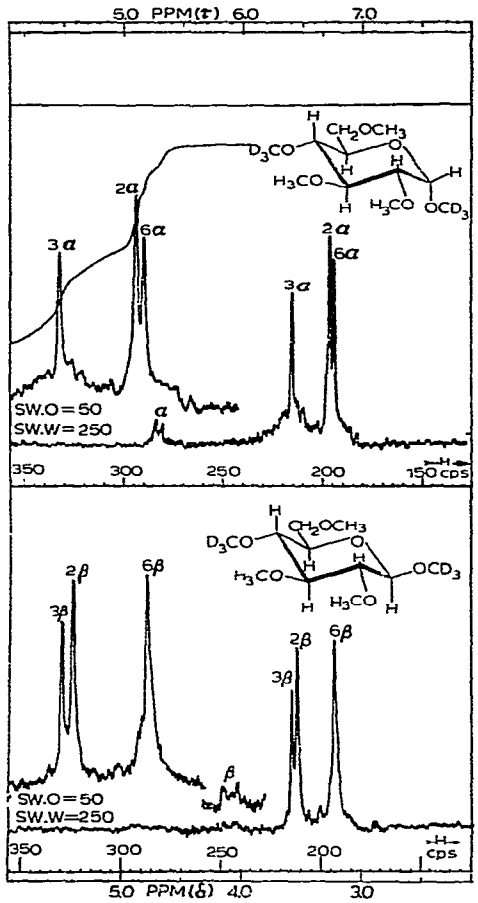


Fig. 4. Spectres de r.m.n. réalisés à 60 MHz, dans le mélange benzène–chloroforme-*d* (6:1) des méthyl-d_3 2,3,6-tri-*O*-méthyl-4-*O*-méthyl-d_3-α et β-D-glucopyranosides.

Les résultats obtenus, 88% de méthylation pour la position 2, 40% de méthylation pour la position 3, 28% de méthylation pour la position 4 et 64% de méthylation pour la position 6, sont conformes à la réactivité pour chaque position.

L'analyse de l'intensité des pics se fait en tenant compte du spectre d'un échantillon où les positions 2, 3, 4 et 6 sont substituées par OCD_3, c'est-à-dire le méthyl 2,3,4,6-tétra-*O*-méthyl-d_3-β-D-glucopyranoside obtenu par épimérisation du méthyl 2,3,4,6-tétra-*O*-méthyl-d_3-α-D-glucopyranoside dans le méthanol acide comme l'a fait Capon[9].

Cette méthode de dosage des groupes méthyles peut constituer une technique très utile dans des études cinétiques de méthylation ou de déméthylation du glucose

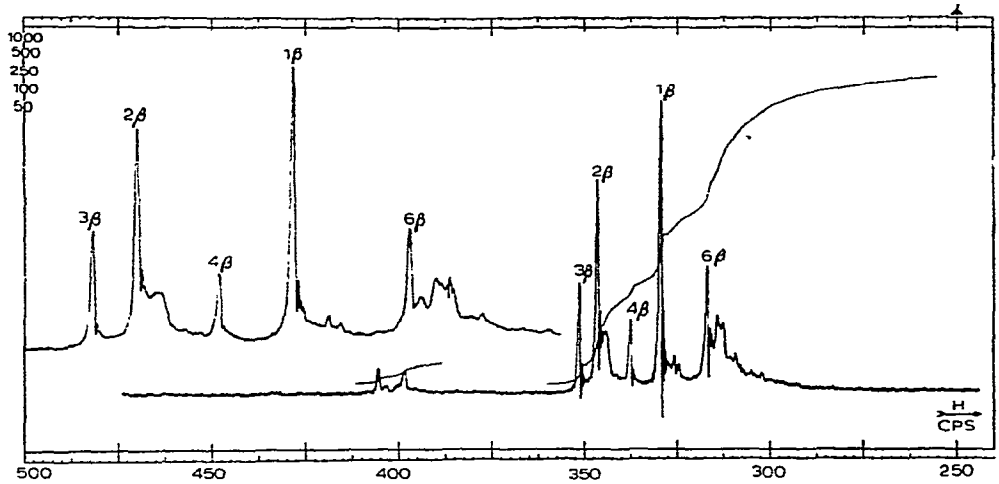


Fig. 5. Spectre de r.m.n. réalisé à 100 MHz, dans le mélange benzène–chloroforme-*d* (6:1) du méthyl 2,3,4,6-tétra-*O*-méthyl-β-D-glucopyranoside partiellement deutérié.

et des glucosides. Elle permet également l'identification immédiate d'un isomère partiellement méthylé des méthyl α- ou β-D-glucopyranosides ou de tester sa pureté. Mais son utilisation principale est dans le domaine des polymères contenant des unités glucosidiques partiellement méthylées en simplifiant considérablement l'analyse des mélanges complexes qui peuvent résulter de leur hydrolyse en conduisant, après perméthylation par ICD_3, à un composé unique (ou deux, compte tenu des formes anomériques). Cette méthode de dosage par voie physique des groupes méthyles est à l'abri de certaines critiques formulées à l'égard des méthodes habituelles.

PARTIE EXPÉRIMENTALE

Méthylation totale. — Les expériences de méthylation sont toutes conduites selon la méthode de Kuhn et Trischmann[3]. Pour obtenir la méthylation avec des groupements OCD_3; on utilise l'iodure de méthyle deutérié commercial (Centre d'Études Nucléaires de Saclay, Service des Isotopes Stables).

Chromatographie préparative des méthyl 2,3,4,6-tétra-O-méthyl-α- et β-D-glucopyranosides (**1** et **2**). — Les couches de silice sont déposées sur des plaques de verre de format 20 cm × 20 cm, selon la technique décrite par Stahl. Pour 5 plaques, on utilise 60 g de silice-G Merck et 120 ml d'eau distillée; les plaques sont ensuite séchées à l'étuve à 130–140° pendant plusieurs heures (activité Brockmann voisine de 11).

Le mélange (50 mg) obtenu par méthylation du D-glucose commercial est dilué par 2 ml de chloroforme. Cette solution est déposée sur la plaque à 2 cm environ du bord inférieur, en une bande continue, obtenue par un jet d'une pipette de verre préalablement étirée. La plaque est ensuite éluée verticalement dans un bac rectangulaire par 100 ml d'un mélange de benzène contenant 4% de méthanol.

Lorsque le front de l'éluant arrive à environ 3 cm du bord supérieur de la plaque, celle-ci est retirée et séchée. La plaque est révélée en y pulvérisant une solution de 3,5,7,2′,4′-pentahydroxyflavone à 1% dans le méthanol. Elle est ensuite éclairée dans une chambre noire par une lampe UV. On observe une fluorescence à l'emplacement des produits. On observe ainsi 4 zones (A, B, C, D, à partir du front) qui sont éluées séparément par 20 ml de chloroforme. On obtient ainsi un sirop (Zone A; 29 mg) qui par trituration dans le pentane conduit à un produit blanc cristallisé (24 mg), le méthyl 2,3,4,6-tétra-*O*-méthyl-*β*-D-glucopyranoside, p.f. 40°, $[\alpha]_D^{20}$ −16°, (*c* 0,8, méthanol), puis un sirop légèrement ambré (Zone B; 6 mg), le méthyl 2,3,4,6-tétra-*O*-méthyl-α-D-glucoside, qui présente un $[\alpha]_D^{20}$ +142° (*c*, 0,73, méthanol). On obtient pour les deux anomères un rendement global de 65% pour l'ensemble des opérations de perméthylation et séparation. La zone C contenait uniquement de la *N*,*N*-diméthylformamide (c.g.l. et r.m.n.) et la zone D fortement colorée, toutes les impuretés du produit brut.

Anomérisation du méthyl 2,3,4,6-tétra-O-*méthyl*-d^3-α-D-*glucoside.* — Le méthyl 2,3,4,6-tétra-*O*-méthyl-d_3-α-D-glucopyranoside (85 mg) est dissous dans 10 ml de méthanol à 70° et 1 g de résine Amberlite IR-120 (H^+), préalablement activée sous forme acide, est ajouté. La suspension est agitée pendant 20 h en maintenant la température à 70°. La résine est éliminée par filtration et lavée plusieurs fois par du méthanol à 70° que l'on joint au filtrat. Le méthanol est évaporé sous pression réduite, le spectre de r.m.n. du mélange d'anomérisation montre que 30% environ de l'anomère α ont été convertis en *β*. Ce mélange est chromatographié sur couche mince de silice par la méthode précédemment décrite : on obtient d'une part le méthyl 2,3,4,6-tétra-*O*-méthyl-d_3-*β*-D-glucopyranoside (18 mg) et d'autre part l'analogue α (44 mg).

Acétolyse d'une tri-O-*méthylcellulose et perméthylation* (d_3) *des produits d'acétolyse.* — La tri-*O*-méthylcellulose (610 mg) est introduite dans un mélange de 12,5 ml d'acide acétique, 12,5 ml d'anhydride acétique et 3,5 ml d'acide sulfurique concentré (d 1,84). On agite pendant 140 h à température ambiante. Le mélange réactionnel est dilué par 100 ml d'eau distillée, neutralisé par le carbonate de sodium, puis extrait par le benzène en continu pendant 72 h. L'évaporation du benzène conduit à un sirop brun (325 mg) que la r.m.n. permet d'identifier à un mélange de 1,4-di-*O*-acétyl-2,3,6-tri-*O*-méthyl-α- et *β*-D-glucoses. Ce produit (215 mg) est dissous dans l'acétone (5 ml) et traité à 60° par 1,35 g de sulfate de méthyle deutérié $[(CD_3)_2SO_4]$ et 3,3 ml d'une solution aqueuse d'hydroxyde de sodium à 30% rajoutée par portions de 1/10 toutes les 10 min. La température est maintenue à 60° ainsi que l'agitation pendant 4 h, puis le mélange est gardé une nuit à température ambiante. L'acétone est chassée du mélange réactionnel et le sulfate de sodium qui précipite est redissout par un peu d'eau distillée. La solution aqueuse est extraite par le chloroforme (3 × 50 ml). Les extraits de chloroforme sont lavés successivement par une solution de carbonate de sodium, une solution saturée de chlorure de sodium et séchés sur sulfate de sodium. L'évaporation du chloroforme conduit à un sirop brun (99 mg) que la r.m.n. permet d'identifier aux deux méthyl-d_3 2,3,6-tri-*O*-méthyl-4-*O*-méthyl-d_3-α- et *β*-D-gluco-

pyranosides. Les deux anomères ont été isolés par chromatographie préparative sur couche mince par la méthode précédemment décrite.

RÉFÉRENCES

1 R. L. WHISTLER, *Methods Carbohyd. Chem.*, 3 (1963) 280.
2 V. W. GOODLETT, J. T. DOUGHERTY ET H. W. PARRON, *Abstracts Papers Amer. Chem. Soc. Meeting*, 152 (1966) E27, et communication personnelle.
3 R. KUHN, A. TRISCHMANN ET I. LÖW, *Angew. Chem.*, 67 (1955) 32.
4 N. K. KOCHETKOV ET O. S. CHIZHOV, *Tetrahedron*, 21 (1965) 2029.
5 L. HOUGH, J. K. N. JONES ET M. S. MAGSON, *J. Chem. Soc.*, (1952) 1525.
6 W. L. GLEN, G. S. MAYERS ET G. A. GRANT, *J. Chem. Soc.*, (1951) 2568.
7 B. CASU, M. REGGIANI, G. G. GALLO ET A. VIGEVANI, *Tetrahedron*, 24 (1968) 803.
8 T. PURDIE ET J. C. IRVINE, *J. Chem. Soc.*, 83 (1903) 1021.
9 B. CAPON, *Chem. Commun.*, 1 (1967) 21.

Note ajoutée à la correction des épreuves. — « Une méthode analogue dans des dérivés acétylés du glucose a été réalisée par D. HORTON et Coll. (*J. Org. Chem.*, 34 (1969) 86 et *Abstracts, 154th National Meeting of the Amer. Chem. Soc., Chicago, Ill.*, Sept. 1967, EO28) ».

Carbohydrate Research
Elsevier Publishing Company, Amsterdam
Printed in Belgium

AN ANALYTICAL STUDY OF GUM EXUDATES FROM THE GENUS *Araucaria* JUSSIEU (GYMNOSPERMAE)

D. M. W. ANDERSON AND A. C. MUNRO
Department of Chemistry, The University, Edinburgh EH9 3JJ (Great Britain)
(Received November 5th, 1968; in revised form, January 30th, 1969)

ABSTRACT

Analytical data are presented for the gum polysaccharides isolated from the resinous exudates from five *Araucaria* species; for three of these, two specimens from different geographical locations have been studied. The data are compared with those available for the only *Araucaria* species examined previously. The results indicate that the analytical differences between species of this Gymnosperm genus are not so great as are known to occur in genera of the Angiospermae.

INTRODUCTION

Chemical studies and comparisons of plant gums have tended, in the past, to reflect an academic interest; their infrequent application in other sciences has indicated that such studies should preferably be conducted and reported on a broader basis.

Chemical comparisons and classifications of plant gums have been made[1,2] on the basis of their oligosaccharide structural units, and general types of gum structure have been compared with the botanical classification of the parent plant at the level of Order and Family[3]. This paper presents an analytical comparison of some gum polysaccharides from the genus *Araucaria* in relation to its botanical divisions; certain correlations on such a basis have recently been proposed[4] for *Acacia* gum exudates.

The genus *Araucaria* (Family, Araucariaceae; Class, Coniferales; Sub-division, Gymnospermae) contains fourteen species[5]. The polysaccharide found in the resinous exudate from *Araucaria bidwillii* has received detailed attention[6,7]; the presence of a polysaccharide in this exudate was first noted by Birch[8]. The presence of a gum polysaccharide in the resin from *A. angustifolia*[9,10] and in a water-soluble fraction of *A. araucana* resin[11] have also been observed. The terpenoid material in *Araucaria* resins has received little attention.

The taxonomy of the *Araucaria* genus is shown in Fig. 1; the genus is divided into three sections on the basis[5] of the morphological characters of the species (Fig. 2). The exudates from five *Araucaria* species, representative of its *Colymbea* and *Eutacta* Sections, have been studied and compared in terms of the accepted

botanical divisions of the genus. Previous studies[6,7] have indicated a large variation in the composition of two different Australian samples of *A. bidwillii* gum. A specimen of this gum from a different geographical source has therefore been examined. In addition, for each of the species *A. araucana*, *A. cunninghamii*, and *A. heterophylla*, two specimens from different sources were also obtained for comparison, in an attempt to ascertain whether the extent of the variation reported[6,7] for *A. bidwillii* gum is a general feature of the genus *Araucaria*.

EXPERIMENTAL

The sources of the exudates studied are detailed below; the full botanical nomenclature involved has been discussed elsewhere[12].

A. araucana I: from a single tree, Camperdown Park, Dundee (collected April 1968). *A. araucana* II: from four trees, Royal Botanic Gardens, Edinburgh (collected November 1967). *A. bidwillii*: from a single tree, Kew Gardens, London (collected November, 1967). *A. columnaris*: from a single tree, Lushoto Arboretum, Tanganyika (collected October, 1962). *A. cunninghamii* I: from a single tree, Kew Gardens, London (collected November, 1967). *A. cunninghamii* II: from a single tree, Longuza, Tanganyika (collected March, 1968). *A. heterophylla* I: from a single tree, Royal Botanic Gardens, Edinburgh (collected February, 1968). *A. heterophylla* II: from a single tree, Tanga, Tanganyika (collected March, 1968).

Extraction and purification of polysaccharides. — Each of the exudates was extracted with cold ethanol (6 × 2-litre portions for *A. araucana* II and *A. columnaris*; 6 × 800-ml portions for the other samples). The residual material was dried, extracted with cold water (to give a *ca.* 2% solution), filtered, dialysed for 48 h, and freeze-dried; in each case, a small proportion of water-insoluble material remained, and it was extracted with 1% aqueous sodium borohydride[13] for 24 h. The borohydride extracts were dialysed and freeze-dried; generally, this fraction contained *ca.* 1% of the total material extracted. For *A. araucana*, however, the borohydride fraction contained *ca.* 5% of the total, extractable material; this fraction (denoted *A. araucana* III) obtained from *A. araucana* sample II was studied in addition to the water-soluble polysaccharides. After isolation, several of the polysaccharides gave cloudy, aqueous solutions which could not be clarified by filtration or centrifugation, probably because trace amounts of resin remained from the ethanol extractions; such solutions were clarified by passage through "Millipore" filters (pore-size 1.2 μm).

Moisture contents were assessed by drying samples to constant weight at 105°. Nitrogen determinations were carried out by a semi-micro Kjeldahl method. Viscosity measurements were made at 25.0° in suspended-level, Ubbelohde, dilution viscometers with polysaccharide concentrations of 4–2% (sequential dilutions) in M sodium chloride. Ultracentrifugation (Beckman "Spinco" Model E ultracentrifuge) was carried out at 44,770 r.p.m. with 0.5% solutions in 0.5M sodium chloride; photographs were taken every 16 min.

Weight-average molecular weights ($\overline{M}_w$) were determined at 25° and 516 nm

Fig. 1. Taxonomy of the genus *Araucaria*[5] (the taxonomy of the *Encephalartos* and *Welwitschia* genera is included for comparison).

A. *COLYMBEA* Endlicher.
A. angustifolia (Bertoloni) O. Kuntze (syn. *A. brasiliensis* A. Richard; Parana Pine; Candelabra Tree).
A. araucana (Molina) K. Koch (syn. *A. imbricata* Pavon; Chile Pine; Monkey Puzzle).
A. bidwillii Hooker (Bunya-bunya).

B. *INTERMEDIA* C.T. White.
A. klinkii Lauterbach.

C. *EUTACTA* Endlicher.
A. balansae Brongniart and Grisebach (syn. *A. elegans* Hort.)
A. beccarii Warburg [syn. *A. cunninghamii* Becarrii (non D. Don); *A. cunninghamii* var. *papuana* Laut.]
A. bernieri Buchholz.
A. biramulata Buchholz.
A. columnaris (Forster) Hooker (syn. *A. cookii* R. Brown ex Lindley).
A. cunninghamii D. Don (Hoop Pine, Moreton Bay Pine).
A. heterophylla (Salisbury) Franco (syn. *A. excelsa* (Lambert) R. Brown; Norfolk Island Pine).
A. humboldtensis Buchholz.
A. muelleri Brongniart and Grisebach.
A. rulei Ferdinand von Mueller (syn. *A. niepratschki* Baumann ex Pynaert; *A. van gaertii* Hort. ex Dallimore).

Fig. 2. Botanical division of the genus *Araucaria* Jussieu[5].

with a "SOFICA" Photogoniodiffusometer. The average of three determinations was taken for solutions (0.2, 0.15, and 0.1% in M sodium chloride) clarified by passage through "Millipore" filters of pore-sizes 0.45 and 0.22 μm.

Molecular-sieve chromatography was carried out on a "Biogel P300" column (47 × 5 cm) precalibrated with dextran fractions[14] of known $\overline{M}_n$. Polysaccharides (15 mg in 1 ml of 2M sodium chloride) were applied carefully at the top of the column; elution was with M sodium chloride. Fractions (2.1 ml) were collected, and examined by the phenol–sulphuric acid method[15]. The values reported for $\overline{M}_n$ correspond to the apex of the main peak. Several of the polysaccharides showed a second, smaller peak at or near the void volume of the column; this value is reported as "$\overline{M}_n$ (second peak)". Because of the method of calibration used, the values obtained for $\overline{M}_n$ cannot be taken as absolute values.

Uronic acid contents, assessed as "hexuronic anhydride", were determined by acid decarboxylation and an infrared technique[16,17].

Chromatography was carried out on Whatman Nos. 1 and 3MM papers, in the following solvent systems: (*a*) benzene–butyl alcohol–pyridine–water (1:5:3:3, upper layer); (*b*) ethyl acetate–pyridine–water (10:4:3); (*c*) acetic acid–ethyl acetate–formic acid–water (3:18:1:4); (*d*) butyl alcohol–ethanol–0.1M phosphoric acid (1:10:5); (*e*) butyl alcohol–ethanol–0.1M hydrochloric acid (1:10:5). Systems (*d*) and (*e*) were developed from a thin-layer technique[18] for the separation of uronic acids; Whatman No. 1 papers which had been pretreated with 0.3M sodium dihydrogen phosphate and then air-dried were used, and good separations of glucuronic acid from galacturonic acid were obtained.

Chromatograms were developed by spraying with a saturated solution of aniline hydrogen oxalate (ethanol–water, 1:1), and then heating for *ca.* 5 min at 120°.

Thin-layer electrophoresis was carried out on "Millipore Phoroslides" in 0.05M ammonium carbonate and 0.05M borate buffers. Polysaccharide solutions (0.5–1.0% in buffer) were applied as a thin band 2 cm from the cathode-end of the strip. Electrophoresis was carried out for 10 min in a "Phoroslide" cell, in conjunction with a Shandon "VOKAM" power unit (model 2541), at a potential gradient of 50 volts along the strip. The polysaccharide bands were located by a modification of the periodate–rosaniline hydrochloride technique[19].

Hydrolyses were effected by heating the polysaccharide (150 mg) with N or 2N sulphuric acid (40 ml) for 7.5 h on a boiling water-bath. The solutions were cooled, neutralised ($BaCO_3$), deionised (Amberlite IR-120 resin, H^+ form) and concentrated to syrups. The 2N acid hydrolysates were examined for uronic acids in solvents (*c*), (*d*), and (*e*); the N acid hydrolysates were examined for neutral sugars in solvents (*a*), (*b*), and (*c*), and for aldobiouronic acids in solvent (*c*). All of the polysaccharides studied showed chromatographic evidence for galactose, arabinose, rhamnose, small proportions of xylose and 3-*O*-methylrhamnose, and the acidic disaccharides 6-*O*-(*β*-D-glucopyranosyluronic acid)-D-galactose and 6-*O*-(4-*O*-methyl-*β*-D-glucopyranosyluronic acid)-D-galactose. Glucuronic acid and its 4-*O*-methyl analogue were the only uronic acids found.

Periodate oxidations of solutions of the polysaccharides (1.5% in 0.25M sodium metaperiodate) were carried out in darkness at room temperature. The formic acid released was estimated by titrating aliquots at intervals with 0.01N sodium hydroxide; the reduction of periodate was estimated after 96 h by back-titration with standard sodium arsenite[20].

The analytical data obtained are recorded in Table I.

DISCUSSION

There have been few studies of gymnosperm exudates, undoubtedly due to the relatively small number of taxa comprising the Gymnospermae. The exudates from *Encephalartos longifolius*[21], *E. latefrons*[22], and *Welwitschia mirabilis*[22] (see Fig. 1) have shown a high degree of complexity, and all contain 3-*O*-methylrhamnose as a component. The presence of this sugar in *A. bidwillii* gum was not previously noted[6,7]. This study indicates that 3-*O*-methylrhamnose is a component of all gymnosperm gums examined to date; since this comparatively rare sugar has not been found in any of the gums from Angiosperms examined so far, it may be a useful taxonomic marker[12].

The gum exudates from different species of Angiospermae genera, *e.g.*, *Acacia*[4,23], *Albizia*[24,25], and *Prunus*[2,26,27] frequently vary quite widely in terms of composition and molecular properties. The *Araucaria* specied studied here are, in comparison, much more closely alike in terms of their analytical parameters. It is particularly interesting that the specimens of *A. heterophylla* from Edinburgh and from Tanganyika should be so closely alike; the same holds for the specimens of *A. cunninghamii* from Kew and from Tanganyika. The main exception to this concerns

TABLE I

ANALYTICAL DATA FOR SPECIES IN *Colymbea* AND *Eutacta* SECTIONS OF THE GENUS *Araucaria*

	Colymbea *species*						Eutacta *species*				
	A. araucana I	*A. araucana* II	*A. araucana* III	*A. bidwillii*	*A. bidwillii*[6]	*A. bidwillii*[7]	*A. columnaris*	*A. cunninghamii* I	*A. cunninghamii* II	*A. heterophylla* I	*A. heterophylla* II
Yield (%)											
Ethanol fraction	81	71	—	18	—	—	41	74	80	67	82
Water fraction	19	24	—	82	—	—	59	25	20	33	18
Borohydride fraction	n.d.[f]	5	—	1	—	—	<1	1	n.d.	<1	n.d.
Moisture (%)	5.8	7.6	10.7	5.4	—	—	10.2	6.8	5.1	6.1	4.7
Specific rotation[a,b] (degrees)	−1.9	−0.7	+11.0	−1.7	+11	−9.5	− 6.8	−1.0	−8.8	−6.3	−4.8
Nitrogen (%)	0.81	0.91	2.50	0.74	—	—	0.28	0.52	0.30	0.48	0.69
Protein (%N × 6.25)	5.1	5.9	16.0	4.7	—	—	1.8	3.3	1.9	3.1	4.4
Intrinsic viscosity[a] (cm^3/g)	16.8	15.1	24.6	12.6	—	—	10.5	16.5	9.8	11.4	13.0
$\overline{M}_w \times 10^3$ [a]	800	300	1,100	185	—	—	145	150	173	120	190
$\overline{M}_n \times 10^3$ [c]	300	49	49	60	—	—	58	57	60	76	60
$\overline{M}_n \times 10^3$ (2nd peak)[c]	—	300	300	*ca.* 130	—	—	—	—	*ca.* 120	—	*ca.* 120
Ultracentrifuge (boundary shape)[d]	AS	AS	AS	AS	—	AS	S	S	AS	S	AS
Electrophoresis (no. components)[e]	2	2	2	1t	—	1	1	1t	1t	1t	1t
Formic acid released[a,b] (mmoles/g)	3.85	3.95	2.20	3.80	4.05[g]	—	4.05	3.85	4.45	4.95	4.55
Periodate reduced[a,b] (mmoles/g)	8.80	9.18	8.53	8.10	9.03[g]	—	8.65	8.08	9.10	10.50	9.45
Uronic anhydride[a,b] (%)	11	10	10	12	10	18	10	10	10	10	11
Galactose (%)	66	67	49	62	72[g]	59[g]	69	64	65	63	60
Arabinose (%)	14	14	33	16	13[g]	15[g]	13	13	13	17	20
Xylose (%)	1	1	1	2	—	trace	1	1	1	2	2
Rhamnose (%)	6	7	7	8	5[g]	8[g]	7	5	5	8	7
3-*O*-Methylrhamnose (%)	2[h]	1[h]	trace	trace	—	—	trace	7[h]	6[h]	trace	trace

[a]Corrected for moisture. [b]Corrected for protein. [c]Average molecular weight by molecular-sieve chromatography with reference to dextran samples of defined $\overline{M}_n$. [d]AS, asymmetric; S, symmetric. [e]t, tailing. [f]n.d. = not done. [g]Value converted from that reported in the literature. [h]Approximate value, based on the calibration curve for L-rhamnose.

the composition reported[6,7] for the two Australian specimens of *A. bidwillii* gum. Paleo-botanical studies indicate[28] that the *Araucaria* genus is much older in origin than Angiosperm genera, and there is histological evidence[29] that it may be in an evolutionary decline. This may account for the small size of the genus, and it may be expected that the surviving species are similar, the more extreme members having become extinct.

The low intrinsic viscosity of the polysaccharides in comparison to $\overline{M}_w$ indicates that the molecules are likely to be globular, rather than rod-shaped[30]. The relatively high ratios of $\overline{M}_w$ to $\overline{M}_n$ indicate a broad range of molecular weight. This is supported by molecular-sieve chromatography, where broad elution peaks were obtained, and several of the samples studied showed small peaks at or near the void volume of the column (denoted "$\overline{M}_n$ second peak" in Table I). Such samples showed slightly asymmetric boundaries on ultracentrifugation, and this second component of higher molecular-weight may be responsible for the higher $\overline{M}_w$ values obtained. Although this evidence tends to indicate the presence of two polysaccharide components, this feature was not shown by the specimens from *A. columnaris*, *A. cunninghamii* I, and *A. heterophylla* I (see Table I). Elution patterns involving 2 peaks have been given[31] by column chomatography of freeze-dried, purified *Acacia* gums on "Sepharose 4B"; these *Acacia* gums are considered to be homogeneous[14,32], and it is significant that the component of apparently higher molecular-weight was not found[31] when the gums concerned were examined before freeze-drying. The possible occurrence of a form of molecular aggregation during the purification and freeze-drying processes cannot therefore be overlooked. It is of interest, however, that, for *A. bidwillii*, *A. cunninghamii* II, and *A. heterophylla* II gums, the higher molecular-weight peak occurred within the molecular-sieving range of the column.

The borohydride-solubilised material, *A. araucana* III, differs from *A. araucana* II in several respects. The large difference in molecular weight observed is in agreement with the effect observed[13] previously for *Acacia drepanolobium* gum. The other main differences between *A. araucana* II and *A. araucana* III, involving the arabinose and protein contents, may have arisen from borohydride extraction of the bark and other debris present in the water-insoluble material.

Calculations of the ratios of neutral sugars assumed that the uronic acid residues were attached solely to galactose, since chromatography of the N acid hydrolysates in solvent (*c*) showed components having the same mobility as reference specimens of the aldobiouronic acids 6-*O*-(*β*-D-glucopyranosyluronic acid)-D-galactose, and 6-*O*-(4-*O*-methyl-*β*-D-glucopyranosyluronic acid)-D-galactose; these acids have been characterised in *A. bidwillii* gum[6], and, as evidence for other aldobiouronic acids was not found, this assumption was presumed to be valid. The possibility that xylose arises as an artefact of hydrolysis and subsequent neutralisation can be discounted; a mixture (in the proportions found) of galactose, arabinose, rhamnose, glucuronic acid, and 4-*O*-methylglucuronic acid was subjected to the conditions of hydrolysis used, and no xylose resulted.

The extent and nature of the heterogeneity previously found[6,7] for *A. bidwillii*

gum would not be expected to be revealed by the techniques used here. The thin-layer electrophoresis experiments indicate that *A. araucana* gum contains two acidic components; all other gums showed one component. This basic differentiation of *A. araucana* gum from the others studied is difficult to explain, although the other species examined, with the exception of *A. columnaris*, showed a slight tendency to "tailing". This phenomenon, under the conditions used by us, has not been observed for *Acacia* or *Prunus* gums, and, although possibly an experimental artefact, the tailing could be due to a second, incompletely resolved, minor component.

From a chemotaxonomic point of view, there are no apparent, large differences between the species in the *Colymbea* and *Eutacta* sections of the genus *Araucaria*, although the *Colymbea* species show slightly higher specific rotations and protein content, and a smaller release of formic acid on periodate oxidation. Further studies directed to discovering the nature of any fine structural differences involved are in progress.

ACKNOWLEDGMENTS

We thank the Science Research Council for a maintenance award (to A.C.M.). We thank Professor R. Brown, F.R.S., for putting a Spinco Ultracentrifuge at our disposal. We are grateful to Mr. D. R. Lyamuya (Tanga), Mr. R. L. Willans (Lushoto), and the Curators of the Royal Botanic Gardens at Kew and Edinburgh for supplying specimens of *Araucaria* resins.

REFERENCES

1 G. O. ASPINALL, *Actes du Symposium International de Grenoble*, July 1964, pp. 89 and 421.
2 E. L. HIRST, 4th International Congress of Biochemistry, Vienna, 1958, Symposium No. 1, Reprint No. 3.
3 E. A. C. L. E. SCHELPE AND A. M. STEPHEN, *S. African Ind. Chemist*, 18 (1964) 12.
4 D. M. W. ANDERSON AND I. C. M. DEA, *Phytochemistry*, 8 (1969) 167.
5 W. DALLIMORE AND A. B. JACKSON, *Handbook of Coniferae and Ginkgoaceae*, 4th Edn., revised S. G. HARRISON, Arnold, 1966.
6 G. O. ASPINALL AND R. M. FAIRWEATHER, *Carbohyd. Res.*, 1 (1965) 83.
7 G. O. ASPINALL AND J. P. MCKENNA, *Carbohyd. Res.*, 7 (1968) 244.
8 A. J. BIRCH, *J. Proc. Roy. Soc. N. S. Wales*, 71 (1938) 259; *Chem. Abstr.*, 32 (1938) 7665 (9).
9 J. L. RANJEL AND H. S. SCHNEIDER, *Rev. Brasil. Chim.* (São Paulo), 2 (1936) 261; *Chem. Abstr.*, 31 (1937) 1522 (8).
10 J. L. RANJEL AND H. S. SCHNEIDER, *Ministerio trabalho ind. com., Inst. nacl. tech., Bol. informacoes*, 7 (1936); *Chem. Abstr.*, 31 (1937) 2028 (3).
11 G. WEISSMANN AND K. BRUNS, *Naturwissenschaften*, 52 (1965) 185.
12 D. M. W. ANDERSON AND A. C. MUNRO, *Phytochemistry*, 8 (1969) 633.
13 D. M. W. ANDERSON AND I. C. M. DEA, *Carbohyd. Res.*, 8 (1968) 440.
14 D. M. W. ANDERSON AND J. F. STODDART, *Carbohyd. Res.*, 2 (1966) 104.
15 M. DUBOIS, K. A. GILLES, J. R. HAMILTON, P. A. REBERS, AND F. SMITH, *Anal. Chem.*, 28 (1956) 350.
16 D. M. W. ANDERSON, *Talanta*, 2 (1959) 73.
17 D. M. W. ANDERSON, S. GARBUTT, AND S. S. H. ZAIDI, *Anal. Chim. Acta*, 29 (1963) 39.
18 Y. S. OVODOV, E. V. EUTUSHENKO, V. A. VASKOVSKY, R. G. OVODOVA, AND T. F. SOLOV'EVA, *J. Chromatog.*, 26 (1967) 111.

19 A. B. S. CONACHER AND D. I. REES, *Analyst*, 91 (1966) 55.
20 R. BELCHER, G. DRYHURST, AND A. M. G. MACDONALD, *J. Chem. Soc.*, (1965) 3964.
21 A. M. STEPHEN AND D. C. DEBRUYN, *Carbohyd. Res.*, 5 (1967) 267.
22 M. KAPLAN, A. M. STEPHEN, AND D. VOGT, *S. African Med. J.*, 40 (1966) 702.
23 D. M. W. ANDERSON AND K. A. KARAMALLA, *J. Chem. Soc.*, (1966) 762.
24 D. M. W. ANDERSON AND I. C. M. DEA, *Advan. Frontiers Plant Sci.*, 23 (1969) 169.
25 D. M. W. ANDERSON, G. M. CREE, J. J. MARSHALL, AND S. RAHMAN, *Carbohyd. Res.*, 2 (1966) 63.
26 G. GUZMAN, A. SOLER, AND M. MAYOL, *Rev. Espan. Fisiol.*, 16 (1960) 239.
27 J. ROSIK AND J. KUBALA, *Collection Czech. Chem. Commun.*, 33 (1968) 1946.
28 A. C. SEWARD, *Fossil Plants*, Vol. 4, Hafner, New York, 1963.
29 F. NYSTERAKIS AND S. QUINTIN, *Compt. Rend.*, 240 (1955) 658.
30 B. JIRGENSONS, *Organic Colloids*, Elsevier, Amsterdam, 1958, p. 145.
31 D. M. W. ANDERSON, I. C. M. DEA, AND A. C. MUNRO, *Carbohyd. Res.*, 9 (1969) 363.
32 D. M. W. ANDERSON, I. C. M. DEA, AND SIR EDMUND HIRST, *Carbohyd. Res.*, 7 (1968) 320.

Carbohydrate Research
Elsevier Publishing Company, Amsterdam
Printed in Belgium

NITROGEN-CONTAINING CARBOHYDRATE DERIVATIVES

PART XXI*. PERIODATE OXIDATION OF METHYL AMINO-4,6-*O*-BENZYLIDENE-DEOXY-α-D-GLYCOSIDES HAVING THE *allo*, *manno*, AND *gluco* CONFIGURATIONS

C. B. BARLOW AND R. D. GUTHRIE**
School of Molecular Sciences, University of Sussex, Brighton BN1 9QJ (Great Britain)
(Received January 15th, 1969)

ABSTRACT

A study has been made of the periodate oxidation of methyl amino-4,6-*O*-benzylidene-deoxy-α-D-glycosides in the *allo*, *gluco*, and *manno* series. The results are compared with those for the oxidation of the corresponding diols and with those for cuprammonium complexing.

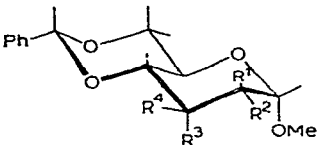
INTRODUCTION

Interest in the chemistry of the amino sugars has been stimulated to a large degree by their widespread distribution as structural components of many naturally occurring substances. Although the periodate oxidation of carbohydrates and their derivatives is of great use in structure investigation[1], little work has been done on amino sugars. In view of the anomalous results of Weiss *et al.*[2], the application of periodate oxidation to the determination of the ring-size of amino-sugar derivatives is of doubtful value. Also, in contrast to that for vicinal diols, the mechanism of the periodate oxidation of vicinal amino-alcohols has received very little attention. McCasland and Smith[3] found that the relative rates of oxidation of *cis*- and *trans*-2-aminocyclohexanol could not be readily distinguished, and that *cis*-2-aminocyclopentanol was oxidised approximately four times faster that the *trans* isomer. To account for the pH dependence of the rate, the assumption was made that only the free, amino group reacted with periodate, and that the protonated form did not. Kovár and his co-workers[4] carried out the first detailed study of the kinetics of the oxidation as a method for the determination of relative configuration of stereoisomers of vicinal amino alcohols. A mechanism, analogous to that for diol oxidations, involving an intermediate complex was proposed. An intermediate complex was also proposed by Dahlgren and Hodsdon[5] for the oxidation of 2-aminoethanol, by analogy with ethylene glycol.

To obtain a fuller understanding of the periodate–amino alcohol reaction, the oxidation of the series of amino-sugar derivatives **1**–**6** has been studied; the

*Part XX: C. B. Barlow, R. D. Guthrie, and A. M. Prior, *Carbohyd. Res.*, 10 (1969) 481–485.
**To whom enquiries should be addressed

oxidation of compounds 7 and 8, which complete the series, will be described elsewhere. The methyl 4,6-*O*-benzylidenehexopyranoside system was chosen in order that there should be only one possible site of oxidation; all of the compounds have one vicinal amino-alcohol grouping present in a pyranoside ring of known conformation. The preparation of these compounds has been described[6]. The course of the reaction was followed by determining the reduction of periodate by means of the titrimetric method[7], and the experimental technique and conditions described by Honeyman and Shaw[8].



	R^1	R^2	R^3	R^4
1	H	NH_2	OH	H
2	H	OH	NH_2	H
3	H	NH_2	H	OH
4	H	OH	H	NH_2
5	NH_2	H	H	OH
6	OH	H	H	NH_2
7	NH_2	H	OH	H
8	OH	H	NH_2	H

EXPERIMENTAL

Kinetic studies. — (*a*) *Experimental conditions.* Analytical grade sodium metaperiodate was used for the oxidations. Because aqueous periodate solutions decompose in the presence of light[9], the solutions of the thermostat were darkened with nigrosine dye.

Most of the kinetic measurements were carried out at 25 $\pm$0.02° or 0 $\pm$0.02°, but some runs were at intermediate temperatures. For the titrimetric determination of second-order rate constants, an approximate, threefold excess of periodate to sugar was used. The solutions were 3.5×10^{-3}M for the sugar and 10×10^{-3}M for periodate. For the isolation of end products, only a small excess of periodate was generally used.

The kinetic experiments were carried out in aqueous buffer solutions of approximately constant ionic strength. Buffer solutions were prepared from analytical or purified reagent-grade chemicals and twice-distilled water. The pH of the buffer solutions was measured with a Pye "Dynacap" pH meter, standardised against potassium hydrogen phthalate (0.05M), pH 4.0 at 0 and 25°, and against potassium dihydrogen phosphate–disodium hydrogen phosphate (0.025M), pH 6.98 at 0° and pH 6.86 at 25°. For pH measurements at 0°, the electrodes were immersed in ice–water for 1 h, and all solutions were precooled to 0°. The buffer solutions were found to be stable over the period of time that they were used. The buffer solutions used were: *A* sodium acetate–acetic acid, pH 4.06, 25° and 0°, ionic strength 0.036 g. ions.litre^{-1}; *B* potassium dihydrogen phosphate(0.065M)–sodium hydroxide(0.035M), pH 6.87, 25°, ionic strength 0.05 g.ions.litre^{-1}; *C* sodium arsenate–hydrochloric acid[10] (0.05M), pH 5.65, 0°; *D* potassium dihydrogen phosphate–disodium hydrogen phosphate, pH 6.03, 8.45°; and *E* β,β'-dimethylglutaric acid–sodium hydroxide[10], pH 5.35, 8.45°.

(*b*) *Analytical determinations.* Measurements were made over the whole of the reaction period, and also for some time after its "completion", in order to determine the rate of over-oxidation. The periodate analysis was carried out by the Müller–Friedberger method[7].

Detection and isolation of products of oxidations. — To obtain the carbohydrate product of the reaction, a slight excess of oxidant was allowed to react with the glycosides in concentrated solution. The product crystallized from the reaction solution and was removed by filtration. The ammonia liberated during the reaction was determined by distillation into standard acid, and back titration with alkali[11].

RESULTS AND DISCUSSION

Comparison of infrared spectra and mixed m.p. with an authentic sample showed that the carbohydrate product from oxidation of compounds **1–6** is identical with that[12] from periodate oxidation of methyl 4,6-*O*-benzylidene-α-D-glucoside. Qualitative and quantitative analysis showed that one equivalent of ammonia was produced in the reaction; overoxidation was very slow. The stoichiometric equation for the amino-glucosides, -mannosides, and -allosides can be written: amino sugar $+P^- \rightarrow$ "dialdehyde" $+IO_3^- + NH_3$ (where P^- represents the oxidising species in solution).

If the reaction between periodate and the vicinal amino-alcohol group involves[3] the non-protonated form and a negative periodate ion[13] (assumed here to be IO_4^-), the reaction may be represented[5] by equation (1).

$$-\frac{d(P)}{dt} = k_2(\mathrm{AS})(IO_4^-) = k_2(\mathrm{AS})(P)_T/(1+1/K_D) \qquad (1)$$

where (AS) and (IO_4^-) are the concentrations of non-protonated, amino sugar and IO_4^-, respectively, k_2 is the true, second-order rate constant, $(P)_T$ is the total periodate concentration as determined by analysis, and the factor $(1+1/K_D)$ is the correction term for the availability of IO_4^- in the pH range 3–7. From the equilibrium $ASH^+ \rightleftharpoons AS + H^+$, the concentration of the non-protonated, amino sugar is given by $(AS) = K_A(ASH^+)/(H^+)$, where (ASH^+) will be essentially the total concentration of amino sugar in the pH range 4–5, since the pK_A values are of the order[14] 6–8. Substitution of this expression into equation (1) gives

$$-d(P)/dt = k_2\ K_A\ (ASH^+)(P)_T/(H^+)(1+1/K_D) \qquad (2)$$

$$= k_2'(P)_T(ASH^+)\,. \qquad (3)$$

Equation (3) is a second-order rate equation, with k_2' being the experimentally determined rate constant. With a small excess of periodate (roughly threefold), all of the reactions studied proved to be strictly first order in both amino sugar and in periodate, at the concentrations of reactants used.

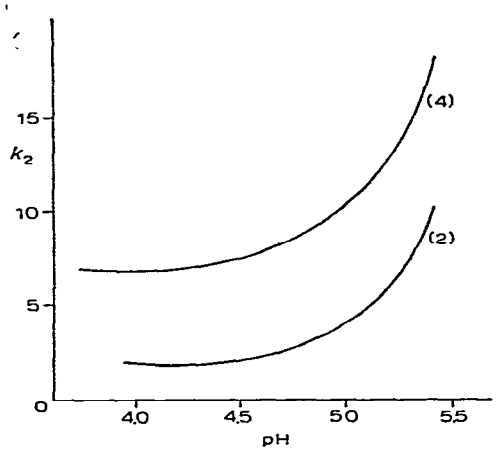


Fig. 1. Variation of the true, second-order rate constant with pH for methyl 3-amino-4,6-*O*-benzylidene-3-deoxy-α-D-glycosides in acetate buffer at 0°.

In a study of the periodate oxidation of 2-aminoethanol, Dahlgren and Hodsdon[5] showed that a plot of the pseudo-first-order rate constant *vs* the pH gave a good straight line of unit slope with the expected intercept; indicating that equation (2) is valid for that reaction. Dahlgren[15] later reported a small increase in the rate due to general base catalysis by the buffer. To test the validity of equation (2) for the amino sugars, it was necessary to determine rate constants at several values of pH. If the increase in rate of reaction with increase in pH is due solely to the increased amount of non-protonated, amino sugar available, the true, second-order rate constant k_2 should be independent of pH. Fig. 1 shows the variation of k_2 with pH for both methyl 3-amino-4,6-*O*-benzylidene-3-deoxy-α-D-glucoside (**4**) and the *allo* analogue (**2**) at 0°. Oxidation of the amino sugars below pH 3.8 was not studied, because of the slowness of the reaction and of the possibility of hydrolysis of the benzylidene grouping. An accurate study at any pH greater than 5.4 was not possible, because the reaction was too fast for the titrimetric technique used. Fig. 1 shows that the rate constants are not independent of pH, especially over the range 4.7–5.4. In the oxidation of many simple 1,2-diols, a cyclic periodate complex is formed rapidly and reversibly from the reactants and decomposes slowly to the products[16,17]. With more-complex 1,2-diols, there is considerable evidence that the formation of the cyclic ester is the rate-limiting step. The variation of the rate with pH for the oxidation of pinacol in the absence of general acids or bases can be explained in terms of this second mechanism[18]. General acid–base catalysis has been observed[19] in the oxidation of some highly substituted 1,2-diols, and it is reasonable to suppose that the catalysed step is the formation of the ester. Senent and Diez[20] found that the second-order rate constant for the oxidation of ethylene glycol, propane-1,2-diol, and butane-2,3-diol for various pH values did not show the same pattern, but showed a trend towards two maxima for the rate constant with increase in methyl substitution. As a simple comparison between a diol and its substituted compounds

is apparently not possible, the difference shown between 2-aminoethanol and the amino sugars is therefore not too surprising. Also, the oxidation of carbohydrates[21] having structures related to those of compounds **1–6** shows a different mechanism to that for ethylene glycol[13]. The degree of substitution alone in the amino sugars does not appear to be the greatest influence on the variation of the rate constant with pH. This is shown by the oxidation of methyl 4,6-*O*-benzylidene-α-D-glucoside at 25°. The second-order rate constant over the pH range 3.8–7.0 showed only a small, gradual decrease with increase in pH (Table I); by analogy with butane-2,3-diol[20], a minimum might have been expected at about pH 5. The similarity between the variation with pH of the second-order rate constants for the amino sugars and several α-dicarbonyl compounds[22] is most striking. It can be inferred from this comparison that the deviation from equation (2) is not due to anomalous behaviour of the $-NH_3^+ \rightleftharpoons -NH_2 + H^+$ equilibrium, but is more likely due to oxidation by *all* periodate species, with possibly each individual reaction being base-catalysed. From Fig. 1, it can be seen that the k_2 values for the two amino sugars do not alter greatly over the pH range 4.0–4.4, the two curves being parallel. For comparative purposes, therefore, it appeared reasonable to study the oxidation of all of the amino-glucosides, -mannosides, and -allosides at a pH within this range. A pH value of 4.06 was chosen, because suitable rates of oxidation were possible, and it also enabled a comparison to be made with other results[8]. The relative rates of oxidation at pH 4.06 are shown in Figs. 2 and 3, and the k_2' and k_2 values are given in Table II.

TABLE I

VARIATION OF THE SECOND-ORDER RATE CONSTANTS FOR THE PERIODATE OXIDATION[a] OF METHYL 4,6-*O*-BENZYLIDENE-α-D-GLUCOSIDE WITH PH AT 25°

pH	3.80	4.06	4.34	4.72	5.01	5.15	5.34	5.73	6.02*	6.16	6.67*	6.93*
$10^3\,k_2$ (*l.mole*$^{-1}$.*sec*$^{-1}$)	3.14	2.88	2.51	2.23	2.20	2.09	2.06	2.18	2.12	2.14	2.02	2.05

[a]In acetate buffer, except those marked* when phosphate buffer was the reaction medium.

TABLE II

EXPERIMENTAL, k'_2 AND TRUE k_2, SECOND-ORDER RATE CONSTANTS (l.MOLE^{-1}.SEC^{-1}) FOR THE PERIODATE OXIDATION OF THE ISOMERIC, AMINO SUGARS AT PH 4.06

Methyl 4,6-O-benzylidene-deoxy-α-D-glycoside	*25°*		*0°*	
	$10^3\,k'_2$	k_2	$10^3\,k'_2$	k_2
2-Amino-alloside (1)	16.7	23.09 ±1.21	1.91	10.5 ±0.52
3-Amino-alloside (2)	1.23	4.90 ±0.3	0.098	1.87 ±0.17
2-Amino-glucoside (3)	15.8	20.86 ±1.04	1.08	6.38 ±0.54
3-Amino-glucoside (4)	14.9	17.95 ±1.03	1.15	6.33 ±0.31
2-Amino-mannoside (5)	97.5	35.6 ±1.7	9.6	13.0 ±1.05
3-Amino-mannoside (6)	69.3	148.4 ±7.1	5.18	47.4 ±3.1

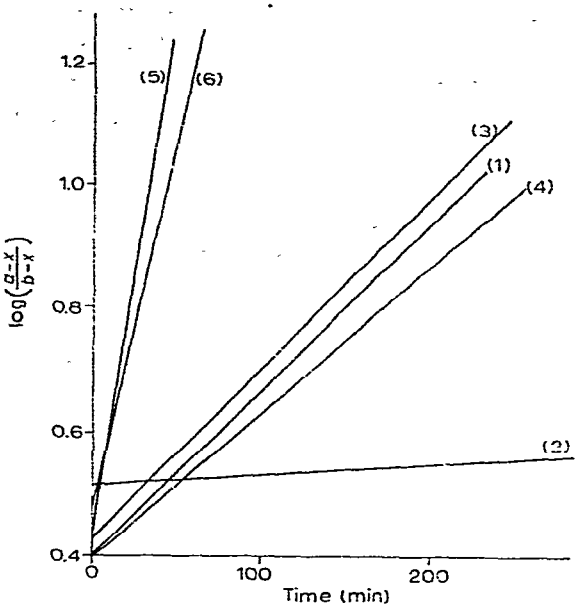


Fig. 2. Oxidation of methyl amino-4,6-*O*-benzylidene-deoxy-α-D glycosides at pH 4.06 and

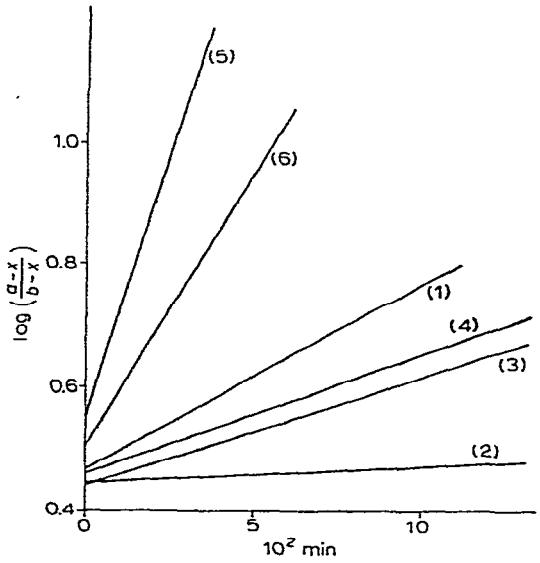


Fig. 3. Oxidation of methyl amino-4,6-*O*-benzylidene-deoxy-α-D-glycosides at pH 4.06 and 0°.

Fig. 4 shows the Arrhenius plot for methyl 3-amino-4,6-*O*-benzylidene-3-deoxy-α-D-glucoside (**4**) and the *allo* analogue (**2**). The graph showed straight lines over the temperature range 0–25°, and thus permitted the activation energy to be obtained for all of the amino sugars, by using the rate constants at 0° and 25°. The

free energy and entropy of activation were calculated by using data obtained at 25°, and are listed in Table III. All of the amino sugars have essentially the same parameters of activation. This is indicative that the rate-determining step of each individual reaction lies on a reaction path that is common to all compounds. The large rate constants for the reaction are borne out by the relatively low value for $\Delta H^{\ddagger}$ of 6–7 kcal.mole^{-1}. The large, negative $\Delta S^{\ddagger}$ for the reaction is not inconsistent with the view that the rate-determining step is the formation of a cyclic intermediate of periodate and amino alcohol. The method used for determining the presence of the complex in the work on ethylene glycol[13] fails with the amino-alcohol group, due to the very low concentration of complex which would be present at any given time[5]. The oxidation of methyl 4,6-*O*-ethylidene-α-D-glucoside and -mannoside appears to proceed through an intermediate. cyclic complex, the formation of which is rate determining[16].

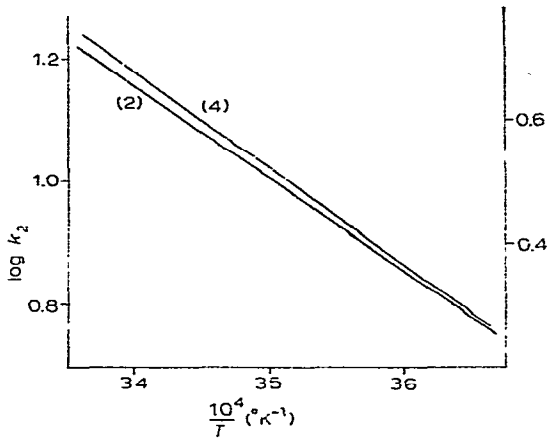


Fig. 4. Arrhenius plot of the true, second-order rate constants for the oxidation of methyl 3-amino-4,6-*O*-benzylidene-3-deoxy-α-D-glycosides at pH 4.06.

TABLE III

THERMODYNAMIC VALUES OF ACTIVATION AT 25° FOR THE OXIDATION OF THE AMINO SUGARS

Methyl 4,6-O-benzylidene-deoxy-α-D-glycoside	E_A (*kcal.mole*$^{-1}$)	$\Delta H^{\ddagger}$ (*kcal.mole*$^{-1}$)	$\Delta S^{\ddagger}$ (*e.u.*)	$\Delta G^{\ddagger}$ (*kcal.mole*$^{-1}$)
2-Amino-alloside (1)	5.09 ±0.73	4.50	37.2 ±1.6	15.6
3-Amino-alloside (2)	6.24 ±1.01	5.65	36.4 ±2.4	16.5
2-Amino-glucoside (3)	7.67 ±1.04	7.08	28.8 ±2.2	15.7
3-Amino-glucoside (4)	6.75 ±1.06	6.16	32.2 ±1.7	15.8
2-Amino-mannoside (5)	6.52 ±0.81	5.93	31.6 ±2.1	15.3
3-Amino-mannoside (6)	7.39 ±0.97	6.80	25.8 ±1.8	15.5

The effect of conformation on the rate of periodate oxidation. — For the purpose of studying the effect of conformation of cyclic amino-alcohols on the rate of

periodate oxidation, second-order rate constants in aqueous buffer solution at pH 4.06 were determined for the amino sugars **1–6**.

The effect of replacing a hydroxyl group in a vicinal diol by an amino group is known[23] to increase the rate of oxidation with periodate, and this has been observed for the amino sugars to an extent of 10^2 to 10^4 (*cf.* ref. 8).

Although the rates of oxidation of the amino-allosides, -mannosides, and -glucosides do not range greatly, significant differences between them can be seen. A consideration of the experimental, second-order rate constants shows that the 3-aminoalloside has a k_2' value at least ten times smaller than those of the next compounds, the two aminoglucosides and the 2-aminoalloside grouped together. The aminomannosides distinctly have the largest rate constants. Correcting for the ionisation constants to give true, second-order rate constants, k_2, does not affect the general order of relative rates, except for an interchange of the mannosides. For periodate cleavage of the C-2–C-3 bond to occur in compounds **1–6**, it is most probable that, as in diol cleavage, an intermediate, cyclic complex will be formed, in which the C–O and the C–N bonds of the hydroxyl and amino groups are rotated towards some degree of coplanarity. The ease with which this complex is formed or broken down will thus depend on the conformation of the amino and hydroxyl groups. However, the exact nature of the complex intermediate in diol oxidations is not known, and for amino-alcohol oxidation there is as yet no evidence for an intermediate complex. It is often difficult to interpret the kinetic effects of structural changes for multistage reactions, and this is particularly so for periodate oxidation of diols, because the rate has a complicated dependence upon equilibrium and rate constants. Details of the reaction mechanism and the non-bonded interactions which may occur in an intermediate complex have to be considered before rigid conclusions about structural effects are made. Levesley *et al.*[24] have stated that kinetic evidence of (glycol–oxidant) complex formation should not be taken as diagnostic of cyclic complex formation.

Cyclisation to form a complex involving equatorial and axial substituents would be expected to take place more easily than that involving two equatorial groups. The rate constants for the amino sugars roughly bear out this hypothesis, and fit the general pattern of diol oxidations in which *cis* groups are oxidised faster than *trans* groups. Both aminomannosides (*eq-ax*) have larger k_2 values than any other amino sugar. The relative rates for the 3-aminomannoside and 2-aminomannoside are of the order of 4:1 at 25°; the reason for this is not known. The k_2 values for the aminoallosides (*eq-ax*) might be expected to be larger than those for the aminoglucosides (*eq-eq*); the value for the 3-aminoalloside is unexpectedly low. If a cyclic intermediate is formed during the reaction, the 1,3-diaxial interaction between the groups attached to C-1 and C-3 should favour a larger degree of complexing for the allosides than for the other amino sugars, because complexing will tend to alleviate this interaction. However, even if this is the case, the rate of breakdown of the complexes of the allosides might be slower than for the other compounds. The results for the oxidation of cyclohexane-1,2-diols in alkaline solution[25] showed that the faster

oxidation of the *cis*- than of the *trans*-diol is due, not to ease of intermediate-complex formation, but to the rapid breakdown of this complex. As well as structural factors, the basicity of the amino group may also have an effect upon the rate of reaction. The increased basicity of an amino group may favour the formation of complex intermediates but at the expense of their rate of breakdown.

Comparison with cuprammonium complexing. — The results from the cuprammonium studies[6] on compounds **1–6** show that the mannosides have the greatest degree of complexing, with the glucosides and allosides complexing to approximately the same degree. This order is the same as that for periodate oxidation. Although the data for cuprammonium complexing concern equilibrium, and the data for oxidation concern relative rates, the close parallel found between the two types of reaction is thus not unexpected, since the geometrical requirements are very similar for the formation of a five-membered ring complex. Reeves[26] found similar relationships between the extent of cuprammonium complexing and rate of oxidations with lead tetra-acetate for several classes of compounds having a vicinal diol grouping. However, too close a parallel must be avoided, since the cleavage reactions are multistage, with the nature of the rate-determining step possibly depending on the structure of the compound and reaction conditions.

ACKNOWLEDGMENTS

We thank Dr. E. R. A. Peeling for helpful discussions, and the Science Research Council for the award of a studentship (to C.B.B.)

REFERENCES

1 J. M. Bobbitt, *Advan. Carbohyd. Chem.*, 11 (1956) 1.
2 M. J. Weiss, J. P. Joseph, H. M. Kissman, A. M. Small, R. E. Schaub, and F. J. McEvoy, *J. Amer. Chem. Soc.*, 81 (1959) 4050.
3 G. E. McCasland and D. M. Smith, *J. Amer. Chem. Soc.*, 73 (1951) 5164.
4 J. Kovár, J. Jarý, and K. Blaha, *Collection Czech. Chem. Commun.*, 28 (1963) 2199.
5 G. Dahlgren and J. M. Hodsdon, *J. Phys. Chem.*, 68 (1964) 416.
6 C. B. Barlow and R. D. G thris, *J. Chem. Soc. (C)*, (1967) 1194.
7 E. Müller and O. Friedberger, *Ber.*, 35 (1902) 2652.
8 J. Honeyman and C. J. G. Shaw, *J. Chem. Soc.*, (1959) 2454.
9 F. S. H. Head, *Nature*, 165 (1950) 236.
10 R. M. C. Dawson, D. C. Elliot, W. H. Elliot, and K. H. Jones, *Data for Biochemical Research*, Oxford University Press, 1959.
11 A. I. Vogel, *Quantitative Inorganic Analysis*, Longmans, Green, London, 1957, p. 246.
12 R. D. Guthrie and J. Honeyman, *J. Chem. Soc.*, (1959) 2441.
13 F. R. Duke, *J. Amer. Chem. Soc.*, 69 (1947) 3054.
14 C. B. Barlow, R. D. Guthrie and A. M. Prior, *Carbohyd. Res.*, 10 (1969) 481.
15 G. Dahlgren and E. M. Rand, *J. Phys. Chem.*, 71 (1967) 1955.
16 G. J. Buist and C. A. Bunton, *J. Chem. Soc.*, (1954) 1406.
17 G. J. Buist, C. A. Bunton, and J. H. Miles, *J. Chem. Soc.*, (1957) 4567.
18 G. J. Buist, C. A. Bunton, and J. Lomas, *J. Chem. Soc. (B)*, (1966) 1094, 1099.
19 P. Zuman, J. Sicher, J. Krupicka, and M. Svoboda, *Nature*, 178 (1956) 1407.
20 S. Senent Perez and B. Diez, *Anales Real Soc. Espan. Fis. Quim.* (Madrid), 60B (1964) 167; *Chem. Abstr.*, 62 (1965) 415.

21 J. HONEYMAN AND C. J. G. SHAW, *J. Chem. Soc.*, (1959) 2451.
22 G. DAHLGREN AND K. L. REED, *J. Amer. Chem. Soc.*, 89 (1967) 1380.
23 Compare, for example, ref. 5 with F. R. DUKE AND V. C. BULGRIN, *J. Amer. Chem. Soc.*, 76 (1954) 3803.
24 P. LEVESLEY, W. A. WATERS, AND A. N. WRIGHT, *J. Chem. Soc.*, (1956) 840.
25 G. J. BUIST, C. A. BUNTON, AND J. H. MILES, *J. Chem. Soc.*, (1959) 743.
26 R. E. REEVES, *Adv. Carbohydrate Chem.*, 6 (1951) 107.

Carbohydrate Research
Elsevier Publishing Company, Amsterdam
Printed in Belgium

TRIFLUOROACETYL AS AN *N*-PROTECTIVE GROUP IN THE SYNTHESIS OF PURINE NUCLEOSIDES OF 2-AMINO-2-DEOXY SACCHARIDES

M. L. WOLFROM AND P. J. CONIGLIARO
Department of Chemistry, The Ohio State University, Columbus, Ohio 43210 (U. S. A.)
(Received March 19th, 1969)

ABSTRACT

Selective *N*-(trifluoroacetyl)ation of 2-amino-2-deoxy-D-glucose, followed by *O*-acetylation, gave the anomeric 1,3,4,6-tetra-*O*-acetyl-2-deoxy-2-(trifluoroacetamido)-α(and β)-D-glucopyranoses (**3** and **4**). Conversion of **3** and **4** into the bromide, followed by condensation with 6-benzamido-9-(chloromercuri)-9*H*-purine, produced 6-benzamido-9-[3,4,6-tri-*O*-acetyl-2-deoxy-2-(trifluoroacetamido)-α-D-glucopyranosyl]-9*H*-purine and the corresponding β-D anomer. Removal of substituent groups was achieved by means of methanolic ammonia, giving the anomeric, crystalline 9-(2-amino-2-deoxy-α(and β)-D-glucopyranosyl)adenines. Application of this procedure to 2-amino-2-deoxy-D-galactose gave, as the final products, 9-(2-amino-2-deoxy-α-D-galactopyranosyl)adenine and the corresponding β-D anomer, characterized as their crystalline dihydrochlorides.

INTRODUCTION

In the synthesis of nucleosides of 2-amino-2-deoxy saccharides, considerable difficulty has been experienced in the selection of an *N*-protective group which can be conveniently introduced and easily removed. The synthesis of purine nucleosides of this type presents special problems, since purine nucleosides, in contrast to pyrimidine nucleosides, are unstable to acid[1]. Thus, the *N*-protective group employed must be removable under basic or neutral conditions. The *N*-acetyl protective group was employed by Baker and co-workers[2] for the synthesis of 9-(2-amino-2-deoxy-β-D-allopyranosyl)-6-(dimethylamino)-9*H*-purine. The removal of the *N*-acetyl group was effected by refluxing with aqueous barium hydroxide, but this procedure failed when applied to nucleoside derivatives wherein the 3-hydroxyl group bore a *trans* relationship to the 2-amino group. Stevens and co-workers[3] also employed the *N*-acetyl protective group for the synthesis of pyrimidine nucleosides, where the stability of these nucleosides to acids allowed the removal of this protective group. The *N*-(benzyloxycarbonyl) and *N*-(methoxycarbonyl) protective groups were also employed by Stevens and co-workers for synthesis of pyrimidine nucleosides. The *N*-bis(phenoxy)phosphinyl[4] and the *N*-(2,4-dinitrophenyl)[5,6] groups have been successfully employed in the synthesis of purine nucleosides of 2-amino-2-deoxy sugars. In all cases, the

N-(2,4-dinitrophenyl) group gave both anomeric nucleosides, since it shows no tendency to participate at C-1. The *N*-bis(phenoxy)phosphinyl[4] and *N*-(2,4-dinitrophenyl)[7] groups have also been employed in the synthesis of pyrimidine nucleosides of 2-amino-2-deoxy saccharides.

We report herein the use of the trifluoroacetyl group as an *N*-protecting group in the synthesis of both anomers of 9-(2-amino-2-deoxy-D-glucopyranosyl)adenine[5] (**6c** and **7c**) and of 9-(2-amino-2-deoxy-D-galactopyranosyl)adenine (**13c** and **14c**). We have previously reported the use of this group in the synthesis of a pyrimidine nucleoside[8].

RESULTS AND DISCUSSION

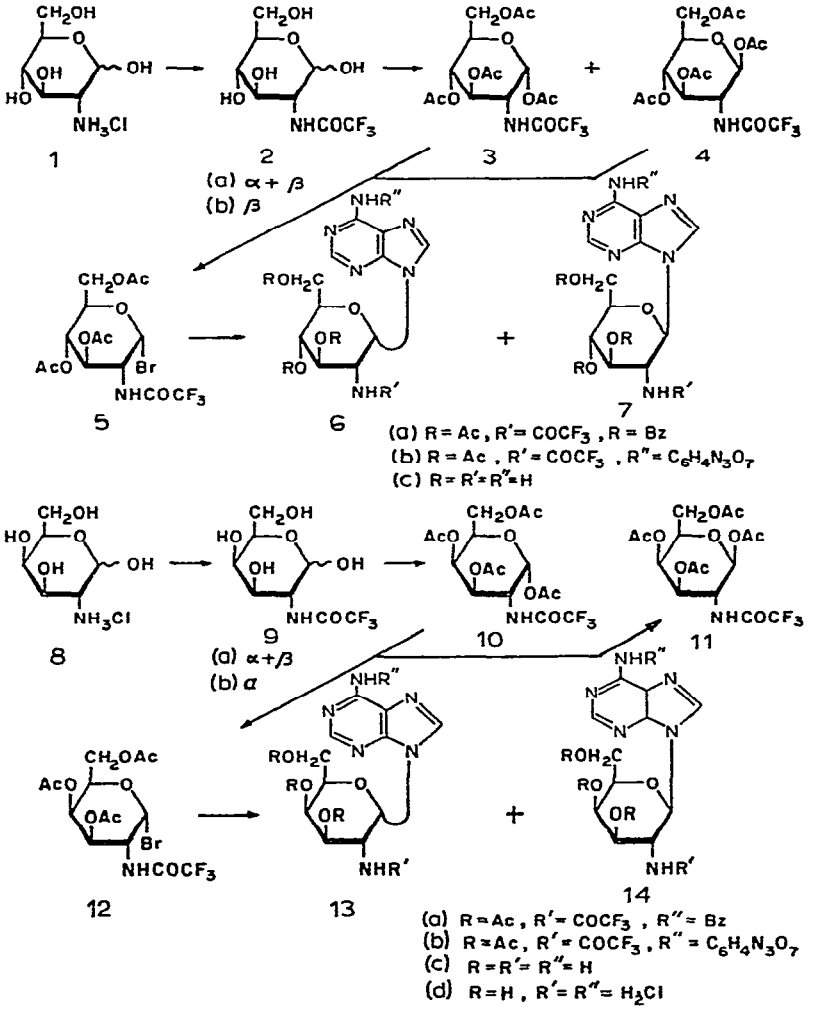
In the present work, direct introduction of the *N*-(trifluoroacetyl) group into the unsubstituted saccharide was accomplished by selective acylation of the amino group with *S*-ethyl trifluorothioacetate. This reagent has been employed for the introduction of the *N*-(trifluoroacetyl) group into amino acids, for peptide synthesis[9]. Previous methods for the introduction of the *N*-(trifluoroacetyl) group in the sugar series[8,10] required prior selective acetylation of the hydroxyl groups.

Treatment of a solution of 2-amino-2-deoxy-D-glucose in methanol with *S*-ethyl trifluorothioacetate produced 2-deoxy-2-(trifluoroacetamido)-D-glucose (**2a**). By repeated recrystallization, the β-D anomer (**2b**) was obtained. Similar treatment of a solution of 2-amino-2-deoxy-D-galactose in methanol produced 2-deoxy-2-(trifluoroacetamido)-D-galactose (**9a**). In this case, the α-D anomer (**9b**) was isolated by repeated recrystallization.

By acetylating **2a** with pyridine–acetic anhydride, crystalline 1,3,4,6-tetra-*O*-acetyl-2-deoxy-2-(trifluoroacetamido)-α-D-glucopyranose (**3**) and the corresponding crystalline β-D anomer (**4**) were obtained in yields of 40 and 45%, respectively. The latter compound was identical with authentic **4** previously prepared in this laboratory[8] from 1,3,4,6-tetra-*O*-acetyl-2-amino-2-deoxy-β-D-glucopyranose hydrochloride[11]. The α-D anomer (**3**) was found to be more dextrorotatory than **4**, indicating qualitative agreement with the Hudson rules of rotation[12]. Anomeric assignments for **2b**, **9b**, **10**, and **11** were thus made on the basis of optical rotation. Acetylation of **9a** produced crystalline 1,3,4,6-tetra-*O*-acetyl-2-deoxy-2-(trifluoroacetamido)-α-D-galactopyranose (**10**) and the corresponding crystalline β-D anomer (**11**) in yields of 46 and 37%, respectively. Treatment of **3** with hydrogen bromide in acetic acid produced the crystalline 3,4,6-tri-*O*-acetyl-2-deoxy-2-(trifluoroacetamido)-α-D-glucopyranosyl bromide (**5**) previously reported[8,10]. Compound **5** was also prepared from a mixture of **3** and **4**, thus avoiding the isolation of the individual anomeric acetates. Similarly, treatment of **10** or **11**, or a mixture of the two, produced syrupy 3,4,6-tri-*O*-acetyl-2-deoxy-2-(trifluoroacetamido)-α-D-galactopyranosyl bromide (**12**).

Condensation of **5** with 6-benzamido-9-(chloromercuri)-2*H*-purine by the general procedure of Davoll and Lowy[13] produced an anomeric mixture of fully substituted nucleoside derivatives which was separable, by preparative t.l.c., into

crystalline 6-benzamido-9-[3,4,6-tri-*O*-acetyl-2-deoxy-2-(trifluoroacetamido)-α-D-glucopyranosyl]purine (**6a**) and the corresponding, amorphous β-D anomer (**7a**) in yields of 6.6 and 26%, respectively. Both **6a** and **7a** formed well defined, crystalline picrates (**6b** and **7b**) on de-*N*-benzoylation with picric acid in isopropyl alcohol–methanol. Complete removal of all of the substituent groups from 6-benzamido-9-[3,4,6-tri-*O*-acetyl-2-deoxy-2-(trifluoroacetamido)-α(and β)-D-glucopyranosyl]purine (**6a** and **7a**) was achieved by treatment with methanolic ammonia at room temperature for 7 days, to give crystalline 9-(2-amino-2-deoxy-α-D-glucopyranosyl)adenine (**6c**) and the crystalline β-D anomer (**7c**), in yields of 89 and 86%, respectively. Both products exhibited physical constants identifiable with those for the compounds previously prepared in this laboratory[5]. For each anomeric pair (**6a**, **7a**; **6b**, **7b**; and **6c**, **7c**), the α-D anomer was the more dextrorotatory.



Condensation of 3,4,6-tri-*O*-acetyl-2-deoxy-2-(trifluoroacetamido)-α-D-galactopyranosyl bromide (**12**) with 6-benzamido-9-(chloromercuri)purine, by the procedure used for the corresponding 2-amino-2-deoxy-D-glucosyl bromide (**5**), produced an anomeric mixture of nucleoside derivatives. Separation by preparative t.l.c. gave crystalline 6-benzamido-9-[3,4,6-tri-*O*-acetyl-2-deoxy-(2-trifluoroacetamido)-α-D-galactopyranosyl]adenine (**13a**) and the corresponding amorphous β-D anomer (**14a**) in yields of 9.3 and 30%, respectively. Both products gave crystalline picrates (**13b** and **14b**) on de-*N*-benzoylation with picric acid in isopropyl alcohol–methanol. Deacylation of **13a** and **14a** with methanolic ammonia yielded 9-(2-amino-2-deoxy-α-D-galactopyranosyl)adenine (**13c**) and the corresponding β-D anomer (**14c**) in yields of 83 and 81%, respectively. Both **13c** and **14c** were amorphous but formed crystalline dihydrochlorides (**13d** and **14d**) on treatment with dilute hydrochloric acid in methanol. Since a qualitative agreement with Hudson's rules of rotation was shown by the nucleoside derivatives of 2-amino-2-deoxy-D-glucose (**6a–c** and **7a–c**), it was assumed that the corresponding derivatives of 2-amino-2-deoxy-D-galactose would show similar agreement. The anomeric assignments of the preceding nucleoside derivatives of 2-amino-2-deoxy-D-galactose were thus made on the basis of optical rotation, **13a–d** being the more dextrorotatory of the respective anomeric pairs.

The *N*-trifluoroacetyl group has thus proved to be a suitable *N*-protective group in synthesis of purine nucleosides of 2-amino-2-deoxy-D-glucopyranose and 2-amino-2-deoxy-D-galactopyranose. As the *N*-trifluoroacetyl group could be directly introduced, without the need for prior selective substitution of the hydroxyl groups, and as it could be removed simultaneously with the *O*-acetyl groups, only five steps were needed to synthesize the nucleoside from the free amino saccharide. The overall yields of 9-(2-amino-2-deoxy-α-D-glucopyranosyl)adenine (**6c**) and the corresponding β-D anomer (**7c**) were 5.9 and 22%, respectively, based on the glycosyl bromide (**5**), or 3.5 and 13%, respectively, based on 2-amino-2-deoxy-D-glucose hydrochloride (**1**). The overall yields of 9-(2-amino-2-deoxy-α-D-galactopyranosyl)adenine (**13c**) and the corresponding β-D anomer (**14c**) were 7.7 and 24%, respectively, based on the bromide (**12**), or 4.5 and 14%, respectively, based on 2-amino-2-deoxy-D-galactose hydrochloride (**8**). The overall yields of **6c** and **7c**, obtained by previous procedures, were 4.0 and 5.2%, respectively[5], and 9.3% for[4] **7c**, based on the particular *N*-protected 2-amino-2-deoxy-D-glucosyl bromides used.

The fact that both anomers of the nucleoside derivatives of 2-amino-2-deoxy-D-glucose and 2-amino-2-deoxy-D-galactose were obtained indicates that the *N*-trifluoroacetyl group does not participate strongly at C-1. The use of such a strongly participating group as *N*-acetyl[14,15] has been found to give only the β-D anomers of purine nucleoside derivatives of 2-amino-2-deoxy-D-glucose. The α:β ratio was 26:100 for the *N*-(trifluoroacetyl)-protected, anomeric nucleoside derivatives of 2-amino-2-deoxy-D-glucose (**6a** and **7a**), and 31:100 for the corresponding derivatives of 2-amino-2-deoxy-D-galactose (**13a** and **14a**). These results indicate that the *N*-(trifluoroacetyl) group probably participates to a slight extent at C-1. Steric factors

might also account for the preponderance of the β-D anomer. However, use of the completely nonparticipating *N*-(2,4-dinitrophenyl) group gave a much higher α:β ratio. Thus, condensation of 3,4,6-tri-*O*-acetyl-2-deoxy-2-(2,4-dinitroanilino)-α-D-glucopyranosyl bromide with 6-acetamido-9-(chloromercuri)purine gave[5] the anomeric nucleoside derivatives in the α:β ratio of 60:100. In view of this result, a much higher α:β ratio for **6a**:**7a** and **13a**:**14a** would be expected were the *N*-(trifluoroacetyl) group completely nonparticipating at C-1.

EXPERIMENTAL

General methods. — Melting points were determined with a Thomas–Hoover apparatus. Specific rotations were determined in a 2-dm polarimeter tube. Infrared spectra were recorded with a Perkin–Elmer Infracord spectrometer. Ultraviolet spectra were recorded with a Bausch and Lomb Spectronic 505 spectrometer. X-Ray powder diffraction data (interplanar spacing, Å; Cu Kα radiation; λ 1.539 Å; Ni filter; camera diameter 114.6 mm; photographic recording) are expressed as relative intensities, estimated visually: m, moderate; s, strong; v, very; w, weak. Parenthetical numbers indicate the order of the most intense lines; 1, most intense; multiple numbers indicate approximately equal intensities. T.l.c. was performed with Desaga equipment, by use of Silica Gel G (E. Merck, Darmstadt, Germany), activated at 110°. Unless otherwise noted, indication was by sulfuric acid; amounts of developers are by volume. Microanalyses were performed by W. N. Rond. Unless otherwise indicated, evaporations were performed under diminished pressure (water aspirator).

2-Deoxy-2-(trifluoroacetamido)-D-glucose (**2**). — A suspension of 2-amino-2-deoxy-D-glucose hydrochloride (**1**, 10 g) in anhydrous methanol (50 ml) was treated with an equivalent amount of sodium methoxide in methanol (1.06 g of sodium dissolved in 10 ml of methanol), and the mixture was swirled until only a small residue (sodium chloride) remained. *S*-Ethyl trifluorothioacetate (10 g) was added, the mixture was kept for 24 h at room temperature, and the solution was evaporated to a solid residue which was extracted with hot acetone (300 ml), the insoluble material being discarded. After the acetone extract had been cooled to room temperature, ether (200 ml) was added, and the mixture was refrigerated overnight. The white, crystalline solid which formed was recrystallized from acetone–ether, giving 9.3 g (73%) of 2-deoxy-2-(trifluoroacetamido)-D-glucose (**2a**): m.p. 193–195° (dec.), $[\alpha]_D^{22}$ +12 ±1 (initial, extrapolated) → +15 ±1° (final; *c* 2.5, water).

Anal. Calc. for $C_8H_{12}F_3NO_6$: C, 34.92; H, 4.40; N, 5.09. Found: C, 35.04; H, 4.70; N, 5.37.

Four additional recrystallizations from acetone gave the β-D anomer (**2b**): m.p. 196–197° (dec.), $[\alpha]_D^{22}$ −28 ±2 (initial, extrapolated) → +15 ±1° (final; *c* 1.5, water); λ_{max}^{KBr} 2.9–3.1 (OH, NH), 5.85 (*N*-trifluoroacetyl carbonyl), 6.4 (NH), 8.6 (CF), 7.35, 7.6, 7.8, 8.23, 8.45, 9.08, 9.28, 9.6, 9.82, 10.1, 11.1, 11.32, 11.44, and 13.64 μm; X-ray powder diffraction data: 10.72 m, 9.41 vw, 6.81 m, 5.32 vs (2), 5.00 m, 4.60 vs (3), 4.23 s, 3.95 vs (1), 3.75 w, 3.68 s, 3.49 vs, 3.19 m, 3.08 w, 2.93 w, 2.84 vs, 2.64 s, 2.50 m, 2.37 m, 2.31 vs, 2.21 w, 2.08 s, 1.99 w, 1.93 w, 1.86 vw,

1.77 w, and 1.66 w. The physical constants of this compound were unchanged by-further recrystallization.

Anal. Calc. for $C_8H_{12}F_3NO_6$: C, 34.92; H, 4.40; N, 5.09. Found: C, 34.55; H, 4.29; N, 5.13.

2-Deoxy-2-(trifluoroacetamido)-D-galactose (**9**). — A suspension of 2-amino; 2-deoxy-D-galactose hydrochloride (**8**, 5 g) in methanol (25 ml) was treated with sodium (0.53 g) in methanol (10 ml) and *S*-ethyl trifluorothioacetate (5 g) by the procedure described in the preceding experiment. The yield, after recrystallization from acetone–ether, was 4.8 g (76%) of 2-deoxy-2-(trifluoroacetamido)-D-galactose (**9a**): m.p. 184–186° (dec.), $[\alpha]_D^{21}$ +68 ±2 (initial, extrapolated) → +59 ±1° (final; *c* 2.9, water).

Anal. Calc. for $C_8H_{12}F_3NO_6$: C, 34.92; H, 4.40; N, 5.09. Found: C, 34.88; H, 4.65; N, 4.88.

Four additional recrystallizations from acetone gave the α-D anomer (**9b**): m.p. 192–193° (dec.), $[\alpha]_D^{22}$ +108 ±2 (initial extrapolated) → +60 ±1.5° (final; *c* 2.0, water); λ_{max}^{KBr} 3.0–3.1 (NH, OH), 5.9 (*N*-trifluoroacetyl carbonyl), 6.42 (NH), 8.62 (CF), 7.42, 7.68, 7.9, 8.28, 8.35, 8.8, 9.03, 9.18, 9.44, 9.56, 9.7, 9.98, 10.28, 10.6, 11.06, 11.42, 11.75, 12.43, and 13.7 μm; X-ray powder diffraction data: 11.26 vw, 9.88 vw, 8.85 vw, 6.63 m, 5.56 s, 5.10 s (2), 4.77 w, 4.50 s (1), 4.31 s (3), 3.96 s, 3.70 s (2), 3.50 m, 3.31 m, 3.16 m, 3.01 m, 2.84 m, 2.68 w, 2.49 w, 2.39 vw, 2.30 m, 2.22 w, and 2.06 w. The physical constants of this compound were unchanged by further recrystallization.

Anal. Calc. for $C_8H_{12}F_3NO_6$: C. 34.92; H, 4.40; N, 5.09. Found: C, 34.79; H, 4.69; N, 4.78.

1,3,4,6-Tetra-O-acetyl-2-deoxy-2-(trifluoroacetamido)-α(and β)-D-glucopyranose (**3** *and* **4**). — 2-Deoxy-2-(trifluoroacetamido)-D-glucose (**2a**, 2.0 g) was dissolved in a pre-cooled (0°) mixture of pyridine (16 ml) and acetic anhydride (9 ml); the solution was kept overnight at room temperature, and then poured into iced water (30 ml), and the mixture extracted with dichloromethane (80 ml). The extract was washed successively with N hydrochloric acid (until the acid was no longer neutralized), 15% aqueous sodium hydrogen carbonate solution, and water, and dried (sodium sulfate). Evaporation of the solvent yielded a clear syrup which, by t.l.c. with ether as the developer, showed two major components (R_F 0.47 and 0.57). The crude product was crystallized and recrystallized from methanol–water, to give 1.0 g (31%) of 1,3,4,6-tetra-*O*-acetyl-2-deoxy-2-(trifluoroacetamido)-β-D-glucopyranose (**4**) as a white, crystalline solid: m.p., and m.m.p. with authentic material[8], 166–167.5°; $[\alpha]_D^{25}$ −12 ±1° (*c* 3.4, chloroform); lit.[8] m.p. 167°, $[\alpha]_D^{22}$ −13° (*c* 2.4, chloroform). The X-ray powder diffraction data were identical with those for this compound previously prepared in this laboratory[8]. The compound was homogeneous by t.l.c., with ether as the developer, and corresponded to the faster-moving component of the crude product (R_F 0.57).

The mother liquors from the two recrystallizations of **4** were evaporated to yield a clear, syrupy residue which, by t.l.c. with ether as the developer, showed a major component (R_F 0.48) and a minor component (R_F 0.56). These two components

were isolated by resolution on 24 chromatoplates (200 × 200 × 1.25 mm), with ether as the developer and indication by iodine vapor. The two zones were removed, and extracted with acetone. Each acetone extract was evaporated, and the residues were dissolved in dichloromethane. The resulting solutions were successively washed with 15% aqueous potassium iodide and water, dried (sodium sulfate), and evaporated. The residue from the faster-moving zone (R_F 0.56) was crystallized from methanol–water, to yield an additional 0.45 g (14%) of **4**: m.p. and m.m.p. 166–167.5°; $[\alpha]_D^{23}$ −11.5 ±2° (*c* 1.5, chloroform).

The material from the slower-moving zone (R_F 0.48) was crystallized from ether–hexane to give 1.3 g (40%) of 1,3,4,6-tetra-*O*-acetyl-2-deoxy-2-(trifluoroacetamido)-α-D-glucopyranose (**3**): m.p. 124.5–126°; $[\alpha]_D^{21}$ +70 ±1° (*c* 4.7, chloroform); λ_{max}^{KBr} 3.0 (NH), 5.7 (*O*-acetyl carbonyl), 5.83 (*N*-trifluoroacetyl carbonyl), 6.4 (NH), 8.1–8.25 (ester), 8.55 (CF), 6.9, 7.0, 7.3, 8.9, 9.4, 9.76, 10.4, 10.62, 10.8, 11.1–11.2, 11.5, 12.95, and 13.7 μm; X-ray powder diffraction data: 16.37 vw, 10.53 s, 9.46 vs (1), 8.21 s, 7.06 m, 6.08 m, 5.49 w, 5.19 s, 4.82 vs (3), 4.66 m, 4.44 w, 4.29 vs (3), 4.03 w, 3.78 vs (2), 3.52 s, 3.45 s, 3.25 vw, 3.02 vw, 2.92 m, 2.79 w, 2.64 w, 2.52 m, 2.34 w, 2.22 vw, 2.17 w, 2.07 w, and 1.90 vw. This compound was homogeneous by t.l.c., with ether as the developer.

Anal. Calc. for $C_{16}H_{20}F_3NO_{10}$: C, 43.35; H, 4.55; N, 3.16. Found: C, 43.19; H, 4.52; N, 3.49.

*1,3,4,6-Tetra-*O*-acetyl-2-deoxy-2-(trifluoroacetamido)-α(and β)-D-galactopyranose* (**10** and **11**). — 2-Deoxy-2-(trifluoroacetamido)-D-galactose (**9a**, 2.0 g) was acetylated with pyridine (16 ml) and acetic anhydride (9 ml) by the procedure described in the preceding experiment. The crude, syrupy product showed two principal components (R_F 0.43 and 0.56) by t.l.c. with ether as the developer. Three recrystallizations from methanol–water gave 1,3,4,6-tetra-*O*-acetyl-2-deoxy-2-(trifluoroacetamido)-α-D-galactopyranose (**10**) as a white, crystalline solid, yield 0.97 g (30%): m.p. 155–156.5°; $[\alpha]_D^{23}$ +84 ±1° (*c* 2.1, chloroform); λ_{max}^{KBr} 3.05 (NH), 5.7 (*O*-acetyl carbonyl), 5.85 (*N*-trifluoroacetyl carbonyl), 6.4 (NH), 8.1–8.3 (ester), 8.68 (CF), 7.32, 9.15, 9.34, 9.6, 9.9, 10.7, 11.1, 11.64, 12.2, 13.1, and 13.8 μm; X-ray powder diffraction data: 7.54 vs (1), 6.33 s, 5.85 m, 5.45 s, 5.01 s, 4.63 m, 4.32 vs (2), 4.10 s, 3.96 s, 3.79 w, 3.66 w, 3.51 s (3), 3.41 vw, 3.34 vw, 3.05 m, 2.91 w, 2.84 m, 2.77 vw, 2.59 vw, 2.51 m, 2.45 vw, 2.31 w, 2.23 vw, 2.17 m, and 2.11 w. This compound was homogeneous by t.l.c. with ether as the developer, and corresponded to the slower-moving component of the crude product (R_F 0.43).

Anal. Calc. for $C_{16}H_{20}F_3NO_{10}$: C, 43.35; H, 4.55; N, 3.16. Found: C, 43.29; H, 4.40; N, 2.95.

The mother liquors from the three recrystallizations of **10** were evaporated to yield a syrupy residue which, on t.l.c. with ether as the developer, showed a major component (R_F 0.57) and a minor component (R_F 0.41). These two components were isolated by resolution on 24 chromatoplates (200 × 200 × 1.25 mm) with ether as the developer, by the procedure described in the preceding experiment. Crystallization of the material obtained from the slower-moving zone (R_F 0.41) from methanol–

water yielded an additional 0.52 g (16%) of **10**: m.p. and m.m.p. 155–156.5°, $[\alpha]_D^{22}$ +84 ±1° (*c* 2.1, chloroform).

The material obtained from the faster-moving zone (R_F 0.57) was crystallized from ether–hexane to give 1.2 g (37%) of 1,3,4,6-tetra-*O*-acetyl-2-deoxy-2-(trifluoroacetamido)-β-D-galactopyranose (**11**): m.p. 130–131°; $[\alpha]_D^{21}$ +11 ±1° (*c* 3.1, chloroform); λ_{max}^{KBr} 3.06 (NH), 5.7 (*O*-acetyl carbonyl), 5.8 (*N*-trifluoroacetyl carbonyl), 6.36 (NH), 8.1–8.3 (ester), 8.65 (CF), 7.33, 8.45, 9.18, 9.66, 10.46, 11.2, 11.7, 13.52, and 13.9 μm; X-ray powder diffraction data: 13.05 m, 7.90 vs (1), 6.25 s, 5.71 s, 5.40 w, 5.17 s (3), 4.80 s, 4.54 m, 4.10 vs (2), 3.83 m, 3.70 m, 3.60 m, 3.43 m, 3.28 s, 3.10 s, 2.98 w, 2.90 m, 2.80 w, 2.70 m, 2.59 m, 2.50 w, 2.44 m, 2.29 m, 2.24 m, 2.18 vw, and 2.08 m. This compound was homogeneous by t.l.c. with ether as the developer.

Anal. Calc. for $C_{16}H_{20}F_3NO_{10}$: C, 43.35; H, 4.55; N, 3.16. Found: C, 43.52; H, 4.58; N, 3.57.

3,4,6-Tri-O-acetyl-2-deoxy-2-(trifluoroacetamido)-α-D-glucopyranosyl bromide[8,10] (**5**). — *Method* (*A*). 1,3,4,6-Tetra-*O*-acetyl-2-deoxy-2-(trifluoroacetamido)-α-D-glucopyranose (**3**, 2.0 g) was moistened with chloroform (1.0 ml), and acetic acid (20 ml) almost saturated at 0° with hydrogen bromide was added. The mixture was kept for 2 h at room temperature in a glass-stoppered flask, and was then dissolved in dichloromethane (30 ml). The solution was successively washed with cold, 20% aqueous sodium acetate solution and water, and dried (magnesium sulfate). Evaporation of the solvent gave a syrupy residue which was crystallized from ether–hexane, yield 2.0 g (96%), m.p. 96–97°, $[\alpha]_D^{20}$ +125 ±1° (*c* 2.7, chloroform); lit.[10] m.p. 95–97°; lit.[8] m.p. 96°, $[\alpha]_D^{21}$ +126° (*c* 2.92, chloroform).

Method (*B*). 2-Deoxy-2-(trifluoroacetamido)-D-glucose (**2a**, 0.8 g) was acetylated with a mixture of pyridine (6.5 ml) and acetic anhydride (3.5 ml) by the procedure described for the preparation of **3** and **4**. The crude, syrupy product was crystallized from ether–hexane, giving a white, crystalline material; yield 1.1 g (85%); m.p. 116–121°. T.l.c. of the product with ether as the developer showed two components (R_F 0.45 and 0.56) which, presumably, were 1,3,4,6-tetra-*O*-acetyl-2-deoxy-2-(trifluoroacetamido)-α-D-glucopyranose (**3**) and its β-D anomer (**4**), the anomers having co-crystallized.

The material was treated with acetic acid (1.1 ml) almost saturated at 0° with hydrogen bromide, according to the procedure used in the preceding experiment. Crystallization of the crude product from ether–hexane gave **5**; yield 1.08 g (94%): m.p. and m.m.p. 96–97°; $[\alpha]_D^{21}$ +127 ±2° (*c* 1.4, chloroform).

3,4,6-Tri-O-acetyl-2-deoxy-2-(trifluoroacetamido)-D-galactopyranosyl bromide (**12**). — *Method* (*A*). 1,3,4,6-Tetra-*O*-acetyl-2-deoxy-2-(trifluoroacetamido)-α-D-galactopyranose (**10**, 1.0 g) was treated with acetic acid almost saturated at 0° with hydrogen bromide, according to the procedure used in the preceding experiment for the preparation of **5**. A clear, syrupy product was obtained which formed a solid foam on removal of the last traces of solvent under diminished pressure in a vacuum desiccator, yield 1.02 g (97%): m.p. 60–62°; $[\alpha]_D^{22}$ +146 ±1° (*c* 1.5, chloroform); λ_{max}^{KBr} 3.05 (NH), 5.7–5.8 (*O*-acetyl and *N*-trifluoroacetyl carbonyl), 6.45 (NH),

8.1–8.3 (ester), 8.65 (CF), 7.3, 9.2, 10.55, 11.1, 11.8, 12.9, and 13.7 μm. This compound was homogeneous by t.l.c. with 2:1 ether–petroleum ether as the developer. Attempts to crystallize the product were unsuccessful.

Anal. Calc. for $C_{14}H_{17}BrF_3NO_8$: C, 36.22; H, 3.69; N, 3.02. Found: C, 36.22; H, 3.90; N, 2.97.

Method (B). 1,3,4,6-Tetra-*O*-acetyl-2-deoxy-2-(trifluoroacetamido)-β-D-galactopyranose (**11**, 0.50 g) was treated with acetic acid (0.50 ml) almost saturated at 0° with hydrogen bromide, according to the procedure used in the preceding experiment; yield 0.49 g (94%) of **12**: m.p. 60–63°; $[\alpha]_D^{20}$ +144 ±2° (*c* 1.1, chloroform).

Method (C). 2-Deoxy-2-(trifluoroacetamido)-D-galactose (**9a**, 0.60 g) was acetylated with a mixture of pyridine (4.5 ml) and acetic anhydride (3.0 ml) by the procedure described for the preparation of **2** and **3**. The crude, syrupy product was dissolved in methanol (20 ml), water (30 ml) was added, and the solution was concentrated to half volume. After refrigeration overnight, a white, crystalline material formed; yield 0.58 g (60%): m.p. 126–130°. Further concentration of the mother liquor to about 15 ml, followed by refrigeration overnight, produced a second crop of crystalline material; yield 0.20 g (21%), m.p. 121–126°. Treatment of the combined material (0.78 g) with acetic acid (0.8 ml) almost saturated at 0° with hydrogen bromide, according to the procedure used in the preceding experiment, produced amorphous **12**, yield 0.76 g (93%), m.p. 59–62°, $[\alpha]_D^{22}$ +143 ±2° (*c* 1.0, chloroform).

6-Benzamido-9-[3,4,6-tri-O-acetyl-2-deoxy-2-(trifluoroacetamido)-α(andβ)-D-glucopyranosyl]-9H-purine (**6a** *and* **7a**). — A mixture of 6-benzamido-9-(chloromercuri)-9*H*-purine[13] (8.0 g), cadmium carbonate (2.0 g), and Celite (2.5 g) in toluene (75 ml) was azeotropically dried by distillation of approximately one third of the solvent. To the hot suspension was added, with stirring, 3,4,6-tri-*O*-acetyl-2-deoxy-2-(trifluoroacetamido)-α-D-glucopyranosyl bromide (**5**, 2.6 g), and the mixture was refluxed for 8 h with stirring, and then kept overnight at room temperature. The mixture was poured into cold (0°) petroleum ether (b.p. 30–60°, 100 ml), and the precipitate that formed was collected by filtration, and extracted with chloroform (300 ml, total). The extract was successively washed with 30% aqueous potassium iodide solution and water, and dried (sodium sulfate). The solution was evaporated to a pale-amber glass (1.65 g) which, on t.l.c. with 5:2 chloroform–acetone as developer, showed a major component (R_F 0.35) and two minor components (R_F 0.6 and 0.8). The crude product was resolved by preparative t.l.c. on 24 chromatoplates (200 × 200 × 1.25 mm), with 5:2 chloroform–acetone as the developer and indication by u.v. light. The two slower-moving zones (R_F 0.35 and 0.6) were excised, and extracted with acetone. Evaporation of the extract from the faster-moving zone (R_F 0.6) gave a clear glass (0.25 g), which was crystallized from chloroform–isopropyl ether to give a gelatinous mixture that yielded 6-benzamido-9-[3,4,6-tri-*O*-acetyl-2-deoxy-2-(trifluoroacetamido)-α-D-glucopyranosyl]-9*H*-purine (**6a**) as a white, crystalline solid, yield 0.23 g (6.6%): m.p. 169–172°, $[\alpha]_D^{22}$ +105 ±2.5° (*c* 0.7, chloroform); λ_{max}^{KBr} 3.1 (NH), 5.75 (*O*-acetyl, and *N*-trifluoroacetyl carbonyl), 6.2, 6.35, 6.65, 6.9 (aryl C=C, purine, NH), 8.1–8.3 (ester), 8.6 (CF), 14.1 (substituted benzene), 7.36,

7.55, 9.15, 9.6, 10.3, 11.2, 12.52, and 13.6 μm; λ_{max}^{EtOH} 211 (ε 21,000), 234 (ε 13,550), and 282 nm (ε 19,500); X-ray powder diffraction data: 13.81 s (1), 11.95 m, 10.43 s (3), 9.69 vw, 8.61 s, 7.45 m, 7.01 vw, 6.50 m, 6.05 m, 5.68 m, 5.28 w, 5.01 w, 4.82 m, 4.60 m, 4.23 w, 3.84 w, 3.60 s (2), 3.32 m, 3.05 w, 2.81 vw, and 2.64 vw. This compound was homogeneous by t.l.c. with 3:1 chloroform–acetone as the developer.

Anal. Calc. for $C_{26}H_{25}F_3N_6O_9$: C, 50.16; H, 4.05; N, 13.50. Found: C, 50.19; H, 4.14; N, 13.70.

Evaporation of the extract from the slower-moving zone (R_F 0.35) gave a clear glass (1.04 g). Attempted crystallization from chloroform–isopropyl ether gave 6-benzamido-9-[3,4,6-tri-*O*-acetyl-2-deoxy-2-(trifluoroacetamido)-β-D-glucopyranosyl]-9*H*-purine (**7a**) as a white, amorphous solid, yield 0.90 g (26%): m.p. 148–153° (softening above 133°); $[\alpha]_D^{21}$ −50 ±1° (*c* 2.6, chloroform); λ_{max}^{KBr} 3.05 (NH), 5.7 (*O*-acetyl and *N*-trifluoroacetyl carbonyl) 6.2, 6.32, 6.65, 6.75, 6.9 (aryl C=C, purine, NH), 8.1–8.3 (ester), 8.6 (CF), 14.1 (substituted benzene), 7.32, 8.7, 9.3, 10.8, 11.2, 12.2, 12.5, and 13.2 μm; λ_{max}^{EtOH} 210 (ε 21,200), 234 (ε 12,500), and 280 nm (ε 19,700). This compound was homogeneous by t.l.c. with 2:1 chloroform–acetone as the developer.

Anal. Calc. for $C_{26}H_{25}F_3N_6O_9$: C, 50.16; H, 4.05; N, 13.50. Found: C, 50.23; H, 4.03; N, 13.80.

9-[3,4,6-Tri-O-acetyl-2-deoxy-2-(trifluoroacetamido)-α-D-glucopyranosyl]adenine picrate (**6b**). — To a solution of 6-benzamido-9-[3,4,6-tri-*O*-acetyl-2-deoxy-2-(trifluoroacetamido)-α-D-glucopyranosyl]-9*H*-purine (**6a**, 60 mg) in isopropyl alcohol (10 ml) was added a solution of picric acid (25 mg) in methanol (5 ml). The solution was concentrated to about one third its volume by boiling (20 min), allowed to cool slowly to room temperature, and then refrigerated overnight. The yellow, crystalline solid which separated was filtered off, and washed with cold hexane; yield 60 mg (83%), m.p. 210–213° (dec.). Further recrystallizations from isopropyl alcohol–methanol–petroleum ether (b.p. 60–110°) afforded pure material: m.p. 216–217° (dec.), $[\alpha]_D^{22}$ +83 ±2° (*c* 0.6, acetone), λ_{max}^{KBr} 3.1–3.3 (NH, NH_3^+), 5.75 (*O*-acetyl and *N*-trifluoroacetyl carbonyl), 5.92, 6.2, 6.4, 6.75, 7.05 (aryl C=C, purine, NH, NH^+), 6.5, 7.6 (NO_2), 8.1–8.25 (ester), 8.6 (CF), 14.0–14.3 (substituted benzene), 7.35, 9.6, 10.96, 12.66, and 13.4 μm; X-ray powder diffraction data: 11.63 s, 10.34 w, 8.23 vs (1), 6.94 m, 6.28 m, 5.79 w, 5.45 m, 5.25 m, 4.81 m, 4.49 m, 4.32 m, 3.99 s (2), 3.81 w, 3.64 w, 3.47 m, 3.35 s (3), 3.20 vw, and 3.02 m.

Anal. Calc. for $C_{25}H_{24}F_3N_9O_{15}$: C, 40.17; H, 3.24; N, 16.86. Found: C, 40.39; H, 3.52; N, 16.68.

9-[3,4,6-Tri-O-acetyl-2-deoxy-2-(trifluoroacetamido)-β-D-glucopyranosyl]adenine picrate (**7b**). — A solution of 6-benzamido-9-[3,4,6-tri-*O*-acetyl-2-deoxy-2-(trifluoroacetamido)-β-D-glucopyranosyl]-9*H*-purine (**7a**, 0.20 g) in isopropyl alcohol (15 ml) was treated with picric acid (0.08 g) in methanol (8 ml) by the procedure described in the preceding experiment, yield 0.22 g (92%); m.p. 207–210° (dec.). Recrystallization from isopropyl alcohol afforded pure material, m.p. 212–214° (dec.), $[\alpha]_D^{21}$ −39 ±1° (*c* 1.9, acetone); λ_{max}^{KBr} 3.0–3.25 (NH, NH_3^+) 5.72 (*O*-acetyl and *N*-trifluoroacetyl

carbonyl), 5.92, 6.2, 6.35, 6.68, 7.04 (aryl C=C, purine, NH, NH^+), 6.5, 7.6 (NO_2), 8.1–8.3 (ester), 8.6 (CF), 14.1 (substituted benzene), 7.35, 9.25, 9.6, 11.0, 12.25, 12.65, and 13.45 μm; X-ray powder diffraction data: 12.63 s (1), 10.28 m, 9.07 m, 7.05 m, 6.44 m, 6.01 vw, 5.47 s (3), 5.00 s, 4.50 s, 4.22 s (2), 4.01 w, 3.83 w, 3.69 vw, 3.52 s, 3.36 m, 3.25 w, 3.16 w, and 3.10 vw.

Anal. Calc. for $C_{25}H_{24}F_3N_9O_{15}$: C, 40.17; H, 3.24; N, 16.86. Found: C, 40.13; H, 3.16; N, 16.79.

9-(2-Amino-2-deoxy-α-D-glucopyranosyl)adenine (**6c**). — 6-Benzamido-9-[3,4,6-tri-*O*-acetyl-2-deoxy-2-(trifluoroacetamido)-α-D-glucopyranosyl]-9*H*-purine (**6a**, 0.20 g) was dissolved in 40 ml of methanol almost saturated at 0° with ammonia. The solution was kept for 7 days at room temperature, after which it was concentrated to 5 ml and an excess of ether (50 ml) was added. The resultant, white, flocculent precipitate was filtered off, washed with ether, and recrystallized from methanol–ethanol; yield 0.085 g (89%): m.p. and m.m.p. with authentic material[5] 242–244° (dec.), $[\alpha]_D^{21}$ +84 ±2° (*c* 1.5, water); lit.[5] m.p. 242–244° (dec.), $[\alpha]_D^{22}$ +83 ±6° (*c* 0.2, water).

9-(2-Amino-2-deoxy-β-D-glucopyranosyl)adenine (**7c**). — 6-Benzamido-9-[3,4,6-tri-*O*-acetyl-2-deoxy-2-(trifluoroacetamido)-β-D-glucopyranosyl]-9*H*-purine (**7a**, 0.50 g) was deacylated with methanol (80 ml) almost saturated at 0° with ammonia, as described in the preceding experiment. The yield, after recrystallization from ethanol, was 0.205 g (86%): m.p. and m.m.p. with authentic material[5] 185–188° (dec.), $[\alpha]_D^{22}$ −17 ±1° (*c* 2.3, water); lit.[5] m.p. 186–188° (dec.), $[\alpha]_D^{23}$ −17 ±2° (*c* 0.2, water).

6-Benzamido-9-[3,4,6-tri-O-acetyl-2-deoxy-2-(trifluoroacetamido)-α(and β)-D-galactopyranosyl]-9H-purine (**13a** *and* **14a**). — 3,4,6-Tri-*O*-acetyl-2-deoxy-2-(trifluoroacetamido)-D-galactosyl bromide (**12**, 2.0 g) was condensed with 6-benzamido-9-(chloromercuri)-9*H*-purine (6.0 g) in refluxing toluene (50 ml) in the presence of cadmium carbonate (2.0 g) and Celite (2.0 g) by the procedure described for the preparation of **6a** and **7a**. A pale-amber glass (1.6 g) was obtained which, by t.l.c. with 5:2 chloroform–acetone as the developer, showed a major component (R_F 0.33) and two minor components (R_F 0.6 and 0.8). The crude product was resolved on 24 chromatoplates (200 × 200 × 1.25 mm), with 5:2 chloroform–acetone as the developer and indication by u.v. light. The two slower-moving zones (R_F 0.33 and 0.6) were removed, and extracted with acetone. Evaporation of the extract from the faster-moving zone gave a clear glass (0.31 g) which was crystallized from chloroform–isopropyl ether to give a gelatinous mixture that yielded 6-benzamido-9-[3,4,6-tri-*O*-acetyl-2-deoxy-2-(trifluoroacetamido)-α-D-galactopyranosyl]-9*H*-purine (**13a**) as a white, crystalline solid upon suction filtration; yield 0.25 g (93%), m.p. 140–144° (softening above 130°), $[\alpha]_D^{22}$ +120 ±3° (*c* 1.0, chloroform); λ_{max}^{KBr} 3.1 (NH), 5.75 (*O*-acetyl and *N*-trifluoroacetyl carbonyl), 6.2, 6.35, 6.65, 6.76, 6.9 (aryl C=C, purine, NH), 8.1–8.3 (ester), 8.65 (CF), 14.1 (substituted benzene), 7.32, 7.55, 9.2, 10.55, 11.1, 11.7, and 12.52 μm; λ_{max}^{EtOH} 210 (ε 22,300), 234 (ε 13,300), and 282 nm (ε 19,500); X-ray powder diffraction data: 13.50 w, 9.98 s (1), 5.17 m, 4.42 m, 3.87 s (2),

3.55 w, and 3.33 s (3). This compound was homogeneous by t.l.c. with 3:1 chloroform–acetone as the developer.

Anal. Calc. for $C_{26}H_{25}F_3N_6O_9$: C, 50.16; H, 4.05; N, 13.50. Found: C, 50.21; H, 4.42; N, 13.42.

Evaporation of the extract from the slower-moving zone (R_F 0.33) gave a clear glass (0.94 g). Attempted crystallization from chloroform–isopropyl ether yielded 6-benzamido-9-[3,4,6-tri-*O*-acetyl-2-deoxy-2-(trifluoroacetamido)-β-D-galactopyranosyl]-9*H*-purine (**14a**) as an amorphous, white solid, yield 0.80 g (30%); m.p. 150–155° (softening above 135°), $[\alpha]_D^{19}$ −34 ±1° (*c* 2.7, chloroform); λ_{max}^{KBr} 3,05, 5.7 (*O*-acetyl and *N*-trifluoroacetyl carbonyl), 6.2, 6.32, 6.65, 6.75, 6.9 (aryl C=C, purine, NH), 8.1–8.3 (ester), 8.65 (CF), 14.1 (substituted benzene), 9.2, 10.5, 10.85, 12.55, and 13.2 μm; λ_{max}^{EtOH} 211 (ε 20,900), 234 (ε 12,600), and 280 nm (ε 19,900). This compound was homogeneous by t.l.c. with 2:1 chloroform–acetone as the developer.

Anal. Calc. for $C_{26}H_{25}F_3N_6O_9$: C, 50.16; H, 4.05; N, 13.50. Found: C, 50.21; H, 4.36; N, 13.56.

*9-[3,4,6-Tri-*O*-acetyl-2-deoxy-2-(trifluoroacetamido)-α-D-galactopyranosyl]adenine picrate* (**13b**). — A solution of 6-benzamido-9-[3,4,6-tri-*O*-acetyl-2-deoxy-2-(trifluoroacetamido)-α-D-galactopyranosyl]-9*H*-purine (**13a**, 50 mg) in isopropyl alcohol (8 ml) was treated with picric acid (20 mg) in methanol (4 ml) as described for the preparation of **6b**. The yield of crude product was 50 mg (83%); m.p. 177–182°. Further recrystallizations from isopropyl alcohol–methanol–petroleum ether (b.p. 60–110°) afforded pure material; m.p. 181–185°, $[\alpha]_D^{22}$ +108 ±4° (*c* 0.3, acetone); λ_{max}^{KBr} 3.1–3.3 (NH, NH_3^+), 5.76 (*O*-acetyl and *N*-trifluoroacetyl carbonyl), 5.92, 6.2, 6.4, 6.7, 7.05 (aryl C=C, purine, NH, NH_3^+), 6.5, 7.6 (NO_2), 8.1–8.3 (ester), 8.65 (CF), 14.1–14.2 (substituted benzene), 7.36, 9.5, 11.0, 12.7, and 13.5 μm; X-ray powder diffraction data: 10.72 w, 9.51 s (2), 7.53 w, 6.73 w, 5.28 w, 4.63 m, 4.34 m, 3.93 s (1), and 3.50 s (3).

Anal. Calc. for $C_{25}H_{24}F_3N_9O_{15}$: C, 40.17; H, 3.24; N, 16.86. Found: C, 40.01; H, 3.35; N, 16.64.

*9-[3,4,6-Tri-*O*-acetyl-2-deoxy-2-(trifluoroacetamido)-β-D-galactopyranosyl]adenine picrate* (**14b**). — A solution of 6-benzamido-9-[3,4,6-tri-*O*-acetyl-2-deoxy-2-(trifluoroacetamido)-β-D-galactopyranosyl]-9*H*-purine (**14b**, 0.18 g) in isopropyl alcohol (12 ml) was treated with picric acid (0.075 g) in methanol (7 ml) as described for the preparation of **6b**. The yield of crude product was 0.19 g (88%); m.p. 203–205° (dec.). Recrystallization from isopropyl alcohol afforded pure material: m.p. 205–207° (dec.), $[\alpha]_D^{19}$ −37 ±1.5° (*c* 1.3, acetone); λ_{max}^{KBr} 3.05–3.3 (NH, NH_3^+), 5.75 (*O*-acetyl and *N*-trifluoroacetyl carbonyl), 5.92, 6.2, 6.4, 6.75, 7.05 (aryl C=C, purine, NH, NH_3^+), 6.5, 7.6 (NO_2), 8.2 (ester), 8.6 (CF), 13.9–14.05 (substituted benzene), 7.36, 9.25, 9.55, 10.85, 12.7, and 13.5 μm; X-ray powder diffraction data: 11.05 w, 8.93 s (1), 7.85 vw, 7.28 s (3), 6.51 m, 5.88 w, 5.44 m, 5.05 m, 4.81 w, 4.59 w, 4.47 s, 4.36 w, 4.19 w, 3.99 vw, 3.74 vw, 3.60 m, 3.40 s (2), 3.28 m, 3.21 vw, 3.09 w, 2.91 vw, 2.83 vw, and 2.73 w.

Anal. Calc. for $C_{25}H_{24}F_3N_9O_{15}$: C, 40.17; H, 3.24; N, 16.86. Found: C, 39.98; H, 3.36; N, 16.79.

9-(2-Amino-2-deoxy-α-D-galactopyranosyl)adenine (**13c**). — 6-Benzamido-9-[3,4,6-tri-*O*-acetyl-2-deoxy-2-(trifluoroacetamido)-α-D-galactopyranosyl]-9*H*-purine (**13a**, 170 mg) was deacylated with methanol (40 ml) almost saturated at 0° with ammonia, as described for the preparation of **6c**. Attempted crystallization of the crude product from methanol–ether gave a white, amorphous solid, yield 67 mg (83%); m.p. 198–208° (softening and swelling above 160°), $[\alpha]_D^{21}$ +134 ±3° (*c* 0.6, water); λ_{max}^{KBr} 2.95–3.1 (OH, NH_2), 6.08, 6.25, 6.35, 6.8 (purine, NH), 7.1, 7.52, 7.7, 8.1, 8.22, 9.2, 11.38, 12.55, 13.55, and 13.95 μm; $\lambda_{max}^{H_2O}$ 210 (ε 18,900) and 262 nm (ε 14,500). This compound was homogeneous by t.l.c. with methanol as the developer.

Anal. Calc. for $C_{11}H_{16}N_6O_5$: C, 44.59; H, 5.44; N, 28.37. Found: C, 44.39; H, 5.48; N, 28.04.

9-(2-Amino-2-deoxy-β-D-galactopyranosyl)adenine (**14c**). — 6-Benzamido-9-[3,4,6-tri-*O*-acetyl-2-deoxy-2-(trifluoroacetamido)-β-D-galactopyranosyl]-9*H*-purine (**14a**, 0.40 g) was deacylated with methanol (80 ml) almost saturated at 0° with ammonia, as described for the preparation of **6c**. Attempted crystallization of the crude product from methanol–ether gave a white, amorphous solid, yield 0.155 g (81%): m.p. 211–220° (softening and swelling above 155°), $[\alpha]_D^{21}$ +19 ±1.5° (*c* 0.12, water); λ_{max}^{KBr} 2.95–3.1 (NH_2, OH), 6.08, 6.25, 6.35, 6.8 (NH, purine), 7.08, 7.3, 7.53, 7.7, 8.0, 8.3, 9.22, 9.86, 11.3, 12.2, 12.55, and 13.75 μm; $\lambda_{max}^{H_2O}$ 210 (ε 19,300) and 261 nm (ε 14,800). This compound was homogeneous by t.l.c. with methanol as the developer.

Anal. Calc. for $C_{11}H_{16}N_6O_5$: C, 44.59; H, 5.44; N, 28.37. Found: C, 44.29; H, 5.58; N, 28.54.

9-(2-Amino-2-deoxy-α-D-galactopyranosyl)adenine dihydrochloride (**13d**). — To a solution of 9-(2-amino-2-deoxy-α-D-galactopyranosyl)adenine (**13c**, 40 mg) in methanol (30 ml) was added 0.40 ml of *N* hydrochloric acid. Several evaporations with methanol (to a volume of 5 ml) were made, to remove the excess of hydrogen chloride. The solution was concentrated to 5 ml, and warmed to about 60°. Isopropyl ether (5 ml) was then slowly added, and the mixture was allowed to cool slowly to room temperature, and then refrigerated overnight. The white, crystalline material that formed was recrystallized from methanol–ethanol, yield 44 mg (88%); dec. at 208–209° (with some charring above 190°), $[\alpha]_D^{22}$ +112 ±3° (*c* 0.6, water); λ_{max}^{KBr} 3.0–3.35 (NH_3^+, OH), 5.78, 5.95, 6.18, 6.3, 6.5, 6.7, 6.85 (purine, NH_3^+), 7.05, 7.38, 7.46, 7.68, 8.2, 8.5, 8.7, 8.85, 9.1, 9.4, 9.62, 9.88, 10.22, 11.43, 12.0, 12.5, and 13.64 μm; $\lambda_{max}^{H_2O}$ 210 (ε 17,800) and 261 nm (ε 14,200); X-ray powder diffraction data: 13.92 s, 9.31 m, 8.41 vw, 7.25 s (1), 6.63 w, 6.33 w, 4.43 s, 4.22 s, 3.89 s (3), 3.60 s, 3.36 m, 3.20 s (2), 3.09 vw, 2.89 vw, 2.78 w, 2.69 vw, 2.55 m, 2.37 m, 2.23 vw, 2.14 w, and 2.01 w.

Anal. Calc. for $C_{11}H_{18}Cl_2N_6O_4$: C, 35.78; H, 4.91; Cl, 19.21; N, 22.76. Found: 35.70; H, 5.18; Cl, 19.27; N, 22.63.

9-(2-Amino-2-deoxy-β-D-galactopyranosyl)adenine dihydrochloride (**14d**). — A

solution of 9-(2-amino-2-deoxy-β-D-galactopyranosyl)adenine (**14c**, 0.10 g) in methanol (35 ml) was treated with M hydrochloric acid (1.0 ml) as described in the preceding experiment. The yield, after recrystallization from methanol–ethanol, was 0.115 g (92%); dec. at 190–192° (with some charring above 180°), $[\alpha]_D^{22}$ +38 ±2° (*c* 0.7, water); λ_{max}^{KBr} 2.95–3.4 (OH, NH_3^+), 5.9, 6.0, 6.28, 6.66, 6.9 (purine, NH_3^+), 7.05, 7.38, 7.54, 7.78, 8.14, 8.75, 8.9, 9.1, 9.45, 9.8, 10.45, 10.66, 11.3, 11.8, 12.3, 12.8, 13.12, and 13.8 μm; $\lambda_{max}^{H_2O}$ 210 (ε 18,500) and 260 nm (ε 14,000); X-ray powder diffraction data: 6.89 vs (1), 6.35 w, 5.93 m, 5.52 w, 5.14 s, 4.81 m, 4.51 s, 4.28 m, 3.72 s, 3.59 s, 3.43 s, 3.25 s (2), 3.13 s (3), 3.00 m, 2.93 vw, 2.77 m, 2.67 vs, 2.52 w, 2.43 m, 3.21 w, 2.26 vw, 2.13 w, 2.02 w, 1.90 vw, 1.84 w, and 1.76 w.

Anal. Calc. for $C_{11}H_{18}Cl_2N_6O_4$: C, 35.78; H, 4.91; Cl, 19.21; N, 22.76. Found: C, 36.02; H, 5.08; Cl, 19.39; N, 22.56.

ACKNOWLEDGMENT

This work was supported by Grants CA-03232-10 and CA-03232-11 from the Department of Health, Education, and Welfare, U. S. Public Health Service, National Institutes of Health, Bethesda, Md. (The Ohio State University Research Foundation Projects 759-I and 759-J.)

REFERENCES

1 R. S. TIPSON, *Advan. Carbohyd. Chem.*, 1 (1945) 193.
2 F. J. MCEVOY, M. J. WEISS, AND B. R. BAKER, *J. Amer. Chem. Soc.*, 82 (1960) 205.
3 C. L. STEVENS AND K. NAGARAJAN, *J. Med. Pharm. Chem.*, 5 (1962) 1124.
4 M. L. WOLFROM, P. J. CONIGLIARO, AND E. J. SOLTES, *J. Org. Chem.*, 32 (1967) 653.
5 M. L. WOLFROM, H. G. GARG, AND D. HORTON, *Chem. Ind.* (London), (1964) 930; *J. Org. Chem.*, 30 (1965) 1556.
6 M. L. WOLFROM AND M. W. WINKLEY, *Chem. Commun.*, (1966) 533; *J. Org. Chem.*, 32 (1967) 1823; M. L. WOLFROM, M. W. WINKLEY, and S. INOUYE, *Carbohyd. Res.*, 10 (1969) 97.
7 M. L. WOLFROM AND H. B. BHAT, *J. Org. Chem.*, 32 (1967) 2757.
8 M. L. WOLFROM AND H. B. BHAT, *Chem. Commun.*, (1966) 146; *J. Org. Chem.*, 32 (1967) 1821.
9 E. E. SCHALLENBERG AND M. CALVIN, *J. Amer. Chem. Soc.*, 77 (1955) 2779.
10 R. G. STRACHAN, W. V. RUYLE, T. Y. SHEN, AND R. HIRSCHMANN, *J. Org. Chem.*, 31 (1966) 507.
11 M. BERGMANN AND L. ZERVAS, *Ber.*, 64 (1931) 975; D. HORTON, *J. Org. Chem.*, 29 (1964) 1776.
12 C. S. HUDSON, *J. Amer. Chem. Soc.*, 31 (1909) 66; *Advan. Carbohyd. Chem.*, 3 (1948) 15.
13 J. DAVOLL AND B. A. LOWY, *J. Amer. Chem. Soc.*, 73 (1951) 1650.
14 B. R. BAKER, J. P. JOSEPH, R. E. SCHAUB, AND J. H. WILLIAMS, *J. Org. Chem.*, 19 (1954) 1786.
15 R. S. TIPSON, *J. Biol. Chem.*, 130 (1939) 55.

Carbohydrate Research
Elsevier Publishing Company, Amsterdam
Printed in Belgium

STRUCTURAL STUDIES ON IRON–DEXTRAN

CHARACTERISATION OF AN ALKALI-DEGRADED DEXTRAN SUITABLE FOR USE IN THE PRODUCTION OF PARENTERAL IRON–DEXTRAN COMPLEXES

I. BREMNER*, J. S. G. COX, AND G. F. MOSS
Research and Development Dept., Fisons Pharmaceuticals Ltd., Bakewell Road, Loughborough (Great Britain)

(Received January 15th, 1969)

ABSTRACT

The structure of the acid-hydrolysed and alkali-degraded dextran used in the production of an iron–dextran complex has been determined. The dextran has acidic end-groups and, after purification by fractionation on anion-exchange columns, was found to possess a low molecular weight (2500–6250) and no reducing properties, and to contain an average of one glucometasaccharinic acid residue per dextran chain. The evidence suggests that this acid group occurs at the former "reducing" end-group and protects the dextran against further attack by alkali. Approximately 12% of the original dextran was not degraded by the alkali and remains as an impurity in the acid dextran. In spite of the extensive acid hydrolysis and alkali degradation, the dextran still contained around 5% of α-(1→3)-D-glucosidic links.

INTRODUCTION

In recent years, interest has increased in the physical and biological properties of several complexes of iron[1]. A polymeric micelle of ferric hydroxide forms the basis of these complexes which are often of high molecular weight. Solids isolated from such complexes contain up to 40% of iron[2].

For many years, complexes of iron and carbohydrates, in particular dextran, have proved useful in the parenteral treatment of iron-deficiency anaemia. To produce a stable, non-toxic, iron–dextran solution, which is well absorbed from an intramuscular injection site, requires the use of a dextran of low molecular weight with the reducing end-group suitably modified. Alkaline treatment of dextran gives a product which meets this requirement.

Alkaline degradation of polysaccharides usually proceeds by a β-alkoxycarbonyl mechanism, involving stepwise depolymerisation from the reducing end-group[3] until an alkali-resistant linkage is encountered or some stable end-group is formed. The alkaline degradation of 6-*O*-substituted sugars has been investigated[4], but apparently not the degradation of dextran. We have examined the product isolated after alkaline-

*Rowett Research Institute, Bucksburn, Aberdeen (Great Britain).

treatment of dextran and determined the nature of the stable end-group and branch-points.

EXPERIMENTAL

General methods. — Paper chromatography was carried out on Whatman No. 1 and 3MM papers. The irrigants (v/v) used were (*a*) ethyl acetate–pyridine–water (2:1:2); (*b*) ethyl acetate–acetic acid–water (3:1:1); (*c*) butanone–water azetrope; (*d*) butyl alcohol–ethanol–acetic acid–water (45:5:1:49). Chromatographic spray reagents were *p*-anisidine hydrochloride, alkaline silver nitrate, hydroxamic acid, and bromophenol blue. Electrophoretic examination of dextrans was carried out on the Antweiler micro-electrophoresis apparatus with barbiturate buffer (pH 8.6). Electrophoretic examination of oligosaccharides and monosaccharides was carried out on Whatman 3MM paper with either 0.1M borax or molybdate buffer[5] (pH 5.0). Optical rotations were determined on aqueous solutions at room temperature with the Bendix NPL automatic polarimeter 143C. Reducing values were determined by the Somogyi method[6]. Equivalent weights were determined by iodometric titration of carboxyl end-groups.

Preparation and isolation of alkali-degraded dextran. — The dextran used had a number-average molecular weight ($\overline{M}_n$) of *ca.* 3,000 and was synthesised by *Leuconostoc mesenteroides* NRRL B.512. To distinguish it from other fractions, this dextran is termed *A*.

A solution of dextran *A* (200 g) in distilled water (final volume, 1 litre) was heated to 60°. While the solution was stirred continuously, 10N sodium hydroxide (110 ml) was added at such a rate as to maintain a basicity of 0.9–1.1N (determined by external titration against N HCl). The rate of uptake of alkali is shown in Fig. 1; no attempt was made to exclude oxygen from the reaction mixture.

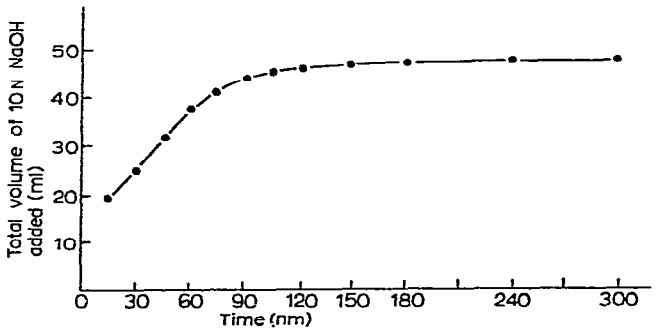


Fig. 1. The uptake of 10N sodium hydroxide during the alkaline degradation of dextran *A* at 60°.

Complete neutralization of the resulting solution caused lactone formation which interfered with later fractionation procedures. Partial neutralization to pH 8 with N hydrochloric acid avoided lactone formation. After dialysis of the partially

neutralised mixture against distilled water for 24 h, the solution was concentrated to *ca.* 400 ml by evaporation *in vacuo* at 40°. Dropwise addition of the solution to ethyl alcohol (2 l) precipitated a light-brown solid (dextran *B*) which was collected, washed with ethanol and ether, and dried over phosphorus pentaoxide to yield material (130 g, 65%) having $[\alpha]_D$ +185° [Found: equivalent wt., 3,500; reducing sugars, 0.49% (*ca.* 10% of that of dextran *A*)].

Separation of acid and neutral fractions of dextran. — Dextran *B* (40 g) dissolved in water (200 ml) was applied to a column (60 × 5 cm) of DEAE cellulose (Ac^-). When the solution had percolated into the column, an interval of 20 h was allowed in order to maximise absorption. The column was then eluted with water (60 ml/h, 2 l) followed by 0.02M potassium acetate (2 l). The carbohydrate content of each fraction (200 ml) was determined by optical rotation.

The eluate was colourless, but a dark-brown material (<0.5%) remained on the column. This could only be removed by subsequent elution with 0.1N sodium hydroxide.

The appropriate fractions were combined and concentrated *in vacuo*. The dextran was then precipitated by dropwise addition to 5 volumes of ethanol. The potassium acetate eluate gave a non-reducing, acidic product [18 g, acid dextran *1* (*AD1*)].

The fraction (22 g) from the water eluate was acidic and reducing. Two more fractionations of this product on DEAE-cellulose gave acid dextran fractions *AD2* (9 g) and *AD3* (4.2 g), and a product (7.6 g) from the water eluate which still had some acid properties. This last fraction was finally separated on a column (24 × 5 cm) of DEAE Sephadex A-50 (OAc^-) by using water followed by 0.02M potassium acetate. The potassium acetate eluate gave a fourth acidic dextran *AD4* (3 g), and the water eluate gave a neutral, reducing dextran, fraction *5* (4.6 g).

Gel filtration of fractions AD1–4 *and* 5. — Samples (5 mg) of each of the dextran fractions were further fractionated by elution from a column (100 × 2.5 cm) of Sephadex G-50 with 0.1M sodium chloride. Fractions (5 ml) were collected, and analysed with the anthrone reagent.

Hydrolysis of the subfractions of dextran B. — A sample (2 g) of dextran *AD1* was hydrolysed with 0.5N H_2SO_4 (100 ml) for 16 h at 100°. The hydrolysate was neutralised with barium carbonate and passed down a column (10 × 1 cm) of Zeo-Karb 225 (H^+) resin. Chromatographic examination [solvents (*b*) and (*c*)] of the column eluate revealed the presence of glucose, small amounts of non-reducing components, and traces of lower oligosaccharides. As initial attempts to resolve these components by chromatography on an anion-exchange resin were unsuccessful, the hydrolysate was treated with excess of calcium carbonate at 50° for 30 min to convert any lactones into the calcium salts. The mixture was centrifuged, and the solution was applied to a column (30 × 1.5 cm) of Amberlite IR-45 (OAc^-) resin. Successive elution with water (600 ml) and 10% acetic acid (500 ml) gave two fractions that contained essentially neutral and acidic sugars, respectively.

The acetic acid eluate was evaporated, and the residue was heated at 80°

in vacuo to convert acids into lactones and then examined by paper chromatography in several solvent systems. In addition to some glucose, components were detected which were chromatographically indistinguishable from α- and β-glucometasaccharinic acid (bromophenol blue spray) and the derived lactones[7] (hydroxamate spray). The lactones in the hydrolysate were isolated by preparative, paper chromatography [solvent (*c*)] and were characterised as follows: α-D-glucometasaccharinolactone, recrystallised from ethyl acetate, had m.p. and mixed m.p. 104° and $[\alpha]_D$ +26° (water); lit[7], m.p. 104° and $[\alpha]_D$ +25° (water); β-D-glucometasaccharinolactone, recrystallised from ethyl acetate, had m.p. and mixed m.p. 91° and $[\alpha]_D$ +6° (water); lit.[7], m.p. 92° and $[\alpha]_D$ +8° (water).

Periodate oxidation of dextrans AD1 *and* AD4. — Samples of *AD1* and *AD4* (97.8 and 92.1 mg, respectively) were oxidised with 0.025M sodium metaperiodate in the dark at room temperature. After 8 days, periodate consumption was measured by the decrease in absorbance of the solution at 223 nm. Formic acid produced was measured by iodometric titration.

The dextrans *AD1* and *AD4* utilised 1.81 and 1.98 moles of periodate per D-glucose residue and liberated 0.87 and 0.98 mole of formic acid per D-glucose residue, respectively.

Smith degradation of dextran AD1. — A sample of dextran *AD1* (2 g) was oxidised in 0.16M sodium metaperiodate (500 ml) for 15 days. After this time, the addition of excess barium carbonate removed the metaperiodate, and centrifugation of the mixture gave a clear supernatant which was deionized with Zeo-Karb 225 (H^+) and IR-45 (HO^-) resins. The solution was then reduced with sodium borohydride (1 g) for 18 h. Cations were removed from the solution with Zeo-Karb 225 (H^+), and, after evaporation, borate was removed from the residue by codistillation with methanol. The resulting polyalcohol was hydrolysed with 0.5N sulphuric acid for 6 h at 100°. The cooled solution was neutralised with barium carbonate, centrifuged, deionised with Zeo-Karb 225 (H^+) and IR-45 (HO^-) resins, and concentrated *in vacuo* at 40°.

Paper chromatography of the hydrolysate in solvents (*a*) and (*b*) showed the presence of a large amount of glycerol and a much smaller amount of glucose.

Enzymic hydrolysis of dextran B. — A solution of dextran *B* (2.1 g) in 0.1M sodium citrate (200 ml) was incubated for 5 days at 37° with an adaptively produced dextranase (66 mg) of *Penicillium funiculosum*[8] (I.M.I. 79195; NRRL 1132). The hydrolysate was heated for 10 min at 100° and then deionised with Zeo-Karb 225 (H^+) and IR-45 (HO^-) resins. The eluate was concentrated and examined by paper chromatography with solvent (*a*). The major components consisted of isomaltodextrins. In addition, there was a second series of oligosaccharides which had R_G values for the lower members of 0.77, 0.42, 0.26, and 0.21. The faster moving of these components corresponded chromatographically to nigerose and *O*-α-D-glucopyranosyl-(1→3)-*O*-α-D-glucopyranosyl-(1→6)-D-glucose.

Graded acetolysis of dextran AD1. — A sample (2 g) of dextran *AD1* was treated with a mixture of acetic anhydride (14.4 ml), acetic acid (9.6 ml), and sulphuric acid (1.8 ml) for 48 h at 37°. The solution was then poured into ice–water (200 ml),

and the precipitate was collected and dissolved in chloroform. Continuous extraction of the aqueous solution with chloroform for 2 days gave additional acetylated material. The combined chloroform solutions were washed with aqueous sodium carbonate and water, dried (Na_2SO_4), and concentrated.

A solution of the syrupy residue in methanol (100 ml) was treated with a solution of 0.2N sodium methoxide (100 ml). After storage for 3 h at 28°, the solution was acidified with acetic acid. Sufficient water was added to redissolve the resulting precipitate, and the solution was passed through a column of Zeo-Karb 225 (H^+) resin. The eluate was evaporated *in vacuo* at 40°.

A sample (0.8 g) of the product (1.62 g) dissolved in water (10 ml) was fractionated by elution with water from a column (60 × 3.8 cm) of Sephadex G-25. Fractions (10 ml) containing carbohydrate (located by polarimetry) formed two peaks, the first of which contained polysaccharides of larger molecular weight. The fractions containing the second peak were combined, concentrated to a small volume *in vacuo*, and further fractionated by preparative, paper chromatography with solvent (*a*).

Two homologous series of oligosaccharides were identified by their chromatographic behaviour in solvent (*a*). Straight-line plots were obtained of log $[(1-R_F)/R_F]$ against the degree of polymerisation. The members of the major series were identified by their chomatographic behaviour as isomaltodextrins. They had R_G values [solvent (*a*)] of 0.64, 0.34, and 0.12. The other oligosaccharides had R_G values of 0.77, 0.46, 0.21, and 0.07. The lower members of this series were characterised as follows: 3-*O*-α-D-glucopyranosyl-D-glucose (nigerose) had $[\alpha]_D$ +137° (lit.[9] +137°), the derived alditol had $M_{Glucitol}$ <0.1 on electrophoresis in molybdate buffer, and the acetate had m.p. 147° (lit.[9] +151°); *O*-α-D-glucopyranosyl-(1→3)-*O*-α-D-glucopyranosyl-(1→6)-D-glucose gave two disaccharides on partial hydrolysis which were chromatographically indistinguishable from nigerose and isomaltose. Borohydride reduction of the trisaccharide gave a product having $M_{Glucitol}$ 0.53 on electrophoresis in molybdate buffer. Partial hydrolysis of the derived alditol gave compounds which were chromatographically and electrophoretically indistinguishable from nigerose and isomaltitol.

RESULTS AND DISCUSSION

The reaction of alkali with a dextran (*A*) of low molecular weight was allowed to proceed until apparently complete, as indicated by cessation of alkali uptake (Fig. 1). Dextran *B* was formed which was acidic and had some reducing power. After fractionation on DEAE-cellulose and DEAE Sephadex A-50, several acidic fractions and one neutral fraction were obtained from Dextran *B*. Table I gives some of the properties of these fractions, and Fig. 2 gives the distribution of molecular size after gel filtration of fractions *AD1*, *AD4*, and fraction 5.

Fraction 5 was indistinguishable from the original dextran *A* in reducing power, on electrophoresis in barbiturate buffer pH 8.6, and on gel filtration on Sephadex G-50.

TABLE I

PROPERTIES OF THE FIVE FRACTIONS ISOLATED AFTER CHROMATOGRAPHY ON DEAE-CELLULOSE OF ALKALI-DEGRADED DEXTRAN

Fraction (%)	$[\alpha]_D$ (*degrees*)	*Equivalent weight*	$\overline{M}_n$
AD1 (46.4)	+174	2,500	1,510
AD2 (23.2)	+188	3,640	
AD3 (10.8)	+192	4,250	
AD4 (7.7)	+197	6,250	4,860
Fraction 5 (12.0)	+199	—	

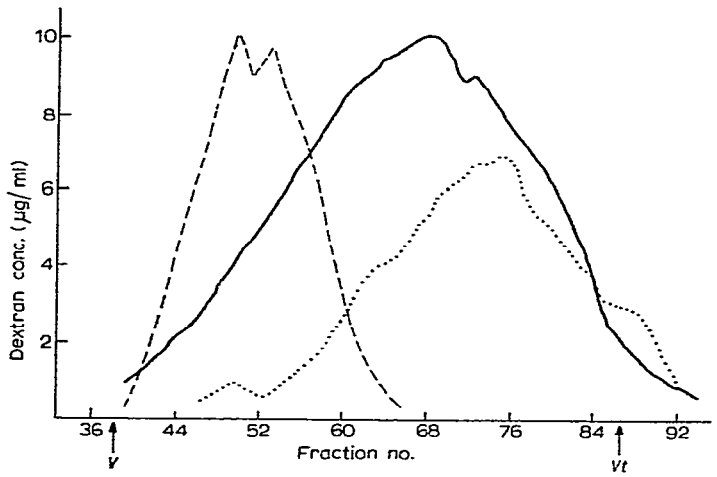


Fig. 2. Gel filtration of dextran *B* and sub-fractions *AD1* and *AD4* on Sephadex G-50. ——— Dextran *B*, ... *AD1*, — — — *AD4*.

Thus, although the uptake of alkali appeared to have reached completion, the fact that 12% of the dextran remained unchanged indicates that this was not the case.

The equivalent weight (3500) of dextran *B* shows an increase over the value (3000) of $\overline{M}_n$ for dextran *A*. This increase probably occurs as a result of the method of preparation of dextran *B*. After acid hydrolysis, dextran *A* gave a proportion of oligosaccharides. The dialysis and the alcohol precipitation during the preparation of dextran *B* will tend to remove these smaller fragments, resulting in an increase in the average molecular weight of the product.

The acid dextran fractions separated from dextran *B* had increasing equivalent weights from *AD1* to *AD4*. The number-average molecular weights determined by osmometry for *AD1* and *AD4* showed similar increases. The reasons for this separation into fractions of increasing molecular weight was probably a result of the method of separation but was not investigated further.

The alkaline treatment of sugars substituted at C-6 has been shown to result

in the formation of metasaccharinic acid[4]. The alkaline treatment of dextran *A* resulted in some degradation with the eventual formation of a glucometasaccharinic acid end-group which stabilised the dextran against further attack by the alkali[2].

The gradation in equivalent weights, molecular weights, and elution volumes from Sephadex G-50 of fractions *AD1–4*, and the presence of the isomeric glucometasaccharinic acids as the sole significant acidic products in the hydrolysates of these fractions, indicate that there is, at the most, only one acid group per dextran molecule. It appears unlikely that the alkaline treatment introduces any other modification into the dextran.

The α-(1→3)-D-glucosidic link present in the original native dextran[10] persisted, despite the extensive acid hydrolysis in the production of dextran *A*. Periodate-oxidation results were consistent with a predominantly (1→6)-linked structure. Inaccuracies inherent in this method did not allow us to make definite conclusions on these values alone. However, the finding of a small proportion of glucose in the hydrolysate after the Smith-degradation of *AD1* indicated some (1→3) branch-points in the molecule. From the value for periodate uptake, this branching must represent only a small percentage (*ca.* 5%) of the total number of D-glucose residues present.

One of the disaccharide products after enzymic hydrolysis of dextran *B* was identified as 3-*O*-α-D-glucopyranosyl-D-glucose (nigerose). This could only have arisen from an α-(1→3)-D-glucosidic link in the dextran chain. This was further confirmed by the identification, after partial acetolysis, of this same disaccharide along with the trisaccharide *O*-α-D-glucopyranosyl-(1→3)-*O*-α-D-glucopyranosyl-(1→6)-D-glucose.

These oligosaccharides were obtained by partial hydrolysis with two different, standard techniques. In view of this, they are unlikely to be artefacts produced by the method. Dextran *B*, therefore, contains (1→3)-D-glucosidic links in the chain. These links must be of the α-D-configuration, since nigerose is an α-D-(1→3)-linked disaccharide.

It is noteworthy that the native dextran elaborated by *Leuconostoc mesenteroides* NRRL B512 contains only 5% of α-(1→3)-D-glucosidic links[10]. The existence of 5% of α-(1→3)-D-glucosidic branches in dextran *B* indicates that these branches are not preferentially hydrolysed during its preparation by hydrolysis and alkaline degradation.

ACKNOWLEDGMENTS

The expert technical assistance of Mr. J. M. Gunn is acknowledged. Thanks are due to Dr. D. Rutherford of these laboratories and Dr. H. Weigel of the Royal Holloway College, London, for their helpful criticism and comments. Our thanks are also due to Dr. Weigel, for kindly supplying a sample of the *P. funiculosum* dextranase, and to Professor W. Hunter, of the Chelsea College of Science and Technology, London, for carrying out determinations of molecular weight.

REFERENCES

1 P. SALTMAN, *J. Chem. Educ.*, 42 (1965) 682.
2 T. G. SPIRO, L. PAPE, AND P. SALTMAN, *J. Amer. Chem. Soc.*, 89 (1967) 5555.
3 R. L. WHISTLER AND J. N. BEMILLER, *Advan. Carbohyd. Chem.*, 13 (1958) 289.
4 J. KENNER AND G. N. RICHARDS, *J. Chem. Soc.*, (1956) 2916.
5 E. J. BOURNE, D. H. HUTSON, AND H. WEIGEL, *J. Chem. Soc.* (1961) 35.
6 M. SOMOGYI, *J. Biol. Chem.*, 160 (1945) 61.
7 W. M. CORBETT, *Methods Carbohyd. Chem.*, 2 (1963) 480.
8 E. J. BOURNE, D. H. HUTSON, AND H. WEIGEL, *Biochem. J.*, 85 (1962) 158.
9 M. STACEY AND J. M. WEBBER, *Methods Carbohyd. Chem.*, 1 (1962) 339.
10 J. C. RANKIN AND A. JEANES, *J. Amer. Chem. Soc.*, 76 (1954) 4435.

Carbohydrate Research
Elsevier Publishing Company, Amsterdam
Printed in Belgium

SYNTHESE VON 2,4-DINITROPHENYLGLYKOSIDEN*

W. HENGSTENBERG UND K. WALLENFELS
Chemisches Laboratorium der Universität Freiburg, Alberstr. 21 (Deutschland)
(Eingegangen den 18. Dezember, 1968; modifiziert den 10. Februar, 1969)

ABSTRACT

2,4-Dinitrophenyl α- and β-D-galactoside, β-D-glucoside, α-D-mannoside, α- and β-L-arabinoside, β-D-xyloside, and 2-chloro-2-deoxy-β-D-galactoside have been synthesized from the respective *O*-trimethylsilylglycosyl halides by the Koenigs–Knorr reaction; all of the glycosides are assumed to have the pyranoid structure. The glycosyl halides were prepared by treatment of the trimethylsilyl derivatives of the ethyl 1-thioglycosides with bromine. The relative amounts of the anomeric forms of the glycosides produced in these syntheses are discussed in the context of mechanistic considerations.

EINFÜHRUNG

Bei der enzymatischen Spaltung von Glykosiden wird ausnahmslos die Bindung zwischen dem glykosidischen Heteroatom und dem C-1 des Glykons gelöst. Man kann sich fragen, ob dies auf der "Spezifität" des Proteins, d.h. der speziellen relativen Position von funktionellen Gruppen am Katalysator zu korrespondierenden am Substrat beruht, oder auf einer unter allen Umständen bevorzugten chemischen Reaktivität der Glykoside. Aus diesem Grunde schien es interessant, Glykoside herzustellen, die nukleophil bevorzugt auf der aglykonischen Seite des glykosidischen Heteroatoms gespalten werden. 2,4-Dinitrophenylglykoside sollten diese interessante Eigenschaft aufweisen. Die Tatsache, daß ihre Herstellung aus den schon vor längerer Zeit beschriebenen Acetylderivaten bisher nicht gelang, mag ein Hinweis hierauf sein. Sie kann aber auch darauf beruhen, daß 2,4-Dinitrophenolat eine extrem gute Austrittsgruppe ist. Die Untersuchung der Solvolyse dieser Verbindungen unter verschiedenen Bedingungen, einschließlich der enzymatischen, machte die Ausarbeitung einer neuen Synthese wünschenswert, bei welcher der Schutz der Hydroxylgruppen unter milderen Bedingungen entfernt werden kann als sie für Entacetylierung und -benzylierung gebraucht werden. Wir berichten in dieser Arbeit über eine Methode, die im Prinzip der Synthese nach Koenigs und Knorr entspricht und als Schutzgruppe für die Herstellung der Halogenzucker die Trimethylsilylgruppe[2,3]

*Kurzmitteilung: Angew. Chem., 77 (1965) 623.

verwendet. Die erhaltenen Trimethylsilyl-glykoside ließen sich hydrolytisch in die freien Glykoside überführen[4], ohne daß ein nennenswerter Angriff an der Glykosidbindung erfolgte.

ERGEBNISSE

Zur Herstellung der Trimethylsilylhalogenosen muß man am C-1 Halogen einführen, ohne daß die extrem säurelabilen Trimethylsilylgruppen[4] entfernt werden. Eine solche Methode hat Bonner[5] zuerst angegeben, sie wurde von Weygand und Ziemann[6], sowie von Wolfrom und Groebke[7] weiter ausgearbeitet und angewendet. Hierbei werden an den Hydroxylfunktionen geschützte 1-Thioglykoside in unpolaren Lösungsmitteln mit elementarem Brom umgesetzt, wobei unter Substitution der Thioalkylfunktion die geschützten Halogenosen entstehen.

Der Mechanismus dieser Reaktion wurde an mehreren Beispielen untersucht. Bonner[5] und Weygand[6] formulieren bei Glucosederivaten einen S_N2 Mechanismus am C-1 des Pyranoserings. Bei den acetylierten 1-Thioglukosiden kann, wie Wolfrom und Groebke[7] zeigen konnten, die Acetylgruppe am C-2 durch Nachbargruppenbeteiligung die Bildung einer β-Acetohalogenose verursachen. Bei der Chlorierung von acetylierten Äthyl-1-thiomannosiden isolierten dieselben Autoren Gemische von anomeren Acetochlormannosen.

Nach diesen experimentellen Befunden sind exakte Voraussagen über den sterischen Verlauf der Halogenierung von 1-Thioglykosiden nicht mit Sicherheit möglich, da diese Reaktion sowohl durch konformative, als auch durch sterische und Nachbargruppeneffekte beeinflußt werden kann.

So konnten bei der Bromierung oder Chlorierung von Äthyl-1-thio-tetra-*O*-trimethylsilyl-β-D-galaktosid und anschließender Glykosidsynthese nach Koenigs und Knorr mit 2,4-Dinitrophenol und Silberoxyd in Benzol nach dem Entfernen der Schutzgruppe säulenchromatographisch 2,4-Dinitrophenyl α- und β-D-galaktosid* (α:β = 1:1) in zusammen 22%iger Ausbeute isoliert werden. Das Reaktionsprodukt der Bromierung des acetylierten Äthyl-1-thio-β-D-galaktosids wurde ebenfalls mit 2,4-Dinitrophenol glykosidiert und lieferte 2,4-Dinitrophenyl-β-D-galaktosidtetraacetat, das gleiche Produkt, das man bei der Glykosidierung von α-Acetobromgalaktose erhält. Die Bromierungsreaktion auf Äthyl-1-thio-tetra-*O*-trimethylsilyl-β-D-glukosid angewandt, liefert nur 2,4-Dinitrophenyl-β-D-glukosid in 10%iger Ausbeute.

Diese Art der Glykosidsynthese wurde auf weitere Hexosen und Pentosen ausgedehnt. Aus Äthyl-1-thio-α-L-arabinosid erhält man ein Gemisch der anomeren 2,4-Dinitrophenyl-L-arabinoside. Äthyl-1-thio-β-D-xylosid liefert nur 2,4-Dinitrophenyl-β-D-xylosid. Aus Äthyl-1-thio-α- und β-D-mannosid konnten wir in beiden Fällen nur das 2,4-Dinitrophenyl-α-D-mannosid aus der Reaktionsmischung isolieren.

Untersuchungen über die Beteiligung der Hydroxylgruppe am C-2 des Galakto-

*Bei diesen und allen weiteren dargestellten Glykosiden wird die Pyranosidstruktur angenommen.

sidmoleküls bei der Spaltung durch Alkali und β-Galaktosidase veranlaßten uns dazu, ein 2,4-Dinitrophenyl-β-D-galaktosid zu synthetisieren, dessen Hydroxylfunktion am C-2 durch eine "schlechte Nachbargruppe" z.B. durch H, OCH_3 oder durch Halogen ersetzt ist. Wir entschieden uns für das Halogen Chlor, da Tri-*O*-acetyl-2-chlor-2-desoxy-α-D-galaktopyranosyl-chlorid [8] (**1**) leicht aus Tri-*O*-acetyl-D-galaktal durch Chlorierung darzustellen ist.

Die Struktur von **1** wurde von Lemieux[8] aus dem Kernresonanzspektrum abgeleitet. Die Glykosidierung dieser Verbindung verläuft unter Bildung des entsprechenden β-D-Glykosids. Denselben Verlauf nimmt die Glykosidierung des Chlorierungsproduktes von Tri-*O*-trimethylsilyl-D-galaktal. Die eindeutige Struktur des 2,4-Dinitrophenyl-2-chlor-2-desoxy-β-D-galaktosids wurde durch das Kernresonanzspektrum festgelegt (Abb. 1).

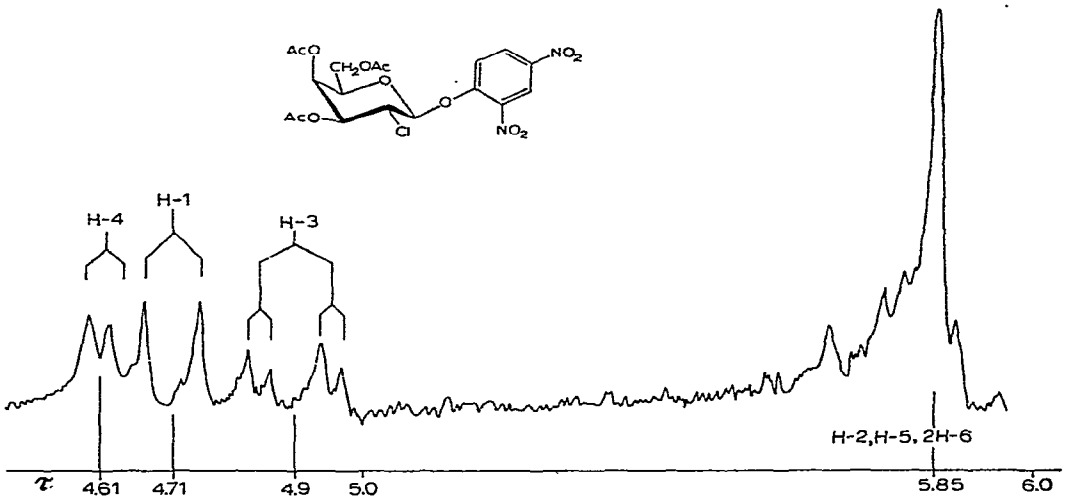


Abb. 1. Kernresonanzspektrum des 2,4-Dinitrophenyl-3,4,6-tri-*O*-acetyl-2-chlor-2-desoxy-β-D-galaktosids bei 100 Megahertz in Deuterochloroform.

Zur Deutung des Spektrums (s, Tabelle I) wurde das analoge Spektrum des Methyl-tri-*O*-acetyl-2-chlor-2-desoxy-β-D-galaktopyranosids herangezogen[8]. Das anomere Proton im entsprechenden 2,4-Dinitrophenyl-galaktosid ist von $\tau \sim 5.5$ nach $\tau = 4.71$ verschoben, während H-4, H-3, H-5, und H-6 unverändert blieben. H-2, das in ein Quadruplett aufspalten sollte, ist schlecht zu vermessen, da es von

TABELLE I

WERTE DER PYRANOSIDISCHEN RINGPROTONEN IN 2,4-DINITROPHENYL-TRI-*O*-ACETYL-2-CHLOR-2-DESOXY-β-D-GALAKTOSID GEGEN TMS ALS INNERER STANDARD

Protonen	1a	2a	3a	4e	5a	6
τ-Werte	4.71	5.80	4.90	4.61	5.85	5.85
	1a 2a	3a 2a	3a 4e	4e 3a	4e 5a	
J (Hz)	8.5	10.5	3.5	3.5	3.5	

TABELLE II

PHYSIKALISCHE EIGENSCHAFTEN DER SYNTHETISCHEN 2,4-DINITROPHENYL-GLYKOSIDE

2,4-Dinitrophenyl-glykosid[a]	*F.P. Zers.*, °	*Ausbeute*[b], %	$[\alpha]_D^{22}$, °
α-D-Galaktosid	158[e]	22	+322 (*c* 0.66, Methanol)
β-D-Galaktosid	150–151[e]		−105 (*c* 1, Methanol)
β-D-Glukosid[c]	100–101[e]	10	−92.8 (*c* 1.06, Methanol)
α-D-Mannosid[d]	149[f]	28	+161 (*c* 1.00, Methanol)
α-L-Arabinosid	167[e]	32	−103 (*c* 1.05, DMF)
β-L-Arabinosid	158[e]		+367 (*c* 0.69, DMF)
β-D-Xylosid	158–159[e]	17	−105 (*c* 1.1, Methanol)
2-Chlor-2-desoxy-β-D-galaktosid	156–157[e]	20[h]	−127 (*c* 0.89, Methanol)
Tri-*O*-acetyl-2-chlor-2-desoxy-β-D-galaktosid	176–177[g]		−52 (*c* 0.98, Chloroform)
Tetra-*O*-acetyl-β-D-galaktosid	174–176[g]		+74 (*c* 1.44, Chloroform)
Tetra-*O*-acetyl-α-D-mannosid	175–176[g]		+137 (*c* 1.69, Chloroform)

Äthyl-1-thio-α-L-arabinosid: Gef.: C, 43.40; H, 7.30; S, 16.10. $C_7H_{14}O_4S$. Ber.: C, 43.20; H, 7.26; N, 16.50%.

[a]Die Zuordnung der Konfiguration am C-1 erfolgte entsprechend den Hudson'schen Regeln. [b]Ausbeute bezogen auf Äthyl-1-thioglykosid. [c]Kristallisiert mit einem Mol Aceton. [d]Enthält nichtstöchiometrische Mengen Aceton. (Acetongehält < 1 mol pro Formeleinheit). [e]Aus Methanol. [f]Aus Aceton. [g]Aus Äthanol. [h]Ausbeute bezogen auf D-Galaktal.

$\tau \sim 6.15$ in die Region von H-5 und H-6 verschoben worden ist. Die Verschiebung von H-1 und H-2 ist wahrscheinlich auf den starken Abschirmungseffekt des 2,4-Dinitrophenyl-Rests am C-1 zurückzuführen. Eine Aufnahme des 2,4-Dinitrophenyl-2-chlor-2-desoxygalaktosids bei 100 Megahertz war nötig, da im 60 Megahertz-Spektrum die Region H-4, H-1, und H-3 nur unvollständig in 6 peaks anstelle von 8 peaks aufgespalten war.

Über die Natur und Eigenschaften der erhaltenen neuen Glykoside unterrichtet Tabelle II.

DISKUSSION

Der sterische Ablauf der Koenigs–Knorr-Reaktion mit Trimethylsilylhalogenosen ist verschieden von dem Reaktionsablauf mit den entsprechenden Acetylhalogenosen. Eine Reaktionslenkung durch einen der Acetylgruppenwirkung entsprechenden Nachbargruppeneffekt ist für die TMS-Gruppe auszuschließen. In welchem Ausmaß sterische Effekte, der anomere Effekt und gegebenenfalls der $\Delta 2$-Effekt[9] für die Bildung von α- und β-Glykosiden verantwortlich gemacht werden können, hängt nicht nur von der Stabilität der Intermediärprodukte, sondern auch von der kinetischen Situation ab, welche sich für die Umsetzung an der Silberoxydoberfläche nur schwer voraussagen läßt.

Die Bildung von α- und β-Glykosiden beim Arabinosid und Galaktosid, sowie das Auftreten des α-D-Mannosids ist wohl durch sterische Effekte kombiniert mit dem anomeren Effekt bedingt[10].

Summenformel Molgewicht	*Ber.*				*Gef.*			
	C	*H*	*N*	*Cl*	*C*	*H*	*N*	*Cl*
$C_{12}H_{14}N_2O_{10}$	41.65	4.08	8.10		41.52	4.04	8.23	
					41.71	4.12	8.21	
$C_{15}H_{20}N_2O_{11}$	44.60	4.98	6.95		44.46	4.94	7.24	
$C_{12}H_{14}N_2O_{10}.0.7C_3H_6O$	43.76	4.74	7.24		43.81	4.63	7.32	
$C_{11}H_{12}N_2O_9$	41.80	3.83	8.86		42.14	4.03	8.86	
					41.92	4.10	8.91	
$C_{11}H_{12}N_2O_9$	41.80	3.83	8.86		41.98	3.95	8.89	
$C_{12}H_{13}ClN_2O_9$	39.47	3.59	7.86	9.72	39.41	3.96	7.83	9.6
$C_{18}H_{19}ClN_2O_{12}$	44.10	3.90	5.71	7.23	44.35	3.91	5.81	7.0
$C_{20}H_{22}N_2O_{14}$	46.65	4.31	5.44		46.80	4.60	5.2	
$C_{20}H_{22}N_2O_{14}$	46.65	4.31	5.44		46.77	4.51	5.67	

Die Koenigs–Knorr-Reaktion verläuft nach Newth und Haynes[11] über einen S_N1 Mechanismus. Das Carboniumion in der E1-Konformation wird im Falle des Galaktosids und Arabinosids durch die voluminöse TMS(Trimethylsilyl)-Gruppe am C-4 so abgeschirmt (Abb. 2), daß der Angriff von der β-D-Seite bzw. von der α-L-Seite leicht gehindert ist. Der anomere Effekt führt dazu, daß das stabilere α-D-Glykosid (β-L-Glykosid) entsteht, wenn am C-1 ein stark polarer Substituent eintritt. Der anomere Effekt wird jedoch beeinträchtigt durch die TMS-Gruppe am C-2, so daß im Falle des Glukosids und Xylosids keine α-D-Glykoside isoliert wurden. Im Falle der 2,4-Dinitrophenyl-α-D-mannosidsynthese können der anomere Effekt und die sterische Beeinflussung des Reaktionsablaufs durch die axiale TMS-Gruppe am C-2 zusammenwirken (Abb. 2).

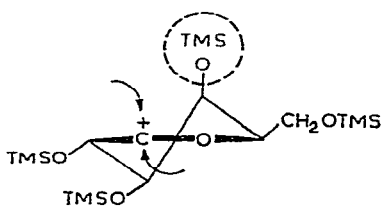


Abb. 2.

EXPERIMENTELLER TEIL

Die als Ausgangsmaterial dienenden 1-Thioglykoside wurden nach oder analog zu Literaturvorschriften hergestellt[12–16]. 2,4-Dinitrophenyl-tetra-*O*-acetyl-

β-D-galaktosid wurde nach Latham *et al.*[1] dargestellt. Das entsprechende Mannosidtetraacetat sowie das Acetat des 2,4-Dinitrophenyl-2-chlor-2-desoxy-β-D-galaktosids wurden aus den freien Glykosiden durch Acetylierung in Pyridin mit Acetanhydrid bei 0° erhalten.

Säulenchromatographie. — Zur Chromatographie eines 10^{-2} molaren Ansatzes benötigt man ungefähr 100 g Kieselgel. 2,4-Dinitrophenol eluierten wir mit Benzol, die 2,4-Dinitrophenyl-glykoside mit Äthylacetat. Die α-D-Verbindungen bzw. die β-L-Verbindungen wandern rascher als die β-D- bzw. α-L-Verbindungen.

Dünnschichtchromatographie. — Trennungen werden auf Glasplatten durchgeführt, die mit Kieselgel G (Merck) beschichtet waren. Für freie 2,4-Dinitrophenylglykoside ist das Laufmittel Benzol–Methanol = 2:1 gut geeignet. Auch Anomerenpaare können so leicht getrennt werden. Entwickelt wurde mit Äthylatlösung oder mit Schwefelsäure im Trockenschrank.

Trimethylsilylierung von Äthyl-1-thioglykosiden und D-Galaktal. — 10^{-2} Mole Substrat werden in 50 ml abs. Pyridin gelöst und mit 2×10^{-2} Mol Hexamethyldisilazan und 2×10^{-2} Mol Chlortrimethylsilan pro freie Hydroxylgruppe versetzt. Die Reaktionsmischung erwärmt sich auf ungefähr 50°. Man läßt 2–3 Stunden stehen und engt am Rotationsverdampfer unter Feuchtigkeitsausschluß ein. Man löst den durch Ammonchlorid getrübten Sirup in Tetrachlorkohlenstoff auf, filtriert ab und entfernt das Lösungsmittel. Es bleibt ein farbloses Öl zurück. Ausbeute: quantitativ.

Bromierung von trimethylsilylierten 1-Thioglykosiden. — 10^{-2} Mole trimethylsilyliertes 1-Thioglykosid werden in 30 ml abs. Tetrachlorkohlenstoff gelöst. Unter Rührung läßt man 1.5×10^{-2} Mole trockenes Brom, gelöst in 10 ml abs. Tetrachlorkohlenstoff unter Feuchtigkeitsausschluß bei Zimmertemperatur zutropfen. Die Bromfarbe verschwindet anfangs rasch, dann bildet sich im Reaktionsgefäß eine gelbe Trübung. Wenn alles Brom zugetropft ist, läßt man noch 45 min weiterrühren. Man engt am Rotationsverdampfer ein. Es bleibt ein von Brom schwach angefärbter, etwas trüber Sirup zurück, der stark nach Äthylsulphenylbromid riecht, das im Ölpumpenvakuum bei 50° abgezogen wird. Der Sirup wird am besten sofort für die Glykosidsynthese weiterverwendet.

Hydrolyse der TMS-Schutzgruppen. — Der sirupöse Glykosidierungsansatz wird in 250 ml Methanol gelöst, man fügt noch etwas Eisessig hinzu und versetzt mit Wasser bis zur bleibenden Trübung. Nach längerem Rühren bei Zimmertemperatur verschwindet die Trübung, die dann wieder bei Zugabe von Wasser auftritt. Nach 4–5 stündigen Rühren tritt beim Zufügen von Wasser keine Trübung mehr auf, die TMS-gruppen sind abgespalten. Die Lösung wird eingeengt, mit Methanol aufgenommen und mit 25 g Hyflo-Supercel versetzt. Das auf Hyflo absorbierte Produkt wird auf eine Kieselgelsäule aufgesetzt.

Chlorierung von Tri-O-trimethylsilyl-D-galaktal. — 2×10^{-2} Mole Tri-*O*-trimethylsilyl-D-galaktal werden in 40 ml abs. Tetrachlorkohlenstoff gelöst. Bei 0° leitet man trockenes Chlor in die Lösung ein, bis eine bleibende Gelbfärbung eintritt. Man entfernt überschüssiges Chlor durch Durchleiten von trockener Luft, engt am

Rotationsverdampfer ein und erhält einen farblosen Sirup, der nach Koenigs und Knorr glykosidiert werden kann.

Glykosidierung mit 2,4-Dinitrophenol und Silberoxyd. — 1.5×10^{-2} Mole 2,4-Dinitrophenol, 6 g Gips, 6 g frisch bereitetes Silberoxyd werden 45 min. mit abs. Benzol geschüttelt. Die Suspension wird 10^{-2} Molen der TMS-halohexose vermischt und unter Feuchtigkeits- und Lichtausschluß 20 Std. gerührt. Man zentrifugiert ab und wäscht den Niederschlag 2 mal mit Benzol und 2 mal mit Methanol. Man säuert mit wenig Eisessig an. Eventuell auftretende Niederschläge werden abfiltriert, das Filtrat wird eingeengt.

LITERATUR

1 H. G. LATHAM, JR., E. L. MAY, UND E. MOSSETTIG, *J. Org. Chem.*, 15 (1950) 884.
2 C. C. SWEELEY, R. BENTLEY, M. MAKITA, UND W. W. WELLS, *J. Amer. Chem. Soc.*, 85 (1963) 2497.
3 L. BIRKOFER, A. RITTER, UND F. BENTZ, *Chem. Ber.*, 97 (1964) 2196.
4 W. G. OVEREND UND E. J. HEDGLEY, *Chem. Ind.* (London), 378 (1960).
5 W. A. BONNER, *J. Amer. Chem. Soc.*, 70 (1948) 3491.
6 F. WEYGAND UND H. ZIEMANN, *Ann*, 657 (1962) 179.
7 M. L. WOLFROM UND W. GROEBKE, *J. Org. Chem.*, 29 (1963) 2986.
8 R. U. LEMIEUX UND B. FRASER-REID, *Can. J. Chem.*, 43 (1965) 1460.
9 E. L. ELIEL, N. L. ALLINGER, S. J. ANGYAL, UND G. A. MORRISON, *Conformational Analysis*, Interscience, New York, 1966, S. 377.
10 Ref. 9, S. 375.
11 F. H. NEWTH AND L. J. HAYNES, *Advan. Carbohyd. Chem.*, 10 (1955) 207.
12 R. U. LEMIEUX, *Can. J. Chem.*, 29 (1951) 1079.
13 B. HELFERICH UND D. TÜRK, *Chem. Ber.*, 89 (1956) 2215.
14 R. U. LEMIEUX UND C. BRICE, *Can. J. Chem.*, 33 (1955) 109.
15 J. FRIED UND D. E. WALZ, *J. Amer. Chem. Soc.*, 71 (1949) 140.
16 J. STANĚK, M. SINDLEROVA, UND M. ČERNÝ, *Collection Czech. Chem. Commun.*, 30 (1965) 297; *Chem. Abstr.*, 62 (1965) 14795.

Carbohydrate Research
Elsevier Publishing Company, Amsterdam
Printed in Belgium

PROOF OF STRUCTURE AND SYNTHESIS OF α-D-GLUCOPYRANOSYL (DIHYDROGEN PHOSPHATE)*

CALVIN L. STEVENS AND ROBERT E. HARMON**
Department of Chemistry, Wayne State University, Detroit, Michigan 48202 (U. S. A.)
(Received March 29th, 1969)

ABSTRACT

The reaction of 3,4,6-tri-*O*-acetyl-2-*O*-(trichloroacetyl)-β-D-glucopyranosyl chloride with silver (dibenzyl phosphate) yielded 3,4,6-tri-*O*-acetyl-2-*O*-(trichloroacetyl)-α-D-glucopyranosyl (dibenzyl phosphate) (**2a**). Hydrolysis of the trichloroacetyl group of **2a**, followed by acetylation, afforded 1,3,4,6-tetra-*O*-acetyl-β-D-glucopyranose 2-(dibenzyl phosphate). However, when **2a** was subjected to debenzylation followed by deacetylation, it furnished α-D-glucopyranosyl (dihydrogen phosphate), which was isolated as the crystalline brucine salt having physical constants identical with those reported in the literature.

INTRODUCTION

Sugar phosphates are known to be intermediates in a large number of biological processes[1,2], but only recently has their synthesis received much attention. So far, only a few methods are available for the preparation of glycosyl phosphates[2-6]. A new route to α-D-glucopyranosyl (dihydrogen phosphate) (**7**) is now described; the configuration at C-1 was proved by known stereochemical transformations.

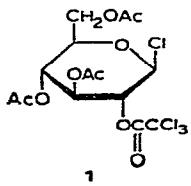
DISCUSSION AND RESULTS

α-D-Glucopyranosyl (dihydrogen phosphate) (**7**) was first synthesized by Cori *et al.*[3] by the reaction of trisilver phosphate with 2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosyl bromide and subsequent saponification of the acetyl groups; the α-D configuration was assigned on the basis of its mutarotation[5]. Since then, two other workers have reported new approaches[7] to the synthesis of **7**, but this is the first proof of the structure based on its stereospecific synthesis. The starting material for this synthesis was 3,4,6-tri-*O*-acetyl-2-*O*-(trichloroacetyl)-β-D-glucopyranosyl chloride[8] (**1**). Reaction of **1** with silver (dibenzyl phosphate) in benzene furnished a

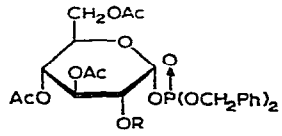
*Robert E. Harmon (U. S. Public Health Research fellow, 1955–59), Ph.D. Dissertation, Wayne State University, Detroit, Michigan, 1959; Calvin L. Stevens and Robert E. Harmon, *Abstracts Papers Amer. Chem. Soc. Meeting*, 149 (1965) 3C.

**Present address: Department of Chemistry, Western Michigan University, Kalamazoo, Michigan.

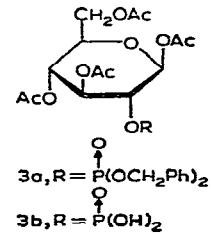
93% yield of 3,4,6-tri-*O*-acetyl-2-*O*-(trichloroacetyl)-α-D-glucopyranosyl (dibenzyl phosphate) (**2a**). Removal of the trichloroacetyl group of **2a** was effected with ammo-



1

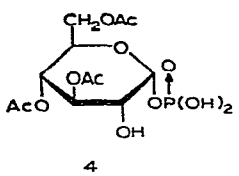


2a, R = COCCl3
2b, R = H

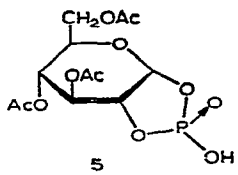


niacal ether to give 3,4,6-tri-*O*-acetyl-α-D-glucopyranosyl (dibenzyl phosphate) (**2b**); this was acetylated with acetic anhydride in the presence of triethylamine. Migration of the (dibenzyl phosphate) group from C-1 to C-2, followed by acetylation of the resulting free hydroxyl group on C-1, afforded **3a** in a yield of 93%. Similar acyl migrations have been observed in several instances[9] when there is a *cis* relationship between the groups on the adjacent carbon atoms involved in the reaction. The structure of **3a** was proved by catalytic hydrogenation to **3b**, which was found to be identical with[10] 1,3,4,6-tetra-*O*-acetyl-β-D-glucopyranose 2-(dihydrogen phosphate).

As this approach did not lead to the desired α-D-glucopyranosyl (dihydrogen phosphate), a new approach was investigated. The benzyl groups in **2a** were removed by catalytic hydrogenation in the presence of palladium-on-carbon. When treated with dicyclohexylcarbodiimide, the resulting 3,4,6-tri-*O*-acetyl-α-D-glucopyranosyl (dihydrogen phosphate) (**4**) formed the cyclic phosphate **5**, isolated as a crystalline brucine salt. The formation of a cyclic phosphate indicated a *cis* relationship between



4

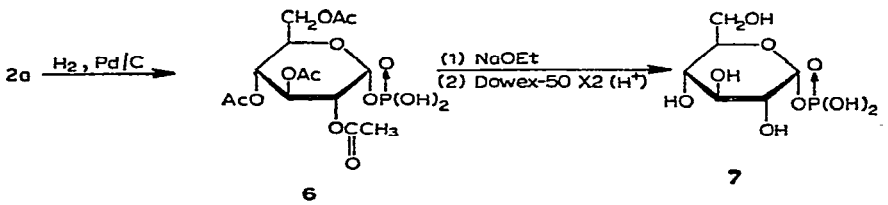


5

the dihydrogen phosphate group on C-1 and the hydroxyl group on C-2 in **4**. However, it did not constitute a rigorous stereochemical proof of structure, as Khorana *et al.*[12] have shown that the pyridinium salts of both α- and β-D-glucopyranosyl (dihydrogen phosphate) give cyclic phosphates when treated with dicyclohexylcarbodiimide.

Finally, **2a** was catalytically hydrogenated in the presence of 5% palladium-on-carbon. The observed uptake of 5 moles of hydrogen per mole was attributed to debenzylation and to hydrogenolysis of the chlorine atoms of the trichloroacetyl group. The resulting compound **6** was deacetylated with a catalytic amount of sodium ethoxide in ethanol, to furnish **7**, which was isolated as a crystalline brucine salt (in 89% overall yield for the two steps). The physical constants of the brucine salt of **7** were identical with those reported for the brucine salt of α-D-glucopyranosyl

(dihydrogen phosphate) by Cori *et al.*[3] and Wolfrom *et al.*[5]. Subsequently, deacetylation of **4** and formation of the brucine salt also furnished the brucine salt of **7**.



6 7

Thus, the structure of α-D-glucopyranosyl (dihydrogen phosphate), tentatively assigned as **7** by previous investigators, has now been confirmed by its stereospecific synthesis.

EXPERIMENTAL*

3,4,6-Tri-O-acetyl-β-D-glucopyranosyl chloride (**1**). — A mixture of 78 g (0.20 mole) of 1,2,3,4,6-penta-*O*-acetyl-β-D-glucopyranose and 208 g (1 mole) of phosphorus pentachloride was heated for 2.5 h under reflux, or until the evolution of hydrogen chloride had ceased. The phosphoryl chloride, phosphorus trichloride, and acetyl chloride were distilled off at 25°/0.2 torr, and the resulting syrup was dissolved in 100 ml of hot isopropyl ether. On being cooled, the solution afforded a colorless, fluffy precipitate, which was filtered off and washed with cold 90% methanol (100 ml). Recrystallization from anhydrous ether furnished 47 g (50%) of colorless crystals of **1**, m.p. 138–138.5°. An analytical sample was obtained by one more recrystallization from ether, m.p. 138–138.5°; $[\alpha]_D^{26}$ +3.0° (*c* 3.0, benzene); $\nu_{max}^{CHCl_3}$ 1770 ($COCl_3$) and 1739 cm^{-1} ($COCH_3$).

Anal. Calc. for $C_{14}H_{16}Cl_4O_9$: C, 35.77; H, 3.43; Cl, 30.10. Found: C, 35.65; H, 3.70; Cl, 29.83.

3,4,6-Tri-O-acetyl-2-O-(trichloroacetyl)-α-D-glucopyranosyl (dibenzyl phosphate) (**2a**). — To a suspension of 4.0 g of powdered Drierite in a solution of 10.25 g (21.8 mmoles) of silver (dibenzyl phosphate) in 100 ml of anhydrous benzene was added 8.4 g (21.8 mmoles) of **1** with stirring. The mixture was heated for 12 h under reflux and cooled, and the silver salts and calcium sulfate were removed by filtration. The filtrate was washed successively with 5% sodium hydrogen carbonate solution and water, dried (sodium sulfate), and evaporated under diminished pressure to a syrup. Crystallization from anhydrous ether yielded 12.5 g (93%) of colorless crystals of **2a**, m.p. 100–101°; $[\alpha]_D^{26}$ +60.5° (*c* 3.38, chloroform); $\nu_{max}^{CHCl_3}$ 1770 ($OCOCl_3$) and 1751 cm^{-1} ($OCOCH_3$); negative Benedict test.

Anal. Calc. for $C_{28}H_{30}Cl_3O_{13}P$: C, 47.24; H, 4.24; Cl, 14.94. Found: C, 47.49; H, 4.30; Cl, 14.92.

*The melting points are uncorrected.

3,4,6-Tri-O-acetyl-α-D-glucopyranosyl (dibenzyl phosphate) (**2b**). — To 100 ml of anhydrous ether presaturated with anhydrous ammonia at 0° was added 2.0 g (2.82 mmoles) of **2a** with stirring. The mixture was stirred for 30 min at 0°, and concentrated under diminished pressure to 20 ml; 5 ml of petroleum ether (b.p. 40–60°) was added, and the mixture was kept overnight at room temperature, giving 1.2 g (96.7%) of colorless crystals of **2b**, m.p. 112–115°; after three recrystallizations from ether–petroleum ether (b.p. 40–60°), it had m.p. 124–125°; $[\alpha]_D^{26}$ +72.0° (*c* 1.1, chloroform); $\nu_{max}^{CHCl_3}$ 3322 (OH) and 1751 cm^{-1} ($OCOCH_3$); negative Benedict test.

Anal. Calc. for $C_{26}H_{31}O_{12}P$: C, 55.11; H, 5.52. Found: C, 55.12; H, 5.77.

1,3,4,6-Tetra-O-acetyl-β-D-glucopyranose 2-(dibenzyl phosphate) (**3a**). — To a solution of 1.0 g (1.76 mmoles) of **2b** in 10 ml of anhydrous triethylamine at 0° was added 6.0 ml of acetic anhydride with stirring. On allowing the mixture to warm to room temperature, 0.8 g of colorless crystals of **3a**, m.p. 140–142°, was obtained. The mother liquor was diluted with chloroform (50 ml), and the solution was washed successively with 10% sodium hydrogen carbonate solution, 3% hydrochloric acid, and a saturated aqueous solution of sodium chloride, dried (sodium sulfate), and evaporated under diminished pressure to afford 0.2 g of **3a**, m.p. 140–142°; total yield of **3a**, 1.0 g (93.4%), m.p. 140–142°. After three recrystallizations from absolute ethanol, it had m.p. 146–147°, $[\alpha]_D^{26}$ +28.8° (*c* 1.36, chloroform); negative Benedict test; its i.r. spectrum in chloroform was consistent with the structure assigned.

Anal. Calc. for $C_{28}H_{33}O_{13}P$: C, 55.58; H, 5.47. Found: C, 55.38; H, 5.45.

1,3,4,6-Tetra-O-acetyl-β-D-glucopyranose 2-(dihydrogen phosphate) (**3b**). — A solution of 0.45 g (0.73 mmole) of **3a** in 25 ml of *p*-dioxane was hydrogenated for 30 min in the presence of 0.45 g of 5% palladium-on-carbon, the catalyst was filtered off, and the filtrate was evaporated to dryness under diminished pressure. Crystallization from anhydrous ethyl acetate–light petroleum (b.p. 40–60°) afforded 0.25 g (80%) of colorless crystals of **3b**, m.p. 157–158°; after two recrystallizations, it had m.p. 159–160°, $[\alpha]_D^{26}$ +14.7° (*c* 1.16, water). A mixture with an authentic sample showed no depression in m.p.

Anal. Calc. for $C_{14}H_{21}O_{13}P$: C, 39.26; H, 4.94; P, 7.09. Found: C, 39.52; H, 4.77; P, 7.23.

3,4,6-Tri-O-acetyl-α-D-glucopyranosyl (dihydrogen phosphate) (**4**) *and its brucine salt.* — A solution of 0.35 g (0.6 mmole) of **2a** in 25 ml of *p*-dioxane was hydrogenated for 30 min in the presence of 0.35 g of 5% palladium-on-carbon, and processed as described for **3b**. Crystallization from ethyl acetate–ether afforded 0.24 g (98%) of crystals of **4**, m.p. 115–117°, $[\alpha]_D^{26}$ +82.5° (*c* 0.855, water).

Anal. Calc. for $C_{12}H_{19}O_{12}P$: C, 37.31; H, 4.96. Found: C, 37.93; H, 5.17.

Treatment of a solution of **4** in anhydrous ether with a solution of brucine tetrahydrate in *p*-dioxane afforded the crystalline brucine salt of **4**, m.p. 166–167° (dec.); $[\alpha]_D^{26}$ +19° (*c* 1.295, water); neutralization equivalent 386 (monobrucine salt).

Anal. Calc. for $C_{35}H_{45}N_2O_{16}P$: C, 53.94; H, 5.81; N, 3.59; P, 3.97. Found: C, 54.02; H, 5.56; N, 3.82; P, 3.97.

Brucine salt of 3,4,6-tri-O-acetyl-α-D-glucopyranosyl 1,2-(dihydrogen phosphate) (**5**). — Catalytic hydrogenation of 0.3 g (0.53 mmole) of **2a** was conducted as described for **4**. The resulting compound (**3b**) was dissolved in 100 ml of anhydrous ether, and dicyclohexylcarbodiimide (118 mg) was added. The mixture was heated for 1 h under reflux and cooled, and the cyclohexylurea (0.12 g, m.p. 211–212°) was filtered off. Treatment of the filtrate with a solution of brucine tetrahydrate (248 mg) in *p*-dioxane afforded 0.36 g of **5**, m.p. 169–171° (dec.); $[\alpha]_D^{26}$ +24.0° (*c* 0.96, water); neutralization equivalent 756 (monobrucine salt).

Anal. Calc. for $C_{23}H_{26}N_2O_4P$: C, 55.10; H, 5.68; N, 3.67; P, 4.06. Found: C, 55.38; H, 5.97; N, 3.45; P, 3.84.

Brucine salt of α-D-glucopyranosyl (dihydrogen phosphate) (**7**). — *Procedure A.* A solution of 2.0 g (2.81 mmoles) of **2a** in 50 ml of *p*-dioxane was hydrogenated in the presence of 2.0 g of 5% palladium-on-charcoal. An uptake of 5 mmoles of hydrogen was achieved after 1 h. The catalyst was filtered off, and the filtrate was evaporated to dryness *in vacuo*. The residue was dissolved in 10 ml of absolute ethanol, and a solution of sodium ethoxide (from 0.69 g of sodium) in 10 ml of absolute ethanol was added, with stirring. After 1 h, the solvent was evaporated off *in vacuo*; the residue was dissolved in 25 ml of distilled water and chromatographed with a column of 5 g of Dowex-50 X2 (H^+), eluted with 100 ml of distilled water. The eluate was made basic with 0.5M brucine in methanol, and the solution was evaporated *in vacuo* at room temperature. The residual solid was washed several times with chloroform (to remove excess of brucine), and the residue was recrystallized from water–acetone, to afford 2.0 g (87%) of colorless crystals of the brucine salt of **7**, m.p. 175–178° (dec.), $[\alpha]_D^{26}$ +0.5° (*c* 1.2, water); lit.[4] m.p. 173–178° (dec.).

Anal. Calc. for $C_{52}H_{65}N_4O_{17}P \cdot 2H_2O$: C, 57.55; H, 6.49; N, 5.16; P, 2.85. Found: C, 57.86; H, 6.34; N, 5.22; P, 2.90.

Procedure B. A solution of 0.30 g (9.53 mmoles) of **2b** in 5 ml of *p*-dioxane was hydrogenated for 30 min in the presence of 0.30 g of 5% palladium-on-charcoal. The catalyst was filtered off, and the filtrate was evaporated to dryness *in vacuo*. The residual syrup was dissolved in 5 ml of absolute ethanol, and the solution was treated with a solution of sodium ethoxide (from 35 mg of sodium) in 5 ml of ethanol. After 1 h, the solution was evaporated to dryness *in vacuo* at room temperature, and the residual sodium salt was dissolved in 10 ml of distilled water and chromatographed with a column of 1.0 g of Dowex-50 X2 (H^+). The column was eluted with 50 ml of water, and the eluate was made basic with 0.5M brucine tetrahydrate in methanol, and processed as described in procedure A, to afford 0.5 g (89%) of colorless crystals of the brucine salt of **7**, m.p. 175–180°, $[\alpha]_D^{26}$ +0.7° (*c* 1.4, water).

Anal. Calc. for $C_{52}H_{65}N_4O_{17}P \cdot 2H_2O$: C, 57.55; H, 6.49; N, 5.16; P, 2.85. Found: C, 57.30; H, 6.34; N, 5.16; P, 2.43.

REFERENCES

1 L. F. LeLoir, *Fortschr. Chem. Org. Naturstoffe*, 8 (1951) 48.
2 A. B. Foster and W. G. Overend, *Quart. Rev.* (London), 11 (1957) 61.

3 C. F. CORI, S. P. COLOWICK, AND G. T. CORI, *J. Biol. Chem.*, 121 (1937) 465.
4 F. R. ATHERTON, H. T. OPENSHAW, AND A. R. TODD, *J. Chem. Soc.*, (1945) 382; P. BRIGL AND H. MÜLLER, *Ber.*, 72 (1939) 2121; H. A. LARDY AND H. O. L. FISCHER, *Biochem. Prepn.*, 2 (1952) 39; E. W. PUTMAN AND W. Z. HASSID, *J. Amer. Chem. Soc.*, 79 (1957) 5057.
5 M. L. WOLFROM, C. S. SMITH, D. L. PLETCHER, AND A. E. BROWN, *J. Amer. Chem. Soc.*, 64 (1942) 23.
6 T. UEDA, *Chem. Pharm. Bull.* (Tokyo), 2 (1960) 459.
7 F. J. REITHEL, *J. Amer. Chem. Soc.*, 67 (1945) 1056; T. POSTERNAK, *ibid.*, 72 (1950) 4824.
8 P. BRIGL, *Z. Physiol. Chem.*, 122 (1922) 245.
9 H. B. WOOD, JR., AND H. G. FLETCHER, JR., *J. Amer. Chem. Soc.*, 78 (1956) 207, 2849.
10 K. R. FARRER, *J. Chem. Soc.*, (1949) 3131.
11 H. G. KHORANA, *Chem. Rev.*, 53 (1953) 145; C. A. DEKKER AND H. G. KHORANA, *J. Amer. Chem. Soc.*, 76 (1954) 3522.
12 H. G. KHORANA, G. M. TENER, R. S. WRIGHT, AND J. G. MOFFATT, *J. Amer. Chem. Soc.*, 79 (1957) 430.

Carbohydrate Research
Elsevier Publishing Company, Amsterdam
Printed in Belgium

STEREOSPECIFIC SYNTHESIS OF β-D-GLUCOPYRANOSYL (DIHYDROGEN PHOSPHATE)*

CALVIN L. STEVENS AND ROBERT E. HARMON**
Department of Chemistry, Wayne State University, Detroit, Michigan 48202 (U. S. A.)
(Received March 29th, 1969)

ABSTRACT

Stereospecific opening of the Brigl anhydride, namely, 3,4,6-tri-*O*-acetyl-1,2-anhydro-α-D-glucopyranose, by hydrogen (dibenzyl phosphate) afforded 3,4,6-tri-*O*-acetyl-β-D-glucopyranosyl (dibenzyl phosphate). This was subjected to debenzylation, followed by deacetylation, to furnish β-D-glucopyranosyl (dihydrogen phosphate), isolated as the crystalline brucine salt having physical constants in agreement with those reported in the literature.

INTRODUCTION

The structure of β-D-glucopyranosyl (dihydrogen phosphate) (**4**) had been assigned by Zervas[1] and Wolfrom *et al.*[2] on the basis of hydrolytic (including enzymic) and mutarotation studies. However, an unequivocal chemical proof of structure has not yet been reported. A proof of structure based on the stereospecific synthesis of **4** is now given.

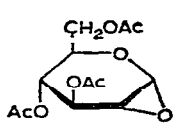
DISCUSSION AND RESULTS

The starting material for this synthesis was the Brigl anhydride, namely, 3,4,6-tri-*O*-acetyl-1,2-anhydro-α-D-glucopyranose[3] (**1**). Reaction of this epoxide with alcohols and carboxylic acids has been shown[3,4] to furnish products resulting from epoxide opening at C-1. Reaction of the Brigl anhydride **1** with hydrogen (dibenzyl phosphate) in benzene at 0° gave **2a**. The structure of **2a** as 3,4,6-tri-*O*-acetyl-β-D-glucopyranosyl (dibenzyl phosphate) was confirmed by comparison with an authentic sample prepared by the reaction of 3,4,6-tri-*O*-acetyl-β-D-glucopyranosyl chloride with silver (dibenzyl phosphate). Catalytic hydrogenation of **2a** afforded crystalline **3** in 82% yield. The *trans* relationship between the dihydrogen phosphate group on C-1 and the hydroxyl group on C-2 was indicated by the inability of **3** to form a

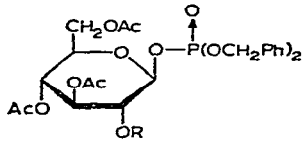
*Robert E. Harmon (U. S. Public Health Research fellow, 1955–59), Ph.D. Dissertation, Wayne State University, Detroit, Michigan, 1959; Calvin L. Stevens and Robert E. Harmon, *Abstracts Papers Amer. Chem. Soc. Meeting*, 149 (1965) 3C.

**Present address: Department of Chemistry, Western Michigan University, Kalamazoo, Michigan.

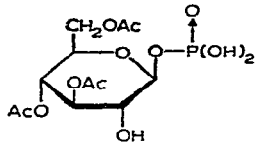
cyclic phosphate on treatment with dicyclohexylcarbodiimide in ether under controlled conditions[5].



1

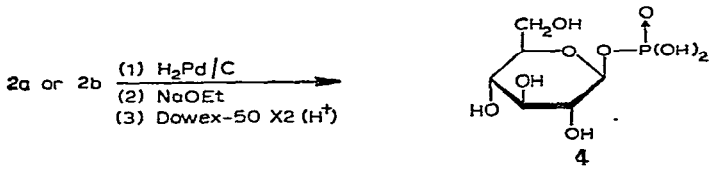


2a, R=H
2b, R=Ac



3

Acetylation of **2a** with acetic anhydride in pyridine afforded **2b**, identical with an authentic sample of 2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl (dibenzyl phosphate) prepared by the method of Zervas[1] and Wolfrom *et al.*[2]. Starting from either **2a** or **2b**, debenzylation by catalytic hydrogenation, followed by deacetylation with a catalytic amount of sodium ethoxide in ethanol, gave the same compound, **4**, isolated as the crystalline brucine salt. The physical constants of the brucine salt of **4**



4

agreed with those reported for the brucine salt of β-D-glucopyranosyl (dihydrogen phosphate) by Zervas[1] and Wolfrom *et al.*[2]. Thus, the structure of β-D-glucopyranosyl (dihydrogen phosphate), tentatively assigned to **4** by Zervas[1] and Wolfrom *et al.*[2] on the basis of physical data, has now been confirmed by its stereospecific synthesis.

EXPERIMENTAL*

3,4,6-Tri-O-acetyl-β-D-glucopyranosyl (dibenzyl phosphate) (**2a**). — *Procedure A.* To a solution of 1.93 g (6.93 mmoles) of hydrogen (dibenzyl phosphate) in 12 ml of benzene at 0° was added a solution of 2.0 g (6.93 mmoles) of 3,4,6-tri-*O*-acetyl-1,2-anhydro-α-D-glucopyranose[3] (**1**) in 12 ml of benzene at 0°. On stirring for 30 min, a colorless precipitate separated out; precipitation was completed by the addition of 10 ml of petroleum ether (b.p. 40–60°). Filtration yielded a colorless solid, m.p. 125–128°, in almost quantitative yield. Recrystallization from ether–petroleum ether (b.p. 40–60°) afforded 3.54 g (90%) of colorless crystals of **2a**, m.p. 135–136°; $[\alpha]_D^{26}$ +27.0° (*c* 2.96, chloroform); positive Benedict test on prolonged heating only.

Anal. Calc. for $C_{26}H_{31}O_{12}P$: C, 55.11; H, 5.52. Found: C, 55.15; H, 5.59.

Procedure B. To a solution of 1.8 g (4.62 mmoles) of silver (dibenzyl phosphate) in 150 ml of tetrahydrofuran was added, with stirring, 1.5 g (4.62 mmoles) of 3,4,6-tri-*O*-acetyl-β-D-glucopyranosyl chloride, and the mixture was stirred in the dark for 8 h at room temperature. The precipitated silver salts were filtered off, and the filtrate

*The melting points are corrected.

was evaporated to dryness under diminished pressure at room temperature. The resulting syrup was crystallized from 10 ml of anhydrous ether, to afford 2.0 g (77%) of 3,4,6-tri-*O*-acetyl-β-D-glucopyranosyl (dibenzyl phosphate), m.p. 134–135°, $[\alpha]_D^{26}$ +0.25° (*c* 1.4, chloroform). A mixture melting point with authentic **2a** showed no depression.

*3,4,6-Tri-*O*-acetyl-β-D-glucopyranosyl (dihydrogen phosphate)* (**3**). — A solution of 5.0 g (8.84 mmoles) of **2a** in 250 ml of ethanol was hydrogenated for 2 h in the presence of 2.5 g of 10% palladium-on-charcoal. The catalyst was removed by filtration, and the filtrate was evaporated to dryness under diminished pressure at room temperature. The syrupy residue was crystallized from anhydrous ether, to afford 2.8 g (82%) of colorless crystals of **3**, m.p. 131–132°, $[\alpha]_D^{26}$ +33.6° (*c* 0.8, water).

Anal. Calc. for $C_{12}H_{19}O_{12}P$: C, 37.32; H, 4.96. Found: C, 37.38; H, 5.17.

*Nonreaction of 3,4,6-tri-*O*-acetyl-β-D-glucopyranosyl (dihydrogen phosphate)* (**3**) *with dicyclohexylcarbodiimide.* — To a solution of 169 mg (0.82 mmole) of dicyclohexylcarbodiimide in 30 ml of anhydrous ether was added 0.3 g (0.82 mmole) of **3**, with stirring, and the mixture was stirred and heated for 1 h under reflux and allowed to cool to room temperature; 0.27 g of colorless crystals, m.p. 128–130°, was obtained. A mixture melting point with **3** showed no depression.

*2,3,4,6-Tetra-*O*-acetyl-β-D-glucopyranosyl (dibenzyl phosphate)* (**2b**). — To a mixture of 50 ml of chloroform, 10 ml of pyridine, and 19 ml of acetic anhydride was added 1 g (1.75 mmoles) of **2a** with stirring, and the mixture was kept for 2 days at room temperature. The solution was washed successively with 10% sodium hydrogen carbonate solution, 10% sodium bisulfite solution, and distilled water, dried (anhydrous sodium sulfate), and evaporated to dryness under diminished pressure at room temperature. The resulting syrup was crystallized from ether–hexane, to afford 0.88 g (80%) of colorless crystals of **2b**, m.p. 73–75°; after three recrystallizations from ether–hexane, it had m.p. 76–78°, $[\alpha]_D^{26}$ −9° (*c* 2.12, chloroform); lit.[2] m.p. 76–78°, $[\alpha]_D^{26}$ −9° (*c* 3.0, chloroform).

Anal. Calc. for $C_{28}H_{33}O_{13}P$: C, 55.26; H, 5.47; P, 5.09. Found: C, 55.20; H, 5.42; P, 5.78.

A mixture melting point with an authentic sample of 2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl (dibenzyl phosphate) prepared by the method of Wolfrom *et al.*[2] showed no depression.

Brucine salt of β-D-glucopyranosyl (dihydrogen phosphate) dihydrate (**4**). — *Procedure A.* A solution of 1.84 g (1.5 mmoles) of **2b** in 75 ml of absolute ethanol was hydrogenated in the presence of 2.0 g of 5% palladium-on-carbon. After the theoretical volume of hydrogen had been taken up, the catalyst was filtered off, and the filtrate was treated with a solution of sodium ethoxide (from 92 mg of sodium) in 10 ml of absolute ethanol. After 1 h, the solution was evaporated *in vacuo* at 40°, and the resulting syrup was dissolved in 25 ml of distilled water. Chromatography with a column of 5 g of Dowex-50 X2 (H^+) by elution with water (100 ml) afforded a colorless solution; this was made basic with a 0.5 M solution of brucine in methanol,

and the solution was evaporated *in vacuo* at 40°. The resulting solid was washed with chloroform (to remove any excess of brucine), and crystallization from water–acetone afforded 1.55 g (98%) of colorless crystals of **4**, m.p. 157–160° (dec.). After two recrystallizations from water–acetone, it had m.p. 163–167° (dec.), $[\alpha]_D^{26}$ −19.7° (*c* 1.0, water); lit.[2] $[\alpha]_D^{26}$ −20° (*c* 1.7, water).

Anal. Calc. for $C_{52}H_{65}N_4O_{17}P \cdot 2H_2O$: C, 57.55; H, 6.49; N, 5.16; P, 2.85. Found: C, 57.30; H, 6.34; N, 5.34; P, 3.07.

Procedure B. A sample of 1.0 g (1.76 mmoles) of **2a** was successively subjected to catalytic hydrogenation, saponification, and brucine salt formation, as described for **2b** (procedure A), affording 1.9 g (94%) of colorless crystals of **4**, m.p. 160–165° (dec.). After one recrystallization from acetone–water, it had m.p. 165–170° (dec.), $[\alpha]_D^{26}$ −19.0° (*c* 1.0, water).

Anal. Calc. for $C_{52}H_{65}N_4O_{17}P \cdot 2H_2O$: C, 57.55; H, 6.49; N, 5.16; P, 2.85. Found: C, 57.75; H, 6.56; N, 5.19; P, 2.43.

A mixture melting point with the product from procedure A showed no depression.

REFERENCES

1 L. ZERVAS, *Naturwissenschaften*, 27 (1939) 317.
2 M. L. WOLFROM, C. S. SMITH, D. E. PLETCHER, AND A. E. BROWN, *J. Amer. Chem. Soc.*, 64 (1942) 23.
3 P. BRIGL, *Z. Physiol. Chem.*, 122 (1922) 245.
4 W. J. HICKINBOTTOM, *J. Chem. Soc.*, (1928) 3149; E. HARDEGGER AND J. DEPASCUAL, *Helv. Chim. Acta*, 31 (1948) 281.
5 H. G. KHORANA, *Chem. Rev.*, 53 (1953) 145; C. A. DEKKER AND H. G. KHORANA, *J. Amer. Chem. Soc.*, 76 (1954) 3522.

Carbohydrate Research
Elsevier Publishing Company, Amsterdam
Printed in Belgium

FRACTIONATION OF ACIDIC GLYCOSAMINOGLYCANS ON DE-ACIDITE RESIN FF

M. J. How, P. J. Wood, and C. N. D. Cruickshank*

Department of Chemistry and Medical Research Council Unit for Research in the Pathology of the Skin, The University, Edgbaston, Birmingham 15 (Great Britain)*

(Received February 19th, 1969)

ABSTRACT

A technique for the separation of the acidic glycosaminoglycans of skin and cartilage by stepwise elution from De-Acidite resin FF has been investigated by using ^{35}S-labelled components isolated from cartilage grown in tissue culture. The overall recovery of radioactivity from the resin was 35–45%. The fractionation patterns obtained with different batches of nominally identical resin varied considerably. Possible explanations for these results are given.

INTRODUCTION

Studies of the changes that occur in mammalian, acidic glycosaminoglycans in pathological conditions, and of biologically induced changes in materials grown in tissue culture have stimulated the development of techniques for the isolation and fractionation of these macromolecules on a micro-scale. Barker, Cruickshank, and Webb[1] reported the separation, from 40 g of rat skin, of the acidic glycosaminoglycans and the sulphate donor molecule, adenosine 3-phosphate 5-phosphosulphate (PAPS) bound to peptide[2], using a single, calibrated column of De-Acidite resin FF (chloride form) by stepwise elution with increasing concentrations of sodium chloride. The small amounts of acidic glycosaminoglycans in individual biopsy specimens of tissue preclude their estimation by colorimetric methods, but the separation pattern, based on scanning for radioactivity of an extract of tissue that had been cultured in the presence of sulphate-^{35}S, was similar to that obtained colorimetrically with larger amounts of tissue extract. Subsequent work on the separation of acidic glycosaminoglycans from guinea-pig ear cartilage also showed[3] that coincident elution profiles were obtained by scanning colorimetrically and for radioactivity. We now report further investigations of this method, with particular reference to the recovery from the resin of the components of ^{35}S-labelled tissue extracts.

MATERIALS AND METHODS

Individual segments of tracheal cartilage from young adult, hooded rats were cultured[1] for 24 h at 37° in a medium containing, among other components, sodium

sulphate-^{35}S (100 μCi/ml). The condition of the cartilage before and after culture was checked histologically. The most efficient method for isolating radioactive components from the tissue involved mechanical disintegration of the defatted tissue with a ground-glass, tissue grinder (Gallenkamp) and 0.1M sodium acetate buffer (pH 7.5) containing calcium chloride (10^{-3}M) and sodium azide (0.002%), followed by proteolytic digestion for 24 h at 37° with Pronase B (Calbiochem., Los Angeles, U. S. A.) on a slow shaker. Residual protein was removed by treatment with a mixture of chloroform and 3-methylbutan-1-ol (10:1, v/v), followed by addition to the cooled, centrifuged, aqueous phase of trichloroacetic acid to 10%. The solution was centrifuged, and the supernatant was dialysed against running water for 16–24 h before concentration under diminished pressure at 25°. Insignificant amounts of radioactive material were extracted from the tissue residue after proteolytic digestion either with 0.5N sodium hydroxide or by further digestion with pronase.

De-Acidite resin FF (batch 1, SRA 71, heteroporous, 100–200 mesh, 7–9% average cross-linking, Permutit Co.) was washed successively with N sodium hydroxide, water, N hydrochloric acid, and finally overnight with distillled water. This regeneration procedure is milder than that reported earlier[1]. Fresh aliquots (6 ml) of resin were packed in columns (diameter, 0.3–0.4 cm) for *each* fractionation experiment, and the same *batch* of resin was used for the fractionation and refractionation experiments described below, and for determining the point of elution of sodium sulphate-^{35}S.

An aliquot of the concentrated tissue extract was assayed for radioactivity by liquid scintillation with a Packard Tricarb 527 scintillation spectrometer. The scintillator solution contained naphthalene (260 g), 2,5-diphenyloxazole (32 g), and 1,4-di-[2-(5-phenyloxazolyl)]benzene (1.6 g) in a mixture of 2-ethoxyethanol (1200 ml), 1,4-dioxane (1200 ml), and xylene (400 ml). A second aliquot was applied to a resin column and eluted sequentially with water (5 ml), 0.5M sodium chloride (8 ml), 1.25M, 1.5M, and 2.0M sodium chloride (10 ml of each), and 4.0M sodium chloride (12 ml) during 3 h under pressure (3–5 lb/sq. in.) of nitrogen. Fractions (1 ml) were collected automatically, and an aliquot (0.5 ml) of each fraction was dialysed, and assayed for radioactivity. A second aliquot was assayed for radioactivity without dialysis, and corrections for quenching were made by the channels-ratio method. The specific activity of the resin after elution in the usual way was determined after washing with water, mixing, and grinding to a fine powder in a tissue grinder. Different amounts of the dried resin were counted in the presence of scintillator and a thixotropic gelling agent, Cab-O-Sil (G.L. Cabot Inc., Boston, Mass., U. S. A.), and the counts were extrapolated to zero mass of resin.

^{35}S-Labelled tissue extracts were also fractionated on resin that had been saturated with a solution of the sulphated polysaccharide, carrageenan (Boots Pure Drug Co.), or with chondroitin 4-sulphate (Evans Biochemicals Ltd.), and eluted in the normal way prior to use. In another series of experiments, an ^{35}S-labelled extract of rat cartilage was fractionated in the normal way, and fractions from each peak (see Fig. 1) were pooled and dialysed, and separate aliquots were assayed for

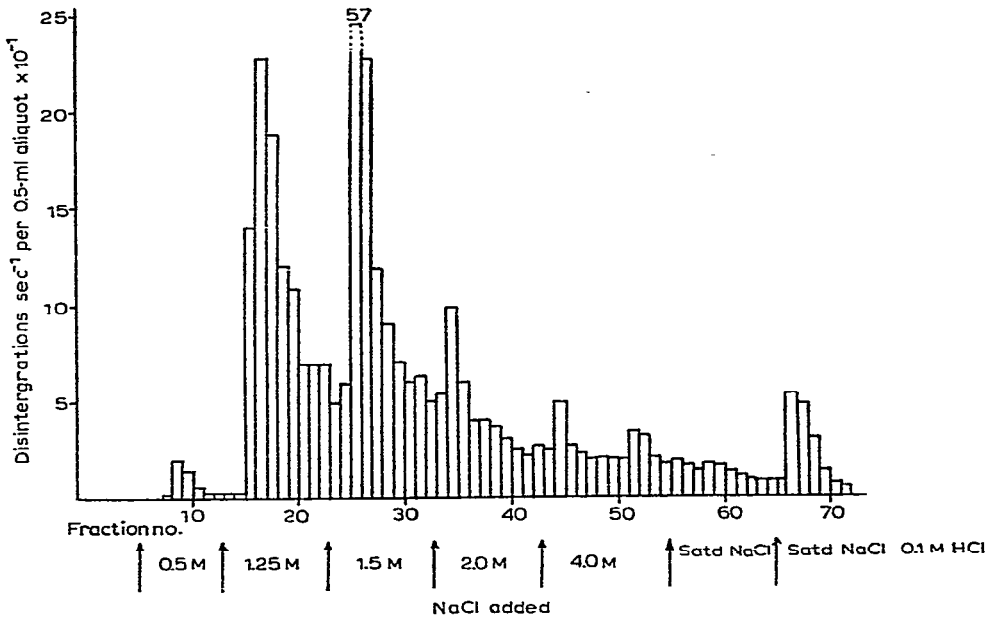


Fig. 1. Fractionation of ^{35}S-labelled components of rat tracheal cartilage on De-Acidite resin FF by elution with increasing concentrations of salt.

TABLE I

REFRACTIONATION ON DE-ACIDITE FF OF ^{35}S-LABELLED COMPONENTS FROM RAT CARTILAGE (SEE FIG. 1)

Tube No.[a]	*Component*[b]	*Uptake of* ^{35}S[c]	*Distribution of* ^{35}S *on refractionation*		
			Tube No.	^{35}S *content*[c]	*% Recovery of* ^{35}S
8–12	PAPS	1.6	9–12	46	100
			13–21		
17–22	CS-4	22.4	17–22	50	42
			27–28	8	
26–31	CS-6	30.7	18–22	9	34
			26–31	59	
35–36	DS	4.5	6–12	28	30
			16–17	21	
			26–27	7	
45	H	1.5	17–21	28	43
			26–28	17	
52–53	KS	1.3	18–20	16	49
			26–29	18	
68–72	Unknown	4.5	18–21	21	38
			27–30	37	
			36–38	6	

[a]Only those tubes that comprised "peaks" of radioactivity have been considered. [b]Tentative identification from elution position (see ref. 1). [c]As % of total ^{35}S eluted from column.

radioactivity and refractionated. The results of these experiments are summarised in Table I.

The point of elution and recovery of sodium sulphate-^{35}S and of mixtures of sodium sulphate-^{35}S with sodium sulphate (218 μg) and with purified heparin (14 μg) were also determined. Heparin (Evans Biochemicals Ltd.) was purified by precipitation with cetyltrimethylammonium bromide[4], and a solution of the purified material in 3M sodium chloride was dialysed against running water and freeze-dried.

In a separate series of experiments, aliquots of an ^{35}S-labelled extract of rat tracheal cartilage were fractionated by using a new batch of heteroporous De-Acidite resin FF (batch 2), nominally identical to that used in the experiments described above. Aliquots of the extract in water, 0.5M, 1.25M, 1.5M, 2.0M, and 4.0M sodium chloride were applied to six separate columns (6 ml of resin), previously equilibrated with water or with sodium chloride of the appropriate molarity. Columns were eluted at atmospheric pressure initially with the solution (20 ml) used for equilibration, followed by stepwise, increasing concentrations of salt as in the normal elution sequence; *e.g.*, the sample applied in 1.5M sodium chloride was eluted with 1.5M (20 ml), 2.0M (10 ml), and 4.0M sodium chloride (12 ml). Fractions (1 ml) were collected automatically and analysed for radioactivity. The recovery of radioactivity from the column was calculated.

RESULTS

Fractionations of ^{35}S-labelled extracts of rat tracheal cartilage indicated the presence of radioactive components whose elution positions from the column were identical with those[1] of PAPS, chondroitin 4- and 6-sulphate (CS-4 and CS-6), dermatan sulphate (DS), heparin (H), and keratan sulphate (KS). These areas in the histogram accounted for approximately 60% of the total radioactivity eluted from the column, the rest appearing as relatively high "background" radioactivity between these areas. Dialysed and non-dialysed aliquots of fractions had identical specific activities, thus showing that the fractions contained no dialysable radioactive components. However, in experiments with the so-called heteroporous resin[5] (batch 1), including several in which tissue extracts from guinea-pig ear cartilage were used, approximately 35% of the radioactivity applied to the column was recovered by using the described elution system. Direct comparison of fractionations on the so-called heteroporous and isoporous resins[5] demonstrated a recovery of 40–45% of radioactivity from the isoporous resin, with the major peak appearing in 1.25M sodium chloride, and a recovery of approximately 35% from the heteroporous resin, with the major peak appearing in 1.5M sodium chloride. Preparation of the resin as described, or by using hot 6N reagents[1], did not significantly alter the fractionation profile or the recovery of radioactivity for the heteroporous resin, but small differences were observed for the isoporous resin.

The fractionation patterns obtained with different batches of nominally identical resin varied considerably, and the resolution of radioactive components on some batches was very poor.

Slower elution of the column at atmospheric pressure with the same solutions during 8 h, and elution with the same solutions at pH 3.5, did not improve the recovery of radioactive material. Approximately 5% of the total radioactivity applied to the column was recovered by elution with saturated sodium chloride (10 ml) and saturated sodium chloride in 0.1N hydrochloric acid (10 ml), subsequent to elution with the normal solutions, but approximately 50% of the radioactivity applied to the resin was not eluted with the solutions described, as was confirmed by analysis of the resin for radioactivity. Approximately 20% of this residual activity was eluted from the resin by prolonged treatment with saturated sodium chloride in 0.1N hydrochloric acid.

Similar recoveries of radioactivity were obtained on fractionation of extracts of guinea-pig skin that had been cultured in a medium containing, among other components, D-glucose-^{14}C (uniformly labelled, 3 μCi/ml). Recoveries of ^{35}S-labelled extracts were not improved by using resin that had been saturated with carrageenan or chondroitin 4-sulphate and eluted in the normal way prior to use. Such treatment might have saturated sites on the resin at which sulphated glycosaminoglycans were attached irreversibly.

The ^{35}S-labelled component of rat tracheal cartilage that had the same elution point as PAPS was refractionated on De-Acidite resin FF (chloride form) with a quantitative recovery of radioactivity, 46% of which was eluted at the same point as in the original fractionation (see Table I). For all other components, however, the total recovery of radioactivity from the column on refractionation was only 30–50%. The refractionation experiments suggested that pooled Fractions 17–22 and 26–31 (Fig. 1) contained components that were incompletely resolved in the initial fractionation. For Fractions 17–22 and 27–31, respectively, 50% and 59% of the radioactivity eluted had the same elution point on refractionation. Apart from additional small "peaks" of radioactivity eluted as indicated in Table I, the rest of the radioactivity recovered from the column on refractionation of Fractions 17–22 and 27–31 was eluted by 2M or higher salt concentrations as a high "background" activity showing no well-defined "peaks".

Refractionation of those components eluted with 2.0M or higher concentrations of salt in the original fractionation (Fig. 1) gave a considerable spread of the point of elution of radioactivity. Furthermore, only a very small percentage of the radioactivity in these components had the same elution point on refractionation. Sodium sulphate-^{35}S, and mixtures of sodium sulphate-^{35}S with unlabelled sodium sulphate or heparin, were fractionated with quantitative recovery of radioactivity. The point of elution (Fractions 13–20) of the radioactive material was the same in each case, thus indicating that no irreversible association occurred between sodium sulphate-^{35}S and heparin. Fractionation of an ^{35}S-labelled extract of rat tracheal cartilage on a new, but nominally identical, batch of resin (batch 2) gave an elution profile essentially similar to that shown in Fig. 1, but a total recovery of radioactivity of only 18%. Quantitative recoveries of radioactivity were obtained when aliquots of the extract were applied to this resin in 1.25M, 1.5M, 2.0M, or 4.0M sodium chloride. A low

recovery was obtained for the sample applied in 0.5M sodium chloride and the elution profile (Fig. 2) was considerably different from that of the control sample applied in water. In all cases of high recovery, 90–100% of the activity was present, as a single peak, in the first few fractions of the eluant. For the sample applied in 1.25M sodium chloride, a second peak (approximately 4% of the total eluted activity) appeared in the 1.5M sodium chloride eluant. An increased "background" of radioactivity was noted in this fractionation. For the sample applied in 0.5M sodium chloride, the "background" activity was of the same order as that observed in the control.

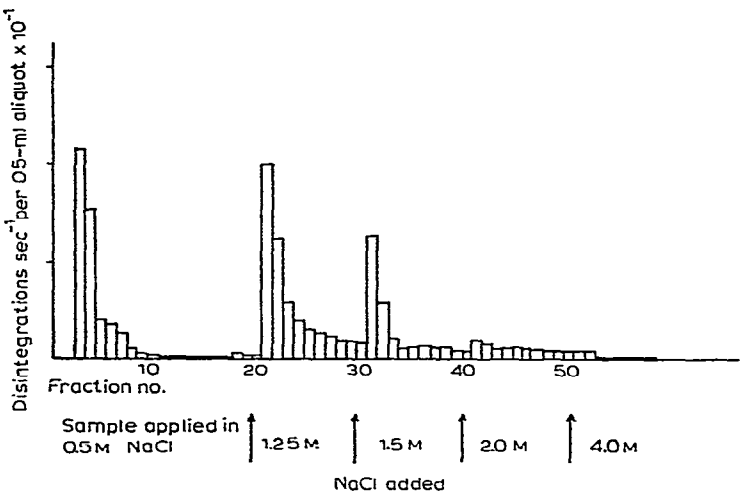


Fig. 2. Fractionation of a solution of ^{35}S-labelled components of rat tracheal cartilage in 0.5M sodium chloride on De-Acidite resin FF equilibrated in 0.5M sodium chloride.

DISCUSSION

It has been shown[1,3] that colorimetric estimation of acidic glycosaminoglycans from skin and cartilage gave a similar elution profile to that obtained from radioactive scanning of extracts of the same tissue that had been cultured in the presence of sodium sulphate-^{35}S or ^{14}C-labelled sugars. Recent work[6] on the fractionation and characterisation of acidic glycosaminoglycans from large amounts of human skin, using the described method on a larger scale, has also confirmed that the elution positions of chondroitin 4- and 6-sulphates, dermatan sulphate, and hyaluronic acid are as previously reported[1].

The amounts of acidic glycosaminoglycans present in tissue extracts used in the present work, however, preclude the use of rigorous methods for their identification in column eluates and thus prevented precise investigations, either of the behaviour of these components on De-Acidite resin in experiments of this scale, or of the relationship between the positions of elution of radioactivity and of acidic glycosaminoglycans. Without such information, the interpretation of elution patterns

that are based solely on radioactive scanning is obviously limited. The low recoveries from the resin of ^{35}S- and ^{14}C-labelled components, their behaviour on refractionation, and the marked differences in the properties of nominally identical resins, however, prompt a detailed consideration of the interaction of polyanions with, and the elution from, such resins.

Ion exchange can be described generally in terms of a number of diffusion steps, and the exchange reaction, which, for the elution of an absorbed polyanion, may be summarised as:

(1) $P^{z-}R^{z+} + zM^+A^- \rightleftharpoons P^{z-}zM^+ + R^{z+}zA^-$ (exchange reaction)
(2) $P^{z-}zM^+$ (exchange site) $\rightleftharpoons P^{z-}zM^+$ (resin bead surface)
(3) $P^{z-}zM^+$ (resin bead surface) $\rightleftharpoons P^{z-}zM^+$ (solution),

where P^{z-} is a polyanion, M^+A^- the electrolyte, and R^{z+} the ion-exchanger. Application of the law of Mass Action to (1) gives:

(4) $[P^{z-}R^{z+}]/[P^{z-}zM^+] = \text{constant}/[M^+A^-]^z$,

assuming that $[R^{z+}zA^-]$ is constant. Equation (4) is similar to that derived[7] for the interaction of large organic cations, *e.g.*, quaternary ammonium ions and certain dyes, with polyanions; $[P^{z-}R^{z+}]/[P^{z-}zM^+]$ is the ratio of polyanion absorbed on the resin to that in solution. Graphical representation of equation (4) conveniently expresses the concept of critical electrolyte concentration (CEC), since the plot of $[P^{z-}R^{z+}]/[P^{z-}zM^+]$ against $1/[M^+A^-]^z$ for large values of z shows an inflexion point, the position of which on the $1/[M^+A^-]^z$ axis determines the value of the CEC.

The chromatographic behaviour of polyions in general on ion-exchange resins may be controlled by diffusion following desorbtion. The times of half exchange with NH_4^+ ions on a polystyrene cation-exchanger for Na^+, 1.25 min; $N^+(CH_3)_4$, 1.75 min; $N^+(C_2H_5)_4$, 3 min; and $C_6H_5N^+(CH_3)_2(CH_2C_6H_5)$, 1 week[8], support the view that the size of the ion is important, although the affinity of the polystyrene matrix for the aromatic nucleus may be partially responsible for the slowness of exchange of the last-named ion. For polyanions, reactions (2) and (3) would be diffusion-controlled.

In the fractionations described, dilute, aqueous solutions of polyanions (acidic glycosaminoglycans) are added to the resin which is in a state of maximum swelling, and the potential between the resin and aqueous phases draws the polyanions into the resin matrix. Most of the absorbed polyanions will be drawn either into "cracks" in the resin beads or into regions which are not highly cross-linked. On addition of the eluting electrolyte, the resin matrix shrinks, tending to trap the absorbed molecules, and the elution processes depicted above begin.

Elution of a polyanion might be delayed or prevented since (*a*) it might be difficult or impossible to achieve a CEC in the region in which the polyanion is absorbed and (*b*) the desorbed polyanion might become entangled with the resin. The quantitative recoveries of radioactivity obtained with samples applied to De-Acidite resin in 1.5, 2.0, and 4.0M sodium chloride indicate that, under these

conditions, the polyanions are not absorbed on, and do not significantly penetrate, the resin matrix. Some penetration and absorption apparently occurred with the sample applied in 1.25M sodium chloride. Samples applied to the resin in 0.5M sodium chloride or water were consistently eluted with low recoveries of radioactivity and considerable "trailing" which may be attributed to slow diffusion of the desorbed polyanion out of the resin bead. Some "trailing" was noted for the sample applied in 1.25M sodium chloride.

The variability of pore and fissure size in the resin, and the constraint placed upon the system both by the semi-rigidity of the resin and by the size of the polyanions, mean that any component polyanion in an extract of cartilage would be distributed on absorption among a number of sites possessing different steric characteristics. The apparent CEC for desorption of a particular polyanionic species, as measured by the ionic strength of the eluant, probably differs from site to site in the resin.

Whereas there is no significant difference in the CEC of the complexes formed by chondroitin 4-sulphate, chondroitin 6-sulphate, and dermatan sulphate with aqueous solutions of quaternary ammonium salts, the ion-exchange resin may be sensitive to the geometrical distribution of anionic sites on these three polysaccharides, and their reported separation on De-Acidite FF resin may be determined in part by this factor.

The differences between batches of nominally identical resin are apparently related to the more-complex series of reactions that have to be considered in ion-exchange processes involving polyanions. A considerable proportion of the "trapped" radioactive components in resin that had been eluted with the described sequence of solutions was eluted on prolonged soaking in strong salt solution for 10 days. This supports the conclusion that elution of some of the polyanions is slow. The higher recovery of radioactive components from isoporous rather than heteroporous resin also suggests that steric factors are involved in the elution step, since the only difference between the two types of resin is the more regular distribution of cross-linking in isoporous resin.

The main features of the separation of cartilage extracts by chromatography on De-Acidite FF as described may be summarised as follows. 1. Polyanions are drawn into the resin matrix and absorbed. 2. On addition of salt, the matrix shrinks and traps absorbed polyanions. The extent and nature of the trapping depends upon the degree of penetration into the resin matrix; the nature of the resin at the exchange site; and the shape of, and distribution of charges on, the polyanion. 3. During elution, peaks of radioactivity appear as the CEC for the particular polyanion and absorption site is reached. The different elution positions for the chondroitin sulphates and dermatan sulphate may depend, in part, on the shape of the polyanions. 4. Complete separation of the polyanions is prevented by (*a*) the steric heterogeneity of the resin sites and (*b*) the slow rate of elution of much of the absorbed material.

Conclusions that are compatible with those noted above have been made by

Knight [9]. Polyanions of defined structure and molecular weight are required for further investigations of these points. It is also particularly important that the structure of the eluted components be rigorously defined.

ACKNOWLEDGMENTS

Drs. S. A. Barker, P. J. Somers, J. F. Kennedy, and S. Tittensor are acknowledged for their valuable discussion, Mrs. S. A. Woodbury for technical assistance, and Professor M. Stacey for his interest. The work was supported, in part, by a grant from the Nuffield Rheumatism Research Council (to P. J. W.).

REFERENCES

1 S. A. BARKER, C. N. D. CRUICKSHANK, AND T. WEBB, *Carbohyd. Res.*, 1 (1965) 52.
2 S. A. BARKER, C. N. D. CRUICKSHANK, AND T. WEBB, *Carbohyd. Res.*, 1 (1965) 62.
3 S. A. BARKER, R. C. E. GUY, AND C. N. D. CRUICKSHANK, *Carbohyd. Res.*, 1 (1965) 312.
4 J. E. SCOTT, *Methods Carbohyd. Chem.*, 5 (1965) 38.
5 T. R. E. KRESSMAN, *Effluent and Water Treatment J.*, 6 (1966) 119.
6 S. A. BARKER, J. F. KENNEDY, AND P. J. SOMERS, *Carbohyd. Res.*, 10 (1969) 57.
7 J. E. SCOTT, in *The Chemical Physiology of Mucopolysaccharides*, G. Quintarelli (Ed.), Churchill, London, 1968, p. 219.
8 T. R. E. KRESSMAN, *J. Phys. Chem.*, 56 (1952) 118.
9 C. S. KNIGHT, *Advan. Chromatog.*, 4 (1967) 61.

Carbohydrate Research
Elsevier Publishing Company, Amsterdam
Printed in Belgium

CORRELATION OF CHEMICAL COMPOSITION AND OPTICAL ROTATION OF WATER-SOLUBLE GALACTOMANNANS

C. LESCHZINER AND A. S. CEREZO

Departamento de Química Orgánica, Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires, Buenos Aires (Argentina)

(Received February 25th, 1969)

ABSTRACT

A quantitative relationship can be obtained between the rotatory power and the molar ratio mannose/galactose for galactomannans having a structure consisting of a backbone of β-(1→4)-linked D-mannopyranose units with side chains of α-(1→6)-linked D-galactopyranose residues, by using an equation based on the principle of optical superposition and derived originally by Timell for native xylans.

By adopting the usually accepted errors for the determination of optical rotations ($\pm 5°$ for polysaccharides) and the percentages of the sugar units ($\pm 5\%$), the natural, water-soluble galactomannans can be grouped into two classes, namely, those for which the differences between the experimental and calculated rotations lie in the range of the experimental error, and those for which the above differences are higher than the experimental error. For the last class, the existence of structural details not considered in the basic pattern used for the correlation is suggested.

INTRODUCTION AND DISCUSSION

There is good evidence that the basic structure of water-soluble galactomannans isolated from different sources, especially seeds of Leguminosae, consists of a backbone of β-(1→4)-linked D-mannopyranose residues with side chains of α-(1→6)-linked D-galactopyranose residues. Since these polysaccharides have specific rotations that differ widely from $-54°$ [the value that is predicted for an infinitely long molecule composed exclusively of (1→4)-linked β-D-mannose residues[1]], it is reasonable to assume that the deviations represent the contribution to the optical rotation by the appended D-galactose residues.

The aim of this study was to use the principle of optical superposition to calculate rotations of hypothetical galactomannans (having the perfect structure mentioned above) as a function of the mannose/galactose ratio (M/G), and to compare these values with those obtained experimentally for natural galactomannans.

Similar calculations have been carried out by Timell[2], on native xylans, using equation 1, which has now been adapted for galactomannans: m is the molar rotation of an unsubstituted D-mannose residue in the chain, μ is the molar rotation of an

α-(1→6)-linked D-galactopyranosyl-D-mannopyranose unit in the chain, and σ is the molar ratio of mannose to the above aldobiose residue,

$$[\alpha]_D \left(163 + \frac{163}{\sigma+1}\right)\left(1 + \frac{1}{\sigma}\right) = m + \mu/\sigma. \quad (1)$$

The factor $[163+(163/\tau+1)]$ is the average residue weight of each mannose unit in the polymer backbone with the aldobiose averaged into each. A plot of the left-hand side of equation 1 for linear galactomannans of different galactose contents should yield a straight line of intercept m and slope μ.

The quantity m, available from the mannan oligosaccharide series[1], is −88.0. The quantity μ is not available at present, since it could only be derived from the difference in molar rotations of the symmetrical tetrasaccharide *O*-β-D-mannopyranosyl-(1→4)-*O*-β-D-mannopyranosyl-[(6→1)-α-D-galactopyranosyl]-(1→4)-D-mannose and mannobiose. Timell[2] obtained the approximate, corresponding value for xylans by using the difference in molar rotations between the tetrasaccharide, *O*-(4-*O*-methyl-α-D-glucopyranosyluronic acid)-(1→2)-*O*-β-D-xylopyranosyl-(1→4)-*O*-β-D-xylopyranosyl-(1→4)-D-xylose, and xylobiose. We tried this procedure, but, on looking at the members of the *O*-α-D-galactopyranosyl-(1→6)-*O*-β-D-mannopyranosyl-(1→4)-*O*-β-D-mannopyranosyl-(1→4) series, we found no relationship between the reported optical rotations and the degree of polymerization (see Table I).

TABLE I

ROTATORY POWERS AND MOLAR ROTATIONS OF OLIGOSACCHARIDES OF THE α-D-GALACTOPYRANOSYL-(1→6)-β-D-MANNOPYRANOSYL-(1→4) SERIES

Oligosaccharide[a]	$[\alpha]_D$ (*water*) (*degrees*)	ϕ	*Ref.*
G→M	+124.6	+426.1	3
G→M→M	+98.4	+495.9	4
G→M→M→M	−7.0	−45.6	1
G→M→M→M→M	−11.0	−89.7	1

[a]G→, α-D-galactopyranosyl-(1→6)-; M→, β-D-mannopyranosyl-(1→4)-.

The quantity μ (+520.9) was then tentatively obtained from the difference of the molar rotations of *O*-α-D-galactopyranosyl-(1→6)-*O*-β-D-mannopyranosyl-(1→4)-D-mannose[4] (+495.9) and M* (−25.0). To be sure that the same equilibrium mixture of anomers is present in solution in both entities, M* was obtained as the difference of the molar rotations of mannotriose[3] (−126.0) and mannotriitol[3] (−101.0).

The galactomannans are listed in Table II, together with the observed optical data, the calculated rotations, and the corresponding values of $(m+\mu/\sigma)$. The experimental rotations were considered to be accurate to ±5° (unless otherwise stated). In the calculations, an error of ±5% in the percentages of mannose and galactose (total error, ±10%) results in a maximum error of ±6° for the calculated

TABLE II

EXPERIMENTAL AND CALCULATED ROTATORY POWERS OF GALACTOMANNANS EXTRACTED WITH WATER FROM DIFFERENT SOURCES

Species	σ[a]	$1/\sigma$	$[\alpha]_D$[b] (*degrees*)	$[\alpha]_D$[c] (*degrees*)	$[\alpha]_D$[d] (*degrees*)	$(m+\mu/\sigma)$[e]	$(m+\mu/\sigma)$[f]	$(m+\mu/\sigma)$[g]	*Ref.*
1 *Medicago sativa* (Var. Provence)[h]	1.00	1.00	89.0	88.5		435.2	432.9		9
2 *Trifolium repens*[i]	1.07	0.94	59.0	85.0	62.8	276.7	398.8	294.5	6
3 *Ipomea muricata*	1.18	0.85	56.0	80.3	57.5	246.3	353.4	252.8	7
4 *Trigonella foenum-graecum*	1.20	0.83	70.0	79.8		303.7	346.1		8
5 *Lotus corniculatus*	1.25	0.80	87.0	77.6		368.7	328.7		9
6 *Medicago sativa* (Var. Provence)[j]	1.25	0.80	118.0[b]	77.6		500.1	328.7		10
7 *Trifolium repens*	1.30	0.77	77.8	75.5		322.1	312.7		11
8 *Trifolium pratense*	1.30	0.77	78.0	75.5		322.9	312.7		10
9 *Anthyllis vulneraria*	1.32	0.76	80.0	74.7		328.4	306.6		12
10 *Glycine soja* I[k]	1.40	0.71	68.0	71.9		268.5	284.1		13
11 *Glycine soja*[l]	1.50	0.67	65.0	68.0		247.7	259.3		14
12 *Sesbania grandiflora*	1.50	0.67	50.0	68.0	44.4	190.6	259.3	169.3	15
13 *Genitsa scoparia* I[m]	1.59	0.63	46.5	65.1	41.2	171.3	239.6	151.7	6
14 *Genitsa scoparia* II[n]	1.66	0.60	36.9	62.9	38.8	132.4	225.8	139.3	6
15 *Cyanopsis tetragonolobus*	2.00	0.50	60.0	52.9		195.6	172.4		16
16 *Glycine soja* II[o]	2.35	0.43	22.0	44.2	18.7	66.6	133.6	56.7	13
17 *Borassus flabellier*	2.40	0.42	8.5	43.1	17.6	25.5	129.0	52.6	17
18 *Gleditsia amorphoides*	3.00	0.33	22.4	31.6		60.7	85.6		18
19 *Gleditsia thriacantos*	3.00	0.30	23.5	31.6		63.7	85.6		19
20 *Ceratonia siliquia*[p]	3.50	0.29	44.0	24.5		113.6	51.9		20
21 *Medicago sativa*[q]	3.61	0.28	57.6	22.1		146.3	56.2		5
22 *Gleditsia ferox*	3.80	0.26	−12.2	19.8	−7.7	−30.3	49.0	−19.1	6
23 *Ceratonia siliquia*[r]	4.00	0.25	9.0	17.3		22.0	42.2		21
24 *Gymnocladus dioica*	4.00	0.25	29.0	17.3		70.9	42.2		22
25 *Gleditsia ferox*[s]	24.00	0.04	−40.0	−37.6		−70.5	−65.3		23

[a]Molar ratio of mannose to the α-(1→6)-D-galactopyranosyl-D-mannopyranose residue in the chain. [b]Experimental data: water solutions were used in all the cases, except in galactomannans 15, 17, 20, and 24, where the samples were dissolved in NaOH solutions. An error of ±5° is inferred for all the values, except in cases 6 and 8, where errors of ±11° are given in the literature[10,12]. [c]Rotations calculated from equation 1 with $m = -88.0$ and $\mu = 520.9$. They have a maximum error of ±6°, except in sample 25, where the error is ±2°. These values arise from adopting errors of ±5% in the percentages of mannose and galactose. [d]Rotations calculated from equation 1 with $m = -142.0$ and $\mu = 462.0$. They have the same error as above. [e]Calculated from the experimental values of $[\alpha]_D$ and σ (left-hand side of equation 1). [f]Calculated from values of $m = -88.0$ and $\mu = 520.9$ and σ (right-hand side of equation 1). [g]Calculated from values of $m = -142.0$ and $\mu = 462.0$ and σ (right-hand side of equation 1). [h]Extracted at room temperature. [i]Extracted from germinated seeds. [j]Hot water extraction. [k]Extracted at room temperature. [l]Commercial sample, extracted at 40°. [m]Extracted at room temperature. [n]Extracted at 50°. [o]Extracted at 60°. [p]Commercial sample. [q]Obtained by enzymic hydrolysis with α-galactosidase from galactomannan 1. [r]Commercial sample. [s]Obtained by enzymic hydrolysis with α-galactosidase from galactomannan 23.

rotations. Accordingly, differences of $\pm 11°$ or less ($\pm 17°$ in samples 6 and 8) between experimental and calculated rotations were considered to be meaningless.

The best straight-line (Fig. 1, line II) calculated (least-square method) by using the experimental rotations of all the galactomannans listed in Table I showed values of m (−93.0) and μ (+502.0) which are in good agreement with those ($m = -88.0$ and $\mu = +520.9$) used in equation **1**. If all the rotations, except those of samples 6 and 20 (galactomannans from *Medicago sativa* and *Ceratonia siliquia* which, when reinvestigated, showed different rotations; see samples 1 and 23, Table II), are used, μ changed to +484.0 (Fig. 1, line III). The above values confirm the well-known, basic structure used in the derivation of equation **1**, but, if $\mu = +484.0$ has any significance, it suggests the presence of fine structural details.

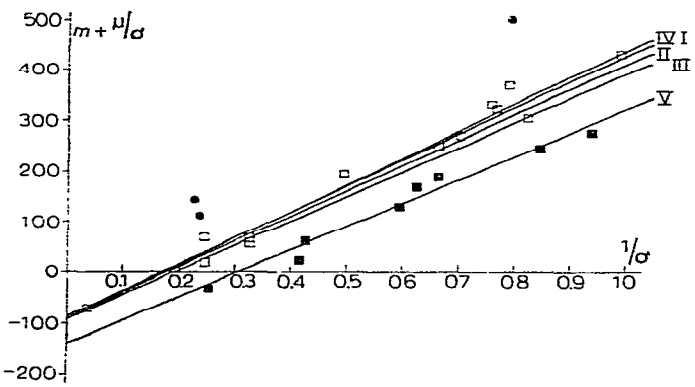


Fig. 1. The $(m+\mu/\sigma)$ values versus the reciprocal $(1/\sigma)$ of the mannose to aldobiose residue ratio for galactomannans extracted with water from different sources. Galactomannans for which the differences between the experimental and calculated rotations (*a*) lie in the range of the experimental error (□); (*b*) are higher than the experimental error (■), and whose rotations are more negative than those predicted by using equation **1**; and (*c*) are higher than the experimental error (●), and whose rotations are more positive than those predicted by using equation **1**. Line I, calculated from equation **1** with $m = -88.0$ and $\mu = +520.9$. Line II, the best straight-line calculated (minimum square-root method) for the $(m+\mu/\sigma)$ values of all the galactomannans listed in Table II. Line III, the best straight-line calculated for the $(m+\mu/\sigma)$ values of all the galactomannans listed in Table II, except for those of *Medicago sativa* and *Ceratonia siliquia* (samples 6 and 20) which, when reinvestigated, showed different rotations. Line IV, the best straight-line calculated for the $(m+\mu/\sigma)$ values of the galactomannans for which the differences between the experimental and calculated rotations lie in the range of the experimental error. Line V, the best straight-line calculated for the $(m+\mu/\sigma)$ values of the galactomannans for which the differences between the experimental and calculated rotations are higher than the experimental error, and whose rotations are more negative than those predicted by using equation **1**.

When the experimental and calculated rotations were compared, it was found that, in fourteen cases (Table II, galactomannans 1, 4, 5, 7–11, 15, 18, 19, and 23–25), the differences lay in the range of the adopted experimental error. The best straight-line (Fig. 1, line IV) calculated (least-square method) with the experimental rotatory powers of the polysaccharides gave values of m (−94.0) and μ (+530.0) which are in very good agreement with those used in equation **1**.

The differences for the other galactomannans (Table II, galactomannans 2, 3, 6, 12–14, 16, 17, and 20–22) are higher than the experimental error and indicate the presence, in these molecules, of structural details not considered in the basic pattern used for equation **1**. Deviation from an exact relationship between the rotatory power and the mannose/galactose ratio has been previously indicated for several galactomannans[6]. In eight cases (Table II, galactomannans 2, 3, 12–14, 16, 17, and 22), the polysaccharides show rotations that are more negative than those predicted. It is noteworthy that the distribution of the points in Fig. 1, corresponding to these substances, indicates a common fine structure that is related to the galactose content. The best straight-line (Fig. 1, line V) calculated for these points gave values of $m = -142.0$ and $\mu = +462.0$, and it is interesting that the differences between the experimental rotations and those predicted on the basis of the above parameters (Table II) lie in the range of the adopted experimental error. The existence of some β-D-galactose residues (which must not be as chain ends) could be one way of explaining these lower rotations. It must be remembered, in this connection, that the galactomannan extracted with water from the kernel of *Cocus nucifera*[24] (which was not included in Table II, because its basic structure is largely different from that accepted for galactomannans extracted with water) has β-(1→4)-linked D-galactopyranose residues in the backbone, as well as β-D-mannopyranose residues as chain ends. These structural variations seem to lower the rotations to unusual values (−89.0°, NaOH solution). Methylation analysis of some of the galactomannans listed in Table II also indicated the presence of small proportions of 6-substituted D-galactose residues in the backbone[17,18,23], and of D-mannopyranose residues as chain ends[18,23].

Three galactomannans[10,20] (Table II; 6, 20, and 21) show higher rotations than those predicted from equation **1**, but when two of them (from *Medicago sativa* and *Ceratonia siliquia*) were reinvestigated, the rotation values (Table II; 1 and 23) were in agreement with those expected from equation **1**.

REFERENCES

1 E. C. A. SCHWARZ AND T. E. TIMELL, *Can. J. Chem.*, 41 (1963) 1381.
2 R. H. MARCHESSAULT, H. HOLAVA, AND T. E. TIMELL, *Can. J. Chem.*, 41 (1963) 1612.
3 J. STANĚK, M. ČERNÝ, AND J. PACÁK, *The Oligosaccharides*, Academic Press, 1965, p. 234.
4 R. L. WHISTLER AND D. F. DURSO, *J. Amer. Chem. Soc.*, 74 (1952) 5140.
5 J. E. COURTOIS, C. ANAGNOSTOPOULOS, AND F. PETEK, *Bull. Soc. Chim. Biol.*, 40 (1958) 1277.
6 J. E. COURTOIS AND P. LE DIZET, *Bull. Soc. Chim. Biol.*, 45 (1963) 731.
7 S. N. KHANNA AND P. C. GUPTA, *Phytochemistry*, 6 (1967) 605.
8 P. ANDREWS, L. HOUGH, AND J. K. N. JONES, *J. Chem. Soc.*, (1952) 2744.
9 R. SØMME, *Acta Chem. Scand.*, 20 (1966) 589.
10 P. ANDREWS, L. HOUGH, AND J. K. N. JONES, *J. Amer. Chem. Soc.*, 74 (1952) 4029.
11 K. FRØYSTAD HORVEY AND A. WICKSTRØM, *Acta Chem. Scand.*, 18 (1964) 833.
12 R. SØMME, *Acta Chem. Scand.*, 21 (1967) 685.
13 G. O. ASPINALL AND J. N. C. WHYTE, *J. Chem. Soc.*, (1964) 5058.
14 R. L. WHISTLER AND J. SAARNIO, *J. Amer. Chem. Soc.*, 79 (1957) 6055.
15 S. RAG AND M. V. L. RAO, *Indian J. Chem.*, 3 (1965) 361.
16 F. SMITH AND R. MONTGOMERY, *The Chemistry of Plant Gums and Mucilages*, Reinhold, 1959, p. 325.

17 A. K. MUKHERJEE, D. CHOUDHURY, AND P. BAGCHI, *Can. J. Chem.*, 39 (1961) 1408.
18 A. CEREZO, *J. Org. Chem.*, 30 (1965) 924.
19 C. LESCHZINER AND A. CEREZO, unpublished data.
20 B. W. LEW AND R. A. GORTNER, *Arch. Biochem.*, 1 (1943) 325.
21 Ref. 16, p. 331.
22 E. B. LARSON AND F. SMITH, *J. Amer. Chem. Soc.*, 77 (1955) 429.
23 J. E. COURTOIS AND P. LE DIZET, *Carbohyd. Res.*, 3 (1966) 141.

Carbohydrate Research
Elsevier Publishing Company, Amsterdam
Printed in Belgium

RAPID, SENSITIVE DETERMINATION OF PERIODATE

GAD AVIGAD*
Department of Biochemistry, Albert Einstein College of Medicine, Bronx, New York 10461 (U. S. A.)
(Received April 10th, 1969)

ABSTRACT

Periodate rapidly oxidizes the violet ferrous–2,4,6-tri-2-pyridyl-*s*-triazine complex to a colorless compound. This reaction serves as the basis for a simple procedure for the quantitative, colorimetric determination of periodate at the nanomolar level. Use of this assay was exemplified by a study of the course of oxidation of 1- to 2-μmolar samples of several representative carbohydrates.

INTRODUCTION

Periodic acid is a powerful oxidant that has found many uses in analytical and organic chemistry[1–4]. Its ability to cleave 1,2-glycols provides a reagent extensively employed in carbohydrate chemistry[5–9].

A selection of methods is available for the determination of periodate in solution[1,2,6,9]. Titrimetric procedures[10–14], which are relatively slow and sensitive only to μmolar levels of periodate per sample, are the most widely employed. Direct determination of periodate by u.v. spectrophotometry provides a sensitive method[15–17], but it often cannot be applied effectively, owing to the presence of other compounds having high absorption in the same spectral region. Another spectrophotometric method, based on the oxidation of 1,2-bis(*p*-dimethylaminophenyl)-1,2-ethanediol, has recently been described[18]. Colorimetric assays for periodate that are based on the oxidation of aromatic amines have also been suggested[19,20]. These procedures are relatively slow, or involve the use of organic solvents and the formation of a colored product that is not very stable.

In the present article, a simple, rapid, sensitive procedure for the assay of periodate is described. This method is based on the very fast oxidation of the stable, violet complex [$Fe(TPTZ)_2^{2+}$] (**1**) of ferrous ion with 2,4,6-tri-2-pyridyl-*s*-triazine[21] (TPTZ) to yield a colorless ferric derivative. The amount of residual violet compound is determined colorimetrically. A convenient range for the assay of 5 to 200 nmoles of periodate is suggested. However, the method can readily be adapted for the determination of one nmole of periodate. Compounds (other than periodate) usually

*On leave from the Hebrew University, Jerusalem, Israel.

found in a standard oxidation system of a carbohydrate do not interfere with the assay. As measurements are made at 593 nm, the presence of such u.v.-absorbing materials as compounds containing unsaturated bonds, aromatic aglycons, purines, pyrimidines, and proteins does not interfere. The method may also be suitable for the assay of periodate in certain water-miscible, organic solvents.

The present assay-procedure was found to be particularly valuable when only a small quantity (one to two μmoles) of the sugar to be examined was available, but a study of the kinetics of its oxidation by periodate was desired.

EXPERIMENTAL

Materials. — 2,4,6-Tri-2-pyridyl-*s*-triazine was obtained from the G. E. Smith Chemical Co., Columbus, Ohio 43223. The preparations of sodium metaperiodate used were analytical reagents from Fluka AG, Buchs, Switzerland, and Fisher Scientific Co., Pittsburgh, Pa.

Spectrophotometry. — Readings were made with a Zeiss Model PMQII spectrophotometer, with cuvets having a 1.0-cm light-path. A Gilford model 300 spectrophotometer was occasionally employed (for rapid assay of a large series of samples).

Reagents. — The violet solution of **1** was prepared as follows. TPTZ (75 mg, 0.24 mmole) was dissolved in acetic acid (46 ml), and M sodium acetate (210 ml) and a freshly prepared solution of $Fe(NH_4)_2(SO_4)_2 \cdot 6H_2O$ (31.4 mg, 0.08 mmole in 100 ml of water) were added. The volume was then made to 1 liter with water. The solution should have pH 4.0–4.2 and $\varepsilon_{1\,cm}^{593} \sim 1.8$. No apparent decrease in absorbance of the solution occurs during one month at room temperature. This reagent may be diluted with M acetate buffer (pH 4.0) to provide solutions of lower absorbance at 593 nm.

A stock solution (50 mM) of sodium metaperiodate was prepared by dissolving $NaIO_4$ (1.0695 g) in water (100 ml) in an amber-colored measuring flask or one wrapped in aluminum foil to ensure complete protection from light. This solution was kept no longer than a week at room temperature. Dilute solutions were freshly made daily with water, and these solutions were kept protected from light.

Assay of the periodate. — In a standard procedure, samples containing 5 to 200 nmoles of periodate were added to tubes containing 4.5 ml of the violet reagent (**1**). The volume was made to 5.0 ml with water, and the solution was well mixed. The absorbance of the solution was read at 593 nm, to determine the amount of residual, violet **1**. As this compound is very stable under the conditions employed, measurement of it can be made at one minute, or many hours, after a sample of periodate has been removed for analysis. This property proved to be especially convenient when several series of samples had to be taken at short time-intervals during a kinetic study of oxidation.

Typical standard-curves for periodate are presented in Fig. 1. The slope of the lines is the same, irrespective of the initial concentration of the violet **1** present

in the reagent (see Fig. 1A). Also, the slope can be proportionally increased when a smaller initial volume of the solution of **1** is employed for the assay of similar amounts of periodate (see Fig. 1B). It is apparent that the range and sensitivity of the method for periodate can readily be adjusted for the needs of any particular experiment over a range of concentrations of oxidant. Thus, the sensitivity can be increased to permit determination of one nmole of periodate.

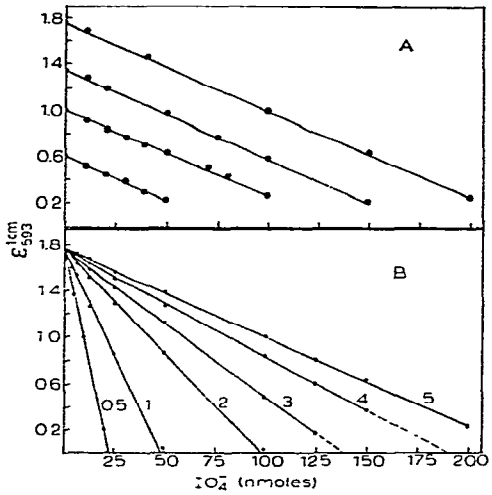


Fig. 1. Oxidation of **1** by periodate. *A*. Reaction conducted in a volume of 5 ml, but containing various initial concentrations of the violet, ferrous complex **1**. *B*. Different volumes of a solution of **1** used. Numbers near each line indicate number of ml of reagent employed.

The decrease in the molar extinction coefficient of the violet **1** that is caused by oxidation with one mole of periodate was found to be 37,000 under the conditions of assay described; this value corresponds to the oxidation of 1.7 g-atoms of ferrous ions[21] (the maximum theoretical equivalent expected is 2.0).

Effect of various compounds on the oxidation of **1** *by periodate.* — At a final concentration of 5 mM, formaldehyde, acetaldehyde, $NaIO_3$, $NaClO_4$, KH_2PO_4, Na_2CO_3, $MgCl_2$, and Tris do not interfere with the assay. Although 5 mM citrate does not decolorize the violet ferrous complex (**1**), concentrations higher than 0.2 mM lessen the sensitivity of its oxidation by periodate. Addition of ethylene(dinitrilotetra-acetate) to the ferrous complex **1** at equimolar concentrations causes instantaneous decolorization[22]. The presence of any one of several such ions as Cu^{2+}, Ag^+, CN^-, and NO_2^- may interfere with[21] the stability of the reagent **1**.

The reaction can also be performed in the presence of any one of several water-soluble, organic solvents. Thus, pyridine (4%), acetonitrile (40%) and acetone (40%) do not interfere significantly with the reaction at concentrations lower than those indicated. *N,N*-Dimethylformamide at concentrations higher than 1% lessens the extent of oxidation, whereas *p*-dioxane at 1% decolorizes the violet, ferrous complex (**1**). Several aliphatic primary alcohols decolorize the ferrous complex **1** and,

at very low concentration, inhibit the periodate reaction. Thus diethylene glycol, diethylene glycol monomethyl ether, methyl Cellosolve, and 2-chloroethanol significantly interfere with the assay when present in a concentration as low as 0.2%.

Methanol, ethanol, and propyl alcohol at final concentrations >0.05% exhibit an inhibitory effect on the extent of oxidation of the ferrous complex **1** by periodate (see refs. 23 and 24).

Kinetics of oxidation of various substrates. — As an example, a representative group of compounds containing the 1,2-glycol grouping was oxidized by periodate, and the course of the consumption of oxidant was estimated by the method already described (see Fig. 2). Each oxidation was conducted at pH 5.2, to ensure slow hydrolysis of any formyl esters formed[25,26]. Similar oxidations at pH 3 to 8 could conveniently be analyzed by the present method, as the reagent was prepared in M acetate buffer at pH 4.0 to 4.2.

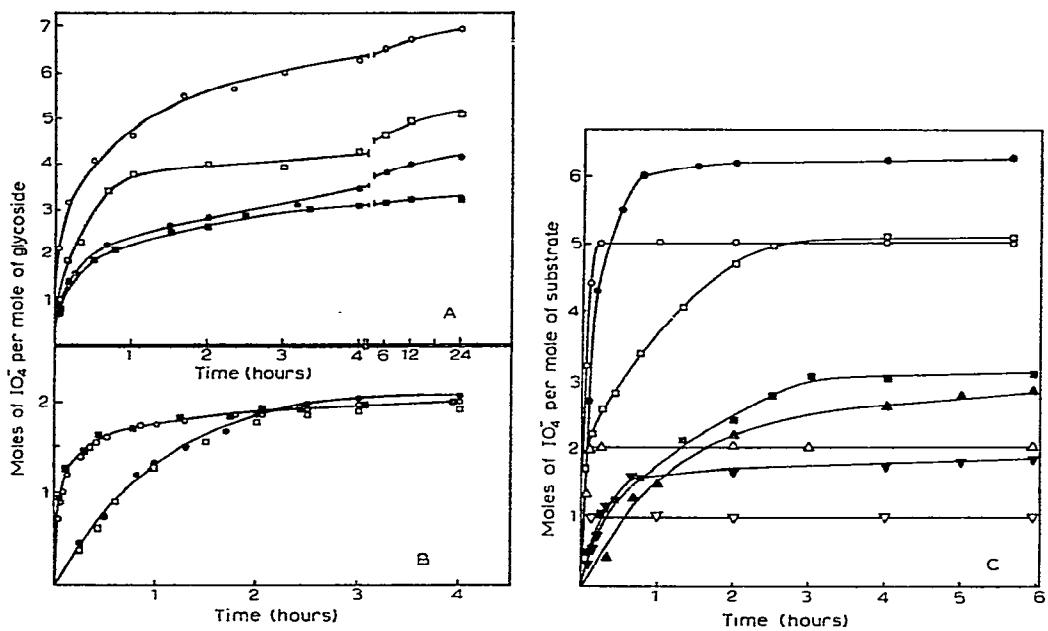


Fig. 2. Course of consumption of periodate during oxidation of different substrates. All reactions were conducted in the dark in 40 mM acetate buffer (pH 5.2). Samples (5 to 25 μl) were withdrawn with a constriction micropipet and directly added to the violet, ferrous reagent (**1**) to determine the amount of residual periodate. *A*. Disaccharides (2 mM) and tetrasaccharide (1 mM) were oxidized with 15.4 mM $NaIO_4$: ○, stachyose; □, lactose; ●, α,α-trehalose; ■, sucrose. *B*. Aldopyranosides (2 mM) were oxidized with 10 mM $NaIO_4$: ●, methyl α-D-xylopyranoside; ○, methyl α-D-arabinopyranoside; □, methyl α-D-glucopyranoside; ■, methyl α-D-mannopyranoside. *C*. Substrates (2 mM) were oxidized with 20.2 mM $NaIO_4$ for inositol (●), D-glucitol (○), and D-glucose (□); with 10.1 mM for 2-deoxy-D-*lyxo*-hexose (■) and 3-*O*-methyl-D-glucose (▲); with 7.9 mM for glycerol (△) and L-serine (▼); and with 5 mM for ethylene glycol (▽).

In all cases (see Fig. 2), the course of the oxidation and the periodate uptake per mole of substrate correspond to the theoretical value predicted. As expected[6,9],

the oxidation of acyclic glycols is very fast compared to that of reducing sugars, involving the intermediary formation of formic esters (see Figs. 2 A, B). Also, cleavage between α-*erythro*-hydroxyl groups (distorted *cis*-hydroxyl groups) is faster than that of α-*threo*-hydroxyl group (distorted *trans*-hydroxyl groups), as is evident from the pattern of oxidation of glycosides (see Fig. 2C).

ACKNOWLEDGMENT

I thank Dr. S. Englard for the hospitality of his laboratory.

REFERENCES

1 C. A. BUNTON, in K. B. WIBERG (Ed.), *Oxidation in Organic Chemistry*, Part A, Academic Press, New York, 1965, p. 367.
2 B. SKLARZ, *Quart. Rev.* (London), 21 (1967) 3.
3 A. J. FATIADI, *J. Res. Nat. Bur. Stand.*, 72A (1968) 361.
4 W. J. BAUMAN, H. H. O. SCHMID, AND H. K. MANGOLD, *J. Lipid Res.*, 10 (1969) 132.
5 R. D. GUTHRIE, *Advan. Carbohyd. Chem.*, 16 (1961) 105.
6 J. STANĚK, M. CERNÝ, J. KOCOUREK, AND J. PACÁK, *The Monosaccharides*, Academic Press, New York, 1963, p. 903.
7 I. J. GOLDSTEIN, G. W. HAY, B. A. LEWIS, AND F. SMITH, *Methods Carbohyd. Chem.*, 5 (1965) 361.
8 L. HOUGH, *Methods Carbohyd. Chem.*, 5 (1965) 370.
9 L. HOUGH, A. C. RICHARDSON, AND C. H. BOLTON, in *Rodd's Chemistry of Carbon Compounds*, Vol. 1F, Elsevier, Amsterdam, 2nd edition, 1967, p. 305.
10 G. F. SMITH, *Analytical Applications of Periodic Acid and Iodic Acid and Their Salts*, G.F. Smith Chemical Co., Columbus, Ohio, 5th edition, 1950.
11 J. R. DYER, *Methods Biochem. Anal.*, 3 (1956) 111.
12 I. M. KOLTHOFF AND R. BELCHER, *Volumetric Analysis*, Vol. III, Interscience Publishers, New York, 1957, p. 475.
13 R. D. GUTHRIE, *Methods Carbohyd. Chem.*, 1 (1962) 435.
14 G. W. HAY, B. A. LEWIS, AND F. SMITH, *Methods Carbohyd. Chem.*, 5 (1965) 357.
15 J. S. DIXON AND D. LIPKIN, *Anal. Chem.*, 26 (1954) 1092.
16 G. V. MARINETTI AND G. ROUSER, *J. Amer. Chem. Soc.*, 77 (1955) 5345.
17 G. O. ASPINALL AND R. J. FERRIER, *Chem. Ind.* (London), (1957) 1216.
18 R. FIELDS AND H. B. F. DIXON, *Biochem. J.*, 108 (1968) 883.
19 M. GUERNET, *Bull. Soc. Chim. France*, (1964) 478.
20 J. ARES PONS, *Publ. Inst. Invest. Microquim., Univ. Nacl. Litoral* (Rosario, Arg.), 26 (1965) 175, 199.
21 P. F. COLLINS, H. DIEHL, AND G. F. SMITH, *Anal. Chem.*, 31 (1959) 1862.
22 B. KRATOCHVIL AND M. C. WHITE, *Anal. Chem.*, 37 (1965) 111.
23 J. F. TAYLOR, B. SOLDANO, AND G. A. HALL, *J. Amer. Chem. Soc.*, 77 (1955) 2656.
24 R. D. GUTHRIE, *Chem. Ind.* (London), (1960) 691.
25 L. HOUGH AND M. B. PERRY, *Chem. Ind.* (London), (1956) 768.
26 L. HOUGH, T. J. TAYLOR, G. H. S. THOMAS, AND B. M. WOODS, *J. Chem. Soc.*, (1958) 1212.

Carbohydrate Research
Elsevier Publishing Company, Amsterdam
Printed in Belgium

4,6-*O*-BENZYLIDENE-1,2-DIDEOXY-D-*erythro*-HEX-1-ENOPYRAN-3-ULOSE

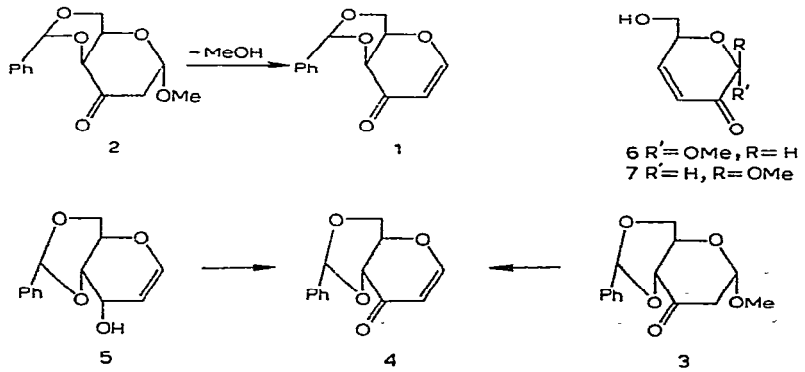
P. M. COLLINS
Chemistry Department, Birkbeck College, Malet Street, London, W.C.1 (Great Britain)
(Received February 18th, 1969)

ABSTRACT

4,6-*O*-Benzylidene-1,2-dideoxy-D-*erythro*-hex-1-enopyran-3-ulose has been prepared in good yield from 4,6-*O*-benzylidene-1,2-dideoxy-D-*ribo*-hex-1-enopyranose. Chromium trioxide in pyridine is the most satisfactory oxidant. The n.m.r. spectra of this and other unsaturated pyranoid derivatives suggest that the magnitude of the *cis* vicinal vinylic coupling constant is reduced when the double bond is between C-1 and C-2. This is attributed to the electronic properties of the ring oxygen atom.

RESULTS AND DISCUSSION

Recent work in this laboratory showed that 4,6-*O*-benzylidene-1,2-dideoxy-D-*threo*-hex-1-enopyran-3-ulose **1** could be readily formed in high yield by mild, acid-catalysed elimination of methanol from the pyranosid-3-ulose **2**. Similar treatment of pyranosid-3-ulose **3** resulted in a low conversion into enone **4**, as indicated by spectroscopic examination of the crude product. Isolation of enone **4** was not achieved, because the chromatographic mobilities of compounds **3** and **4** on silica gel were identical.



In connection with a photochemical project[2], it became necessary to obtain enone **4**, and the preparation of this compound is now reported. Two methods of synthesis appeared to be suitable. One method involved selective oxidation[3] of the

axial C-3 hydroxyl group in 1,2-dideoxy-D-*ribo*-hex-1-enopyranose, followed by benzylidenation. Alternatively, the C-3 hydroxyl group in 4,6-*O*-benzylidene-1,2-dideoxy-D-*ribo*-hex-1-enopyranose **5** could be oxidized. The latter method seemed favourable, since compound **5** is now readily available[4a,b].

The most satisfactory reagent was found to be chromium trioxide in pyridine[5,6]. Treatment of compound **5** with this reagent gave a 75–80% yield of product that was virtually uncontaminated with starting material. Recrystallisation gave pure 4,6-*O*-benzylidene-1,2-dideoxy-D-*erythro*-hex-1-enopyran-3-ulose **4** which showed an intense ultraviolet absorption at 262 nm, typical of α,β-unsaturated ketones[1], and infrared maxima at 1700 and 1600 cm^{-1}, also characteristic of this structural feature. The n.m.r. spectrum ($CDCl_3$) is consistent with the structure assigned to compound **4**: H-2 appeared at τ 4.52 as a doublet ($J_{1,2}$ 6.0 Hz), and the diagnostic[7] enone β hydrogen H-1, at τ 2.70, was partly obscured by the signals for the phenyl protons. In C_6D_6 (Table I), the signal for H-1 (τ 3.60, doublet, $J_{1,2}$ 6 Hz) is separated from the phenyl signals, and the same splitting is found in the H-2 doublet now observed at τ 4.95. In all these respects, the compound clearly resembles the enone with the *threo* structure **1**, and, for comparison, the n.m.r. parameters of enone **1** in $CDCl_3$ and C_6D_6 are recorded in Table I.

TABLE I

N.M.R. PARAMETERS[a] OF *erythro*- AND *threo*-4,6-*O*-BENZYLIDENE-1,2-DIDEOXY-D-HEX-1-ENOPYRAN-3-ULOSES

Enone	C_6H_5	*H-7*	*H-1*	*H-2*	*H-4*	*H-5*	*H-6*	*H-6'*
4[b]($CDCl_3$)	2.3–2.8 m	4.4 s	2.7 d $J_{1,2}$ (6.0)	4.52 d $J_{2,1}$ (6.0)	5.3–6.2 m			
4(C_6D_6)	2.2–2.9 m	4.82 s	3.6 d $J_{1,2}$ (6.0)	4.95 d $J_{2,1}$ (6.0)	5.85–6.7 m			
1($CDCl_3$)	2.4–2.7 m	4.35 s	2.47 d $J_{1,2}$ (6.5)	4.44 d $J_{2,1}$ (6.5)	5.30–6.0 m			
1(C_6D_6)	2.3–3.0 m	4.80 s	3.33 d $J_{1,2}$ (6.5)	4.80 q $J_{2,1}$ (6.5) $J_{2,4}$ (1.3)	6.26 bt $J_{4,5}$ (2.0) $J_{4,2}$ (1.3)	6.88 bs 5.0†	6.06 AB (q of d) $J_{6,6'}$ (13.0) $J_{6,5}$ (2.0)	6.78 $J_{6',5}$ (2.0)

[a]Measured with a Varian A-60D spectrometer; chemical shifts on τ scale; s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet † = width at half height, b = broad. Values in parentheses are splittings in Hz; these will be very close to *J* values, since Δ/J is large enough for first-order analysis to apply[16]. [b]These chemical shifts supersede those reported earlier[1].

A significant difference in the n.m.r. spectra of enones **1** and **4** is the long-range splitting of 1.3 Hz observed in the H-2 quartet of compound **1**, compared to the smaller splitting (less than 0.2 Hz) in the H-2 doublet of compound **4**. This splitting

is due to long-range coupling between H-2 and H-4. The difference in magnitude of the couplings must arise from the change in configuration at C-4 in these compounds. Anet [8] has observed a difference, similar to that described above, for $J_{1,3}$ in the α **6** and β anomers **7** of methyl 3,4-dideoxy-6-*O*-methyl-D-*glycero*-hex-3-enopyranosidulose; the values reported [8] were 0.7 and 0.2 Hz, respectively. Thus, a quasi equatorial* proton shows strong (~1.0 Hz) ^4J coupling through the carbonyl group to the vinylic proton, as illustrated by compounds **1** and **6**, whereas the quasi axial* proton couples only weakly, as shown by compounds **4** and **7**. These changes in coupling constant with changes in orientation of protons are in agreement with observations in cyclohexanones [9].

There is a striking difference between the magnitude of the vicinal vinyl couplings reported [8] for compounds **6** and **7** and the enones **1** and **4**. Compounds **6** and **7** have $J_{3,4}$ 10.7 Hz. This is a reasonable value for a *cis* vicinal coupling constant. For compounds **1** and **4**, however, the values for $J_{1,2}$ are only 6.5 and 6.0 Hz, respectively. Thus, the splitting is diminished by *ca.* 4 Hz when the double bond is between C-1 and C-2. We find that this difference is not restricted to the enones discussed above. The reported [4b,10] vicinal vinylic couplings ($J_{2,3}$) in several isomeric-hex-2-enopyranosides are 10 Hz, whereas, for several derivatives of 1,2-dideoxyhex-1-enopyranoses, the values for $J_{1,2}$ are all very close to 6 Hz, and for tri-*O*-acetyl-D-glucal [11] $J_{1,2}$ is 6.4 Hz, again a difference of *ca.* 4 Hz.

The electronegativity of the ring oxygen atom is probably responsible for the diminished coupling. This conclusion is supported by the observation [12] that coupling of the *cis*-vicinal protons in substituted ethylenes ($CH_2=CHX$) is 10.3 Hz when X is an alkyl group, but decreases to 6.7 Hz when X is an alkoxyl group.

The suitability of other oxidants for the conversion of compound **5** into **4** was examined. Manganese dioxide, a reagent recommended [13] for oxidation of allylic alcohols, had little effect, and ruthenium dioxide attacked the double bond [14]. Methyl sulphoxide and the sulphur trioxide–pyridine complex, recently introduced by Parikh and Doering [15], did produce some enone, but the yield of **4** could not be increased above 30%.

EXPERIMENTAL

4,6-O-Benzylidene-1,2-dideoxy-D-ribo-*hex-1-enopyranose* (**5**). — This compound was prepared in two steps [4b] from methyl 4,6-*O*-benzylidene-2,3-anhydro-α-D-allopyranoside.

4,6-O-benzylidene-1,2-dideoxy-D-erythro-*hex-1-enopyran-3-ulose* (**4**). — A solution of compound **5** (5 g) in pyridine (10 ml) was added to the oxidant prepared from chromium trioxide (15 g) and pyridine (500 ml) at −5°. The mixture was

*Dreiding models of compounds **1** and **4** indicated these orientations for H-4. The conformation is more certain in the case of compound **4**, where the two six-membered rings are *trans* fused. Anet has proposed these orientations for H-1 in compounds **6** and **7**.

stirred for 18 h at room temperature, ether (700 ml) was added, and the mixture was filtered. The filtrate was evaporated to low bulk, methylene dichloride (100 ml) was added followed by ether (200 ml), and the mixture was filtered. The filtrate was again treated as described above. This afforded an off-white solid (3.8 g, 76%). Recrystallisation from isopropyl alcohol afforded compound **4**, identical to the material prepared photochemically[2]. It had m.p. 128–129°, $[\alpha]_D$ +189° (chloroform); ν_{max} 1700 and 1600 cm^{-1} (C=C–C=O); λ_{max} 262 nm (ε 8.4×10^3, ethanol); and the n.m.r. parameters are shown in Table I (Found: C, 67.5; H, 5.3. $C_{13}H_{12}O_4$ calc.: C, 67.2; H, 5.2%).

ACKNOWLEDGMENT

I thank Professor W. G. Overend for his encouragement.

REFERENCES

1 P. J. Beynon, P. M. Collins, P. T. Doganges, and W. G. Overend, *J. Chem. Soc. (C)*, (1966) 1131.
2 P. M. Collins and P. Gupta, *Chem. Commun.*, (1969) 90.
3 K. Heyns and H. Gottschalck, *Ber.*, 99 (1966) 3718.
4 (*a*) A. A. Feast, W. G. Overend, and N. A. Williams, *J. Chem. Soc.*, (1965) 7378; (*b*) R. U. Lemieux, E. Fraga, and K. A. Watanabe, *Can. J. Chem.*, 46 (1968) 59.
5 G. I. Poos, G. E. Arth, R. E. Beyler, and L. H. Sarett, *J. Amer. Chem. Soc.*, 75 (1953) 422.
6 J. S. Burton, W. G. Overend, and N. R. Williams, *J. Chem. Soc.*, (1965) 3433.
7 J. N. Shoolery and M. T. Rogers, *J. Amer. Chem. Soc.*, 80 (1958) 5121.
8 E. F. L. J. Anet, *Carbohyd. Res.*, 1 (1966) 348.
9 N. S. Bhacca and D. H. Williams, *Applications of NMR Spectroscopy in Organic Chemistry*, Holden-Day, San Francisco, 1964, p. 121.
10 M. Sharma and R. K. Brown, *Can. J. Chem.*, 44 (1966) 2825.
11 L. D. Hall and L. F. Johnson, *Tetrahedron*, 20 (1964) 883.
12 W. Brügel, Th. Angel, and F. Krückeberg, *Z. Elektrochem.*, 64 (1960) 1121.
13 R. M. Evans, *Quart. Rev.* (London), 13 (1959) 61.
14 L. W. Berkowitz and P. N. Rylander, *J. Amer. Chem. Soc.*, 80 (1958) 6682.
15 J. R. Parikh and W. von E. Doering, *J. Amer. Chem. Soc.*, 89 (1967) 5505.
16 D. E. McGreer and M. M. Mocek, *J. Chem. Educ.*, 40 (1963) 358.

Carbohydrate Research
Elsevier Publishing Company, Amsterdam
Printed in Belgium

DETERMINATION OF SULPHATE AND OF BARIUM IN CARBOHYDRATE SULPHATES BY FLAME PHOTOMETRY

P. F. LLOYD, B. EVANS, AND R. J. FIELDER
Department of Chemistry, University College of North Wales, Bangor, Caernarvonshire (Great Britain)
(Received February 4th, 1969)

ABSTRACT

Methods are described for the determination of barium and of sulphate in carbohydrates by using an EEL flame photometer. The intensity of the barium flame is unaffected by the presence of carbohydrates, and the barium content is determined by direct examination of a solution of the carbohydrate. Prior to sulphate assay, the carbohydrate ester is heated with nitric acid; hydrolysis with hydrochloric acid is unsatisfactory in some cases. The sulphate is then determined by precipitation as barium sulphate, followed by dissolution of the latter in ethylenediamine tetra-acetate solution and photometric assay. An alternative, more-direct method, in which the concentration of barium remaining after introduction of sulphate and centrifugation is measured, is less accurate. The methods have been successfully applied to a range of sugar and polysaccharide sulphates with 2–5 mg samples.

INTRODUCTION

A variety of methods[1] have been used to determine the ester sulphate content of carbohydrates. Applied to suitable hydrolysates, the gravimetric ($BaSO_4$) method is accurate but requires a skilful operator and a large sample. When sulphuric acid is liberated enzymically, it can often be determined satisfactorily by the colorimetric benzidine method[2] or by turbidimetric[3] or automatic titrimetric[4] methods; in our hands, the barium chloroanilate method[5] did not give reproducible results, possibly on account of its marked susceptibility to cationic interference. However, none of these methods used in enzyme studies was considered suitable for the analysis of carbohydrate sulphates themselves, and recourse was usually made to combustion analysis or to the 4′-chlorobiphenyl-4-ylamine method of Jones and Letham[6]. When, as a preliminary to the latter method, the initial hydrolysis was conducted in hydrochloric acid, low results were often obtained[7]; more accurate values were recorded when the sample was subjected to an oxygen-flask combustion or treatment with fuming nitric acid.

The need for a more convenient method for the routine determination of sulphates in carbohydrates led us to investigate procedures based upon the flame photometry of barium. The results are reported below. Recently, a flame photometric

method for (carbohydrate) sulphate assay based upon the calcium flame[8] was reported. Although this method can be used to analyse for very small quantities of sulphate (0–30 μg), supplementary assay and treatments are necessary before it can be applied to substances containing uronic acids and interfering cations.

RESULTS AND DISCUSSION

Barium determination. — Initially, it was established, with standard solutions, that there was a linear relationship between Ba^{2+} concentration and the scale reading on an EEL flame photometer over the range 0.05–0.6 mg of Ba^{2+}/ml. The addition to barium chloride solutions of a representative range of sugars (D-glucose, D-galactose, 2-amino-2-deoxy-D-glucose, and D-galacturonic acid) caused no alteration in the photometric responses, except at very high concentrations (36 mg/ml), well beyond the range normally encountered. This suggested that the barium content of barium salts of carbohydrate sulphates could be obtained directly by flame photometric examination of their solutions. That this was so was established experimentally; the values for barium content determined photometrically for a range of carbohydrate sulphates were in excellent agreement with those obtained gravimetrically and with the theoretical values (Table I).

TABLE I

FLAME PHOTOMETRIC DETERMINATION OF BARIUM IN BARIUM SALTS OF CARBOHYDRATE SULPHATES

Compound	*Barium (%) determined by*		*Calc. (%)*
	Flame photometric method	*Gravimetric method*	
D-Galactose 6-(barium sulphate)	20.9	21.3	20.9
D-Galactose 2,3-di-(barium sulphate)	28.5	28.5	28.8
Methyl α-D-galactoside 4-(barium sulphate)	20.0	20.4	20.1
Methyl α-D-galactoside 2,3-di-(barium sulphate)	27.6	27.5	27.9

Sulphate determination. — Our first approach to sulphate assay was a direct one. The unknown solution containing sulphate ions was treated with a standard solution of barium chloride, and, after removal of the precipitated barium sulphate, the decrease in barium flame of the supernatant was measured. A series of such determinations is shown graphically in Fig. 1; in this experiment, the precipitate, formed at 100° under neutral conditions, was removed by centrifugation. In subsequent runs, some variation in experimental procedure was made, including precipitation under weakly acid conditions and/or in the presence of added barium sulphate, precipitation at room temperature, and accelerated ageing of the precipitate by alternative heating and cooling, and also removal of the precipitate by filtration. The results were essentially similar.

The flame photometric readings shown in Fig. 1 were higher than expected. This

was due to the presence of sodium ions; the non-linear effect of added Na^+ (as NaCl) is shown in Fig. 2. The effect of potassium ions was even more marked, but the addition of various carbohydrates had no effect on the photometer reading.

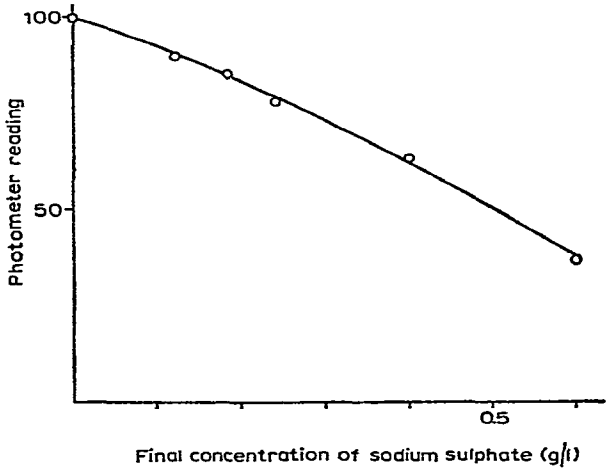


Fig. 1. Barium flame of a barium chloride solution (dihydrate 1.0 g/l) after partial removal of barium as barium sulphate.

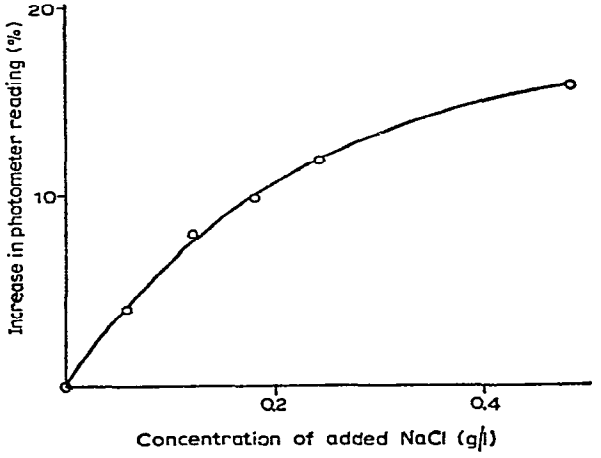


Fig. 2. Effect on barium flame of added sodium chloride [NaCl added to a solution of $BaCl_2 \cdot 2H_2O$ (0.5 g/l); initial photometer reading: 50.0].

This simple difference method of sulphate assay is applicable to solutions containing carbohydrate, and sulphate in the range 50–500 p.p.m. However, since replicate values may differ by as much as 15 p.p.m., the method can be regarded as semi-quantitative only. Moreover, application of this method to sulphate assay of (nitric acid-hydrolysed) chondroitin sulphate gave grossly anomalous results, and this is attributed to the presence of traces of K^+. The latter had been introduced by exchange with Na^+ during a deproteinisation procedure involving kaolin.

Quantitative sulphate determination. — An alternative method of greater accuracy which was not susceptible to interference by adventitious cations was required, and methods involving measurement of the barium content of the precipitated barium sulphate, rather than the supernatant, were considered. Callum and Thomas[9] have described the solubilisation, for flame photometry, of barium sulphate in an aqueous solution of ammonium ethylenediamine tetra-acetate, and this procedure was investigated.

Solutions containing known amounts of inorganic sulphate were treated with barium chloride, and the resultant precipitates were separated by centrifugation, washed, dissolved in ethylenediamine tetra-acetate (EDTA) solutions, and examined photometrically. Reproducible curves relating (linearly) sulphate concentration (over the range of 0.2–1.0 mg of sulphate/ml) and flame intensity were obtained. The values were not influenced by the presence of carbohydrate (up to 20 mg/ml) in the solution from which precipitation was effected.

Application to carbohydrate sulphates. — Published data[10] suggest that the susceptibility to acid hydrolysis of the ester linkage in carbohydrate sulphates differs widely. It was clearly desirable that a standard method of sulphate hydrolysis applicable to all carbohydrate sulphates should be evolved for use with the flame photometric assay. Earlier indications that liberation of sulphate ion is not maximal when hydrochloric acid is employed for hydrolysis were confirmed by applying the flame photometric method to suitable hydrolysate residues. The results are shown in Table II. Although some variations, in both acid strength and duration of hydrolysis at 100°, were made, the results were low by comparison with those derived from elemental combustion analysis.

TABLE II

FLAME PHOTOMETRIC DETERMINATION OF SULPHATE: HYDROLYSIS WITH HYDROCHLORIC ACID

Compound	*Normality of acid*	*Time of hydrolysis at 100° (h)*	*Sulphate content (%)*	
			Flame photometry	*Elemental analysis*
D-Glucose 6-(barium sulphate)[a]	2	16	22.0	23.7
Chondroitin 4-(sodium sulphate)	3	12	6.3	12.8
Chondroitin 4-(sodium sulphate)	6	12	11.2	12.8
Heparin (sodium salt)	6	24	24.9	30.0
Mucilage from *Laurencia pinnatifida*	6	16	11.3	15.1

[a]Containing some free D-glucose.

These data led us to employ the less specific but, as subsequently shown, completely reliable method of oxidative hydrolysis with fuming nitric acid. A standard procedure was developed, in which, following the hydrolysis for 24 h at 100°, the nitric acid was removed by evaporation, and the residue was shaken with water

and treated with barium chloride. The precipitated barium sulphate was dissolved in EDTA and assayed as described above.

To establish the total method, it was applied to a range of sugar and polysaccharide sulphates. In each case, a second value for sulphate content, based upon total sulphur analysis by an alternative method, was obtained. The results (Table III) show an excellent correlation and are in good agreement with the theoretical values in the case of the synthetic compounds. The method has been applied to both sodium and barium salts. Determinations were usually carried out in duplicate or triplicate; applied to compounds with a sulphate content of 10–40%, the method gave replicate values for sulphate content, which usually, to within 0.2%, were identical, and which rarely differed by more than 0.3%.

TABLE III

DETERMINATION OF SULPHATE IN CARBOHYDRATE SULPHATES BY THE $BaSO_4$–EDTA–FLAME PHOTOMETRIC METHOD

Compound	*Sulphate (%) as determined by*		*Calc. (%)*
	Flame photometry	*Alternative method*	
D-Galactose 4-(barium sulphate)	29.0	29.4[a]	29.1
D-Galactose 2,3-di-(barium sulphate)	40.0	40.5[a]	40.2
Methyl α-D-galactoside 4-(barium sulphate)	28.4	28.1[a]	28.1
Methyl α-D-galactoside 2,3-di-(barium sulphate)	38.9	39.2[a]	39.1
Chondroitin 4-(sodium sulphate)	15.0	15.4[b]	
Heparin (sodium salt)	39.0	38.0[b]	
Heparin (commercial sample)	29.9	30.0[b]	
Mucilage from *Laurencia pinnatifida*	15.7	15.1[b]	
Porphyran from *Porphyra umbilicalis*	11.7	11.7[b]	

[a]Digestion with fuming nitric acid, followed by the method of Jones and Letham[6]. [b]Oxygen-flask combustion, followed by titration with barium perchlorate using sulphonazo III indicator in acetone[12].

The method was also applied to mucopolysaccharides containing native protein. With bovine albumin as standard, an estimate of the protein content was made by a quantitative biuret method[11]. In Table IV, the results are compared with those obtained by an alternative method of sulphur determination.

DISCUSSION

The EDTA method described has been adopted as a routine procedure in our laboratories. The apparatus and procedures are uncomplicated and accurate, and reproducible results are obtained immediately. The sample required, 2–5 mg in most cases, can be diminished if the final volume of EDTA solution is kept below 2 ml or if its concentration is diminished, but there may be some loss in accuracy. It seems certain that further scaling down could be achieved by the use of a more refined photometer.

TABLE IV

FLAME PHOTOMETRIC DETERMINATION OF SULPHATE: EFFECT OF PROTEIN

	Protein content (%)	*Sulphate content* (%)	
		Flame photometry	*Combustion*[12]
Chondroitin sulphate from trachea	15.2	13.7	12.8
	2.1	14.5	14.0
	0.5	15.0	15.4
Chondroitin sulphate from nasal septum	13.6	13.9	13.0

Some of the procedures described merit further comment. Digestion of the barium salt of a sugar sulphate with nitric acid is expected to furnish equimolar amounts of barium sulphate and sulphuric acid. It was our intention, originally, to add sodium chloride to the digest to convert free acid into sulphate, in order to minimize losses by vaporisation during evaporation of the nitric acid. In practice, such an addition has proved unnecessary, even though temperatures as high as 360° (sand-bath) have been applied; sulphuric acid (98.3%) has b.p. 338°. This may be due to chemical or physical interaction of barium sulphate and sulphuric acid to form, substantially, a homogeneous matrix, with diminution of the vapour pressure. This cannot be ascribed to the formation of $BaSO_4 . H_2SO_4$, as this acid salt decomposes[13] at 160°, but may be simply dissolution of the salt by the parent acid. Barium sulphate dissolves in sulphuric acid, even at 25° (15.9 g in 100 g of solution[14]), and is readily soluble[15] at 100°. Presumably, water is removed from the system, azeotropically, during the evaporation of the nitric acid.

When, after removal of nitric acid, the residuum is cooled, some barium sulphate will remain in solution, probably as an equilibrium mixture of salts and complex acids[13]; the remainder may separate from solution as acid salts[13,16] together with barium sulphate. Some species will decompose when water is added. The results show that, whatever the form and number of phases present, transfer of sulphate (in salt or free acid form) from the reaction vessel is effected, quantitatively, by a brief treatment with water. Barium sulphate at this stage may be colloidal or in very fine suspension.

Nitric acid, but not hydrochloric acid, has proved reliable for oxidative hydrolysis of samples prior to sulphate assay. The lack of specificity of this reagent must be borne in mind when, in addition to *O*- and *N*- sulphate groups, other sulphur-containing residues are present in the molecule. Though it has not yet been established by us, it seems that, in most cases, the method will provide a suitable means for total sulphur assay. It is possible that, in the case of some sulphate esters, *e.g.*, those of simple sugars, prolonged hydrolysis with very dilute hydrochloric acid, or ion-exchange resins, will lead to complete liberation of ester groups as SO_4^{2-}. This is being investigated. The use, as hydrolysing agent, of hydrochloric acid of higher

strength often leads to charring, and the unexpectedly low SO_4^{2-} content of such hydrolysates may be due to reduction rather than to incomplete scission of the ester linkage.

The semi-quantitative method described is more direct and has applications in routine monitoring and sulphatase studies, but, in its present form, lacks the accuracy of the alternative procedure. An obvious drawback is the interference observed with sodium and potassium, and probably other cations, consequent on the simple light-filtering device employed in the photometer. Preliminary studies on a Zeiss spectrophotometer (PMQ11) fitted with a flame attachment (FA1), which utilises a quartz prism monochromator, suggest that the method has greater potential when allied to a more refined photometer.

EXPERIMENTAL

Flame photometer. — An EEL flame photometer (Evans Electroselenium Ltd.) fitted with a Wratten No. 77 filter and an EEL air compressor was used in this work.

Determination of barium in barium salts of carbohydrate sulphates. — A sample containing 0.1–1.0 mg of Ba^{2+} was dissolved in water and made up to 2 ml in a volumetric flask. The flame photometer was set to give zero deflection with distilled water, and full-scale deflection with the reference solution ($BaCl_2 \cdot 2H_2O$) of highest concentration (usually containing 0.6 mg of Ba^{2+}/ml), and a calibration curve was constructed by using standard solutions of lower concentration. The unknown solution was then sprayed, and the deflection noted; the barium concentration was read from the calibration curve. Determinations made in duplicate or triplicate furnished identical results. The zero and full-scale deflections were checked between determinations by using water and the appropriate standard solution.

Determination of sulphate in carbohydrate sulphates. — (*a*) *Semi-quantitative method.* The solution (1 ml) of carbohydrate and SO_4^{2-} (0.1–0.8 mg) was heated in a water bath at 100°, and a solution of barium chloride dihydrate (0.2% w/v, 1 ml), also at 100°, was added. The solutions were mixed, maintained for 10 min at 100°, cooled, and, after a further 10 min, centrifuged at 3000 r.p.m. (10 min). The sulphate content in the supernatant (1.5 ml) was then determined by flame photometry. Prior to each determination, the zero and full-scale deflections were set against water and an appropriate barium chloride solution. A calibration curve was constructed by treatment of standard solutions of sodium sulphate with barium chloride.

(*b*) *EDTA solution method.* — Ethylenediamine tetra-acetic acid (H^+ form, 5.0 g) was dissolved in a mixture of water (100 ml) and ammonia (sp.gr. 0.880, 50 ml), and the volume was made up to 500 ml with water.

The carbohydrate sulphate (2–8 mg) was treated with fuming nitric acid (1.5 ml) in a sealed, hard-glass tube (7 × 0.5 in.) for 24 h at 100°. The cooled tube was opened, and the nitric acid was removed by being heated for *ca.* 3 h at 330–360° on a sand-bath. The residue was shaken with water (1 ml) and transferred to a 10-ml conical centrifuge tube, quantitative transfer being effected by further washings

(2 × 1 ml). Aqueous barium chloride (dihydrate 1% w/v, 5 ml) was added, with mixing, and then 1 drop of conc. hydrcohloric acid (A.R.). After storage for 5 min, the tube was centrifuged at 3000 r.p.m. (10 min), and the supernatant was rejected. The precipitate was broken up, and washed with water (5 ml), and the washings were removed by centrifugation. The precipitate was dissolved in aqueous ammonium ethylenediamine tetra-acetate, and the volume was made up to 2 ml.

Ammonium EDTA solution was used to set the zero of the flame photometer, and a calibration curve was constructed by using standard solutions of sodium sulphate, precipitating SO_4^{2-} as $BaSO_4$, and dissolving the latter in ammonium EDTA solution, as described above. The instrument was set to give full-scale deflection with the most concentrated solution (usually 1 mg of SO_4^{2-}/ml). While maintaining the instrument settings (checking between readings), the readings were recorded for the unknown solutions, and the sulphate contents were deduced from the calibration curve.

ACKNOWLEDGMENTS

The interest and encouragement of the late Professor Peat, F.R.S., is gratefully acknowledged. The authors thank Professor W. Charles Evans for facilities for flame photometry. The award of a studentship by the Science Research Council (to R.J.F.), and an Imperial Chemical Industries Fellowship (to B.E.), are gratefully acknowledged. The authors also thank Dr. J. R. Turvey and Dr. M. J. Harris for samples of monosaccharide disulphates and algal polysaccharides and, Evans Medical Ltd. for the heparin.

REFERENCES

1 J. P. Dixon, *Modern Methods in Organic Microanalysis*, Van Nostrand, London, 1968.
2 K. S. Dodgson and B. Spencer, *Biochem. J.*, 55 (1953) 436.
3 P. F. Lloyd, K. O. Lloyd, and Olive Owen, *Biochem. J.*, 85 (1962) 193.
4 P. F. Lloyd and C. H. Stuart, *Biochem. J.*, 99 (1966) 37p.
5 A. G. Lloyd, *Biochem. J.*, 72 (1959) 133.
6 A. S. Jones and D. S. Letham, *Analyst*, 81 (1956) 15.
7 D. A. Rees and J. R. Turvey, unpublished results.
8 S. A. Barker, J. F. Kennedy, P. J. Somers, and M. Stacey, *Carbohyd. Res.*, 7 (1968) 361.
9 D. C. Callum and D. B. Thomas, *Analyst*, 85 (1960) 688.
10 For a review see J. R. Turvey, *Advan. Carbohyd. Chem.*, 20 (1965) 200.
11 G. R. Kingsley, *J. Biol. Chem.*, 131 (1939) 197.
12 B. Budesinsky, *Anal. Chem.*, 37 (1965) 1159.
13 J. W. Mellor, *A Comprehensive Treatise on Inorganic and Theoretical Chemistry*, Vol. 3, Longmans Green, London, 1923, p. 784.
14 A. Seidell, *Solubilities of Inorganic and Metal Organic Compounds*, 3rd Edn., Van Nostrand, New York, 1940, p. 184.
15 J. F. C. Thorpe and M. A. Whiteley, *Thorpes Dictionary of Applied Chemistry*, 4th Edn., Longmans Green, London, 1937, p. 633.
16 J. Kendall and A. W. Davidson, *J. Amer. Chem. Soc.*, 43 (1921) 979.

Note

A thiophene derivative from 1,2,3,5-tetra-*O*-acetyl-4-thio-D-ribofuranose*

R. L. WHISTLER AND D. J. HOFFMAN
Department of Biochemistry, Purdue University, Lafayette, Indiana 47907 (U. S. A.)
(Received March 7th, 1969)

During the course of synthesis of a nucleoside by condensation of 2,3,5-tri-*O*-acetyl-4-thio-D-ribofuranosyl chloride with chloromercuri-6-(benzamido)purine[1], we have observed an interesting aromatization reaction leading to an optically inactive elimination product. The product has been identified as 4-acetoxy-2-(acetoxymethyl)-thiophene (**1**) by its i.r., n.m.r., and mass spectra. The i.r. spectrum lacks hydroxyl absorptions at 3300–3600 cm^{-1}, and shows the presence of two acetyl bands at 1745 cm^{-1} and 1765 cm^{-1} (the latter, characteristic of an aryl acetate) and has strong absorptions at 1550 cm^{-1} and 3100 cm^{-1}, characteristic of thiophene derivatives[2]. The n.m.r. spectrum of compound **1** is shown in Fig. 1. This spectrum integrates for 10 protons, and is composed of three singlet signals: two acetyl-methyl resonances at τ 7.89 and 8.04, and a methylene resonance at 5.00. Two aromatic doublets at τ 3.07 and 3.24 ($J_{3,5}$ 1.5 Hz, H-3 and H-5) are characteristic of 3,5-disubstituted thiophene derivatives[3]. Other disubstituted thiophene isomers have $J>3$. All of the principal, mass-spectral peaks expected of structure **1** are present in the mass

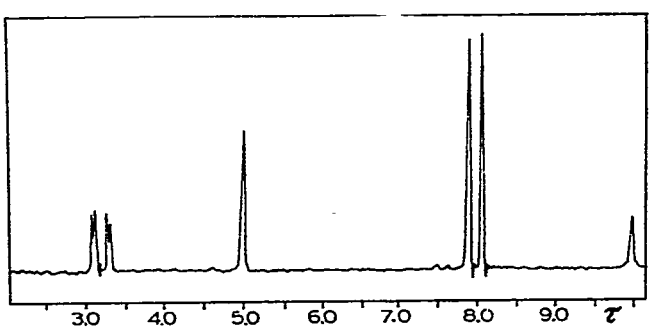
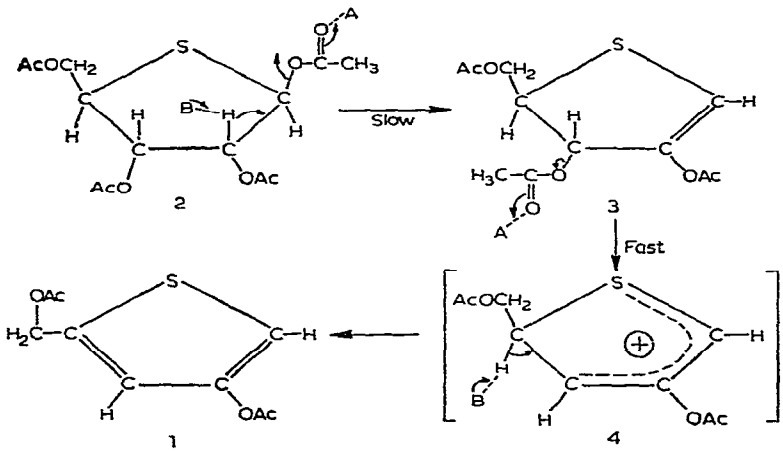


Fig. 1. The 60-MHz n.m.r. spectrum of 4-acetoxy-2-(acetoxymethyl)thiophene (**1**) in carbon tetrachloride.

*This work was supported by the National Institutes of Health, Department of Health, Education, and Welfare, Bethesda, Md. Grant No. 5 RO1 AM 11463 and by the Agricultural Research Service, U. S. Department of Agriculture, Grant No. 12-14-100-7662 (71), administered by the Northern Regional Research Laboratory, Peoria, Ill. Journal Paper No. 3637 of the Purdue Agricultural Experimental Station, Lafayette, Indiana.

spectrum (*m/e* 43, 84, 112, 130, 155, 172, and 214). One mode of cleavage is the loss of the acetylium ion to produce a strong *m/e* 43 peak, and another is the loss of ketene from the molecular ion to produce an intense M-42 peak, characteristic of enol acetates[4].

Alkaline hydrolysis of **1** produces an unstable compound that has a phenolic odor and that gives a red color with ferric chloride. Thiophene-3-ol behaves in a similar way with ferric chloride[5]. The formation of **1** from 1,2,3,5-tetra-*O*-acetyl-4-thio-D-ribofuranose (**2**) requires, for significant yields, the presence of both an acid and a base. Mercuric chloride, or di-(*p*-nitrophenyl) phosphate, as the acid, and 6-(benzamido)purine or piperidine as the base, in *N,N*-dimethylformamide at 115°, gives **1** in 40–50% yield. A yield of ~6% of **1** is obtained during the nucleoside synthesis in benzene at 80°. The formation of **1** takes place very slowly (as indicated by t.l.c.) at 115° in the presence of acid, and does not occur in the presence of base only. The requirement for both an acid and a base suggests that the mechanisms may occur through a C-1 carbonium ion (or, less probably, by a concerted elimination) to yield a glyc-1-enose (**3**), which undergoes further elimination to produce the thiophene derivative (**1**). Since the postulated intermediate **3** was not revealed by



t.l.c. examination during the reactions, it must react rapidly under the conditions selected.

The acid-catalyzed, alkyl–oxygen cleavage of the 3-acetate would be expected to be facile, due to the formation of a comparatively stable carbonium ion (**4**) as the intermediate; from **4** thus produced, the stable product **1** is obtained by elimination of the C-4 proton. No comparable reaction is observed for a related oxygen analog. Treatment of 1-*O*-acetyl-2,3-5-tri-*O*-benzoyl-D-ribofuranose[6] with piperidine and mercuric chloride in *N,N*-dimethylformamide for 1 h at 115° gives a dark mixture that, in t l.c., remains at the origin. However, it is possible that a furan derivative is formed, and is rapidly degraded to products that do not move under the chromatographic conditions employed.

EXPERIMENTAL

General methods. — T.l.c. was performed with 7:3 (v/v) hexane–ethyl acetate as the irrigant, and detection by spraying with 5% sulfuric acid in ethanol and charring. The i.r. spectra (Nujol) were recorded with a Perkin–Elmer Model 337 spectrophotometer. Optical rotations were determined with a Perkin–Elmer Model 141 automatic polarimeter. N.m.r. spectra were recorded with a Varian Associates A-60 spectrometer for solutions in carbon tetrachloride containing tetramethylsilane as the internal standard. Mass spectra were measured by Dr. F. Regnier with an LKB 9000A gas chromatograph–mass spectrometer.

4-Acetoxy-2-(acetoxymethyl)thiophene (**1**). — To a solution of 1,2,3,5-tetra-*O*-acetyl-4-thio-D-ribofuranose[1] (2 g) in dry *N,N*-dimethylformamide (100 ml) were added mercuric chloride (110 mg) and 0.6 ml of freshly distilled piperidine. After the mixture had been refluxed for 6 h, the *N,N*-dimethylformamide was removed under diminished pressure, leaving a dark liquid that was applied to a silica gel column and eluted with 7:3 (v/v) hexane–ethyl acetate. Compound **1** moved faster than the starting material, and gave a dark-blue color on charring on a t.l.c. plate. After the solvent had been removed under diminished pressure, the product was further purified by passing it through another silica gel column with the same eluant. Removal of the solvent left a light-yellow liquid; yield 610 mg (46%); $[\alpha]_D^{25}$ 0 (*c* 1.2, chloroform); R_F 0.60.

Anal. Calc. for $C_9H_{10}O_4S$: S, 15.70. Found: S, 15.77.

REFERENCES

1 E. J. Reist, D. E. Gueffroy, and L. Goodman, *J. Amer. Chem. Soc.*, 86 (1964) 5658.
2 H. A. Szymanski, *Infrared Band Handbook*, Plenum Press, New York, 1963.
3 R. A. Hoggman and S. Gronowitz, *Ark. Kemi*, 16 (1960) 563.
4 N. K. Kochetkov and O. S. Chizhov, *Advan. Carbohyd. Chem.*, 21 (1966) 53.
5 M. C. Ford and D. Mackay, *J. Chem. Soc.*, (1956) 4985.
6 R. K. Ness, H. W. Diehl, and H. G. Fletcher, Jr., *J. Amer. Chem. Soc.*, 76 (1964) 763.

Note

2-Deoxy sugars
Part XVI. Improved preparation of methyl 2-deoxy-α-D-*arabino*-hexofuranoside*

W. WERNER ZORBACH, CLARITA C. BHAT, AND K. VENKATRAMANA BHAT

Department of Bio-Organic Chemistry, Gulf South Research Institute, New Iberia, Louisiana 70560 (U. S. A.)

(Received April 1st, 1969)

Methyl 2-deoxy-α-D-*arabino*-hexofuranoside[1] (**2**) is the starting compound for the preparation of 5,6-*O*-carbonyl-2-deoxy-3-*O*-*p*-nitrobenzoyl-α-D-*arabino*-hexosyl bromide[1], which has utility in the preparation of pyrimidine nucleosides that contain 2-deoxy-β-D-*arabino*-hexofuranose as the carbohydrate residue[1b,c]. The furanoside may be readily prepared in *ca.* 30% yield by the direct methyl glycosidation of 2-deoxy-D-*arabino*-hexose, but its separation from the glycosidation mixture requires column chromatography on powdered cellulose, in which only about 1 g of the mixture can be handled at one time. The method is not suitable, therefore, for large-scale preparations of the furanoside (**2**).

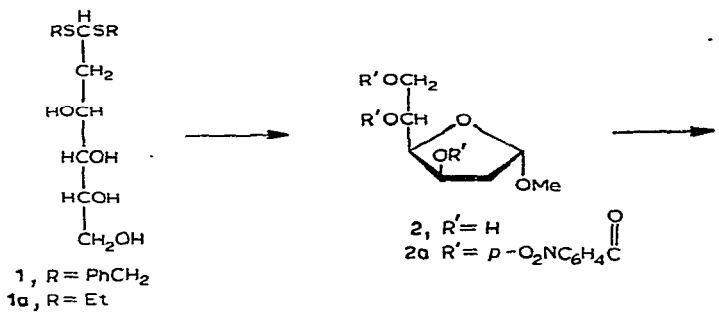
In order to find a way to facilitate the isolation of **2**, we restudied a procedure described by Overend *et al.*[2]. These workers converted 2-deoxy-D-*arabino*-hexose into its dibenzyl dithioacetal (**1**), and demercaptalated the latter in dry methanol[3] to afford (it was claimed) solely "methyl 2-deoxyglucofuranoside" (methyl 2-deoxy-D-*arabino*-hexofuranoside). We repeated their experiment, and our results showed, with the aid of paper-chromatographic analysis, that the product was a mixture, consisting of approximately 50% of methyl 2-deoxy-α-D-*arabino*-hexofuranoside (**2**), the remainder being isomeric pyranoside(s). A similar demercaptalation of the diethyl dithioacetal[4] **1a** gave comparable results.

Nevertheless, the yield of **2** from **1** or **1a** was a marked improvement over that obtained by the direct methyl glycosidation[1] of 2-deoxy-D-*arabino*-hexose, and, because of the greater proportion of **2** formed in the demercaptalation procedure, it was possible to recover about half of the compound by direct crystallization from a solution of the reaction mixture. Technically, in terms of time and effort, this procedure is a more efficient means for large-scale preparations of **2**, *if* total recovery is not a desideratum. The remainder of **2** may be recovered, however, by *p*-nitrobenzoylating the syrupy residue to give the tris-*p*-nitrobenzoate (**2a**), which separates in almost quantitative yield from an acetone solution of the reaction mixture.

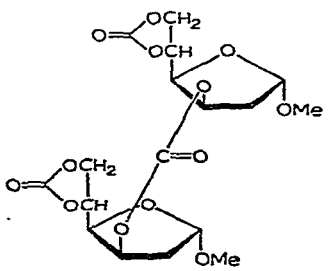
*This work was supported by Grant No. GSRI-NS257, from the Louisiana State Science Foundation.

If total recovery of **2** is the objective, it is more efficient to *p*-nitrobenzoylate the demercaptalation product, without prior separation by crystallization; an almost quantitative recovery (corresponding to the proportion of **2** estimated by chromatography) of **2a** is obtained. Deacylation of **2a** to afford crystalline **2** is a simple procedure[1b] to execute; therefore, the conversion consisting of 2-deoxy-D-*arabino*-hexose→dialkyl dithioacetal (**1** or **1a**) →tris-*p*-nitrobenzoate (**2a**)→methyl 2-deoxy-α-D-*arabino*-hexofuranoside (**2**) is a practical method for large-scale preparations of **2**.

By the latter method, we were able to secure an adequate supply of **2**, making possible a scaled-up preparation of methyl 5,6-*O*-carbonyl-2-deoxy-α-D-*arabino*-hexofuranoside, an intermediate in the synthesis of the previously reported furanosyl halide[1] employed in nucleoside syntheses. As more product was formed on this occasion during the carbonylation of **2**, another water-insoluble product was isolated which, from its elemental composition and i.r. spectrum, has been tentatively identified as 3,3′-*O*-carbonylbis(methyl 5,6-*O*-carbonyl-2-deoxy-α-D-*arabino*-hexofuranoside) (**3**).



1, R = $PhCH_2$
1a, R = Et



3

EXPERIMENTAL

Demercaptalation of the dithioacetals (**1** *or* **1a**). — To 54 mmoles of either **1** or **1a** in 200 ml of dry methanol were added 8 g of Drierite, 30 g of mercuric chloride, and 16 g of yellow mercuric oxide. The mixture was stirred for 3 h at room temperature, and filtered through a pad of Celite, and to the filtrate was added 25 ml of pyridine. The mixture was kept in a refrigerator overnight, the pyridine–mercuric chloride complex was filtered off, and the filtrate was stirred for 7 min with a small

amount of Rexyn 300 (H^+, OH^-) ion-exchange resin*. The mixture was filtered through a pad of decolorizing carbon, the filtrate was evaporated to dryness under diminished pressure, and the resulting clear syrup crystallized on storage in a vacuum desiccator (phosphorus pentaoxide). Paper chromatography of the syrupy product with water-saturated 9:1 butyl alcohol–toluene disclosed about 50% of methyl 2-deoxy-α-D-*arabino*-hexofuranoside (**2**) (R_F 0.43); the remainder consisted of the isomeric α-D-pyranoside (R_F 0.35), slightly contaminated with what was most probably its anomer.

Methyl 2-deoxy-α-D-arabino-hexofuranoside (**2**). — (*a*) Via *its tris-p-nitrobenzoate* (**2a**). To 8.3 g (47 mmoles) of the syrupy product (obtained from the preceding experiment) in 190 ml of dry pyridine at 0° was added 40 g (220 mmoles) of *p*-nitrobenzoyl chloride. The mixture was stirred for 1 h at 0° and for 1 h at room temperature, and was then kept in a refrigerator for 3 days. After being warmed to room temperature, the mixture was slowly added, with efficient stirring, to 300 ml of saturated, aqueous sodium hydrogen carbonate. Ice-cold water (1.5 l) was added, the mixture was stirred for 1 h, and the resulting precipitate was filtered off, washed with water, and dried in a vacuum desiccator (phosphorus pentaoxide). Three recrystallizations from acetone gave 14.5 g of the *p*-nitrobenzoylated furanoside **2a** (m.p. 142–144° and also 168–169°), corresponding to 4.1 g (*ca.* 50%) of the unsubstituted furanoside (**2**) present in the original syrup. Deacylation of **2a** was readily accomplished[1b], affording **2** in almost quantitative yield.

(*b*) *By direct crystallization.* The syrupy mixture of methyl glycosides (8 g) (obtained from the demercaptalation of **1a**) was dissolved in 80 ml of tetrahydrofuran, and ether was added to incipient turbidity, followed by the addition of one drop of tetrahydrofuran. The solution was nucleated, and the inside of the flask was vigorously scratched to prevent the separation of syrupy material. The solution was kept overnight at room temperature, and the crystals that formed were filtered off and recrystallized from tetrahydrofuran–ether to which a few drops of pentane were added; yield of **2**, 1.9 g (23% based on **1a**), m.p. 76–80°. The syrup that remained after separation of crystalline **2** was *p*-nitrobenzoylated as described in the preceding experiment. The resulting tris-*p*-nitrobenzoate (**2a**) was recrystallized three times from acetone, to afford 6.2 g of pure product, corresponding to 1.8 g (21%) of the unsubstituted furanoside (**2**). The total yield of **2** was, therefore, 44%.

3,3'-O-Carbonylbis(methyl 5,6-O-carbonyl-2-deoxy-α-D-arabino-hexofuranoside) (**3**). — A solution of 976 mg (5.5 mmoles) of **2** in 11 ml of dry pyridine and 8 ml of dry carbon tetrachloride was cooled to −10°, and to this solution was slowly added dropwise, with stirring, 4.2 ml of a 15% (w/w) solution of carbonyl chloride in dry toluene. The mixture was stirred for 1 h at −10° and for 1 h at room temperature, and was then poured, with stirring, into a mixture of 4 g of freshly prepared barium carbonate with 150 ml of crushed ice. After the ice had melted, the mixture was filtered through a bed of Celite. (The filtrate contained the desired methyl 5,6-*O*-

*Fisher Scientific Co.

carbonyl-2-deoxy-α-D-*arabino*-hexofuranoside.) The solid residue, which contained Celite, barium carbonate, and by-product (**3**), was repeatedly extracted with hot tetrahydrofuran. The extracts were combined and evaporated to dryness under diminished pressure, and the residue was recrystallized from acetone–ether–pentane to yield 300 mg of **3**, m.p. 163–164.5°, $[\alpha]_D^{23}$ +83.8° (*c* 1.35, acetone); ν_{max}^{KBr} 1815 (cyclic carbonate carbonyl) and 1755 cm^{-1} (acyclic carbonate carbonyl).

Anal. Calc. for $C_{17}H_{22}O_{13}$: C, 47.01; H, 5.10. Found: C, 46.75; H, 4.97.

REFERENCES

1 (a) K. V. BHAT AND W. W. ZORBACH, *Carbohyd. Res.*, 1 (1965) 93; (b) 6 (1968) 63; (c) K. V. BHAT, in W. W. ZORBACH AND R. S. TIPSON (Eds.), *Synthetic Procedures in Nucleic Acid Chemistry*, Vol. 1, Interscience Publishers, Inc., New York, N.Y., 1968, pp. 303–308.
2 W. G. OVEREND, M. STACEY, AND J. STANĚK, *J. Chem. Soc.*, (1949) 2841.
3 E. PACSU AND J. W. GREEN, *J. Amer. Chem. Soc.*, 58 (1936) 1823.
4 I. W. HUGHES, W. G. OVEREND, AND M. STACEY, *J. Chem. Soc.*, (1949) 2846.

Note

Gluconic acid end-groups in unbleached cotton cellulose

KENNETH LARSSON AND OLOF SAMUELSON
Department of Engineering Chemistry, Chalmers Tekniska Högskola, Göteborg (Sweden)
(Received February 12th, 1969)

Great difficulties are encountered in the determination of the structure of the end groups in unbleached cellulose. Firstly, the number of end groups is very small, and secondly, appreciable proportions of polysaccharides other than cellulose are present in the unbleached cotton[1]. These polysaccharides contain uronic acid units which interfere with the determination of acidic end-groups present in the cellulose. In most cotton samples, the number of carboxyl groups is larger than the number of end groups calculated from molecular weight determinations, even in samples subjected to purification by extraction with hot alkali. No conclusions about the presence of carboxyl end-groups in undegraded cellulose can therefore be drawn from experiments of this type.

A three-channel analyzer coupled with chromatography on anion-exchange resins makes it possible to detect small amounts of aldonic acids in the presence of large amounts of uronic acids[2]. In one channel, all oxidizable acids are determined by chromic acid oxidation. In a second channel, the eluate is treated with carbazole which gives a strong reaction with uronic, but no reaction with aldonic, acids. The third channel is employed for periodate oxidation with subsequent determination of formaldehyde. Aldonic acids give a strong response, whereas uronic acids give no, or a very slight, response.

A chromatographic study of the non-volatile, monoprotic acids isolated from a hydrolyzate of unbleached cotton revealed that a variety of acids were present. A chromatogram from a run in 0.08M sodium acetate is reproduced in Fig. 1. As expected, a large amount of levulinic acid (L) was recorded. Another artefact is an anhydrosaccharinic acid (AS) found to be present in all hydrolyzates from cellulose prepared by the applied method[3]. Galacturonic acid (Ga) was the preponderant uronic acid, which confirms that raw cotton contains appreciable proportions of pectic substances[1]. As expected from previous work[4,5], 2-*O*-(4-*O*-methyl-α-D-glucopyranosyluronic acid)-D-xylose (MGX) and 4-*O*-methylglucuronic acid (MG) were present in large amounts. The chromatograms confirm, moreover, the earlier observation that cellobiouronic (C) and glucuronic (Gu) acids were present[5]. Another biouronic acid, with properties similar to those of 2-*O*-(4-*O*-methyl-α-D-glucopyranosyluronic acid)-D-xylose, was indicated as well. No attempts were made to identify this acid.

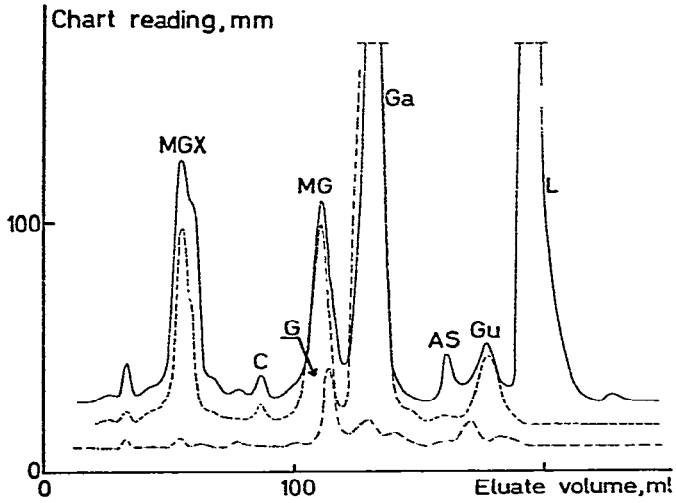


Fig. 1. Separation of monoprotic acids isolated from 1.5 g of solvent-extracted Peruvian cotton. Column: Dowex 1-x8; 23–24 μm; 1150 × 3.8 mm. Eluent: 0.08M sodium acetate with acetic acid added to obtain pH 5.9; ————, chromic acid method; – – – –, carbazole method; —•—·—·—, periodate–formaldehyde method.

A distinct peak with the position of gluconic acid (G) was recorded in the periodate channel. In 0.5M acetic acid, gluconic acid appears at a retention volume very close to that of 2-*O*-(4-*O*-methyl-α-D-glucopyranosyluronic acid)-D-xylose[5], and a run in this medium showed that a distinct peak was recorded with periodate at the relevant position. Gluconic acid was isolated on a preparative scale from 100 g of cotton by chromatography in 0.08M sodium acetate, which gave a mixture containing mainly 4-*O*-methylglucuronic acid. This mixture was rechromatographed in 0.5M acetic acid. A cleancut separation was obtained[5].

The purity of the isolated gluconic acid was checked on the automatic column. The distribution coefficients and the response indices[2] agreed with those recorded with an authentic sample. The 1,4-lactone was trimethylsilylated and studied by gas chromatography–mass spectrometry[6]. The results confirmed that the isolated acid consisted of gluconic acid.

To study whether the gluconic acid was present as end groups in the cellulose or in some non-cellulosic impurity, the raw cotton was purified by boiling with alkali[7]. The chromatograms showed that galacturonic acid had almost completely disappeared, but that appreciable amounts of the other uronic acids were still present. Peaks corresponding to gluconic acid were recorded in runs both in 0.08M sodium acetate and in 0.5M acetic acid. Chromatography of the sugar fraction obtained after hydrolysis revealed that appreciable amounts of xylose were present[8].

The alkali-boiled cellulose was further purified by subjecting it to a mild treatment with acid and a subsequent extraction with 6% sodium hydroxide at room temperature. The chromatograms indicated that gluconic acid was present, although in a smaller amount than in the non-hydrolyzed sample. Likewise, 4-*O*-

methylglucuronic acid and 2-*O*-(4-*O*-methyl-α-D-glucopyranosyluronic acid)-D-xylose were present in about the same amounts as gluconic acid. The other uronic acids were not present in detectable amounts. The sugar analysis showed that small amounts of xylose were still present[8], and that the total amount of aldoses other than glucose was about 0.05%. The gluconic acid was isolated on a preparative scale and identified.

To ascertain that gluconic acid was not an artefact formed by oxidation during the hydrolysis or during the procedure used for its isolation, blank experiments were made with glucose under identical conditions. No gluconic acid was detected in the acid fraction which contained large amounts of levulinic acid, minor amounts of the anhydrosaccharinic acid referred to above, and trace amounts of some unidentified acids. Blank tests with gluconic acid showed that the losses were less than 10% during the whole procedure. The results indicate that the applied method gives a reliable determination of gluconic acid groups in cellulose materials.

The amount of gluconic acid in the hydrolyzate from the raw cotton (purified by solvent extraction only) was 16 mg/100 g of cotton. The corresponding figure obtained with the alkali-boiled, hydrolyzed, and cold alkali-extracted cotton was 4 mg.

As shown previously, gluconic acid end-groups present in large amounts in bleached cellulose are split off fairly easily during a partial hydrolysis of the cellulose[4]. In addition, low-molecular fragments of the cellulose molecules are extracted together with the non-cellulosic material during the extraction with cold alkali. For these reasons, a lower figure was expected with the hydrolyzed and cold alkali-extracted sample. Unfortunately, the removal of non-cellulosic material from the cotton was unsatisfactory, unless the sample was subjected to an acid treatment before the final extraction with alkali. Since the hydrolyzed and alkali-extracted sample contained only traces of other polysaccharides, it can be concluded that the presence of gluconic acid in the hydrolyzate can be ascribed to gluconic acid end-groups in the cellulose chains.

The experimental results do not permit a decision about whether all, or only a fraction, of the gluconic acid groups in the raw cotton originate from the terminal units in the cellulose chains. It is likely that other end groups can be present as well. For these reasons, it is risky to apply determinations of gluconic acid groups in cotton to the determination of the degree of polymerization.

EXPERIMENTAL

The cotton samples were extracted with ethanol for 18 h and with dichloromethane for 18 h, and air dried at room temperature. One of the samples (Peruvian cotton) was not purified further. Another sample (American cotton) was purified by alkali cooking[7], then subjected to acid hydrolysis in boiling 0.05M sulfuric acid for 3 h, and finally extracted for 2 min at room temperature with 6% sodium hydroxide.

The cellulose samples were dissolved in hydrochloric acid and, after hydrolysis, the non-volatile, monoprotic acids were isolated as a group according to a method

described previously[9]. Chromatographic separations of the organic acids were carried out both on a preparative scale (100 g of cotton) and on automatic columns[2]. The chromic acid oxidation and the periodate oxidation (0.015M sodium metaperiodate buffered at pH 7.0; 25°; residence time, 2 min) were carried out as described previously[2]. The length of the flow cell in the carbazole channel was increased to obtain a higher response with uronic acids. The identifications were made as described in the papers referred to above[3-5].

ACKNOWLEDGMENTS

The authors express their thanks to the Swedish Council for Applied Research for financial support.

REFERENCES

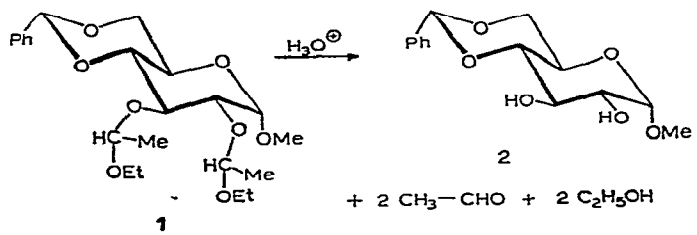
1 G. v. HORNUFF AND H. RICHTER, *Faserforsch. Textiltech.*, 15 (1964) 115.
2 B. CARLSSON, T. ISAKSSON, AND O. SAMUELSON, *Anal. Chim. Acta*, 43 (1968) 47.
3 S. PETTERSSON AND O. SAMUELSON, *Svensk Papperstid.*, 72 (1969) 261.
4 I. NORSTEDT AND O. SAMUELSON, *Svensk Papperstid.*, 68 (1965) 565.
5 S. PETTERSSON AND O. SAMUELSON, *Svensk Papperstid.*, 71 (1968) 429.
6 G. PETERSSON, O. SAMUELSON, K. ANJOU, AND E. v. SYDOW, *Acta Chem. Scand.*, 21 (1967) 1251.
7 C. DOREE, *Methods of Cellulose Chemistry*, Chapman and Hall, London, 1950.
8 L.-I. LARSSON AND O. SAMUELSON, *Svensk Papperstid.*, 71 (1968) 432.
9 O. SAMUELSON AND L. THEDE, *Tappi*, 52 (1969) 99.

Note

Reaction of carbohydrates with vinyl ethers; a differential hydrolysis*

M. L. WOLFROM**, S. S. BHATTACHARJEE, AND ROSA M. DE LEDERKREMER
Department of Chemistry, The Ohio State University, Columbus, Ohio 43210 (U. S. A.)
(Recived March 4th, 1969)

In continuation of work on the reaction of vinyl ethers with carbohydrates[1-4], we report herein two crystalline, fully substituted derivatives of glycosides, namely, methyl 4,6-*O*-benzylidene-2,3-bis-*O*-(1-ethoxyethyl)-α-D-glucopyranoside (**1**) and methyl 2,3,4-tris-*O*-(tetrahydro-2*H*-pyran-2-yl)-β-D-arabinopyranoside. In addition, we describe an analytically pure, but syrupy, pentakis-*O*-(1-methoxyethyl)-D-glucose, the ring size and anomeric form of which have not yet been established. An amorphous, tetrakis(tetrahydro-2*H*-pyran-2-yl) ether of D-glucose has been reported[5].



1

The products of reaction of vinyl ethers with carbohydrates are acetals, and are therefore subject to acid-catalyzed hydrolysis; this hydrolysis has been studied for the vinylation products formed from starch[4]. We now report that the two 1-ethoxyethyl groups of compound **1** may be removed almost quantitatively, without cleavage of the benzylidene group, by proper selection of the conditions of hydrolysis with acid. Such a differential hydrolysis may prove useful in the carbohydrate field.

EXPERIMENTAL

*Methyl 4,6-O-benzylidene-2,3-bis-*O*-(1-ethoxyethyl)-α-D-glucopyranoside* (**1**). — Methyl 4,6-*O*-benzylidene-α-D-glucopyranoside[6] (**2**, 18.0 g) was suspended in benzene (50 ml), and a 2.5% solution (6 ml, dried over anhydrous sodium sulfate) of *p*-toluenesulfonic acid in ether was added. The mixture was stirred magnetically, and ethyl vinyl ether (70 ml, 13 molar equiv.) was added slowly. Complete dissolution occurred

*The opinions expressed in this article are those of the authors, and are not necessarily those of the supporting agency.
**Decaesed, June 20th, 1969.

in ~10 min, whereupon the reaction mixture was poured into a cold, aqueous solution of sodium hydrogen carbonate. The nonaqueous layer was dried (potassium carbonate) and evaporated to dryness under diminished pressure. The product was obtained crystalline from hexane; yield 6.8 g (24.9%), m.p. 104°. Pure material was obtained on recrystallization from ether–hexane, m.p. 127–129°, $[\alpha]_D^{20}$ +17° (*c* 0.5, chloroform).

Anal. Calc. for $C_{22}H_{34}O_8$: C, 61.95; H, 8.04. Found: C, 61.83; H, 8.15.

Partial, acid hydrolysis of methyl 4,6-O-benzylidene-2,3-bis-O-(1-ethoxyethyl)-α-D-glucopyranoside (**1**). — Methyl 4,6-*O*-benzylidene-2,3-bis-*O*-(1-ethoxyethyl)-α-D-glucopyranoside (**1**, 100 mg, m.p. 127–129°) was dissolved in *p*-dioxane (22.5 ml), 2.5 ml of 0.1M hydrochloric acid was added, and the mixture was stirred for 2 min at 60°. An excess of silver carbonate was added, and the material obtained on removal of solvent was crystallized from *p*-dioxane–water; yield 59 mg (90%), m.p. 158–160° [undepressed on admixture with methyl 4,6-*O*-benzylidene-α-D-glucopyranoside (**2**)].

Pentakis-O-(1-methoxyethyl)-D-glucose. — To a suspension of anhydrous α-D-glucose (5 g) in 15 ml of *N,N*-dimethylformamide was added *p*-toluenesulfonic acid (170 mg), followed by methyl vinyl ether (12.9 g, 8 molar equiv.). The mixture was stirred magnetically for 30 min at 20° in a stoppered flask, and was then poured into an excess of a saturated, aqueous solution of sodium hydrogen carbonate; a thick oil separated. The mixture was extracted with ether, and the extract was washed with water, dried (sodium sulfate), and evaporated to dryness; yield of syrup, 12.4 g (95%), $[\alpha]_D^{22}$ +36° (*c* 1.3, ethanol), hydroxyl group absent from the i.r. spectrum. The substance liberated 98% of the theoretical amount of acetaldehyde on treatment[1] with acid.

Anal. Calc. for $C_{21}H_{42}O_{11}$: C, 53.61; H, 8.93. Found: C, 53.42; H, 8.80.

Methyl 2,3,4-tris-O-(tetrahydro-2H-pyran-2-yl)-β-D-arabinopyranoside. — To a suspension of 1.64 g of methyl β-D-arabinopyranoside[7] in a mixture of *p*-dioxane (5 ml) and 3,4-dihydro-2*H*-pyran (10 ml, 10 molar proportions) was added 80 mg of *p*-toluenesulfonic acid portionwise, with stirring; the reaction was exothermic. The clear solution resulting after 1 h was diluted with ether, and washed with 5 ml of a saturated, aqueous solution of sodium hydrogen carbonate. The nonaqueous layer was evaporated to dryness, and the residue was crystallized from hexane by standing overnight at 15°; yield 510 mg (12.4%), m.p. 128°. Pure material was obtained on recrystallization from hexane; m.p. 141–142°, $[\alpha]_D^{20}$ −122° (*c* 0.09, chloroform).

Anal. Calc. for $C_{21}H_{36}O_8$: C, 60.55; H, 8.71. Found: C, 60.77; H, 8.71.

Investigation of the mother liquor by t.l.c. showed the presence of another compound; this was not studied.

ACKNOWLEDGMENTS

This work was supported by the Agricultural Research Service, U. S. Department of Agriculture, Grant No. 12-14-100-7652(71) (The Ohio State University

Research Foundation Project 1856), administered by the Northern Regional Research Laboratory, Peoria, Illinois. Acknowledgment is also made of the assistance of G. G. Parekh.

REFERENCES

1 M. L. WOLFROM, S. S. BHATTACHARJEE, AND G. G. PAREKH, *Staerke*, 18 (1966) 131.
2 M. L. WOLFROM, A. BEATTIE, AND S. S. BHATTACHARJEE, *J. Org. Chem.*, 33 (1968) 1067.
3 M. L. WOLFROM, A. BEATTIE, S. S. BHATTACHARJEE, AND G. G. PAREKH, *J. Org. Chem.*, 33 (1968) 3990.
4 M. L. WOLFROM AND S. S. BHATTACHARJEE, *Staerke*, 21 (1969), in press.
5 S. A. BARKER, J. S. BRIMACOMBE, J. A. JARVIS, AND J. M. WILLIAMS, *J. Chem. Soc.*, (1962) 3158.
6 W. ALBERDA VAN EKENSTEIN AND J. J. BLANKSMA, *Rec. Trav. Chim.*, 25 (1906) 153; K. FREUDENBERG, H. TOEPFFER, AND C. C. ANDERSEN, *Ber.*, 61 (1928) 1750.
7 T. PURDIE AND R. E. ROSE, *J. Chem. Soc.*, 89 (1906) 1204; J. E. CADOTTE, F. SMITH, AND D. SPRIESTERBACH, *J. Amer. Chem. Soc.*, 74 (1952) 1501; C. S. HUDSON, *ibid.*, 47 (1925) 265.

Preliminary communication

Gas–liquid chromatography in the study of the Maillard browning reaction

M. L. WOLFROM* and N. KASHIMURA
Department of Chemistry, The Ohio State University, Columbus, Ohio 43210 (U.S.A.)
(Received June 19th, 1969)

In attempting to follow reactions of the Maillard browning type by g.l.c. techniqu the involatility of the amino compounds and their reaction products has presented difficulties. Development of new trimethylsilylating agents[1] has aroused our interest, and we find that a suitable trimethylsilylating agent for components of the Maillard browning reaction is 5:5:1 (v/v) *N,O*-bis(trimethylsilyl)acetamide[2], *N*-trimethylsilylimidazole[3], and chlorotrimethylsilane.

As a model system pertinent to studies on the nonenzymic browning of dried citrus juice, 1:1 molar proportions of D-glucose and 4-aminobutyric acid were dissolved in the minimal volume of water at room temperature, and the solution was imme-

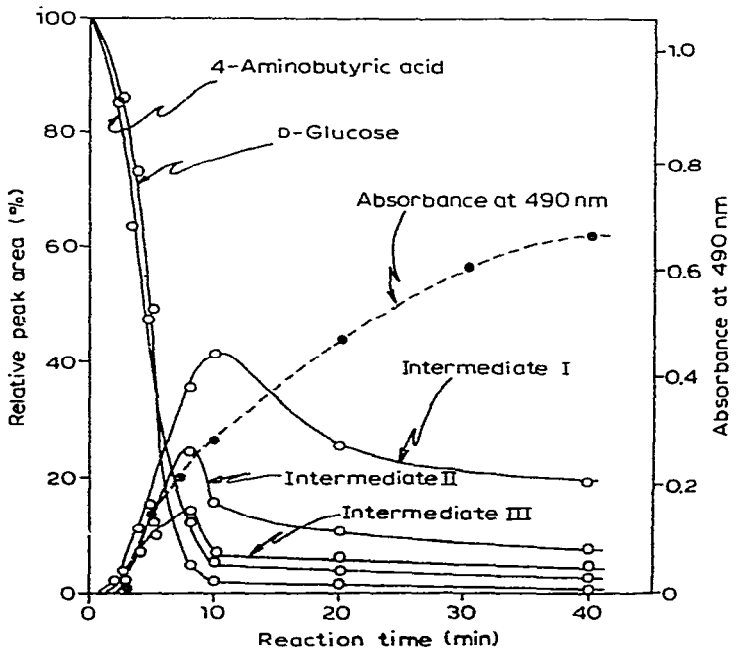


Fig.1. Rates of change of g.l.c. relative peak areas and of optical absorbance at 490 nm.

*Deceased June 20th, 1969.

diately freeze-dried. The solid product (containing ~ 2.5% of water) was then heated at 100°. Portions of the mixture were trimethylsilylated in pyridine, at selected times, and the products were analyzed by g.l.c., with D-glucitol as the internal standard, by means of a Beckman GC-5 instrument (equipped with a hydrogen flame detector) and a stainless-steel column (6 ft × 0.125 in) packed with 3% SE-30 on Chromosorb G (42–60 mesh). The results are shown in Figure 1. Three intermediates were detectable; they are arbitrarily designated I, II, and III.

For convenient isolation, the reaction was interrupted at the point of maximal formation of intermediate I, and a suitable amount was trimethylsilylated and subjected to chromatography on a column of silicic acid, under strictly anhydrous conditions, by successive use of benzene, 99:1 (v/v) benzene–methanol, and methanol as developers, with monitoring by g.l.c. Intermediate I as its trimethylsilyl derivative was so isolated in 99% purity. The chemical nature of this intermediate is under investigation.

ACKNOWLEDGMENT

This work was supported by the Agricultural Research Service, U.S. Department of Agriculture, Contract No. 12-14-100-8934 (72), administered by the Southern Regional Research Laboratory, New Orleans, Louisiana, and the Fruit and Vegetable Products Laboratory, Winter Haven, Florida.

REFERENCES

1 A. E. Pierce, *Silylation of Organic Compounds*, Pierce Chemical Co., Rockford, Illinois, 1968.
2 J. F. Klebe, H. Finkbeiner, and D. M. White, *J. Amer. Chem. Soc.*, 88 (1966) 3390.
3 M. G. Horning, A. M. Moss, and E. C. Horning, *Biochim. Biophys. Acta*, 148 (1967) 597.

Preliminary communication

Studies of ^{15}N-labeled amino sugars. Synthesis and spectroscopy of derivatives of 6-amino-6-deoxy-D-glucose-*6*-^{15}N

B. COXON

Institute for Materials Research, National Bureau of Standards, Washington, D.C. 20234 (U.S.A.)

(Received June 9th, 1969)

The proton and fluorine magnetic resonance parameters of carbohydrates and their derivatives have provided invaluable information for structural and conformational analysis. However, the potential of the magnetic resonance parameters of other nuclei for such analyses remains largely unexplored. As part of a program for the synthesis and spectral and conformational characterization of isotopically labeled carbohydrate reference materials of biomedical interest, a study of amino sugars labeled with ^{15}N (enrichment > 99%) has been commenced. This program has been initiated by a synthesis of derivatives of 6-amino-6-deoxy-D-glucose-*6*-^{15}N, which in its non-labeled form, occurs as a component of the antibiotic Kanamycin[1].

Reactions of either 1,2:3,5-di-*O*-isopropylidene-6-*O*-*p*-tolylsulfonyl-α-D-glucofuranose[2] (**1**) or 6-deoxy-6-iodo-1,2:3,5-di-*O*-isopropylidene-α-D-glucofuranose[3] (**2**) with potassium phtalimide (1.05–1.2 molar equivalents) in either *N*,*N*-dimethylformamide or hexamethylphosphoramide gave 6-deoxy-1,2:3,5-di-*O*-isopropylidene-6-phthalimido-α-D-glucofuranose (**3**) in yields of 80–83%. After two recrystallizations, **3** had a purity of 99.6 mole %, as determined by differential scanning calorimetry[4]. Treatment of **3** with hydrazine hydrate in ethanol gave 6-amino-6-deoxy-1,2:3,5-di-*O*-isopropylidene-α-D-glucofuranose (**4**) characterized as its known[5], crystalline, *p*-toluenesulfonate salt. The free amine (**4**) was also converted into its *N*-acetyl derivative (**5**).

Treatment of **1** with potassium phthalimide-^{15}N (1.05–1.2 molar equivalents) in hexamethylphosphoramide afforded 3-^{15}N in 84% yield. The mass spectrum of the amine (**4**) contained prominent peaks at *m/e* 244 (M-15; loss of Me), 229 (M-30; loss of $H_2\,{}^{14}NCH_2$), and 30 ($H_2\,{}^{14}N^{\oplus}{=}CH_2$), whereas a similar spectrum of 4-^{15}N showed intense peaks at *m/e* 245 (M-15; loss of Me), 229 (M-31; loss of $H_2\,{}^{15}NCH_2$), and 31 ($H_2\,{}^{15}N^{\oplus}{=}CH_2$). A pressure-sensitive M+1 ion was observed in some cases.

The magnetic resonance parameters of compounds **3**–**5** and their ^{15}N-labeled derivatives were studied initially by p.m.r. spectroscopy at 100 MHz. Spectra of the *N*-acetyl derivatives **5** and **5**-^{15}N in pyridine-d_5 are shown in Fig.1 (*a* and *b*, respectively). It may be seen that, even in the presence of a basic solvent, NH proton exchange is sufficiently slow to allow observation of the $J_{6\ N,H}$ and $J_{6'N,H}$ couplings of **5**, and additionally of $J_{15N,H}$ 91.3 Hz in **5**-^{15}N. The absence of quadrupolar broadening in the spectrum of the ^{15}N derivative facilitates measurement of the NH couplings. A selection of proton–^{15}N coupling constants is given in Table 1.

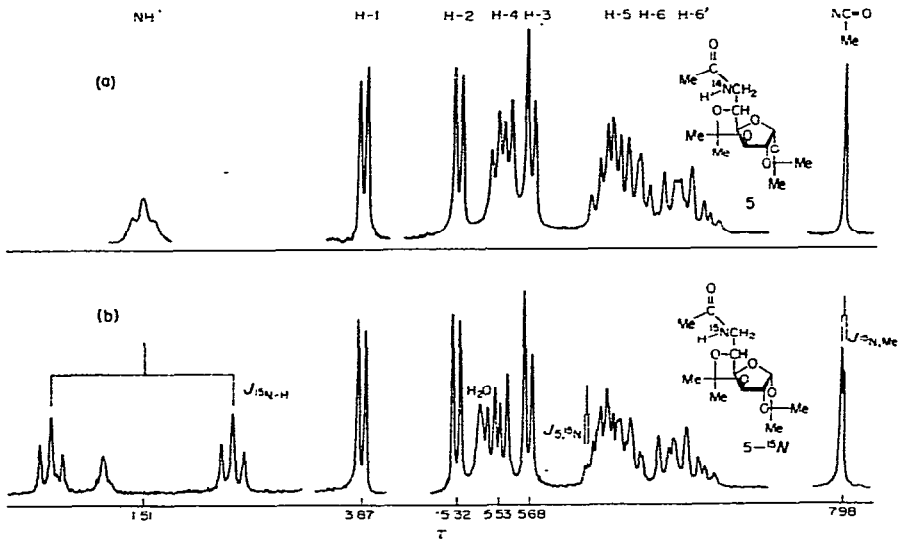


Fig. 1. P.m.r. spectra of solutions in pyridine-d_5 at 100 MHz; the isopropylidene methyl resonances are omitted, (*a*) 6-Acetamido-6-deoxy-1,2:3,5-di-*O*-isopropylidene-α-D-glucofuranose (**5**), (*b*) 6-acetamido-6-deoxy-1,2:3,5-di-*O*-isopropylidene-α-D-glucofuranose-*6*-^{15}N (**5**-^{15}N)

It has been suggested previously[8] that the magnitudes of coupling constants between directly bonded ^{1}H and ^{15}N nuclei are determined mainly by Fermi contact interaction[9], and hence, by analogy with ^{13}C—H couplings[10], are proportional to the amount of *s*-character of the nitrogen-bond orbital. Substitution of the value $J_{^{15}N,H}$ 91.3 Hz of **5**-^{15}N in the empirical equation[8]

$$S = 0.43J_{^{15}N,H} - 6$$

indicates that the *s*-character of the ^{15}N-to-hydrogen bond orbital is 33.3%. This suggests that the dipolar, canonical form of the acetamido group of **5**-^{15}N makes a predominant contribution (that is, the nitrogen atom is essentially fully sp^2-hybridized). From Table I, it may be seen that the ^{15}N—^{1}H couplings over three bonds are two to three times as large

TABLE I

COUPLING CONSTANTS (Hz, FIRST-ORDER) OF 6-AMINO-6-DEOXY-1,2:3,5-DI-*O*-ISOPROPYLIDENE-α-D-GLUCOFURANOSE-*6*-^{15}N DERIVATIVES AT 100 MHz

Derivative	*Solvent*	*^{15}N-coupling constants*[a,b]
4-^{15}N	$CDCl_3$	$^3J_{5\ ^{15}N}$ 1.5, $^2J_{6\ ^{15}N}$ ~0.5, $^2J_{6\ ^{15}N}$ ~0.5
5-^{15}N	C_5D_5N	$^3J_{5\ ^{15}N}$ 1.5, $^2J_{6\ ^{15}N}$ ~0.5, $^1J_{^{15}N\ H}$ 91.3, $^3J_{^{15}N\ Me}$
5-^{15}N-*d*[c]	C_5D_5N–D_2O (5:2 v/v)	$^3J_{5\ ^{15}N}$ 1.7, $^2J_{6\ ^{15}N}$ ~0.7, $^2J_{6\ ^{15}N}$ ~0.6, $^3J_{^{15}N\ Me}$ 1.3

[a] Measurements from proton spectra. [b] Superscripts before *J* denote number of bonds separating the coupled nuclei. [c] *N*-deuterated.

as those over two bonds. However, previous work on other systems suggests that the magnitudes of such couplings are often enhanced considerably by the introduction of sp^2- or *sp*-hybridized atoms[8,11]. Dependence on configuration[12,13], hydrogen bonding[14] and [15] pH is also possible.

REFERENCES

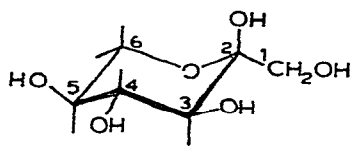
1 K. L. Rinehart, Jr., *The Neomycins and Related Antibiotics*, E. R. Squibb Lectures on Chemistry of Microbial Products, Wiley, New York (1964).
2 H. Ohle and L. von Vargha, *Ber.*, 61 (1928) 1203.
3 *cf.* K. Freudenberg, H. Toepffer, and C. C. Andersen, *Ber.*, 61 (1928) 1750.
4 C. Plato and A. R. Glasgow, Jr., *Anal. Chem.*, 41 (1969) 330.
5 H. Ohle and L. von Vargha, *Ber.*, 62 (1929) 2425.
6 B. Coxon, *Carbohyd. Res.*, 8 (1968) 125.
7 B. Coxon, to be published.
8 G. Binsch, J. B. Lambert, B. W. Roberts, and J. D. Roberts, *J. Amer. Chem. Soc.*, 86 (1964) 5564.
9 F. A. Bovey, *Nuclear Magnetic Resonance Spectroscopy*, Academic Press, New York (1969), p.88.
10 N. Muller and D. E. Pritchard, *J. Chem. Phys.*, 31 (1959) 768.
11 A. K. Bose and I. Kugajevsky, *Tetrahedron*, 23 (1967) 1489.
12 J. P. Kintzinger and J. M. Lehn, *Chem. Commun.*, (1967) 660.
13 T. Axenrod, P. S. Pregosin, and G. W. A. Milne, *Chem. Commun.*, (1968) 702; *Tetrahedron Lett.*, (1968) 5293.
14 E. D. Becker, H. T. Miles, and R. B. Bradley, *J. Amer. Chem. Soc.*, 87 (1965) 5575.
15 K. Tori, M. Ohtsuru, K. Aono, Y. Kawazoe, and M. Ohnishi, *J. Amer. Chem. Soc.*, 89 (1967) 2765.

Preliminary communication

The structure of α-D-tagatose and comparison with crystal structures of other ketohexoses

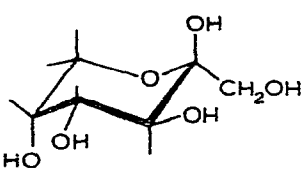
S. TAKAGI and R. D. ROSENSTEIN
Crystallography Laboratory, University of Pittsburgh, Pittsburgh, Pennsylvania 15213 (U.S.A.)
(Received May 31st, 1969)

As part of a program in crystallography of carbohydrates, a series of crystal structures of ketohexoses has been determined in this laboratory, starting with α-L-sorbopyranose[1], which was found to have the *1C* (L) conformation (**1**). This conformation

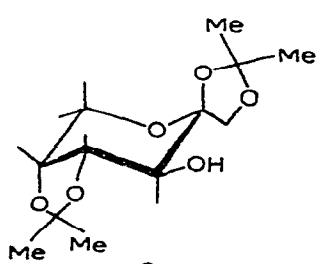


1

was subsequently found[2] in 1:1 methyl sulfoxide:acetone-d_6 solution by n.m.r. The crystal structure of β-D-fructopyranose[3] also showed the *1C* (D) conformation (**2**), the same as **1** except for the hydroxyl group on C-5, and as the *1C* (D) form **3** in the crystal structure of 1,2:4,5-di-*O*-isopropylidene-β-D-fructopyranose[4].



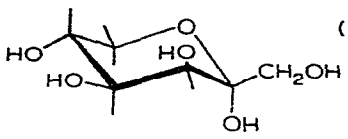
2



3

We have now determined the crystal structure of α-D-tagatose, obtained from Koch–Light Laboratories and recrystallized from aqueous solution by slow evaporation to dryness. The crystals are orthorhombic $P2_12_12_1$ with $a = 6.237$, $b = 6.529$, $c = 17.757$ Å, and $Z = 4$ molecules per unit cell. CuKα diffractometer intensities were collected with θ–2θ scans of 2° up to 130° in 2θ. Out of 755 independent reflections, not including systematic absences, 9 were below 2σ. The structure was solved by the non-centrosymmetric, direct method[5] and refined by anisotropic full-matrix least squares[6] to an $R = 0.07$.

for all atoms except hydrogens. The conformation found is *C1* (D) as shown in **4** and the bond lengths and angles are given in **5** and **6**. Hence, the D-tagatose sample studied, crystallized in this way, is pure α-D with the *C1* (D) pyranose conformation. High-resolution n.m.r. measurements[2] of tagatose in methyl sulfoxide solution showed about 80 percent of this anomer together with about 15 percent of β-D-tagatose having the *1C* (D) conformation.

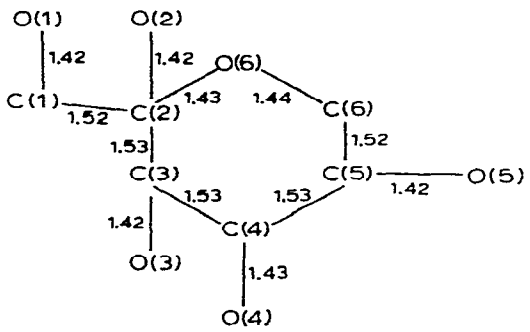


4

Torsion angles
(+ signifies turning A→B to the right)

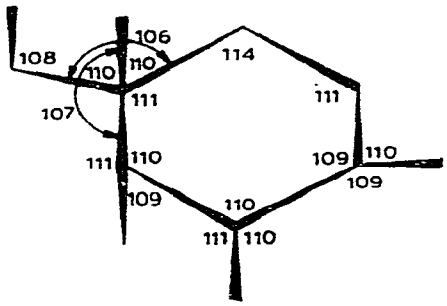
O-1—O-2	+51°
O-2—O-3	+174°
O-3—O-4	−56°
O-4—O-5	−61°

Thus all three ketohexoses have an equatorial primary alcoholic group and an axial hydroxyl group attached to C-2, and both D-fructose and D-tagatose have two axial hydroxyl groups, on C-2 and C-5, and on C-2 and C-3 respectively, on opposite sides of the mean plane of their pyranose rings. The primary hydroxyl group in L-sorbose was found to be in two orientations, with unequal occupancy, namely, in the Klyne and Prelog[7] and Cahn, Ingold and Prelog[8] notation, 63 percent Psc and 37 percent Msc with respect to the ring-oxygen atom. The primary hydroxyl groups of D-fructose and D-tagatose are exclusively Msc with respect to the ring-oxygen atom. With respect to the hydroxyl group on C-2, however, D-fructose is ap and D-tagatose is Psc while L-sorbose is 3:2 Msc:ap. The conformation of 1,2:4,5-di-*O*-isopropylidene-D-fructopyranose (**3**) is constrained in the Psc form.



5

Bond distances (Å)



6

Bond angles(degrees)

ACKNOWLEDGMENTS

This research was supported by the U.S. Public Health Service, National Institutes of Health, under Grant number GM-11293. We are grateful to Dr. Helen Berman for her kind help in the use of the direct method for the solution of the structure.

REFERENCES

1 S. H. Kim and R. D. Rosenstein, *Acta Cryst.*, 22 (1967) 648.
2 J. C. Jochimo, G. Taigel, A. Sediger, P. Lutz and H. E. Driesen, *Tetrahedron Lett.*, (1967) 4363.
3 R. D. Rosenstein, *Am. Crystallographic Assoc., Abstracts*, 1968, Summer Meeting.
4 S. Takagi and R. D. Rosenstein, *Intern. Union Crystallography, Abstracts*, (1969) XV-61.
5 S. R. Hall, Directed Phasing Methods, UWAC-17, Crystallographic Program for the PDP-6 Computer, adapted to the IBM 7090 by H. M. Berman.
6 R. Shiono (1966) Modification of the Oak Ridge Least Squares Program, ORNL-TM-305 (1962) of Busing, Martin and Levy.
7 W. Klyne and V. Prelog, *Experientia*, 16 (1960) 521.
8 R. S. Cahn, C. K. Ingold and V. Prelog, *Angew. Chem. Intern. Ed. Engl.*, 5 (4) (1966) 385.

Preliminary communication

9-(β-D-Apio-L-furanosyl)-2-chloroadenine*

F. PERINI**, F. A. CAREY***, and L. LONG, Jr.

Pioneering Research Laboratory, U.S. Army Natick Laboratories, Natick, Massachusetts 01760 (U.S.A.)

(Received June 10th, 1969; in revised form July 10th, 1969)

The preparation of C-3'-modified purine nucleosides has both chemical and pharmacological interest in view of the recent syntheses of such compounds. These systems often exhibit antibiotic and/or antitumor activity. Some of these compounds are cordycepin (3'-deoxyadenosine)[1,2], puromycin[3], 3'-*C*-alkylerythrofuranosyl nucleosides[4,5] and, most recently, a nogalose (a 3'-*C*-methyl-D-allopyranose) nucleoside[6]. Our program on the chemistry of apiose[7] includes the production of nucleosides from this compound, which is one of the most widespread among naturally occurring branched-chain sugars.

We now wish to report the first synthesis of an apiosyl nucleoside of unequivocal structure.

The configuration at C-3 and C-5 of the D-apio-L-furanose system was locked by cyclocarbonation. Treatment of 1,2-*O*-isopropylidene-α-D-apio-L-furanose[8] (**1**) with *N,N'*-carbonyldiimidazole in tetrahydrofuran gave (92%) 3,5-*O*-carbonyl-1,2-*O*-isopropylidene-α-D-apio-L-furanose† (**2**), m.p. 113–114°; $[\alpha]_D^{22}$ + 63.7°, (*c* 1.9, chloroform); λ_{max}^{Nujol} 5.52 μm (-O-CO-O-). Sulfuric acid-catalyzed acetolysis of **2** in acetic acid–acetic anhydride yielded (42–59%) crystalline 1,2-di-*O*-acetyl-3,5-*O*-carbonyl-α and β-D-apio-L-furanose (**3**), m.p. 141.5–145°; $[\alpha]_D^{23}$ + 38.6° (*c* 1.1, chloroform); λ_{max}^{Nujol} 5.50 μm (-O-CO-O-) and 5.71 μm (OAc). The n.m.r. spectrum†† (chloroform-*d*) included: 6.44 (0.3-proton doublet, $J_{1,2}$ 4.5 Hz, H-1, 30% α-anomer) and 6.13 (0.7-proton singlet, H-1, 70% β-anomer). A syrupy product, partially characterized as 1,1,2,4-tetra-*O*-acetyl-3,5-*O*-carbonylapiose (**4**). (~40–49%) λ_{max} 5.52 μm (-O-CO-O-) and 5.71 μm (OAc); n.m.r. (chloroform-*d*): 6.96 (1-proton doublet, $J_{1,2}$ 3 Hz, H-1), was also formed during acetolysis of **2**.

Fusion of 2,6-dichloropurine (DCP) and **3** in the presence of dichloroacetic acid ($Cl_2CHCOOH$)[9] at 160° was successful. This technique, as found in other systems[10,11], gave a mixture (58%, after silica gel column chromatography) of α,β-anomers, 2,6-dichloro-9-(2'-*O*-acetyl-3', 5'-*O*-carbonyl-α-and β-D-apio-L-furanosyl)purine (**5a** and **5b**), m.p.

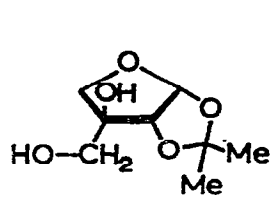
*Paper VI of a series of publications from this laboratory concerning the chemistry of apiose.

**PRL Postdoctoral Research Fellow, 1967–1969.

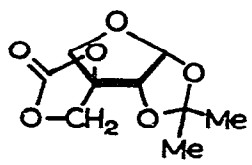
***Present address: Department of Chemistry, University of Virginia, Charlottesville, Virginia 22901, U.S.A.

†Acceptable elemental analyses were obtained for all crystalline compounds reported. Melting points are uncorrected.

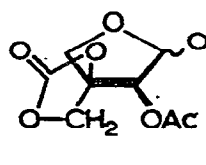
††100 MHz, with chemical shifts reported in δ units from a tetramethylsilane internal standard.



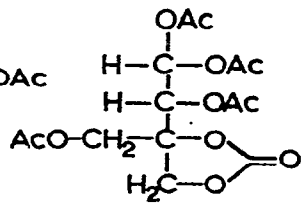
1



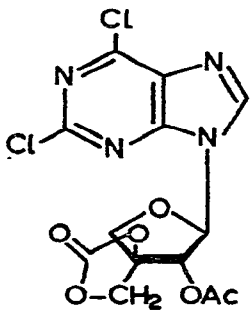
2



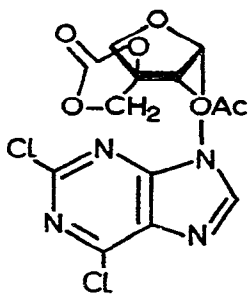
3



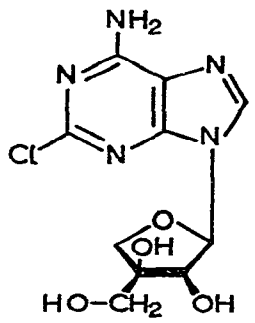
4



5a



5b



6

115–120°; n.m.r. (chloroform-*d*): 6.68 (0.32-proton doublet, $J_{1',2'}$ 5.5 Hz, H-1', 32% α-anomer)[12], and 6.26 (0.68-proton doublet, $J_{1',2'}$ 2.5 Hz, H-1', 68% β-anomer) [12]. The chromatographic mobilities of **5a** and **5b** were different but too similar to allow preparative-scale separation.

Condensation of DCP with **3** in nitromethane at reflux[13, 14], in the presence of dichloroacetic acid, gave only the pure branched-chain sugar nucleoside **5a** (51%, after silica gel column chromatography); m.p. 177–177.5°; $[\alpha]_D^{25}$ +55.0° (*c* 0.7, chloroform); λ_{max}^{Nujol} 5.49 (-O-CO-O-), 5.70 (OAc) and 6.25, 6.41 μm (C=N, C=C); λ_{max}^{EtOH} (pH 6) 255 shoulder (ε, 5660) and 274.5 nm (ε, 9650). Formation of **5a** in nitromethane, a solvent which promotes β-D anomer production in glycoside synthesis[15], was virtually stereospecific, as shown by the n.m.r. data (chloroform-*d*): 8.40 (1-proton singlet, H-8), 6.20 (1-proton doublet, $J_{1',2'}$ 2.5 Hz, H-1'), 5.67 (1-proton doublet, $J_{1',2'}$ 2.5 Hz, H-2'), 4.70, 4.18 (2-proton AB quartet, $J_{4',4'}$ 11 Hz, H-4'), 4.69, 4.40 (2-proton AB quartet, $J_{5',5'}$ 10 Hz, H-5'), and 2.22 (3-proton singlet, OAc). The β-D anomeric configuration was assigned to **5a** due to the observation of a narrow spacing (2.5 Hz) of the doublet[12] ascribable to H-1'. Predominant formation of a *trans* nucleoside would be expected from 2-*O*-acyl group participation[16].

Other Lewis acids, such as sulfamic acid[14] (76%) and boron trifluoride etherate (57%), also catalyzed the highly stereoselective formation of **5a**. Heating DCP with **3** in nitromethane for 1.5 day at 120° without catalyst, however, gave no product. It is known[17] that, in acidic media, DCP is hydrolyzed to xanthine. Under dry conditions,

during the synthesis of **5a** in the presence of dichloroacetic acid 27% of the DCP was converted into xanthine.

The convenient synthesis of **5a** opens the way to the production of various apiose nucleosides modified on the purine ring[18]. Ammonolysis of **5a** with saturated methanolic ammonia in a sealed tube[18,19], followed by picrate formation and liberation of the base with an aqueous suspension of AG 1–X4 (CO_3^{2-}) anion exchange resin gave the useful deblocked apioside (49%), 9-(β-D-apio-L-furanosyl)-2-chloroadenine (**6**), m.p. 137–138°; $[\alpha]_D^{26}$ - 16.0° (*c* 0.5, methanol); λ_{max}^{Nujol} 3.00, 3.20 (OH, NH), and 6.02, 6.27, 6.30 μm (NH, C=N, C=C); λ_{max}^{EtOH} (pH 6) 266 nm (ε, 15300); n.m.r. (deuterium oxide): 8.60 (1-proton singlet, H-8), 6.34 (1-proton doublet, $J_{1',2'}$ 2 Hz, H-1')[12], 4.90 (1-proton doublet, $J_{1',2'}$ 2 Hz, H-2'), 4.75 (2-proton singlet, H-5'), and 4.67, 4.63 (2-proton singlets, H-4').

Work on the synthesis of several derivatives of **5a** and **6** is in progress, and will be reported in full elsewhere.

ACKNOWLEDGMENT

The authors thank Mr. F. H. Bissett and Dr. R. C. Chalk for the measurement of n.m.r. spectra.

REFERENCES

1 E. Walton, F. W. Holly, G. E. Boxer, R. F. Nutt,and S. R. Jenkins, *J. Med. Chem.*, 8 (1965) 659.
2 J. Prokop and D. H. Murray, *J. Pharm. Sci.*, 54 (1965) 1468.
3 J. J. Fox, K. A. Watanabe,and A. Bloch, *Progr. Nucleic Acid Res. and Mol. Biol.*, 5 (1966) 253.
4 E. J. Reist, D. F. Calkins,and L. Goodman, *J. Amer. Chem. Soc.*, 90 (1968) 3852.
5 R. F. Nutt, M. J. Dickinson, F. W. Holly,and E. Walton, *J. Org. Chem.*, 33 (1968) 1789.
6 G. B. Howarth, W. A. Szarek,and J. K. N. Jones, *Can. J. Chem.*, 46 (1968) 3691.
7 D. H. Ball, F. A. Carey, I. L. Klundt,and L. Long, Jr., *Carbohyd. Res.*, 10 (1969) 121 and references cited therein.
8 F. A. Carey, D. H. Ball,and L. Long, Jr., *Carbohyd. Res.*, 3 (1966) 205.
9 Y. H. Pan, R. K. Robins,and L. B. Townsend, *J. Heterocycl. Chem.*, 4 (1967) 246.
10 W. W. Lee, A. P. Martinez, G. L. Tong,and L. Goodman, *Chem. Ind.*, (London), (1963) 2007.
11 J. A. M. Montgomery and K. Hewson, *Chem. Commun.*, (1969) 15.
12 J. A. Montgomery and K. Hewson, *J. Med. Chem.*, 11 (1968) 48, and references cited therein.
13 M. Honjo, S. Furukawa,and K. Kobayashi, Japanese Patent 42-11077 (June 20, 1967); *Chem. Abstr.*, 67 (1967) 188958r.
14 Y. Ishido, H. Tanaka, T. Yoshino, M. Sekiya, K. Iwabuchi,and T. Sato, *Tetrahedron Lett.*, (1967) 5245.
15 P. F. Lloyd and G. P. Roberts, *J. Chem. Soc.*, (1963) 2962.
16 T. Sato, T. Simadate, and Y. Ishido, *Nippon Kagaku Zasshi*, 81 (1960) 1440.
17 E. H. Rodd, ed., *Chemistry of Carbon Compounds, Heterocyclic Compounds*, Vol. IVc, Elsevier, Amsterdam, 1960, p. 1672.
18 H. J. Schaeffer and H. J. Thomas, *J. Amer. Chem. Soc.*, 80 (1958) 3738.
19 Y. Ishido, Y. Kikuchi,and T. Sato, *Nippon Kagaku Zasshi*, 86 (1965) 240; (also, T. Sato, *Synth. Procedures Nucleic Acid Chem.*, 1 (1968) 267)

Book review

Synthetic Procedures in Nucleic Acid Chemistry. Volume 1; Preparation of Purines, Pyrimidines, Nucleosides, and Nucleotides: edited by W. WERNER ZORBACH AND R. STUART TIPSON, Wiley, New York, 1968, v + 570 pp., 159*s* ($ 16.95).

The extensive literature in synthetic nucleic acid chemistry is dispersed among a wide range of publications, some of them not readily accessible to the reader who lacks a knowledge of Russian. There has been an urgent requirement for the unification of the more important, preparative procedures in book form. The editors of this volume, the first of a series, have done much to satisfy this long-felt need.

The volume is divided into five sections: Purines and Analogs (52 pages); Pyrimidines and Analogs (62 pages); Nucleosides [A. Purine Nucleosides and Analogs (168 pages); B. Pyrimidine Nucleosides and Analogs (160 pages)]; Nucleotides and Oligonucleotides (72 pages); Reagents, Intermediates, and Miscellaneous Compounds (20 pages). Within each section are numerous short chapters, written by experts in the chosen field, in which the preparation of individual compounds is used to illustrate a general procedure. The experimental details are more complete than is customary in the journals. Each chapter also includes a short introduction detailing the synthetic method and mentioning any biological activity, and a bibliography covering the literature to 1968. In addition to the usual Author and Subject Index, the latter listing the individual compounds described, there is a useful Index of General Reactions.

Books of this type are primarily sources of information on the preparation of known compounds and the application of these methods to related problems. However, they can also serve as a pointer to those areas in which further research is needed. From the numerous examples in the first two sections of the alkylation of purine and pyrimidine derivatives, it is clear, as one author states (D. T. Browne, p. 98), that the course of these reactions is often difficult to predict. A related topic, the prediction of the site of attack on the base by a sugar derivative, is only one aspect of the problem of nucleoside synthesis by such routes. The need for a general synthesis, which is totally stereoselective for one anomer, is apparent from a study of the many methods in the sections on nucleosides.

Other major topics of synthetic nucleic acid chemistry are adequately represented, with the possible exception of the synthesis of oligonucleotides, to which only two chapters, representing the work of the Czech school, are devoted. The final section could, perhaps, have been omitted, since only one of its eight chapters could not have been dispersed among the other sections, and that one (p. 527) could have been if the authors had chosen a pyrimidine, rather than a pyridine, glycoside as the end product. However, these are minor points. This volume is essential to all engaged in synthetic nucleic acid chemistry, and is highly recommended to the carbohydrate chemist, who will find the sections on nucleosides of particular interest.

Chester Beatty Research Institute, London M. JARMAN

Book review

Carbohydrate Chemistry — Volume 1; A Specialist Periodical Report: The Chemical Society, London, 1968, x +293 pp., 8½×5½", cloth, £3.10*s* (£2 — Fellows).

A recent innovation by the Chemical Society is the publication of Specialist Periodical Reports. Amongst the first to appear is Volume I of the series devoted to Carbohydrate Chemistry, and it is the intention that subsequent volumes will appear at yearly intervals.

The report on Carbohydrate Chemistry not only constitutes a very significant development in the specialist field but is of importance to general organic chemistry, since it collates a wealth of data and presents it in a form which is readily assimilable to workers in other areas of organic chemistry.

The Report has been compiled by Dr. R. D. Guthrie (University of Sussex), with the assistance of Dr. R. J. Ferrier (Birkbeck College, University of London) and Dr. M. J. How (University of Birmingham), and contains a review of the literature published during 1967; there are almost 1200 literature citations. The reporters are to be warmly congratulated on the extent and thoroughness of the coverage, and on the high standard of presentation that has assured the success of the publication.

The Report is divided into two parts devoted to (1) mono-, di-, and tri-saccharides, and their derivatives, and (2) macromolecules. In the first and major section of Part I, the literature data are presented according to 21 molecular types, *e.g.*, free sugars, glycosides, ethers and anhydro sugars, acetals, and esters. Subsequently, there appear sections devoted to physical methods. Although other styles of presentation might have been used, the policy of liberal cross-referencing will largely deprive the critics of their ammunition. Part I of the report is profusely illustrated with formulae, and although the emphasis is placed on new facts, attention is appropriately given to the mechanistic implications wherever there is an element of novelty.

The opening section of Part II is devoted to general methods and is followed, principally, by accounts of plant and bacterial polysaccharides, glycopeptides and proteins, and carbohydrate sulphates.

Two criticisms can be made of an otherwise, first-class, specialist report. Firstly, the delay in publication; the preface is dated April 1968, but the volume apparently did not appear until 1969. Rapid publication is mandatory, in order to ensure the maximum impact of the specialist reports, and one hopes that the publication time for Volume 2 will be much shortened. Secondly, whereas the text is singularly free from errors, the index is liberally sprinkled; the Reporters were not responsible for the index.

Chester Beatty Research Institute, London A. B. FOSTER

ANNOUNCEMENT

The Editors note with profound regret the death of Professor Melville L. Wolfrom on June 20th, 1969. Professor Wolfrom was a highly valued member of the Advisory Board from the inception of *Carbohydrate Research* in 1965, and he continued to give the journal the benefit of his advice when this Board was merged with the Editorial Board in 1969.

We feel that his passing marks the end of an epoch; hence, as a tribute to his accomplishments, we intend to publish an issue in his memory in the near future.

CORRIGENDUM

Carbohydrate Research, 10 (1969) page 474 line 3 and 4 should read:

IC conformation) is the mirror image of that of compound 8 (α-L configuration, *C1* conformation), as shown in Fig. 1. An analogous situation obtains (Fig. 3) for

Carbohydrate Research
Elsevier Publishing Company, Amsterdam
Printed in Belgium

SYNTHESIS OF NEW SUGAR DERIVATIVES HAVING POTENTIAL ANTITUMOUR ACTIVITY

PART XII*. 1,2:5,6-DIEPITHIO-L-IDITOL AND SOME DERIVATIVES THEREOF

J. KUSZMANN AND L. VARGHA
*Research Institute for Pharmaceutical Chemistry, Budapest (Hungary)***

(Received December 18th, 1968; in revised form, January 20th, 1969)

ABSTRACT

The synthesis of 1,2:5,6-diepithio-L-iditol and some of its derivatives, starting from 1,6-dibromo-1,6-dideoxy-D-mannitol, is described.

INTRODUCTION

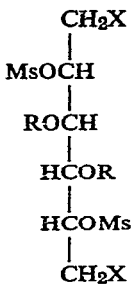
It is well known that some diepoxides possess cytostatic activity and inhibit the growth of experimental tumours[1]. In the carbohydrate field, 1,2:5,6-dianhydro-D-mannitol[2], in contrast to its 3,4-*O*-isopropylidene derivative[3], shows significant cytostatic activity. Episulphides, which are analogous to epoxides, have been described in the carbohydrate field[4], but diepithio compounds have not been reported. The possibility that diepithio derivatives might show biological and chemical behaviour similar to that of diepoxides prompted the synthesis of 1,2:5,6-diepithiohexitols.

RESULTS AND DISCUSSION

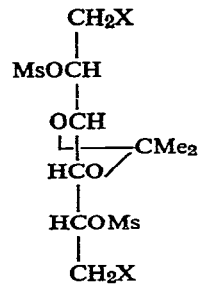
Treatment of 1,6-dibromo-1,6-dideoxy-2,5-di-*O*-methanesulphonyl-D-mannitol[5] (**1**) with potassium thiolbenzoate gave 1,6-di-*S*-benzoyl-2,5-di-*O*-methanesulphonyl-1,6-dithio-D-mannitol (**2**), the structure of which was proved by methanesulphonylation to give 1,6-di-*S*-benzoyl-2,3,4,5-tetra-*O*-methanesulphonyl-1,6-dithio-D-mannitol (**3**), which was also synthesised by another route described below. Treatment of compound **2** with methanolic sodium methoxide gave a mixture of products, from which the desired diepithiohexitol could not be separated. Similarly, the reaction of the diacetate **5** of compound **2** with sodium methoxide gave a mixture, which was shown by t.l.c. [development with 4-(*p*-nitrobenzyl)pyridine] to contain not less than 6 components, indicating the presence of different alkylating agents which could be formed partly by the participation of the hydroxyl groups at C-3 and C-4.

Because of these results, 1,6-dibromo-1,6-dideoxy-3,4-*O*-isopropylidene-2,5-di-*O*-methanesulphonyl-D-mannitol[5] (**6**) was chosen as a starting material in which the hydroxyl groups at positions 3 and 4 are protected by a base-stable group.

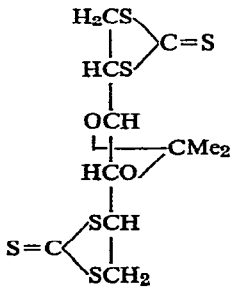
*Part XI: *Carbohyd. Res.*, 8 (1968) 157.
**Budapest IV, Szabadságharcosok u. 47.



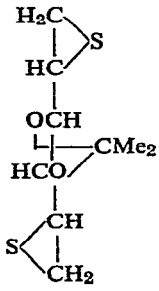
1 X = Br; R = H
2 X = SBz; R = H
3 X = SBz; R = Ms
4 X = Br; R = Ac
5 X = SBz; R = Ac
13 X = Br; R = Ms



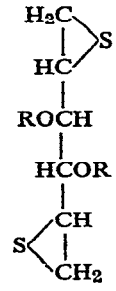
6 X = Br
7 X = SBz



9



8



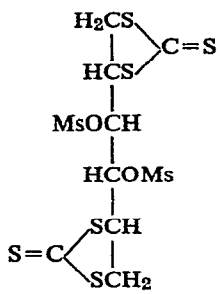
10 R = H
11 R = Ac
12 R = Ms

In this case, the isopropylidene group has to be removed subsequently, without splitting the labile epithio groups. Although the isopropylidene group could not be removed from 5,6-anhydro-1,2-*O*-isopropylidene-α-D-glucofuranose or 1,2:5,6-dianhydro-3,4-*O*-isopropylidene-D-mannitol without cleavage of the epoxide rings, the formation[6] of a moderate yield of 5,6-epithio-L-idose by mild, acidic hydrolysis of 5,6-epithio-1,2-*O*-isopropylidene-α-L-idofuranose indicated that epithio rings are somewhat more resistant towards acids than are epoxide rings. Confirmation of this supposition has now been obtained.

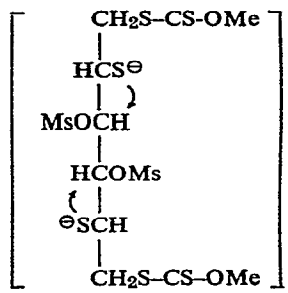
The bromine atoms could be easily displaced from compound **6** by the benzoylthio group to give 1,6-di-*S*-benzoyl-3,4-*O*-isopropylidene-2,5-di-*O*-methanesulphonyl-1,6-dithio-D-mannitol (**7**). With methanolic sodium methoxide, compound **7** gave crystalline compound **8** in good yield. Compound **8** was identified as 1,2:5,6-diepithio-3,4-*O*-isopropylidene-L-iditol on the basis of analytical data, chemical properties, and the likely mechanism of formation involving Walden inversion at C-2 and C-5. The presence of strained epithio rings, indicated by an infrared band at 3075 cm^{-1}, was proved by conversion of compound **8** into the known 3,4-*O*-isopropylidene-1,2,5,6-tetrathio-L-iditol 1,2:5,6-bis(trithiocarbonate) (**9**).

Compound **8** is very sensitive to bases and acids. In methanol solution, in the presence of sodium methoxide or triethylamine, it rapidly polymerised to an amorphous product having a high optical rotation (−314°) and a molecular weight corresponding to a tetramer. Since the i.r. spectrum of this polymeric product showed no bands for SH or OH groups, its units must be linked by thioether bonds.

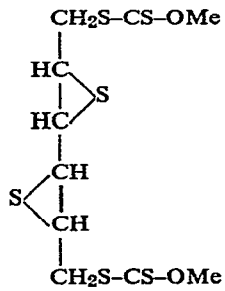
The isopropylidene group of compound **8** could be removed, without splitting the epithio rings, by a short treatment with N methanolic hydrogen chloride to give 1,2:5,6-diepithio-L-iditol (**10**) in 70% yield. The structure of compound **10** was verified by its easy polymerisation in aqueous solution, by the positive reaction with 4-(*p*-nitrobenzyl)pyridine, and by the i.r. spectrum which showed a band at 3060 cm^{-1} (strained rings). The n.m.r. data of the di-*O*-acetyl derivative **11** further supported the assigned structure. Methanesulphonylation of compound **10** gave 1,2:5,6-diepithio-3,4-di-*O*-methanesulphonyl-L-iditol (**12**), identical with the product obtained from 1,6-di-*S*-benzoyl-2,3,4,5-tetra-*O*-methanesulphonyl-1,6-dithio-D-mannitol (**3**) by treatment with sodium methoxide. Compound **3** was prepared from 1,6-dibromo-1,6-dideoxy-2,3,4,5-tetra-*O*-methanesulphonyl-D-mannitol (**13**) by displacing the bromine atoms with thiolbenzoate groups. The di-*O*-methanesulphonyl derivative **12** reacts with carbon disulphide in the presence of 2 equivalents of sodium methoxide to give, not the expected bis(trithiocarbonate) **14**, but sodium methanesulphonate (quantitative yield) and a syrupy compound, to which the structure 2,3:4,5-diepithio-1,6-bis[*S*-(methoxythiocarbonyl)]-1,6-dithio-L-mannitol (**15**) was assigned on the basis of analytical data, its alkylating properties, and the probable mechanism of formation, involving compound **16** as an intermediate.



14



16



15

The epithio derivatives **8**, **10**, and **12** showed no cytostatic activity on Yoshida sarcoma (solid and ascites forms), Walker 256 carcinosarcoma, and Ehrlich ascites sarcoma in doses of $LD_{50}/10$. The absence of *in vivo* activity may be due to the ease of polymerisation of the episulphides.

EXPERIMENTAL

Melting points are uncorrected. Thin-layer chromatography (t.l.c.) was carried out on Kieselgel G with chloroform–ethyl acetate, 3:1 (*A*), 3:2 (*B*), and 5:1 (*C*) as

solvent systems. Detection reagents used were 0.1M potassium permanganate–2N sulphuric acid (1:1), and 4-(*p*-nitrobenzyl)pyridine[8]. I.r. spectra were recorded with a U.R.10 instrument, and n.m.r. spectra with a JEOL J.N.M.-C-60 spectrometer. All evaporations were carried out in a rotary evaporator under diminished pressure. Light petroleum had b.p. 60–80°.

*1,6-Di-*S*-benzoyl-2,5-di-*O*-methanesulphonyl-1,6-dithio-*D*-mannitol* (**2**). — A solution of compound[5] **1** (5 g) and potassium thiolbenzoate (10 g) in acetone (100 ml) was heated on a steam bath for 15 min, cooled, and filtered, and the filtrate was evaporated to a syrup. The residue was dissolved in chloroform, and the solution was washed with 5% aqueous sodium hydrogen carbonate and water, dried (Na_2SO_4), and evaporated. The crystalline residue was triturated with ether and recrystallized from benzene (15 ml); yield, 3.8 g (61.3%); m.p. 106–107°; $[\alpha]_D^{20}$ −107.7° (*c* 1, chloroform); R_F 0.4 (solvent *B*) (Found: C, 45.22; H, 4.73; S, 22.39. $C_{22}H_{26}O_{10}S_4$ calc.: C, 45.66; H, 4.53; S, 22.16%).

Methanesulphonylation of compound **2**. — A solution of compound **2** (0.5 g) in dry pyridine (5 ml) was treated at 0° with methanesulphonyl chloride (0.5 ml). After 24 h at room temperature, the reaction mixture was poured onto ice, and the precipitate was filtered off, dried, and recrystallized from ethanol to yield compound **3** (0.4 g, 63%), m.p. 156–158° alone and in admixture with the compound described below.

*1,6-Di-*S*-benzoyl-2,3,4,5-tetra-*O*-methanesulphonyl-1,6-dithio-*D*-mannitol* (**3**). — A solution of compound **13** (31 g) and potassium thiolbenzoate (18 g) in acetone (500 ml) was heated on a steam bath for 30 min. The precipitate, which formed immediately, was filtered off after cooling, and the filtrate was evaporated to 100 ml, treated with water until turbidity, filtered with charcoal, and diluted with water. The precipitated syrup solidified on storage at 0°, and, after filtration and washing with water and ethanol, gave a crude product (35.5 g). Recrystallization from acetone–ethanol yielded compound **3** (26.5 g, 72%), m.p. 150–152°. This preparation was pure enough for the next step. Further purification was achieved by recrystallization from 150 volumes of ethanol; m.p. 156–158°, $[\alpha]_D^{20}$ +77.5° (*c* 1, chloroform), R_F 0.4 (solvent *A*) (Found: C, 38.96; H, 4.62; S, 25.98. $C_{24}H_{30}O_{14}S_6$ calc.: C, 39.23; H, 4.12; S, 26.18%).

*3,4-Di-*O*-acetyl-1,6-dibromo-1,6-dideoxy-2,5-di-*O*-methanesulphonyl-*D*-mannitol* (**4**). — To a suspension of the dimethanesulphonate **1** (4.65 g) in acetic anhydride (5 ml), one drop of conc. HBr was added. A gentle, exothermic reaction took place, and the solution became clear in a few minutes. Next day, it was poured onto ice, and the precipitate was filtered off, washed with water, dried, and recrystallized from methanol (12 ml) to yield compound **4** (5.2 g, 95%), m.p. 99–100°, $[\alpha]_D^{20}$ +13.7° (*c* 1, chloroform) (Found: C, 26.42; H, 3.85; Br, 28.82; S, 11.53. $C_{12}H_{20}Br_2O_{10}S_2$ calc.: C, 26.28; H, 3.68; Br, 29.16; S, 11.69%).

*3,4-Di-*O*-acetyl-1,6-di-*S*-benzoyl-2,5-di-*O*-methanesulphonyl-1,6-dithio-*D*-mannitol* (**5**). — A solution of compound **4** (5.5 g) and potassium thiolbenzoate (3.6 g) in acetone (50 ml) was treated as described for compound **2**. Evaporation of the

chloroform solution gave an amorphous solid (6 g), $[\alpha]_D^{20}$ +24.0° (*c* 1, chloroform), R_F 0.3 (solvent *C*) (Found: S, 18.61, $C_{26}H_{30}O_{12}S_4$ calc.: S, 19.35%).

*1,6-Di-*S*-benzoyl-3,4-*O*-isopropylidene-2,5-di-*O*-methanesulphonyl-1,6-dithio-*D*-mannitol* (7). — A solution of compound **6** (25.2 g) and potassium thiolbenzoate (17.6 g) in acetone (250 ml) was treated according to the previous procedure. The resulting syrupy **7** (31 g) was pure enough for the next step (Found: S, 20.0%). For further purification, it was eluted from a column of silica gel, first with carbon tetrachloride to remove a contaminant (R_F 0.9, solvent *A*), and then with carbon tetrachloride–ethyl acetate (8:2) to give compound **7** as a colourless syrup, $[\alpha]_D^{20}$ +31.5° (*c* 1, chloroform), R_F 0.45 (solvent A) (Found: S, 19.85. $C_{25}H_{30}O_{10}S_4$ calc.: S, 20.73%).

*1,2:5,6-Diepithio-3,4-*O*-isopropylidene-*L*-iditol* (**8**). — Crude compound **7** (from 25.2 g of compound **6**) was dissolved in dry chloroform (250 ml) and, during 5 min, N sodium methoxide (100 ml) was added, with stirring and cooling, to the solution at 0–10°. The stirring was continued without cooling for 5 min, and then the reaction mixture was washed twice with ice-cold water. The chloroform layer was dried (Na_2SO_4) and evaporated, and methyl benzoate was distilled off at 1 mmHg. The residue was dissolved in methanol (5 ml), and the solution was chilled to −10°. The crystals formed were filtered off and washed with cold methanol; yield, 4.4 g, m.p. 79–80°. The filtrate was evaporated, and the residue was distilled (b.p. 90–92°, 0.05 mm) to yield a further 3.3 g. Recrystallization of the combined crops from methanol (15 ml) afforded compound **8** (7.45 g, 68%), m.p. 80–81°, $[\alpha]_D^{20}$ +89.8° (*c* 1, chloroform), R_F 0.40 (solvent *C*) (Found: C, 49.07; H, 6.40; S, 28.94. $C_9H_{14}O_2S_2$ calc.: C, 49.51; H, 6.46; S, 29.37%). Compound **8** had ν_{max}^{KBr} 3075 (epithio CH and CH_2), 2990, 2980, 1390, 1380, 1180, 1170 (isopropylidene CH_3), 1080, and 870 cm^{-1} (*O*-isopropylidene). N.m.r. data ($CDCl_3$): δ 3.6 (2-proton multiplet, H-3 and H-4), 2.95 (2-proton multiplet, H-2 and H-5), 2.8 (4-proton multiplet, H-1 and H-6), and 1.35 (6-proton singlet, CMe_2).

Polymerization of compound **8**. — A solution of the diepithio compound **8** (1.1 g) in methanol (10 ml) containing N sodium methoxide (1 ml) or triethylamine (1 ml) was refluxed for 2 h. The precipitate was filtered off, after cooling, and washed with methanol, to yield a product (1.1 g), which gradually softened above 160°, $[\alpha]_D^{20}$ −280° (*c* 1, chloroform). The polymer was dissolved in chloroform (5 ml), and the solution was treated with light petroleum until turbidity and then filtered through charcoal into light petroleum (200 ml). The precipitate was filtered off and washed with light petroleum and methanol. The "softening" point increased to 180°; $[\alpha]_D^{20}$ −314° (*c* 1, chloroform). The i.r. spectrum (KBr) had no absorption between 3600–3000 (OH) and 2600–2500 cm^{-1} (SH) (Found: C, 48.97; H, 6.74; S, 28.26; mol. wt. (Rast in borneol), 875. $(C_9H_{14}O_2S_2)_n$ calc.: C, 49.51; H, 6.46; S, 29.37%; mol. wt. (tetramer), 883.

*3,4-*O*-Isopropylidene-1,2,5,6-tetrathio-*L*-iditol 1,2:5,6-bis(trithiocarbonate)* (**9**). — Potassium hydroxide (2.3 g) was dissolved in methanol (10 ml), and carbon disulphide (2.9 ml) and, after cooling, compound **8** (1.5 g) were added. The reaction mixture

was kept for 48 h at room temperature, and the crystalline precipitate was filtered off, and washed with methanol and water to yield a product (0.5 g), m.p. 142–146°. Recrystallization from benzene (6 ml) and light petroleum (3 ml) afforded compound **9** as yellow crystals (0.3 g, 11.6%, m.p. 147–148°), $[\alpha]_D^{20}$ +200° (*c* 1, chloroform); the m.p. was not depressed on admixture with the authentic trithiocarbonate[4].

*1,2:5,6-Diepithio-*L*-iditol* (**10**). — A solution of compound **8** (6.6 g) in N methanolic hydrogen chloride (400 ml) was kept for 10 min at room temperature, and then poured with vigorous stirring and cooling onto an excess (40 g) of solid sodium hydrogen carbonate. The filtered solution was evaporated below 30°, and the residue was treated three times with hot ethyl acetate. The united extracts were evaporated, and the residue was recrystallized from dry chloroform (100 ml) to yield colorless needles of compound **10** (3.2 g). Evaporation of the mother liquor gave a second crop (0.5 g); total yield, 69.3%. On heating, the compound suffered a rearrangement at 118–119° to give material (probably polymeric) having no definite m.p.; a slow decomposition started about 280°. The product had $[\alpha]_D^{20}$ +86.4° (*c* 1, chloroform), R_F 0.15 (solvent *A*), ν_{max}^{KBr} 3500–3300 (OH) and 3060 cm^{-1} (epithio CH) (Found: C, 40.51; H, 5.84; S, 35.67. $C_6H_{10}O_2S_2$ calc.: C, 40.42; H, 5.65; S, 35.97%).

On boiling a solution of compound **10** (0.1 g) in water (5 ml), an insoluble, presumably polymeric material was formed. After 20 min, starting material was not detectable (t.l.c.), and, after cooling, the polymeric material (0.1 g) was filtered off. It had no i.r. absorption in the region of 3000–3100 cm^{-1} (epithio CH) (Found: S, 33.70%).

Acetylation of compound **10**. — A solution of compound **10** (1.8 g) in pyridine (10 ml) and acetic anhydride (3 ml) was kept at room temperature overnight, and then poured onto ice. The solid precipitate was filtered off, washed with water, and dried to yield the crude ester **11** (2 g). This was extracted with hot methanol (20 ml), and water (10 ml) was added to the filtered extract to give compound **11** as needles (1.2 g, 46%), m.p. 124–125°, $[\alpha]_D^{20}$ +35.4° (*c* 1, chloroform), R_F 0.8 (solvent *A*), ν_{max}^{KBr} 3085 (epithio CH), 1725, 1225, 1085, 1060, and 1035 cm^{-1} (ester groups) (Found: C, 45.64; H, 5.44; S, 24.02. $C_{10}H_{14}O_4S_2$ calc.: C, 45.78; H, 5.38; S, 24.45%). N.m.r. data: δ 4.90 (2-proton multiplet, H-3 and H-4), 3.05 (2-proton multiplet, H-2 and H-5), 2.35 (4-proton multiplet, H-1 and H-6), 2.10 (6-proton singlet, acetyl Me).

Methanesulphonylation of compound **10**. — A solution of compound **10** (0.1 g) in dry pyridine (1 ml) was treated with methanesulphonyl chloride (0.1 ml) at 0°. The solution was kept at room temperature for 4 h and was then poured onto ice, and the precipitate was filtered off and washed with water. The crude product (0.15 g, m.p. 109–112°) was recrystallized from ethanol (2 ml) to yield compound **12** (0.1 g, 53.2%), m.p. 114–116° alone and in admixture with the compound described below.

*1,2:5,6-Diepithio-3,4-di-*O*-methanesulphonyl-*L*-iditol* (**12**). — To a stirred solution of compound **3** (14.8 g) in dry chloroform (200 ml), N methanolic sodium methox-

ide (40 ml) was added during a period of 10 min at 0°. The reaction mixture was stirred for 5 min and then poured onto a mixture of 5% aqueous sodium hydrogen carbonate–ice (1:1). The chloroform layer was washed with ice–cold, aqueous sodium hydrogen carbonate and twice with cold water, dried (Na_2SO_4), and evaporated. The semisolid residue was treated with ether to give a crude product (4.8 g, 71.5%). Recrystallization from acetone–light petroleum gave compound **12** (4.4 g), m.p. 114–116°, $[\alpha]_D^{20}$ +36.3° (*c* 1, chloroform), R_F 0.5 (solvent *A*); ν_{max}^{KBr} 3100, 3040, 3025 (epithio CH and CH_2), 3010, and 2935 cm^{-1} (methanesulphonyl CH_3) (Found: C, 28.75; H, 4.50; S, 38.10. $C_8H_{14}O_6S_4$ calc.: C, 28.73; H, 4.22; S, 38.35%). N.m.r. data ($CDCl_3$): δ 4.4 (2-proton multiplet, H-3 and H-4), 3.2 (2-proton multiplet, H-2 and H-5), 2.6 (4-proton multiplet, H-1 and H-6), 3.15 (6-proton singlet, two methanesulphonyl methyl groups).

Treatment of compound **12** *with sodium* O-*methyl dithiocarbonate.* — A solution of compound **12** (3.4 g) in dry chloroform (100 ml) was treated with carbon disulphide (6 ml) and N sodium methoxide (20 ml). The reaction mixture became turbid after a few minutes, and after 3 h, the precipitated sodium methanesulphonate was filtered off. The solution was washed three times with water, dried (Na_2SO_4), and evaporated. Traces of solvent were removed from the syrupy compound **15** at 10^{-2} mmHg and 100°; $[\alpha]_D^{20}$ −31.5° (*c* 1, chloroform), R_F 0.9 (solvent *A*) (Found: S, 51.10; CH_3O, 18.8. $C_{10}H_{14}O_2S_6$ calc.: S, 53.65; CH_3O, 17.28%).

*1,6-Dibromo-1,6-dideoxy-2,3,4,5-tetra-*O*-methanesulphonyl-*D*-mannitol* (**13**). — A solution of 1,6-dibromo-1,6-dideoxy-D-mannitol (MYELOBROMOL®, 30.8 g) in pyridine (200 ml) was treated at 0° with methanesulphonyl chloride (38.5 ml). The reaction mixture was kept at room temperature overnight and then poured onto ice. The precipitated oil solidified on treatment with fresh water, and the product (58.5 g) was dissolved in acetone (50 ml). The solution was treated with charcoal, and diluted with ether (150 ml). The colorless crystals formed were filtered off and washed with ether; yield, 40.7 g. Evaporation of the mother liquor gave a further crop (9.5 g); total yield, 80.5%; m.p. 105–107°, $[\alpha]_D^{20}$ +29.1° (*c* 1, chloroform), R_F 0.15 (solvent A) (Found: C, 19.73; H, 3.67; Br, 25.87; S, 20.88. $C_{10}H_{20}Br_2O_{12}S_4$ calc.: C, 19.36; H, 3.25; Br, 25.78; S, 20.68%).

ACKNOWLEDGMENTS

The authors are indebted to Dr. P. Sohár and Dr. G. Tóth for determining and interpreting the i.r. and n.m.r. spectra, and to Dr. E. Csányi and Dr. M. Halász for the antitumour assays.

REFERENCES

1 W. C. J. Ross, *Biological Alkylating Agents*, Butterworths, London, 1962, p. 107.
2 M. Jarman and W. C. J. Ross, *Chem. Ind.* (London), (1967) 1789.
3 L. Vargha and E. Kasztreiner, *Ber.*, 92 (1959) 2506.
4 A. M. Creighton and L. N. Owen, *J. Chem. Soc.*, (1960) 1024.
5 L. Vargha and T. Horváth, *Acta Unio Intern. Contra Cancrum*, 20 (1964) 76.
6 L. D. Hall, L. Hough, and R. A. Pritchard, *J. Chem. Soc.*, (1961) 1537.
7 S. M. Iqbal and L. N. Owen, *J. Chem. Soc.*, (1960) 1030.
8 D. M. Friedman and E. Boger, *Anal. Chem.*, 33 (1961) 906.

Carbohydrate Research
Elsevier Publishing Company, Amsterdam
Printed in Belgium

SYNTHESIS OF METHYL D-MANNOFURANOSIDES AND OF 5-*O*-METHYL-D-MANNOSE

M. H. RANDALL*
School of Chemistry, The University of New South Wales, Kensington, N. S. W. (Australia)
(Received February 10th, 1969)

ABSTRACT

Kuhn methylation of 2,3:5,6-di-*O*-isopropylidene-D-mannofuranose affords a mixture of methyl 2,3:5,6-di-*O*-isopropylidene-α-D-mannofuranoside and methyl 2,3:5,6-di-*O*-isopropylidene-β-D-mannofuranoside, in the ratio 99:1. Treatment of the sodio derivative of the di-acetal with methyl iodide gave the same products, but the ratio was reversed and was 10:1 in favour of the β-D anomer. Convenient procedures for the synthesis of methyl α-D-mannofuranoside and methyl β-D-mannofuranoside from the corresponding di-acetals are described, and a synthesis of 5-*O*-methyl-D-mannose is also presented.

INTRODUCTION

The methyl D-mannofuranosides are difficult compounds to prepare and have only been obtained by tedious methods in low yield. For example, methanolysis[1] of D-mannose yields, after extensive chromatography on cellulose powder, both anomers in low yield. Methyl α-D-mannofuranoside[2] has been prepared by Purdie methylation of D-mannofuranose 2,3:5,6-dicarbonate. More recently, derivatives of methyl β-D-mannofuranoside[3] have been obtained by treating 5,6-di-*O*-acetyl-α-D-mannofuranosyl bromide 2,3-carbonate with silver oxide and methanol, whereas the α-D anomer could be obtained by treating the compound with sodium methoxide in benzene.

As part of a programme involving the investigation of the furanoid forms of carbohydrates, some mannofuranosides were required, since, in the β-D anomer, the all *cis* disposition of the hydroxyl groups and the side chain could affect the conformation of the furanoid ring and also the anomeric equilibrium. It was therefore decided to attempt a synthesis of 5-*O*-methyl-D-mannose, since this compound cannot assume a pyranoid form. Perlin[3] obtained 5-*O*-methyl-D-mannose by methylation of 6-*O*-trityl-D-mannose 2,3-carbonate, followed by treatment with base, to yield methyl 5-*O*-methyl-6-*O*-trityl-α-D-mannofuranoside. Acid treatment then gave the required product. An alternative approach is now reported.

*Present address: Department of Chemistry, Massachusetts Institute of Technology, Cambridge, Mass., U. S. A.

RESULTS AND DISCUSSION

Kuhn methylation of the easily obtained 2,3:5,6-di-*O*-isopropylidene-α-D-mannofuranose[4] gave two products (well separated by g.l.c.) in the ratio *ca.* 99:1. Separation of these products by column chromatography on silicic acid gave first methyl 2,3:5,6-di-*O*-isopropylidene-α-D-mannofuranoside as the main component, followed by methyl 2,3:5,6-di-*O*-isopropylidene-β-D-mannofuranoside. Both of these compounds have previously been reported crystalline[5,6], but, in our hands, only the α-D anomer crystallised. The structures of these compounds were confirmed when acid hydrolysis of each di-acetal gave the corresponding methyl D-mannofuranoside. Haworth and co-workers[5] have previously prepared methyl 2,3:5,6-di-*O*-isopropylidene-α-D-mannofuranoside by treating methyl α-D-mannofuranoside with acetone and anhydrous copper sulphate.

Partial, acid hydrolysis of methyl 2,3:5,6-di-*O*-isopropylidene-α-D-mannofuranoside afforded syrupy methyl 2,3-*O*-isopropylidene-α-D-mannofuranoside, characterised as the 5,6-diacetate. Treatment of methyl 2,3-*O*-isopropylidene-α-D-mannofuranoside with 1.1 moles of benzoyl chloride afforded an oily mixture of two components (t.l.c.), presumably the mono- and di-benzoates of methyl 2,3-*O*-isopropylidene-α-D-mannofuranoside. Kuhn methylation of this mixture afforded an oil, which, on treatment with ethanolic sodium hydroxide, gave methyl 2,3-*O*-isopropylidene-5-*O*-methyl-α-D-mannofuranoside. From the yield (56%) of this material, the major product of benzoylation was the expected methyl 6-*O*-benzoyl-2,3-*O*-isopropylidene-α-D-mannofuranoside. Acid hydrolysis of methyl 2,3-*O*-isopropylidene-5-*O*-methyl-α-D-mannofuranoside gave 5-*O*-methyl-D-mannose which gave a phenylosazone identical with that prepared from 5-*O*-methyl-D-glucose.

Levene and Meyer[7] investigated the Purdie methylation of 2,3:5,6-di-*O*-isopropylidene-α-D-mannofuranose and found that the α-D anomer was the major product; however, they also observed that if the sodio derivative of the diacetal was allowed to react with methyl iodide, the major product was the β-D anomer, together with a small proportion of the α-D anomer. This reversal of product ratios is quite remarkable. It is known that the crystalline starting material exists in the α-D form[8] and mutarotates very slowly, and it would thus be expected that Kuhn or Purdie methylation would afford mainly the α-D anomer. It is difficult to explain why the sodio derivative should give the sterically less-favorable β-D anomer on reaction with methyl iodide, unless some effect is causing the negatively charged oxygen atom to assume the β position.

In our hands, the product ratio for the methylation of the sodio derivative was $\beta:\alpha = 10:1$, thus confirming the work of Levene and Meyer. On using the same separation technique as for the Kuhn methylation, we were able to obtain methyl 2,3:5,6-di-*O*-isopropylidene-β-D-mannofuranoside. As expected (since all the substituents on the furanose ring are *cis*), the latter compound was very sensitive to acid and underwent partial hydrolysis with acid when stored in daylight in chloroform solution.

A comparison of the acid labilities of the α and β anomers of methyl 2,3:5,6-di-*O*-isopropylidene-D-mannofuranoside was obtained by storing each compound in 0.1N hydrochloric acid for 65 h at 20°, the β anomer gave D-mannose and methyl β-D-mannofuranoside, whereas the α anomer gave methyl α-D-mannofuranoside and methyl 2,3-*O*-isopropylidene-α-D-mannofuranoside. It is therefore clear that the β-D anomer is more acid-labile than the α-D anomer.

As stated previously, methyl mannofuranosides are difficult compounds to synthesise in good yield. However, if the acid hydrolysis of the above diacetals could be stopped at the glycoside stage, an easy route would be obtained for the synthesis of these compounds. Thus, treatment of methyl 2,3:5,6-di-*O*-isopropylidene-α-D-mannofuranoside with 0.05N ethanolic hydrogen chloride for 120 h at 20° afforded a mixture of methyl α-D-mannofuranoside and methyl 2,3-*O*-isopropylidene-α-D-mannofuranoside. Chloroform extraction of the mixture afforded the monoacetal, and concentration of the aqueous solution gave methyl α-D-mannofuranoside. Similarly, mild, acid hydrolysis of methyl 2,3:5,6-di-*O*-isopropylidene-β-D-mannofuranoside afforded methyl β-D-mannofuranoside which was characterised as its calcium chloride complex.

EXPERIMENTAL

Melting points were determined on a Kofler microstage apparatus and are uncorrected. Analytical t.l.c. was performed with silica gel G on microscope slides with various solvents. The compounds were detected by treatment with 1:9 chlorosulphonic acid–acetic acid for *ca.* 10 min at 110°.

For g.l.c., a 4-ft. polyester (1.5% LAC-1-R-296 on Celite) column was used in a custom-built instrument. The carrier gas was nitrogen, at a flow rate of 30–40 ml/min. The best operating temperature was 145°, with the injection block at 200°. The retention times of methyl 2,3:5,6-di-*O*-isopropylidene-α-D-mannofuranoside and methyl 2,3:5,6-di-*O*-isopropylidene-β-D-mannofuranoside were 4.3 and 9.5 min, respectively.

*Methylation of 2,3:5,6-di-*O*-isopropylidene-α-D-mannofuranose.* — (*a*) The title compound (1 g), *N*,*N*-dimethylformamide (4 ml), methyl iodide (6 ml), and silver oxide (2 g) were stirred together for 14 h at 20°. After the filtered solids had been washed with chloroform, the combined filtrate and washings were concentrated *in vacuo* to yield an oil (1.2 g), which contained (t.l.c., benzene–ether, 7:3) two components (ratio *ca.* 99:1, g.l.c.) with higher mobility than the starting material, and the complete absence of the latter. The compounds were separated by using CC4 100–200 mesh Mallinckrodt silicic acid (15 g) and benzene–ether (7:3) as irrigant; the first component, methyl 2,3:5,6-di-*O*-isopropylidene-α-D-mannofuranoside (0.84 g), b.p. 85°/0.1 mmHg, crystallised completely on storage at 0° and had m.p. 23°, $[\alpha]_D^{25}$ +58.2° (*c*, 3.8, 1,1:2,2-tetrachloroethane), $[\alpha]_D^{25}$ +50.1° (*c* 4.65, chloroform), $[\alpha]_D$ +70.3° (*c*, 1.85, methanol); lit.[5], b.p. 125°/0.04 mmHg (bath temp.), m.p. 24°, $[\alpha]_D^{21}$ +68° (*c* 2.8 in methanol).

Further elution then afforded methyl 2,3:5,6-di-*O*-isopropylidene-β-D-mannofuranoside (0.02 g), b.p. 115°/0.5 mmHg, $[\alpha]_D^{25}$ −49.3° (*c* 0.83, 1,1:2,2-tetrachloroethane), $[\alpha]_D^{25}$ −58.9° (*c* 2.0, chloroform); lit.[7] $[\alpha]_D^{20}$ −42.2° (*c* 5.36, 1,1:2,2-tetrachloroethane). P.m.r. data (chloroform-*d*): methyl 2,3:5,6-di-*O*-isopropylidene-α-D-mannofuranoside, δ 1.33 (singlet, 3 protons), 1.38 (singlet, 3 protons), 1.47 (singlet, 6 protons), 3.33 (singlet, OCH_3 protons), 3.8–4.9 (multiplet, 7 protons); methyl 2,3:5,6-di-*O*-isopropylidene-β-D-mannofuranoside; δ 1.37 (singlet, 6 protons), 1.43 (singlet, 3 protons), 1.53 (singlet, 3 protons), 3.53 (singlet, OCH_3 protons), 3.55–4.9 (multiplet 7 protons). The two compounds are readily distinguished by the glycosidic methyl signals or the isopropylidene signals.

(*b*) The title compound (1 g) was dissolved in benzene (20 ml), and a few pieces of sodium were added. After storage for 15 h at 20°, the unreacted sodium was removed, and the solution was concentrated to yield an oil, to which methyl iodide (10 ml) was added. After refluxing for 24 h, water (20 ml) was added, and the resulting solution was extracted with benzene (3 × 20 ml). The extract was washed with water (2 × 15 ml), dried (Na_2SO_4), and concentrated to yield an oil (0.7 g) which contained (t.l.c.) the same products as in (*a*), but in the reversed ratio, *viz.* α:β = 10:1 (g.l.c.). The compounds were separated as described in experiment (*a*).

*Comparison of acid lability of anomeric methyl 2,3:5,6-di-*O*-isopropylidene-D-mannofuranosides.* — Each glycoside (0.1 g) was dissolved in 3:1 aqueous ethanol (1.5 ml), which was 0.1N with respect to HCl. After storage for 65 h at 20°, the solutions were neutralized with IRA-400 resin (HCO_3^-) and concentrated to yield, in each case, an oil which was examined by p.m.r. in D_2O with acetone as internal standard.

For the β-D anomer, three resonances were observed in the anomeric region: (*a*) δ 5.1, $J_{1,2}$ 1.5 Hz; (*b*) 4.8, $J_{1,2}$ 1.0 Hz; (*c*) 4.8, $J_{1,2}$ 4.5 Hz. The first two resonances correspond to those reported[9] for D-mannose (δ 5.25, $J_{1,2}$ 1.7 Hz; δ 4.97, $J_{1,2}$ 1.0 Hz), and the latter corresponds to that for methyl β-D-mannofuranoside[10] (δ 4.8, $J_{1,2}$ 4.2 Hz).

For the α-D anomer, no resonances corresponding to mannose were observed, but a strong resonance at δ 4.9, $J_{1,2}$ 4.0 Hz, indicated that the hydrolysis had stopped at the methyl α-D-mannofuranoside stage.

Methyl 2,3-O-isopropylidene-α-D-mannofuranoside. — Methyl 2,3:5,6-di-*O*-isopropylidene-α-D-mannofuranoside (4 g) was dissolved in ethanol (50 ml), and 0.2N HCl (50 ml) was added. After storage for 30 h at 25°, examination of the reaction mixture by t.l.c. [benzene–ether (1:1)] showed traces of starting material, together with a single component of lower mobility. Neutralisation of the mixture with IRA-400 resin (HCO_3^-), followed by concentration of the solution after separation of the resin, gave an oil which was dissolved in benzene and extracted with water (3 × 15 ml). Concentration of the aqueous extract gave syrupy methyl 2,3-*O*-isopropylidene-α-D-mannofuranoside (2.5 g, 73%).

Acetylation with pyridine–acetic anhydride gave methyl 5,6-di-*O*-acetyl-2,3-*O*-isopropylidene-α-D-mannofuranoside, which was recrystallised from water or light

petroleum (b.p. 40–60°); m.p. 54–55°, $[\alpha]_D$ +57° (*c* 1.3, chloroform). *Anal.* Calc. for $C_{14}H_{22}O_8$: C, 52.8; H, 7.0. Found: C, 53.1; H, 6.97. P.m.r. data: δ 5.3 (octet, H-5), 3.3 (singlet, 3 protons, OMe), 2.05 (singlet, 6 protons, acetyl groups), 1.44 and 1.33 (singlets, CMe_2).

Methyl 2,3-O-isopropylidene-5-O-methyl-α-D-mannofuranoside. — A cooled solution of methyl 2,3-*O*-isopropylidene-α-D-mannofuranoside (1.35 g) in pyridine (40 ml) was treated with benzoyl chloride (0.84 ml). After storage for 3 days at 0°, the solution was concentrated under diminished pressure at 60°, and water (25 ml) was added. After extraction of the aqueous solution with chloroform (3 × 20 ml), the organic extract was washed with dilute HCl until the washings were acid, and then with dilute, aqueous sodium hydrogen carbonate and water. The dried ($MgSO_4$) extract was concentrated to yield an oil (1.97 g) which was shaken for 14 h with *N,N*-dimethylformamide (15 ml), methyl iodide (30 ml), and silver oxide (5 g). The solids were filtered off and well washed with chloroform, and the resulting filtrate and washings were concentrated to yield an oil (1.9 g) which was dissolved in ethanol (15 ml), and 20% sodium hydroxide solution (5 ml) was then added. After heating on the steam bath for 4 h, the solution was neutralised with carbon dioxide and extracted with chloroform (3 × 20 ml). The dried ($MgSO_4$) extract was concentrated to yield an oil (1 g) which crystallised spontaneously. Examination of the material by t.l.c. [benzene–ether (3:2)] showed the presence of two components, the minor of which corresponded to methyl 2,3-*O*-isopropylidene-α-D-mannofuranoside. The material was fractionated by using CC7 100–200 mesh Mallinckrodt silicic acid as absorbent and ethyl acetate as irrigant. Fractionation was monitored by t.l.c., and the initial fraction (0.8 g) crystallised upon evaporation of the solvent and was recrystallised from light petroleum (b.p. 40–60°) to yield methyl 2,3-*O*-isopropylidene-5-*O*-methyl-α-D-mannofuranoside (0.55 g, 56%), m.p. 54°, $[\alpha]_D^{24}$ +76° (*c*, 0.25 chloroform).

Anal. Calc. for $C_{11}H_{20}O_6$: C, 53.2; H, 8.1. Found: C, 53.5; H, 8.2.

Methyl α-D-mannofuranoside. — Methyl 2,3:5,6-di-*O*-isopropylidene-α-D-mannofuranoside (0.67 g) was dissolved in absolute ethanol (4 ml), and 0.1N hydrochloric acid (4 ml) added. After storage for 5 days at 20°, the solution was neutralised with IRA-400 resin (HCO_3^-), filtered, and extracted continuously with chloroform to yield, on concentration, an oil (0.41 g). Concentration of the aqueous solution gave an oil (0.12 g) which crystallised spontaneously; recrystallisation from propyl alcohol yielded methyl α-D-mannofuranoside (0.1 g), m.p. 119°, $[\alpha]_D^{20}$ +114.4° (*c* 0.7, water); lit.[11], m.p. 120–121°, $[\alpha]_D^{24}$ +109° (*c* 1.0, water). Investigation of the chloroform-soluble component showed it to be homogeneous and to have the same mobility as methyl 2,3-*O*-isopropylidene-α-D-mannofuranoside.

Methyl β-D-mannofuranoside. — Methyl 2,3:5,6-di-*O*-isopropylidene-β-D-mannofuranoside (1.79 g) was dissolved in absolute ethanol (20 ml), and 0.033N hydrochloric acid (50 ml) was added. The reaction mixture was stored at 25° and monitored by t.l.c. [ethyl acetate–methanol (24:1), detection with iodine vapour]. After 22.5 h, the starting material had disappeared, and three components, all having lower mobility than the starting material, had appeared. The reaction mixture

was neutralised with IRA-400 resin (HCO_3^-) and then extracted continuously with chloroform for 1.5 h. Concentration of the extract yielded an oil (0.12 g), which was shown by t.l.c. to contain the more-mobile components of the hydrolysate and none of the slow-moving material. The aqueous extract was concentrated *in vacuo* at 50° to yield crude methyl β-D-mannofuranoside (1.06 g), $[\alpha]_D^{20}$ −80.6° (*c* 2.2, water). The p.m.r. spectrum in D_2O showed a doublet at δ 5.0, $J_{1,2}$ 4.2 Hz.

Preparation of the calcium chloride complex of methyl β-D-mannofuranoside. — Crude methyl β-D-mannofuranoside (0.94 g) was mixed with 70% calcium chloride solution (3 ml), whereupon precipitation began almost immediately. After addition of cold propyl alcohol (2 ml), the mixture was stored overnight at 0° and then filtered, and the crystals were washed with absolute ethanol and dried *in vacuo* over anhydrous calcium chloride to give the complex (1.31 g), $[\alpha]_D^{20}$ −54.7° (*c* 1.35, water); lit.[12] $[\alpha]_D^{20}$ −58.5° (*c* 1.71, water).

5-O-Methyl-D-mannose. — Methyl 2,3-*O*-isopropylidene-5-*O*-methyl-α-D-mannofuranoside (0.25 g) was dissolved in 0.2N HCl and heated for 1.5 h at 80–90°. The hydrolysate was then neutralised with IRA-400 resin (HCO_3^-) and concentrated to yield syrupy 5-*O*-methyl-D-mannose (0.18 g) which, on treatment with phenylhydrazine hydrochloride and sodium acetate, gave 5-*O*-methyl-D-*arabino*-hexose phenylosazone, m.p. 131°; in admixture with the phenylosazone formed from 5-*O*-methyl-D-glucose, the product had m.p. 130–131°.

ACKNOWLEDGMENTS

This investigation was supported by a grant from The Australian Research Grants Committee. It is a pleasure to acknowledge helpful discussions with Professor S. J. Angyal, and the assistance of Mrs. R. Milfull with many of the experiments.

REFERENCES

1 D. F. Mowery, *Methods Carbohyd. Chem.*, 2 (1963) 328.
2 E. L. Hirst and E. Percival, *Methods Carbohyd. Chem.*, 2 (1963) 351.
3 A. S. Perlin, *Can. J. Chem.*, 42 (1964) 1365.
4 O. T. Schmidt, *Methods Carbohyd. Chem.*, 2 (1963) 319.
5 R. G. Ault, W. N. Haworth, and E. L. Hirst, *J. Chem. Soc.*, (1935) 1012.
6 K. Freudenberg, W. Dürr, and H. von Hochstetter, *Ber.*, 61 (1928) 1735.
7 P. A. Levene and G. M. Meyer, *J. Biol. Chem.*, 59 (1924) 145.
8 S. J. Angyal, V. A. Pickles, and R. Ahluwalia, *Carbohyd. Res.*, 3 (1967) 300.
9 R. U. Lemieux and J. D. Stevens, *Can. J. Chem.*, 44 (1966) 249.
10 Personal communication from Professor A. S. Perlin to Professor S. J. Angyal.
11 W. N. Haworth and C. R. Porter, *J. Chem. Soc.*, (1930) 649.
12 A. Scattergood and E. Pacsu, *J. Amer. Chem. Soc.*, 62 (1940) 903.

Carbohydrate Research
Elsevier Publishing Company, Amsterdam
Printed in Belgium

ON THE DISTRIBUTION OF SULFATE IN HEPARIN[1]

M. L. WOLFROM*, P. Y. WANG, AND S. HONDA
Department of Chemistry, The Ohio State University, Columbus, Ohio 43210 (U. S. A.)
(Received February 20th, 1969)

ABSTRACT

The crystalline barium acid salt of heparin (after conversion into the sodium salt) was oxidized with periodate, whereby its D-glucuronic acid residues were destroyed. The oxidized material was reduced with borohydride; the product was treated with acid, and then deaminated with nitrous acid. From the deamination mixture, 2,5-anhydro-*aldehydo*-D-mannose 6-sulfate was isolated as its crystalline brucinium salt, and identified by comparison with authentic material. This work definitively places the two sulfate groups (per tetrasaccharide unit) on C-6 of the 2-amino-2-deoxy-D-glucose residues, and shows that the D-glucuronic acid residues are not sulfated.

INTRODUCTION

It has been established[2] that, in the heparin molecule, D-glucuronic acid units are polymerically condensed with 2-amino-2-deoxy-D-glucose units in α-D-(1→4)-linkage, and that 2-amino-2-deoxy-D-glucose residues are likewise attached to D-glucuronic acid residues by α-D-(1→4)-linkages. It is now apparent that a considerable proportion of L-iduronic acid residues is also present in heparin[3,4]. There is some evidence for the placement of some of the sulfate groups in heparin, although not all of this evidence is definitive. It is generally accepted that there are five sulfate groups per tetrasaccharide unit in this heteropolysaccharide. This number agrees best with the sulfate content found[5] and with the fact that the periodate consumption of the crystalline barium acid salt[6] of heparin (after conversion into the sodium salt) indicates the presence of one glycol group per tetrasaccharide unit[5], necessarily located in a uronic acid component. Two of the five sulfuric acid groups are present as sulfoamino groups[5,7-9] that are readily hydrolyzed by acid, and, consequently, samples of processed heparin have a very small content of free amino group[7,9]. By isolation and characterization of 2-amino-2-deoxy-3-*O*-methyl-D-glucose after hydrolysis of a partially methylated heparin, Nominé and co-workers[10] have shown that the 3-hydroxyl groups of the 2-amino-2-deoxy-D-glucose residues are not sulfated. In confirmation of this conclusion, they also found that, on periodate oxidation, all of the nitrogen content of a de-*N*-sulfated heparin is liberated as

*Deceased, June 20th, 1969.

ammonia. The strong anionic character of the polyelectrolyte heparin renders its complete methylation impossible. Accordingly, these methylation techniques cannot be used for the location of all of the substituent sulfate groups in heparin. Deaminative scission (with nitrous acid) of adjacent glycosidic bonds in polysaccharides containing 2-amino-2-deoxyhexose residues[11] has been applied by Foster, Stacey, and co-workers[12] to de-*N*-sulfated heparin. They obtained, presumably, an equimolar mixture of mono- and di-sulfated disaccharides, and the results they obtained on periodate oxidation of the mixture suggested the presence of 2- and 6-sulfate groups in heparin.

Any substituent group, such as a sulfuric ester, on C-6 of the 2-amino-2-deoxy-D-glucose residues in heparin, being antiparallel to the amino function, should not alter the course of the deamination with nitrous acid[13], and sulfated derivatives of 2,5-anhydro-*aldehydo*-D-mannose would be the products expected.

RESULTS AND DISCUSSION

The sodium salt of heparin, purified through the crystalline, barium acid salt[3], was oxidized with sodium metaperiodate[5] until exactly one molar proportion of the oxidant had been consumed per tetrasaccharide unit. After the inorganic salts had been removed by dialysis, the oxidized heparin was treated with sodium borohydride, to reduce the dialdehyde polymeric units, and the product was dialyzed. The periodate-oxidized, borohydride-reduced heparin was recovered as a white powder (*A*) by lyophilization.

Acid hydrolysis of the barium acid salt of heparin (after conversion into the sodium salt), by a modification of the method of Monier-Williams[14], had shown[3] that both D-glucuronic and L-iduronic acid residues are present in the heparin molecule. When the same procedure was applied to the periodate-oxidized, borohydride-reduced product from the crystalline barium acid salt of heparin, only L-iduronic acid was found; D-glucuronic acid was absent. Therefore, all of the D-glucuronic acid residues in the polymer had been cleaved by the periodate oxidation; and, from this, it follows that all of the D-glucuronic acid residues in heparin contain periodate-oxidizable α-glycol groups and therefore are not sulfated. This proof is based on the results of chromatography, but both D-glucuronic acid and L-iduronic acid have been identified[2,3] as crystalline derivatives, and paper chromatography is known to be a sensitive method for the detection of compounds of established identity.

It is known that linkages in periodate-oxidized, borohydride-reduced polysaccharides are more readily hydrolyzed than those in the original polysaccharide[15]. It was therefore possible to obtain the residual heparin material free from the periodate-oxidized, borohydride-reduced heparin by mild hydrolysis of *A* with acid, which caused removal of the sulfoamino groups and the oxidized unit, but no significant cleavage of the remaining linkages. These linkages were then cleaved further by nitrous acid deamination[11,12], and the resultant mixture was investigated.

Paper electrophoresis of the deamination product in borate solution revealed the presence of four distinct components having M_G 1.27, 1.02, 0.84, and 0.65. When de-*N*-sulfated heparin was similarly deaminated, paper electrophoresis showed the presence of only two components, having M_G 1.27 and 1.02, presumably corresponding to the mono- and di-sulfated disaccharides[12]. The presence, in the deamination product of the heparin modification, of the fraction of M_G 1.02, inferred from its mobility[16] to be the monosulfated disaccharide, probably resulted from the removal of the secondary sulfate groups during the hydrolysis with dilute acid[17].

The fraction of M_G 0.84 was identical in paper electrophoretic and chromatographic mobility with 2,5-anhydro-*aldehydo*-D-mannose 6-(sodium sulfate) (**1**) prepared by direct sulfation of 2-amino-2-deoxy-D-glucose hydrochloride[18] followed by deamination with nitrous acid[12]. This fraction was then isolated from the deamination product of heparin by preparative paper-chromatography, and was converted into the crystalline brucinium salt, whose X-ray powder diffraction pattern was identical with that of the corresponding authentic brucinium salt. The i.r. spectrum of **1** showed a strong, clearly delineated band at 816 cm^{-1} characteristic of an equatorial sulfated primary hydroxyl group, and thus of a 6-sulfate[19] of a D-aldohexopyranose in the *C1* (D) conformation.

EXPERIMENTAL

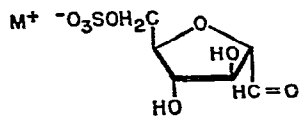
Periodate oxidation of heparin (sodium salt) followed by reduction with sodium borohydride. — The sodium salt of heparin (0.49 g, 0.4 mmole), purified[2] through the barium acid salt, was dissolved in water (4 ml) in a 10-ml volumetric flask, aqueous sodium metaperiodate solution (5 ml, 0.2M) was added, and the total volume was brought to 10 ml with water. A blank was prepared similarly. The oxidation proceeded at room temperature in the dark. The periodate consumption was followed spectrophotometrically[20] at 223 nm by use of 0.1-ml aliquots of the solution diluted to 250 ml. After deduction of absorption due to iodate ion, the results were (h, mole of IO_4^- consumed per mole): 19, 0.5; 42.5, 0.77; 48, 0.86; and 94, 1.22. In a preparative experiment, 2.46 g of the sodium salt of purified heparin was treated for 65 h with periodate solution, as just described, the excess of oxidant was decomposed with ethylene glycol, and the solution was dialyzed for 3 days against running water. The nondialyzable fraction, reducing to Fehling solution, was concentrated to a small volume and freeze-dried, to yield 2.02 g (83%) of a white powder. Reduction of this material with sodium borohydride (2 g) in water (100 ml), followed by dialysis, concentration, and freeze-drying, gave 1.69 g (84%) of a nonreducing material (*A*), $[\alpha]_D^{23}$ +51° (*c* 1.0, water).

Anal. Calc. for $C_{24}H_{33}N_2Na_7O_{35}S_5 \cdot H_2O$: C, 23.08; H, 2.86; N, 2.24; S, 12.84. Found: C, 23.82; H, 3.54; N, 2.48; S, 12.46.

Hydrolysis of periodate-oxidized, borohydride-reduced heparin. — The periodate-oxidized, borohydride-reduced product (100 mg) was hydrolyzed for 2 h at room temperature with 0.4 ml of 72% sulfuric acid. The solution was then diluted with

water to give a concentration of 3% of sulfuric acid, and was refluxed for 2.5 h, as previously described[3] for the barium acid salt of heparin (after conversion into the sodium salt). Paper chromatography of the hydrolyzate was performed in two solvent systems by use of two indicators[3]. Whereas, previously, both D-glucuronic acid and L-iduronic acid and their lactones had been detected[3], only L-iduronic acid and its lactone were now found.

*Deamination of partially acid-hydrolyzed, periodate-oxidized, borohydride-reduced heparin (sodium salt), and isolation of 2,5-anhydro-*aldehydo-D-*mannose 6-(brucinium sulfate)* (1). — The heparin modification *A* (0.24 g) was refluxed for 3 h with 0.04M sulfuric acid (20 ml), to effect de-*N*-sulfation and hydrolysis of the acetal



1

M^+ = Na^+ or brucinium$^+$

linkages in the oxidized units. After the solution had been cooled to room temperature, a solution of barium nitrite monohydrate (0.50 g) in water (10 ml), was slowly added, with gentle shaking. More sulfuric acid (4 ml, 0.5M) was added to the solution, to generate nitrous acid, and the stoppered flask was kept undisturbed for 2 h. The excess of nitrous acid was then removed under diminished pressure, and sufficient barium carbonate was added to neutralize the acids. After removal of the precipitated inorganic salt by filtration, the clear filtrate was passed through a column (1 × 15 cm) of Amberlite IR-120 (H^+) ion-exchange resin. The acidic effluent was made neutral with 0.01M sodium hydroxide, and was concentrated to 10 ml. Paper electrophoresis[21] on Whatman No. 3 MM paper (12 cm × 22 cm) for 4 h at 500 V (0.1M borate) gave four distinct components as revealed by indication with alkaline silver nitrate solution: M_G 1.27, 1.02, 0.84, and 0.67; the relative intensities of these spots, as estimated visually, were 45, 30, 15, and 10%, respectively. The zone having M_G 0.84 was identical in mobility with 2,5-anhydro-D-mannose 6-(sodium sulfate), prepared as described later. The solution was then applied to 8 sheets of Whatman No. 3 MM paper (23 cm × 57 cm), and these were developed for 3 days with 9:3:2 (v/v) ethyl acetate–acetic acid–water[22]. Because of the presence of nitrate, the resolution of the four components was very poor. Narrow strips were then excised from the middle of that band similar in mobility to authentic 2,5-anhydro-*aldehydo*-D-mannose 6-(sodium sulfate) on guide strips, and these were eluted with water. Evaporation to dryness under diminished pressure at 25° gave a white powder (13 mg) whose i.r. spectrum* showed poor resolution in the 6–15-μm region. The remaining material (11 mg) was then extracted with hot 95% ethanol (20 ml), and the extract was centrifuged. Evaporation of the alcoholic solution afforded a pale-yellow gum (7 mg)

*Recorded with a Perkin–Elmer Infracord infrared spectrometer.

which, on paper electrophoresis, showed a single spot having M_G 0.84; λ_{max}^{KBr} 6.18 (CHO), 7.90 (OH), 8.83, 9.90 (SO_4 ester), 9.30, 10.60, 10.40, 10.18, 13.70 (equatorial primary SO_4 ester), and 13.00 μm. An aqueous solution (10 ml) of this gum was de-ionized with Amberlite IR-120 (H^+) ion-exchange resin (1 × 4 cm), and the acidic effluent was brought to pH 8 with alcoholic brucine solution and concentrated, without warming, to about 1 ml by means of a stream of nitrogen. Water (15 ml) was then added, and the excess of the alkaloid was removed by successive extraction with chloroform (3 × 20 ml) and ether (2 × 20 ml). The aqueous solution was decolorized with carbon, the suspension was filtered, and the filtrate was concentrated, as before, to about 0.5 ml. Acetone was added to incipient turbidity, and the solution was refrigerated. Tiny clusters of bladed needles formed within three days. The supernatant mother-liquor was withdrawn by means of a small pipet. The crystals were successively washed with 60% aqueous acetone (3 × 1 ml) and acetone, and dried under diminished pressure over Drierite to yield 4 mg, m.p. and m.m.p. 200–201° (dec.); X-ray powder diffraction data identical with those of the authentic 2,5-anhydro-*aldehydo*-D-mannose 6-(brucinium sulfate) (**1**) described next.

Authentic 2,5-anhydro-aldehydo-D-*mannose*[23] *6-(brucinium sulfate)* (**1**). — Following essentially the sulfation procedure of Saito and co-workers[18], 2.16 g of 2-amino-2-deoxy-D-glucose hydrochloride was slowly added, at 0°, to 15 ml of sulfuric acid. Chlorosulfonic acid (1.3 ml) was then added dropwise, with stirring, during 2 h. The sulfated product was precipitated by adding the solution to ether (100 ml, cooled with solid carbon dioxide), washed with cold ether (3 × 100 ml), and dissolved in iced water (80 ml). After neutralization of the solution with barium carbonate and centrifugation, the resultant clear, supernatant liquor was concentrated under diminished pressure at 40° to a small volume, and freeze-dried; yield 1.6 g. The sulfated amino sugar (800 mg) was dissolved in sulfuric acid (0.025M, 50 ml), and the solution was refluxed for 2 h. After being cooled to room temperature, the solution was treated with barium nitrite monohydrate (1.75 g), followed by 0.5M sulfuric acid until no further precipitation occurred. The flask was swirled gently and stoppered, and the solution kept for 2 h. The excess of nitrous acid was removed under diminished pressure, the acid solution was made neutral with barium carbonate, the suspension was centrifuged, and the clear, supernatant liquor was passed through a Millipore filter. After concentration of the solution to about 20 ml, paper electrophoresis (0.1M borate, at 500 V for 3 h) showed three components, having M_G 0.01 (2,5-anhydro-*aldehydo*-D-mannose), 0.84, and 1.47, the second preponderating. The barium salt of the deaminated sugar sulfate was de-ionized with Amberlite IR-120 (H^+) ion-exchange resin, and the acidic effluent was made neutral with sodium hydroxide (0.01M). The solution was concentrated to 10 ml, and spotted on 12 sheets (23 cm × 57 cm) of Whatman 3 MM paper; these were developed for 3 days, as before. The middle portion of the band having R_G 0.24 was excised, and eluted with water. The aqueous solution was evaporated to dryness, and extracted with hot 95% ethanol, the extract was centrifuged, and the clear, alcoholic, supernatant liquor was evaporated to a pale-yellow gum (47 mg). On paper electrophoresis, this material

showed a single spot, M_G 0.84; λ_{max}^{KBr} 6.18 (CHO), 7.90 (OH), 8.83, 9.90 (SO_4 ester), 9.30, 10.60, 10.40, 10.18, 13.70 (816 cm^{-1}, equatorial primary SO_4 ester), and 13.00 μm.

The substance (44 mg) so purified by paper chromatography was de-ionized with Amberlite IR-120 (H^+) ion-exchange resin, and the column was washed with water until the final effluent was neutral. The eluate was brought to pH 8 with alcoholic brucine solution, and the solution was concentrated to a small volume at 25° (to remove the alcohol), and diluted with water (20 ml). The solution was decolorized with carbon, the suspension was filtered, the filtrate was successively extracted with chloroform (4 × 30 ml) and ether (3 × 30 ml), and the aqueous phase was concentrated to about 1 ml. Acetone was added to incipient turbidity, and the solution was warmed at 40° until it became clear, cooled to room temperature, and refrigerated. Crystals formed the next day; yield 25 mg, m.p. 199–201° (dec.); X-ray powder diffraction data*: 12.81 (vs, 3), 8.67 (s), 7.50 (vs, 1), 7.03 (s), 6.46 (m), 5.98 (m), 5.68 (m), 5.37 (m), 4.96 (w), 4.37 (s), 4.02 (m), 3.90 (m), 3.77 (s), 3.55 (vs, 2), 3.38 (w), 3.21 (w), and 3.06 (m).

Anal. Calc. for $C_{29}H_{36}N_2OS$: C, 54.80; H, 5.66; S, 5.04. Found: C, 55.05; H, 6.23; S, 5.40.

ACKNOWLEDGMENTS

This work was supported, in part, by the National Science Foundation Grant No. GB 1685 (The Ohio State Research Foundation Project 1755) and, in part, by funds from the State of Ohio administered by the Office of Research, The Ohio State University. The commercial heparin used in this investigation was kindly furnished by The Upjohn Company, Kalamazoo, Michigan.

REFERENCES

1 Preliminary communication: M. L. WOLFROM AND P. Y. WANG, *Chem. Commun.*, (1967) 241.
2 M. L. WOLFROM, J. R. VERCELLOTTI, AND G. H. S. THOMAS, *J. Org. Chem.*, 26 (1961) 2160, 29 (1964) 536; M. L. WOLFROM, J. R. VERCELLOTTI, AND D. HORTON, *ibid.*, 27 (1962) 705; 28 (1963) 278, 279; 29 (1964) 540, 547; M. L. WOLFROM, H. EL KHADEM, AND J. R. VERCELLOTTI, *ibid.*, 29 (1964) 3284; M. L. WOLFROM, H. TOMOMATSU, AND W. A. SZAREK, *ibid.*, 31 (1966) 1173.
3 M. L. WOLFROM, S. HONDA, AND P. Y. WANG, *Chem. Commun.*, (1968) 505; *Carbohyd. Res.*, 10 (1969) 259.
4 A. S. PERLIN, M. MAZUREK, L. B. JAQUES, AND L. W. KAVANAGH, *Carbohyd. Res.*, 7 (1968) 369.
5 M. L. WOLFROM, D. I. WEISBLAT, J. V. KARABINOS, W. H. MCNEELY, AND J. MCLEAN, *J. Amer. Chem. Soc.*, 65 (1943) 2077.
6 A. F. CHARLES AND D. A. SCOTT, *Biochem. J.*, 30 (1936) 1927.
7 M. L. WOLFROM AND W. H. MCNEELY, *J. Amer. Chem. Soc.*, 67 (1945) 748.
8 J. E. JORPES, H. BOSTROM, AND V. MUTT, *J. Biol. Chem.*, 183 (1950) 607.
9 R. A. GIBBONS AND M. L. WOLFROM, *Arch. Biochem. Biophys.*, 98 (1962) 374.
10 G. NOMINÉ, R. BUCOURT, AND D. BERTIN, *Bull. Soc. Chim. France*, (1961) 561.

*X-Ray powder diffraction pattern data refer to interplanar spacings in Å, CuKα radiation, λ 1.539 Å, Ni filter, 114.6-mm camera diameter. Relative intensities were recorded photographically, and estimated visually: m, medium; s, strong; v, very; w, weak. The first three strongest lines are numbered (1, strongest).

11 K. H. MEYER AND H. WEHRLI, *Helv. Chim. Acta*, 20 (1937) 353.
12 A. B. FOSTER, R. HARRISON, T. D. INCH, M. STACEY, AND J. M. WEBBER, *J. Chem. Soc.*, (1963) 2279.
13 F. SHAFIZADEH, *Advan. Carbohyd. Chem.*, 13 (1958) 45.
14 G. W. MONIER-WILLIAMS, *J. Chem. Soc.*, 119 (1921) 803.
15 Personal communication from the late F. SMITH.
16 J. R. TURVEY, *Advan. Carbohyd. Chem.*, 20 (1965) 190.
17 D. A. REES, *Biochem. J.*, 88 (1963) 343.
18 T. SAITO AND J. NOGUCHI, *Nippon Kagaku Zasshi*, 82 (1961) 471; T. SAITO, J. NOGUCHI, AND K. KOMATSU, *ibid.*, 82 (1961) 472; *Chem. Abstr.*, 56 (1962) 11678, 11679.
19 A. G. LLOYD AND K. S. DODGSON, *Biochim. Biophys. Acta*, 46 (1961) 116; M. B. MATHEWS, *Nature*, 181 (1958) 421.
20 G. O. ASPINALL AND R. J. FERRIER, *Chem. Ind.* (London), (1957) 1216.
21 A. B. FOSTER, *Chem. Ind.* (London), (1952) 1050.
22 J. R. TURVEY AND T. P. WILLIAMS, *J. Chem. Soc.*, (1962) 2119.
23 E. FISCHER AND F. TIEMANN, *Ber.*, 27 (1894) 138; B. C. BERA, A. B. FOSTER, AND M. STACEY, *J. Chem. Soc.*, (1956) 4531.

Carbohydrate Research
Elsevier Publishing Company, Amsterdam
Printed in Belgium

POLYSACCHARIDE METHYLATION STUDIES

N. W. H. CHEETHAM AND R. J. MCILROY
Department of Agriculture, University of Queensland, St. Lucia, Brisbane 4067 (Australia)
(Received March 3rd, 1969)

ABSTRACT

Comparisons have been made, on two polysaccharides, of three methylation procedures. On the evidence of molecular weights determined by vapour-phase osmometry, and methanolysis products identified by g.l.c., it is concluded that the sodium hydride–methyl sulphoxide–methyl iodide system has advantages over the classical Haworth–Purdie procedures for the polysaccharides studied. The sodium hydroxide–methyl sulphate–methyl sulphoxide procedure gave products having lower molecular weights and extra g.l.c. peaks, and should be viewed with some caution.

INTRODUCTION

Methylation studies continue to play an important role in polysaccharide structure determination. In addition to the classical Haworth[1] and Purdie[2] techniques, which often require many additions of reagent, more recent methods have been reported to give improved yields with fewer treatments[3–6].

The sodium hydride–methyl sulphoxide–methyl iodide system has been shown to have advantages for some neutral and acidic[6] polysaccharides. It has led to at least one correction of earlier data[9]. However, with *Acacia senegal* gum, which has significant proportions of L-rhamnopyranose residues as end groups attached to C-4 of D-glucuronic acid residues, loss of 2,3,4-tri-*O*-methyl-L-rhamnose and probably 2,3-di-*O*-methyl-D-glucuronic acid occurs[7].

It has been suggested[6,7] that the performance of the above-mentioned system be assessed on a wider range of polysaccharides. Also, methylations of the same polysaccharide by different methods, for comparison, should be informative. In accordance with these ideas, we have used three different procedures to methylate the hemicelluloses of the tropical pasture species *Cynodon plectostachyus* and *Setaria sphacelata*. The methods employed were: (*1*) Haworth[1] followed by the Purdie[2] procedure, (*2*) sodium hydride–methyl sulphoxide–methyl iodide[6] (completion by Purdie method), and (*3*) sodium hydroxide–methyl sulphoxide–methyl sulphate[5] (completion by the Purdie method).

The methanolysis products of the methylated polysaccharides were examined

by gas–liquid chromatography (g.l.c.), and the number-average molecular weights (M_n) were determined by vapour-phase osmometry (v.p.o.).

RESULTS AND DISCUSSION

Full structural studies are still in progress, but work done to date indicates that both hemicelluloses consist essentially of a backbone of β-linked D-xylopyranose residues, to which are attached, as side-chains, residues of L-arabinofuranose and D-glucuronic acid or one of its methyl ethers.

After methylation, the polysaccharide samples were precipitated from chloroform solution by pouring into light petroleum (b.p. 60–80°). No fractionation of the precipitated material was carried out, as it was desired to compare by vapour-phase osmometry the M_n values of all the molecular species present. The v.p.o. results are summarised in Table I.

TABLE I

NUMBER-AVERAGE MOLECULAR WEIGHTS (M_n) OF *Setaria* AND *Cynodon* POLYSACCHARIDES

Sample	*Methylation procedure*	*Mean* M_n	*Methoxyl* (%)	*Yield* (%)
Setaria	1[a]	9,300 ±350[b]	38.8	39
Setaria	2	9,400 ±350[b]	38.9	100
Setaria	3	8,500 ±350[b]	38.3	65
Cynodon	1	6,700 ±300[c]	38.6	42
Cynodon	2	7,500 ±300[b]	38.8	90
Cynodon	3	5,300 ±300[c]	38.9	62

[a]Numbers refer, respectively, to the methods listed in the introduction. [b]Mean of two samples. [c]Mean of three samples.

The methanolysis products of all six samples included the methyl glycosides of the following sugars (as shown by g.l.c.): 2,3,4-tri-*O*-methyl-D-xylose, 2,3,5-tri-*O*-methyl-L-arabinose, 3,5-di-*O*-methyl-L-arabinose, 2,3-di-*O*-methyl-D-xylose, and 2-*O*-methyl-D-xylose.

In the samples methylated by procedure *3*, there also appeared three minor but definite peaks; two were between those of the tri- and di-*O*-methylglycosides, and one between the di- and mono-*O*-methylglycoside peaks. These could not be identified. Yields from methods *2* and *3* were higher than from method *1*.

It is concluded that the sodium hydride–methyl sulphoxide–methyl iodide procedure is superior to the Haworth–Purdie procedures for the polysaccharides studied. Yields for the former were much higher, and, in the case of *Cynodon*, the molecular weight was markedly higher. In the sodium hydroxide–methyl sulphoxide–methyl sulphate method, the lower molecular weights and extra g.l.c. peaks indicate that some degree of degradation occurred. The method would appear to be unsuitable for such polysaccharides, at least under the conditions described herein.

EXPERIMENTAL

Paper chromatography was carried out on Whatman No. 1 paper with the following solvent systems (v/v): (*a*) ethyl acetate–pyridine–water (10:4:3), (*b*) butyl alcohol–ethanol–water (4:1:1), (*c*) butyl alcohol–benzene–pyridine–water (5:1:3:3, upper layer). Alkaline silver nitrate and *p*-anisidine hydrochloride were used as spray reagents.

G.l.c. was carried out isothermally at 160° on columns (5 ft. × 1/8 in.) of (*a*) ethylene glycol succinate (14% by wt.) on acid-washed Chromosorb W, 80–100 mesh, and (*b*) butane-1,4-diol succinate (15% by wt.) on the same support. The carrier gas was nitrogen at a flow rate of 35 ml/min, and the chromatograph a Varian–Aerograph model 1520, fitted with flame-ionization detectors.

Vapour-phase osmometry readings were carried out on a Hewlett–Packard Model 302 with a 37° non-aqueous probe. The solvent was recrystallizable benzene. A standard curve was constructed by using various molalities of monodisperse polystyrene (M_n 4,800; $M_w/M_n < 1.10$. Pressure Chemical Co., Pittsburgh, Pa.).

Isolation of the polysaccharides. — Isolation and purification procedures for both hemicelluloses were essentially as described by McIlroy[8].

Cynodon hemicellulose had $[\alpha]_D^{25}$ −97.3° (*c* 0.51, 4% sodium hydroxide) (Found: uronic anhydride by decarboxylation, 3–4; ash, 2; OMe, 0.46%). Hydrolysis by N sulphuric acid, followed by paper chromatography in solvents (*a*)–(*c*), indicated the presence of xylose, arabinose, traces of glucose, and an aldobiouronic acid. *Setaria* hemicellulose had $[\alpha]_D^{24}$ −92° (*c* 0.3, 4% sodium hydroxide) (Found: uronic anhydride, 3–4; ash, 3; OMe, 1.3%). Hydrolysis and chromatography, as before, established the presence of xylose, arabinose, traces of glucose, and an aldobiouronic acid.

Partial hydrolysis by 0.02N oxalic acid for 3 h at 100° yielded, for both polysaccharides, hydrolysates containing only arabinose (paper chromatography). Complete hydrolysis of the degraded polysaccharides, precipitated by ethanol (5 vol.), yielded xylose, a trace only of arabinose, and aldobiouronic acids.

Methylations. — (*1*) *Haworth–Purdie procedure.* For *Cynodon* hemicellulose, 5 additions of the Haworth and 4 of the Purdie reagents were required for complete methylation, whereas *Setaria* required 5 treatments of each.

(2) *Sodium hydride–methyl sulphoxide–methyl iodide.* The following procedure gave the best results, *e.g.*, for *Setaria*. Freshly distilled methyl sulphoxide (150 ml) was allowed to stand in contact with dried Linde type 4A molecular sieve for 1 h. It was run slowly from the drying column directly into a 250-ml three-necked flask, fitted with mechanical stirrer and condenser, and flushed slowly with dry nitrogen. The hemicellulose (2 g) was added quickly, and dissolved by stirring and warming to 50°. Sodium hydride (4 g) was added in portions over 2.5 h (the vessel containing the sodium hydride was attached to the reaction flask and was thus flushed with dry nitrogen during this time; additions were made by tilting the sodium hydride vessel and tapping the vessel sharply).

Stirring was continued for a further 4 h at 50°, by which time evolution of hydrogen had ceased. To the solution, cooled to room temperature, was added methyl iodide (20 ml) during 75 min. Stirring was continued overnight (14 h). Two further additions of sodium hydride (2 g) and methyl iodide (10 ml) were made on successive days. Chloroform (200 ml) was then added to the reaction mixture, a drop of which gave a neutral reaction when added to water and spotted on indicator paper. The chloroform solution was filtered free of sodium iodide and extracted thoroughly with water (total volume, 2 litres) to remove methyl sulphoxide. This procedure avoided emulsion formation, which occurred when the reaction mixture was poured into water and extracted with chloroform.

The chloroform solution was dried ($MgSO_4$) and evaporated to yield 2.03 g of product (Found: OMe, 37.0%), which still showed some hydroxyl absorption in the infrared. A further methylation by the Purdie method, followed by precipitation from chloroform solution by pouring into cold, light petroleum (b.p. 60–80°) yielded 2.0 g of nearly white, fluffy product (Found: OMe 38.9%), showing virtually no absorption in the hydroxyl region of the infrared.

For *Cynodon* hemicellulose, the yield was 1.8 g from 2.0 g of starting material (Found: OMe 38.8%); no hydroxyl absorption in the infrared.

(*3*) *Sodium hydroxide–methyl sulphoxide–methyl sulphate.* The procedure adopted was similar to that of Srivastava *et al.*[5], *e.g.*, for *Setaria* hemicellulose. The polysaccharide (1 g) was dissolved (under nitrogen at 60°) in freshly distilled methyl sulphoxide (50 ml). Sodium hydroxide (25 g) and methyl sulphate (40 ml) were added over 8 h at room temperature. Stirring at room temperature in an atmosphere of dry nitrogen was carried out for a further 16 h. The mixture was then heated for 1 h at 80–90° to destroy excess of methyl sulphate, cooled to about 5°, and neutralized with 20% sulphuric acid to pH 7. Sodium sulphate was removed by filtration and washed with chloroform. The yellow, aqueous methyl sulphoxide solution was extracted with chloroform in a liquid–liquid extractor for 24 h. The chloroform extract was re-extracted with water (5 × 200 ml) to remove methyl sulphoxide, dried over anhydrous magnesium sulphate, and evaporated to yield 0.7 g of polysaccharide (Found: OMe 32%). This required three Purdie treatments to complete methylation (Yield: 0.65 g; OMe, 38.3%; no hydroxyl absorption in the infrared).

For *Cynodon* hemicellulose, the final yield was 0.62 g from 1.0 g of starting material. One Purdie treatment was needed to raise the methoxyl content to 38.9% and remove hydroxyl absorption in the infrared.

Vapour-phase osmometry. — Readings were carried out on duplicate or triplicate samples (see Table I). Benzene was added to a known mass of polysaccharide, a small, weighed stopper was inserted, and reweighing was carried out immediately. Samples were taken rapidly into the osmometer syringes; ΔR readings for each individual sample were repeated until three consecutive readings were within 1%.

Methanolysis was effected in 3% methanolic hydrogen chloride for 18 h at 60° in a sealed tube. After neutralization by silver carbonate, samples were injected directly into the gas chromatograph.

ACKNOWLEDGMENTS

The authors express their gratitude to Dr. R. F. Evans, Department of Chemistry, University of Queensland, for his help and permission to use the Hewlett–Packard Osmometer; and to Dr. J. H. O'Donnell of the same Department for supplying polystyrene for the standard curve.

REFERENCES

1 W. N. HAWORTH, *J. Chem. Soc.*, 107 (1915) 8.
2 T. PURDIE AND J. C. IRVINE, *J. Chem. Soc.*, 83 (1903) 1021.
3 R. KUHN, H. TRISCHMANN, AND I. LÖW, *Angew. Chem.*, 67 (1955) 32.
4 K. WALLENFELS, G. BECHTLER, R. KUHN, H. TRISCHMANN, AND H. EGGE, *Angew. Chem. Intern. Ed. Engl.*, 2 (1963) 515.
5 H. C. SRIVASTAVA, P. P. SINGH, S. N. HARSHE, AND K. VIRK, *Tetrahedron Lett.*, (1964) 493.
6 D. M. W. ANDERSON AND G. M. CREE, *Carbohyd. Res.*, 2 (1966) 162.
7 D. M. W. ANDERSON, I. C. M. DEA, P. A. MAGGS, AND A. C. MUNRO, *Carbohyd. Res.*, 5 (1967) 489.
8 R. J. MCILROY, *J. Chem. Soc.*, (1963) 6067.
9 P. A. SANDFORD AND H. E. CONRAD, *Biochemistry*, 5 (1966) 1508.

Carbohydrate Research
Elsevier Publishing Company, Amsterdam
Printed in Belgium

APPLICATION OF THE WITTIG REACTION TO THE SYNTHESIS OF 3-DEOXY-D-*manno*-OCTULOSONIC ACID

N. K. KOCHETKOV, B. A. DMITRIEV, AND L. V. BACKINOWSKY
N. D. Zelinsky Institute of Organic Chemistry, Moscow (U. S. S. R.)
(Received March 3rd, 1969)

ABSTRACT

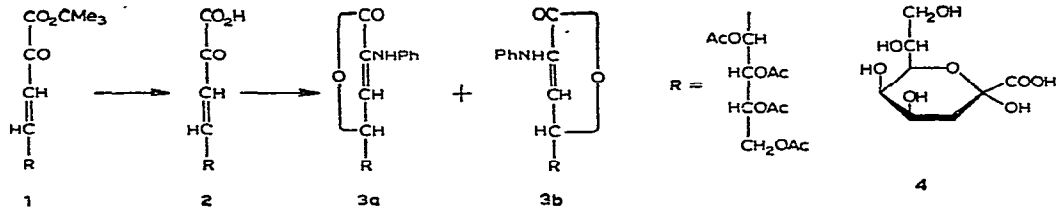
A synthesis of the naturally occurring 3-deoxy-D-*manno*-octulosonic acid by the Wittig reaction is described. Condensation of tetra-*O*-acetyl-*aldehydo*-D-arabinose with (*tert*-butoxyoxalyl)methylenetriphenylphosphorane leads to an unsaturated acid (**2**), which, with aniline hydrochloride in the presence of *N*-benzylideneaniline, affords the epimeric enaminolactones **3**. The enaminolactone **3a** having the D-*manno* configuration was converted into 3-deoxy-D-*manno*-octulosonic acid. The g.l.c. properties of derivatives of 3-deoxyoctulosonic acids are discussed.

INTRODUCTION

3-Deoxy-D-*manno*-octulosonic acid is a constituent of the cell-wall lipopolysaccharides of gram-negative bacteria. This acid is chemically bonded to the polysaccharide and lipid components[1] and is responsible for the immunospecificity[2] of the biopolymer. Previously, it has been synthesised by the condensation of D-arabinose with oxaloacetic acid[3,4] or its di(tert-butyl) ester[5], in a manner similar to that used[6,7] for the preparation of *N*-acetylneuraminic acid. We now describe a new synthesis of 3-deoxy-D-*manno*-octulosonic acid.

RESULTS AND DISCUSSION

The condensation of tetra-*O*-acetyl-*aldehydo*-D-arabinose with (*tert*-butoxyoxalyl)methylenetriphenylphosphorane in boiling toluene gave tert-butyl 5,6,7,8-tetra-*O*-acetyl-3,4-dideoxy-D-*arabino*-octulos-3-enonate (**1**). Upon treatment with trifluoroacetic acid, this ester was converted into the free acid **2**, which was used in the following reaction step without additional purification. The reaction of the L-enantiomer of acid **2** with aniline hydrochloride in glacial acetic acid affords[8] a mixture of enaminolactones, accompanied by degradation products. An attempt to remove the hydrogen chloride and water that are formed in this reaction, by using the molecular sieve Linde 5A (the reaction was carried out in *N*,*N*-dimethylformamide), was unsuccessful, because of the ion-exchange properties of the absorbent; the free arylamine reacts with acid **2**, with the predominant formation of an enaminolactam (*cf.* ref. 9).



The removal of hydrogen chloride and water from the reaction mixture was therefore accomplished by addition of *N*-benzylideneaniline; this Schiff's base readily reacts with water under acidic conditions to form aniline hydrochloride and benzaldehyde. The lactonisation of acid **2** was carried out in the presence of 0.2–0.5 mol. of aniline hydrochloride and 1 mol. of *N*-benzylideneaniline, under mild conditions, to give *ca.* 40% of a nearly equimolar mixture of the D-*manno*- (**3a**) and D-*gluco*-enaminolactones (**3b**).

From the mixture of enaminolactones, the individual C-4 epimers were isolated by fractional crystallization. Their properties correspond to those of the L-enantiomers[8]. Methanolysis of the D-*manno*-enaminolactone **3a**, followed by acid hydrolysis, led to 3-deoxy-D-*manno*-octulosonic acid (**4**), which was identical with the natural product (comparison of ammonium salts[4]).

Difficulties are encountered in the chemical and biochemical[3] identification of 3-deoxyglyculosonic acids. Thus, for example, ammonium 3-deoxy-D-*manno*-octulosonate[5], and penta-*O*-acetyl-3-deoxy-D-*manno*-octulosonic acid[4] or its methyl ester[3] are obtainable in the crystalline state in only 5–10% yield. We therefore studied the possibility of identifying 3-deoxyoctulosonic acids by gas–liquid chromatography.

Methanolysis of individual enaminolactones or of the corresponding acids resulted in homogeneous (paper chromatography and electrophoresis) products having protected carbonyl and carboxyl groups. Several compounds, namely, methyl esters of methyl furanosides and pyranosides, and possibly the γ-lactone dimethyl acetal of a 3-deoxyoctulosonic acid (*cf.* ref. 10), might be expected to be formed upon methanolysis. Indeed, the i.r. spectrum of the methanolysate had a band at 1780 (γ-lactone) in addition to that at 1745 cm^{-1} (ester group). Trimethylsilylation of the methanolysis product and examination by g.l.c. revealed three products which were tentatively identified as the compounds mentioned above. On methanolysis, followed by silylation, the *manno* and *gluco* epimers gave three components on g.l.c. when a polar, stationary-phase column was used (Fig. 1). The first two peaks had different retention times (Table I), thus enabling 3-deoxy-D-*manno*- and -D-*gluco*-octulosonic acids to be distinguished. The third components possessed almost the same retention times for both epimers and appear to have a γ-lactone dimethyl acetal structure. An attempt to prevent lactone–acetal formation, by replacement of enaminolactone by the corresponding acid, resulted only in a decrease in the intensity of the third component. With a nonpolar stationary phase, both isomers gave the same g.l.c. pattern, consisting of two peaks.

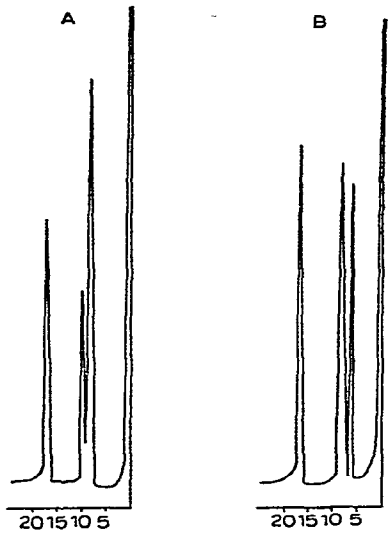


Fig. 1. G.l.c. separation of derivatives of 3-deoxy-D-*manno*- (A) and -D-*gluco*-octulosonic acids (B).

TABLE I

RETENTION TIMES OF 3-DEOXY-D-*manno*- AND -*gluco*-OCTULOSONIC ACID DERIVATIVES, RELATIVE TO METHYL TETRA-*O*-TRIMETHYLSILYL-α-D-GLUCOPYRANOSIDE (T = 2.6 MIN)

Compound	*Retention time*		
manno isomer	2.71	3.32	5.74
gluco isomer	2.16	2.82	5.73

EXPERIMENTAL

General. — Thin-layer chromatography (t.l.c.) was performed on silica gel KSK, and detection was effected with conc. sulphuric acid or aqueous potassium permanganate. Melting points were determined on a Kofler micro-heating stage. G.l.c. was performed with a Pye Argon Chromatograph, equipped with a β-ionisation detector, and glass columns (120 × 0.5 cm) packed with 5% (w/w) of poly(neopentyl-glycol succinate) on Chromosorb W (80–100 mesh) (polar phase) or with 5% of silicone gum rubber SE-30 on DMCS-treated Chromosorb W (60–80 mesh) (nonpolar stationary phase) at 180°; the flow rate of argon was 50 ml per min. Solutions were evaporated under diminished pressure at 40°.

tert-*Butyl 5,6,7,8-tetra*-O-*acetyl-3,4-dideoxy*-D-arabino-*octulos-3-enonate* (**1**). — A solution of tetra-*O*-acetyl-*aldehydo*-D-arabinose (2.50 g) and (*tert*-butoxyoxalyl)-methylenetriphenylphosphorane[11] (3.82 g, 1.2 mol.) in dry toluene (100 ml) was heated under reflux for 4 h. The pale-yellow solution was taken to dryness, the residue was treated with ether (50 ml), and the precipitated triphenylphosphine oxide was removed by filtration. The filtrate was evaporated, and the residue placed on a

column of silicic acid (250 g). The column was washed with regularly increasing concentrations of ether in benzene (0–30%). Fractions containing pure substance (R_F 0.8, benzene–ether 1:1) were combined and evaporated to give the ester **1** (2.93 g, 84%) as a syrup, $[\alpha]_D^{25}$ +12.1° (*c* 5.04, benzene) (Found: C, 54.33; H, 6.42. $C_{20}H_{28}O_{11}$ calc.: C, 54.05; H, 6.35%).

2-Anilino-4-(R)- *and -4-*(S)-(D-arabino-*tetra-acetoxybutyl)-2-buten-4-olides* (**3a** and **3b**). — A solution of ester **1** (0.95 g) in trifluoroacetic acid (10 ml) was kept for 40 min at room temperature and then evaporated to dryness. Benzene (3 × 5 ml) was distilled from the residue, and the yellow, syrupy acid **2** obtained was dissolved in glacial acetic acid (30 ml). Aniline hydrochloride (55 mg, 0.2 mol.) and *N*-benzylideneaniline (390 mg, 1 mol.) were added, and the solution was heated for 2.5 h at 60°. The red solution was taken to dryness, the residue was dissolved in benzene (50 ml), and the filtered solution was washed with 2N hydrochloric acid (3 × 15 ml), water, saturated, aqueous sodium hydrogen carbonate, and water, dried ($MgSO_4$), and evaporated. The residue was chromatographed on a column (30 × 1.5 cm) of silica gel by elution with benzene (100 ml) and benzene–ether 7:3 (150 ml). The appropriate fractions were combined and evaporated to yield a syrupy mixture of enaminolactones **3a** and **3b** (440 mg, 44.5%) which was treated with ether (5 ml). After storage overnight, the enaminolactone **3b** was collected by filtration, and the crystals were washed with boiling ether (2 × 3 ml) to give the analytical sample of **3b** (150 mg); m.p. 145–146°, $[\alpha]_D^{24}$ +23° (*c* 2.42, chloroform); the L-enantiomer[8] has m.p. 145–146°, $[\alpha]_D^{23}$ −25° (*c* 1.94, chloroform) (Found: C, 57.10; H, 5.50; N, 3.16. $C_{22}H_{25}NO_{10}$ calc.: C, 57.01; H, 5.44; N, 3.02%).

The mother liquor from the separation of **3b** was evaporated, and a solution of the residue in ether (2 ml) was kept overnight at room temperature to give an additional crop of **3b**. The residue obtained from the resulting mother liquor was dissolved in benzene and chromatographed on a small column of silica gel by elution with benzene–ether (7:3). Crystallisation of the product from ethyl acetate–light petroleum gave enaminolactone **3a** (120 mg), m.p. 115–117°, $[\alpha]_D^{21}$ −53° (*c* 2.14, chloroform); the L-enantiomer[8] has m.p. 115–117°, $[\alpha]_D^{20}$ +50° (*c* 2.5, chloroform) (Found: C, 57.08; H, 5.51. $C_{22}H_{25}NO_{10}$ calc.: C, 57.01; H, 5.44%).

*Ammonium 3-deoxy-*D-manno-*octulosonate*. — A solution of enaminolactone **3a** (100 mg) in 10 ml of 0.2N methanolic hydrogen chloride was heated under reflux for 2 h, water (2.5 ml) was added, and the solution was heated for 1 h at 80°. The cooled solution was neutralised with silver carbonate, filtered through a pad of Filter Cel, and passed through a column (3 × 0.7 cm) of cation-exchanger KU-2(H^+) (prewashed with 80% methanol) by elution with 80% methanol (50 ml). Methanol was distilled from the eluate under diminished pressure, and the residual, aqueous solution was treated with 0.1N NH_4OH to pH 0.9. After storage for 24 h at 0°, the solution was carefully neutralised with KU-2 resin to pH 7.0, filtered, decolorised with charcoal, and freeze-dried. The residue crystallised slowly (2 months) from 85% aqueous acetone (20 ml) at 0° to give several mg of ammonium 3-deoxy-D-*manno*-octulosonate, m.p. 120–123° (dec.); lit.[4] m.p. 121–123°.

Methanolysis of enaminolactones. — Enaminolactone **3a** or **3b** (10–15 mg) was heated under reflux with 5 ml of 0.2N methanolic hydrogen chloride for 2 h. The cooled solution was neutralised with silver carbonate, filtered, passed through a column (1.5 × 0.7 cm) of KU-2(H^+) (prewashed with methanol), eluted with methanol (10 ml), and evaporated. The product obtained migrated as a single spot (R_F 0.65) on a paper chromatogram in the solvent system butyl alcohol–acetic acid–water (4:1:1); it could be detected with periodate–benzidine and hydroxylamine–ferric chloride reagents. This product was trimethylsilylated according to the literature procedure[12].

REFERENCES

1 O. LÜDERITZ, A. M. STAUB, AND O. WESTPHAL, *Bacteriol. Rev.* 30 (1966) 192.
2 K. W. KNOX, *Biochem. J.*, 100 (1966) 73.
3 M. A. GHALAMBOR, E. M. LEVINE, AND E. C. HEATH, *J. Biol. Chem.*, 241 (1966) 3207.
4 C. HERSHBERGER, M. DAVIS, AND S. B. BINKLEY, *J. Biol. Chem.*, 243 (1968) 1585.
5 C. HERSHBERGER AND S. B. BINKLEY, *J. Biol. Chem.*, 243 (1968) 1578.
6 P. M. CARROL AND J. W. CORNFORTH, *Biochim. Biophys. Acta*, 39 (1960) 161.
7 R. KUHN AND G. BASCHANG, *Ann.*, 659 (1962) 156.
8 N. K. KOCHETKOV, B. A. DMITRIEV, AND L. V. BACKINOWSKY, *Izv. Akad. Nauk SSSR, Otd. Khim. Nauk*, (1968) 2341.
9 N. K. KOCHETKOV, B. A. DMITRIEV, AND L. V. BACKINOWSKY, *Carbohyd. Res.*, 5 (1967) 399.
10 E. D. STECHER AND E. GELBLUM, *J. Org. Chem.*, 26 (1961) 2693.
11 B. A. DMITRIEV, N. E. BAIRAMOVA, L. V. BACKINOWSKY, AND N. K. KOCHETKOV, *Dokl. Akad. Nauk SSSR*, 173 (1967) 350.
12 C. C. SWEELEY AND B. WALKER, *Anal. Chem.*, 36 (1964) 1461.

Carbohydrate Research
Elsevier Publishing Company, Amsterdam
Printed in Belgium

FURTHER STUDIES OF RUTHENIUM TETROXIDE AS AN OXIDANT FOR CARBOHYDRATE DERIVATIVES

P. M. COLLINS, P. T. DOGANGES, A. KOLARIKOL, AND W. G. OVEREND

Chemistry Department, Birkbeck College (University of London), Malet Street, London, W.C.1 (Great Britain)

(Received March 10th, 1969)

ABSTRACT

An extension is reported of the evaluation of ruthenium tetroxide as an oxidant for the conversion of partially protected carbohydrates into glycosulose derivatives. It has been demonstrated that free hydroxyl groups in benzoic and toluene-*p*-sulphonic esters, and in *N*-acetamido derivatives of glycosides can all be oxidised successfully by the reagent. It has been shown also that the oxidant converts 1,4:3,6-dianhydrohexitols into the corresponding 2,5-dione; no significant selectivity in the oxidation of *endo* and *exo* hydroxyl groups was noted.

Comment is made on the abnormally high optical rotation of the bis(2,4-dinitrophenylhydrazone) of 1,4:3,6-dianhydro-D-*threo*-hexo-2,5-diulose.

INTRODUCTION

Recently, we reported[1,2] on ruthenium tetroxide as an oxidant for a single hydroxyl group in partially protected sugars. Generally, yields of oxidation products were very good with alkylidene or arylidene derivatives, and this has been confirmed by other workers[3]. Apart from our report[1] of the successful oxidation of methyl 3,4,6-tri-*O*-benzoyl-α-D-glucoside to methyl 3,4,6-tri-*O*-benzoyl-α-D-*arabino*-hexopyranosidulose, less work seems to have been done with sugars partially protected by acyl groups. In this paper, we report on the oxidation of some partially acylated glycosides and of some 1,4:3,6-dianhydrohexitols.

RESULTS AND DISCUSSION

Both methyl 2,3,6-tri-*O*-benzoyl-α-D-galactopyranoside[4] (**1**) and the analogous glucoside[5] **2** could be oxidised with ruthenium tetroxide to give pure, crystalline methyl 2,3,6-tri-*O*-benzoyl-α-D-*xylo*-hexopyranosid-4-ulose (**3**) in good yield (81 and 79%, respectively). The pyranosidulose **3** afforded a 2,4-dinitrophenylhydrazone **4**. These yields of the ulose **3** were better than that obtained when methyl sulphoxide and acetic anhydride were used; it is noteworthy that Gabriel[6] recently reported difficulty in isolating this pyranosidulose from the Me_2SO–Ac_2O oxidising medium.

These oxidations further exemplify the conclusion[2] that ruthenium tetroxide appears to oxidise axial and equatorial hydroxyl groups with equal ease.

Other benzoates which can be oxidised successfully with ruthenium tetroxide are methyl 3,6-di-*O*-benzoyl-2-deoxy-α-D-*arabino*-hexopyranoside and phenyl 3,6-di-*O*-benzoyl-2-deoxy-α-D-*lyxo*-hexopyranoside.

The toluene-*p*-sulphonyl group is stable towards the oxidant, since methyl 4,6-*O*-benzylidene-2-*O*-toluene-*p*-sulphonyl-α-D-glucoside can be converted into the hexopyranosidulose **5** with ruthenium tetroxide. However, in this case, the oxidation procedure has no advantage over the method used previously[7].

The reagent can be used to oxidise acetamidoglycosides. For example, methyl 2-acetamido-4,6-*O*-benzylidene-2-deoxy-α-D-glucoside yielded the acetamidoglycosidulose **6**, in this case, by use of a catalytic amount of the tetroxide in the presence of metaperiodate solution. The method gives a reasonable yield, but not so good as that reported[8] by the use of the Pfitzner–Moffatt reagent.

Although ruthenium tetroxide will oxidise two suitably located hydroxyl groups within a molecule, attempts[9] to thus prepare an 'α-diketone' from methyl 4,6-*O*-benzylidene-α-D-glucopyranoside have failed so far. The product remains strongly attached to the ruthenium oxide. On the other hand, 1,4:3,6-dianhydrohexitols are oxidised, but there is no useful discrimination between *endo* and *exo* hydroxyl groups. This lack of selectivity contrasts with the selective, platinum-catalysed oxidation of *endo*-hydroxyl groups[10].

We were interested in studying the oxidation of the dianhydrohexitols, because of the dearth of information available about the related carbocyclic system, bicyclo-[3.3.0]octa-2,6-dione. Consequently, 1,4:3,6-dianhydro-D-mannitol (**7**), -L-iditol (**8**), and -D-glucitol (**9**) were oxidised by RuO_4 under identical conditions, and, in each case, the oxidation product (**10**) was isolated as the bis(2,4-dinitrophenylhydrazone) (**11**) (yields, 53, 43, and 48%, respectively). Although a trend indicating some preference for oxidation of *endo* over *exo* hydroxyl groups might be read into these results, we prefer not to do so, because the variation is only *ca.* 10% for the oxidation of the *endo–endo* derivative **7** and the *exo–exo* compound **8**, and, in no case, was a 2,4-dinitrophenylhydrazone of a mono-ulose obtained.

The molecular rotations for some anils derived from the diulose **10** are listed in Table I. It is clear that the bis(2,4-dinitrophenylhydrazone) **11** has a remarkably high optical rotation and one much larger than that ($[\alpha]_D$ +466°) of the *p*-nitrophenylhydrazone of 4,6-*O*-ethylidene-1,2-*O*-isopropylidene-D-*xylo*-hexopyran-3-ulose, which was the subject of recent comment[11]. Also, it is much larger than that ($[\alpha]_D$ +100°) of the 2,4-dinitrophenylhydrazone (**4**) derived from the uloside **3** or that ($[\alpha]_D$ +238°) of the 2,4-dinitrophenylhydrazone prepared from methyl 3,4,6-tri-*O*-benzoyl-α-D-*xylo*-hexopyranosidulose[1]. The explanation of the large rotation of compound **11** must be that the light used (sodium D-line, λ 589 nm) for the measurement was of a wavelength close to that at which the compound exhibited a Cotton effect. Compound **11** gives a typical ultraviolet spectrum for the 2,4-dinitrophenylhydrazone chromophore (Fig. 1). The o.r.d. curve (also shown in Fig. 1)

shows several Cotton effects in the wavelength range 220–330 nm, but, at 385 nm, a positive Cotton effect of large amplitude (a = 111,300) is centred*. This appears to be associated with the electronic transition which gives rise to the shoulder on the

TABLE I

SPECIFIC AND MOLECULAR ROTATIONS OF SOME NITROGENOUS DERIVATIVES OF 1,4:3,6-DIANHYDRO-D-*threo*-HEXO-2,5-DIULOSE

Derivative	$[\alpha]_D$ (*degrees*)	*Solvent*	$[M]$ (*degrees*)
Bis(2,4-dinitrophenylhydrazone)	+1670	$CHCl_3$ (*c* 0.1)	8383
Bis(*p*-nitrophenylhydrazone)	+ 582	Me_2CO (*c* 0.02)	2400
Bis(phenylhydrazone)	+ 770	$CHCl_3$ (*c* 0.1)	2480
Bis(1-naphthylhydrazone)	+ 934	$CHCl_3$ (*c* 0.04)	3940
Dioxime	+ 207	EtOH (*c* 0.06)	356

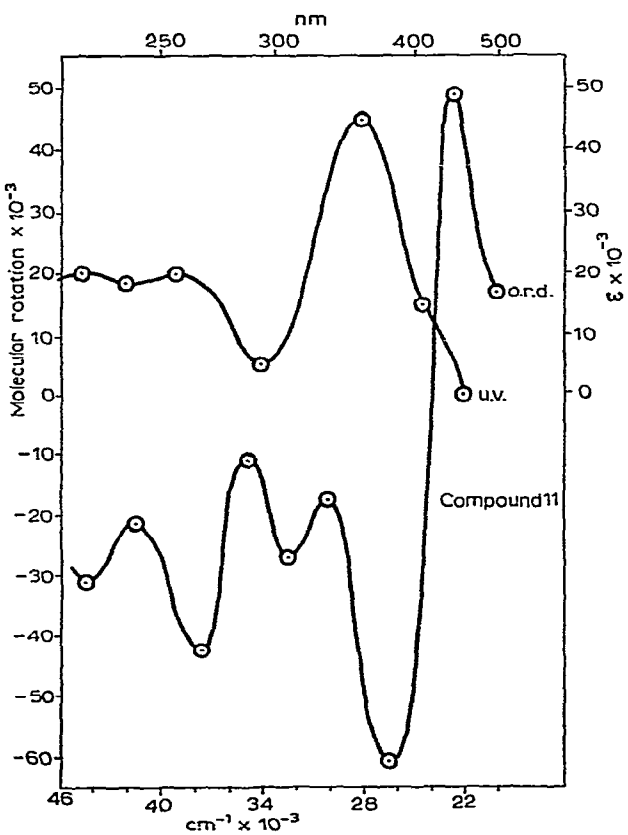


Fig. 1. Ultraviolet and o.r.d. curves of compound **11** in *p*-dioxane.

*It is because of the large amplitude that this o.r.d. spectrum could be measured with such a strongly absorbing compound.

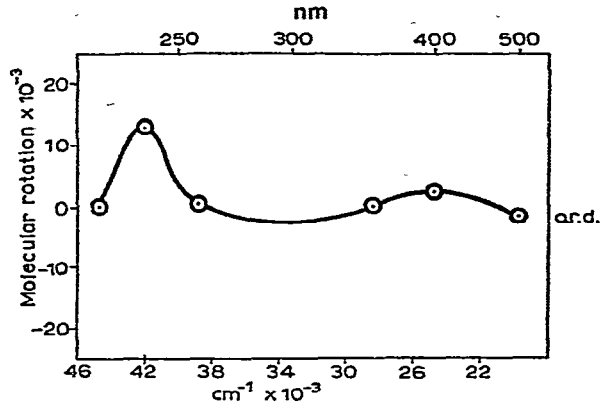


Fig. 2. O.r.d. curve of the bis(*p*-nitrophenylhydrazone) of the diulose **10**, measured in *p*-dioxane.

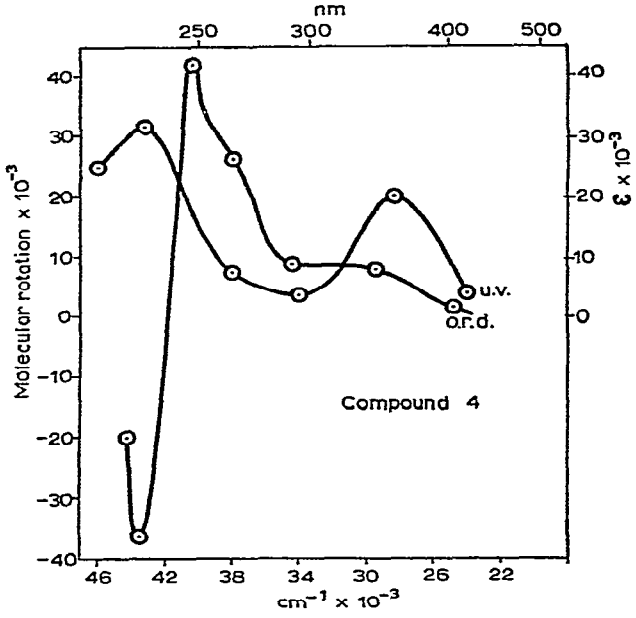


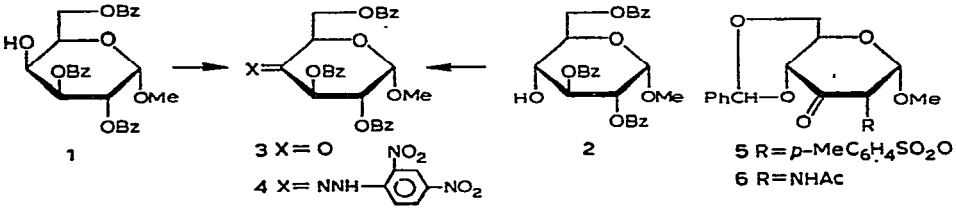
Fig. 3. Ultraviolet and o.r.d. curves of compound **4** in *p*-dioxane.

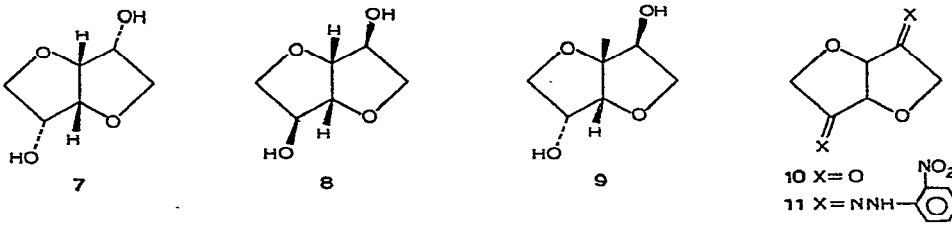
long-wavelength absorption band. The bis(*p*-nitrophenylhydrazone) of the diulose **10** shows only a weak Cotton effect at shorter wavelength (Fig. 2).

The 2,4-dinitrophenylhydrazone of the uloside **3** has the ultraviolet spectrum shown in Fig. 3. The o.r.d. curve of the compound shows a Cotton effect with a large amplitude (a = 78,000), but this is centred at shorter wavelength (*i.e.*, 238 nm) than for compound **11**.

Although the large, specific rotation of compound **11** is explicable by reference to its o.r.d. curve, the reason why this compound should exhibit a long-wavelength Cotton effect is, at present, a matter for speculation. Little work in this connection

has been done with 2,4-dinitrophenylhydrazones. Although Guthrie and co-workers[12] have found interesting results with phenylazo sugars, comparisons with our work are not meaningful, because of the difference in complexity of the two types of chromophore.





In some way, the large optical rotation of compound **11** would seem to be attributable to the *o*-nitro group, since the bis(*p*-nitrophenylhydrazone) of the diulose **10** does not have such a large rotation. However, the effect is not due solely to the presence of the 2,4-dinitrophenylhydrazone residue, regardless of its molecular environment, since the optical rotation of compound **4** is unexceptional. It is noteworthy that *o*-nitrophenyl glycosides have been found to have abnormally high optical rotations which exhibit a marked dependence on temperature, but the reason for this behaviour is not understood[13]. The phenomenon is being further investigated.

EXPERIMENTAL

Unless stated to the contrary, optical rotations were measured on chloroform solutions. Infrared spectra were determined with a Perkin–Elmer Infracord model 137; solids were dispersed in KBr discs, and gums were smeared on such discs. Ultraviolet spectra were measured with a Unicam SP 500 or 700 spectrometer on solutions in 'Spectrosol' grade solvents. N.m.r. spectra were obtained with a Varian A-60 spectrometer. O.r.d. curves were measured (solutions in *p*-dioxane) with a Bellingham and Stanley (Polarmatic 62) instrument. T.l.c. plates were coated with silica gel G (Merck), and compounds were located[14] with an ethanolic solution of anisaldehyde (0.1%) and sulphuric acid (5%).

Methyl 2,3,6-tri-O-benzoyl-α-D-xylo-hexopyranosid-4-ulose (**3**). — (*a*) Methyl 2,3,6-tri-*O*-benzoyl-α-D-glucopyranoside (**2**) (6 g) in methylene chloride (50 ml) was oxidised with ruthenium tetroxide (from 3.1 g of RuO_2) in carbon tetrachloride

(200 ml) for 4 h. Recrystallisation of the product from light petroleum (b.p. 60–80°)–ether gave the title compound (4.8 g, 79%) as needles, m.p. 121°, $[\alpha]_D$ +172° (*c* 1.8, methylene dichloride), ν_{max} 1700 cm^{-1}.

Anal. Calc. for $C_{28}H_{24}O_9$: C, 66.6; H, 4.8. Found: C, 65.6; H, 5.0.

The same compound was obtained in 64% yield by carrying out the oxidation of the glucoside **2** (5 g) with methyl sulphoxide (25 ml) and acetic anhydride (18 ml).

(*b*) Likewise, methyl 2,3,6-tri-*O*-benzoyl-α-D-galactopyranoside (**1**, 3 g) could be oxidised in methylene chloride with ruthenium tetroxide (from 1.5 g of RuO_2) in carbon tetrachloride. After 4 h at room temperature, excess of oxidant was destroyed by addition of isopropyl alcohol (1 ml), and the filtered solution was washed with water (2 × 50 ml), dried (Na_2SO_4), and evaporated. The residue was recrystallised as above to give the ulose (2.3 g, 81%), m.p. 121°. Oxidation of compound **1** (2 g) with methyl sulphoxide (8 ml) and acetic anhydride (5 ml) for 8 h gave the ulose (1.41 g) in 72% yield.

Treatment of crude ulose **3** (0.4 g) with 2,4-dinitrophenylhydrazine (0.16 g) in ethanol (50 ml) containing hydrochloric acid (0.002M) for 1 h at 75° gave the 2,4-dinitrophenylhydrazone **4** (0.2 g) as yellow crystals, m.p. 131–132°, $[\alpha]_D$ +100°; ν_{max} 3370 (NH), 1730 (ester C=O), and 1600 cm^{-1}; n.m.r. data [$(CD_3)_2SO$]: τ 6.41 (3H, methoxyl); 5.0 (3H, H-6, H-6′, and H-5); 4.67 (1-proton doublet, 3.5 Hz, H-1); 4.2 (1-proton quartet, 3.5 and 7.8 Hz, H-2); 3.62 (1-proton doublet, 7.8 Hz, H-3); 1.7–2.8 (17-proton multiplet, three benzoyl groups and two protons of the dinitrophenylhydrazino residue); 0.97 (1-proton doublet, 2.5 Hz, proton situated between the two nitro groups); δ 11.8 (broad NH signal).

Anal. Calc. for $C_{34}H_{28}N_4O_{12}$: C, 59.6; H, 4.1; N, 8.2. Found: C, 59.6; H, 4.4; N, 7.8.

Methyl 3,6-di-O-benzoyl-2-deoxy-α-D-threo-hexosid-4-ulose. — A solution of methyl 3,6-di-*O*-benzoyl-2-deoxy-α-D-*arabino*-hexopyranoside[15] (5 g) in dry carbon tetrachloride (40 ml) at 0° was treated for 5 h with ruthenium tetroxide (from 3 g of the dioxide) in carbon tetrachloride. Excess of oxidant was eliminated by addition of isopropyl alcohol (1.5 ml), and the filtered solution was dried (Na_2SO_4) and concentrated to a syrup which was triturated with 60% ethanol. The resulting prisms were recrystallised from light petroleum (b.p. 60–80°)–ether to afford the title compound (3.4 g, 68%), m.p. 88–89°, $[\alpha]_D$ +125° (*c* 1, methylene dichloride), ν_{max} 1700 cm^{-1}.

Anal. Calc. for $C_{21}H_{20}O_7$: C, 65.6; H, 5.3. Found: C, 65.5; H, 5.3.

Phenyl 3,6-di-O-benzoyl-2-deoxy-α-D-threo-hexosid-4-ulose. — A solution of phenyl 3,6-di-*O*-benzoyl-2-deoxy-α-D-*lyxo*-hexopyranoside (2 g) in methylene chloride (20 ml) was treated at 0° with a solution of ruthenium tetroxide (from 1.2 g of the dioxide) in carbon tetrachloride for 45 min. The product was isolated as described previously and was recrystallised from light petroleum (b.p. 60–80°)–ether to give the title compound (0.91 g, 48%) as needles, m.p. 118°, $[\alpha]_D$ +153° (*c* 1, methylene dichloride), which soon became hydrated.

Anal. Calc. for $C_{26}H_{22}O_7$: C, 69.7; H, 5.0. Found: C, 69.7; H, 5.1.

Methyl 4,6-O-benzylidene-2-O-toluene-p-sulphonyl-α-D-ribo-hexopyranosid-3-ulose (**5**). — Methyl 4,6-*O*-benzylidene-2-*O*-toluene-*p*-sulphonyl-α-D-glucopyranoside (2 g) was oxidised for 20 h in the usual manner with ruthenium tetroxide (prepared from 0.66 g of the dioxide). The crude material (1.46 g) isolated was recrystallised from methylene dichloride (8 ml) and light petroleum (b.p. 40–60°) (25 ml) to yield starting material (0.91 g). The mother liquors were concentrated to a residue which, after recrystallisation (twice) from ethanol, gave compound **5** (0.3 g), m.p. 164–166°, $[\alpha]_D$ +42° (*c* 0.5, *N,N*-dimethylformamide); lit.[7], m.p. 165–167°, $[\alpha]_D$ +45° (*N,N*-dimethylformamide).

Treatment of the starting material with sodium metaperiodate and a catalytic amount of ruthenium dioxide afforded less product.

Methyl 2-acetamido-4,6-O-benzylidene-2-deoxy-α-D-ribo-hexopyranosid-3-ulose (**6**). — A solution of sodium metaperiodate (2.3 g) in water (150 ml) containing ruthenium dioxide (0.06 g) was mixed with methyl 2-acetamido-4,6-*O*-benzylidene-2-deoxy-α-D-glucoside (1.5 g) in methylene chloride (300 ml). The mixture was stirred for 4 h at room temperature, and the pH was maintained at 6–7 by addition of 2N aqueous sodium hydroxide. When t.l.c. monitoring showed the absence of starting material, the solution was treated with isopropyl alcohol (1 ml), filtered, and concentrated to a solid residue (1.1 g) which, on recrystallisation from acetone–light petroleum (b.p. 40–60°), afforded compound **6** (0.87 g, 58%), m.p. 227–229° (dec.), $[\alpha]_D^{20}$ +88° (*c* 1.1); ν_{max} 3400 (NH), 1770 (C=O), 1660 and 1565 (amide) cm^{-1}; lit.[8], m.p. 227–228° (dec.), $[\alpha]_D$ +110° ±4° (*N,N*-dimethylformamide).

Anal. Calc. for $C_{16}H_{19}NO_6$: C, 59.8; H, 5.96; N, 4.4. Found: C, 59.4; H, 6.45; N, 4.2.

1,4:3,6-Dianhydro-D-threo-hexo-2,5-diulose bis(2,4-dinitrophenylhydrazone) (**11**). — This compound was prepared from the 1,4:3,6-dianhydrides of D-mannitol[16] (**7**), L-iditol[17] (**8**), and D-glucitol[18] (**9**), in separate experiments under identical conditions. The dianhydride (0.58 g) in methylene chloride (500 ml) was treated, in the usual way, with ruthenium tetroxide prepared from ruthenium dioxide (1.1 g). After 4 h, the reaction was terminated by addition of isopropyl alcohol (0.5 ml). The mixture was filtered, and the dioxide was washed with acetone. The combined filtrate and washings were evaporated to a residue which was treated with 2,4-dinitrophenylhydrazine. The bis(dinitrophenylhydrazone) had m.p. 245–246° (dec.), $[\alpha]_D$ +1670° (*c* 1), irrespective of the starting material; lit.[10] m.p. 248°. N.m.r. data [$(CD_3)_2SO$]: τ 5.24 (4-proton, broad signal, pair of methylene groups); 4.16 (2-proton, broad signal, two bridge-head methine protons); 2.12 (1-proton doublet, 10 Hz, proton adjacent to the hydrazino substituent); 1.09 (1-proton doublet, 2.5 Hz, the proton between the two nitro-groups); 1.54 (1-proton quartet, 10 and 2.5 Hz, the remaining aromatic ring proton); δ 11.6 (broad NH signal).

Anal. Calc. for $C_{18}H_{14}N_8O_{10}$: C, 43.0; H, 2.8; N, 22.3. Found: C, 43.0; H, 2.7; N, 21.95.

ACKNOWLEDGMENTS

We thank Dr. P. M. Scopes of Westfield College (University of London), for measuring the o.r.d. spectra, and Mr. D. Marshall of West Ham College of Technology for helpful discussions.

REFERENCES

1 P. J. BEYNON, P. M. COLLINS, AND W. G. OVEREND, *Proc. Chem. Soc.*, (1964) 342; P. J. BEYNON, P. M. COLLINS, P. T. DOGANGES, AND W. G. OVEREND, *J. Chem. Soc.*, (1966) 1131.
2 P. J. BEYNON, P. M. COLLINS, D. GARDINER, AND W. G. OVEREND, *Carbohyd. Res.*, 6 (1968) 431.
3 R. F. NUTT, B. ARISON, F. W. HOLLY, AND E. WALTON, *J. Amer. Chem. Soc.*, 87 (1965) 3273; R. F. NUTT, M. J. DICKINSON, F. W. HOLLY, AND E. WALTON, *J. Org. Chem.*, 33 (1968) 1789; V. M. PARIKH AND J. K. N. JONES, *Can. J. Chem.*, 43 (1965) 3452; G. B. HOWARTH, W. A. SZAREK, AND J. K. N. JONES, *Carbohyd. Res.*, 7 (1968) 284; D. HORTON AND J. S. JEWELL, *ibid.*, 5 (1967) 149.
4 A. C. RICHARDSON AND J. M. WILLIAMS, *Chem. Commun.*, (1965) 104; *Tetrahedron*, 23 (1967) 1369.
5 E. J. REIST, R. R. SPENCER, D. F. CALKINS, B. R. BAKER, AND L. GOODMAN, *J. Org. Chem.*, 30 (1965) 2312.
6 O. GABRIEL, *Carbohyd. Res.*, 6 (1968) 319.
7 B. R. BAKER AND D. H. BUSS, *J. Org. Chem.*, 30 (1965) 2304.
8 B. R. BAKER AND D. H. BUSS, *J. Org. Chem.*, 30 (1965) 2308.
9 Personal communication from D. GARDINER.
10 K. HEYNS, W. P. TRAUTWEIN, AND H. PAULSEN, *Ber.*, 96 (1963) 3195.
11 G. J. F. CHITTENDEN, *Chem. Commun.*, (1968) 779.
12 E. O. BISHOP, G. J. F. CHITTENDEN, R. D. GUTHRIE, A. F. JOHNSON, AND J. F. MCCARTHY, *Chem. Commun.*, (1965) 93.
13 B. CAPON, W. G. OVEREND, AND M. SOBELL, *J. Chem. Soc.*, (1961) 5172.
14 E. STAHL AND U. KALTENBACH, *J. Chromatog.*, 5 (1961) 351.
15 A. KOLARIKOL, Ph.D Thesis, London University, 1968.
16 L. F. WIGGINS, *J. Chem. Soc.*, (1945) 4.
17 L. F. WIGGINS, *J. Chem. Soc.*, (1947) 1403.
18 R. MONTGOMERY AND L. F. WIGGINS, *J. Chem. Soc.*, (1946) 390.

Carbohydrate Research
Elsevier Publishing Company, Amsterdam
Printed in Belgium

COMPARATIVE STUDIES OF LORENZINI JELLY FROM TWO SPECIES OF ELASMOBRANCH

PART I. PREPARATION OF GLYCOPEPTIDES

M. J. How*, J. V. S. Jones
Department of Chemistry, The University, Edgbaston, Birmingham 15 (Great Britain)
AND (IN PART) J. Doyle
The Scottish Marine Biological Association, Millport (Great Britain)

(Received March 12th, 1969)

ABSTRACT

Glycopeptides from the Lorenzini jelly of two species of elasmobranch have been prepared and their sugar and amino acid components assayed. Preliminary structural studies are reported.

INTRODUCTION

The ampullae and tubes of the organs of Lorenzini, specialised sense organs that occur in elasmobranch fish, are filled with a hyaline jelly that contains a protein–polysaccharide complex designated[1] as 'Lorenzan sulphates'. An electrophoretically homogeneous polysaccharide purified from papain digests of the jelly from *Squalus acanthias* was reported to contain 2-amino-2-deoxyglucose, 2-amino-2-deoxygalactose, galactose, sulphate, acetyl groups, and peptide, and traces of hexuronic acid. It was considered to be structurally more closely related to keratan sulphate than to the chondroitin sulphates. Subsequent, comparative, analytical studies[2] showed that the crude jelly from a number of elasmobranch species contained different proportions of the above components, and that the ratio 2-amino-2-deoxyglucose:2-amino-2-deoxygalactose varied from 8:1 to 1:8. The present paper describes more-detailed analytical studies of glycopeptides obtained by pronase-digestion of the jellies from *Raja clavata* (Ray) and *Cetorhinus maximus* (Basking shark).

MATERIALS AND METHODS

Collection of jelly. — Jelly from freshly caught specimens of *Raja clavata* was expressed from the pores by pressure on the skin surface with a spatula. Contamination with surface mucins was avoided, and material from several fish was pooled. A sample of the jelly from a single specimen of *Cetorhinus maximus* was collected by dissection. In both cases, the wet jelly was stored at −25° prior to homogenisation and dehydration with acetone in a high-speed macerator.

*Present address: Unilever Research Laboratory, Colworth House, Sharnbrook, Bedford, Great Britain.

Preparation and purification of glycopeptides from acetone-dried jelly. — Pronase B (Calbiochem., 60 mg) in 0.1M Tris (pH 8, 250 ml) containing $CaCl_2$ (10mM) was added to acetone-dried Lorenzini jelly (2.5 g), and the solution was incubated for 24 h at 37° and then clarified by centrifugation, dialysed, and freeze-dried. The product was re-incubated with pronase as described. The centrifuged, dialysed product-mixture was concentrated by freeze-drying, and purified by gel chromatography on Sephadex G-100 (Figs. 1a, 1b), by stepwise elution from DEAE-Sephadex (Cl^- form) in 0.1M Tris buffer, pH 8, with increasing concentrations of sodium chloride (Figs. 2a, 2b), and finally by gel chromatography on Bio-Gel P-300 (Figs. 3a, 3b). Fractions of column eluates were collected automatically and scanned for carbohydrate with phenol–sulphuric acid[3] (Method A, absorbance measured at 490 nm), for protein by the method of Lowrie *et al.*[4] (Method B, absorbance measured at 650 nm), and for absorption at 260 nm (Method C). The fractionation schemes, with yields, are summarised in Fig. 4. Glycopeptides from *Raja clavata* and *Cetorhinus maximus* are designated by R and S, respectively, followed by a number related to the elution point of the glycopeptide from DEAE-Sephadex.

Analytical methods. — The electrophoretic homogeneity of 1% (w/v) solutions of the glycopeptides at pH 5, 7, and 8 was investigated by using a Carl Zeiss Jena IDDR electrophoresis apparatus. I.r. spectra were determined on discs containing glycopeptide (1.5 mg) and KBr (300 mg). The following solvent systems were used for paper chromatography (Whatman No. 1 paper): (*A*) pyridine–ethyl acetate–acetic acid–water[5] (5:5:1:3); (*B*) butanone–acetic acid–saturated, aqueous boric acid[6] (9:1:1).

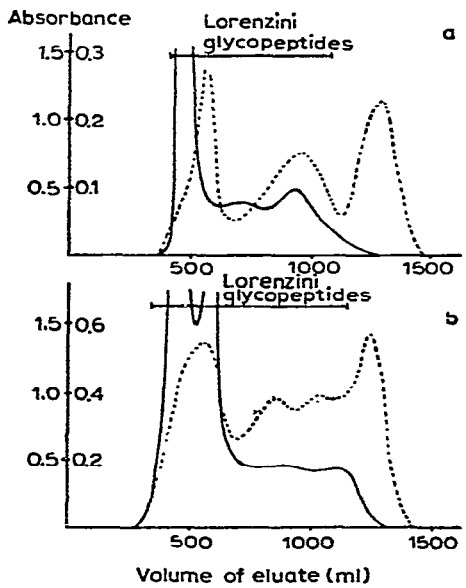


Fig. 1. Elution of pronase-digested Lorenzini jelly from Sephadex G-100 with 0.9 per-cent sodium chloride solution. Fractions assayed by methods A (————) and B (– – – – –). (a) *Raja clavata*, (b) *Cetorhinus maximus*.

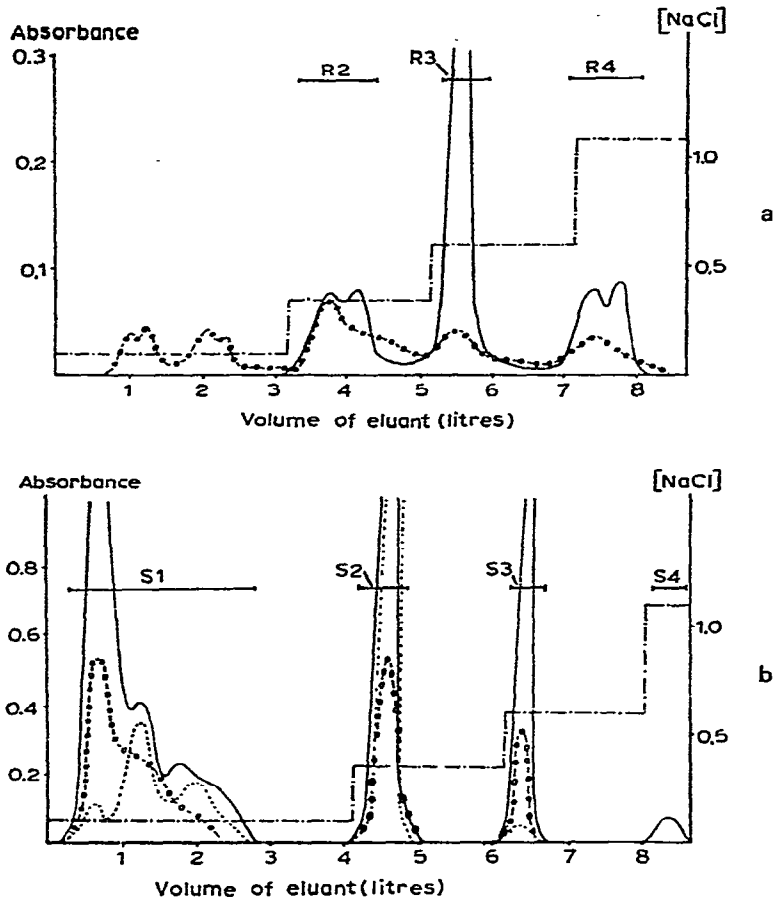


Fig. 2. Elution of Lorenzini glycopeptides (see Figs. 1a, 1b) from DEAE-Sephadex. Fractions assayed by methods A (————), B (– – – –), and C (—°—°—°—). Sodium chloride concentration (—•—•—). (a) *Raja clavata*, (b) *Cetorhinus maximus*.

Glycopeptides (1–2 mg) were hydrolysed in 2N sulphuric acid (1 ml) at 100° (sealed tube) for 6 h, and the neutralised [$Ba(OH)_2$] hydrolysate was analysed by paper chromatography with solvent *A*. Part of the hydrolysate was heated with ninhydrin solution[7], concentrated, and analysed by paper chromatography in solvent *B*. Paper electrophoresis[8] (Whatman 3MM paper) was carried out in acetate buffer, pH 6.0.

Gas-liquid chromatography (g.l.c.). Basic components in glycopeptide hydrolysates were absorbed on Dowex-50 (H^+) resin. Neutral components, eluted from the resin with water, were converted[9] into alditol acetates and analysed by using a temperature-programmed, Pye 104 gas chromatograph and a column packing of 2% ECNSS-M on Gas Chrom Q (60–80 mesh, Applied Science Lab. Inc., State College, Pa., U. S. A.) heated from 150–200° at 1.5°/min.

Chemical analyses. Reducing sugars were determined[10] by using alkali ferri-

resin. Total 2-amino-2-deoxyhexose was determined by the method of Gatt and Berman[13]. To determine the enantiomeric nature of galactose and 2-amino-2-deoxygalactose, glycopeptides were hydrolysed (2N hydrochloric acid, 100°, 6 h) in sealed tubes, and neutral and basic components of the hydrolysate were separated by using Dowex-50 × 8 (H^+) resin. Neutral components were eluted with water and analysed for galactose by the cysteine–sulphuric acid method, and for D-galactose by using D-galactose oxidase[14]. Basic components were eluted with 2N hydrochloric acid, and the acid was removed by rotary evaporation with addition of water, followed by concentration to dryness *in vacuo* over potassium hydroxide. An aliquot of an aqueous solution of the residue was analysed for content of 2-amino-2-deoxy-D-galactose by reaction with D-galactose oxidase. Hexuronic acid was determined by a modification[15] of the Dische[16] carbazole reaction. Acetyl group analysis was by the method of Ludowieg and Dorfman[17], and sulphate analysis by the method of Jones and Letham[18]. Phosphorus was determined by the method of Dryer *et al.*[19], and ash (as sodium) by flame photometry. Amino acid residues present after hydrolysis of glycopeptides with 6N hydrochloric acid for 22 h at 111° under nitrogen in a sealed tube were determined by using a Technicon autoanalyser. Prior to hydrolysis, the glycopeptides were treated[20] with performic acid to oxidise cysteine and methionine residues to cysteic acid and methionine sulphone, respectively.

Rechromatography of glycopeptides S1, S2, S3, and R3 on DEAE-Sephadex. Solutions of glycopeptides (2 mg) in 0.1M Tris buffer, pH 8 (S2, S3, and R3), or 0.05M Tris buffer, pH 8 (S1), were eluted from DEAE-Sephadex (Cl^- form, 75 × 1 cm) in the appropriate buffer, by using buffer (30–60 ml) followed by a salt gradient determined by the elution properties of the original glycopeptides on DEAE-Sephadex. Fractions (2 ml) were collected automatically, and aliquots were scanned by the phenol–sulphuric acid assay. The salt concentration in the eluate was determined conductimetrically. The elution profiles obtained are shown in Fig. 5. For S2, the contents of appropriate tubes were pooled, dialysed, and freeze-dried to give fractions S2a,b,c,d,e.

RESULTS

Preparation of glycopeptides. — Free protein was removed from the pronase-digest of pooled Lorenzini jelly (*Raja clavata*) by fractionation on Sephadex G-100 (Fig. 1a). Three glycopeptides (R2, R3, and R4), obtained from the carbohydrate-containing components of the digest by stepwise elution of the mixture from DEAE-Sephadex with 0.25, 0.5, and M sodium chloride (Fig. 2a), each contained a component that absorbed strongly at 260 nm. This material was removed from glycopeptides R3 and R4 by chromatography on Bio-Gel P-300, but purification of glycopeptide R2, of apparently lower molecular weight than R3 or R4, was not possible by this method (Fig. 3a). Glycopeptides R3 and R4, thus purified, were electrophoretically homogeneous at pH 5, 7, and 8, and were totally excluded from Bio-Gel P-300. Fractionation on DEAE-Sephadex of the pronase digest of an individual sample of jelly from

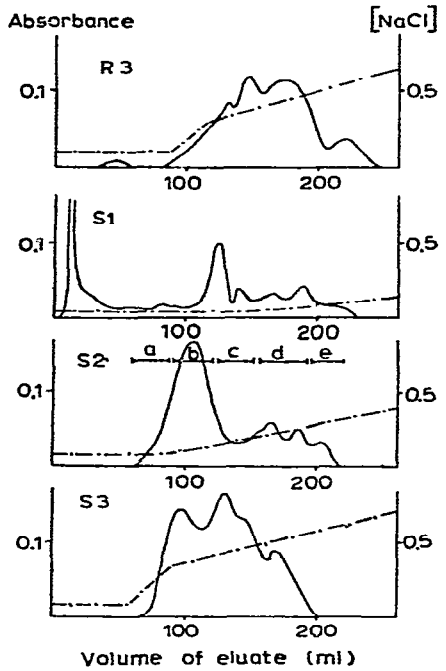


Fig. 5. Elution of Lorenzini glycopeptides R3, S1, S2, and S3 (see Figs. 3a, 3b) from DEAE-Sephadex Fractions assayed by method A (————). Sodium chloride concentration (—•—•—).

Cetorhinus maximus gave four components (Fig. 2b), one (S1) of which was eluted with buffer alone. The elution points of the three other components (S2, S3, S4) of the digest corresponded with those of glycopeptides R2, R3, and R4, respectively. Glycopeptides S1, S2, and S3 contained an impurity, apparently similar to that found in glycopeptides from *Raja clavata*, which was removed by fractionation on Bio-Gel P-300 (Fig. 3b). The purified glycopeptide S3 was totally excluded from Bio-Gel P-300, and the apparently high molecular weight fractions of S2 and S3 were selected for further investigations. Glycopeptides S1, S2, and S3, thus purified, were electrophoretically homogeneous at pH 5, 7, and 8.

Infrared analysis. — The spectrum of the crude jelly from both species showed absorption bands at 1240 (S=O stretching, characteristic of sulphate), and 1640 and 1540 cm^{-1} (strong, acetamido groups). The latter absorption bands were present in the spectra of all of the purified glycopeptides, but the intensity of the absorptions due to sulphate in the glycopeptides from *Cetorhinus maximus* increased in the order S1<S2<S3, and in the glycopeptides from *Raja clavata* in the order R2<R3<R4. Absorption bands in the region 780–820 cm^{-1} in the spectra of all of the glycopeptides may be diagnostic of equatorial, rather than axial, sulphate ester residues.

Chemical analyses. — Acid hydrolysis of glycopeptides R3, R4, S1, S2, and S3 liberated components indistinguishable (paper chromatography) from galactose, 2-amino-2-deoxyglucose, and 2-amino-2-deoxygalactose, together with a component,

tentatively identified, from its paper-chromatographic mobility, as a disaccharide. Visual estimation of chromatograms of hydrolysates of identical amounts of the glycopeptides indicated that the ratio of the two 2-amino-2-deoxyhexoses in all of the glycopeptides was different. This was subsequently confirmed by quantitative analyses (Table I). Treatment of glycopeptide hydrolysates with ninhydrin yielded components indistinguishable (paper chromatography) from lyxose and arabinose.

TABLE I

CHEMICAL ANALYSES OF GLYCOPEPTIDES OBTAINED FROM PRONASE-DIGESTED JELLY OF *Cetorhinus maximus* (S1, S2, S3) AND *Raja clavata* (R3, R4)

Component (μmoles/mg)[a]	*Glycopeptide*				
	S1	*S2*	*S3*	*R3*	*R4*
D-Galactose[c]	1.85	1.77	1.99	2.17	1.44
6-Deoxyhexose (as fucose)[c]	0.12	0.20	0.25	0.04	0.04
Total hexosamine[d]	1.40	1.39	1.70	1.65	1.33
2-Amino-2-deoxyglucose[e]	0.31	0.68	1.43	1.38	1.21
2-Amino-2-deoxy-D-galactose[f]	1.09	0.71	0.27	0.27	0.12
Reducing sugar	1.61	0.97	0.37	0.45	[b]
Acetyl	1.74	1.58	1.84	1.74	1.26
Sulphate	0.18	0.31	1.31	1.74	[b]
Ash (as Na)	0.23	0.87	2.87	2.48	2.91
Phosphorus	0.10	0.06	0.04	0.04	[b]
Threonine	1.05	0.84	0.14	0.26	0.16
Glutamic Acid	0.03	0.05	0.06	0.16	0.10
Proline	0.29	0.22	0.13	0.07	0.16
Alanine	0.06	0.07	0.13	0.08	0.04
Valine	0.21	0.18	0.06	0.17	0.03
Isoleucine	0.31	0.22	0.02	0.06	0.04
Lysine	0.33	0.23	0.03	0.05	0.03
Peptide	2.28	1.81	0.57	0.85	0.56

[a]Values are not corrected for ash content. [b]Not determined. [c]Cysteine–sulphuric acid assay. [d]Gatt and Berman assay. [e]By difference. [f]By D-galactose oxidase.

A minor component of hydrolysates of S1, S2, and S3 had a similar mobility to that of fucose or xylose. The presence of fucose, and absence of xylose, in S2 was confirmed by g.l.c. Paper-electrophoretic analysis of glycopeptide hydrolysates did not reveal anionic, reducing sugars.

The glycopeptides examined gave unexpectedly high values for reducing sugar, as estimated from their reactions with alkaline ferricyanide. Quantitative analyses of the purified glycopeptides for sugars, amino acids, and other components are summarised in Table I. Maximal release of 2-amino-2-deoxyhexose was obtained by hydrolysis of glycopeptides with 4N hydrochloric acid for 4.5 h at 100° or with 2N hydrochloric acid for 10 h at 100°.

Analysis, using D-galactose oxidase, of the neutral and basic sugars liberated on hydrolysis and subsequently separated on an ion-exchange resin showed that galactose and 2-amino-2-deoxygalactose were present exclusively as the D-enan-

tiomer. The molar ratio of galactose to hexosamine in all of the glycopeptides analysed was *ca.* 1.2:1 and that of hexosamine to acetyl was *ca.* 1:1.

The glycopeptides from *Cetorhinus maximus* contained less sulphate than those from *Raja clavata*, consistent with the infrared spectroscopic data. Colorimetric analyses of the glycopeptides from both species indicated a hexuronic acid content of 2–3% (w/w). The colour produced had λ_{max} 530 nm, typical of that given by authentic D-glucuronic acid. Similar analyses of galactose, in amounts found in the glycopeptides, gave an identical colour equivalent to 2–3% hexuronic acid. The absorption spectra given by the glycopeptides and of galactose in the assay were identical. Thus, the colour reaction given by the glycopeptides was probably due to components other than hexuronic acid.

The peptide contents of the whole jelly from *Raja clavata* and *Cetorhinus maximus* were 20 and 40%, respectively. The distribution of amino acids in hydrolysates of the jelly from both species was notable for the absence of cysteine, methionine, and tyrosine, and for the presence in the jelly of *Cetorhinus maximus* of excessive amounts of threonine. The most-sulphated glycopeptides contained the least peptide (Table I), and all of the glycopeptides showed the expected disproportionate distribution of amino acids. 2-Amino-2-deoxy-D-galactose and threonine were present in approximately equimolar amounts in all of the glycopeptides, and the amount of these components was particularly large in glycopeptides S1 and S2.

Chemical analyses suggested that the glycopeptides derived from each species could be represented by a spectrum of molecules from those of high 2-amino-2-deoxy-D-galactose and peptide contents and low sulphate and 2-amino-2-deoxyglucose contents, to those of low 2-amino-2-deoxy-D-galactose and peptide contents and high sulphate and 2-amino-2-deoxyglucose contents. Further evidence for this trend was obtained by fractionation of glycopeptide S2 by gradient elution from DEAE-Sephadex. The analyses (Table II) showed a similar spectrum to that given by S1, S2, and S3, but with a narrower range. Insufficient material was available for amino acid analyses of these subfractions of S2. The elution profiles for all other

TABLE II

CHEMICAL ANALYSES OF SUBFRACTIONS OBTAINED FROM GLYCOPEPTIDE S2 BY CHROMATOGRAPHY ON DEAE-SEPHADEX

Subfraction	*Total hexosamine*	*Component (molar ratio to galactose)*			
		2-Amino-2-deoxyglucose	*2-Amino-2-deoxy-D-galactose*	*Sulphate*	*Fucose*
$S2_a$	0.77	0.18	0.59	0.11	0.08
$S2_b$	0.78	0.19	0.59	0.12	0.09
$S2_c$	0.80	0.37	0.43	0.19	0.10
$S2_d$	0.83	0.36	0.46	0.40	0.11
$S2_e$	0.84	0.58	0.27	0.43	0.11
S2	0.79	0.38	0.40	0.18	0.11

glycopeptides showed similar variations, apparently related to charge density, to those observed with S2, but chemical analyses of subfractions of glycopeptides other than S2 were not made.

DISCUSSION

The glycopeptides obtained by pronase-digestion of the Lorenzini jelly from both *Raja clavata* and *Cetorhinus maximus* are sulphated, and all contain the same monosaccharide components, namely D-galactose, 2-amino-2-deoxyglucose and 2-amino-2-deoxy-D-galactose (probably as the *N*-acetylated derivatives), and fucose, and the same amino acids. Quantitative analyses, however, suggest that, for each species, the glycopeptides can be represented by a spectrum ranging from molecules of high 2-amino-2-deoxy-D-galactose and peptide contents and low 2-amino-2-deoxyglucose and sulphate contents, to those of low 2-amino-2-deoxy-D-galactose and peptide contents and high 2-amino-2-deoxyglucose and sulphate contents. Further evidence for this is provided by analyses of subfractions of S2 obtained by chromatography of that glycopeptide on DEAE-Sephadex. The similarity of the analytical values for glycopeptides S3 and R3 may reflect the presence of certain similar structural features in the jelly from the two species, which was not evident from analyses of the whole jelly[1]. Analytical data indicate that the polysaccharide–protein complex(es) in the Lorenzini jelly are structurally more complicated than those of the glycosaminoglycans of ground substance.

The conclusion that terminal, non-reducing, D-galactosyl residues occur in all of the glycopeptides, as indicated by reaction with D-galactose oxidase, was supported by qualitative, immunochemical studies of S2, which precipitated part of the antibody from Type XIV antipneumococcal serum[21]. The high values given by the glycopeptides in the alkaline ferricyanide reaction for reducing sugars indicate the presence of alkali-labile linkages which, on cleavage, expose reducing sugar residues. Highest "reducing sugar" values were given by those glycopeptides containing large amounts of 2-amino-2-deoxy-D-galactose and threonine, and these glycopeptides probably contain alkali-labile 2-acetamido-2-deoxy-D-galactosylthreonine linkages between carbohydrate and peptide. The structural importance of *O*-glycosylated hydroxyamino acids in glycoproteins and sulphated glycosaminoglycans is well known[22]. Detailed studies of the alkali-lability of the glycopeptides isolated from the two species of elasmobranch are reported in the following paper[23].

The composition of the Lorenzini jelly from fish of the same species may vary in a number of ways, *e.g.*, age-dependent variations, variations among the ampullae or groups of ampullae of one specimen, and variations along the length or across the diameter of the ampullary tubes. All of these factors are relevant when considering analyses of the pooled jelly from *Raja clavata* used in this work, although Doyle[1] observed no major difference in the composition of jelly from four specimens of *Squalus acanthias*. The use of jelly from one specimen of *Cetorhinus maximus* eliminates one such variable. The observed difference in charge density of glyco-

peptides obtained from the two species may reflect the different methods used to extract the whole jelly. Thus, jelly from *Cetorhinus maximus* was taken from the whole length of the ampullary tube, whereas that from *Raja clavata* was inevitably rich in jelly from the distal(pore) end. Variations in the potassium and urea contents of the Lorenzini jelly along the ampullary tube have been reported[24].

Glycopeptides produced by proteolytic digestion of the whole jelly are amenable to structural analysis, but whether such products are derived from one (or more) protein–polysaccharide complex(es) in the whole jelly cannot be determined from the present work.

ACKNOWLEDGMENTS

We thank Professor M. Stacey, C.B.E., F.R.S. for his interest in this work; Dr. Michael Heidelberger, New York University Medical School, for immunochemical studies; and the Science Research Council for financial support.

REFERENCES

1 J. DOYLE, *Biochem. J.*, 88 (1963) 7P; 103 (1967) 325.
2 J. DOYLE, *Comp. Biochem. Physiol.*, 24 (1968) 479.
3 M. DUBOIS, K. A. GILLES, J. K. HAMILTON, P. A. REBERS, AND F. SMITH, *Anal. Chem.*, 28 (1956) 350.
4 O. H. LOWRIE, N. J. ROSEBROUGH, A. L. FARR, AND R. J. RODELL, *J. Biol. Chem.*, 193 (1957) 265.
5 F. G. FISCHER AND H. DORFEL, *Z. Physiol. Chem.*, 301 (1955) 224.
6 W. R. REES AND T. REYNOLDS, *Nature*, 181 (1958) 767.
7 P. J. STOFFYN AND R. W. JEANLOZ, *Arch. Biochem. Biophys.*, 52 (1954) 373.
8 A. B. FOSTER, *Chem. Ind.* (London), (1952) 1050.
9 P. ALBERSHEIM, D. J. NEVINS, P. D. ENGLISH, AND A. KARR, *Carbohyd. Res.*, 5 (1967) 340.
10 O. FOLIN AND H. MALMROS, *J. Biol. Chem.*, 83 (1929) 115.
11 Z. DISCHE, *Methods Biochem. Anal.*, 2 (1955) 313.
12 S. GARDELL, *Acta Chem. Scand.*, 7 (1953) 207.
13 R. GATT AND E. R. BERMAN, *Anal. Biochem.*, 15 (1966) 167.
14 G. AVIGAD, C. ASENIO, D. AMARAL, AND D. HORECKER, *Biochem. Biophys. Res. Commun.*, 4 (1961) 474.
15 T. BITTER AND H. M. MUIR, *Anal. Chem.*, 34 (1962) 330.
16 Z. DISCHE, *J. Biol. Chem.*, 167 (1947) 189.
17 J. LUDOWIEG AND A. DORFMAN, *Biochim. Biophys. Acta*, 38 (1960) 212.
18 A. S. JONES AND D. S. LETHAM, *Analyst*, 81 (1956) 15.
19 R. L. DRYER, A. R. TAMMES, AND J. I. ROUTH, *J. Biol. Chem.*, 225 (1957) 177.
20 D. S. BIDMEAD AND F. J. LEY, *Biochim. Biophys. Acta*, 29 (1958) 562.
21 M. HEIDELBERGER. personal communication.
22 For a review, see R. D. GUTHRIE, R. J. FERRIER, AND M. J. HOW, *Carbohydrate Chemistry*, Volume 1, The Chemical Society, London, 1967, p. 240.
23 M. J. HOW, J. V. S. JONES, AND M. STACEY, *Carbohyd. Res.*, in press.
24 R. W. MURRAY AND W. T. W. POTTS, *Comp. Biochem. Physiol.*, 2 (1961) 65.

Carbohydrate Research
Elsevier Publishing Company, Amsterdam
Printed in Belgium

STUDIES ON STARCHES OF HIGH AMYLOSE-CONTENT

PART X*. AN IMPROVED METHOD FOR THE FRACTIONATION OF MAIZE AND AMYLOMAIZE STARCHES BY COMPLEX FORMATION FROM AQUEOUS DISPERSION AFTER PRETREATMENT WITH METHYL SULPHOXIDE

G. K. ADKINS AND C. T. GREENWOOD
Department of Chemistry, The University of Edinburgh (Great Britain)
(Received March 19th, 1969)

ABSTRACT

A method has been developed of separating maize starches of different genotypes quantitatively into their component material. Pretreatment of the granular starches with methyl sulphoxide is followed by the formation of a stable, aqueous methyl sulphoxide dispersion from which the amylose component can be rapidly precipitated as an easily centrifugeable complex with butan-1-ol. The residual starch material has been subfractionated by the formation of the iodine complex at low temperatures. Properties of the different starch fractions from this fractionation scheme have been examined, and the results are briefly considered with regard to current theories of maize starches of high amylose-content.

INTRODUCTION

The most successful procedures which have been suggested for fractionating amylomaize starch appear to involve dispersion of the starch into aqueous solution as an essential first-stage[1]. Separation of the components can be made then by the addition of a complexing agent[1–3], by ultracentrifugation[4], and by self-precipitation of the linear component[5], but quantitative fractionation is difficult to achieve.

Efficient and effective fractionation of a starch depends on the completeness with which the granular structure can be disrupted without degradation occurring. We have found that pretreatment of the hydrated granules with methyl sulphoxide is the most satisfactory method of achieving this for amylomaize starch. This pretreatment has been used here, and fractionation conditions have been developed to ensure rapid precipitation of the amylose–butan-1-ol complex in a form that can be isolated by low-speed centrifugation. In this manner, amylose products have been obtained in high yield and purity from maize starch, and from amylomaizes having apparent contents of amylose varying between 57 and 75%. Starch material which did not form a complex with butan-1-ol has been subfractionated with iodine at low temperatures. The properties of the components resulting from this fractionation scheme have been examined.

*For Part IX, see *Carbohyd. Res.*, 6 (1968) 241.

EXPERIMENTAL

Materials. — The *laboratory* isolation and purification of the starches used in this work have been described in detail[6]. Starches were stored in water under toluene at 20°, and were fractionated within a few months of isolation.

Fractionation procedure. — (*a*) *Pretreatment and dispersion of starch.* Defatted starch granules were thoroughly washed in, and centrifuged from, distilled water, to yield a well-drained sediment containing *ca.* 40% of moisture. They were then dissolved in methyl sulphoxide (0.4% of water) with continuous stirring for 3 h at room temperature, the concentration being adjusted for the starch type, *i.e.*, amylostarches 4–5%, regular maize 2.5–3%, and waxy maize 1.5–2% w/v.

Water (2 vol.) was then added, and the starch was precipitated with acetone (3.5 vol.), dehydrated with acetone, and dried to a fine, friable powder in a stream of nitrogen. The pretreated starch was then redissolved in methyl sulphoxide to the above concentrations.

(*b*) *Formation of butan-1-ol complex.* The methyl sulphoxide solution was stirred continuously while the complexing agent [7 vol.; water containing butan-1-ol (6% by volume), and sodium chloride (0.1% by weight)] was added. The mixture was allowed to stand at room temperature until there was incipient sedimentation of the complex under gravity (*ca.* 30 min). Centrifugation at 1500 *g* for 15 min then yielded the butan-1-ol complex (*C*1) and supernatant liquor (*S*1).

(*c*) *Recrystallization of complex C1.* A solution of the complex, *C*1, in water (0.2–0.3%) was warmed to 50–55°, and then butan-1-ol (to saturation) and sodium chloride (0.1%) were added with stirring. After 4 h at room temperature, the complex (*C*2) was removed by centrifugation (20 min; 1500 *g*). The supernatant liquor contained fraction *S*2.

This recrystallization procedure was repeated to yield complex *C*3 and supernatant liquor *S*3. The final butan-1-ol complex (*C*3) was stored at room temperature.

Fractions *S*2 and *S*3 were combined, the butan-1-ol was removed by concentration on a rotary evaporator at 35° under diminished pressure, and the polysaccharide, *S*(2+3), was precipitated, and washed and dried with acetone. For examination, the product was dissolved in methyl sulphoxide to 2% concentration.

(*d*) *Subfractionation of supernatant liquor S1.* The supernatant liquor was immediately concentrated 8-fold on a rotary evaporator at 35°, and the polysaccharide was precipitated with acetone, washed, and redissolved in methyl sulphoxide to yield a 2% solution. Iodine (1.3% in methyl sulphoxide) was then added in an amount equivalent to 25% by weight of the polysaccharide content of the mixture. The iodine–glucan complex was produced on the addition of two volumes of buffer (pH 6.8; phosphate, 0.04M), and the mixture was aged for 18 h at 4°. The dense, black iodine-complex (*S*1*a*) was removed by centrifugation at 4° (10 min at 1500 *g*). *S*1*a* was dissolved in water, then precipitated and washed several times with acetone to remove iodine, and finally dried with acetone. The supernatant liquors (*S*1*b*)

were treated similarly. Both polysaccharides were dissolved in methyl sulphoxide before examination.

(*e*) *Subfractionation of* S(*2+3*). The methyl sulphoxide solution of fraction *S*(2+3) was treated with iodine as above to yield the iodine-complexable fraction *S*(2+3)*a* and the non-complexable fraction *S*(2+3)*b*.

Characterization of the fractionation products. — The methods used to characterize the starch products have been described earlier[3,7-9]. Concentrations of the starch fractions at different stages in the fractionation scheme were determined by hydrolyzing aliquots to D-glucose, and estimating the reducing power with alkaline ferricyanide.

RESULTS AND DISCUSSION

The starches used in this work were waxy maize (WM), regular maize (RM), and five samples of high-amylose maize starch (HA57, HA62, HA67, HA70, and HA75), where the number represents the reputed percentage of amylose.

Fractionation procedure

Preliminary ultracentrifuge experiments showed that *re-solution* of methyl sulphoxide-pretreated starch in methyl sulphoxide is essential to ensure complete disorganization of the granular structure of amylomaize starches. Viscosity measurements showed that solutions of the starches in this solvent are stable, and that molecular degradation is unlikely. In the initial dispersion, a high starch:solvent ratio was required, in order to ensure that interaction between the swollen granules would occur under the mild shearing effects of stirring. The optimum ratio varied with starch type.

Addition of water to these methyl sulphoxide solutions of amylomaize starch produced an aqueous dispersion, which was stable for several hours at room temperature if the polysaccharide concentration was in the range of 0.4–0.5%. The further addition of aqueous butan-1-ol containing sodium chloride then caused amylose–butan-1-ol complex formation which was complete within one hour; the presence of a trace of electrolyte caused rapid formation of a complex which could be removed by low-speed centrifugation. These butan-1-ol complexes were readily soluble, which enabled the recrystallized complexes to be obtained in high yield and purity.

The material in the supernatant liquors from the recrystallizations [fraction *S*(2+3)] was unstable in aqueous solution, and it was removed by precipitation and dissolved in methyl sulphoxide for further study.

The clear, supernatant liquors after removal of the butan-1-ol complexes (fraction *S*1) were unstable at room temperature, and tended to develop turbidity. This instability was avoided by immediately concentrating the solution to yield a stable, aqueous methyl sulphoxide dispersion. These solutions were then separated into an iodine-complex forming and non-forming fraction. The complex formed more rapidly at low temperatures, and control of pH was required to avoid degradation,

but a high ionic strength had to be avoided as it caused total precipitation of the polysaccharide. Iodine had to be very carefully removed from all of the subfractions, in order to avoid its interference in later characterization experiments.

Fractionation yields

Small-scale fractionations of *ca.* 1 g of starch gave reproducible yields of the different fractions as shown in Table I. Larger scale preparations carried out to obtain additional amounts of the minor fractions for detailed analysis occasionally showed significant physical losses.

TABLE I

YIELDS OF COMPONENTS OBTAINED ON FRACTIONATION

Fraction[a]	*Percentage of original starch*[b]						
	WM	*RM*	*HA57*	*HA62*	*HA67*	*HA70*	*HA75*
C1	0	27	55	59	64	68	71
C3	0	25	51	55	59	63	65
S(2+3)	0	2	4	4	5	5	6
S(2+3)a	0	n.d.[c]	3	n.d.	4	4	n.d.
S(2+3)b	0	n.d.	1	n.d.	1	1	n.d.
S1	99	73	45	41	36	32	29
S1a	2	4	36	35	30	27	25
S1b	96	68	9	6	6	4	4

[a]See Experimental section. [b]WM = waxy-maize starch; RM = regular maize starch; HA = high-amylose maize starch, with the number representing the reputed amylose-content. [c]n.d. = not determined.

Properties of the fractions

The properties of the *butan-1-ol complexes, fraction C3*, shown in Table II, are those of normal amylose. The high degree of purity of each sample was shown by its almost complete conversion into maltose under the concurrent action of β-amylase and Z-enzyme, and the high levels (*ca.* 20%) of iodine affinity. The value of the limiting-viscosity number was found to decrease with increase in the reputed amylose content of the starch, which may indicate a corresponding trend in change of molecular size.

It is also significant that, although the yields of normal amylose were directly proportional to the apparent amylose content of the parent starch, the amounts for the amylomaize starches were much smaller than those reputed to be present.

The properties of the *supernatant liquors from the recrystallization of the amylose, fraction S(2+3)* are shown in Table III. This fraction was only a minor component of the starches, and was difficult to assess accurately; the iodine affinities were apparently high, but incomplete conversion into maltose by the concurrent action of β-amylase and Z-enzyme suggested that the material consisted of amylose-like polymer with some branched component. Subfractionation with iodine confirmed

TABLE II

PROPERTIES OF THE RECRYSTALLIZED AMYLOSE–BUTAN-1-OL COMPLEXES, FRACTION *C*3

Starch[a]	*RM*	*HA57*	*HA62*	*HA67*	*HA70*	*HA75*
Percentage of starch	25	51	55	59	63	65
Iodine affinity, %, 20°	19.8	20.1	20.4	20.6	19.9	20.7
Iodine affinity[8], %, 2°	20.1	20.4	n.d.	21.0	20.8	n.d.
[β]-Limit[b]	83	81	79	84	79	80
[β+Z]-Limit[c]	100	97	98	97	98	98
[η] (ml.g^{-1}) in Me_2SO	142	115	106	97	94	89
[η] (ml.g^{-1}) in 0.15M KOH	266	155	130	138	130	122

[a]As in Table I. [b][β]-Limit = % conversion into maltose under action of β-amylase. [c][β+Z]-Limit = % conversion into maltose under concurrent action of β-amylase and Z-enzyme.

this concept and showed that the sample was probably a mixture of short-chain amylose and amylopectin-like material (see Table III).

TABLE III

PROPERTIES OF FRACTIONS *S*(2+3) AND THEIR SUBFRACTIONS *S*(2+3)*a* AND *S*(2+3)*b*

Starch[a]	*Fraction*	*Percentage of starch*	*I.A.*[b], %, 2°	[β][a] *limit*	[β+Z][a] *limit*	*Chain length (enzymic)*
RM	*S*(2+3)	2	11.9	74	92	n.d.
HA57	*S*(2+3)	4	n.d.	78	87	n.d.
	S(2+3)*a*	3	16.0	83	99	n.d.
	S(2+3)*b*	1	0.2	n.d.	n.d.	n.d.
HA67	*S*(2+3)	5	n.d.	n.d.	n.d.	n.d.
	S(2+3)*a*	4	16.5	80	96	90
	S(2+3)*b*	1	n.d.	51.5	n.d.	19
HA70	*S*(2+3)	5	n.d.	75	88	n.d.
	S(2+3)*a*	4	17.8	86	97	120
	S(2+3)*b*	1	0.2	54	n.d.	22

[a]As in Table II. [b]I.A. = Iodine affinity.

TABLE IV

PROPERTIES OF THE TOTAL NON-BUTAN-1-OL-COMPLEXING COMPONENTS, FRACTION *S*1

Starch[a]	*WM*	*RM*	*HA57*	*HA62*	*HA67*	*HA70*	*HA75*
Percentage of starch	99	73	45	41	36	32	29
Iodine affinity, %, 20°	0.05	0.15	*ca.* 2.3	n.d.	n.d.	*ca.* 4.2	n.d.
[β]-Limit[a]	56	58	53	n.d.	65	64	65
[β+Z]-Limit[a]	57	61	68	n.d.	77	77	79
Chain length, enzymic	18	29	34	n.d.	n.d.	45	44
Chain length, periodate	23	26	38	n.d.	42	37	45
[η] (ml.g^{-1}) in Me_2SO	170	120	84	n.d.	70	70	68

[a]As in Table II.

Table IV shows the properties of the *materials from the supernatant*, S*1*. The waxy and regular maize fractions behaved as typical, normal amylopectins, whereas, in agreement with earlier reports in the literature[1,3], the corresponding fractions from the amylomaize starches had iodine affinities, β-amylolysis limits, and average chain-lengths which were abnormally high.

In agreement with earlier studies[3], the subfractionation of these anomalous samples by the formation of an iodine complex resulted in the isolation of material which behaved as essentially short-chain, linear amylose (*fraction S1a*), as shown in Table V, and material having the characteristics of normal amylopectin (*fraction S1b*; see Table VI).

TABLE V

PROPERTIES OF SUB-FRACTIONS, *S*1a

Starch[a]	*WM*	*RM*	*HA57*	*HA62*	*HA67*	*HA70*	*HA75*
Percentage of starch	2	4	36	35	30	27	25
Iodine affinity, %, 2°	5.2	4.1	10.8	n.d.	13.8	14.7	14.7
[β]-Limit[a]	95	94	92	95	89	92	90
[β+Z]-Limit[a]	96	96	93	95	93	93	90
Chain length, enzymic	46	58	88	52	52	110	61
[η] (ml.g^{-1}) in Me_2SO	18	22	n.d.	24	n.d.	48	46
Instability[b], %	68	76	70	n.d.	n.d.	88	95

[a]As in Table II. [b]Expressed as the percentage of centrifugable material after 8-h ageing, 6°, 2 mg/ml in 10% v/v aqueous methyl sulphoxide.

TABLE VI

PROPERTIES OF SUB-FRACTIONS, *S*1*b* (AMYLOPECTINS)

Starch[a]	*WM*	*RM*	*HA57*	*HA62*	*HA67*	*HA70*	*HA75*
Percentage of starch	96	68	9	6	6	4	4
Iodine affinity, %, 20°	0.05	0.2	0.1	n.d.	n.d.	n.d.	0.15
Iodine affinity, %, 2°	0.2	0.3	0.3	0.4	0.3	0.4	0.3
[β]-Limit[a]	54	55	57	56	54	n.d.	54
[β+Z]-Limit[a]	54	56	57	n.d.	55	57	54
Chain length, enzymic	16	24	26	24	n.d.	27	22
[η] (ml.g^{-1}) in Me_2SO	175	159	120	160	152	n.d.	154
Instability[9], %	2	2	3	n.d.	2	5	n.d.

[a]As in Table II.

The amount of normal amylopectin in the amylomaize starches was low and decreased with increase in the reputed amylose-content of the starch. In contrast, fraction *S1b* became a major component of the starches of high amylose-content.

Solution stability

The stabilities of aqueous solutions of the starch fractions as a function of time and temperature were measured in terms of the proportion of material remaining

in solution after centrifugation. The results for the major fractions are shown in Table VII.

TABLE VII

STABILITY OF AQUEOUS SOLUTIONS[a] OF STARCH FRACTIONS

Fraction	*Temp.* (*degrees*)	*Time* (*h*)	*Solubility*[b]						
			WM	*RM*	*HA57*	*HA62*	*HA67*	*HA70*	*HA75*
Amylose (*C3*)	20	8	—	94	96	90	94	88	90
	20	24	—	88	81	80	86	80	88
	6	20	—	75	69	71	68	57	70
Control[c]	35	<1	—	100	100	100	100	100	100
Intermediate (*S1a*)	20	8	78	64	79	n.d.	n.d.	72	75
	20	20	58	60	69	59	48	46	59
	6	8	32	24	30	n.d.	n.d.	12	41
	6	20	20	16	27	19	28	12	29
Control[c]	35	<1	98	97	98	99	n.d.	94	96
Amylopectin (*S1b*)	20	26	100	98	98	97	98	96	96
	6	8	98	98	97	n.d.	98	95	n.d.
Control[c]	35	<1	100	98	99	100	100	96	100

[a]Conditions: methyl sulphoxide solution (*c*, 20 mg.ml^{-1}) diluted to 10 vol. with buffer (pH, 6.8; 0.01M McIlvaine). [b]Percentage of polysaccharide in supernatant liquor after centrifuging for 20 min at 1500 *g*. [c]Solution centrifuged immediately after dilution of methyl sulphoxide solution.

All of the amylose fractions, even from the aqueous methyl sulphoxide solutions, showed degrees of stability which were time and temperature dependent. This has been observed also in aqueous solutions of the butan-1-ol complexes.

The short-chain, amylosic materials, fraction *S1a*, were very unstable in these neutral, aqueous solutions, whereas the amylopectin fractions, *S1b*, were relatively highly stable.

The minor fraction *S*(2+3)*a* was not examined in detail, but experiments on this material from regular maize and HA70 showed the percentage solubility after ageing for 6 h at 60° to be 25 and 16%, respectively, indicating a high degree of instability.

In qualitative terms, it was evident that the instability of aqueous amylostarch dispersions[1,5] is mainly due to the presence and behaviour of intermediate, short-chain material, and, to a lesser extent, to the behaviour of the amylose-type component.

Iodine-titration behaviour

It was found that the anomalous iodine-titration curves[8,10] of theamylomaize starches could be readily reproduced from admixtures of the fractionation products. For example, for starch HA70, a 64:5:27:4 mixture of fractions *C3*:*S*(2+3):*S1a*:*S1b* was found to give a curve identical to that for the parent starch.

In agreement with earlier results[8,10], although the titration curves of the whole starch were abnormal, mixtures of the normal amylose and amylopectin components

possessed experimental iodine affinities which were in agreement with calculated values; for starch HA70, a 70:30 and 30:70 mixture of fractions *C*3 and *S*1*b* had observed iodine affinities of 13.7 and 6.2%, respectively, and the theoretical values calculated from the iodine affinity of the individual components were 14.0% and 6.1%.

Conclusions

The results of the new fractionation procedure presented here are in general agreement with the concept that we have developed earlier in this series[3-5,7,8,10]. We regard the abnormal properties of amylomaize starches as being due, almost exclusively, to the presence of short-chain, amylosic material. The amount of this fraction increases with increase in the reputed amylose-content of the starch, and it may account for as much as 30% of the total. This material is inherently unstable in aqueous solution and is difficult to handle and adequately characterize.

ACKNOWLEDGMENT

This work was supported, in part, by a grant from the U. S. Department of Agriculture, under P. L. 480.

REFERENCES

1 For a review, see G. K. ADKINS AND C. T. GREENWOOD, *Stärke*, 18 (1966) 171.
2 I. A. WOLFF, B. T. HOFREITER, P. R. WATSON, W. L. DEATHERAGE, AND M. M. MACMASTERS, *J. Amer. Chem. Soc.*, 77 (1955) 1654.
3 C. T. GREENWOOD AND S. L. MACKENZIE, *Carbohyd. Res.*, 3 (1966) 7.
4 G. K. ADKINS AND C. T. GREENWOOD, *Stärke*, 18 (1966) 237.
5 G. K. ADKINS AND C. T. GREENWOOD, *Stärke*, 18 (1966) 240.
6 G. K. ADKINS AND C. T. GREENWOOD, *Stärke*, 18 (1966) 213.
7 G. K. ADKINS, W. BANKS, AND C. T. GREENWOOD, *Carbohyd. Res.*, 2 (1966) 502.
8 G. K. ADKINS AND C. T. GREENWOOD, *Carbohyd. Res.*, 3 (1966) 152.
9 W. BANKS AND C. T. GREENWOOD, *Carbohyd. Res.*, 6 (1968) 177.
10 G. K. ADKINS AND C. T. GREENWOOD, *Carbohyd. Res.*, 3 (1966) 81.

Carbohydrate Research
Elsevier Publishing Company, Amsterdam
Printed in Belgium

THE AUTOMATED SPECTROFLUORIMETRIC DETERMINATION OF FORMALDEHYDE IN THE PERIODATE OXIDATION OF CARBOHYDRATES AND AMINO ACIDS

H. CHO TUN, J. F. KENNEDY, M. STACEY, AND R. R. WOODBURY
Department of Chemistry, University of Birmingham, P. O. Box 363, Birmingham 15 (Great Britain)
(Received March 19th, 1969)

ABSTRACT

The automated spectrofluorimetric determination of formaldehyde, released by periodate oxidation, as 3,5-diacetyl-1,4-dihydro-2,6-dimethylpyridine is described. The application of the method, with modifications where necessary, to the monitoring of various procedures for fractionation of carbohydrates is outlined, and the sensitivity of the automated method is compared with the less-sensitive colorimetric method. The response of various amino acids in the assay system has been investigated and reveals the applicability of the method to serine in particular and the caution necessary in periodate oxidation of carbohydrates in the presence of free amino acids.

INTRODUCTION

Since the reaction was first noted[1], periodate oxidation has become an extremely useful tool for the identification and structural elucidation of many carbohydrates.

Formaldehyde has been quantitatively measured by a variety of methods, although not all of these are suited to use in conjunction with small-scale, periodate-oxidation studies. Although it is commonly identified and determined by its reaction with dimedone[2-5], the method is not very quantitative when compared with colorimetric and fluorimetric procedures. Formaldehyde has been determined colorimetrically by using chromotropic acid[6], phenylhydrazine–potassium ferricyanide[7], and the Hantzsch pyridine reaction[8]. The last method has been automated[9]. Fluorimetric determination has been carried out manually[10] by using J-acid (6-amino-1-naphthol-3-sulphonic acid) and by the Hantzsch reaction[11].

The present paper describes the automated fluorimetric determination of formaldehyde by a modification of the Hantzsch reaction. The application of the devised method to the identification and determination of carbohydrates, glycoproteins, and amino acids is discussed.

EXPERIMENTAL AND RESULTS

The automated fluorimetric determination of formaldehyde. — *(a) Instrumentation.* Formaldehyde released by periodate oxidation was determined by condensation with

ammonia and pentane-2,4-dione, and the product, 3,5-diacetyl-1,4-dihydro-2,6-dimethylpyridine, was determined fluorimetrically. Technicon Modular Autoanalyser equipment was used to automate the whole process, and a schematic representation of the assembly is shown in Fig. 1. The various reagents were pumped, at the rates shown, by using a peristaltic pump. Sample, air, and periodic acid reagent were mixed, and oxidation was allowed to proceed for 7 min at 20°, before excess of periodate was destroyed by the addition of arsenite reagent. Ammonium acetate and pentane-2,4-dione reagents were separately added to the flow stream in that order, and the mixture was heated for 30 min at 70°. The mixture was cooled to 20°, and the fluorescence intensity was measured continuously by passage through the fluorimeter flow-cell. The excitation and emission filters used were 420 and 480 nm, respectively; these were the nearest filters obtainable to the actual values, found by an independent fluorimeter, of 410 and 510 nm, respectively.

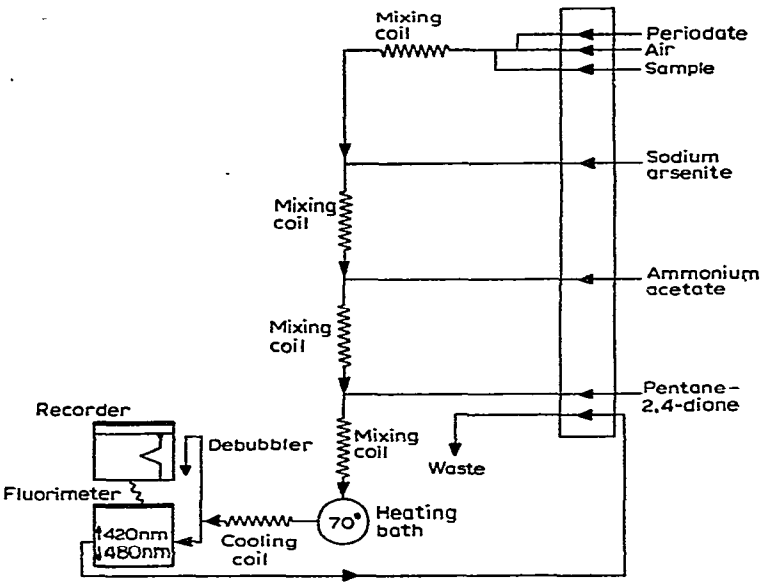


Fig. 1. Schematic representation of automated system for the fluorimetric determination of formaldehyde. Sample, 0.10; air, 0.16; periodate, 0.015; sodium arsenite, 0.10; ammonium acetate, 0.05; pentane-2,4-dione, 0.05; flow cell, 0.32 ml/min.

(*b*) *Reagents.* 0.015M Periodic acid solution neutralised to pH 7.5 with conc. ammonia (sp. gr. 0.880), mixed in the ratio 10:1 with M sodium phosphate buffer, pH 7.5 (the solution, when stored in a dark-coloured bottle away from direct sunlight, was stable for one month); 0.25M sodium arsenite, adjusted to pH 8.0 with conc. hydrochloric acid; 2M ammonium acetate; 0.06M pentane-2,4-dione (freshly redistilled) in 0.07M acetic acid.

Application of the automated method to carbohydrates. — (*a*) *Neutral carbohydrates.* A mixture of maltose and D-glucose (2 μg each) were separated[12] on an

ion-exclusion column of AG-50W x2 (Li^+, 200–400 mesh, 150 × 0.6 cm) resin, and the aqueous eluate was monitored automatically for formaldehyde released on periodate oxidation. The elution profile was identical to that obtained by colorimetric monitoring of 100-μg samples of the same compounds, fractionated under identical conditions.

(*b*) *Basic carbohydrates.* Separation of a mixture of 2-amino-2-deoxy-D-glucose and 2-amino-2-deoxy-D-galactose was carried out by fractionation[13] on a column of AG-50W x8 (H^+, 200–400 mesh, 79 × 0.6 cm) anion-exchange resin pre-equilibrated with 0.3N HCl. The sample was eluted with 0.3N HCl at a flow rate of 0.16 ml/min, and the eluate was monitored automatically for formaldehyde released on periodate oxidation. On account of the low pH of the eluate, an additional stage was introduced into the analytical system, the column eluate being neutralised with 0.3N NaOH prior to periodate oxidation. This application has been extended to the separation and identification of the constituent 2-amino-2-deoxyhexoses of follicle-stimulating hormone. Follicle-stimulating hormone (100 μg) was hydrolysed in 3.9N HCl for 9 h at 100°, and the hydrolysate was fractionated as described above (Fig. 2a). The method was also applied to the determination of the constituent 2-amino-2-deoxyhexoses of acidic mucopolysaccharides and their protein complexes by hydrolysis, under the same conditions, followed by fractionation; *e.g.*, dermatan sulphate (Fig. 2b) which contains 2-amino-2-deoxy-D-galactose.

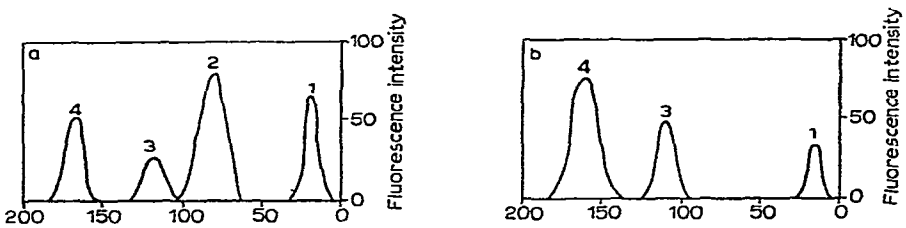


Fig. 2. Fractionation of hydrolysates of (*a*) follicle-stimulating hormone and (*b*) dermatan sulphate–protein on AG-50W x8 (H^+ form): (1) Neutral or acidic sugars, (2) 2-amino-2-deoxy-D-glucose, (3) 2-amino-2-deoxy-D-galactose, and (4) amino acids.

Fractions corresponding to peak 4 (Fig. 2a) were combined, and concentration and removal of HCl was effected by rotatory evaporation *in vacuo* (bath temperature 37°). Fractionation on the Technicon amino acid autoanalyser showed that all of the naturally occurring amino acids were present, but application of the Elson–Morgan assay[14,15] for 2-amino-2-deoxyhexoses showed that the sample did not contain a 2-amino-2-deoxyhexose disaccharide.

Application of the automated method to alditols. — A mixture of ethylene glycol, propane-1,2-diol, glycerol, erythritol, and mannitol in 86% ethanol (v/v) was chromatographed[16] on a column (60 × 0.45 cm) of AG-1 x8 resin (SO_4^{2-}) resin which had been pre-equilibrated with deaerated 86% ethanol at 50°. The eluate was monitored automatically for formaldehyde released on periodate oxidation. The arsenite reagent in the analytical system was replaced by a solution of butane-2,3-diol

(20 ml/litre). The elution profile was identical to that obtained by colorimetric monitoring of larger samples of the same compounds fractionated under identical conditions.

Application of the automated method to polysaccharides and glycoproteins. — Synovial fluid (100 μl) from a psoriatic arthritis patient was diluted with 0.2M NaCl (600 μl) and fractionated on a Porasil F column (75–100 mesh, average pore-diameter >1500 Å, 100×1 cm). The Porasil was deaerated under water before packing into the column, and was then equilibrated with 0.2M NaCl. By using the same solution, the samples were eluted at a flow rate of 0.7 ml/min, and one seventh of the eluate was monitored automatically for formaldehyde released on periodate oxidation (Fig. 3a). A further portion of the eluate was monitored automatically[17] by the carbazole assay[18] for uronic acids. The magnitude of peak B decreased by 84% when an equal aliquot of this synovial fluid was fractionated after dialysis against 0.005M phosphate buffer pH 6.9 (5×1-litre) for 24 h at 4°. An authentic sample of hyaluronic acid (Koch–Light Laboratories) was similarly fractionated (Fig. 3b).

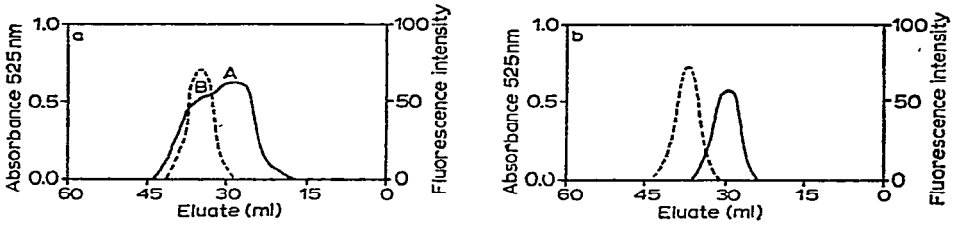


Fig. 3. Fractionation ot (*a*) synovial fluid, and (*b*) hyaluronic acid on Porosil F column: (— — — —), fluorimetric formaldehyde assay; (————), colorimetric carbazole assay.

Fractionation of pure samples of orosomucoid and human-serum albumin on the same column showed single peaks in the high molecular weight region when the eluate was monitored by the periodate oxidation–formaldehyde assay, whereas alginic acid gave no response. Concentrations as low as 20 μg of glycoprotein/ml could be determined by the analytical method.

Application of the automated method to amino acids. — Solutions of fifteen amino acids, representing the various classes of amino acids, were subjected to the automatic analysis of formaldehyde released by periodate oxidation, by direct sampling into the assay system. The periodate reagent in the assay system was then replaced with water, and the solutions were resampled (Table I).

DISCUSSION

The numerous advantages of automation of analytical methods include increased accuracy over the corresponding manual methods. On the other hand, automation does not necessarily lead to increased sensitivity, but the pentane-2,4-dione assay for formaldehyde has the advantage that the chromophore, which is usually measured colorimetrically, is also fluorescent, and this added property is a great advantage when increased sensitivity is required.

Although the colorimetric method has been adopted previously for the direct

TABLE I

RESPONSE OF AMINO ACIDS IN THE ASSAY

Amino acid	*Moles of formaldehyde/mole of amino acid*		*Amino acid*	*Moles of formaldehyde/mole of amino acid*	
	Oxidised	*Unoxidised*		*Oxidised*	*Unoxidised*
Isoleucine	0.0000	0.0000	Arginine	0.0031	0.0000
Proline	0.0018	0.0000	Lysine HCl	0.0018	0.0000
Histidine	0.0250	0.0017	Valine	0.0009	0.0000
Aspartic acid	0.0250	0.0007	Hydroxyproline	0.0094	0.0000
Serine	1.1500	0.0224	Cysteine HCl	0.1120	0.0027
Tyrosine	0.0000	0.0000	Methionine	0.0027	0.0000
Tryptophan	0.0045	0.0000	Threonine	0.0032	0.0006
Leucine	0.0000	0.0000			

fluorimetric assay[11] of formaldehyde, certain modifications were necessary to facilitate its application to the *in situ* measurement of formaldehyde released on periodate oxidation. The principal problem is the undesirably high, background fluorescence which can arise from the pentane-2,4-dione reagent, and from heating at too high a temperature before determining the fluorescence intensity. To overcome this problem, the pentane-2,4-dione reagent [9] was added in two stages (ammonium acetate and pentane-2,4-dione reagent in acetic acid); the background fluorescence of these freshly prepared reagents was less than that of freshly prepared, combined reagent, and background fluorescence did not increase on storage. Also, the heating-bath temperature was lowered to 70°; it has been shown[11] that the Hantzsch reaction will proceed at lower temperatures, but the temperature selected here is consistent with a reaction time that is acceptable for automated analysis. Iodate ion is known to interfere in fluorescence studies, and this will affect the sensitivity of the method. The present method is 100 times more sensitive than the colorimetric version.

The method is suited to the continuous monitoring of column eluates, and when applied to acidic (or basic) eluates, it is necessary to introduce a neutralisation stage, since the 0.1M buffer of the periodate reagent is unable to cope with pH extremes. A suitable increase in the buffer concentration is not practicable, since it leads to the formation of a precipitate on addition of arsenite. In the case of eluates containing organic solvents, arsenite is not a suitable reagent for destruction of excess of periodate, but is best replaced by an agent that is soluble in the organic solvent. Although all ion-exchange resins tend to leak continuously, these small amounts of ultraviolet-active material did not have any adverse effect upon the assay method.

The application of the method to analysis of the fractionations described requires little enlargement. The increased sensitivity is of obvious advantage, *e.g.*, the constituent 2-amino-2-deoxyhexose of an acidic mucopolysaccharide can now be identified and determined quantitatively on as little as 2 μg; this is an improvement on the method we have previously described[13].

On account of the increased sensitivity of the method, the possibility existed of

detecting certain polysaccharides by liberation of formaldehyde from the terminal reducing sugars. Such a response would be dependent on the nature of these reducing units. Direct assay of authentic hyaluronic acid gave an apparent yield of 0.5 mole of formaldehyde/mole of disaccharide repeating-unit. This is far too high to be accounted for by oxidation of a terminal, reducing 2-amino-2-deoxy-D-glucose residue. Hyaluronic acid is susceptible to oxidation only at the C-2–C-3 glycol group[19], since the amino group of the 2-amino-2-deoxyhexose residue is protected by the acetyl group. Because the oxidation time in the method is only 7 min, there is no question of over-oxidation of hyaluronic acid which is oxidised only very slowly, even in the presence of added, inert electrolyte to diminish the electrostatic effect produced by the carboxylate groups[19]. Fractionation of synovial fluid on Porasil F showed two components (Fig. 3a), of which A gave a true reaction and B a non-specific reaction in the automated carbazole assay[17]. Clearly, in this case, as in the case of dialysed synovial fluid and authentic hyaluronic acid, compounds other than hyaluronic acid were giving rise to a formaldehyde response. Since the hyaluronic acids gave no response, it seems that the hyaluronic acid is (*a*) protected at its reducing residue by an amino acid or peptide not giving a formaldehyde response, or (*b*) possesses D-glucuronic acid as the terminal, reducing residue. In the latter case, the conclusion that such an end unit would not contribute positive material on oxidation is supported by the lack of response of alginic acid. Orosomucoid, which contains 10–12% of *N*-acetylneuraminic acid at its non-reducing terminals, and serum albumin, which contains 2-amino-2-deoxy-D-glucose and mannose, both responded.

However, it was evident, from the fractionation of hydrolysates of glycoproteins, that amino acids were also responding to some extent in the assay system. Table I shows that almost all amino acids tested were giving some response, although, in many cases, this was quite small. The response of serine is expected, because it contains the $HOH_2C–CH(NH_2)–$ group which is oxidisable to formaldehyde. The response of cysteine hydrochloride can similarly be attributed to the presence of the α-aminothiol group. Thus, thioformaldehyde will be released and could condense with the ammonia and pentane-2,4-dione reagents to give the usual condensation product, 3,5-diacetyl-1,4-dihydro-2,6-dimethylpyridine, although at a different reaction rate. Threonine, which is also an α-aminoalcohol, gives acetaldehyde on oxidation, which can condense with the ammonia and pentane-2,4-dione reagents, but at a lower rate, to give a similar kind of condensation product, namely, 3,5-diacetyl-1,4-dihydro-2,4,6-trimethylpyridine[8,20]. This compound has an absorption maximum at 388 nm[8]. Since the response is very small, the condensation product may be regarded as having excitation and emission spectra which do not coincide with that of 3,5-diacetyl-1,4-dihydro-2,6-dimethylpyridine. The slight response of some of the other amino acids may be due to fluorescence of small amounts of impurities or due to the condensation of products from non-specific oxidation with the pentane-2,4-dione reagent. Since a substituted ammonia can participate in the Hantzsch reaction, these fluorescences, and those of the unoxidised acids, may also be due to involvement of the amino acid nitrogen atom in ring formation.

Clamp and Hough[21] have shown that all α-amino acids are oxidised by periodate, but at rates that depend upon pH. Of the amino acids that gave formaldehyde response in the present work, only cysteine is oxidised when both the carboxyl and amino groups are substituted, as in a polypeptide chain; however, serine at an *N*-terminal chain-end would respond. Therefore, careful attention must be given to interpretation of the results of periodate oxidation when free amino acids are present, particularly with respect to possible degradation of glycoproteins, by processes other than periodate oxidation, during the automated reaction.

The method described for the determination of formaldehyde released on periodate oxidation should prove useful in structural studies involving monitoring of carbohydrates, glycoproteins, and some amino acids, particularly in work on a microgram scale.

ACKNOWLEDGMENTS

The authors thank Dr. S. A. Barker, for his interest in this work, which was supported financially by the Medical Research Council (J.F.K. and R.R.W.), the British Council (H.C.T.), and the Nuffield Foundation.

REFERENCES

1 M. L. MALAPRADE, *Bull. Soc. Chim. France*, 43 (1928) 683.
2 D. J. BELL, *J. Chem. Soc.*, (1948) 992.
3 P. FLEURY AND J. COURTOIS, *Bull. Soc. Chim. France*, 8 (1941) 75.
4 R. E. REEVES, *J. Amer. Chem. Soc.*, 63 (1941) 1476.
5 D. VORLANDER, *Z. Anal. Chem.*, 77 (1929) 241.
6 D. A. MACFADYEN, *J. Biol. Chem.*, 158 (1945) 107.
7 P. DESNUELLE AND M. NAUDET, *Bull. Soc. Chim. France*, 12 (1945) 871.
8 T. NASH, *Biochem. J.*, 55 (1953) 416.
9 O. SAMUELSON AND H. STROMBERG, *Carbohyd. Res.*, 3 (1966) 89.
10 E. SAWICKI, T. W. STANLEY, AND J. PFAFF, *Anal. Chim. Acta*, 28 (1963) 156.
11 S. BELMAN, *Anal. Chim. Acta*, 29 (1963) 120.
12 S. A. BARKER, B. W. HATT, J. F. KENNEDY, AND P. J. SOMERS, *Carbohyd. Res.*, 9 (1969) 327.
13 S. A. BARKER, J. F. KENNEDY, AND P. J. SOMERS, *Carbohyd. Res.*, 8 (1968) 482.
14 L. A. ELSON AND W. T. J. MORGAN, *Biochem. J.*, 28 (1933) 1824.
15 L. SVENNERHOLM, *Acta Soc. Med. Upsalien.*, 61 (1956) 287.
16 S. A. BARKER, M. J. HOW, P. V. PEPLOW, AND P. J. SOMERS, *Anal. Biochem.*, 26 (1968) 219.
17 R. G. JONES AND A. ZAMIR, unpublished observations.
18 T. BITTER AND H. M. MUIR, *Anal. Chem.*, 4 (1962) 330.
19 J. E. SCOTT AND R. J. HARBINSON, *Histochemie*, 14 (1968) 215.
20 G. M. BREARLEY AND J. B. WEISS, *Biochem. J.*, 110 (1968) 413.
21 J. R. CLAMP AND L. HOUGH, *Biochem. J.*, 94 (1965) 17.

Carbohydrate Research
Elsevier Publishing Company, Amsterdam
Printed in Belgium

BRANCHED-CHAIN SUGARS

PART IX*. THE SYNTHESIS OF HAMAMELITANNIN

A. D. EZEKIEL, W. G. OVEREND, AND N. R. WILLIAMS

Chemistry Department, Birkbeck College (University of London), Malet Street, London W.C.1 (Great Britain)

(Received March 20th, 1969)

ABSTRACT

A synthesis of hamamelitannin (the 1′,5-di-*O*-galloyl derivative of 2-*C*-hydroxymethyl-D-ribofuranose) is reported.

INTRODUCTION

Recently, we described a synthesis of D-hamamelose[1], which is a branched-chain sugar occurring naturally in hamamelitannin. Originally, this tannin was isolated from the bark of Witch Hazel (*Hamamelis virginiana* L.)[2], but, more recently, it has been obtained from Spanish Chestnut (*Castanea sativa*)[3] and the American Red Oak (*Quercus rubra*)[4]. Degradative studies[5,6] have established that the tannin is the 1′,5-di-*O*-galloyl derivative (**1**) of 2-*C*-hydroxymethyl-D-ribofuranose (hamamelofuranose). Now, we report a synthesis of hamamelitannin which confirms this structural assignment.

DISCUSSION

Treatment of D-hamamelose[1] with 0.1% methanolic hydrogen chloride under reflux gave methyl 2-*C*-hydroxymethyl-D-ribofuranoside (**2**) in almost quantitative yield. Since this product consumed two molecular equivalents of sodium periodate, the furanoside structure was indicated. The p.m.r. spectrum of the material was consistent with a mixture of α- and β-D anomers in approximately equal amount. Attempts to separate the anomers by cellulose or basic ion-exchange chromatography were unsuccessful.

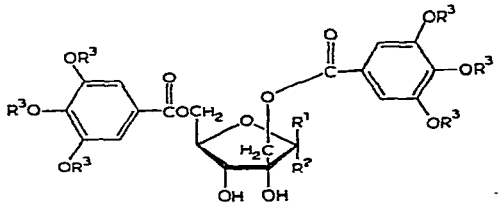
Acylation of the furanoside mixture **2** with tri-*O*-benzylgalloyl chloride (2.05 mol. equivalents) in pyridine at $-25°$ led to a mixture containing mainly di-*O*-acylated material, with traces of tri- and mono-*O*-acylated products. Since it is well known[7] that, under these conditions, primary hydroxyl groups are more reactive than those that are secondary or tertiary, the major products were expected to be the

*Part VIII: R. J. Ferrier, W. G. Overend, G. A. Rafferty, H. M. Wall, and N. R. Williams, *J. Chem. Soc.* (C), (1968) 1091.

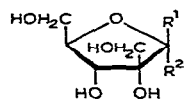
1′,5-di-*O*-galloyl derivatives **3** and **4**. These isomers were isolated and separated by fractional crystallisation and column chromatography.

Catalytic hydrogenation of compounds **3** and **4** yielded crystalline methyl 1′,5-di-*O*-galloyl-2-*C*-hydroxymethyl-α-D-ribofuranoside (**5**) and the β-D anomer (**6**), respectively. These compounds had the same melting points and R_F values as were recorded by Mayer *et al.*[6] for the two methyl glycosides prepared from hamamelitannin. The identity of the α-D anomer and the minor glycoside prepared by Mayer and his co-workers[6] was further confirmed by co-chromatography.

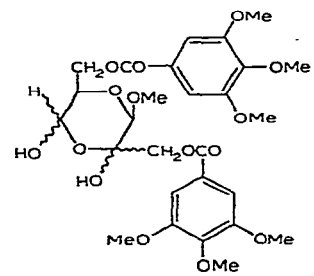
The phenolic hydroxyl groups in these hamamelitannin glycosides were methylated smoothly with diazomethane to give the corresponding bis(tri-*O*-methylgalloyl) derivatives **7** and **8**. Compound **8** consumed one mol. equivalent of periodate to afford the cyclic derivative **9**, the melting point and optical rotation of which are identical with the values reported in the literature[6] for this substance.



1 R^1, R^2=H, OH, R^3=H
3 R^1=H, R^2=OMe, R^3=CH_2Ph
4 R^1=OMe, R^2=H, R^3=CH_2Ph
5 R^1=H, R^2=OMe, R^3=H
6 R^1=OMe, R^2=H, R^3=H
7 R^1=H, R^2=OMe, R^3=Me
8 R^1=OMe R^2=H R^3=Me
11 R^1=OCH_2Ph, R^2=H, R^3=CH_2Ph



2 R^1, R^2=H, OMe
10 R^1, R^2=H, OCH_2Ph



9

Attempts to hydrolyse selectively the glycofuranosidic linkage in the glycoside **6** or its hexabenzyl ether **4** under mild conditions were unsuccessful, since, in each case, extensive, concomitant hydrolysis of the ester groups occurred. This difficulty was avoided by applying the same sequence of reactions to the benzyl glycoside derivatives and finally producing the free furanose sugar by catalytic hydrogenolysis of the glycosidic linkage.

An anomeric mixture of benzyl 2-*C*-hydroxymethyl-D-ribofuranosides (**10**) was obtained by treating D-hamamelose with 1% hydrogen chloride in benzyl alcohol for 120 h at 50°. Oxidation with sodium periodate served to confirm the furanoside structure of this mixture (2.01 mol. equiv. of oxidant consumed in 1 h). On catalytic hydrogenation, the mixture of glycosides was converted into D-hamamelose. On storage, partial crystallisation of the syrupy glycosides occurred, and the β-D anomer could be separated in solid form. Selective acylation of this crystalline glycoside with tri-*O*-benzylgalloyl chloride yielded the crystalline benzyl β-D-glycoside (**11**) of hexa-*O*-benzylhamamelitannin, which, on hydrogenation over 10% palladium-

on-charcoal, gave crystalline hamamelitannin (1); this was shown to be identical with the natural product.

EXPERIMENTAL

All liquid ratios are volume/volume. Melting points are uncorrected. Infrared spectra were obtained on a Perkin–Elmer Infracord Spectrophotometer, model 137: syrups were measured as liquid films on potassium bromide discs. P.m.r. spectra were recorded on a Varian A-60 spectrometer with deuteriochloroform as solvent (unless otherwise stated) and tetramethylsilane as internal standard. Paper chromatography was performed on Whatman No. 1 paper with the following solvent systems: (*A*) butanone saturated with water; (*B*) the organic phase of butyl alcohol–acetic acid–water (4:1:5), (*C*) N acetic acid. Ammoniacal silver nitrate spray was used to locate spots on the chromatograms. Thin-layer chromatography (t.l.c.) was performed with Kieselgel G (Stahl) activated for 12 h at 50°. The following solvent systems were used: (*a*) benzene–acetone (9:1), (*b*) ethyl ether–ethyl acetate (9:1); (*c*) ethyl acetate; (*d*) ethyl acetate–ethanol (4:1); (*e*) chloroform–ethyl acetate (7:1). Spots were detected by using either anisaldehyde–sulphuric acid–ethanol (1:1:20) or sulphuric acid–ethanol (1:1) at 150°. Periodate consumption was estimated by the spectrophotometric method[8]. D-Hamamelose was prepared essentially by the method previously described[1], but modified to improve the overall yield from D-arabinose by the use of the toluene-*p*-sulphonic acid–molecular sieve method [9] for isopropylidenation of methyl β-D-arabinopyranoside, and by replacing chromic oxide by ruthenium tetroxide[10] in the oxidation stage.

Methyl 2-C-hydroxymethyl-D-ribofuranoside (2). — 2-*C*-Hydroxymethyl-D-ribose (4.6 g) was heated under reflux in methanol (230 ml) containing hydrogen chloride (0.1%) for 17.5 h. (Trial experiments indicated that these were optimal conditions for the preferential formation of furanosides.) The cooled solution was neutralised with lead carbonate, filtered, and concentrated under diminished pressure to a colourless syrup (4.9 g, 99%), $[\alpha]_D$ −5.2° (*c* 1.0, methanol), R_F 0.12 (solvent A), single spot (3 h) showing slight separation after 24 h. P.m.r. (methyl sulphoxide-d_6) data: τ 5.36 and 5.46 (α- and β-anomeric protons, ratio 1:1). On oxidation 1.98 mol. equiv. of sodium periodate were consumed in 2 h at room temperature.

Anal. Calc. for $C_7H_{14}O_6$: C, 43.3; H, 7.3; OMe, 16.0. Found: C, 43.0; H, 7.0; OMe, 15.75.

Hexa-O-benzylhamamelitannin methyl α- and β-D-glycoside (3 *and* 4). — A solution of tri-*O*-benzylgalloyl chloride[11] (22.3 g, 0.0485 mole) in dry tetrahydrofuran (270 ml) was added dropwise over 1.75 h to a magnetically stirred solution of compound 2 (4.6 g, 0.0236 mole) in dry pyridine (150 ml) at −25°. The solution was stored at this temperature for 67 h, allowed to attain room temperature, and stored for a further 6 h. Crystalline pyridinium chloride was removed by filtration, and the filtrate was concentrated under diminished pressure (bath temperature, 40°) to a syrup which was dissolved in dichloromethane (1500 ml). The solution was washed

successively with 0.5N hydrochloric acid (2 × 250 ml) and water, dried ($MgSO_4$), and concentrated to a semi-solid residue (24 g) which was shown by t.l.c. (solvent *a*) to contain at least four components. A partial separation of the mixture was effected by column chromatography on silica gel (1.85 kg) with solvent *a*. Three fractions were obtained: (*i*) tri-*O*-acylglycosides as a syrup (5.85 g, 17%); (*ii*) di-*O*-acylglycosides as a waxy solid (14.4 g, 57%); (*iii*) mono-*O*-acylglycosides as a syrup (2.0 g, 13%). The di-*O*-acyl fraction yielded crystals from ethyl acetate–ethanol (2:1) which, on recrystallisation from the same solvent, gave the β-D-glycoside **4** as feathery needles (6.0 g, 23%), m.p. 169.5–171°, $[\alpha]_D$ −8.0° (*c* 1.0, chloroform); ν_{max} 3500 (OH), 2900 (C–H), 1720 (C=O), 1600, 760, 740 and 700 (aromatic) cm^{-1}; n.m.r. data: τ 2.50 (*m*) and 2.82 (*m*) (aromatic protons), 4.88 (*s*) (benzyl CH_2), 5.20 (*s*) (H-1), 6.72 (*s*) (CH_3–O), and 8.30 (*s*, absent on addition of D_2O) (OH).

Anal. Calc. for $C_{63}H_{58}O_{14}$: C, 72.8; H, 5.6; OMe, 3.0. Found: C, 72.5; H, 5.4; OMe, 2.5.

The mother liquors from the crystallisations were combined and concentrated under diminished pressure to a syrup (7.5 g) which was shown by t.l.c. (solvent *b*) to contain two components: the more-mobile component was the β-D anomer. Rechromatography on silica gel (1.5 kg) (solvent *b*) afforded two fractions, the second being pure methyl α-D-glycoside **3**. This was obtained as an amorphous solid (1.8 g, 7.3%) after reprecipitation from ethanol containing a trace of acetone. It had m.p. 132–134°, $[\alpha]_D$ +19.9° (*c* 1.1, carbon tetrachloride); ν_{max} 3450 (OH), 2900 (C–H), 1725 (C=O), 1600, 760 and 735 (aromatic) cm^{-1}, n.m.r. data: τ 2.45(*m*) and 2.80(*m*) (anomeric proton), 4.90(*s*) (benzyl CH_2), 5.08(*s*) H-1), 6.48(*s*) (CH_3–O), and 7.35 (*m*, absent on addition of D_2O) (OH).

Anal. Calc. for $C_{63}H_{58}O_{14}$: C, 72.8; H, 5.6; OMe, 3.0. Found: C, 72.5; H, 5.45; OMe, 3.0.

Hamamelitannin methyl α-D-glycoside (**5**). — A solution of compound **3** (0.7 g) in ethyl acetate (150 ml) was hydrogenated over 10% palladium-on-charcoal (0.7 g) at room temperature and pressure, uptake of hydrogen being complete within 1 h. The catalyst was removed, and the solution was concentrated under diminished pressure to a syrup which crystallised on trituration with water. The product was recrystallised from water as colourless needles (0.25 g, 72%) which were obtained as a hydrate even after drying *in vacuo* at 64°, m.p. 147–150°, $[\alpha]_D$ +33.0° (*c* 2.0, ethanol), R_F 0.49 (solvent *B*), 0.43 (solvent *C*); lit.[6], m.p. 146–148°, R_F 0.49 (solvent *B*), 0.43 (solvent *C*); ν_{max} 3300 (OH), 1690 (C=O), 760 (aromatic) cm^{-1}; n.m.r. data (methyl sulphoxide-d_6, containing D_2O): τ 2.93(*s*) (aromatic protons), 5.12(*s*) (H-1), and 6.56(*s*) (CH_3–O). An authentic sample behaved identically to this sample on paper chromatography.

Anal. Calc. for $C_{21}H_{22}O_{14} \cdot H_2O$: C, 48.8, H, 4.7; OMe, 6.0. Found: C, 48.6; H, 4.7; OMe, 6.25.

Hamamelitannin methyl β-D-glycoside (**6**). — A solution of compound **4** (1 g) in ethyl acetate (400 ml) was hydrogenated similarly (uptake of hydrogen complete in 3 h) to yield a solid product which was recrystallised from water as a hydrate

(stable even on drying at 64° *in vacuo*) (0.4 g, 76%), m.p. 206–208°, $[\alpha]_D$ −30.6° (*c* 2, ethanol), R_F 0.54 (solvent *B*), 0.35 (solvent *C*); ν_{max} 3300 (OH), 1700 (C=O), 760 and 740 (aromatic) cm^{-1}; lit.[6], m.p. 206–209°, R_F 0.54 (solvent *B*), 0.35 (solvent *C*), $[\alpha]_D$ −29.4° (*c* 2.0, acetone); n.m.r. data (methyl sulphoxide-d_6, containing D_2O): τ 2.90(*s*) (aromatic protons), 5.20(*s*) (H-1), and 6.75(*s*) (CH_3-O).

Anal. Calc. for $C_{21}H_{22}O_{14} \cdot H_2O$: C, 48.8; H, 4.7; OMe, 6.0. Found: C, 49.1; H, 4.4; OMe, 6.2.

Hexa-O-methylhamamelitannin methyl α-D-glycoside (7). — A solution of the glycoside **5** (0.1 g) in acetone (6 ml) was methylated with an ethereal solution of diazomethane (10 ml, ~1%) at 0°. After storage for 16 h at 0° and for 2 h at room temperature, the solvent was evaporated to give a syrup which was fractionated on a column of silica gel (30 g) with solvent *c* to yield compound **7**, in syrupy form, as the major fraction. On trituration with acetone, this fraction slowly crystallised. Recrystallisation from ethyl ether–acetone (20:1) yielded compound **7** as colourless needles (0.06 g, 53%), m.p. 94–95°, $[\alpha]_D$ +43.0° (*c* 0.5, ethanol).

Anal. Calc. for $C_{27}H_{34}O_{14}$: C, 55.7; H, 5.9; OMe, 37.2. Found: C, 55.6; H, 6.0; OMe, 37.0.

Hexa-O-methylhamamelitannin methyl β-D-glycoside (8). — A solution of compound **6** (0.5 g) in acetone (8 ml) was treated similarly with ethereal diazomethane (30 ml, ~0.1%) to afford a syrup which was purified on a column of silica gel (60 g) with solvent *c*. Compound **8** was obtained as a syrup which slowly crystallised on trituration with methanol. Colourless prisms (0.27 g, 47%) were obtained on recrystallisation from acetone; m.p. 162.5–163.5°, $[\alpha]_D$ −28.5° (*c* 2, acetone); n.m.r. data: τ 2.63(*m*) and 2.71(*m*) (aromatic protons), 5.04(*s*) (H-1), 6.09(*m*) (aromatic O-Me), and 6.62(*s*) (OMe at C-1). Mayer *et al.*[6] gave m.p. 161–162°, $[\alpha]_D$ −20.5° for this compound.

Anal. Calc. for $C_{27}H_{34}O_{14}$: C, 55.7; H, 5.9; OMe, 37.2. Found: C, 55.5; H, 6.0; OMe, 37.1.

When compound **8** (0.95 g, 1.63 mmoles) in *p*-dioxane (20 ml) was treated with sodium periodate (0.46 g, 2.03 mmoles) in water (6.6 ml), oxidation occurred. After storage for 24 h at room temperature, the solution was diluted with water (70 ml) and kept at 0°. The crystals which separated were recrystallised from acetone–ether (1:2) to give the oxidation product **9** (0.6 g, 61%), m.p. 120–122°, $[\alpha]_D$ −17.3° (*c* 1.0, *p*-dioxane); n.m.r. data: τ 2.66(*m*) and 2.72(*m*) (aromatic protons), 5.30(*s*) (H-1), 6.08(*m*) (aromatic CH_3-O), and 6.47(*s*) (acetal CH_3-O). Mayer *et al.*[6] gave m.p. 120–121°, $[\alpha]_D$ −17.0° for this compound.

Anal. Calc. for $C_{27}H_{34}O_{15}$: C, 54.2; H, 5.7; OMe, 36.3. Found: C, 54.25; H, 5.8; OMe, 36.1.

Attempted partial hydrolysis of compounds **5** *and* **6**. — The glycoside (**5** or **6**) was heated under reflux in aqueous sulphuric acid of varied concentration in the range 0.1 to 2.0N, and, in each case, the solution was analysed at hourly intervals by neutralising a sample with barium carbonate and examining it by paper chromatography (solvent *B*). Under all of the conditions examined, hydrolysis to hamameli-

tannin (R_F 0.32) was accompanied by extensive formation of gallic acid (R_F 0.60) and hamamelose (R_F 0.27). Similar treatment of the hexabenzyl ether (**4**) of compound **6**, followed by catalytic hydrogenation of the benzyl groups, again yielded gallic acid as a major product, with only traces of the tannin.

Benzyl 2-C-hydroxymethyl-β-D-ribofuranoside (**10**). — A suspension of 2-*C*-hydroxymethyl-D-ribose (8.04 g) in benzyl alcohol (400 ml) containing hydrogen chloride (1%, w/v) was stirred for 120 h at 50–55° (bath). The cooled solution was neutralised with potassium carbonate and filtered, and the benzyl alcohol was removed by distillation at 80° (bath)/0.1 mm. Chromatographic separation on silica gel (1.5 kg) (solvent *d*) of the residue afforded benzyl 2-*C*-hydroxymethyl-D-ribofuranoside (10 g, 80%) as a syrup, $[\alpha]_D$ −4.8° (*c* 0.9, ethanol), R_F 0.74 (solvent *A*) (Found: C, 57.6; H, 6.9%). On storage of the syrup, partial crystallisation occurred, and the crystals were collected and recrystallised from ethyl acetate as colourless needles (2.0 g, 16%), m.p. 95.5–96.5°, $[\alpha]_D$ −115° (*c* 1.0, ethanol); ν_{max} 3300 (OH), 750 and 730 (aromatic) cm^{-1}; n.m.r. data (methyl sulphoxide-d_6, containing D_2O): τ 2.66(*s*) (aromatic protons), 5.18(*s*) (H-1), and an AB system centred at τ 5.40(*q*) (benzyl CH_2, coupling constant 13 Hz). From Hudson's rules, the crystalline glycoside was assumed to be the β-D anomer.

Anal. Calc. for $C_{13}H_{18}O_6$: C, 57.8; H, 6.7. Found: C, 58.0; H, 6.4.

On sodium periodate oxidation at room temperature, the syrupy glycoside consumed 2.01 molar equivalents of oxidant in 1 h. In the same time, the crystalline glycoside consumed 2.0 molar equivalents of the oxidant.

Hydrogenation of benzyl 2-*C*-hydroxymethyl-β-D-ribofuranoside (0.1 g) in ethanol (20 ml) in the presence of 10% palladium-on-charcoal (2 g) gave 2-*C*-hydroxymethyl-D-ribose as the only product.

Hexa-O-benzylhamamelitannin benzyl β-D-glycoside (**11**). — Tri-*O*-benzylgalloyl chloride (4.67 g) in dry tetrahydrofuran (45 ml) was added dropwise to a stirred solution of crystalline benzyl 2-*C*-hydroxymethyl-β-D-ribofuranoside (1.34 g) in dry pyridine (30 ml) at −40°. After storage for 65 h at −30°, then for 3 h at 0°, and finally for 3 h at room temperature, the reaction mixture was worked up in the usual way to afford a syrup (containing crystals) which was shown (t.l.c., solvent *a*) to contain three components. Fractionation on silica gel (550 g) with solvent *a* gave (*i*) a waxy solid (1.5 g), assumed to be a tri-*O*-galloyl derivative, and (*ii*) another waxy solid (3.0 g), assumed to be the required di-*O*-galloyl derivative (**11**), which, after recrystallisation twice from ethyl acetate–acetone–chloroform (6:3:5), was obtained as colourless, fibrous needles (1.2 g, 22%), m.p. 174.5–176°, $[\alpha]_D$ −20.6° (*c* 0.9, ethyl acetate); ν_{max} 3300 (OH), 1700 (C=O), 1575, 760 and 730 (phenyl groups) cm^{-1}; n.m.r. data: τ 2.47–2.87(*m*) (aromatic protons), 4.78–4.96(*m*) (benzyl CH_2), and 5.03(*s*) (H-1). Refractionation of the mother liquors on silica gel yielded a further 0.6 g (11%) of product.

Anal. Calc. for $C_{69}H_{62}O_{14}$: C, 74.3; H, 5.6. Found: C, 74.0; H, 5.4.

Hamamelitannin. — A solution of β-D-glycoside **11** (1.0 g) in ethyl acetate (450 ml) was hydrogenated over 10% palladium-on-charcoal (1.0 g). When hydrogen

uptake ceased (after 1 h), the filtered solution was concentrated under diminished pressure to a syrup which crystallised on trituration with water. Recrystallisation from water furnished colourless needles (0.25 g, 58%), m.p. 145–147.5° (after drying *in vacuo* at 100°), $[\alpha]_D$ +31.3° (*c* 1.5, water), R_F 0.26 (solvent *B*), 0.32 (solvent *C*); ν_{max} 3400 (OH), 1700 (C=O), 760 (phenyl) cm^{-1}; n.m.r. data (methyl sulphoxide-d_6, containing D_2O): τ 2.95(*s*) (aromatic protons). Mayer *et al.*[6] reported m.p. 145–147°, $[\alpha]_D$ +32.6°, for this compound. An authentic sample of hamamelitannin showed the same R_F values on paper chromatography, gave an identical infrared spectrum, and was identical by mixed m.p. with the synthetic material.

Anal. Calc. for $C_{20}H_{20}O_{14}$: C, 49.6; H, 4.2. Found: C, 49.4; H, 4.3.

ACKNOWLEDGMENTS

The authors thank Professor W. Mayer for generous samples of hamamelitannin and its methyl α-D-glycoside, and the S.R.C. for a maintenance award (to A.D.E.).

REFERENCES

1 W. G. Overend and N. R. Williams, *J. Chem. Soc.*, (1965) 3446.
2 F. Grüttner, *Arch. Pharm.*, 236 (1898) 278.
3 W. Mayer and W. Kunz, *Naturwissenschaften*, 46 (1959) 206.
4 W. Kunz, Dissertation, University of Heidelberg, 1961; see also ref. 6.
5 For a review of earlier studies, see F. Shafizadeh, *Advan. Carbohyd. Chem.*, 11 (1956) 263.
6 W. Mayer, W. Kunz, and F. Loebich, *Ann.*, 688 (1965) 232.
7 J. M. Sugihara, *Advan. Carbohyd. Chem.*, 8 (1953) 1.
8 G. O. Aspinall and R. J. Ferrier, *Chem. Ind.* (London), (1957) 1216.
9 E. J. Hedgley and H. G. Fletcher, Jr., *J. Org. Chem.*, 30 (1965) 1282.
10 P. J. Beynon, P. M. Collins, P. T. Doganges, and W. G. Overend, *J. Chem. Soc.* (*C*), (1966) 1131.
11 R. Armitage, G. S. Bayliss, J. W. Gramshaw, E. Haslam, R. D. Haworth, K. Jones, H. J. Rogers, and T. Searle, *J. Chem. Soc.*, (1961) 1842.

Carbohydrate Research
Elsevier Publishing Company, Amsterdam
Printed in Belgium

CHEMICAL SYNTHESIS OF *p*-NITROPHENYL β-SOPHOROSIDE AND *p*-NITROPHENYL β-LAMINARABIOSIDE*

R. N. IYER† AND I. J. GOLDSTEIN‡

Department of Biological Chemistry, The University of Michigan, Ann Arbor, Michigan 48104 (U. S. A.)

(Received March 21st, 1969)

ABSTRACT

Condensation of tetra-*O*-acetyl-α-D-glucopyranosyl bromide with *p*-nitrophenyl 4,6-*O*-benzylidene-β-D-glucopyranoside followed by appropriate deblocking reactions gives rise to an approximately equimolar mixture of *p*-nitrophenyl β-sophoroside and *p*-nitrophenyl β-laminarabioside. The two glycosides are readily separated and isolated in pure form by cellulose-column chromatography.

INTRODUCTION

Our interest in carbohydrate–protein conjugates as model substrates for investigating the interaction of phytohemagglutinins[1,2] and for studying the immunogenicity of carbohydrates[3] prompted us to prepare the β-sophorosyl and β-laminarabiosyl-*p*-azophenyl–protein conjugates. Carbohydrate–protein conjugates are generally prepared by the diazo coupling of *p*-aminophenyl glycosides to tyrosyl, histidyl, and lysyl side chains of a protein carrier[4]. *p*-Nitrophenyl glycosides, precursors for the synthesis of *p*-aminophenyl glycosides, are generally prepared by the method of Helferich[5] or that of Koenigs and Knorr[6]. Since sophorose and laminarabiose are relatively rare oligosaccharides, the general methods for the preparation of the *p*-nitrophenyl glycosides of these oligosaccharides were found unsuitable. This communication describes a facile, simultaneous preparation of the *p*-nitrophenyl β-glycosides of sophorose and laminarabiose.

EXPERIMENTAL

Melting points were determined on a Fisher–Johns melting-point apparatus and are uncorrected. All evaporations were conducted *in vacuo* at 35–40° with a rotary evaporator. Silica gel (type G) for t.l.c. was purchased from Brinkmann

*This research was supported by Grant AM-10171 from the National Institutes of Health.
†Present address: Department of Pediatrics, Medical Center, Case-Western Reserve University, Cleveland, Ohio.
‡This work was done while this author was an Established Investigator of the American Heart Association. To whom enquiries concerning this paper should be sent.

Instruments; components were detected by spraying with 50% (v/v) ethanol-sulfuric acid followed by charring in an oven at 120°. Paper chromatography was carried out on Whatman No. 1 paper with butanone–water azeotrope as the solvent system. Localization of *p*-nitrophenyl glycosides was accomplished by examination of paper chromatograms under ultraviolet light.

p-*Nitrophenyl 4,6-O-benzylidene-β-D-glucopyranoside.* — To a solution of *p*-nitrophenyl β-D-glucopyranoside (10 g, 33.2 mmoles) in formic acid (50 ml) was added benzaldehyde[7] (60 ml). The reaction mixture was stirred vigorously for 5.5 min and was immediately neutralized with 30% aqueous potassium carbonate. Petroleum ether (b.p. 30–60°, 2 l) was added and the product that separated at the interface was filtered, and washed throughly with petroleum ether and subsequently with water. The product (10.8 g, 88.5%), was recrystallized from 95% ethanol; m.p. 184–185°, $[\alpha]_D^{25}$ −46.2° (*c* 1.0, acetone). Lit.[8] m.p. 184–185°, $[\alpha]_D^{20}$ −44.9° (*c* 2, acetone).

p-*Nitrophenyl 4,6-O-benzylidene-2-O-(2,3,4,6-tetra-O-acetyl-β-D-glucopyranosyl)-β-D-glucopyranoside.* — To a mixture of *p*-nitrophenyl 4,6-*O*-benzylidene-β-D-glucopyranoside (10 g, 25.2 mmoles), Drierite (15 g), and silver carbonate (15 g) in dichloromethane (150 ml, dried over molecular sieve), which had been stirred for 0.5 h, was added tetra-*O*-acetyl-α-D-glucopyranosyl bromide (13.4 g, 32.7 mmoles) and iodine (1.7 g). The reaction mixture, protected from light, was shaken on a Burrell Wrist-Action Shaker at room temperature. The progress of the reaction was followed by testing for ionizable bromide and by t.l.c. (solvent: benzene–methanol, 9:1, v/v). After 28 h no further ionizable bromide was detectable, and the reaction mixture was filtered through a thin layer of Norite and concentrated to a syrup. Purification of *p*-nitrophenyl 4,6-*O*-benzylidene-2-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)-β-D-glucopyranoside (henceforth called sophorose adduct) by crystallization proved to be difficult inasmuch as the sophorose adduct had a strong tendency to co-crystallize with *p*-nitrophenyl 4,6-*O*-benzylidene-β-D-glucopyranoside. However a small amount of sophorose adduct (760 mg) was obtained as fine needles by dissolving the reaction mixture in hot ethanol and adding water until the first sign of turbidity. Crystallization ensued at room temperature, and the crystals that formed within 5 min were filtered immediately. The process was repeated (2 or 3 times) until the product could be shown to be chromatographically homogeneous; yield 760 mg, m.p. 170–171°.

Anal. Calc. for $C_{33}H_{37}NO_{17}\cdot H_2O$: C, 53.7; H, 5.29; N, 1.9. Found: C, 53.6; H, 5.69; N, 1.51.

p-*Nitrophenyl 2-O-(2,3,4,6-tetra-O-acetyl-β-D-glucopyranosyl)-β-D-glucopyranoside.* — The sophorose adduct (100 mg) was dissolved in 50% aqueous acetic acid (5 ml) and refluxed for 20 min. Acetic acid and benzaldehyde were removed by repeated evaporation under diminished pressure with frequent additions of water. The white crystalline solid that formed was recrystallized from 95% ethanol; yield 40 mg (46%), m.p. 207–208°.

Anal. Calc. for $C_{26}H_{33}NO_{17}$: C, 49.5; H, 5.24; N, 2.2. Found: C, 49.29; H, 5.33; N, 2.09.

p-*Nitrophenyl β-sophoroside and* p-*nitrophenyl β-laminarabioside.*—The reaction was effected as described earlier and the reaction mixture was filtered through charcoal–Celite and concentrated to a syrup. No attempt was made to crystallize the sophorose adduct at this stage. The syrup was dried *in vacuo* for 8–10 h at 40° in a vacuum oven (yield 21 g) and dissolved in anhydrous methanol (50 ml). Sodium methoxide in methanol (1%, 1 ml) was added, and the mixture was kept for 90 min at room temperature. The reaction mixture was neutralized (acetic acid) and concentrated to a syrup. The deacetylated product was debenzylidenated by dissolving it in 50% aqueous acetic acid (100 ml) and refluxing the solution for 30 min. Acetic acid and benzaldehyde were removed by repeated evaporation in the presence of water, and the reaction product was concentrated to a syrup. The syrup was dissolved in water (100 ml) and extracted with chloroform; the chloroform layer was discarded. Cations were removed from the aqueous phase by treatment with Amberlite IR-120 (H^+) resin and the water was removed by evaporation to afford a thick syrup (10.2 g) (*p*-nitrophenyl *β*-laminarabioside and *p*-nitrophenyl *β*-sophoroside may be co-crystallized at this stage from aqueous ethanol). Paper-chromatographic examination (butanone–water azeotrope) showed the presence of *p*-nitrophenyl *β*-D-glucopyranoside (R_F 0.47), *p*-nitrophenyl *β*-laminarabioside (R_F 0.16), *p*-nitrophenyl *β*-sophoroside (R_F 0.11), and glucose (at the origin). The *p*-nitrophenyl glycosides were readily detected by examination of the chromatogram under ultraviolet light; D-glucose was visualized by an alkaline silver nitrate spray.

Separation of p-*nitrophenyl β-sophoroside and* p-*nitrophenyl β-laminarabioside by chromatography on cellulose.* — The deblocked reaction mixture (0.5 g) was applied to a cellulose powder (Whatman cellulose powder, standard grade) column (48 cm × 1 cm), and the column was developed with butanone–water azeotrope at a flow-rate of 20 ml per h. Fractions (5 ml) were collected and monitored for the presence of carbohydrate by paper chromatography. The following components were identified: *p*-nitrophenyl *β*-D-glucopyranoside (fractions 10–29), *p*-nitrophenyl *β*-laminarabioside (fractions 35–56), *p*-nitrophenyl *β*-laminarabioside and *p*-nitrophenyl *β*-sophoroside (fractions 58–62), *p*-nitrophenyl *β*-sophoroside (fractions 63–80).

p-*Nitrophenyl β-laminarabioside.*—Fractions (35–56), which contained *p*-nitrophenyl *β*-laminarabioside (R_F 0.16), were pooled and concentrated to afford a crystalline mass. Recrystallization from 90% ethanol gave the pure product (105 mg), m.p. 235–236°, $[\alpha]_D^{25}$ −87° (*c* 0.5, water).

Anal. Calc. for $C_{18}H_{25}NO_{13}$: C, 46.7; H, 5.41; N, 3.02. Found: C, 46.7; H, 5.53; N, 3.13.

The compound was characterized as *p*-nitrophenyl *β*-laminarabioside on the basis of the following experiments: partial acid hydrolysis (0.33N H_2SO_4 for 30 min at 100°) gave products migrating with mobilities corresponding to laminarabiose, *p*-nitrophenyl *β*-D-glucopyranoside, D-glucose, *p*-nitrophenol, and unhydrolyzed product. Treatment with almond emulsin gave glucose, *p*-nitrophenol, and a trace of *p*-nitrophenyl *β*-D-glucopyranoside.

p-*Nitrophenyl β-sophoroside.* — Tubes (63–80), containing the slower-moving

component (R_F 0.11), were combined and concentrated to yield a crystalline product. The compound was recrystallized from 90% aqueous ethanol giving the pure substance (87 mg), m.p. 261–262°, $[\alpha]_D^{25}$ −67.9° (*c* 1, water).

Anal. Calc. for $C_{18}H_{25}NO_{13}$: C, 46.7; H, 5.41; N, 3.02. Found: C, 46.53; H, 5.65; N, 2.85.

The product was identical by m.p., mixed m.p., and specific rotation with a sample of *p*-nitrophenyl β-sophoroside prepared by condensing hepta-*O*-acetylsophorosyl bromide and *p*-nitrophenol. Partial acid hydrolysis (0.33N H_2SO_4 for 30 min at 100°) gave products migrating with sophorose (neither the reference nor the sample reacted with triphenyltetrazolium chloride reagent[9]), D-glucose, *p*-nitrophenol, *p*-nitrophenyl β-D-glucopyranoside, and the unhydrolyzed product. Treatment with almond emulsin gave D-glucose and *p*-nitrophenol.

p-*Aminophenyl β-sophoroside.* — *p*-Aminophenyl β-sophoroside was prepared by catalytic hydrogenation of *p*-nitrophenyl β-sophoroside. Platinum oxide (100 mg) was added to a solution of *p*-nitrophenyl β-sophoroside (500 mg) in 50% aqueous methanol (150 ml). The reduction was conducted at atmospheric pressure for 1 h. The catalyst was filtered and washed, and the filtrate was concentrated to a syrup under diminished pressure. The syrup was dissolved in a minimum quantity of water, abs. ethanol was added, and crystallization was allowed to proceed in the cold; yield, 380 mg; m.p. 211–212°.

Anal. Calc. for $C_{18}H_{27}NO_{11}$: C, 47.9, H, 6.45; N, 3.1. Found: C, 47.66; H, 6.63; N, 2.87.

Determination of the ratio of p-*nitrophenyl β-sophoroside to* p-*nitrophenyl β-laminarabioside.* — Unfractionated, deblocked reaction mixture (1 mg) was applied to Whatman No. 1 paper, and the chromatogram was developed with butanone–water azeotrope. The regions corresponding to *p*-nitrophenyl β-sophoroside and *p*-nitrophenyl β-laminarabioside were excised and equilibrated with 10 ml of water. Sugar concentrations were determined by the phenol–sulfuric acid procedure[10], employing *p*-nitrophenyl β-sophoroside and *p*-nitrophenyl β-laminarabioside as standards. The ratio of *p*-nitrophenyl β-laminarabioside to *p*-nitrophenyl β-sophoroside was found to be 1:1.

DISCUSSION

Although sophorose occurs in Nature (pods of *Sophora japonica*[11]) and as residues in a (1→2)-β-D-glucan[12], it is more readily prepared by chemical synthesis[13–15]. Laminarabiose, also a rare saccharide, has been prepared by the partial acid hydrolysis of laminaran[16] and by the Koenigs–Knorr condensation of tetra-*O*-acetyl-α-D-glucopyranosyl bromide with 4,6-*O*-benzylidene-1,2-*O*-isopropylidene-D-glucose[17], 1,2,4,6-tetra-*O*-acetyl-β-D-glucose[18], or 5,6-di-*O*-acetyl-1,2-*O*-isopropylidene-D-glucose[19], with subsequent removal of protecting groups.

A comparative study of the protecting groups available for the synthesis of sophorose was recently reported by Koeppen[20]. This author claimed that the Hel-

ferich and Zirner procedure[14], of condensing 1,3,4,6-tetra-*O*-acetyl-α-D-glucopyranose with tetra-*O*-acetyl-α-D-glucopyranosyl bromide in the presence of a suitable catalyst, is the method of choice despite the lower yields, because of the advantage of producing a peracetylated glycosyl halide as an intermediate, useful for the synthesis of glycosides. As shown herein, the Freudenberg[21,22] procedure is quite versatile inasmuch as use of the preformed glucoside obviates a further condensation step in the synthesis of the *p*-nitrophenyl glycoside of sophorose. Furthermore the method has the advantage of yielding at the same time the *p*-nitrophenyl glycoside of laminarabiose.

In a previous report[13] on the synthesis of sophorose from the condensation of tetra-*O*-acetyl-α-D-glucopyranosyl bromide with methyl 4,6-*O*-benzylidene-α-D-glucopyranoside, no mention was made concerning the possibility that laminarabiose was also formed. However, when the corresponding benzyl glucoside was employed as one of the reactants, laminarabiose as well as a trisaccharide [3,6-di-*O*-(β-D-glucopyranosyl)-D-glucopyranose] was formed[15]. In the present synthesis we have shown that equivalent amounts of *p*-nitrophenyl laminarabioside and sophoroside are formed, and it is quite probable that the aglycon moiety has a directive influence in affording the equimolar mixture of these two glycosides.

REFERENCES

1 I. J. GOLDSTEIN AND R. N. IYER, *Biochim. Biophys. Acta*, 121 (1966) 197.
2 I. J. GOLDSTEIN, R. N. IYER, E. E. SMITH, AND L. L. SO, *Biochemistry*, 6 (1967) 2373.
3 P. Z. ALLEN, I. J. GOLDSTEIN, AND R. N. IYER, *Biochemistry*, 6 (1967) 3029.
4 W. F. GOEBEL, O. T. AVERY, AND F. H. BABERS, *J. Exp. Med.*, 60 (1934) 599.
5 B. HELFERICH AND E. SCHMITZ-HILLEBRECHT, *Ber.*, 66 (1933) 378.
6 W. KOENIGS AND E. KNORR, *Ber.*, 34 (1901) 957.
7 J. G. BUCHANAN AND J. C. P. SCHWARZ, *J. Chem. Soc.*, (1962) 4770.
8 M. A. JERMYN, *Aust. J. Chem.*, 10 (1957) 448.
9 K. WALLENFELS, *Naturwissenschaften*, 37 (1950) 491.
10 M. DUBOIS, K. A. GILLES, J. K. HAMILTON, P. A. REBERS, AND F. SMITH, *Anal. Chem.*, 28 (1956) 350.
11 M. J. CLANCY, *Methods Carbohyd. Chem.*, 1 (1962) 345.
12 F. C. MCINTIRE, W. H. PETERSON, AND A. J. RIKER, *J. Biol. Chem.*, 143 (1942) 491.
13 B. COXON AND H. G. FLETCHER, JR., *J. Org. Chem.*, 26 (1961) 2892.
14 B. HELFERICH AND J. ZIRNER, *Ber.*, 2 (1963) 2604.
15 A. KLEMER AND K. HOMBERG, *Ber.*, 94 (1961) 2747.
16 V. C. BARRY AND J. E. MCCORMICK, *Methods Carbohyd. Chem.*, 1 (1962) 328.
17 P. BACHLI AND E. G. V. PERCIVAL, *J. Chem. Soc.*, (1952) 1243.
18 K. MATSUDA, *Chem. Ind.* (London), (1958) 1627.
19 K. FREUDENBERG AND K. V. OERTZEN, *Ann.*, 574 (1951) 37.
20 B. H. KOEPPEN, *Carbohyd. Res.*, 7 (1968) 410.
21 K. FREUDENBERG AND H. KNAUBER, *Naturwissenschaften*, 34 (1947) 344.
22 K. FREUDENBERG, H. KNAUBER, AND F. CRAMER, *Ber.*, 84 (1951) 144.

Carbohydrate Research
Elsevier Publishing Company, Amsterdam
Printed in Belgium

DETERMINATION OF THE STRUCTURE OF DISACCHARIDES AS *O*-TRIMETHYLSILYL DERIVATIVES OF DISACCHARIDE ALDITOLS BY GAS–LIQUID CHROMATOGRAPHY–MASS SPECTROMETRY*

JORMA KÄRKKÄINEN
Department of Medical Chemistry, University of Helsinki, Helsinki (Finland)
(Received March 21st, 1969)

ABSTRACT

A method is described for the structural analysis of disaccharides by gas–liquid chromatography (g.l.c.)–mass spectrometry. A disaccharide mixture is reduced to the corresponding alditols, and analyzed as the *O*-trimethylsilyl derivatives by g.l.c. Simultaneous recording of the mass spectra allows the determination of the position of the intermolecular bond, the molecular weight, and certain other structural details. The use of borodeuteride instead of borohydride in the reduction stage is essential for the interpretation of the mass spectra. The monosaccharide components are identified by g.l.c. after isolation of the *O*-trimethylsilyl derivatives of the disaccharide alditols by small-scale, preparative g.l.c. The method is applied to the analysis of disaccharides containing both simple and amino sugars.

INTRODUCTION

Although analysis of small oligosaccharides as their *O*-trimethylsilyl (TMS) derivatives[1] by gas–liquid chromatography (g.l.c.) offers several advantages as compared to the use of other chromatographic techniques, this method has not been widely employed in the study of biochemical problems. Gas-chromatographic retention times of the anomers of reducing sugars and of their borohydride-reduction products have ordinarily formed the basis for qualitative identifications[2,3]. This type of identification usually requires some previous knowledge of the composition of the mixture and availability of reference materials. Effective utilization of the sensitivity, good resolution, and automation offered by g.l.c. has not been possible in oligosaccharide analysis, because of the lack of reliable small-scale methods for the characterization of the compounds separated by g.l.c.

Recent studies on mass spectrometry (m.s.) of the TMS derivatives of simple mono- and oligo-saccharides[4,5] as well as on combined gas–liquid chromatography–mass spectrometry (g.l.c.–m.s.) of the TMS derivatives of amino sugars and sugar alditols[6] have shown that direct analysis of the gas-chromatographic effluents with

*Presented in part at the Third Annual Meeting of Finnish Medical Societies, March 28–29, 1969, Helsinki.

m.s. can be successfully used for the identification of carbohydrates. However, because of the insensitivity of m.s. towards the stereochemical differences in monosaccharides, conclusive determination of the structure of an unknown oligosaccharide is not yet possible without its preparative isolation.

Separation of the TMS derivatives of anomeric disaccharides by g.l.c. approximately doubles the number of the peaks in the chromatograms and increases the probability of peak overlapping. If the mixture is reduced to the corresponding alditols with borohydride or borodeuteride prior to analysis, single peaks are obtained. As the anomeric composition of sugars is seldom of interest in biochemical problems, analysis of oligosaccharide alditols gives practically the same information as analysis of reducing oligosaccharides. The use of alditols in structural analysis diminishes the amount of work and, in addition, leads to considerable simplification of mass-spectral interpretations and monosaccharide analysis.

The method described below involves the reduction of a disaccharide mixture with borodeuteride, and analysis of the derived alditols as TMS derivatives by g.l.c.–m.s., followed by small-scale, preparative g.l.c. and identification of the monosaccharide components of the purified fractions with g.l.c. That the method can also be applied to the analysis of disaccharides containing amino sugars is of special importance, because of the widespread occurrence of amino sugars in the complex carbohydrates of animal and bacterial origin.

EXPERIMENTAL

D-Glucose, D-galactose, D-mannose, 2-acetamido-2-deoxy-D-glucose, 2-acetamido-2-deoxy-D-galactose, 4-*O*-β-D-galactopyranosyl-D-glucose (lactose), 4-*O*-β-D-glucopyranosyl-D-glucose (cellobiose), 4-*O*-α-D-glucopyranosyl-D-glucose (maltose), 6-*O*-β-D-glucopyranosyl-D-glucose (gentiobiose), and 6-*O*-α-D-galactopyranosyl-D-glucose (melibiose) were obtained from commercial sources. 2-Acetamido-2-deoxy-4-*O*-(β-D-galactopyranosyl)-D-glucose (*N*-acetyllactosamine) and 4-*O*-(2-acetamido-2-deoxy-D-galactopyranosyl)-D-galactose (D-GalNAc-(1→4)-D-Gal) were gifts from Dr. Adeline Gauhe, Heidelberg, Germany, and Dr. D. Shapiro, Rehovoth, Israel, respectively. All of these sugars were equilibrated in water for a minimum of two days, and their purity (as TMS derivatives) was checked by g.l.c. 3-*O*-β-D-Glucopyranosyl-D-glucose (laminaribiose) was prepared from laminarin by hydrolysis with 0.1N H_2SO_4 for 3 h at 100°. Analysis by g.l.c.–m.s. showed that, in addition to laminaribiose[5], a small amount of an unknown disaccharide alditol was also liberated by the hydrolysis. As the presence of the latter compound (10% of the amount of laminaribiose) did not interfere with g.l.c., the hydrolyzate was not further purified.

Disaccharide alditols were prepared by treatment of the reducing sugars (0.1 mg) with 4 mg of KBH_4 in an aqueous borate buffer (1 ml, pH 8.2) for 18 h at 4°, followed by acidification with acetic acid, removal of cations with Dowex-50 (H^+), and evaporation with dry methanol. Deuterated analogues[7] were prepared similarly with $NaBD_4$ in buffered deuterium oxide. However, if H_2O was substituted

for D_2O, the yield of the deuterated derivative was not lowered to a noticeable degree, indicating that the use of D_2O is probably unnecessary.

TMS derivatives were made by treatment of the carbohydrates with a mixture of chlorotrimethylsilane, hexamethyldisilazane, and pyridine (1:2:10) for 3 h at 70°, followed by evaporation in a nitrogen stream and extraction into hexane. All silylation reagents were redistilled before use.

Perkin–Elmer Model 900 and F & M Model 400, gas chromatographs, equipped with flame-ionization detectors, were used in the experiments. G.l.c.–m.s. was performed with an LKB Model 9000 Gas Chromatograph–Mass Spectrometer with helium as carrier gas. The energy of the bombarding electrons was 70 eV; the ionizing current was 60 μamps. The total mass-range covered was from *m/e* 40–1000. Glass columns (2 or 3.5 m × 3.5 mm), filled with packings of 2.2% SE-30 on Gas Chrom S (100–120 mesh), 3% QF-1 on Gas Chrom Q (80–100 mesh), 3% XE-60 on Gas Chrom Q (80–100 mesh) (supports and phases from Applied Science Laboratories Inc., State College, Pennsylvania), and 1% OV-22 on Supelcoport (80–100 mesh, Supelco Inc., Bellefonte, Pennsylvania), were employed in g.l.c. and g.l.c.–m.s.

In preparations with g.l.c., the fractions were collected by means of a Pasteur pipette attached to the jet of the flame-ionization detector on the basis of relative retention times established by a previous test run. After the purity of the fractions had been checked with g.l.c., they were methanolyzed in N dry methanolic HCl for 16 h at 70°, and the methanolysis products were acetylated with pyridine–acetic anhydride (1:1) for 1 h at 70°. Retention times of alditols and methyl glycosides prepared from the corresponding reference monosaccharides served as a basis for t.l.c. identifications. No correction factors for the peak areas were used in the quantigative calculations.

RESULTS

Analysis of disaccharide alditols with g.l.c. — Table I shows the relative retention times of a number of disaccharide alditols analyzed as their TMS derivatives with g.l.c., using four different stationary phases. Although complete separation of all of the derivatives could not be achieved with any single column, analysis with two columns, *e.g.*, OV-22 and XE-60, permitted the quantitative estimation of every disaccharide alditol shown in Table I from their mixture. The effect of deuterium substitution on the retention times of the disaccharide alditols was negligible.

Analysis of disaccharide alditols with g.l.c.–m.s. — Partial mass spectra of the TMS derivatives of the disaccharide alditols are presented in Table II. Ions below *m/e* 450 and above *m/e* 700 (see below) are largely omitted from the list.

Peaks of highest intensity (*m/e* 73, 103, 147, 204, 217, and 361) were found in the low mass-range of the spectra. Because the relative intensity of the ion at *m/e* 217 showed the least variation, as compared to that of ions of greater *m/e*, all intensities are expressed as a percent of this ion.

TABLE I

RELATIVE RETENTION TIMES OF TMS DERIVATIVES OF DISACCHARIDE ALDITOLS IN G.L.C.

Compound	*Stationary phase*			
	2.2% SE-30 (263°)	*3% QF-1 (227°)*	*3% XE-60 (230°)*	*1% OV-22 (218°)*
Lactitol	0.62	0.64	0.60	0.54
Cellobiitol	0.64	0.66	0.61	0.59
Maltitol	0.76	0.77	0.72	0.68
Laminaribiitol	0.67	0.71	0.65	0.63
Gentiobiitol	0.85	0.87	0.89	0.89
Melibiitol	1.00	1.00	1.00	1.00
N-Acetyllactosaminitol	0.89	1.97	1.45	1.20
D-GalNAc-(1→4)-D-Gal-ol	0.80	1.46	1.47	1.01

TABLE II

PARTIAL MASS SPECTRA OF TMS DERIVATIVES OF DISACCHARIDE ALDITOLS

m/e	*Lactitol*	*Cellobiitol*	*Maltitol*	*Laminaribiitol*	*Gentiobiitol*	*Melibiitol*	N-*Acetyllactosaminitol*	D-*GalNAc*-(*1*→*4*)-D-*Gal*-ol
173	0.5	0.2	1.0	1.0	1.0	1.0	2.1	59
174	—	—	—	—	—	—	4.7[a]	—
204	210	310	147	245	326	195	90	24
205	45	58	51[a]	44	45[a]	49[a]	18	43
217	100	100	100	100	100	100	100	100
276	—	—	—	—	—	—	7.6[a]	—
307	7.5[a]	12[a]	22[a]	9.0	22[a]	27[a]	—	10[a]
330	—	—	—	—	—	—	—	80
361	64	99	206	129	108	76	44	2
404	—	—	—	—	—	—	2.4[a]	4
420	—	—	—	—	—	—	—	96
435	2.0[a]	2.2[a]	2.8[a]	3.0[a]	2.0[a]	1.5[a]	—	—
451	9.6	12	8.0	8.1	4.2	5.1	3.0	7.4
494	—	—	—	—	—	—	68[a]	—
508	—	—	—	—	—	—	—	2.0
525	8.6[a]	9.0[a]	7.4[a]	9.2[a]	9.0[a]	13[a]	—	1.4[a]
539	1.5	2.9	2.1	3.1	2.2	3.2	1.6	—
564	—	—	—	—	—	—	2.0[a]	—
576	—	—	—	—	—	—	—	1.5
595	2.9[a]	0.9[a]	0.3[a]	0.4[a]	0.3	0.2	—	10[a]
607	0.4	0.1	0.3	0.2[a]	—	—	—	—
612	—	—	—	—	—	—	28[a]	—
643	2.3[a]	2.0[a]	2.0[a]	2.7[a]	0.3[a]	0.6[a]	—	—
654	—	—	—	—	—	—	9.4[a]	—
666	—	—	—	—	—	—	—	1.4
685	6.2[a]	1.3[a]	0.4[a]	0.9[a]	0.8	0.2	—	16[a]
697	0.6	2.5	0.4	0.2[a]	0.1	0.1	0.3	—

[a]In the deuterated derivative, > 50% of the ion is converted into the next higher *m/e*.

The molecular ions (at *m/e* 992 for simple, and 961 for amino sugar-containing disaccharide alditols) were seen only exceptionally. Fragments at M–15, M–90, M–(15+90), M–103, M–(103+90), and M–147 were present in most of the spectra. Although the intensity of these ions was low (less than 8% of the intensity of *m/e* 217, usually less than 0.5%), they permit the assignment of the molecular weight. As was to be expected[6], the intensity of these ions was greater in the spectra of the nitrogen-containing disaccharide alditols.

The molecular weights of the hexose and alditol units can be established by examining peaks analogous to those at *m/e* 451 and 361 in the spectra of the TMS derivatives of disaccharides[4,5]. Disaccharide alditols composed of a simple hexose and alditol show, in addition to the ions at *m/e* 451 and 361, prominent peaks at *m/e* 525 (451+74) and at 435 (361+74), corresponding to the calculated difference between the molecular weights of the two units. In the spectrum of *N*-acetyl-lactosaminitol, the two latter peaks are replaced by ions at *m/e* 494 (525−31) and at 404 (435−31), owing to the presence of the *N*-acetylamino group in the alditol moiety. In the spectrum of D-GalNAc-(1→4)-D-Gal-ol, the presence of the *N*-acetylamino group in the hexose unit analogously leads to the appearance of intense peaks at *m/e* 420 (451−31) and 330 (361−31). In the spectra of the two nitrogen-containing disaccharide alditols, the ions arising from the simple hexose or simple alditol units were of weak or only moderate intensity. Reduction with borodeuteride increased the *m/e* of the ions at 525, 435, 494, and 404 by one unit, which further confirms that they must arise from the alditol moiety.

For the assignment of the position of the intermolecular bond, the ion at *m/e* 307 (276 in the spectrum of *N*-acetyllactosaminitol) is of great importance, since it seems to arise exclusively through cleavage of the bond between C-3 and C-4 in the alditol moiety (Fig. 1). The change *m/e* 307→308 (*m/e* 276→277) observed after borodeuteride reduction of (1→4)- and (1→6)-linked disaccharides clearly distinguishes these from (1→3)-linked disaccharides. The presence of the ion at *m/e* 276, and the absence of the ion at *m/e* 307, in the spectrum of *N*-acetyllactosaminitol is, even without deuterium labeling, sufficient to exclude the possibility of a (1→3)-bond in the molecule.

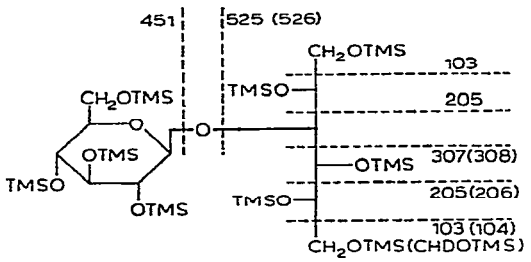


Fig. 1. *O*-Trimethylsilyl derivative of cellobiitol.

Although the intense ions at *m/e* 685 and 595 could arise through primary cleavage of the C-3–C-4 bond of the alditol unit, this path of fragmentation seems

to play a subordinate role in the generation of these ions. Firstly, these ions were absent from the spectrum of *N*-acetyllactosaminitol, which, on the other hand, displayed peaks at *m/e* 654 and 564 (655 and 565 in the deuterated species). Secondly, the peaks at *m/e* 685 and 595 (686 and 596) were present in the spectrum of D-GalNAc-(1→4)-D-Gal-ol, which correspondingly lacked the ions at *m/e* 654 and 564. Finally, reduction of simple (1→4)- and (1→3)-linked disaccharides with borodeuteride resulted, in both cases, in almost complete conversion of the ion at *m/e* 685→686 and of the ion at *m/e* 595→596. As the ions at *m/e* 685 and 595 are present in the mass spectrum, in spite of the *N*-acetylamino group attached to C-2 of the hexose moiety, it is evident that this substituent and probably also C-2 are eliminated during the generation of these ions. The fragmentation mechanism is probably the same as the one that leads to the ions at *m/e* 611 (M–307) and 521 (M–307–90) in the spectra of TMS derivatives of simple disaccharides. The ions at *m/e* 685 and 595 in the spectra of (1→6)-linked disaccharide alditols were not affected by deuterium labeling, which clearly differentiates (1→6)- from (1→4)- and (1→3)-linked structures. In this particular case, primary cleavage of the C-3–C-4 bond in the alditol unit probably presents the greatest contribution to the formation of these fragments.

A fragment of 205 mass units can be formed by primary cleavage of two different C–C bonds of (1→3)- and (1→4)-linked disaccharide alditols. Approximately half of the intensity of this ion (somewhat less in the case of laminaribiitol) was converted into the ion at *m/e* 206 by deuterium labeling, which further indicates that a C–C bond adjacent to the glycosidic bond is not easily cleaved. The proportion of the deuterated species was higher in the spectra of (1→6)-linked disaccharide alditols. Although the presence of the isotope peaks of the prominent ion at *m/e* 204 somewhat complicates the estimation of the intensities, the ions at *m/e* 205 and 206 can be used in the differentiation of (1→6)- from (1→4)- and (1→3)-linked structures. The ion at *m/e* 174 in the spectrum of *N*-acetyllactosaminitol was changed to *m/e* 175 in the deuterated species and is evidently formed by cleavage of the C-2–C-3 bond of the alditol moiety.

Examination of ions at *m/e* 787 (M–205), 697 (M–205–90), and 607 (M–205–2×90) as well as at *m/e* 666 and 576 (M–205–90 and M–205–2×90 from D-GalNAc-(1→4)-D-Gal-ol) showed that conversion of these ions into the next higher *m/e* in the deuterated derivatives was not detectable in the spectra of (1→6)-linked, and less than 50% in the spectra of (1→4)-linked, disaccharide alditols, but almost complete in the spectrum of laminaribiitol. This result gives additional support to the hypothesis that cleavage of a C–C bond next to the glycosidic linkage does not occur readily. These ions are useful in the differentiation of (1→6)-, (1→4)-, and (1→3)-linked disaccharide alditols.

The ions at *m/e* 643 in the spectra of simple disaccharide alditols and at *m/e* 612 in the spectrum of *N*-acetyllactosaminitol were completely converted into *m/e* 644 and 613, respectively, after reduction with borodeuteride. These ions must contain the alditol unit and are probably analogous to the ion at *m/e* 569 in the spectra of simple disaccharides[5]. The intensity of the prominent ion at *m/e* 539 (508 in the

TABLE III

RELATIVE RETENTION TIMES OF ACETATES OF ALDITOLS AND METHYL GLYCOSIDES OF MONOSACCHARIDES IN G.L.C.

Compound	*Stationary phase*			
	2.2% SE-30 (212°)	*3% QF-1 (220°)*	*3% XE-60 (220°)*	*1% OV-22 (202°)*
Methyl D-glucoside	0.55	0.52	0.49[a], 0.53	0.50
Methyl D-galactoside	0.55	0.40, 0.47[a]	0.43, 0.45[a], 0.53	0.45[a], 0.50
Methyl D-mannoside	0.51	0.46	0.45	0.44
Methyl 2-amino-2-deoxy-D-glucoside	1.05	1.39	1.50	1.25
Methyl 2-amino-2-deoxy-D-galactoside	1.03	1.29	1.48	1.14
D-Glucitol	1.00	1.00	1.00	1.00
D-Galactitol	1.04	0.88	0.91	1.04
D-Mannitol	0.98	0.84	0.84	0.96
2-Amino-2-deoxy-D-glucitol	1.73	2.47	3.35	2.13
2-Amino-2-deoxy-D-galactitol	1.89	2.57	3.81	2.41

[a]Major peak.

spectrum of D-GalNAc-(1→4)-D-Gal-ol) was unchanged by deuterium labeling. As the ion at *m/e* 539 was also found to be constantly present in the spectra of simple disaccharides, it seems reasonable to assume that it contains the monosaccharide unit of the molecule. Peaks at *m/e* 668, 656, 655, 566, and 565 were found in the spectra of simple (1→3)- and (1→4)-linked disaccharide alditols. Their intensities (0–2.6% of the intensities of *m/e* 217), as well as the extent of deuterium substitution, showed differences, which can possibly be of value in the determination of the position or configuration of the glycosidic bond. The origin of these ions is unknown.

Because the majority of the ions below *m/e* 450 are produced by independent fragmentation of the two units, their examination gives relatively little information about the overall structure of the disaccharide alditol. Some of these ions (*e.g.*, *m/e* 173), however, may be useful in obtaining additional information about the structure of the monosaccharide units.

Differences in the stereochemistry of the monosaccharide units had very little influence on the mass spectra of the disaccharide alditols. On the other hand, as was to be expected on the basis of studies with disaccharides[4,5], the configuration of the glycosidic bond was found to have a more distinct effect on the intensity of a number of ions. However, it was not possible to establish general rules for the assignment of the configuration of the glycosidic bond, owing to the limited number of sugars analyzed and to lack of knowledge of the detailed fragmentation mechanisms.

Analysis of the monosaccharide components of disaccharide alditols. — Table III shows that the acetates are suitable derivatives for the g.l.c. identification of the methanolysis products of disaccharide alditols. The procedure was tested by reducing a mixture of melibiose, *N*-acetyllactosamine, and D-GalNAc-(1→4)-D-Gal (0.05 mg of each) with borohydride and subjecting the disaccharide alditols as TMS derivatives to preparative g.l.c. (stationary phase: QF-1). The purity of the fractions was checked by g.l.c. immediately after isolation of the peaks, using two different stationary phases. The methanolysis products obtained from the purified TMS derivatives were identified by g.l.c. as methyl D-galactoside and D-glucitol (1:1), methyl D-galactoside and 2-amino-2-deoxy-D-glucitol (0.9:1), and methyl 2-amino-2-deoxy-D-galactoside and D-galacitol (0.8:1), respectively.

DISCUSSION

The suitability of mass-spectrometric techniques for the analysis of the TMS derivatives of reducing oligosaccharides[4,5] provides a definite improvement in the methods for the study of polymeric carbohydrates. The molecular weight of a disaccharide, and the position and possibly the configuration of the intermolecular bond, as well as the sequence of monosaccharides of different molecular weight can be deduced from careful examination of the spectra of reducing disaccharides. However, preliminary studies by g.l.c.–m.s. of several simple and nitrogen-containing disaccharides indicated that the rules proposed previously[4,5], *e.g.*, for the assignment of the intermolecular bond, may not always be easily applicable. Furthermore, difficulties

will be encountered in the interpretation of these spectra if more than one disaccharide is present in the same chromatographic peak.

Determination of the sequence of monosaccharide units of equal molecular weight requires, after preparative isolation of the TMS derivative of a reducing disaccharide by g.l.c., hydrolysis of the silyl groups without breakage of the glycosidic bond. An experiment with the TMS derivative of lactose showed that it is possible to accomplish such hydrolysis. The liberated disaccharide was reduced with borohydride and hydrolyzed, and the products were identified as galactose and glucitol. However, the process is complicated; also, the hydrolysis of trimethylsilyl groups may present difficulties, owing to the acid lability of some amino sugar-containing disaccharides and the low solubility of TMS derivatives in polar solvents.

Analysis of disaccharides as their reduction products instead of reducing disaccharides was found to offer several advantages. The mass spectra of the disaccharide alditols are simpler to interpret, because of the possibility of deuterium labeling, which is of special importance in the analysis of unknown biological mixtures, where overlapping of peaks in g.l.c. cannot always be predicted and avoided. The presence of disaccharide alditols as single peaks also diminishes the possibility of overlapping. Furthermore, the component monosaccharides and their sequence can be determined by g.l.c., after complete hydrolysis or methanolysis of the TMS derivatives following their purification by g.l.c.

In the present work, methanolysis was employed instead of hydrolysis, because of the low solubility of the TMS derivatives in water, although the latter alternative is probably also feasible. The monosaccharide components were analyzed as acetates because of the poor g.l.c. resolution of the TMS derivatives of simple alditols. The g.l.c. analyses of monosaccharides can be supplemented, if considered necessary, with simultaneous recording of the mass spectra. Although disaccharides containing (1→2)- or (1→5)-linkages, or disaccharides composed of pentoses or deoxy sugars were not studied, it is probable that such sugars are equally suitable for structural analysis by the present method.

ACKNOWLEDGMENTS

I am grateful to Dr. H. Adlercreutz and Dr. T. Luukkainen for permission to use the g.l.c.–m.s. instrument at the Department of Clinical Chemistry, University of Helsinki.

REFERENCES

1 C. C. SWEELEY, R. BENTLEY, M. MAKITA, AND W. W. WELLS, *J. Amer. Chem. Soc.*, 85 (1963) 2497.
2 E. PERCIVAL, *Carbohyd. Res.*, 4 (1967) 441.
3 J. HUTTUNEN AND T. MIETTINEN, *Anal. Biochem.*, in press.
4 O. S. CHIZHOV, N. V. MOLODTSOV, AND N. K. KOCHETKOV, *Carbohyd. Res.*, 4 (1967) 273.
5 N. K. KOCHETKOV, O. S. CHIZHOV, AND N. V. MOLODTSOV, *Tetrahedron*, 24 (1968) 5587.
6 J. KÄRKKÄINEN AND R. VIHKO, *Carbohyd. Res.*, 10 (1969) 113.
7 H. BJÖRNDAL, B. LINDBERG, AND S. SVENSSON, *Carbohyd. Res.*, 5 (1967) 433.

Carbohydrate Research
Elsevier Publishing Company, Amsterdam
Printed in Belgium

REACTION OF METHYL 4,6-*O*-BENZYLIDENE-3-*C*-METHYL-2-*O*-*p*-TOLYLSULFONYL-α-D-ALLOPYRANOSIDE WITH SODIUM METHOXIDE IN METHYL SULFOXIDE: SYNTHESIS OF 6-DEOXY-3-*C*-METHYL-3-*O*-METHYL-D-ALLOSE (2-HYDROXY-D-CLADINOSE)

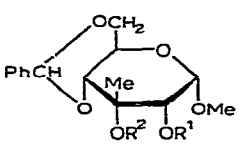
G. B. HOWARTH, W. A. SZAREK, AND J. K. N. JONES
Department of Chemistry, Queen's University, Kingston, Ontario (Canada)
(Received March 27th, 1969)

ABSTRACT

Treatment of methyl 4,6-*O*-benzylidene-3-*C*-methyl-2-*O*-*p*-tolylsulfonyl-α-D-allopyranoside (**1**) with an excess of sodium methoxide in dry methyl sulfoxide gave a complex mixture from which four crystalline compounds have been isolated: methyl 4,6-*O*-benzylidene-3-*C*-methyl-α-D-allopyranoside (**2**) and its 3-*O*-methyl (**3**), 2,3-di-*O*-methyl (**4**), and 2-*O*-methyl (**5**) derivatives. Compound **3** was converted into 6-deoxy-3-*C*-methyl-3-*O*-methyl-D-allose (2-hydroxy-D-cladinose, **8**) by a route which involved opening of the benzylidene ring by *N*-bromosuccinimide.

INTRODUCTION

In the course of our studies on the synthesis of branched-chain sugars, it was necessary to prepare methyl 3-*C*-methyl-2-*O*-methyl-α-D-allopyranoside[1]. A report by Ball *et al.*[2] that equatorial methanesulfonates of carbohydrates can be converted into ethers with retention of configuration, by reaction with alkoxides in methyl sulfoxide, led us to apply this reaction to methyl 4,6-*O*-benzylidene-3-*C*-methyl-2-*O*-*p*-tolylsulfonyl-α-D-allopyranoside[1] (**1**); the anticipated 2-*O*-methyl derivative (**5**), after de-*O*-benzylidenation, would give the required compound. The present report describes our results from the reaction with sodium methoxide, and the conversion of one of the products (**3**) into 6-deoxy-3-*C*-methyl-3-*O*-methyl-D-allose (2-hydroxy-D-cladinose, **8**).



1 R^1 = Ts, R^2 = H
2 R^1 = R^2 = H
3 R^1 = H, R^2 = Me
4 R^1 = R^2 = Me
5 R^1 = Me, R^2 H

RESULTS AND DISCUSSION

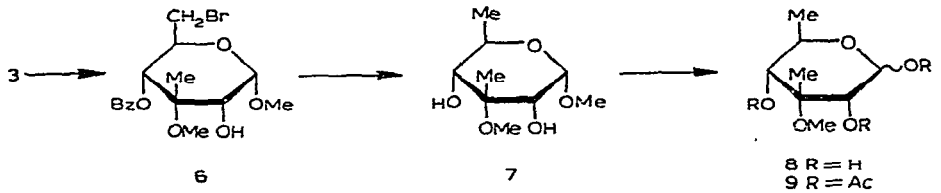
Treatment of **1** with an excess of sodium methoxide in anhydrous methyl sulfoxide gave a complex mixture from which four compounds were isolated in crystalline form by chromatography on silica gel. These compounds are methyl 4,6-*O*-benzylidene-3-*C*-methyl-α-D-allopyranoside (**2**) and its 3-*O*-methyl (**3**), 2,3-di-*O*-methyl (**4**), and 2-*O*-methyl (**5**) derivatives. Compound **2** was identified by comparison of its physical constants and infrared and n.m.r. spectra with those of an authentic sample prepared previously[1]; the structure was confirmed by resulfonation of **2** to give the starting material **1**. Treatment of **2** with methyl sulfate and sodium hydroxide in tetrahydrofuran gave a di-*O*-methyl derivative that was identical with compound **4**; the structure of the latter compound is, therefore, that of methyl 4,6-*O*-benzylidene-3-*C*-methyl-2,3-di-*O*-methyl-α-D-allopyranoside. Methylation of compounds **3** and **5**, which were shown to be monomethyl ethers by n.m.r. spectroscopy and elemental analysis, gave in each case compound **4**, a result that establishes that **3** and **5** are positional isomers. Compound **3** was assigned the structure of methyl 4,6-*O*-benzylidene-3-*C*-methyl-3-*O*-methyl-α-D-allopyranoside, since the same compound was obtained by methylation of **1** and removal of the sulfonic ester by base. It follows that compound **5** must be methyl 4,6-*O*-benzylidene-3-*C*-methyl-2-*O*-methyl-α-D-allopyranoside, an assignment which was corroborated by the conversion of an authentic sample[1] of methyl 3-*C*-methyl-2-*O*-methyl-α-D-allopyranoside* into **5** by treatment with benzaldehyde and zinc chloride. The n.m.r. spectra of compounds **2**, **3**, **4**, and **5** were consistent with the assigned structures; in each case $J_{1,2}$ was equal to 4 Hz, which indicated that the equatorial–axial orientation of H-1 and H-2 had been maintained.

The formation of the four compounds above can be accommodated by the mechanism proposed by Ball *et al.*[2] for the reaction of methanesulfonates with alkoxides in methyl sulfoxide. According to this mechanism, the initial step in the reaction with **1** would be a nucleophilic attack by methoxide on the sulfur atom of the *p*-tolylsulfonyloxy group to produce methyl *p*-toluenesulfonate and liberate a carbohydrate oxide anion, which would exist as a dianion in the basic medium. The second stage of the reaction involves a competition between methoxide and the carbohydrate anion for the methyl *p*-toluenesulfonate. The formation of more of the 3-methyl ether (**3**) than the 2-methyl ether (**5**) (see Experimental section) is consistent with the greater nucleophilicity of the tertiary oxide anion.

Recently, a synthesis of the branched-chain sugar L-cladinose (2,6-dideoxy-3-*C*-methyl-3-*O*-methyl-L-*ribo*-hexose) was reported[3]. In the present work, compound **3** was converted very readily into 2-hydroxy-D-cladinose (6-deoxy-3-*C*-methyl-3-*O*-methyl-D-allose, **8**). Treatment of **3** with *N*-bromosuccinimide[4,5] in boiling carbon

*Although, as indicated in the Introduction, the reaction with sodium methoxide in methyl sulfoxide was applied to **1** with a view to the preparation ultimately of this compound, its synthesis was achieved also by another route[1].

tetrachloride, in the presence of barium carbonate, gave a high yield of methyl-4-*O*-benzoyl-6-bromo-6-deoxy-3-*C*-methyl-3-*O*-methyl-α-D-allopyranoside (**6**). Removal of the benzoyl group at C-4 by catalytic hydrolysis, followed by debromination with lithium aluminum hydride, afforded methyl 6-deoxy-3-*C*-methyl-3-*O*-methyl-α-D-



6 7 8 R = H 9 R = Ac

allopyranoside (**7**). Acid-catalyzed hydrolysis of the glycoside **7** gave syrupy 2-hydroxy-D-cladinose (**8**), which formed a crystalline tri-acetate (**9**). Paper-chromatographic data for compound **8** are given in Table I.

EXPERIMENTAL

General methods. — Melting points were determined on a Fisher–Johns apparatus and are uncorrected. Optical rotations were measured with a Bendix ETL-NPL automatic polarimeter, Type 143A, at 20 ±2°. I.r. spectra were measured on a Beckman-IR5A spectrophotometer. N.m.r. spectra were determined at 60 MHz in chloroform-*d* with tetramethylsilane as internal standard. Silica Gel G was used for column chromatography. The term "petroleum ether" refers to the fraction of b.p. 60–80°.

Reaction of methyl 4,6-O-benzylidene-3-C-methyl-2-O-p-tolylsulfonyl-α-D-allopyranoside (**1**) *with sodium methoxide in methyl sulfoxide.* — To a solution of compound **1** (ref. 1) (3 g) in dry (molecular sieves) methyl sulfoxide (300 ml) was added sodium methoxide (3.1 g), and the mixture was stirred for 1 h at 70°. The dark-brown reaction mixture was poured into water (500 ml), and the aqueous solution was extracted with chloroform (3 × 300 ml). Concentration of the extracts, after they had been washed with water and dried (magnesium sulfate), gave a syrup (1.6 g) which yielded from chloroform–petroleum ether a crystalline product (0.66 g), m.p. 196–198°. Two recrystallizations from this solvent mixture gave analytically pure methyl 4,6-*O*-benzylidene-3-*C*-methyl-α-D-allopyranoside (**2**), having the same physical constants and i.r. and n.m.r. spectra as those previously reported[1] for this compound. A portion of **2** (20 mg) was treated with *p*-toluenesulfonyl chloride (30 mg) in pyridine (0.2 ml). After 24 h at room temperature, the product was isolated in the usual manner and recrystallized from ether–petroleum ether; the crystals had m.p. 138–139°, not depressed on admixture with authentic methyl 4,6-*O*-benzylidene-3-*C*-methyl-2-*O*-*p*-tolylsulfonyl-α-D-allopyranoside (**1**). The i.r. and n.m.r. spectra of the product and of **1** were identical.

Concentration of the chloroform–petroleum ether mother liquors above yielded a syrup, which was chromatographed on silica gel, with 2:3 ethyl acetate–

petroleum ether as eluent. The first component was a solid, which was recrystallized from *n*-pentane–petroleum ether to give methyl 4,6-*O*-benzylidene-3-*C*-methyl-3-*O*-methyl-α-D-allopyranoside (**3**) as needles; yield 250 mg (12%), m.p. 96–97°, $[\alpha]_D$ +86° (*c* 0.3, chloroform); λ_{max}^{KBr} 2.85 μm (OH); n.m.r. data τ 2.5–2.9 (5-proton multiplet, Ph), τ 4.7 (1-proton singlet, benzylidene-methine H), τ 5.4 (1-proton doublet, $J_{1,2}$ 4 Hz, H-1), τ 6.56, 6.61 (3-proton singlets, C-1 OMe and C-3 OMe), τ 8.6 (3-proton singlet, C-3 Me).

Anal. Calc. for $C_{16}H_{22}O_6$: C, 62.0; H, 7.1. Found: C, 61.8; H, 7.2.

The structure of compound **3** was confirmed by its preparation from **1** as follows. A mixture of **1** (100 mg), sodium hydroxide (600 mg), and methyl sulfate (1 ml) in tetrahydrofuran (2 ml) was stirred at room temperature overnight. The methylated product was obtained as a syrup; λ_{max}^{film} 6.25, 7.4, 8.4 μm (sulfonate), no absorption attributable to OH. A solution of this material in dry methanol (5 ml) containing sodium (200 mg) was heated for 1 h at reflux temperature. The product was isolated in the usual manner, and after recrystallization from *n*-pentane–petroleum ether was identical with compound **3** (m.p., and i.r. and n.m.r. spectra).

The second component eluted from the silica gel column was methyl 4,6-*O*-benzylidene-3-*C*-methyl-2,3-di-*O*-methyl-α-D-allopyranoside (**4**), and was obtained as needles after recrystallization from *n*-pentane; yield 52 mg (2%), m.p. 94–95°, $[\alpha]_D$ +90° (*c* 0.4, chloroform); no absorption in its i.r. spectrum (KBr) attributable to OH or sulfonate; n.m.r. data: τ 2.5–2.9 (5-proton multiplet, Ph), τ 4.55 (1-proton singlet, benzylidene-methine H), τ 5.2 (1-proton doublet, $J_{1,2}$ 4 Hz, H-1), τ 6.5–6.6 (9 protons, C-1 OMe, C-2 OMe, and C-3 OMe), τ 8.5 (3-proton singlet, C-3 Me).

Anal. Calc. for $C_{17}H_{24}O_6$: C, 63.0; H, 7.4. Found: C, 62.7; H, 7.4.

Compound **4** was obtained also by methylation of methyl 4,6-*O*-benzylidene-3-*C*-methyl-α-D-allopyranoside (**2**) with methyl sulfate and sodium hydroxide in tetrahydrofuran.

The third component eluted from the column was methyl 4,6-*O*-benzylidene-3-*C*-methyl-2-*O*-methyl-α-D-allopyranoside (**5**). Two recrystallizations from ether–petroleum ether gave prisms; yield 105 mg (5%), m.p. 142–143°, $[\alpha]_D$ +89° (*c* 0.2, chloroform); λ_{max}^{KBr} 2.85 μm (OH); n.m.r. data: τ 2.5–3.0 (5-proton multiplet, Ph), τ 4.6 (1-proton singlet, benzylidene-methine H), τ 5.2 (1-proton doublet, $J_{1,2}$ 4 Hz, H-1), τ 6.55, 6.6 (3-proton singlets, C-1 OMe and C-2 OMe), τ 8.6 (3-proton singlet, C-3 Me).

Anal. Calc. for $C_{16}H_{22}O_6$: C, 62.0; H, 7.1. Found: C, 61.7; H, 7.2.

Methylation of compounds **3** and **5** with methyl sulfate and sodium hydroxide in tetrahydrofuran gave in each case methyl 4,6-*O*-benzylidene-3-*C*-methyl-2,3-di-*O*-methyl-α-D-allopyranoside (**4**).

Methyl 4-O-benzoyl-6-bromo-6-deoxy-3-C-methyl-3-O-methyl-α-D-allopyranoside (**6**). — Methyl 4,6-*O*-benzylidene-3-*C*-methyl-3-*O*-methyl-α-D-allopyranoside (**3**) (126 mg) and *N*-bromosuccinimide (80 mg) in dry carbon tetrachloride (5 ml) containing barium carbonate (350 mg) were heated for 30 min at reflux temperature. The reaction mixture was filtered, and the filtrate was concentrated to a syrup, which

crystallized from ether–petroleum ether. Recrystallization from this solvent mixture gave **6** as needles; yield 125 mg (79%), m.p. 124–125°, $[\alpha]_D$ +84° (*c* 0.7, chloroform); λ_{max}^{KBr} 2.85 (OH), 5.8 (OBz), 6.25 μm (Ph); n.m.r. data : τ 1.8–2.0, 2.3–2.6 (multiplets, 5 protons, Ph), τ 5.1 (1-proton doublet, $J_{1,2}$ 4 Hz, H-1), τ 6.5, 6.7 (3-proton singlets, C-1 OMe and C-3 OMe), τ 8.8 (3-proton singlet, C-3 Me).

Anal. Calc. for $C_{16}H_{21}BrO_6$: C, 49.4; H, 5.4; Br, 20.6. Found: C, 49.3; H, 5.1; Br, 19.9.

Methyl 6-deoxy-3-C-methyl-3-O-methyl-α-D-allopyranoside (**7**). — A solution of compound **6** (50 mg) in dry methanol (2 ml) in which sodium (20 mg) had been dissolved was kept overnight at room temperature. The debenzoylated product was isolated in the usual manner as a syrup which was heated then for 5 h at reflux temperature with lithium aluminium hydride (50 mg) in tetrahydrofuran (2 ml). The reduced product was purified by chromatography on silica gel, with ether as eluent. Compound **7** was obtained as a syrup; yield 18 mg (68%), $[\alpha]_D$ +101° (*c* 0.7, chloroform); n.m.r. data: τ 5.15 (1-proton doublet, $J_{1,2}$ 4 Hz, H-1), τ 6.5, 6.55 (3-proton singlets, C-1 OMe and C-3 OMe), τ 8.7 (3-proton singlet, C-3 Me), τ 8.72 (3-proton doublet, *J* 6 Hz, C-5 Me).

Anal. Calc. for $C_9H_{18}O_5$: C, 52.5; H, 8.8. Found: C, 52.3; H, 8.6.

6-Deoxy-3-C-methyl-3-O-methyl-D-allose (*2-hydroxy-D-cladinose*) (**8**). — A solution of the glycoside **7** (18 mg) in 2M hydrochloric acid (1 ml) was heated for 4 h at 90°. The solution was neutralized with Duolite A-4 resin (OH^-) and concentrated to give the free sugar **8** as a clear viscous syrup; yield 14 mg (83%), $[\alpha]_D$ +5° (*c* 0.5, water). The syrup was shown to be chromatographically homogeneous in five solvent systems (see Table I).

TABLE I

CHROMATOGRAPHIC DATA FOR 2-HYDROXY-D-CLADINOSE[a] (**8**)

Solvent system (*v/v*)	R_{Rha}
3:1:3 Butyl alcohol–ethanol–water	1.74
3:1:3 Ethyl acetate–acetic acid–water	2.24
6:4:3 Butyl alcohol–pyridine–water	1.54
4:1:5 Butyl alcohol–ethanol–water	2.01
10:4:3 Ethyl acetate–pyridine–water	1.16

[a]Paper chromatography was carried out by the descending method on Whatman No. 1 filter paper. The chromatograms were sprayed with a 1:1 mixture of vanillin (1% in ethanol) and perchloric acid (3% in water), and heated[6]; a pink spot appeared which changed to grayish blue within 24 h.

1,2,4-Tri-O-acetyl-6-deoxy-3-C-methyl-3-O-methyl-D-allopyranose (**9**). — A solution of **8** (10 mg) in 2:3 acetic anhydride–pyridine (0.5 ml) was heated for 30 min at 90°. The product was isolated in the usual manner, and recrystallized from ether–petroleum ether to give prisms; yield 12 mg, m.p. 138–139°; λ_{max}^{KBr} 5.65, 5.7 μm (OAc).

Anal. Calc. for $C_{14}H_{22}O_8$: C, 52.9; H, 6.9. Found: C, 52.8; H, 6.9.

ACKNOWLEDGMENTS

The authors thank the National Research Council of Canada for financial support of this research. Some of the n.m.r. spectra were determined at the Royal Military College, Kingston, Ontario, and we are indebted to Dr. D. G. M. Diaper for the use of the spectrometer.

REFERENCES

1 G. B. HOWARTH, W. A. SZAREK, AND J. K. N. JONES, *Can. J. Chem.*, 46 (1968) 3375.
2 E. D. M. EADES, D. H. BALL, AND L. LONG, JR., *J. Org. Chem.*, 31 (1966) 1159.
3 G. B. HOWARTH AND J. K. N. JONES, *Can. J. Chem.*, 45 (1967) 2253.
4 S. HANESSIAN, *Carbohyd. Res.*, 2 (1966) 86; *Advan. Carbohyd. Chem.*, 21 (1966) 143.
5 G. B. HOWARTH, W. A. SZAREK, AND J. K. N. JONES, *Carbohyd. Res.*, 7 (1968) 284.
6 A. P. MACLENNAN, H. M. RANDALL, AND D. W. SMITH, *Anal. Chem.*, 31 (1959) 2020.

Carbohydrate Research
Elsevier Publishing Company, Amsterdam
Printed in Belgium

Note

Benzylidenation in basic medium: the reaction of methyl 2,3-di-*O*-methyl-6-*O*-toluene-*p*-sulphonyl-α-D-glucopyranoside and 3-chloro-1-propanol with benzaldehyde

N. Baggett, Md. Mosihuzzaman, and J. M. Webber
Chemistry Department, The University, P.O. Box 363, Birmingham 15 (Great Britain)
(Received March 10th, 1969)

Cyclic acetals are generally prepared by the acid-catalysed condensation of carbonyl compounds with hydroxylic substrates, and the nature of the product is thermodynamically controlled[1]. Under these conditions, both diastereoisomers of a 2-phenyl-1,3-dioxane derivative are not usually obtained[2], and the formation of cyclic acetals spanning vicinal, *trans*-hydroxyl groups is rare[3]. However, it has been shown that benzylidenation under basic conditions is kinetically controlled and gives products that are unusual because of their thermodynamic instability. Thus, diastereoisomeric 4,6-*O*-benzylidene derivatives have been prepared by the reaction of benzylidene halides with certain methyl 2,3-di-*O*-methylhexopyranosides in the presence of base[4]. Methylene derivatives of vicinal *trans*-diols have been obtained in analogous reactions with methylene halides[5]. The yields of acetals in these reactions were not high (*ca.* 30%), and it was therefore of interest to seek an alternative method of preparation subject to kinetic control.

The proposed route, involving the intramolecular displacement of a suitable leaving group (*e.g.*, chloride or sulphonate) by a hemiacetal anion, is shown in Fig. 1.

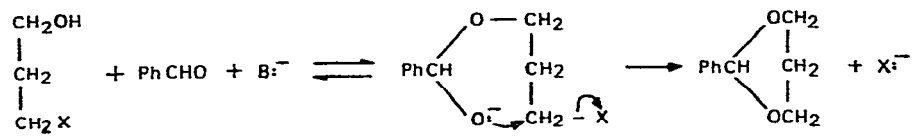


Fig. 1.

It has been suggested[6] that this route is followed in the formation of cyclic acetals by treatment of α-fluoroketones with halohydrins in the presence of potassium carbonate, but it was not clear whether the method could be extended to reactions of benzaldehyde, which forms hemiacetals to only a small extent[7]. However, the feasibility of the proposed scheme was demonstrated when 2-phenyl-1,3-dioxane was obtained, albeit in low yield, by treatment of 3-chloro-1-propanol with a 3-molar excess of benzaldehyde and potassium *tert*-butoxide in *tert*-butyl alcohol at 37°; a major by-product was benzyl alcohol. Similar treatment of methyl 2,3-di-*O*-methyl-6-*O*-toluene-*p*-sulphonyl-α-D-glucopyranoside (1) gave approximately equal amounts

(*ca.* 35% combined yield) of the diastereoisomeric forms of methyl 4,6-*O*-benzylidene-2,3-di-*O*-methyl-α-D-glucopyranoside. The acetals were fractionated by chromatography on silica gel and were identical with the known diastereoisomers[4]. The yield of acetals was not significantly affected by use of a larger excess of reagents (12 molar proportions), and this indicated that sufficient benzaldehyde had been employed to overcome the losses by conversion into benzyl alcohol. The use of a longer reaction time or a higher reaction temperature also had no significant effect on the yield of acetals.

Under the strongly basic conditions used in these reactions, the halide or sulphonate may also (*a*) undergo elimination to give an olefin or (*b*) form an anhydro compound by intramolecular displacement, and these side-reactions probably account for the low yield of acetals. It has been shown[8] that, for certain cyclic derivatives, these displacement reactions are much slower than for acyclic analogues, and, in particular, (*trans*-2-hydroxycyclohexyl)methyl *p*-bromobenzenesulphonate gave no oxetane. This observation may account, in part, for the fact that the yield of methyl 4,6-*O*-benzylidene-2,3-di-*O*-methyl-α-D-glucopyranoside was higher than that of 2-phenyl-1,3-dioxane.

The foregoing method of benzylidenation is complementary to the benzylidene halide method[4], but the low yield of acetals makes it of limited preparative value. In the examples described above, the displacement occurs at a primary carbon atom, and attempts at a similar displacement of the secondary sulphonyloxy group in *cis*-2-hydroxycyclohexyl *p*-bromobenzenesulphonate were unsuccessful. This reaction was of interest as a possible route to cyclic acetals spanning vicinal *trans*-diol groups. Provided that acetal formation involves intramolecular displacement by the hemiacetal group, the lack of reaction agrees with the finding of Sicher *et al.*[9] that neighbouring-group participation by the benzamido group does not take place in a series of *cis*-cyclohexyl derivatives.

Attempts to prepare *cis*-2-hydroxycyclohexyl *p*-bromobenzenesulphonate by the published[10] method, using 2–3 molar amounts of acid chloride for 30 min at 5–10°, gave a product that was not chromatographically homogeneous. Chromatographic separation gave crystalline *cis*-1,2-di-*p*-bromobenzenesulphonyloxycyclohexane, m.p. 125–126°, followed by *cis*-2-hydroxycyclohexyl *p*-bromobenzenesulphonate, m.p. 75–76°. Mori[10] gave m.p. 110.6° for the latter compound. Use of molar proportions of *p*-bromobenzenesulphonyl chloride for 10–15 min at 0° gave the monosulphonate in 60% yield. Similar conditions of sulphonylation were used to prepare the syrupy 6-toluene-*p*-sulphonate **1** [characterised as the 4-(*p*-phenylazobenzoate)] from methyl 2,3-di-*O*-methyl-α-D-glucopyranoside; longer reaction times produced mixtures of mono- and di-sulphonates, as indicated by t.l.c.

EXPERIMENTAL

T.l.c. was performed with silica gel G (Merck 7731), and separated components were detected with iodine vapour. Silica gel for column chromatography refers to Davison (U. S. A.) grade 950, 60–200 mesh, and alumina refers to the type H material

supplied by P. Spence Ltd. Optical rotation at 5461 Å was determined with an Ericsson ETL-NPL 143A automatic polarimeter at a path length of 1 cm. N.m.r. spectra were recorded with a Varian A–60 instrument. G.l.c. was performed with a Pye Argon instrument [poly(ethylene glycol adipate) column at 150°].

2-Phenyl-1,3-dioxane. — Freshly distilled benzaldehyde (8 ml) and a M solution of potassium *tert*-butoxide in *tert*-butyl alcohol (85 ml) were added batchwise (0.8 and 8.5 ml, respectively, at 30-min intervals) to a stirred solution of 3-chloro-1-propanol (2.5 g) in *tert*-butyl alcohol (20 ml) at 37°, the solvents were evaporated, and the residue was partitioned between water and ether. The ether extract was dried ($MgSO_4$) and evaporated to a liquid (5 g). Examination by g.l.c. revealed peaks corresponding to those for the title compound and benzyl alcohol, in the ratio of *ca.* 1:5. The liquid was fractionated on alumina (500 g), and elution with hexane–benzene (1:2) afforded the title compound (0.43 g, 10%), m.p. 42–43° (from hexane), mixed[11] m.p. 42–43°. The infrared spectrum of the product was indistinguishable from that of an authentic specimen. Further elution with benzene gave a liquid product that was indistinguishable from benzyl alcohol.

Methyl 2,3-di-O-methyl-6-O-toluene-p-sulphonyl-α-D-glucopyranoside (**1**). — Toluene-*p*-sulphonyl chloride (1.9 g, 10 mmoles) was added to a cooled solution of methyl 2,3-di-*O*-methyl-α-D-glucopyranoside[12] (2.22 g, 10 mmoles) in dry pyridine (10 ml), and the mixture was stirred for 10 min at 0°. The solution was diluted with water (200 ml) and extracted with chloroform (2 × 100 ml). The chloroform solution was washed with dilute hydrochloric acid, dried ($MgSO_4$), and evaporated to give the title compound as a colourless, chromatographically homogeneous syrup (2.5 g, 68%), ν_{max} (3500 (OH), 1360 and 1190 cm^{-1} (OSO_2) (Found: S, 8.7. $C_{16}H_{24}O_8S$ calc.: S, 8.5%).

By the usual method[13], the product was converted into the *p*-phenylazobenzoate (73%, m.p. 112–113° (from ethanol), $[\alpha]_{5461}^{21}$ +16.2° (*c* 1.0, acetone) (Found: C, 59.5; H, 5.5; N, 5.0; S, 5.5. $C_{29}H_{32}N_2O_9S$ calc.: C, 59.55; H, 5.5; N, 4.8; S, 5.5%).

Diastereoisomeric forms of methyl 4,6-O-benzylidene-2,3-di-O-methyl-α-D-glucopyranoside. — Benzaldehyde (14 ml, 10% solution in *tert*-butyl alcohol) and a M solution of potassium *tert*-butoxide in *tert*-butyl alcohol (16 ml) were added in portions during 4 h to a solution of methyl 2,3-di-*O*-methyl-6-*O*-toluene-*p*-sulphonyl-α-D-glucopyranoside (2.5 g) in *tert*-butyl alcohol (50 ml). The temperature was maintained at 37° overnight. After evaporation of the solvents, the crude reaction product was partitioned between saturated aqueous sodium chloride and ether. The ether extract was dried ($MgSO_4$) and evaporated to a syrup (2 g) which was then fractionated on a column of alumina (150 g). Elution with benzene–ether (9:1) gave a crystalline product *A* (0.4 g, 34% on the basis of reacted starting material), m.p. 115–118°, which showed (t.l.c.; benzene–ether, 1:1) components having R_F 0.65 and 0.73. Subsequent elution with ether–methanol mixtures gave syrupy starting material (1.0 g). The n.m.r. spectrum of product *A* (CCl_4) showed benzyl proton signals at τ 4.09 and 4.65, in the ratio of 3:5. Product *A* (780 mg) was refrac-

tionated on silica gel (100 g) with benzene–ether mixtures, and fractions (50 ml) were examined by t.l.c. Fractions 17–19 (242 mg, eluted with benzene–ether, 4:1) contained a single component (R_F 0.73), and recrystallisation from ether–hexane gave the title compound ["unusual", (*S*) diastereoisomer[4]], m.p. and mixed m.p. 126°, $[\alpha]_D^{29}$ +182° (*c* 0.4, acetone); the n.m.r. spectrum (CCl_4) showed a single benzyl proton signal at τ 4.09. Fractions 20–22 (373 mg) contained both components, but fractions 23–28 (183 mg; eluted with benzene–ether, 4:1) contained a single component (R_F 0.65), and recrystallisation from ether–hexane gave the title compound ["usual", (*R*) diastereoisomer[4]], m.p. and mixed m.p. 121–122°, $[\alpha]_D^{29}$ +95° (*c* 0.5, acetone); the n.m.r. spectrum showed a single benzyl proton signal at τ 4.65.

cis-*2-Hydroxycyclohexyl* p-*bromobenzenesulphonate*. — *p*-Bromobenzenesulphonyl chloride (2.56 g) was added to a solution of cyclohexane-*cis*-1,2-diol[14] (1.16 g) in dry pyridine (6 ml). The mixture was shaken for 10 min at *ca.* 0°, diluted with water, and extracted with chloroform. The chloroform solution was washed repeatedly with dilute hydrochloric acid, dried ($MgSO_4$), and evaporated to a syrup (2.54 g) which was fractionated on a column of silica gel (200 g). Elution with benzene gave *cis*-1,2-di-*p*-bromobenzenesulphonyloxycyclohexane (0.43 g, 8%); m.p. 125–126° (from ether); ν_{max} 1590 (aryl), 1370 and 1190 cm^{-1} (OSO_2) (Found: C, 39.0 ; H, 3.3; Br, 29.0; S, 11.6. $C_{18}H_{18}Br_2O_6S_2$ calc.: C, 39.0; H, 3.25; Br, 28.9; S, 11.55%). Subsequent elution with benzene–ether (4:1) gave the title compound (1.73 g, 60%); m.p. 75–76° (from ethyl acetate–hexane); ν_{max} 3500 (OH), 1590 (aryl), and 1370 and 1190 cm^{-1} (OSO_2) (Found: C, 42.8; H, 4.6; S, 9.5. $C_{12}H_{15}BrO_4S$ calc.: C, 43.0; H, 4.5; S, 9.55%).

ACKNOWLEDGMENTS

The authors thank Professor M. Stacey, C.B.E., F.R.S., for his interest, and the Association of Commonwealth Universities for a Commonwealth Scholarship (M.M.).

REFERENCES

1 J. A. Mills, *Advan. Carbohyd. Chem.*, 10 (1955) 1.
2 B. Dobinson, A. B. Foster, and M. Stacey, *Tetrahedron Lett.*, (1959) 1.
3 M. E. Evans and F. W. Parrish, *Tetrahedron Lett.*, (1966) 3805.
4 N. Baggett, J. M. Duxbury, A. B. Foster, and J. M. Webber, *Carbohyd. Res.*, 1 (1965) 22.
5 J. S. Brimacombe, A. B. Foster, B. D. Jones, and J. J. Willard, *J. Chem. Soc.* (*C*), (1967) 2404.
6 H. E. Simmons and D. W. Wiley, *J. Amer. Chem. Soc.*, 82 (1960) 2288.
7 J. M. Bell, D. G. Kubler, P. Sartwell, and R. G. Zepp, *J. Org. Chem.*, 30 (1965) 4284.
8 H. B. Henbest and B. B. Millward, *J. Chem. Soc.*, (1960) 3575.
9 J. Sicher, M. Tichý, F. Šipoš, and M. Pánková, *Collection Czech. Chem. Commun.*, 26 (1961) 2418.
10 N. Mori, *Bull. Chem. Soc. Japan*, 33 (1960) 1144.
11 E. Fischer, *Ber.*, 27 (1894) 1524.
12 J. C. Irvine and J. P. Scott, *J. Chem. Soc.*, 103 (1913) 575.
13 N. Baggett, A. B. Foster, A. H. Haines, and M. Stacey, *J. Chem. Soc.*, (1960) 3528.
14 F. V. Brutcher, Jr., and G. Evans III, *J. Org. Chem.*, 23 (1958) 618.

Carbohydrate Research
Elsevier Publishing Company, Amsterdam
Printed in Belgium

Note

Synthesis of 3-*O*-α-D-glucopyranosyl-L-serine

KICHANG KUM

Department of Pediatrics, University of California, San Francisco Medical Center, San Francisco, California 94122 (U.S.A.)

(Received January 27th, 1969; in revised form, March 7th, 1969).

In previous work[1], the author's interest in serine glycosides was stimulated by enzymic studies on the biosynthesis of glycoproteins[2] and sphingoglycolipids[3]. In the gangliosides and cerebrosides, the hydroxyl group at C-1 of *N*-acylsphingosine is linked to either D-glucose or D-galactose. Earlier *in vivo* isotope-experiments had shown that C-1, C-2, and the amino group are derived from C-3, and C-2, and the amino group of serine, respectively[4]. *In vivo* studies suggested that L-serine was condensed with hexadecanaldehyde, with concomitant loss of the carboxyl group, to yield sphingosine[5]. However, it appeared possible that an alternative pathway existed, namely the condensation of an *O*-glycosyl-L-serine with hexadecanaldehyde to give psychosine. In order to examine this hypothesis, the author has earlier synthesized serine glycoside derivatives as potential substrates[1].

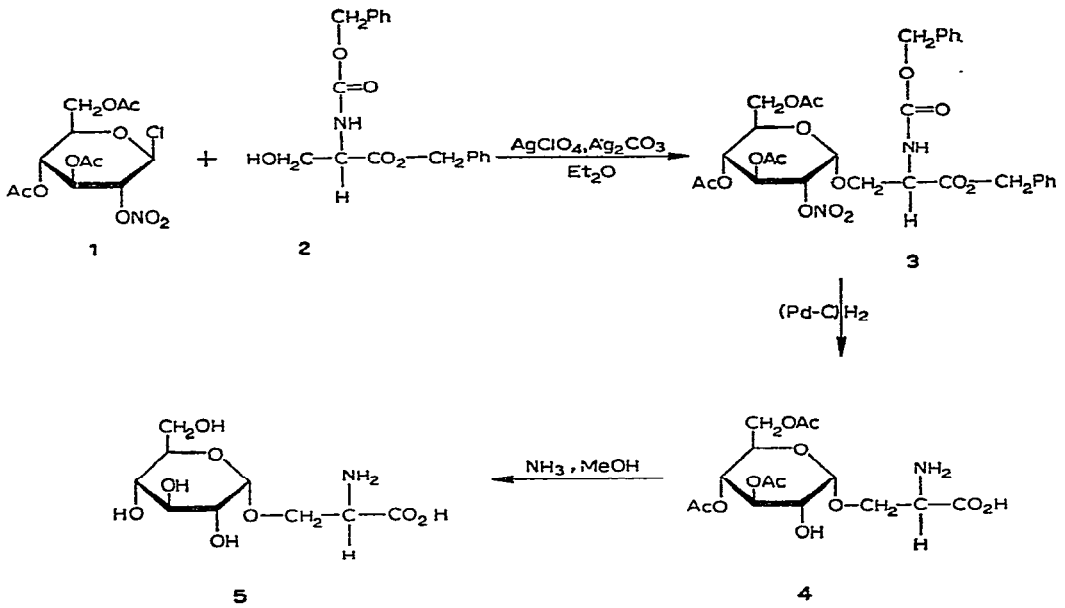
In 1966, Davidson *et al.*[7] coupled 2,3,4-tri-*O*-acetyl-α-D-xylopyranosyl bromide with *N*-benzyloxycarbonyl-L-serine benzyl ester by the Koenigs–Knorr procedure to obtain 3-*O*-β-D-xylopyranosyl-L-serine. The α-D-anomer was isolated from the mother liquor as a by-product. More recently, Derevitskaya *et al.*[11] synthesized 3,4,6-tri-*O*-acetyl-α-D-glucopyranose 1,2-*O*-[2′-(benzyloxycarbonylamino)-2′-(methoxycarbonyl)ethyl orthoacetate] by condensing tetra-*O*-acetyl-α-D-glucopyranosyl bromide with *N*-benzyloxycarbonyl-DL-serine methyl ester. In the present work, the synthesis of 3-*O*-α-D-glucopyranosyl-L-serine was undertaken to determine the configurational relationship between the hydroxyl group at C-1 of *N*-acylsphingosine, and D-glucose in the ganglioside.

The procedure consisted of the following steps.

(*a*) *N*-Benzyloxycarbonyl-L-serine benzyl ester (**2**) was condensed with 3,4,6-tri-*O*-acetyl-2-*O*-nitro-β-D-glucopyranosyl chloride (**1**) in the presence of silver carbonate, a catalytic quantity of silver perchlorate[6], and Drierite. The product was obtained in 75% yield by column chromatography, and was crystallized.

(*b*) Hydrogenolysis of the crystalline mass removed both benzyl and benzyloxycarbonyl protecting groups, and the resulting serine derivative was isolated as a crystalline solid in 90% yield.

(*c*) After deacetylation with methanolic ammonia, the desired 3-*O*-D-glucopyranosyl-L-serine was obtained as an amorphous, white solid in 75% yield. The overall yield was 50%.



Under the conditions used for the condensation, essentially the Koenigs–Knorr reaction, the D-glucosylserine derivative was expected to be the α-D-anomer[6]. As shown in Table I, a comparison of the optical rotations of the synthetic compounds with those of the corresponding methyl α- and β-D-glycosides and 3-*O*-β-D-glucopyranosyl-L-serine indicates that the product was indeed the α-D-glucoside.

TABLE I

COMPARISON OF MOLAR ROTATIONS OF KNOWN GLUCOSIDES AND SYNTHETIC SERINE GLUCOPYRANOSIDES[a]

Compound	$[\alpha]_D^{20}$ (*degrees*)	*Mol. Wt.*	$[M]_D$ (*degrees*)
3-*O*-β-D-Glucopyranosyl-L-serine[1]	−23.0	267.2	−6,146
Methyl β-D-glucopyranoside			−6,640
Methyl α-D-glucopyranoside			+30,860
3-*O*-D-Glucopyranosyl-L-serine	+93	267.2	+24,850

[a]The molar rotations of the D-glucosyl-L-serines were not corrected for the contribution by the L-serine ($[M]_D = -717°$ in water); the effect of pH on the rotations was not studied. The rotations of the methyl D-glucopyranosides are literature values[8].

EXPERIMENTAL

General methods. — Melting points are uncorrected. Optical rotations were measured with a Perkin–Elmer Model 141 polarimeter and a Rudolph Model 80 polarimeter. The thin-layer chromatograms were examined with a Chromato-Vue (Ultra-Violet Products, Inc.).

Determination of benzyl groups was performed by measuring the absorbancy

of the compound at 257 nm, with 4% chloroform in ethanol. Carroll's anthrone method[10] was used for determining sugar concentrations. Activated silicic acid, 200–325 mesh (Unisil, Clarkson Chemical Co.) was employed, without pretreatment, for chromatography. T.l.c. was conducted with 4% (v/v) methanol in benzene on fluorescent Silica Gel GF254. Benzyl groups were detected on the chromatograms in u.v. light as dark areas on a fluorescent, yellow background. The acetylated sugars gave orange spots on a pale yellow background with the spray reagent of Tate and Bishop[9].

N-Benzyloxycarbonyl-3-O-(3,4,6-tri-O-acetyl-2-O-nitro-α-D-glucopyranosyl)-L-serine benzyl ester (3). — *N*-Benzyloxycarbonyl-L-serine benzyl ester (6.6 g, 20 mmoles) was dissolved in dry ether (200 ml), and stirred for 10 min with anhydrous silver carbonate (9.9 g, 33 mmoles) and Drierite (20 g, 150 mmoles). The 3,4,6-tri-*O*-acetyl-2-*O*-nitro-β-D-glucopyranosyl chloride (7.4 g, 20 mmoles) and silver perchlorate (1 g) were then added, and the mixture was stirred in the dark, with the exclusion of moisture, for 48 h. The reaction mixture was filtered, and the filtrate was diluted with benzene, washed with cold, saturated, sodium hydrogen carbonate solution (100 ml), and then washed at least three times with water, dried (sodium sulfate), and concentrated to a syrup under vacuum. T.l.c. of the syrup showed zones that had R_F values (given in parentheses) identical with those of the compounds indicated: 3,4,6-tri-*O*-acetyl-2-*O*-nitro-β-D-glucopyranosyl chloride(0.83), *N*-benzyloxycarbonyl-3-*O*-(3,4,6-tri-*O*-acetyl-2-*O*-nitro-α-D-glucopyranosyl)-L-serine benzyl ester (0.64), 2,2′-di-*O*-nitrotrehalose hexaacetate* (0.42), *N*-benzyloxycarbonyl-L-serine benzyl ester (0.32), 3,4,6-tri-*O*-acetyl-2-*O*-nitro-D-glucose (0.24).

The desired condensation product, characterized by the presence of both aromatic and acetyl groups, migrated between the unreacted 3,4,6-tri-*O*-acetyl-2-*O*-nitro-β-D-glucopyranosyl chloride and the *N*-benzyloxycarbonyl-L-serine benzyl ester. Isolation of the desired product was achieved by chromatography on a silicic acid column similar to that reported by Kum and Roseman[1]. The product was obtained as a syrup; yield 9.9 g (75%). The final product crystallized during a 3-month period, but it could not be recrystallized. The product was analyzed for the sugar moiety by the anthrone method, with the corresponding sugar as standard, and for the benzyl group by measurement of the absorbance at 257 nm, with *N*-benzyloxycarbonyl-L-serine benzyl ester as standard. It was found that the product contained 1 mole of a glucose per mole of serine.

*By-products having R_F values similar to those of octa-*O*-acetyl-β-D-glucopyranosyl β-D-glucopyranoside (trehalose octaacetate) and 2,3,4,6-tetra-*O*-acetyl-β-D-glucose were observed in the reaction mixture of 3,4,6-tri-*O*-acetyl-2-*O*-nitro-β-D-glucopyranosyl chloride and *N*-benzyloxycarbonyl-L-serine benzyl ester. In the previous work[1], examination of the syrup obtained from the tetra-*O*-acetyl-α-D-glucopyranosyl bromide–*N*-benzyloxycarbonyl-L-serine benzyl ester reaction mixture by t.l.c. (silica gel G) showed the following components (R_F): tetra-*O*-acetyl-α-D-glucopyranosyl bromide 0.84; trehalose octaacetate 0.34; *N*-benzyloxycarbonyl-L-serine benzyl ester 0.33; and 2,3,4,6-tetra-*O*-acetyl-β-D-glucose, 0.18. In contrast to the findings of the previous work, the by-product having R_F of 0.42 appeared to be 2,2′-di-*O*-nitrotrehalose hexaacetate.

3-O-(3,4,6-Tri-O-acetyl-α-D-glucopyranosyl)-L-serine (**4**). — N-Benzyloxycarbonyl-3-*O*-(3,4,6-tri-*O*-acetyl-2-*O*-nitro-α-D-glucopyranosyl-L-serine benzyl ester (3.5 g) was dissolved in a mixture containing ethanol (or *p*-dioxane) (80 ml) and water (20 ml), and hydrogenated at a slight overpressure in the presence of a 10% palladium-on-charcoal catalyst (0.2 g). The absorption of hydrogen essentially ceased after about 3–4 h (corresponding to consumption of 3 moles per mole in removing 1 nitro group and 2 benzyl residues). Following filtration to remove the catalyst, the solution was concentrated, giving a syrup that crystallized spontaneously. The crude, crystalline product, yield 1.87 g (90%), which was recrystallized from water–acetone, had m.p. 186–188°, $[\alpha]_D^{20}$ +134.9° (*c* 2, water).

Anal. Calc. for $C_{15}H_{23}NO_{11}$ (393.34): C, 45.80; H, 5.85; N, 3.56. Found: C, 45.82; H, 6.11; N, 3.56.

3-O-α-D-Glucopyranosyl-L-serine (**5**). — 3-*O*-(3,4,6-Tri-*O*-acetyl-α-D-glucopyranosyl)-L-serine (0.7 g) was suspended in anhydrous methanol (50 ml), cooled to 0°, and mixed with methanol (50 ml) saturated with ammonia at 0°. The mixture was slowly brought to room temperature, and was maintained for 5 h at room temperature. The colorless solution was concentrated in a vacuum, at 30–35°, whereupon the residue solidified. The amorphous white solid, yield 0.36 g (75%), which could not be crystallized, had $[\alpha]_D^{20}$ +93° (*c* 2, water).

Anal. Calc. for $C_9H_{17}NO_8$ (267.24): N, 5.24. Found: N, 5.19.

T.l.c. (4:1:5 *sec*-butyl alcohol–acetic acid–water) revealed only one ninhydrin-positive component.

REFERENCES

1 K. Kum and S. Roseman, *Biochemistry*, 5 (1966) 3061.
2 D. M. Carlson, E. J. McGuire, G. W. Jourdian, and S. Roseman, *Federation Proc.*, 23 (1964) 380.
3 S. Basu, B. Kaufman, and S. Roseman, *J. Biol. Chem.*, 240 (1965) PC 4115.
4 D. B. Sprinson and A. Coulon, *J. Biol. Chem.*, 207 (1954) 585.
5 R. O. Brady and G. J. Koval, *J. Biol. Chem.*, 233 (1958) 26.
6 M. L. Wolfrom, A. O. Pittet, and I. C. Gillam, *Proc. Nat. Acad. Sci. U. S.*, 47 (1961) 700.
7 K. Brendel and E. A. Davidson, *Carbohyd. Res.*, 2 (1966) 42.
8 F. J. Bates, and Associates, *Polarimetry, Saccharimetry, and the Sugars*, National Bureau of Standards Circular *C440*, (1942).
9 M. E. Tate and C. T. Bishop, *Can. J. Chem.*, 40 (1962) 1043.
10 K. K. Carroll, *J. Lipid Res.*, 1 (1960) 171.
11 V. A. Derevitskaya, M. G. Vafina, and N. K. Kochetkov, *Carbohyd. Res.*, 3 (1967) 377.

Carbohydrate Research
Elsevier Publishing Company, Amsterdam
Printed in Belgium

Note

Synthesis of 3-*O*-α-D-glucopyranosyl-L-serine

KICHANG KUM

Department of Pediatrics, University of California, San Francisco Medical Center, San Francisco, California 94122 (U.S.A.)

(Received January 27th, 1969; in revised form, March 7th, 1969).

In previous work[1], the author's interest in serine glycosides was stimulated by enzymic studies on the biosynthesis of glycoproteins[2] and sphingoglycolipids[3]. In the gangliosides and cerebrosides, the hydroxyl group at C-1 of *N*-acylsphingosine is linked to either D-glucose or D-galactose. Earlier *in vivo* isotope-experiments had shown that C-1, C-2, and the amino group are derived from C-3, and C-2, and the amino group of serine, respectively[4]. *In vivo* studies suggested that L-serine was condensed with hexadecanaldehyde, with concomitant loss of the carboxyl group, to yield sphingosine[5]. However, it appeared possible that an alternative pathway existed, namely the condensation of an *O*-glycosyl-L-serine with hexadecanaldehyde to give psychosine. In order to examine this hypothesis, the author has earlier synthesized serine glycoside derivatives as potential substrates[1].

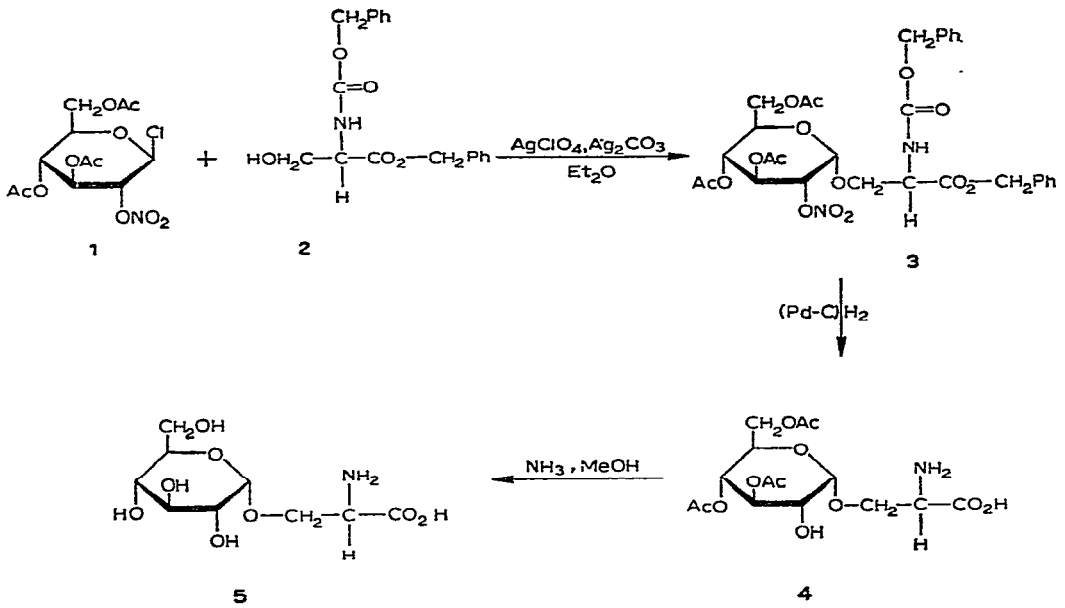
In 1966, Davidson *et al.*[7] coupled 2,3,4-tri-*O*-acetyl-α-D-xylopyranosyl bromide with *N*-benzyloxycarbonyl-L-serine benzyl ester by the Koenigs–Knorr procedure to obtain 3-*O*-β-D-xylopyranosyl-L-serine. The α-D-anomer was isolated from the mother liquor as a by-product. More recently, Derevitskaya *et al.*[11] synthesized 3,4,6-tri-*O*-acetyl-α-D-glucopyranose 1,2-*O*-[2′-(benzyloxycarbonylamino)-2′-(methoxycarbonyl)ethyl orthoacetate] by condensing tetra-*O*-acetyl-α-D-glucopyranosyl bromide with *N*-benzyloxycarbonyl-DL-serine methyl ester. In the present work, the synthesis of 3-*O*-α-D-glucopyranosyl-L-serine was undertaken to determine the configurational relationship between the hydroxyl group at C-1 of *N*-acylsphingosine, and D-glucose in the ganglioside.

The procedure consisted of the following steps.

(*a*) *N*-Benzyloxycarbonyl-L-serine benzyl ester (**2**) was condensed with 3,4,6-tri-*O*-acetyl-2-*O*-nitro-β-D-glucopyranosyl chloride (**1**) in the presence of silver carbonate, a catalytic quantity of silver perchlorate[6], and Drierite. The product was obtained in 75% yield by column chromatography, and was crystallized.

(*b*) Hydrogenolysis of the crystalline mass removed both benzyl and benzyloxycarbonyl protecting groups, and the resulting serine derivative was isolated as a crystalline solid in 90% yield.

(*c*) After deacetylation with methanolic ammonia, the desired 3-*O*-D-glucopyranosyl-L-serine was obtained as an amorphous, white solid in 75% yield. The overall yield was 50%.



Under the conditions used for the condensation, essentially the Koenigs–Knorr reaction, the D-glucosylserine derivative was expected to be the α-D-anomer[6]. As shown in Table I, a comparison of the optical rotations of the synthetic compounds with those of the corresponding methyl α- and β-D-glycosides and 3-*O*-β-D-glucopyranosyl-L-serine indicates that the product was indeed the α-D-glucoside.

TABLE I

COMPARISON OF MOLAR ROTATIONS OF KNOWN GLUCOSIDES AND SYNTHETIC SERINE GLUCOPYRANOSIDES[a]

Compound	$[\alpha]_D^{20}$ (*degrees*)	*Mol. Wt.*	$[M]_D$ (*degrees*)
3-*O*-β-D-Glucopyranosyl-L-serine[1]	−23.0	267.2	−6,146
Methyl β-D-glucopyranoside			−6,640
Methyl α-D-glucopyranoside			+30,860
3-*O*-D-Glucopyranosyl-L-serine	+93	267.2	+24,850

[a]The molar rotations of the D-glucosyl-L-serines were not corrected for the contribution by the L-serine ($[M]_D = -717°$ in water); the effect of pH on the rotations was not studied. The rotations of the methyl D-glucopyranosides are literature values[8].

EXPERIMENTAL

General methods. — Melting points are uncorrected. Optical rotations were measured with a Perkin–Elmer Model 141 polarimeter and a Rudolph Model 80 polarimeter. The thin-layer chromatograms were examined with a Chromato-Vue (Ultra-Violet Products, Inc.).

Determination of benzyl groups was performed by measuring the absorbancy

of the compound at 257 nm, with 4% chloroform in ethanol. Carroll's anthrone method[10] was used for determining sugar concentrations. Activated silicic acid, 200–325 mesh (Unisil, Clarkson Chemical Co.) was employed, without pretreatment, for chromatography. T.l.c. was conducted with 4% (v/v) methanol in benzene on fluorescent Silica Gel GF254. Benzyl groups were detected on the chromatograms in u.v. light as dark areas on a fluorescent, yellow background. The acetylated sugars gave orange spots on a pale yellow background with the spray reagent of Tate and Bishop[9].

N-Benzyloxycarbonyl-3-O-(3,4,6-tri-O-acetyl-2-O-nitro-α-D-glucopyranosyl)-L-serine benzyl ester (3). — *N*-Benzyloxycarbonyl-L-serine benzyl ester (6.6 g, 20 mmoles) was dissolved in dry ether (200 ml), and stirred for 10 min with anhydrous silver carbonate (9.9 g, 33 mmoles) and Drierite (20 g, 150 mmoles). The 3,4,6-tri-*O*-acetyl-2-*O*-nitro-β-D-glucopyranosyl chloride (7.4 g, 20 mmoles) and silver perchlorate (1 g) were then added, and the mixture was stirred in the dark, with the exclusion of moisture, for 48 h. The reaction mixture was filtered, and the filtrate was diluted with benzene, washed with cold, saturated, sodium hydrogen carbonate solution (100 ml), and then washed at least three times with water, dried (sodium sulfate), and concentrated to a syrup under vacuum. T.l.c. of the syrup showed zones that had R_F values (given in parentheses) identical with those of the compounds indicated: 3,4,6-tri-*O*-acetyl-2-*O*-nitro-β-D-glucopyranosyl chloride(0.83), *N*-benzyloxycarbonyl-3-*O*-(3,4,6-tri-*O*-acetyl-2-*O*-nitro-α-D-glucopyranosyl)-L-serine benzyl ester (0.64), 2,2′-di-*O*-nitrotrehalose hexaacetate* (0.42), *N*-benzyloxycarbonyl-L-serine benzyl ester (0.32), 3,4,6-tri-*O*-acetyl-2-*O*-nitro-D-glucose (0.24).

The desired condensation product, characterized by the presence of both aromatic and acetyl groups, migrated between the unreacted 3,4,6-tri-*O*-acetyl-2-*O*-nitro-β-D-glucopyranosyl chloride and the *N*-benzyloxycarbonyl-L-serine benzyl ester. Isolation of the desired product was achieved by chromatography on a silicic acid column similar to that reported by Kum and Roseman[1]. The product was obtained as a syrup; yield 9.9 g (75%). The final product crystallized during a 3-month period, but it could not be recrystallized. The product was analyzed for the sugar moiety by the anthrone method, with the corresponding sugar as standard, and for the benzyl group by measurement of the absorbance at 257 nm, with *N*-benzyloxycarbonyl-L-serine benzyl ester as standard. It was found that the product contained 1 mole of a glucose per mole of serine.

*By-products having R_F values similar to those of octa-*O*-acetyl-β-D-glucopyranosyl β-D-glucopyranoside (trehalose octaacetate) and 2,3,4,6-tetra-*O*-acetyl-β-D-glucose were observed in the reaction mixture of 3,4,6-tri-*O*-acetyl-2-*O*-nitro-β-D-glucopyranosyl chloride and *N*-benzyloxycarbonyl-L-serine benzyl ester. In the previous work[1], examination of the syrup obtained from the tetra-*O*-acetyl-α-D-glucopyranosyl bromide–*N*-benzyloxycarbonyl-L-serine benzyl ester reaction mixture by t.l.c. (silica gel G) showed the following components (R_F): tetra-*O*-acetyl-α-D-glucopyranosyl bromide 0.84; trehalose octaacetate 0.34; *N*-benzyloxycarbonyl-L-serine benzyl ester 0.33; and 2,3,4,6-tetra-*O*-acetyl-β-D-glucose, 0.18. In contrast to the findings of the previous work, the by-product having R_F of 0.42 appeared to be 2,2′-di-*O*-nitrotrehalose hexaacetate.

3-O-(3,4,6-Tri-O-acetyl-α-D-glucopyranosyl)-L-serine (**4**). — N-Benzyloxycarbonyl-3-*O*-(3,4,6-tri-*O*-acetyl-2-*O*-nitro-α-D-glucopyranosyl-L-serine benzyl ester (3.5 g) was dissolved in a mixture containing ethanol (or *p*-dioxane) (80 ml) and water (20 ml), and hydrogenated at a slight overpressure in the presence of a 10% palladium-on-charcoal catalyst (0.2 g). The absorption of hydrogen essentially ceased after about 3–4 h (corresponding to consumption of 3 moles per mole in removing 1 nitro group and 2 benzyl residues). Following filtration to remove the catalyst, the solution was concentrated, giving a syrup that crystallized spontaneously. The crude, crystalline product, yield 1.87 g (90%), which was recrystallized from water–acetone, had m.p. 186–188°, $[\alpha]_D^{20}$ +134.9° (*c* 2, water).

Anal. Calc. for $C_{15}H_{23}NO_{11}$ (393.34): C, 45.80; H, 5.85; N, 3.56. Found: C, 45.82; H, 6.11; N, 3.56.

3-O-α-D-Glucopyranosyl-L-serine (**5**). — 3-*O*-(3,4,6-Tri-*O*-acetyl-α-D-glucopyranosyl)-L-serine (0.7 g) was suspended in anhydrous methanol (50 ml), cooled to 0°, and mixed with methanol (50 ml) saturated with ammonia at 0°. The mixture was slowly brought to room temperature, and was maintained for 5 h at room temperature. The colorless solution was concentrated in a vacuum, at 30–35°, whereupon the residue solidified. The amorphous white solid, yield 0.36 g (75%), which could not be crystallized, had $[\alpha]_D^{20}$ +93° (*c* 2, water).

Anal. Calc. for $C_9H_{17}NO_8$ (267.24): N, 5.24. Found: N, 5.19.

T.l.c. (4:1:5 *sec*-butyl alcohol–acetic acid–water) revealed only one ninhydrin-positive component.

REFERENCES

1 K. Kum and S. Roseman, *Biochemistry*, 5 (1966) 3061.
2 D. M. Carlson, E. J. McGuire, G. W. Jourdian, and S. Roseman, *Federation Proc.*, 23 (1964) 380.
3 S. Basu, B. Kaufman, and S. Roseman, *J. Biol. Chem.*, 240 (1965) PC 4115.
4 D. B. Sprinson and A. Coulon, *J. Biol. Chem.*, 207 (1954) 585.
5 R. O. Brady and G. J. Koval, *J. Biol. Chem.*, 233 (1958) 26.
6 M. L. Wolfrom, A. O. Pittet, and I. C. Gillam, *Proc. Nat. Acad. Sci. U. S.*, 47 (1961) 700.
7 K. Brendel and E. A. Davidson, *Carbohyd. Res.*, 2 (1966) 42.
8 F. J. Bates, and Associates, *Polarimetry, Saccharimetry, and the Sugars*, National Bureau of Standards Circular *C440*, (1942).
9 M. E. Tate and C. T. Bishop, *Can. J. Chem.*, 40 (1962) 1043.
10 K. K. Carroll, *J. Lipid Res.*, 1 (1960) 171.
11 V. A. Derevitskaya, M. G. Vafina, and N. K. Kochetkov, *Carbohyd. Res.*, 3 (1967) 377.

Carbohydrate Research
Elsevier Publishing Company, Amsterdam
Printed in Belgium

Note

Synthesis of the 2,3- and 3,4-dimethyl ethers of D-mannose*

V. L. N. MURTY AND I. R. SIDDIQUI
Food Research Institute, Canada Department of Agriculture, Ottawa (Canada)
(Received, March 31st, 1969)

In a synthesis[1] of 2,4-di-*O*-methyl-D-mannose from the 3-*O*-tosyl** derivative of methyl 6-*O*-trityl-α-D-mannopyranoside, a fraction (17%) containing the 2- and 4-*O*-tosyl derivatives was also reported. 3,4-Di-*O*-methyl-D-mannose has now been obtained from the faster-moving, minor component (methyl 2-*O*-tosyl-6-*O*-trityl-α-D-mannopyranoside) by successive methylation, detosylation, detritylation, and hydrolysis, and 2,3-di-*O*-methyl-D-mannose was similarly prepared from the slower-moving component (methyl 4-*O*-tosyl-6-*O*-trityl-α-D-mannopyranoside).

The constitution of the di-*O*-methyl sugars was deduced from their mode of synthesis. Confirmation was provided as follows: (*a*) demethylation in each case produced mannose; (*b*) the high M_G value (0.48) in borate buffer for the former di-*O*-methyl sugar showed that position 2 was unsubstituted, and the lower M_G value (0.17) for the latter sugar showed that this position was substituted. 3,4-Di-*O*-methyl-D-mannose was obtained crystalline, and 2,3-di-*O*-methyl-D-mannose was characterized as the known 1,4,6-tris(*p*-nitrobenzoate).

Although the 3,4- and 2,3-dimethyl ethers have long been known as constituents of the hydrolysis products of methylated polysaccharides containing mannose residues involved in 1,2,6 and 1,4,6 branch points, respectively, the synthesis of 3,4-di-*O*-methyl-D-mannose has not been reported previously. However, during the progress of this study, alternative syntheses of these and several other di- and trimethyl ethers of D-mannose have been achieved by Bhattacharjee and Gorin[2].

EXPERIMENTAL

The general experimental methods have been described previously[3]. Rotations were measured on a Perkin–Elmer 141 polarimeter.

Fractionation and methylation of the mono-O-tosyl derivatives of methyl 6-O-trityl-α-D-mannopyranoside. — The mixture of monosulphonates[1] (3.4 g) was separated by t.l.c. (60 plates, 20×20×0.3 cm) with ethyl acetate–light petroleum (b.p. 60–80°) (4:6). After three developments, the sugars were located by spraying with

*Contribution No. 125 of the Food Research Institute, Canada Department of Agriculture.
**Tosyl = toluene-*p*-sulphonyl.

water; the plates were dried at room temperature, and the two components were recovered, after discarding the overlapping middle zone, by extraction with acetone–chloroform (1:1). Filtration and evaporation gave as the faster-moving component, methyl 2-*O*-tosyl-6-*O*-trityl-α-D-mannopyranoside (*A*, 0.954 g), $[\alpha]_D^{17}$ +5.4° (*c* 2.06, chloroform); and methyl 4-*O*-tosyl-6-*O*-trityl-α-D-mannopyranoside (*B*, 0.922 g), $[\alpha]_D^{17}$ +45.6° (*c* 2.11, chloroform).

Treatment of *A* (0.954 g) with methyl iodide (10 ml) and silver oxide (1 g) gave a product which, after seven additional methylations, yielded an impure solid (0.77 g, 77%), which was purified by t.l.c. in acetone–chloroform (1:15) and then crystallized from isopropyl ether–ether to give methyl 3,4-di-*O*-methyl-2-*O*-tosyl-6-*O*-trityl-α-D-mannopyranoside (**1**), m.p. 160–161°, $[\alpha]_D^{17}$ +3.6° (*c* 1.31, chloroform).

Anal. Calc. for $C_{35}H_{38}O_8S$: C, 67.94; H, 6.19; S, 5.18; OCH_3, 15.05. Found: C, 68.24; H, 6.51; S, 5.28; OCH_3, 15.24.

On similar methylation, *B* (0.912 g) yielded methyl 2,3-di-*O*-methyl-4-*O*-tosyl-6-*O*-trityl-α-D-mannopyranoside (**2**) (0.882 g, 91%), which, after crystallization from isopropyl ether, had m.p. 146–147°, $[\alpha]_D^{20}$ +35.1° (*c* 1.44, chloroform) (Found: C, 67.92; H, 6.34; S, 5.12; OCH_3, 14.96).

*Methyl 3,4-di-*O*-methyl-6-*O*-trityl-α-D-mannopyranoside* (**3**). — A solution of ether **1** (0.439 g) in benzene (7 ml) was mixed with a solution of sodium methoxide [(sodium (0.70 g) in dry methanol (15 ml)], and the solution was boiled under reflux for 22 h. Isolation of the product in the usual way[3] yielded compound **3** (0.307 g, 89%), $[\alpha]_D^{20}$ +51.2° (*c* 1.75, chloroform).

Anal. Calc. for $C_{28}H_{32}O_6$: C, 72.39; H, 6.94; OCH_3, 20.04. Found, C, 72.71; H, 7.04; OCH_3, 19.91.

*Methyl 2,3-di-*O*-methyl-6-*O*-trityl-α-D-mannopyranoside* (**4**). — Ether **2** (0.569 g) was similarly detosylated to give compound **4** (0.38 g, 90%) which crystallized from ethanol and had m.p. 177–178°, $[\alpha]_D^{19}$ +76.1° (*c* 1.05, chloroform) (Found: C, 72.45; H, 6.99; OCH_3, 19.97).

*Methyl 3,4-di-*O*-methyl-α-D-mannopyranoside* (**5**). — Trityl ether **3** (0.305 g) was dissolved in acetic acid (7 ml), water (3 ml) was added, and the turbid suspension was stirred for 7 h. Isolation of the product, in the usual way[3], gave glycoside **5** (0.139 g, 94%), $[\alpha]_D^{18}$ +72.5° (*c* 0.9, water) (Found: OCH_3, 41.86; Di-*O*-methyl hexoside calc.: 41.9).

*Methyl 2,3-di-*O*-methyl-α-D-mannopyranoside* (**6**). — Trityl ether **4** (0.360 g) was similarly detritylated to yield a syrup (0.194 g, 93%), $[\alpha]_D^{18}$ +37.1° (*c* 1.31, chloroform) (Found: OCH_3, 41.9); lit.[4] $[\alpha]_D$ +43.5° (chloroform).

*3,4-Di-*O*-methyl-D-mannose.* — Glycoside **5** (0.125 g) was hydrolyzed with N sulphuric acid (2.5 ml) for 16 h at 100°. Neutralization (barium carbonate), filtration, and evaporation gave a syrup (0.113 g, 96%). Paper chromatography (solvent *A*) and paper electrophoresis showed a single component having R_G and M_G values of 0.39 and 0.48, respectively. Demethylation[5] with boron trichloride gave mannose. The sugar crystallized from ethyl acetate, on seeding with an authentic specimen,

and had m.p. and mixed m.p. 73–74°, $[\alpha]_D^{19}$ +12.2 (5 min) → +5° (2 h, equil.); lit.[6,7], m.p. 70–73°, $[\alpha]_D$ +18 → +6°.

2,3-Di-O-methyl-D-mannose. — Glycoside **6** (0.174 g) was similarly hydrolyzed with N sulphuric acid (4 ml) to yield a syrup (0.145 g, 89%), $[\alpha]_D^{17}$ −21.7° (*c* 1.17, water); lit.[4] $[\alpha]_D$ −15.8°. Paper chromatography (solvent *A*) and paper electrophoresis showed a single component having R_F, R_G, and M_G values of 0.25, 0.33, and 0.17, respectively; demethylation produced[5] mannose.

To a solution of 2,3-di-*O*-methyl-D-mannose (0.032 g) in dry pyridine (1 ml), was added freshly crystallized *p*-nitrobenzoyl chloride (0.090 g). After 24 h at 40°, the product was isolated[8] and purified on a small column of silica gel with acetone–chloroform (1:15), giving a product which, after crystallization from methanol, had m.p. and mixed m.p. 194–195°, $[\alpha]_D^{20}$ +42° (*c* 0.9, chloroform); lit.[8], m.p. 194°, $[\alpha]_D$ +65° (chloroform). An authentic sample of 2,3-di-*O*-methyl-1,4,6-tri-*O*-*p*-nitrobenzoyl-D-mannose showed $[\alpha]_D^{21}$ +41.6° (*c* 0.9, chloroform).

REFERENCES

1 V. L. N. MURTY AND I. R. SIDDIQUI, *Carbohyd. Res.*, (in press).
2 S. S. BHATTACHARJEE AND P. A. J. GORIN, personal communication.
3 I. R. SIDDIQUI AND B. URBAS, *Carbohyd. Res.*, 5 (1967) 210.
4 G. J. ROBERTSON, *J. Chem. Soc.*, (1934) 330.
5 S. ALLEN, T. G. BONNER, E. J. BOURNE, AND N. M. SAVILLE, *Chem. Ind.* (London), (1958) 630.
6 P. A. J. GORIN AND A. S. PERLIN, *Can. J. Chem.*, 34 (1956) 1796.
7 G. O. ASPINALL AND G. ZWEIFEL, *J. Chem. Soc.*, (1957) 2271.
8 F. SMITH AND R. MONTGOMERY, *The Chemistry of Plant Gums and Mucilages*, Reinhold, New York, 1959, Appendix II.

Carbohydrate Research
Elsevier Publishing Company, Amsterdam
Printed in Belgium

Note

On the catalysis of the Amadori rearrangement

J. YOSHIMURA, M. FUNABASHI,

Laboratory of Chemistry for Natural Products, Tokyo Institute of Technology, Meguroku, Tokyo (Japan)

AND H. SIMON

Chemisches Institut der Fakultät für Landwirtschaft und Gartenbau Weihenstephan, Technische Hochschule München, D 8050 Freising-Weihenstephan (Deutschland)

(Received March 21st, 1969)

Catalysis of the Amadori rearrangement[1] has been effected by a variety of acids[2–4], such compounds as ethyl malonate or phenylacetone (which contain activated methylene groups[5]), and tertiary amine salts[6]. A mechanism involving *N*-protonation of the glycosylamine, followed by prototropic shifts and loss of a proton from the tautomeric cation, has been proposed[3,7]. Other suggested mechanisms involve addition of the proton to the glycosylamine ring oxygen[8], and rearrangement of the Schiff's base of 3,4:5,6-tetra-*O*-benzoyl-*aldehydo*-D-glucose and *p*-toluidine through the corresponding 1,1-bis(*p*-toluidino) derivative. By tracer techniques, it was proved, however, that this mechanism is not valid in the case of glycosylamines having free hydroxyl groups[10].

We now report that complexes of amines with such Lewis acids as boron trifluoride and its derivatives accelerate the rearrangement, and that 1,2:5,6-di-*O*-isopropylidene-D-*arabino*-hex-3-ulose and acetoin also react with amines and different catalysts in an Amadori rearrangement.

The rates of the rearrangements of 4,6-*O*-benzylidene-*N*-phenyl- (**1**) and -*N*-(*p*-tolyl)-D-glucosylamine (**2**) in absolute *p*-dioxane at 85° in the presence of different catalysts were measured by titration with Tillman's reagent[6] under a nitrogen atmosphere. In the case of an acid–base-catalyzed rearrangement, the rate should be accelerated by the addition compounds of amines with Lewis acids. As shown in Fig. 1, triethylborane has no effect, whereas difluoro(phenyl)borane shows a weaker catalytic activity than acetic acid. The first-order rate constant of the rearrangement of glycosylamine **2** was increased from 4.2×10^{-4} min^{-1} to 9.0 and to 12.5×10^{-4} min^{-1} in the presence of difluoro(phenyl)borane by addition of pyridine and triethylamine, respectively. The rate constant (1.3×10^{-3} min^{-1}) for compound **1** in the presence of acetic acid was increased about 1.3 times by the addition of aniline, pyridine, or triethylamine. The linearity between the amount of product formed and the reaction time indicates that the simultaneous destructive reactions[6] are essentially being supressed under the conditions used, especially in the initial reaction stage.

As shown in Fig. 2, boron trifluoride and ethoxydifluoroborane showed stronger

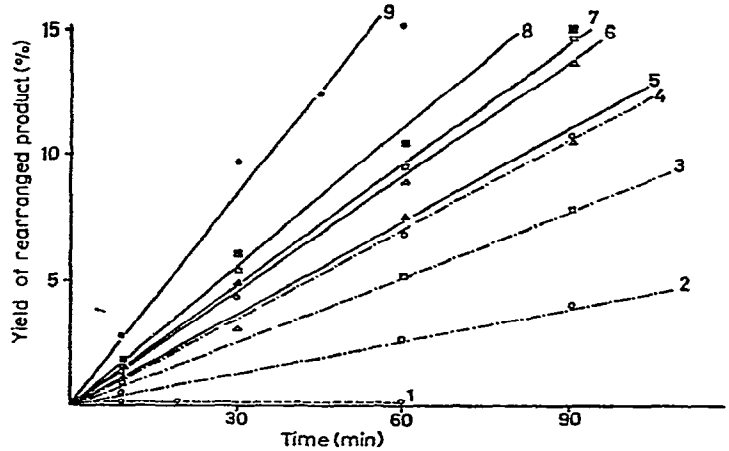


Fig. 1. Amadori rearrangement of 2 mmoles of **1** (lines 5–9) and **2** (lines 1–4) in 50 ml of *p*-dioxane at 85 ±0.5° in the presence of (1) 1 mmole of triethylborane; (2) 3 mmoles of difluoro(phenyl)-borane; (3) 9 mmoles of pyridine in addition to (2); (4) 3 mmoles of triethylamine in addition to (2); (5) 1 mmole of acetic acid; (6) 3 mmoles of triethylamine in addition to (5); (7) 4 mmoles of pyridine in addition to (5); (8) 5 mmoles of aniline in addition to (5); (9) 2 mmoles of acetic acid.

catalytic action than acetic acid in the initial reaction stage, but color formation occurred due to the destruction of glycosylamine **2** and the product. The addition of pyridine, however, caused a decrease in both the initial reaction and decomposition

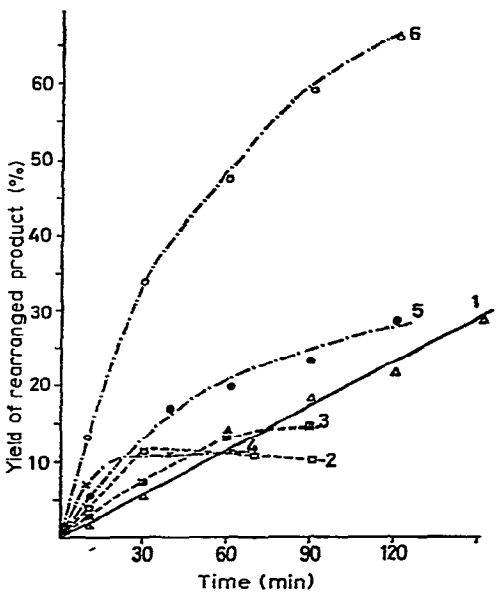
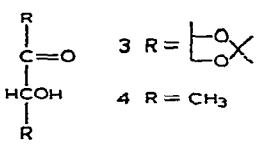


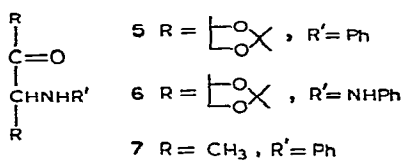
Fig. 2. Amadori rearrangement of 2 mmoles of **2** in 50 ml of *p*-dioxane at 85 ±0.5° in the presence of (1) 0.5 mmole of acetic acid; (2) 0.2 mmole of boron trifluoride; (3) 0.4 mmole of boron trifluoride and 2 mmoles of pyridine; (4) 0.5 mmole of ethoxydifluoroborane; (5) 4.75 mmoles of pyridine in addition to (4); (6) 1.7 mmoles of triethylamine in addition to (4).

rates. Triethylamine showed a remarkable acceleration of the initial rate and the suppression of decomposition reactions. From a consideration of the results mentioned above, it seems most reasonable to conclude that the Amadori rearrangement is an acid–base-catalysed reaction in which the balance of the acidity and basicity in the reaction system controls simultaneous and consecutive reactions.

On the other hand, the Amadori rearrangement is considered to be general in the reaction of α-hydroxycarbonyl compounds with amines, wherein a carbonyl group is generated in place of an α-hydroxyl group. Such a structural change has been conclusively proved in the reaction of benzoin[11] and ketoses[12] with amines. Moreover, Paulsen *et al.*[13] reported that 5-amino-5-deoxy-D-xylose rearranges easily to 1,5-dideoxy-1,5-imino-D-*threo*-pentulose hydrate through a cyclic Schiff's base structure in a neutral or acidic solution. In the course of a study of the dimerisation of *N*-arylaldimines[14], the authors obtained evidence that the Amadori rearrangement occurs with 1,2:5,6-di-*O*-isopropylidene-D-*arabino*-hex-3-ulose (**3**) in the reaction with amines.

By partial acetylation of 1,2:5,6-di-*O*-isopropylidene-D-mannitol, we obtained the corresponding monoacetate which was oxidized with methyl sulfoxide and acetic anhydride[15] to the 4-*O*-acetyl derivative of compound **3**, which was then deacetylated with methanolic ammonia. Because of its instability, compound **3** was used without further purification. Condensation of ketose **3** with an excess of aniline or phenylhydrazine in methanol, in the presence of ammonium chloride or acetic acid, followed by chromatography on silica gel, gave the corresponding 4-anilino-4-deoxy (**5**) or





4-deoxy-4-phenylhydrazino derivatives (**6**). Similar condensation of acetoin (**4**) and two molar equivalents of aniline gave 3-anilino-2-butanone (**7**). The structures of compounds **5**, **6**, and **7** were ascertained by the characteristic i.r. absorptions of the carbonyl group and the hydrogen atom attached to nitrogen. Direct condensation of acetoin and excess of aniline in the presence of boron trifluoride, under a nitrogen atmosphere, at higher temperature afforded *N*-(3-anilino-2-butylidene)aniline (the disubstituted derivative), which was gradually oxidized by air to the Schiff's base of biacetyl. These results, together with Carson's observation[16] that the biacetyl Schiff's base is produced in the reaction of acetoin with cyclohexylamine, indicate that other examples of the Amadori rearrangement of α-hydroxycarbonyl compounds could result from suitable selection of reaction conditions.

EXPERIMENTAL

Melting points are uncorrected. Solutions were evaporated under diminished pressure at a bath temperature not exceeding 40°. Optical rotations were measured

in a 0.5-dm tube. Triethylborane[17], difluoro(phenyl)borane[18], and ethoxydifluoroborane[19] were prepared according to the literature procedures. Boron trifluoride etherate was a commercial product. 4,6-*O*-Benzylidene-*N*-phenyl-D-glucosylamine[20] had m.p. 145.5–147°, $[\alpha]_D$ −75.2° (*c* 0.5, *p*-dioxane), and the corresponding *N*-(*p*-tolyl) derivative had m.p. 144.5–145°, $[\alpha]_D$ −89° (*c* 0.5, pyridine); lit.[21] m.p. 142–143°.

Measurement of the rate of the Amadori rearrangement. — A solution of glycosylamine **1** or **2** (2 mmoles) in absolute *p*-dioxane[22] (50 ml) was kept at 85 ±0.5° under dry nitrogen. After addition of the catalysts, a 5-ml aliquot was transferred at intervals (usually, 10, 30, 60, 90, and 120 min) to a flask which was immersed in an ice–water bath. The surface of the solution was covered with nitrogen, 5 ml of 0.2N sodium hydroxide was added, and the solution was then immediately titrated with dichlorophenyl-indophenol (Tillman's reagent)[6]. The mean value of two titrations, differing usually less than 1%, was adopted. The Tillman's reagent was checked before each experiment by titration of 1 mg of the standard sample of 1-anilino-4,6-*O*-benzylidene-1-deoxy-D-fructose[4] or 4,6-*O*-benzylidene-1-deoxy-1-(*p*-toluidino)-D-fructose[21]. The results were shown in Figs. 1 and 2.

3-O-Acetyl-1,2:5,6-di-O-isopropylidene-D-mannitol. — Acetic anhydride (6.5 ml, 69 mmoles) was added dropwise during 25 min to a solution of 1,2:5,6-di-*O*-isopropylidene-D-mannitol (18 g, 69 mmoles) in pyridine (30 ml). After standing overnight at room temperature, the reaction mixture was poured into ice–water. The resulting crystals (5.0 g; 20%), m.p. 125°; lit.[23] m.p. 123°; were the corresponding diacetate. The filtrate was extracted with methylene chloride, and the extract was evaporated. Distillation of the residue gave the title compound (14.5 g, 66.7%), b.p. 144–150°/4 mm, $[\alpha]_D^{28}$ 2.4° (*c* 3.34, methanol).

Anal. Calc. for $C_{14}H_{24}O_7$: C, 55.25; H, 7.95. Found: C, 54.94; H, 8.13%.

4-O-Acetyl-1,2:5,6-di-O-isopropylidene-D-arabino-hex-3-ulose (**3**). — A solution of the above monoacetate (14.5 g, 48 mmoles) in methyl sulfoxide (150 ml) and acetic anhydride (100 ml) was kept overnight at room temperature, poured into ice–water, and neutralized with sodium hydrogen carbonate. The solution was extracted with methylene chloride, and the extract was washed twice with water, dried, and evaporated. Distillation of the resulting syrup gave a fraction (9.3 g, 64.2%), b.p. 126–128°/1 mm, $[\alpha]_D^{28}$ −5.1° (*c* 1.77, methanol), which showed no i.r. absorption band for hydroxyl groups.

Anal. Calc. for $C_{14}H_{22}O_7$: C, 55.62; H, 7.34. Found: C, 55.50; H, 7.86%.

4-Anilino-4-deoxy-1,2:5,6-di-O-isopropylidene-D-arabino- (and/or D-lyxo)-hex-3-ulose (**5**). — A solution of the above hexulose (9.3 g, 38 mmoles) in 60 ml of methanol saturated with dry ammonia was kept for 2 h at room temperature, and evaporated. The syrup obtained was immediately mixed with toluene (30 ml), aniline (7.8 g, 84 mmoles), and ammonium chloride (0.5 g), and the mixture was refluxed for 4 h and then evaporated. The resulting syrup was fractionated on a column packed with 150 g of silica gel (Wakogel 100) by eluting with benzene–acetone of successively increasing concentration in acetone. The fraction eluted with solvent ratios of 10:1 and 10:2 was rechromatographed in the same manner to give the title compound

as a pale-brown syrup (2.4 g, 20.6%); t.l.c., R_F 0.42 (benzene–ligroin–acetone, 3:1:1); $[\alpha]_D^{22}$ −44.5° (*c* 0.72, methanol); ν_{max}^{NaCl} 3350 (N–H), 1742 and 1670 cm^{-1} (C=O).

Anal. Calc. for $C_{18}H_{25}NO_5 \cdot H_2O$: C, 58.20; H, 7.87; N, 4.07. Found: C, 58.50; H, 7.79; N, 3.77%.

*4-Deoxy-1,2:5,6-di-O-isopropylidene-4-phenylhydrazino-*D-arabino*(and/or* D-lyxo*)-hex-3-ulose* (**6**). — A solution of ketose **3** (2.5 g, 9.6 mmoles) and phenylhydrazine (3.1 g, 28 mmoles) in 10 ml of methanol was refluxed for 3 h in the presence of a few drops of acetic acid. The syrup obtained by evaporation was chromatographed on a silica gel column by elution, in turn, with benzene–ligroin, benzene, benzene–acetone, and acetone. Evaporation of the last fraction gave compound **6** as a pale-brown syrup (1.8 g, 54.5%); $[\alpha]_D^{22}$ −70° (*c* 1.08, methanol); ν_{max}^{NaCl} 3350 and 3250 (N–H), 1740 and 1680 cm^{-1} (C=O).

Anal. Calc. for $C_{18}H_{26}N_2O_5$: C, 61.70; H, 7.48; N, 8.00. Found: C, 61.40; H, 7.49; N, 8.12%.

3-Anilino-2-butanone (**7**). — A solution of acetoin (4.4 g, 50 mmoles), aniline (9.9 g, 106 mmoles), and one drop of boron trifluoride etherate in ethanol (30 ml) was refluxed for 2 h and evaporated. Distillation of the residual syrup gave compound **7** (2.7 g, 33.4%) as a pale-yellow liquid; b.p. 114–115°/1 mm; ν_{max}^{NaCl} 3350 (N–H), 1710 and 1690 cm^{-1} (C=O).

Anal. Calc. for $C_{10}H_{13}NO$: C, 73.59; H, 8.03; N, 8.08. Found: C, 73.59; H, 8.36; N, 8.15%.

N-(3-Anilino-2-butylidene)aniline. — A mixture of acetoin (4.4 g, 50 mmoles), aniline (18.6 g, 200 mmoles), and one drop of boron trifluoride etherate was refluxed for 3 h under nitrogen, and immediately distilled to give the title compound as a pale-yellow liquid (2.9 g, 24.3%); b.p. 162–163°/2 mm; ν_{max}^{NaCl} 3360 (N–H) and 1663 cm^{-1} (C=N).

Anal. Calc. for $C_{16}H_{18}N_3$: C, 80.63; H, 7.61; N, 11.76. Found: C, 80.06; H, 7.72; N, 11.67%.

Gradual oxidation of this liquid by air produced the Schiff's base of biacetyl[24], m.p. and mixed m.p. 138°.

REFERENCES

1 J. E. HODGE, *Advan. Carbohyd. Chem.*, 10 (1955) 169.
2 Y. INOUE AND K. ONODERA, *J. Agr. Chem. Soc. Japan*, 22 (1948) 120.
3 F. WEYGAND, *Ber.*, 73 (1940) 1259.
4 F. WEYGAND, H. SIMON, AND R. VON ARDENNE, *Ber.*, 92 (1959) 3117.
5 J. E. HODGE AND C. E. RIST, *J. Amer. Chem. Soc.*, 75 (1953) 316.
6 L. ROSEN, J. W. WOODS, AND W. PIGMAN, *Ber.*, 90 (1957) 1038; *J. Amer. Chem. Soc.*, 80 (1958) 4697.
7 R. KUHN AND F. WEYGAND, *Ber.*, 70 (1937) 769.
8 A. GOTTSCHALK, *Biochem. J.*, 52 (1952) 455.
9 F. MICHEEL AND J. DIJONG, *Ann.*, 658 (1962) 120.
10 D. PALM AND H. SIMON, *Z. Naturforsch.*, 20b (1965) 32.
11 C. N. CAMERON, *Trans. Roy. Soc. Can.*, 23 (1929) 53; C. N. CAMERON, A. C. NIXON, AND S. BASTAFIELD, *ibid.*, 25 (1931) 145; W. STÜHHEN, G. MÄSSWARB, AND K. D. LEDWOCH, *Arch. Pharm.*, 286 (1953) 418; C. D. HURD AND C. M. BUESS, *J. Amer. Chem. Soc.*, 78 (1956) 5667.

12 J. G. ERICKSON, *J. Amer. Chem. Soc.*, 75 (1953) 2784; 77 (1955) 2839; J. F. CARSON, *ibid.*, 77 (1955) 1881; K. HEYNS, H. PAULSEN, R. EICHSTEDT, AND M. ROLLE, *Ber.*, 90 (1957) 2039.
13 H. PAULSEN, F. LEUPOLD, AND K. TODT, *Ann.*, 692 (1966) 200.
14 M. FUNABASHI AND J. YOSHIMURA, *Bull. Chem. Soc. Japan*, 41 (1968) 2735.
15 J. D. ALBRIGHT AND L. GOLDMAN, *J. Amer. Chem. Soc.*, 87 (1965) 4214.
16 J. F. CARSON, *J. Amer. Chem. Soc.*, 75 (1953) 4337.
17 R. KÖSTER, *Ann.*, 618 (1958) 31.
18 K. TORSELL, *Acta Chem. Scand.*, 8 (1954) 1779.
19 P. A. MCCUSKER AND M. L. KILZER, *J. Amer. Chem. Soc.*, 82 (1960) 372.
20 F. MICHEEL AND J. DIJONG, *Ann.*, 669 (1963) 143.
21 F. MICHEEL AND A. FROWEIN, *Ber.*, 90 (1957) 1603.
22 HOUBEN-WEYL, *Methoden der organischen Chemie*, Vol. 1/2, Georg Thieme Verlag, Stuttgart, p. 816.
23 L. V. VARGHA, *Ber.*, 66B (1933) 1394.
24 H. V. PECHMANN, *Ber.*, 21 (1888) 1452.

Carbohydrate Research
Elsevier Publishing Company, Amsterdam
Printed in Belgium

Note

The action of sulfur nucleophiles on methyl 2-bromo-2-deoxy-β-D-glucopyranoside

T. VAN ES
Department of Biochemistry, Rutgers, The State University, New Brunswick, New Jersey 08903 (U. S. A.)

(Received March 19th, 1969)

The action of potassium methanethioxide on methyl 2-bromo-2-deoxy-β-D-glucopyranoside[1] (**1**) gave a crystalline compound[2] which, after hydrolysis and treatment with phenylhydrazine, did not give an osazone. On the basis of this evidence it was assumed that the compound was the 2-methylthio derivative. Presumably this compound would be methyl 2-*S*-methyl-2-thio-β-D-mannopyranoside, formed by an S_N2 reaction. Alternatively, the compound could be either methyl 2-*S*-methyl-2-thio-β-D-glucopyranoside or methyl 3-*S*-methyl-3-thio-β-D-altropyranoside, formed by ring opening of the intermediate methyl 2,3-anhydro-β-D-mannopyranoside, assuming normal trans ring-opening of epoxides. This latter possibility was favored by the author, as it is known that treatment of **1** with ammonia[3] gives methyl 3-amino-3-deoxy-β-D-altropyranoside.

The reaction was repeated by treating the triacetate of **1** with ethane-, ω-toluene-, and triphenylmethane-thiol. Different thiols were used as it had been found[4] that the products of opening the epoxide ring in certain pentofuranose derivatives gave different products depending on the nature of the thiol. The products of the reactions were desulfurized and the resulting deoxy compounds were shown to be identical in chromatographic behavior, i.r. spectra and optical rotation. N.m.r. spectral analysis of the deoxy sugar and its acetate[5] showed the H-1 signal as a doublet. It was concluded that the deoxy sugar is methyl 3-deoxy-β-D-*arabino*-hexopyranoside and that the reaction products with the thiols are methyl 3-*S*-alkyl-3-thio-β-D-altropyranosides.

EXPERIMENTAL

General methods. — T.l.c. was performed on Kieselgel G (Merck) with 5 or 20% methanol in benzene as irrigant. Paper chromatography was run on Whatman No. 1 paper with 8:2:1 ethyl acetate–pyridine–water as solvent. Melting points were determined on an electrically heated Kofler block. I.r. spectra were measured with a Perkin–Elmer Model 137 spectrophotometer. N.m.r. spectra were measured with a Varian A-60 spectrophotometer. Chemical shifts are given in τ units at 60 MHz

downfield from tetramethylsilane as internal standard. All evaporations were done under vacuum below 50°.

Methyl 3-S-ethyl-3-thio-β-D-altropyranoside. — Methyl 3,4,6-tri-*O*-acetyl-2-bromo-2-deoxy-β-D-glucopyranoside[1] (3.83 g, 10 mmoles), ethanethiol (3.10 g, 50 mmoles) and methanol (150 ml) containing sodium (1.15 g, 50 mmoles) were refluxed overnight. The solution was neutralized with acetic acid and evaporated. The residue was taken up in water (50 ml), and the solution was extracted continuously with chloroform. The extracts were evaporated to give the title compound (2.4 g, 100%), m.p. 108–109°. It was recrystallised from ethyl acetate; m.p. 112°, $[\alpha]_D^{25}$ −60.4° (*c* 1.01, water); n.m.r. data (D_2O); τ 5.23 (1-proton doublet, $J_{1,2}$ 1.8 Hz, H-1), τ 6.50 (3-proton singlet, $OC\underline{H}_3$), τ 7.31 (2-proton quartet, *J* 7.0 Hz, $SC\underline{H}_2CH_3$), τ 8.76 (3-proton triplet, $SCH_2C\underline{H}_3$).

Anal. Calc. for $C_9H_{18}O_5S$: C, 45.4; H, 7.8. Found: C, 45.3; H, 7.6.

Methyl 2,4,6-tri-O-acetyl-3-S-ethyl-3-thio-β-D-altropyranoside. — The foregoing compound was acetylated in pyridine with acetic anhydride to give a syrupy acetate, which was purified by chromatography on a column of silica gel with 5% methanol in benzene as the eluant; $[\alpha]_D^{25}$ −64° (*c* 1.39, chloroform); n.m.r. data ($CDCl_3$): τ 5.11 (1-proton doublet, $J_{1,2}$ 2.0 Hz, H-1), τ 6.50 (3-proton singlet, $OC\underline{H}_3$), τ 7.35 (2-proton quartet, *J*, 7.0 Hz), τ 7.84, 7.90 (3- and 6-proton singlets, $OCOC\underline{H}_3$), τ 8.74 (3-proton triplet, $SCH_2C\underline{H}_3$).

Anal. Calc. for $C_{15}H_{24}O_8S$: C, 49.4; H, 6.6. Found: C, 49.6; H, 6.8.

Methyl 3-S-benzyl-3-thio-β-D-altropyranoside. — The triacetate of **1** (3.53 g, 10 mmoles), ω-toluenethiol (1.83 g, 15 mmoles) and methanol (30 ml) containing sodium (0.345 g, 15 mmoles) were refluxed overnight. The solution was processed in the same manner as described above. The title compound was obtained as a syrup. T.l.c. (20% methanol in benzene) showed one main spot, R_F 0.30, and two minor spots, R_F 0.49 and 0.68. The product was chromatographed on a column of silica gel with 15% methanol in benzene as eluant to give a syrupy product; yield 2.70 g (90%); $[\alpha]_D^{25}$ −29.5° (*c* 4.26, chloroform).

Anal. Calc. for $C_{14}H_{20}O_5S$: C, 56.0; H, 6.7. Found: C, 56.1; H, 6.7.

Methyl 3-S-triphenylmethyl-3-thio-β-D-altropyranoside. — The triacetate of **1** (2.50 g, 7.1 mmoles), triphenylmethanethiol (1.96 g, 7.1 mmoles) and methanol (20 ml) containing sodium (0.18 g, 7.1 mmoles) were refluxed overnight. The compound was isolated as described above; it was a syrup which subsequently crystallized. It was recrystallized from ethyl acetate; yield 2.51 g (80%), m.p. 165–166°, $[\alpha]_D^{25}$ −155° (*c* 1.1, pyridine).

Anal. Calc. for $C_{26}H_{28}O_5S$: C, 68.9; H, 6.2. Found: C, 69.2; H, 6.3.

Methyl 3-deoxy-β-D-arabino-hexopyranoside. — The alkylthio compounds prepared above were refluxed in ethanol with Raney nickel (30 × weight) for 6 h. The solutions were filtered through Celite, and the filter was repeatedly washed with hot ethanol. The filtrates were evaporated to syrups which showed one main component, R_F 0.11 (t.l.c., 20% methanol in benzene). The products were chromatographed on a column of silica gel with 20% methanol in benzene as the eluant to

give the desulfurized glycoside; $[\alpha]_D^{25}$ −49° (*c* 1.07, methanol), n.m.r. data (D_2O): τ 5.45 (1-proton doublet, $J_{1,2}$ 1.8 Hz, H-1), τ 6.51 (3-proton singlet, $OC\underline{H}_3$).

Anal. Calc. for $C_7H_{14}O_5$: C, 47.2; H, 7.9. Found: C, 47.3; H, 7.8.

Methyl 2,4,6-tri-O-acetyl-3-deoxy-β-D-arabino-hexopyranoside. — The above compound was acetylated in pyridine with acetic anhydride to give a syrupy acetate. T.l.c. (5% methanol in benzene) showed one main component, R_F 0.38. The syrup was purified by chromatography on a column of silica gel by using 5% methanol in benzene as the eluant to give the product, $[\alpha]_D^{25}$ −63° (*c* 1.2, chloroform); n.m.r. data ($CDCl_3$): τ 5.39 (1-proton doublet, $J_{1,2}$ 2.0 Hz, H-1), τ 6.50 (3-proton singlet, $OC\underline{H}_3$), τ 7.83 and 7.92 (3- and 6-proton singlets, $OCOC\underline{H}_3$).

Anal. Calc. for $C_{13}H_{20}O_8$: C, 51.3; H, 6.6. Found: C, 51.6; H, 6.9.

ACKNOWLEDGMENT

The author thanks the South African Council for Scientific and Industrial Research for the measurement of n.m.r. spectra.

REFERENCES

1 R. U. LEMIEUX AND B. FRASER-REID, *Can. J. Chem.*, 42 (1964) 532.
2 A. L. RAYMOND, *J. Biol. Chem.*, 107 (1934) 85.
3 E. FISCHER, M. BERGMANN, AND H. SCHOTTE, *Ber.*, 53B (1920) 509.
4 G. CASINI AND L. GOODMAN, *J. Amer. Chem. Soc.*, 86 (1964) 1427. E. J. REIST, D. F. CALKINS, AND L. GOODMAN, *J. Org. Chem.*, 32 (1967) 2538.
5 C. D. ANDERSON, W. W. LEE, L. GOODMAN, AND B. R. BAKER, *J. Amer. Chem. Soc.*, 83 (1961) 1900.

Carbohydrate Research
Elsevier Publishing Company, Amsterdam
Printed in Belgium

Preliminary communication

4-Deoxy-4-fluoro-D-glucose

A. D. BARFORD, A. B. FOSTER, J. H. WESTWOOD

Chester Beatty Research Institute, Institute of Cancer Research: Royal Cancer Hospital, Fulham Road, London, S. W. 3 (Great Britain)

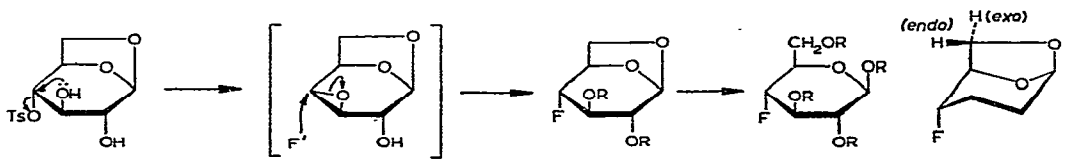
AND L. D. HALL

Department of Chemistry, University of British Columbia, Vancouver 8, B. C. (Canada)

(Received June 24th, 1969)

In connection with a study of structure–activity relationships[1] involving substrates of hexokinase isozymes of normal and cancerous tissue[2], the complete series of deoxyfluoro-D-glucopyranose derivatives was required. The 2-, 3-, and 6-fluoro derivatives have been described[3], and the α- and β-D-glucopyranosyl fluorides are known[4]. We now report on the sole remaining member of the series, 4-deoxy-4-fluoro-D-glucose.

Treatment of 1,6-anhydro-4-*O*-toluene-*p*-sulphonyl-β-D-glucopyranose[5] (**1**) with KHF_2 in boiling ethylene glycol for 75 min [monitoring by t.l.c., Kieselgel (Merck, 7731), ether–ethyl acetate (3:1)] gave 1,6-anhydro-4-deoxy-4-fluoro-β-D-glucopyranose (**3**, 47%; presumably *via* the epoxide **2**, *cf.* ref. 6), m.p. 118–120° (from acetone–ether), $[\alpha]_D$ −53° (*c* 2, water) (Found: C, 44.35; H, 5.45; F, 11.3. $C_6H_9FO_4$ calc.: C, 43.9; H, 5.5; F, 11.7%). The diacetate **4** (85%, using pyridine–acetic anhydride) was a liquid, b.p. 120–140° (bath)/0.15 mmHg, $[\alpha]_D$ −49° (*c* 1.7, chloroform) (Found: C, 48.35; H, 5.25; F, 7.35. $C_{10}H_{13}FO_6$ calc.: C, 48.4; H, 5.25; F, 7.65%).



Hydrolysis of the fluoro-anhydride **3** with refluxing N hydrochloric acid for 16 h [t.l.c. monitoring, ether–ethyl acetate (5:1)], followed by neutralisation with silver carbonate, gave 4-deoxy-4-fluoro-D-glucose (**5**, 56%), m.p. 187–189° [from ethanol–light petroleum (b.p. 40–60°)], $[\alpha]_D$ +26 (9 min)→ +49° (76 h, equil., *c* 1, water), R_G 2.53 on Whatman No. 1 paper with butyl alcohol–acetic acid–water (5:2:3) and detection with alkaline silver nitrate (Found: C, 39.85; H, 5.95; F, 10.3. $C_6H_{11}FO_5$ calc.: C, 39.55; H, 6.05; F, 10.45%). Treatment of the fluoro sugar **5**

with sodium acetate in boiling acetic anhydride for 20 min, followed by elution of the isolated product mixture from Kieselgel (Merck, 7734) with light petroleum (b.p. 40–60°)–ether (1:2), gave the β-D-tetra-acetate **6** (31%), m.p. 127–129° (from ethanol), $[\alpha]_D$ −32° (*c* 0.5, chloroform) (Found: C, 48.3; H, 5.65; F, 5.7. $C_{14}H_{19}FO_9$ calc.: C, 48.0; H, 5.4; F, 5.4%).

The structure of 4-deoxy-4-fluoro-D-glucose was unequivocally established on the basis of n.m.r. data for the β-D-tetra-acetate **6**, for which the relevant coupling constants (Hz) were obtained from a spectrum, for a solution in deuteriochloroform, measured with a Varian HA-100 spectrometer operating in the frequency-sweep mode for ^1H resonances and (at 94 MHz) in the locked, field-sweep mode for ^{19}F resonances: $J_{1,2}$ 8.1, $J_{2,3}$ 9.5, $J_{3,4}$ 8.8, $J_{4,5}$ 10.0, $J_{5,6'}$ 2.5, $J_{5,6''}$ 4.4, $J_{6',6''}$ 12.4, $J_{F,3}$ 14.5, $J_{F,4}$ 49.5, $J_{F,5}$ ~2.6, $J_{F,6'}$ ~1.6, $J_{F,6''}$ ~1.5. The magnitude of the vicinal H–H couplings are indicative[7] of *trans* di-axial relationships and therefore of the *gluco* configuration. Also of importance are the relatively small magnitudes of $J_{F,3}$ and $J_{F,5}$ which are consistent with an *eq* fluorine atom flanked by *ax* protons.

A complete analysis of the n.m.r. spectrum of the fluoro-anhydride **4** was also possible. With the exception of the ^{19}F–^1H couplings, the ^1H n.m.r. spectrum is remarkably similar to that of 2,3,4-tri-*O*-acetyl-1,6-anhydro-β-D-glucopyranose [8]. Although, for compound **4**, the vicinal F–H and H–H couplings do not permit unequivocal assignment of configuration at C-4, an assignment is possible on the basis of ^4J values. The values $J_{F,6\,endo}$ 0.7 and $J_{F,6\,exo}$ 4.4 Hz accord with the known[9] stereospecificity of ^4J, ^{19}F–^1H couplings, provided that the fluorine atom at C-4 is assigned to an axial position (7). This would give rise to a near-planar W arrangement of F-4 and *exo*-H-6, with the consequent, relatively large coupling as observed. The absence of a $J_{F,2}$ coupling in compound **4** is also consistent with an axial disposition of F-4.

A full analysis of the n.m.r. data will be presented elsewhere.

The low yield in the conversion of 1,6-anhydro-D-glucose into the 4-toluene-*p*-sulphonate largely deprives the above synthesis of convenience as a route for the large-scale preparation of 4-deoxy-4-fluoro-D-glucose, and alternative synthetic approaches are being investigated.

REFERENCES

1 J. ADAMSON AND A. B. FOSTER, *Carbohyd. Res.*, 10 (1969) 517.
2 R. T. SCHMIKE AND L. GROSSBARD, *Ann. New York Acad. Sci.*, 151 (1968) 332.
3 J. PACÁK, Z. TOĆIK, AND M. ČERNÝ, *Chem. Commun.*, (1969) 77; J. ADAMSON, A. B. FOSTER, L. D. HALL, AND R. HESSE, *ibid.*, (1969) 309; B. HELFERICH AND A. GNÜCHTEL, *Ber.*, 74 (1941) 1035; A. B. FOSTER, R. HEMS, AND J. M. WEBBER, *Carbohyd. Res.*, 5 (1967) 292.
4 B. HELFERICH, K. BÄUERLEIN, AND F. WEYGAND, *Ann.*, 447 (1926) 27; B. HELFERICH AND O. PETERS, *ibid.*, 494 (1932) 101; B. HELFERICH AND R. GOOTZ, *Ber.*, 62 (1929) 2505; F. MICHEEL AND E. KLEMER, *ibid.*, 85 (1952) 187.
5 R. W. JEANLOZ, A. M. C. RAPIN, AND S. HAKOMORI, *J. Org. Chem.*, 26 (1961) 3939.
6 M. ČERNÝ, J. STANĚK, AND J. PACÁK, *Collection Czech. Chem. Commun.*, 34 (1969) 849.
7 L. D. HALL, *Advan. Carbohyd. Chem.*, 19 (1964) 51.
8 K. HEYNS AND J. WEYER, *Ann.*, 718 (1968) 224; L. D. HALL AND A. TRACEY, unpublished data.
9 A. B. FOSTER, R. HEMS, L. D. HALL, AND J. F. MANVILLE, *Chem. Commun.*, (1968) 158.

Carbohydrate Research
Elsevier Publishing Company, Amsterdam
Printed in Belgium

Preliminary communication

4-Deoxy-4-fluoro-D-glucose

A. D. BARFORD, A. B. FOSTER, J. H. WESTWOOD

Chester Beatty Research Institute, Institute of Cancer Research: Royal Cancer Hospital, Fulham Road, London, S. W. 3 (Great Britain)

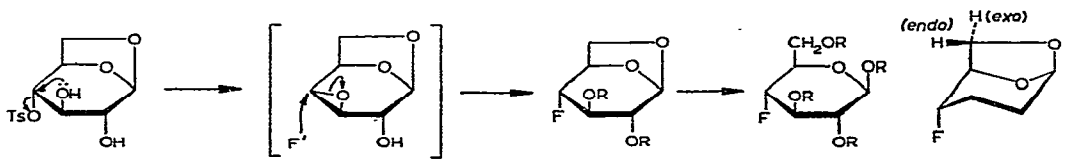
AND L. D. HALL

Department of Chemistry, University of British Columbia, Vancouver 8, B. C. (Canada)

(Received June 24th, 1969)

In connection with a study of structure–activity relationships[1] involving substrates of hexokinase isozymes of normal and cancerous tissue[2], the complete series of deoxyfluoro-D-glucopyranose derivatives was required. The 2-, 3-, and 6-fluoro derivatives have been described[3], and the α- and β-D-glucopyranosyl fluorides are known[4]. We now report on the sole remaining member of the series, 4-deoxy-4-fluoro-D-glucose.

Treatment of 1,6-anhydro-4-*O*-toluene-*p*-sulphonyl-β-D-glucopyranose[5] (**1**) with KHF_2 in boiling ethylene glycol for 75 min [monitoring by t.l.c., Kieselgel (Merck, 7731), ether–ethyl acetate (3:1)] gave 1,6-anhydro-4-deoxy-4-fluoro-β-D-glucopyranose (**3**, 47%; presumably *via* the epoxide **2**, *cf.* ref. 6), m.p. 118–120° (from acetone–ether), $[\alpha]_D$ −53° (*c* 2, water) (Found: C, 44.35; H, 5.45; F, 11.3. $C_6H_9FO_4$ calc.: C, 43.9; H, 5.5; F, 11.7%). The diacetate **4** (85%, using pyridine–acetic anhydride) was a liquid, b.p. 120–140° (bath)/0.15 mmHg, $[\alpha]_D$ −49° (*c* 1.7, chloroform) (Found: C, 48.35; H, 5.25; F, 7.35. $C_{10}H_{13}FO_6$ calc.: C, 48.4; H, 5.25; F, 7.65%).



Hydrolysis of the fluoro-anhydride **3** with refluxing N hydrochloric acid for 16 h [t.l.c. monitoring, ether–ethyl acetate (5:1)], followed by neutralisation with silver carbonate, gave 4-deoxy-4-fluoro-D-glucose (**5**, 56%), m.p. 187–189° [from ethanol–light petroleum (b.p. 40–60°)], $[\alpha]_D$ +26 (9 min)→ +49° (76 h, equil., *c* 1, water), R_G 2.53 on Whatman No. 1 paper with butyl alcohol–acetic acid–water (5:2:3) and detection with alkaline silver nitrate (Found: C, 39.85; H, 5.95; F, 10.3. $C_6H_{11}FO_5$ calc.: C, 39.55; H, 6.05; F, 10.45%). Treatment of the fluoro sugar **5**

with sodium acetate in boiling acetic anhydride for 20 min, followed by elution of the isolated product mixture from Kieselgel (Merck, 7734) with light petroleum (b.p. 40–60°)–ether (1:2), gave the β-D-tetra-acetate **6** (31%), m.p. 127–129° (from ethanol), $[\alpha]_D$ −32° (*c* 0.5, chloroform) (Found: C, 48.3; H, 5.65; F, 5.7. $C_{14}H_{19}FO_9$ calc.: C, 48.0; H, 5.4; F, 5.4%).

The structure of 4-deoxy-4-fluoro-D-glucose was unequivocally established on the basis of n.m.r. data for the β-D-tetra-acetate **6**, for which the relevant coupling constants (Hz) were obtained from a spectrum, for a solution in deuteriochloroform, measured with a Varian HA-100 spectrometer operating in the frequency-sweep mode for ^1H resonances and (at 94 MHz) in the locked, field-sweep mode for ^{19}F resonances: $J_{1,2}$ 8.1, $J_{2,3}$ 9.5, $J_{3,4}$ 8.8, $J_{4,5}$ 10.0, $J_{5,6'}$ 2.5, $J_{5,6''}$ 4.4, $J_{6',6''}$ 12.4, $J_{F,3}$ 14.5, $J_{F,4}$ 49.5, $J_{F,5}$ ~2.6, $J_{F,6'}$ ~1.6, $J_{F,6''}$ ~1.5. The magnitude of the vicinal H–H couplings are indicative[7] of *trans* di-axial relationships and therefore of the *gluco* configuration. Also of importance are the relatively small magnitudes of $J_{F,3}$ and $J_{F,5}$ which are consistent with an *eq* fluorine atom flanked by *ax* protons.

A complete analysis of the n.m.r. spectrum of the fluoro-anhydride **4** was also possible. With the exception of the ^{19}F–^1H couplings, the ^1H n.m.r. spectrum is remarkably similar to that of 2,3,4-tri-*O*-acetyl-1,6-anhydro-β-D-glucopyranose [8]. Although, for compound **4**, the vicinal F–H and H–H couplings do not permit unequivocal assignment of configuration at C-4, an assignment is possible on the basis of ^4J values. The values $J_{F,6\,endo}$ 0.7 and $J_{F,6\,exo}$ 4.4 Hz accord with the known[9] stereospecificity of ^4J, ^{19}F–^1H couplings, provided that the fluorine atom at C-4 is assigned to an axial position (7). This would give rise to a near-planar W arrangement of F-4 and *exo*-H-6, with the consequent, relatively large coupling as observed. The absence of a $J_{F,2}$ coupling in compound **4** is also consistent with an axial disposition of F-4.

A full analysis of the n.m.r. data will be presented elsewhere.

The low yield in the conversion of 1,6-anhydro-D-glucose into the 4-toluene-*p*-sulphonate largely deprives the above synthesis of convenience as a route for the large-scale preparation of 4-deoxy-4-fluoro-D-glucose, and alternative synthetic approaches are being investigated.

REFERENCES

1 J. ADAMSON AND A. B. FOSTER, *Carbohyd. Res.*, 10 (1969) 517.
2 R. T. SCHMIKE AND L. GROSSBARD, *Ann. New York Acad. Sci.*, 151 (1968) 332.
3 J. PACÁK, Z. TOĆIK, AND M. ČERNÝ, *Chem. Commun.*, (1969) 77; J. ADAMSON, A. B. FOSTER, L. D. HALL, AND R. HESSE, *ibid.*, (1969) 309; B. HELFERICH AND A. GNÜCHTEL, *Ber.*, 74 (1941) 1035; A. B. FOSTER, R. HEMS, AND J. M. WEBBER, *Carbohyd. Res.*, 5 (1967) 292.
4 B. HELFERICH, K. BÄUERLEIN, AND F. WEYGAND, *Ann.*, 447 (1926) 27; B. HELFERICH AND O. PETERS, *ibid.*, 494 (1932) 101; B. HELFERICH AND R. GOOTZ, *Ber.*, 62 (1929) 2505; F. MICHEEL AND E. KLEMER, *ibid.*, 85 (1952) 187.
5 R. W. JEANLOZ, A. M. C. RAPIN, AND S. HAKOMORI, *J. Org. Chem.*, 26 (1961) 3939.
6 M. ČERNÝ, J. STANĚK, AND J. PACÁK, *Collection Czech. Chem. Commun.*, 34 (1969) 849.
7 L. D. HALL, *Advan. Carbohyd. Chem.*, 19 (1964) 51.
8 K. HEYNS AND J. WEYER, *Ann.*, 718 (1968) 224; L. D. HALL AND A. TRACEY, unpublished data.
9 A. B. FOSTER, R. HEMS, L. D. HALL, AND J. F. MANVILLE, *Chem. Commun.*, (1968) 158.

CORRIGENDUM

Carbohydrate Research, 11 (1969)

Page 33, line 12 of ABSTRACT should read:
indicated that the degree of methylation at positions 2, 3, 4, and 6 was 88%, 40%,

Page 36, 3rd line below formulae should read:
réalisés à 60 MHz donnent (quoique moins bien résolus qu'à 100 MHz) des pics

CORRIGENDUM

Carbohydrate Research, 11 (1969)

Page 33, line 12 of ABSTRACT should read:
indicated that the degree of methylation at positions 2, 3, 4, and 6 was 88%, 40%,

Page 36, 3rd line below formulae should read:
réalisés à 60 MHz donnent (quoique moins bien résolus qu'à 100 MHz) des pics

Carbohydrate Research
Elsevier Publishing Company, Amsterdam
Printed in Belgium

PREPARATION OF L-ARABINOSE-CONTAINING POLYSACCHARIDES AND THE ACTION OF AN α-L-ARABINOFURANOSIDASE ON THESE POLYSACCHARIDES

KIYOSHI TAGAWA AND AKIRA KAJI*
Department of Food Science, Faculty of Agriculture, Kagawa University, Kagawa (Japan)
(Received December 23rd, 1968; in revised form April 16th, 1969)

ABSTRACT

A method, involving chromatography on DEAE-cellulose and gel-filtration on Sephadex G-50, is described for purification of the L-arabinan from a crude beet arabinan preparation. From the purified L-arabinan a (1→5)-linked L-arabinan was prepared by treatment with an α-L-arabinofuranosidase from *Aspergillus niger* and was characterized as a linear polymer. The L-arabinofuranose residues in these L-arabinans were hydrolyzed by action of the α-L-arabinofuranosidase, and the L-arabinose residues in wheat L-arabino-D-xylan and a small proportion of those in gum arabic were also susceptible to hydrolysis by the enzyme.

INTRODUCTION

Although polysaccharides containing L-arabinose residues are widely distributed in plant hemicellulose fractions, enzymes that degrade these polysaccharides have not been studied so extensively as the pectolytic enzymes.

An L-arabinan is commonly found in pectic substances and its structure has been well characterized by Hirst and Jones[1], and by others[2,3]. Enzymic cleavage of L-arabinan was first described by Ehrlich and Schubert[4]. Recently, we have studied enzymes produced by *Aspergillus niger*[5,6] and other microorganisms[7]. One enzyme was highly purified from the culture filtrate of *A. niger*, and was characterized as an α-L-arabinofuranosidase[6]. When L-arabinan was hydrolyzed with α-L-arabinofuranosidase, L-arabinose was liberated and the hydrolytic data showed that α-(1→3)-linked L-arabinose residues of the L-arabinan molecule, attached along a main L-arabinose chain as a single unit side-chains, are selectively cleaved. However, the mode of action of this enzyme on the (1→5)-α-L-arabinosyl linkages in the substrate was not clearly established. This report deals with the preparation and characterization of (1→5)-L-arabinan, together with an improved procedure for preparation of the parent L-arabinan. The results obtained from the action of the α-L-arabinofuranosidase on various polysaccharides containing L-arabinose residues are also described.

*Department of Agricultural Chemistry, Kagawa University.

RESULTS AND DISCUSSION

Hirst and Jones[1] reported that acetylation and deacetylation are effective procedures for separation of L-arabinan from D-galactan. These procedure are, however, rather tedious and could also cause a marked change in the L-arabinan preparation, if moisture is not carefully excluded. We set out to prepare a pure sample of L-arabinan, with a minimum of degradation, for preparing (1→5)-L-arabinan and for investigation as a substrate for the α-L-arabinofuranosidase.

Crude beet arabinan was used as the starting material. It had $[\alpha]_D^{25}$ −87° and contained residues of L-arabinose (73.7%), D-galactose (22.0%), and a small proportion of D-galacturonic acid. Preliminary experiments showed that fractionation of the crude arabinan by alcohol was not successful, little separation of the L-arabinan from contaminating polysaccharide being achieved. Chromatography on *O*-[2-(diethylamino)ethyl]cellulose (DEAE-cellulose), however, led to successful fractionation, as shown in Fig. 1. The crude arabinan gave two main polysaccharide fractions by DEAE-cellulose chromatography. The polysaccharides that were not adsorbed had an L-arabinose content of 93.6% and $[\alpha]_D^{25}$ −128°, whereas the fraction eluted with 0.1M hydrochloric acid contained L-arabinose, D-galactose, and D-galacturonic acid residues. The latter fraction was not examined further.

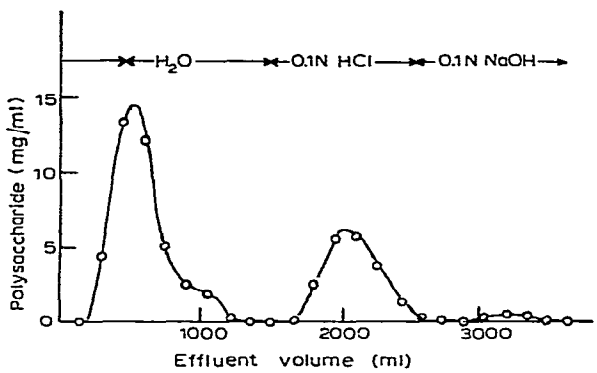


Fig. 1. Fractionation of the crude beet arabinan on DEAE-cellulose (OH^- form).

The L-arabinan preparation thus obtained contained a small proportion of D-galactose residues, as revealed by paper chromatography of an acid hydrolyzate. Removal of this contamination was effectively achieved by gel-filtration on Sephadex G-50. As shown in Fig. 2, polysaccharides having a high content of L-arabinose were eluted faster than those containing D-galactose residues, although they were not completely separated from each other. The polysaccharide obtained from the appropriate fraction showed the accepted characteristics[1, 8] for a pure L-arabinan (see Table I), and methylation analysis indicated that it had a branched structure, as first proposed by Hirst and Jones[1]. Fig. 3A shows the gas–liquid chromatogram of methanolysis products from the methylated L-arabinan (OMe, 38.8%), wherein

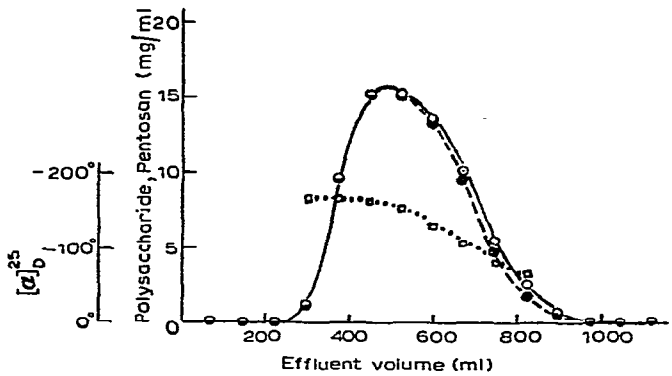


Fig. 2. Fractionation of the partially purified L-arabinan on Sephadex G-50. Polysaccharide content —O—O—, Pentosan content —●— – —●—, Optical rotation $[\alpha]_D^{25}$. □ . . □ .

TABLE I

PROPERTIES OF THE L-ARABINAN AND THE (1→5)-L-ARABINAN

	L-Arabinan	(1→5)-L-*Arabinan*
Sugar components	L-arabinose (97.8%) D-galactose (1.1%)	L-arabinose (98.3%)
Solubility in water	very soluble, ~0.2 g/ml	3.5×10^{-3} g/ml at 25°, soluble after heating at above 90°
$[\alpha]_D^{25}$ (water, *c*, 1.0)	−161°	−148°
Intrinsic viscosity [η]	19.5 $g.cm^{-1}$	23.7 $g.cm^{-1}$
Density	1.593 $g.cm^{-3}$	1.524 $g.cm^{-3}$
Periodate consumption (per sugar residue, in 48 h)	0.697 mole	1.067 mole

methyl glycosides of 2,3,5-tri-*O*-methyl-, 2,3-di-*O*-methyl- and mono-*O*-methyl-L-arabinoses were detected in molar proportions of 34.3:31.7:34.0. From the observed

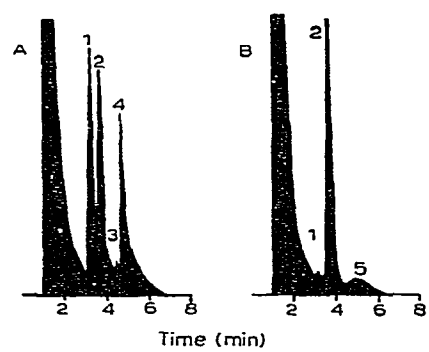


Fig. 3. Gas–liquid chromatograms of methanolyzed products obtained from the fully methylated L-arabinan, (A), and the fully methylated (1→5)-L-arabinan, (B). (1) Methyl 2,3,5-tri-*O*-methyl-L-arabinoside, (2) Methyl 2,3-di-*O*-methyl-L-arabinoside, (3) Methyl 2-*O*-methyl-β(?)-L-arabinoside, (4) Methyl 2-*O*-methyl-α(?)-L-arabinoside, (5) Methyl *O*-methyl-L-arabinoside.

behavior on the gel, it is considered that the fraction of lower molecular weight probably has a linear or slightly branched structure, consisting mainly of galactose residues, as suggested by Hough and Powell[3]. When the arabinan was incubated with α-L-arabinofuranosidase, the side-chains of the L-arabinan molecule were easily hydrolyzed, and the resulting linear polymer of L-arabinose was precipitated from the reaction mixture by lowering the temperature after the extent of hydrolysis reached 35% or a little more. The linear polysaccharide, designated (1→5)-L-arabinan, consisted of only L-arabinose (98.3%) and had a negative optical rotation of $[\alpha]_D^{25}$ −148°. Its properties, contrasted with those of the parent L-arabinan, are listed in Table 1. The data show that the (1→5)-L-arabinan has a straight-chain structure with α-L-glycosidic linkages, and this structural evidence was indicated more clearly by a gas-chromatographic examination of methanolysis products from the methylated (1→5)-L-arabinan (OMe, 37.1%). As will be seen in Fig. 3B, a large proportion of methyl 2,3-di-*O*-methyl-L-arabinoside (91.3%, in molar proportion) was detected, while methyl 2,3,5-tri-*O*-methyl- and methyl mono-*O*-methyl-L-arabinosides were present in small proportions (2.3 and 6.5%, respectively, in molar proportion). With this evidence for a (1→5)-L-arabinan, the specificity of the α-L-arabinofuranosidase was examined.

Some properties of the α-L-arabinofuranosidase from *A. niger*[6] have been reported already. The present study was undertaken to gain more knowledge of the action and the specificity of the enzyme. The enzyme preparation used was highly purified and its homogeneity was proved by ultracentrifugal analysis[6]. Beside the parent L-arabinan and the (1→5)-L-arabinan described above, wheat L-arabino-D-xylan and gum arabic (*Acacia senegal* gum) were used as substrates. Wheat L-arabino-D-xylan consists of L-arabinose (35%), D-xylose (61%), and D-galactose (4%) residues, and gum arabic of L-arabinose (27%), L-rhamnose, D-galactose, D-galacturonic acid, and a trace of unidentified sugar residues. Hydrolysis curves of these polysaccharides as a result of enzyme action are shown in Fig. 4.

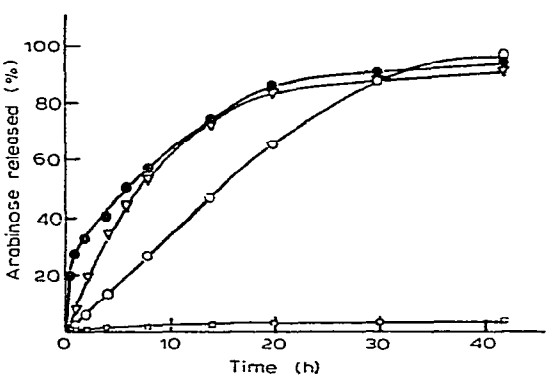


Fig. 4. Hydrolysis curves of polysaccharides containing L-arabinose residues with the α-L-arabinofuranosidase from *A. niger*.
L-Arabinan —●—●— , (1→5)-L-Arabinan —○—○— ,
L-Arabino-D-xylan —▽—▽— , Gum arabic —□—□— .

The parent L-arabinan was hydrolyzed rapidly to the extent of about 30% and thereafter hydrolysis was slow. On the other hand, the (1→5)-L-arabinan was hydrolyzed linearly with time, at a rate less than one tenth of the initial rate of hydrolysis of the parent L-arabinan. In both cases, hydrolysis to >90% was attained after incubation for 30 h and L-arabinose was detected (papergram) as the sole product. From these results it is clearly established that the α-L-arabinofuranosidase has hydrolytic activity for both α-(1→3)- and α-(1→5)-linked, non-reducing, terminal L-arabinofuranose residues, and does not act on the internal α-L-arabinofuranosyl linkages. On this account, it will be appreciated[9] that the difference in the rate between hydrolysis of the L-arabinan and (1→5)-L-arabinan depends largely on the number of non-reducing, terminal groups in each polysaccharide.

Wheat L-arabino-D-xylan was also hydrolyzed with the enzyme and the release of L-arabinose in the digest was observed by paper chromatography. As the reaction progressed an amorphous precipitate formed, which was found to be a polysaccharide consisting mainly of D-xylose residues. There is thus convincing evidence that the enzyme is capable of hydrolyzing L-arabinosyl linkages in L-arabino-D-xylan, because L-arabinose residues in this polysaccharide are present as non-reducing, terminal groups, in the furanose form, and must be attached to the main D-xylose chain by α-L-glycosidic linkages[10,11]. Since this work was concluded[12], substantial evidence for an α-L-arabinofuranosidase (partially purified from Pectinol R-10) has been reported by Neukom, *et al.*[13] They observed that incubation of L-arabino-D-xylan with their enzyme preparation caused formation of a white precipitate, which consisted of a D-xylan containing only traces of L-arabinose.

When the enzyme acted on gum arabic, a small proportion of L-arabinose was released and the reducing value increased. After prolonged incubation the hydrolysis accounted for about 5% of the L-arabinose residues of this gum. There are some reports[14–16] suggesting that the most of the L-arabinose residues are present in the furanose form, but nothing is yet known about the localization of these residues. The result, however, strongly suggests that some linkages in the gum are terminated by α-L-linked, arabinofuranose residues.

On the basis of these enzyme data, susceptibility to the α-L-arabinofuranosidase can be used as evidence for non-reducing, terminal, L-arabinofuranose residues in polysaccharides.

EXPERIMENTAL

Materials. — DEAE-Cellulose was purchased from Brown Co., U. S. A., and Sephadex G-50 (fine grade) from Pharmacia Fine Chemicals, Sweden. Crude beet arabinan was prepared by the method of Hirst and Jones[1]. Wheat L-arabino-D-xylan was obtained according to the method of Neukom *et al*[17,18]. Gum arabic was purchased from Wako Pure Chemical Industries, Japan. α-L-Arabinofuranosidase was obtained from a culture filtrate of *Aspergillus niger* by means of fractional precipitation with ammonium sulfate, gel-filtration on Sephadex G-100, and column

chromatography on DEAE-cellulose, DEAE-Sephadex, SE-Sephadex, and hydroxyapatite[5]. The enzyme had a specific activity of 420 units per mg of protein. One activity unit has been defined as that amount of enzyme which will hydrolyze 1 μmole of phenyl α-L-arabinofuranoside per min at 40°, pH 4.0.

General analytical methods. — Quantitative determination of polysaccharides was made by the phenol–sulfuric acid method[19]. The pentosan contents were determined by the colorimetric orcinol method[20]. The reducing values of enzymic digests were measured by the Somogyi–Nelson method[21,22]. Total acid hydrolysis of polysaccharides was carried out in 0.25M sulfuric acid for 16 h at 100°. Paper chromatography of sugars was effected on Toyo filter paper No. 51A by the ascending method with the following solvent systems: 12:5:4 ethyl acetate–pyridine–water; and the upper layer of a 4:1:5 butyl alcohol–acetic acid–water. Spots were developed with aniline hydrogen phthalate[23] or alkaline silver nitrate[24]. Gas-liquid chromatography of methyl glycosides was conducted with a Hitachi gas chromatograph, type K53, with a flame ionization detector, and a stainless-steel column (0.3 × 200 cm), which was packed with 5% dimethyl silicone gum (SE-30) on 80–100 mesh, acid-washed Chromosorb W. Nitrogen was used as the carrier gas and the pressure at the inlet was kept at 0.5 $kg.cm^{-2}$. The temperatures of the injection block and the oven were maintained at 250° and 200°, respectively. For quantitative analysis, the detector response to the amount of material applied was tested by using authentic samples of methyl 2,3,5-tri-*O*-methyl- and methyl 2,3-di-*O*-methyl-L-arabinosides. The peak areas on the charts were confirmed to be directly proportional to concentration, irrespective of the nature of the glycoside.

Purification of the L-*arabinan.* — Crude beet arabinan (10 g) was dissolved in water (200 ml) and applied to a column (3.8 × 53 cm) of DEAE-cellulose (OH^- form) and eluted successively with water, 0.1M hydrochloric acid and 0.1M sodium hydroxide (1000 ml of each). Fractions were analyzed for polysaccharide content (Fig. 1). The polysaccharide eluted at 300–900 ml was dialyzed against water, concentrated, and precipitated with alcohol. On vacuum dryness it gave a pale yellow glass (5.4 g). Further purification was performed by gel-filtration on Sephadex G-50. The arabinan (5 g), dissolved in water (200 ml), was passed through a column (3.8 × 60 cm) of the gel under gravity flow with water. Fractions were analyzed for polysaccharide and pentosan contents, and for optical rotation ($[\alpha]_D^{25}$). The elution pattern is shown in Fig. 2. On the basis of pentosan content and optical rotation, the fraction eluted at 320–560 ml was pooled, concentrated, and the product was precipitated with alcohol. Drying the precipitate gave a hygroscopic material (2.1 g).

Preparation of (1→5)-L-*arabinan.* — The purified L-arabinan (10 g) was dissolved in 0.02 M citrate–phosphate buffer, pH 4.0 (400 ml), and the α-L-arabinofuranosidase (500 units) was added. The reaction allowed to proceed at 40° and was monitored by the increase in reducing value. After the extent of hydrolysis attained 30% (about 6 h of incubation) the mixture was cooled to 20° and incubated at this temperature for a further 16 h. These treatments led to formation of an amorphous precipitate and the extent of hydrolysis was 38%. The reaction mixture

was then heated for 5 min at 100° to inactivate the enzyme, and alcohol was added to 80% concentration. The precipitated polysaccharide was dissolved in hot water (200 ml) and reprecipitated by keeping the solution for 24 h at 2°. Drying the product gave a white powder (5.6 g).

Characterization of the L-*arabinan and the* (1→5)-L-*arabinan.* — *Sugar components.* A sample (100 mg) was hydrolyzed in 0.25M sulfuric acid (5 ml) for 16 h at 100°. After neutralization with barium carbonate, the clear hydrolyzate was subjected to paper chromatography. Each of the sugars located on the paper was eluted with water and determined by the phenol–sulfuric acid method[19].

Solubility. Solubility in water was measured polarimetrically at 25°.

Optical rotation. This was observed by a direct-reading polarimeter at 25 ±2°.

Intrinsic viscosity. The viscosity was measured at 25° in an Ostwald viscometer with a water flow-time of 57 sec. Measurements at several dilutions were made. From a graph showing the variation of η_{sp}/c with c, the intrinsic viscosity, $[\eta]$, was determined by extrapolation to zero concentration (c is the concentration and η_{sp} the specific viscosity).

Density. The determination was made in a pycnometer of 50-ml capacity at 20°.

Periodate consumption. A sample (100 mg), dissolved in water (25 ml), was treated in the dark at 20° with 0.2M sodium metaperiodate (25 ml). Samples (5 ml) were withdrawn at intervals, and the amount of periodate consumed was determined by the Fleury–Lange method[25].

Methylation analysis. The L-arabinan and the (1→5)-L-arabinan were first acetylated, and then methylated by the Haworth method[26] followed by the Purdie method[27]. A sample (5 g) was suspended in pyridine (200 ml) and acetic anhydride (60 ml) was added. The mixture was heated for 5 h at 80° and then poured into ice–water (1000 ml). The resulting precipitate was washed with cold water and dried under diminished pressure. The acetylated L-arabinan, dissolved in acetone (50 ml), was simultaneously deacetylated and methylated at 40° by the dropwise addition of 30% aqueous sodium hydroxide (100 ml) and methyl sulfate (40 ml). The reaction was conducted for 20 h in an atmosphere of nitrogen. After cooling in an ice bath, the solution was neutralized with M sulfuric acid and dialyzed against water for 24 h. Evaporation of this dialyzed solution gave a sticky material, which was dissolved in acetone and methylated three additional times in the same manner with methyl sulfate and sodium hydroxide. The reaction mixture was then extracted with chloroform (4 × 100 ml). The chloroform solution was dried on anhydrous magnesium sulfate and concentrated under diminished pressure. The partially methylated L-arabinan so obtained was dissolved in methyl iodide (30 ml), and silver oxide (25 g) was added during 4 h to the stirred solution at 50°. After it had been stirred for a further 2 h, the methylated product was extracted as mentioned above. Four treatments with methyl iodide and silver oxide were required to give a fully methylated L-arabinan (the methoxyl content was not raised on further attempted methylation). Each sample (100 mg) of the methylated L-arabinans was hydrolyzed in a sealed tube with 3% methanolic hydrochloric acid (4 ml) for 7 h at 100°. After neutralization

with silver carbonate, the methyl glycosides were extracted with chloroform (3 × 5 ml) and the chloroform solutions were concentrated to a small volume (1 ml). Portions (0.5–1.0 μl) of the concentrates were analyzed by gas–liquid chromatography, with authentic samples of methylated methyl arabinosides as reference compounds (Fig. 3).

Action of α-L-arabinofuranosidase on polysaccharides containing L-arabinose residues. — Samples (100 mg) of the polysaccharides: L-arabinan, (1→5)-L-arabinan, wheat L-arabino-D-xylan, and gum arabic, were dissolved in water (10 ml). To each of the solutions, 0.1M citrate–phosphate buffer, pH 4.0 (5 ml), α-L-arabinofuranosidase (60 units in 5 ml) and a few drops of toluene were added, and the mixture was incubated at 40°. At intervals, portions (1 ml) were removed and analyzed for reducing value and sugars released by the Somogyi-Nelson method[21,22] and paper chromatography, respectively. In all experiments, L-arabinose was the only product, and its amount corresponded to the values calculated from the increase in reducing value. Therefore, in Fig. 4, the extents of hydrolysis are expressed as equivalents of L-arabinose released by the action of the enzyme on the polysaccharides.

ACKNOWLEDGMENTS

The authors thank Professor K. Onodera and Dr. S. Hirano of Kyoto University for valued counsel, and Mr. K. Matsubara and Miss T. Ichimi for technical assistance.

REFERENCES

1 E. L. HIRST AND J. K. N. JONES, *J. Chem. Soc.*, (1938) 496, (1939) 452, 454, (1947) 1221, (1948) 2311.
2 G. H. BEAVAN AND J. K. N. JONES, *J. Chem. Soc.*, (1947) 1218.
3 L. HOUGH AND D. B. POWELL, *J. Chem. Soc.*, (1960) 16.
4 F. EHRLICH AND F. SCHUBERT, *Biochem. Z.*, 203 (1928) 343.
5 A. KAJI, K. TAGAWA, AND K. MATSUBARA, *Agr. Biol. Chem.* (Tokyo), 31 (1967) 1023.
6 A. KAJI, K. TAGAWA, AND T. ICHIMI, *Biochim. Biophys. Acta*, 171 (1969) 186.
7 A. KAJI, K. TAGAWA, AND K. MOTOYAMA, *J. Agr. Chem. Soc. Japan*, 39 (1965) 352.
8 T. K. GAPONENKOV, *Colloid J.* (U. S. S. R.), 2 (1936) 561; *Chem. Abstr.*, 31 (1937) 1649, *J. Gen. Chem.* (U. S. S. R.), 7 (1937) 1729; *Chem. Abstr.*, 31 (1937) 8307, *Colloid J.* (U. S. S. R.), 3 (1937) 663; *Chem. Abstr.*, 32 (1938) 6927.
9 K. TAGAWA, in preparation.
10 A. S. PERLIN, *Cereal Chem.*, 28 (1951) 370, 382.
11 R. MONTGOMERY AND F. SMITH, *J. Amer. Chem. Soc.*, 77 (1955) 3325.
12 A. KAJI, K. TAGAWA, K. MATSUBARA, AND K. TORIGOE, *Annual Meeting of Agr. Chem. Soc. Japan*, 1967.
13 H. NEUKOM, L. PROVIDOLI, H. GREMLI, AND P. A. HUI, *Cereal Chem.*, 44 (1967) 238.
14 F. SMITH, *J. Chem. Soc.*, (1939) 1724, (1940) 1035.
15 R. L. WHISTLER AND C. L. SMART, *Polysaccharide Chemistry*, Academic Press, New York, 1953.
16 F. SMITH AND R. MONTGOMERY, *The Chemistry of Plant Gums and Mucilages*, Reinhold, New York, 1959.
17 H. NEUKOM, H. DEUEL, W. J. HERI, AND W. KÜNDIG, *Helv. Chim. Acta*, 43 (1960) 64.
18 W. KÜNDIG, H. NEUKOM, AND H. DEUEL, *Helv. Chim. Acta*, 44 (1961) 823.
19 M. DUBOIS, K. A. GILLIS, J. K. HAMILTON, P. A. REBERS, AND F. SMITH, *Nature*, 168 (1951) 167, *Anal. Chem.*, 28 (1956) 350.

20 W. MEJBAUM, *Z. Physiol. Chem.*, 258 (1939) 117.
21 M. SOMOGYI, *J. Biol. Chem.*, 195 (1952) 19.
22 N. NELSON, *J. Biol. Chem.*, 153 (1944) 375.
23 S. M. PARTRIDGE, *Nature*, 164 (1949) 443.
24 W. E. TREVELYAN, D. P. PROCTER, AND J. S. HARRISON, *Nature*, 166 (1950) 444.
25 P. F. FLEURY AND J. LANGE, *J. Pharm. Chim.*, 17 (1933) 107, 196.
26 W. N. HAWORTH, *J. Chem. Soc.*, 107 (1915) 8.
27 T. PURDIE AND J. C. IRVINE, *J. Chem. Soc.*, 83 (1903) 1021.

Carbohydrate Research
Elsevier Publishing Company, Amsterdam
Printed in Belgium

PHENYLHYDRAZONO–PHENYLAZO TAUTOMERISM.

PART III*. REACTIONS OF PHENYLFORMAZANS AND CERTAIN BIS(PHENYLHYDRAZONES) WITH STRONG ACIDS**

HORACE S. ISBELL*** AND ALEXANDER J. FATIADI

Division of Analytical Chemistry, Institute for Materials Research, National Bureau of Standards, Washington, D. C. 20234 (U. S. A.)

(Received February 24th, 1969; in revised form, May 12th, 1969)

ABSTRACT

The behavior of diphenylformazans, 2-oxo-1,3-bis(phenylhydrazones), 1,2-bis-(phenylazo)ethylene, and the phenylhydrazone of 4-oxo-1-phenyl-5-phenylazo-3-pyridazinecarboxaldehyde on protonation has been examined spectrophotometrically. These compounds form purple, blue, or green protonated cations. The nature of the spectral changes suggests that the highly colored cations have resonance-stabilized structures. Phenylosazones and bis(phenylhydrazono) compounds that cannot form resonance-stabilized cations on protonation do not usually give the blue-color reaction. Structures are proposed for the cations derived from diphenylformazans and certain 2-oxo-1,3-bis(phenylhydrazones).

Treatment of the red, enolic tautomer of 2-oxo-1,3-bis(phenylhydrazono)-cyclohexane with perchloric acid in acetic acid yields a new, stable, dark-blue, crystalline salt. Dissolution of this salt in acetic acid, followed by addition of ice–water, yields a previously described yellow hydrate of the *keto* tautomer. Dissolution of the hydrate in ethanol and warming regenerates the red, enolic tautomer.

E.s.r. measurements of colored solutions of 2-oxo-1,3-bis(phenylhydrazones) or diphenylformazans did not show the presence of radical species, thus indicating the ionic character of the products formed on protonation.

INTRODUCTION

Ketazine bis(hydrazones), azo dyes, enolic phenylhydrazono–phenylazo compounds[1–5], and similar compounds show characteristic absorption bands that change in both wavelength and intensity on addition of strong acids; and these changes provide a means of studying the compounds in solution. All of the compounds that give intense colors with bathochromic shifts are, on protonation, capable of forming resonance-stabilized cations, whereas the compounds that do not give intense colors presumably lack the capacity to form such resonance-stabilized structures.

*For Part II, see ref. 2.

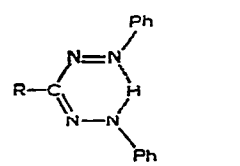
**Presented, in part, at the 150th National Meeting of the American Chemical Society, Atlantic City, New Jersey, September, 1965.

***Present address: Chemistry Department, American University, Washington, D. C. 20016 (U. S. A.).

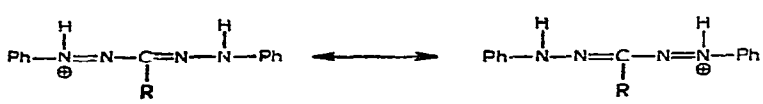
The bathochromic shifts and intense colors could arise either from resonance-stabilized cations or from free-radical structures. E.s.r. measurements of a solution of protonated 1,3-bis(phenylhydrazones) (compounds **2** to **7**) showed no radical intermediates; hence, the colors must arise from polar structures similar to resonance-stabilized salts of azine bis(phenylhydrazones)[6], other 1,3-bis(phenylhydrazones)[7], or the azonium salts of 4-aminoazobenzenes[8,9]. The bathochromic shift is due to the energy difference between the normal and excited states being less for the cation than for the parent compound. This is explained in terms of more extensive delocalization of the electron in the excited state of the cation. Phenylhydrazones, phenylosazones, and certain phenylazo compounds lacking auxochromic groups do not usually show substantial bathochromic shifts on treatment with strong acid. In marked contrast, *p*-aminoazobenzene and related azo compounds show considerable spectral changes under similar conditions. Thus, *p*-aminoazobenzene in ethanol absorbs in the region at 380 to 440 nm; addition of a strong acid to this solution shifts the visible band to 500–540 nm and gives another band at 320 nm. From studies of the properties of a series of related compounds, Lewis[8] concluded that the band at 500 to 540 nm arises from the azonium ion and the band at 320 nm from the ammonium cation.

Recently, Eistert and co-workers[7] studied a series of related 1,3-bis(phenylhydrazono) compounds and obtained similar salts. The compounds that we have studied are listed in Table I, and are described in the sections that follow.

D-*Mannose diphenylformazan*[18] (**1**). — Treatment of **1** in acetic acid with perchloric acid results in a bathochromic shift from λ_{max} 455 to 550 nm; the color changes from orange-red to purple-blue. Removal of the strong acid, or lessening of the acidity by dilution with alcohol or water restores the orange-red color. In the course of time, the original purple-blue color of a solution of **1** fades. This was to be expected, because, on treatment with warm mineral acids, phenylformazans rearrange to benzotriazines[19], and, on oxidation, yield colorless tetrazolium salts[20,21]. Furthermore, the formazans exist in several geometric forms depending on different orientation about the C=N and N=N bonds, and isomerization is reported to take place on irradiation with visible light[14].



1 R = $-(CHOH)_4CH_2OH$



1a R = $-(CHOH)_4CH_2OH$

TABLE I

CHANGES IN VISIBLE ABSORPTION BANDS[a] AND THEIR INTENSITIES FOR PHENYLHYDRAZONO–PHENYLAZO COMPOUNDS ON ADDITION OF STRONG ACID

Compound No.	*References*	*Color in acetic acid*	*Visible spectrum in AcOH*		*Color in perchloric acid*	*Visible spectrum in $HClO_4$*		*Bathochromic shift (nm)*
			λ_{max} (*nm*)	ε		λ_{max} (*nm*)	ε	
1	10	orange-red	455	18,000	purple-blue	550	27,500	+ 95
2	7, 11	orange-red	439	5,600	blue	605	11,650	+136
						640sh	11,000	+201
3	12	wine-red	485	37,000	blue	565	78,500	+ 80
4	2	wine-red	485	34,500	purple	490	33,000	+ 15
						595	5,800	+110
5	13	wine-red	486	34,200	purple-blue	562	68,800	+ 76
6	14	orange-red	475	55,000	purple-blue	570	106,100	+ 95
7	15, 16	yellow-orange	442	3,500	purple-blue	554	5,800	+112
8	17	red	357	30,400	red	No		
			423sh	14,800		change[b]		
			485	28,500				
9	1	red	468	23,500	purple-green	442	14,700	− 26
						610	13,000	+145
12	2, 18	purple-red	470	1,450	yellow-orange	450	5,300	− 20

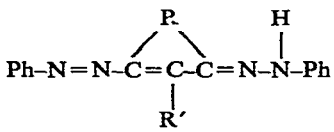
[a]The spectra were recorded 2 min after the sample had dissolved. [b]On heating 8 in the presence of a strong acid, the red solution slowly changed to purple-red.

Diphenylformazans have a phenylhydrazono group and a phenylazo group attached to the same carbon atom. The neutral molecule, for example, D-mannose diphenylformazan, has a chelate structure **1** in which the imino hydrogen atom spans the phenylhydrazono group and the phenylazo group[10]. Apparently, strong acids rupture this chelate, and the resulting, resonance-stabilized cation may have a structure encompassing the resonance forms depicted in **1a**.

The electron system for the cation of a diphenylformazan, **1a**, encompasses 17 atoms, but only two of the resonance forms are illustrated. Delocalization of the positive charges over the resonance system in **1a** may account for the bathochromic shift found in the spectrum of **1** on protonation. A labile proton may be attached to any one of the four nitrogen atoms. In **1a**, the protons are shown attached to the *alpha* nitrogen atoms (*i.e.*, adjacent to the phenyl rings), because, when the protons are in this position there is less separation of unlike charges than when the protons are attached to the *beta* nitrogen atoms. Sharing of the proton between the nitrogen atoms is possible, but not probable, because this would require a *cis* structure for the phenylazo moiety instead of the more stable *trans* structure. The resonance-stabilized structure for the cation includes both of the phenylhydrazono groups.

Phenylhydrazono–phenylazo compounds (**2** *through* **8**). — These compounds, like the phenylformazans, have a phenylhydrazono group joined in a resonance structure with a phenylazo group. On treatment with strong acids in acetic acid, they give purple, blue, or green colors. In 1-phenylazo-3-phenylhydrazono-1-cyclohexene (**2**), the phenylazo and phenylhydrazono functions are joined through a vinyl group, whereas, in compounds **3** through **7**, these functions are joined through an enol group.

Compounds **2** through **8** have the following structures.



2 R = $-CH_2CH_2CH_2-$; R′ = H
3 R = $-CH_2CH_2CH_2-$; R′ = OH
4 R = $-CH(OH)CH(OH)CH(OH)-$; R′ = OH
5 R = $-CH_2CH(CO_2H)CH_2-$; R′ = OH
6 R = $-CH_2CH_2-$; R′ = OH
7 R = –H, –H; R′ = OH

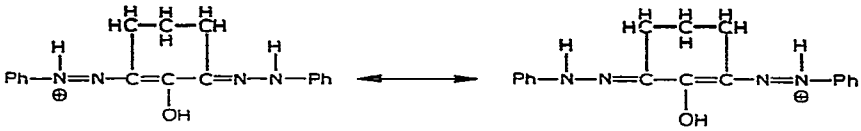
8 R =  ; R′ = OH

Compound **3** differs from **2** merely in the presence of a hydroxyl group, instead of a hydrogen atom, attached to C-2 and, as might be expected, the absorption spectra of the two compounds are similar, both in the absence and presence of a strong acid.

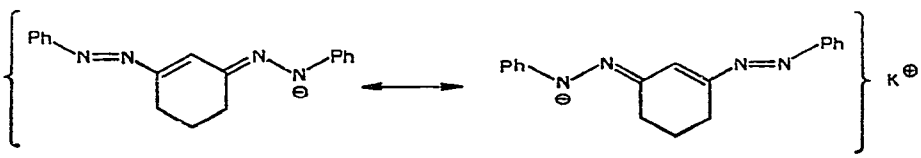
Compound **3**, a typical 2-oxo-1,3-bis(phenylhydrazono) derivative, exists in two forms, a red enolic form **3a** and a yellow hydrate[2] **3b**. When perchloric acid was added to a concentrated solution of **3** in acetic acid, a crystalline, blue salt was obtained. By mixing the blue, crystalline salt **3c** with cold, aqueous acetic acid, yellow hydrate **3b** was regenerated. As reported previously[2], on heating in toluene, the yellow hydrate changes to the red, enolic form. The salt **3c** contains, by analysis and titration, one equivalent of perchloric acid per mole of **3**; hence, the formation of the blue cation presumably involves addition of one proton.

Introduction of perchloric acid (approx. 75 mM) to **3** in acetic acid caused a shift of the main peak in the visible spectrum from λ_{max} 485 nm (37,000)* to λ_{max} 565 nm (78,500). Strong bathochromic shifts of this character were found for other compounds having conjugated phenylhydrazono–phenylazo structures, with the possible exception of compound **8**.

Presumably, the bathochromic shifts and the intense colors found for compounds **2** through **7** arise from conjugated, resonance-stabilized cations having structures of the type depicted.



In the light of recent studies by Eistert and co-workers[7], treatment of Merling's[11] azo compound **2** with strong base (potassium hydroxide in an aprotic solvent, namely, *N*,*N*-dimethylformamide or methyl sulfoxide) also produces a deep-blue solution; the amphoteric character of compound **2** and other formazan vinylogs was ascribed[7] to the formation of resonance-stabilized anions **2**↔**2a**. The absorption spectrum of anion **2**↔**2a** should resemble the resonance-stabilized cation spectra of 2-oxo-1,3-bis(phenylhydrazones), indicating some amphoteric character of compounds **2** to **7**.

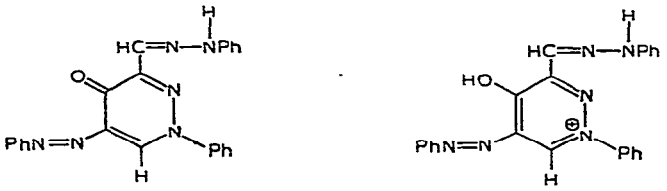


2 **2a**

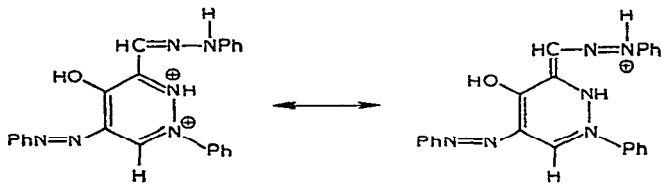
Surprisingly, compound **8** did not give a strong bathochromic shift under the conditions that gave a strong bathochromic shift with compounds **2** to **7**. On heating in acid solution, **8** gave a colored solution which may have involved a resonance-stabilized cation. The inertness of **8** presumably arises from restriction of resonance by steric hindrance in accord with the conjugated, bicyclic indane structure. However,

*Molecular extinction coefficients are given in parentheses.

restriction of conjugation by steric hindrance has also been noted[22]. Inspection of a model of **8** revealed that a structure consisting of three carbon atoms fused to a phenyl ring involves considerable angular strain, and that a resonance-stabilized structure of the type postulated for compounds **1** to **7** can be formed only by distortion of the normal bond-angles. This fact accounts for the unusual behavior of **8**, and demonstrates the usefulness of the particular spectral measurements for structural studies.



9 **9a**



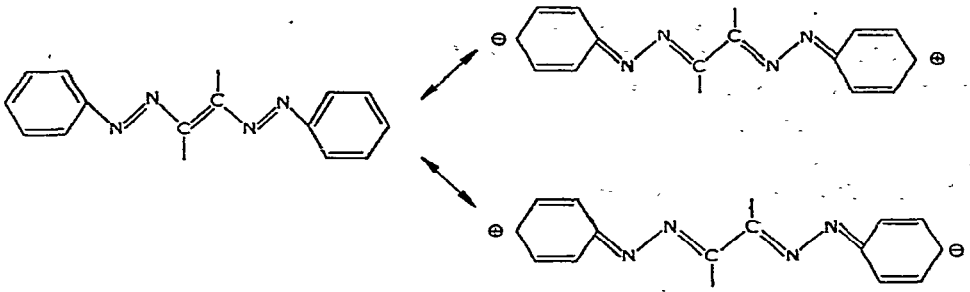
9b

The results for compound **9** (4-oxo-1-phenyl-5-phenylazo-3-pyridazinecarboxaldehyde[1]) also illustrate utilization of the color reaction in structural studies. On treatment with perchloric acid in acetic acid, compound **9** gave a purple color which changed to an olive-green. By protonation of **9**, a resonance-stabilized, monovalent cation **9a** and a divalent cation might be expected. The monovalent cation **9a** has a conjugated phenylhydrazono–phenylazo structure that, on further protonation, may yield a resonance-stabilized structure **9b**. Delocalization of the positive charges in the resonance forms of **9b** may account for the intense purple color found experimentally.

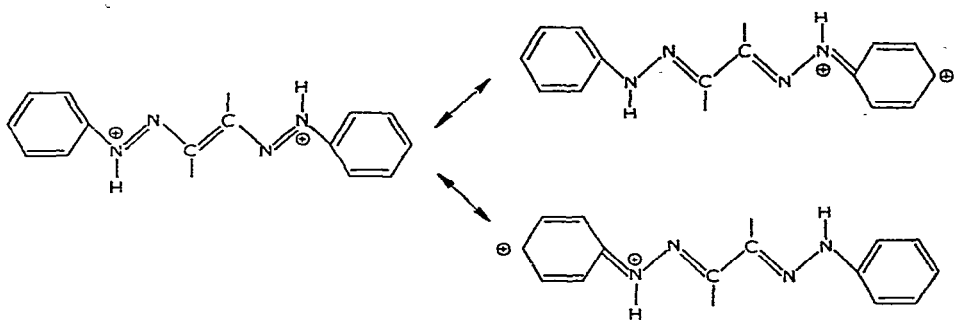
In some instances, the lack of a strong bathochromic shift in the spectrum of a compound on treatment with strong acid serves as an indication of structure. Thus, the lack of a strong bathochromic shift in the spectrum of the bis(phenylhydrazone) of tetrahydro-5-hydroxy-5,6-diphenylpyran-2,3,4-trione was our first evidence that this compound is not a 3-oxo-2,3-bis(phenylhydrazono) derivative[2,23].

In continuation of this study, we have examined the behavior of 1,2-bis-(phenylazo)ethylene (**12**) on protonation. This compound has an extended, conjugated, resonance structure both as the free base and as the divalent cation (**12a**). The π-electron system extends over 18 atoms, and many resonance forms may be involved.

The principal resonance form of base **12** is somewhat like those of the cation **12a**. In forming the cation, protonation of the nitrogen atom would be expected to cause displacement of the absorption bands to shorter wavelengths, because of the restrictive effect of the protons attached to the nitrogen atoms on the resonance



12



12a

structure of **12a** as compared with **12**. Protonation of **12** was, indeed, accompanied by a hypsochromic shift and slightly higher intensity (See Table I.) Presumably, protonation of either the *alpha-* or the *beta*-nitrogen could occur. The protons are shown attached to the *alpha*-nitrogen atoms in **12a** because, in this compound, there is a wider separation of charge in the principal resonance form than in the other isomer. In addition to the resonance-stabilized structure depicted, a radical structure is possible. In fact, e.s.r. monitoring of the protonation reaction of **12** (perchloric acid in acetic acid) at room temperature revealed the presence of a radical; the one-line spectrum (width 4 gauss, $g = 2.0032$) developed rather slowly. The low intensity of the e.s.r. spectrum, the lack of hyperfine structure, and the slow development suggest that resonance may arise from a side reaction.

EXPERIMENTAL

The previously known compounds listed in Table I were prepared by the methods described in the references cited and their properties were checked as described in our earlier paper[2]. Visible spectra were recorded with either a Beckman*

*Mention in this article of certain commercial instruments or chemical compounds does not constitute endorsement by the National Bureau of Standards.

DK-2 or a Cary 14 Spectrophotometer. The spectrum of each compound in acetic acid (mM to 10μM solution) was first recorded. It was then recorded in a perchloric acid mixture prepared by adding 0.1 ml of 70% aqueous perchloric acid to 5 ml of a solution of the compound in acetic acid. The spectra were recorded 2 to 3 min after addition of the perchloric acid. I.r. spectra were recorded with a Perkin–Elmer grating Model 257 spectrophotometer, and e.s.r. spectra with a Varian Model 4500 EPR Spectrometer, with 100-kHz field modulation and use of a Varian Model V-4548 aqueous-solution sample-cell.

2-Oxo-1,3-bis(phenylhydrazono)cyclohexane perchlorate (**3c**). — A solution of **3** (307 mg, 1 mmole) in warm acetic acid (5 ml) was cooled in an ice–water bath, and a solution of perchloric acid (0.5 ml of 70% aq. $HClO_4$) in 2 ml of acetic acid was added dropwise, with stirring. The dark reaction mixture was stirred for an additional 15 min, whereupon dark-blue crystals of **3c** (330 mg, 81%) formed. The crystals were separated, and washed successively with acetic acid (2 ml) and ether (3 ml). The analytical sample was recrystallized from a small volume of acetic acid by concentration *in vacuo*. The compound can also be recrystallized from acetic acid–ether–acetone containing a trace of perchloric acid; a sample dried for 2 h at 78°/0.1 torr had m.p. 136–137° (red melt).

Anal. Calc. for $C_{18}H_{18}N_4O \cdot HClO_4$: C, 53.14; H, 4.71; N, 13.77. Found: C, 53.30; H, 4.65; N, 13.70.

Compound **3** (30.6 mg, 0.1 mmole) in 15 ml of 90% aqueous acetic acid required 10.08 ml of 10 mM perchloric acid for neutralization; this indicates that the salt contains one equivalent of perchloric acid per mole of **3**.

Perchlorate **3c** showed ν_{max}^{KBr} 3240s (NH, OH), 1600s (phenyl ring), 1572w (N=N), 1555w (N–H, bending), 1508s (phenyl ring), 1490s (phenyl ring), 1460s (phenyl ring), 1421w (N=N), and 1130m and 1160m cm^{-1} (Ph–N)[25]. Fingerprint region: 1358s, 1335s, 1240s, 1180w, 1070s, 1010w, 940s, 920w, 880m, 868w, 840w, 760s, 740sh, and 700s cm^{-1}.

Treatment of **3** in glacial acetic acid with concentrated sulfuric acid gave a dark-blue, crystalline salt (shift from λ_{max}^{HOAc} 485 to 562 nm); treatment of **3** with trifluoroacetic acid gave a deep-green salt (shift from λ_{max}^{HOAc} 485 to 560 nm). Although the salts were not analyzed, it is presumed that they have a resonance-stabilized structure.

ACKNOWLEDGMENTS

The authors thank Professor Dr. Bernd Eistert, University of Saarland, for a copy of the Ph. D. Dissertation of G. Kilpper and for use of some information therefrom prior to its publication[26]; Norman Adams (NBS) for making the e.s.r. measurements and the referees for their helpful suggestions and comments.

REFERENCES

1 H. S. ISBELL AND A. J. FATIADI, *Carbohyd. Res.*, 2 (1966) 204.
2 A. J. FATIADI AND H. S. ISBELL, *Carbohyd. Res.*, 5 (1967) 302.

3 H. S. ISBELL AND A. J. FATIADI, *NBS Technical Note 274*, (1965) 65.
4 H. S. ISBELL AND A. J. FATIADI, *NBS Technical Note 405*, (1966) 69.
5 H. S. ISBELL AND A. J. FATIADI, *Abstracts Papers Amer. Chem. Soc. Meeting*, 150 (1965) 22D.
6 G. HENSEKE AND H. J. BINTE, *Ann.*, 612 (1958) 205.
7 G. KILPPER, Ph. D. Dissertation, University of Saarland, 1968.
8 G. E. LEWIS, *Tetrahedron*, 10 (1960) 129.
9 E. SAWICKI, *J. Org. Chem.*, 21 (1956) 605; 22 (1957) 365, 621, 1084.
10 (a) L. MESTER AND A. MAJOR, *J. Amer. Chem. Soc.*, 78 (1956) 1403; (b) L. MESTER, *Advan. Carbohyd. Chem.*, 13 (1958) 105.
11 G. MERLING, *Ann.*, 278 (1893) 1403.
12 B. PECHERER, L. M. JAMPOLSKEY, AND H. M. WUEST, *J. Amer. Chem. Soc.*, 70 (1948) 2587.
13 W. MAYER, R. BACHMANN, AND F. KRAUS, *Chem. Ber.*, 88 (1955) 316.
14 T. REICHSTEIN AND R. OPPENAUER, *Helv. Chim. Acta*, 16 (1933) 988; 17 (1934) 390.
15 H. VON PECHMANN AND K. JENISCH, *Ber.*, 24 (1891) 3255.
16 W. HAHN, R. BARTNIK, AND J. EPSZTAJN, *Rocz. Chem.*, 36 (1962) 1645.
17 S. RUHEMANN, *J. Chem. Soc.*, 97 (1910) 1438.
18 H. VON PECHMANN, *Ber.*, 21 (1888) 2751.
19 R. F. ROBBINS AND K. SCHOFIELD, *J. Chem. Soc.*, (1957) 3186; R. A. ABRAMOVITCH AND K. SCHOFIELD, *ibid.*, (1955) 2326.
20 L. HOUGH AND A. C. RICHARDSON, in S. COFFEY (Ed.), *Rodd's Chemistry of Carbon Compounds*, Vol. 1, Part F, 2nd edition, Elsevier, New York, 1967, p. 439.
21 F. E. SMITH, *Science*, 113 (1951) 753.
22 B. M. WEPSTER, in G. M. RAY (Ed.), *Steric Effects in Conjugated Systems*, Academic Press Inc., New York, 1958, Chapter 8.
23 H. DAHN AND H. HAUTH, *Helv. Chim. Acta*, 40 (1957) 2249, 2261.
24 A. J. FATIADI AND H. S. ISBELL, *Abstracts Papers Amer. Chem. Soc. Meeting*, 145 (1963) 1D.
25 A. J. FATIADI, *J. Res. Nat. Bur. Stand.*, 71A (1967) 277.
26 B. EISTERT, G. KILPPER, AND J. GÖRING, *Chem. Ber.*, 102 (1969) 1379.

Carbohydrate Research
Elsevier Publishing Company, Amsterdam
Printed in Belgium

AN IMPROVED SYNTHESIS OF *N*-ACETYLNEURAMINIC ACID

M. J. How*, M. D. A. Halford‡, M. Stacey,
Department of Chemistry, The University, Edgbaston, Birmingham 15 (Great Britain)
and E. Vickers (in part)
Koch-Light Laboratories, Colnbrook, Bucks (Great Britain)
(Received March 11th, 1969)

ABSTRACT

The overall yield and stereoselectivity of the aldol condensation reactions of 2-acetamido-2-deoxy-D-glucose and 2-acetamido-2-deoxy-D-mannose with oxalacetate at pH 10 are improved by the incorporation of borate ion in the reaction mixture. Borate ion inhibits the alkaline epimerisation of various 2-acylamino-2-deoxyaldoses.

INTRODUCTION

5-Acetamido-3,5-dideoxy-D-*glycero*-D-*galacto*-nonulosonic acid (*N*-acetylneuraminic acid, **1**) has been synthesised by reaction of 2-acetamido-2-deoxy-D-mannose (**2**) and pyruvate or oxalacetate at pH 10. Under these conditions, **2** partially epimerises to 2-acetamido-2-deoxy-D-glucose (**3**), and four diastereoisomeric *N*-acetylnonulosaminic acids (**1**, **4**–**6**) are obtained. Yields of **1** of 11.5% have been reported[1], but none of the other isomers has been isolated in the crystalline state.

The present paper describes a simple modification of this aldol reaction that results in improved yield and greater stereoselectivity.

EXPERIMENTAL

Spectrophotometric analyses. — *N*-Acetylnonulosaminic acids were determined by the method of Warren[2] using acid **1** (Koch–Light Labs., Ltd.) as standard. The amount of acid **1** in mixtures of *N*-acetylnonulosaminic acids was calculated from analyses of the mixture before and after treatment with *N*-acetylneuraminic acid aldolase (*N*-acetylneuraminate pyruvatelyase, E.C. 4.1.3.3.) prepared[3] from pigs' kidneys. 2-Acetamido-2-deoxyhexoses were determined by the method of Reissig *et al.*[4], and pyruvate was determined[5] by using lactic dehydrogenase (L-lactate: NAD oxidoreductase, E.C. 1.1.1.27., Koch–Light Labs., Ltd.).

Paper chromatograms (Whatman No. 52) were developed with solvent *A* (butyl alcohol–pyridine–water, 6:4:3), and components were detected with alkaline silver

*Present address: Unilever Research Laboratory, Colworth House, Sharnbrook, Bedford (Great Britain).
‡Present address: Laboratory for Carbohydrate Research, Massachusetts General Hospital, Boston, Massachusetts 02114 (U. S. A.).

nitrate, Ehrlich, or periodate–thiobarbituric acid reagents. R_{NANA} values of sugars refer to distances moved relative to acid **1**.

2-Amino-2-deoxyaldose derivatives. — 2-Acetamido-2-deoxy-D-mannose monohydrate, {m.p. 128°, $[\alpha]_D^{25}$ +10.2° (*c* 5.0, water); lit.[6], m.p. 128°, $[\alpha]_D^{25}$ +9.8°} was prepared from 2-acetamido-2-deoxy-D-glucose by epimerisation in dilute aqueous NaOH at pH 11. 2-Deoxy-2-propionamido-D-glucose (**7**), 2-deoxy-2-propionamido-D-mannose (**8**), 2-butyramido-2-deoxy-D-glucose (**9**), 2-deoxy-2-formamido-D-glucose (**10**), and 2-acetamido-2-deoxy-D-xylose (**11**) were kindly provided by Dr. R. G. Plevey.

Oxalacetic acid was prepared[7] from ethyl sodio-oxalacetate (B.D.H. Ltd.).

Investigation of the alkaline epimerisation of sugars **2** *and* **3**. — Solutions (final volume, 1 ml) of sugars **2** (25 mg) or **3** (25 mg) in (*a*) water and (*b*) 0.1M sodium tetraborate, each containing pentaerythritol (1 mg) and adjusted to pH 10 with 10N sodium hydroxide, were incubated for 18 h at room temperature. Aqueous solutions of sugars **2** and **3** without alkali were treated similarly. The solutions were eluted from columns of Dowex-50 x8 (H^+, 2 ml) with water (4 ml), and the eluates were concentrated under diminished pressure to 2 ml, and methanol (3 × 1 ml) was evaporated from the residue. The *O*-trimethylsilyl derivatives[8] of the dried products were analysed by g.l.c. with a Pye 104 temperature-programmed chromatograph and a 10% SE 30 column packing, maintained at 150° for 5 min after injection of the sample and then heated to 210° at 4°/min.

Polarimetry. — The changes in $[\alpha]_D$ of solutions (*c* 2.5) of various 2-acylamino-2-deoxyhexoses in water, aqueous sodium hydroxide (pH 10), and 0.1M sodium tetraborate (pH 10) were determined. Initial readings were obtained 2–4 min after the addition of solvent.

Reaction of 2-acylamino-2-deoxyaldoses with oxalacetate at pH 10 in the presence or absence of borate ion. — (*a*) *Reactions of sugars* **2** *and* **3**. In a typical experiment, a solution of sugar **2** or **3** (50 mg, 0.23 mmole) in water (1 ml) was added to a solution

or absence of borate ion. — (*a*) *Reactions of acids* **2** *and* **3**. In a typical experiment a solution of acid **2** or **3** (50 mg, 0.23 mmole) in water (1 ml) was added to a solution of oxalacetic acid (100 mg, 0.76 mmole) in water (1 ml) or 0.2M sodium tetraborate (1 ml) adjusted to pH 10 with 5N sodium hydroxide. Solutions were incubated for 18 h at 18°, and the pH was maintained at 10.0 during the first 3 h. After 18 h, the pH of the solution was adjusted to pH 6 with acetic acid, and the solution was diluted to 250 ml with water. Solutions containing sugar **2** or **3**, and oxalacetate, in alkali and in alkaline borate, were incubated, acidified, and diluted similarly. Aliquots from test and control solutions were analysed by the method of Warren (Table I). In a similar experiment, the product mixture after acidification with acetic acid was eluted from a column (10 × 1 cm) of Dowex-1 x8 resin (formate form) with water, until the eluate gave a negative colour reaction for 2-acetamido-2-deoxyhexose, followed by 0.3N formic acid. Fractions (10 ml) of the acid eluate were collected automatically, and aliquots were assayed by the method of Warren using 0.3N formic acid as blank. Fractions that gave positive reactions for *N*-acetylnonulosaminic acids were pooled, and concentrated under diminished pressure to one-half volume, and formic acid

was removed by repeated evaporation with water and finally by freeze-drying. An aliquot of a solution of the product in 0.05M potassium phosphate buffer (pH 7.2) was analysed for *N*-acetylnonulosaminic acids, and separate aliquots were incubated for 30 min at 37° with a solution containing sufficient *N*-acetylneuraminic acid aldolase, in the same buffer, to degrade completely all of the *N*-acetylnonulosaminic acids expressed as acid **1**. Solutions containing (*a*) acid **1** plus enzyme in buffer, (*b*) acid **1** in buffer, (*c*) enzyme in buffer, and (*d*) buffer alone were treated similarly. After incubation, all solutions were heated for 2 min at 100° and centrifuged, prior to analysis of an aliquot of the supernatant for content of *N*-acetylnonulosaminic acids. The results are summarised in Table II. Aliquots of the solutions before and after incubation with *N*-acetylneuraminic acid aldolase were also analysed for pyruvate.

(*b*) *Reactions of sugars* **7–11**. 2-Acylamino-2-deoxyaldoses (**7–11**) were treated with oxalacetic acid, in the molar proportions described above, in dilute sodium hydroxide (pH 10) or 0.1M sodium tetraborate (pH 10). After 18 h, the solutions were

TABLE I

REACTIONS OF OXALACETATE AND PYRUVATE WITH 2-ACYLAMINO-2-DEOXYALDOSES, IN THE PRESENCE AND ABSENCE OF 0.1M SODIUM TETRABORATE, AT pH 10

Sugar	*Nucleophile*	*Warren-positive material*[a] (%)	
		NaOH, pH 10	*0.1M Sodium tetraborate, pH 10*
2	Oxalacetate	6	15
3	Oxalacetate	3	20
7	Oxalacetate	0.5	13
8	Oxalacetate	8	20
9	Oxalacetate	2.5	15
10	Oxalacetate	1	10
11	Oxalacetate	4	25
2	Pyruvate	0.5	3.6
3	Pyruvate	0.5	4.2

[a]Expressed as *N*-acetylneuraminic acid.

TABLE II

PERCENTAGE YIELDS OF *N*-ACETYLNONULOSAMINIC ACIDS AND *N*-ACETYLNEURAMINIC ACID FROM REACTIONS OF SUGARS **2** AND **3** WITH OXALACETATE IN THE PRESENCE AND ABSENCE OF 0.1M SODIUM TETRABORATE

Sugar	N-*acetylnonulosaminic acids*[a] *in* (A) (%)		N-*acetylneuraminic acid*[b] *in* (A) (%)	
	NaOH, pH 10	*0.1M Sodium tetraborate, pH 10*	*NaOH, pH 10*	*0.1M Sodium tetraborate, pH 10*
2	6	15	34	87
3	1	22	74	15

[a]By Warren assay. [b]By Warren assay after incubation with *N*-acetylneuraminic acid aldolase.

adjusted to pH 6 and eluted from Dowex-1 x8 (formate form) as described, and the formic acid eluate was analysed by the method of Warren. The results are summarised in Table I.

Reactions of sugars **2** *and* **3** *with pyruvate at pH 10 in the presence or absence of borate ion.* — Solutions of sugars **2** and **3** (25 mg, 0.11 mmole) in water were separately mixed with solutions of sodium pyruvate (B.D.H. Ltd., 45 mg, 0.41 mmole) in dilute sodium hydroxide (1 ml) or 0.2M sodium tetraborate solution (1 ml) at pH 10. After incubation for 18 h at room temperature with addition of alkali to maintain pH 10, the solutions were adjusted to pH 6 with acetic acid, and diluted to 25.0 ml with water, and aliquots were analysed for *N*-acetylnonulosaminic acids (Table I).

Large-scale preparation of acid **1** *and isolation of an isomer.* — A solution of oxalacetic acid (390 g) in water (2600 ml) was adjusted to pH 10.0 by addition of 10N sodium hydroxide, with cooling to maintain the temperature at 20°. Sodium tetraborate (230 g) and the monohydrate of sugar **2** (650 g) were added, and the pH was adjusted to 10 and maintained at this value by autotitration. Additional oxalacetic acid (95 g) was added after 3 and 4.5 h, and the pH was adjusted to 10.0. After 46 h, the reaction mixture was neutralised (pH 6) with glacial acetic acid, and the solution was eluted from Zeokarb 225 (H^+ form, 45 × 17 cm) and De-Acidite FF (formate form, 45 × 15 cm) resins. The latter resin was washed with water (200 l) prior to elution with 0.3N formic acid (250 l). Fractions that gave a positive reaction for *N*-acetylnonulosaminic acids were pooled and concentrated under diminished pressure at 25° with frequent addition of water. The resulting syrup (490 g) was dissolved in solvent *A* by addition of extra pyridine and eluted from a cellulose column (52 × 45 cm, 33 kg of cellulose) with solvent *A*. Paper-chromatographic analysis of the eluate showed four components, the major of which had R_{NANA} 1.0. Fractions that contained mainly acid **1**, as shown by paper chromatography, were pooled and concentrated under diminished pressure with frequent addition of water. Crystallisation of the residue from 80% acetic acid gave acid **1** (181 g, 21.6%), m.p. 183° (dec.), $[\alpha]_D^{25}$ −34° (*c* 1.0, water); lit.[9], m.p. 183° (dec.), $[\alpha]_D$ −32° (Found: C, 42.73; H, 5.90; N, 4.59; $C_{11}H_{19}NO_9$ calc.: C, 42.73; H, 6.15; N, 4.53%).

Isolation of an isomer of acid **1.** — Approximately one-tenth of the acetic acid mother liquors from the large-scale synthesis of acid **1** were concentrated under diminished pressure at 25° with frequent addition of water to remove acetic acid. Paper-chromatographic analysis of the resultant solution, which contained *ca.* 4.5 g of *N*-acetylnonulosaminic acids (Warren assay), showed two major components, R_{NANA} 1.0 and 1.3 in solvent *A*, and three minor components, R_{NANA} 0.2, 0.7, and 1.6. Only the major components reacted with all three spray reagents. A chromatographically homogeneous product, R_{NANA} 1.3, was isolated as a syrup by chromatography of an aliquot of the solution on a column of cellulose (Whatman Chromedia CF 11, 54 × 4 cm) previously washed with water, 0.1N hydrochloric acid, and water, and equilibrated with solvent *A*.

RESULTS AND DISCUSSION

The Warren assay for N-*acetylnonulosaminic acids.* — Periodate oxidation of *N*-acylnonulosaminic acids and *N*-acyloctulosaminic acids in 3M phosphoric acid, and of 2-deoxy-2-ketoaldonic acids having more than 5 carbon atoms, *e.g.*, isosaccharinic acid [3-deoxy-2-*C*-(hydroxymethyl)pentonic acid], yields β-formylpyruvic acid which reacts with 2-thiobarbituric acid to give a chromophore having λ_{max} 549 nm. Malonaldehyde and formic acid, which may be produced on periodate oxidation of certain carbohydrates, also give coloured products having λ_{max} 532 and 450 nm, respectively, with 2-thiobarbituric acid. Solutions of acid **1** in water, 0.1M sodium tetraborate, and 0.2M boric acid gave identical molar extinction coefficients and absorption spectra (λ_{max} 549 nm). Solutions of sugars **2** and **3**, after incubation for 18 h in aqueous sodium hydroxide (pH 10) or in 0.1M sodium tetraborate (pH 10), gave a chromophore having λ_{max} 450 nm in the Warren assay, attributable to the production of formic acid on periodate oxidation, but no chromophores due to β-formylpyruvic acid or malonaldehyde, thus indicating that alkaline rearrangement of sugars **2** and **3** to isosaccharinic acid or metasaccharinic acid (3-deoxyhexonic acid) had not occurred. Analyses of solutions of sodium oxalacetate, after incubation at pH 10 for 18 h in the presence and absence of 0.1M sodium tetraborate, showed weak absorption (λ_{max} 450 nm).

Effect of borate ion on alkaline epimerisation of 2-acylamino-2-deoxyaldoses. — The specific optical rotations of solutions of various 2-acylamino-2-deoxyhexoses in 0.1M borate (pH 10) were constant with time, whereas they changed rapidly in aqueous sodium hydroxide (pH 10) and more slowly in water (pH 5.5). The *O*-(trimethylsilyl) (TMS) derivatives of the α- and β-D anomers of sugars **2** and **3** (presumably in the pyranoid ring form) were separated by g.l.c. Analysis of the TMS derivative of the crystalline α-D anomer of sugar **3** showed one component, whereas analysis of mutarotated **3** showed two components, the major of which had the same retention time as the TMS derivative of the α-D anomer of **3**. G.l.c. analysis of the TMS derivative of the crystalline β-D anomer[10] of sugar **2** showed two components, the major of which was assumed to be the TMS derivative of the β-D anomer*. Analysis of mutarotated sugar **2** showed that the α-D anomer was preponderant. Treatment of sugars **2** or **3** with aqueous sodium hydroxide (pH 10) gave a mixture of products shown by g.l.c. to contain the α-D anomer of **2** and the α- and β-D anomers of **3**. Analysis of a solution of sugar **2** in 0.1M sodium tetraborate (pH 10), after 18 h at room temperature, showed only one component, indistinguishable by g.l.c. from the α-D anomer of **2**. Analysis of a solution of sugar **3** treated similarly showed the presence of the α- and β-D anomers of **3** as major components, with a small proportion of the α-D anomer of **2**.

These results indicate that, in the presence of tetraborate ion, the alkaline epimerisation of 2-acylamino-2-deoxyaldoses is inhibited, or, in some cases, prevented

*It is assumed that the second component arises by mutarotation of **2** in pyridine solution.

completely, and suggest that, in alkaline borate solutions, sugar **2** exists mainly as the α-D anomer. Previous workers[11] have studied the changes in the equilibrium specific rotation caused by the addition of either sodium or potassium tetraborate to solutions of such simple sugars as D-glucose, and concluded that D-glucose, for example, forms three borate complexes, according to the concentration and the tetraborate–D-glucose ratio. The effect of tetraborate on the mutarotation of such sugars was not reported, and the studies were not extended to such sugars as **2** and **3**.

Effect of borate ion on aldol reaction of oxalacetate or pyruvate with 2-acylamino-2-deoxyaldoses. — Initial experiments showed that the overall yields of *N*-acylnonulosaminic acids were increased in the presence of borate ion, as was the yield of *N*-acetyloctulosaminic acids from the reaction of sugar **11** with oxalacetate under similar conditions (see Table I). Improved yields of *N*-acetylnonulosaminic acids were obtained from the reactions of sugars **2** and **3** with pyruvate in the presence of borate at pH 10, but the overall yields were less than those obtained in similar reactions using oxalacetate rather than pyruvate.

The observed effect of borate in suppressing or preventing the alkaline epimerisation of sugars **2** and **3**, and other 2-acylamino-2-deoxy aldoses, suggested that the reaction between such sugars and oxalacetate or pyruvate at pH 10 would be more stereoselective in the presence of borate ion.

No quick method was available for the analysis of the relative amounts of each of the diastereoisomeric *N*-acyl-nonulosaminic or -octulosaminic acids obtained by condensation of sugars **7**–**11** with oxalacetate as described. All of the isomeric *N*-acetylnonulosaminic acids arising from the reaction of sugars **2** or **3** with oxalacetic acid would be expected to react in the Warren assay. Only isomer **1**, however, is degraded by *N*-acetylneuraminic acid aldolase and, assuming that none of the other isomers inhibited the action of the enzyme, it was possible to analyse reaction mixtures of isomeric *N*-acetylnonulosaminic acids for content of acid **1** by using the Warren reaction before and after incubation with *N*-acetylneuraminic acid aldolase. In all cases, treatment of mixtures of *N*-acetylnonulosaminic acids with *N*-acetylneuraminic acid aldolase resulted in the production of pyruvic acid equivalent to the amount of acid **1** degraded. In the reaction of sugar **2** with oxalacetate at pH 10, the overall yield of *N*-acetylnonulosaminic acids obtained in the presence of borate ion was 15%, of which 87% was acid **1**, whereas, in the absence of borate ion, the overall yield was 6%, of which 34% was acid **1**. In similar reactions using sugar **3**, the overall yields of *N*-acetylnonulosaminic acids obtained in the presence and absence of borate ion were 22 and 1%, respectively, of which 15 and 74%, respectively, were acid **1** (Table II). These results show that the stereoselectivity of the reactions in the presence of borate ion was greatly increased, but that the epimerisation between sugars **2** and **3** was not completely supressed.

Large-scale synthesis of acid **1** *and isolation of an isomeric* N-*acetylnonulosaminic acid.* — Crystalline acid **1** was isolated in 21.6% yield from the reaction of sugar **2** with oxalacetate at pH 10 in the presence of 0.1M sodium tetraborate. The yield obtained routinely[12] under similar conditions in the absence of borate is

approximately 10%. The increased yield, with respect to that obtained during the small-scale reactions, probably reflects differences in the reaction conditions. Chromatographic analysis of the mother liquors from the large-scale synthesis revealed components, having paper-chromatographic mobilities (relative to that of acid **1**) of 1.0 and 1.3, which reacted with alkaline silver nitrate, Elson–Morgan reagents, and periodic acid–thiobarbituric acid reagents. The latter component, isolated as a glass by chromatography on cellulose, had the same extinction coefficient as acid **1** in the Warren assay. Its infrared spectrum (KBr disc) was very similar to that of a sample of freeze-dried acid **1**, but not so well-defined. The isomer was not degraded by *N*-acetylneuraminic acid aldolase and did not inhibit the action of the enzyme. The stereochemistry of this isomer at C-4 and C-5 was not determined.

Both the overall yield and the stereoselectivity of the aldol condensation reactions of sugars **2** and **3** with oxalacetate at pH 10 have been improved by the incorporation of borate ion in the reaction mixture. In addition, the yield of products from the reaction of a series of other 2-acylamino-2-deoxyaldoses was similarly increased. The influence of borate ion in such reactions is not easily defined, due to its possible involvement in complex formation with starting material, products, or reaction intermediates. One effect of borate is to diminish markedly the alkaline epimerisation of sugars **2** and **3**. Böeseken[13] inferred that, for cyclic carbohydrates, only vicinal *cis*-hydroxyl groups could complex with borate ion in aqueous boric acid, but the observation that 2,3-di-*O*-methyl-D-glucose, the furanoid and pyranoid forms of which have no vicinal *cis*-hydroxyl groups, can form a complex with borate in alkali suggested that other types of interaction with borate ion can occur[14]. From a systematic study of the electrophoretic mobility of derivatives of simple sugars in alkaline borate buffer, Bouveng and Lindberg[15] suggested that the *aldehydo* form of certain sugars is the principal one involved in complex formation, with the hydroxyl groups at C-2 and C-4 sterically most favourable for complex formation. For some sugars, however, *e.g.*, methyl α-D-glucofuranoside and 1,2-*O*-isopropylidene-α-D-glucofuranose, it has been proposed[13] that complex formation involves the hydroxyl groups at C-3, C-5, and C-6, and that the vicinal *cis*-hydroxyl groups at C-1 and C-2 of 4-*O*-methyl-α-D-glucopyranose were most probably involved in complex formation for that sugar.

Recent studies in these laboratories have indicated the value of ^{1}H and ^{11}B n.m.r. studies in the elucidation of the structure of complexes between simple sugars and borate ion at a range of pH values, and it is hoped that such studies will give information on the mode of action of borate ion in the syntheses described in this paper.

REFERENCES

1 P. M. CARROLL AND J. W. CORNFORTH, *Biochim. Biophys. Acta*, 39 (1960) 161.
2 L. WARREN, *J. Biol. Chem.*, 234 (1959) 1971.
3 F. BRUNETTI, G. W. JOURDIAN, AND S. ROSEMAN, *J. Biol. Chem.*, 237 (1962) 2447.
4 J. L. REISSIG, J. L. STROMINGER, AND L. F. LELOIR, *J. Biol. Chem.*, 217 (1955) 959.
5 S. A. BARKER, A. R. LAW, P. J. SOMERS, AND M. STACEY, *Carbohyd. Res.*, 3 (1967) 435.

6 C. T. SPIVAK AND S. ROSEMAN, *J. Amer. Chem. Soc.*, 81 (1959) 2403.
7 C. HEIDELBERGER AND R. B. HURLBERT, *J. Amer. Chem. Soc.*, 72 (1950) 4704.
8 C. C. SWEELEY, R. BENTLEY, M. MAKITA, AND W. W. WELLS, *J. Amer. Chem. Soc.*, 85 (1963) 2497.
9 R. KUHN AND G. BASCHANG, *Ann.*, 659 (1962) 156.
10 D. HORTON, J. S. JEWELL, AND K. D. PHILIPS, *J. Org. Chem.*, 31 (1966) 4022.
11 H. S. ISBELL, J. F. BREWSTER, N. B. HOLT, AND H. L. FRUSH, *J. Res. Natl. Bur. Std.*, 40 (1948) 129.
12 E. VICKERS, unpublished results.
13 J. BÖESEKEN, *Advan. Carbohyd. Chem.*, 4 (1949) 189.
14 For a review, see A. B. FOSTER, *Advan. Carbohyd. Chem.*, 12 (1957) 81.
15 H. BOUVENG AND B. LINDBERG, *Acta Chem. Scand.*, 10 (1956) 1283.

Carbohydrate Research
Elsevier Publishing Company, Amsterdam
Printed in Belgium

RING-OPENING REACTIONS OF *trans*-CARBONATES AND THIONOCARBONATES

W. M. DOANE, B. S. SHASHA, E. I. STOUT, C. R. RUSSELL, AND C. E. RIST
Northern Regional Research Laboratory, Peoria, Illinois 61604 (U. S. A.)*
(Received March 20th, 1969)

ABSTRACT

The reaction of methyl 4,6-*O*-benzylidene-α-D-glucopyranoside 2,3-carbonate (**1**) and the corresponding 2,3-thionocarbonate (**2**) with various nucleophiles was investigated. Under proper conditions, **1** and **2** reacted with methanol, benzyl alcohol, α-toluenethiol, ammonia, piperidine, and glycine to give the corresponding 2-*O*- and 3-*O*-carbonyl and thiocarbonyl adducts, which were obtained in crystalline form. In each reaction product the 2-isomer was preponderant.

INTRODUCTION

The vicinal diol group at C-2–C-3 of D-glucopyranose rings can be converted into a five-membered cyclic carbonate[1] or thionocarbonate[2]. Although formation of the carbonyl bridge between the *trans*-diequatorial hydroxyl groups introduces considerable torsional strain into the pyranose ring, the conversion proceeds readily, and excellent yields of the crystalline, cyclic derivatives result. With slight modification of the procedure developed for the formation of such groups in simple glucoside derivatives, the *trans*-fused cyclic group can also be introduced into polysaccharides[2,3]. We considered that these cyclic derivatives, because of the nature of the carbonate group and because of the strain introduced in their formation, should provide highly reactive centers that would react with a variety of alcohols, thiols, and amines.

A preliminary report[4] noted the reaction of methyl 4,6-*O*-benzylidene-α-D-glucopyranoside 2,3-carbonate (**1**) with methanol, α-toluenethiol, and piperidine. Now the ring opening reaction of **1** has been extended to include benzyl alcohol, ammonia, and glycine. In addition, the reaction of the various nucleophiles with methyl 4,6-*O*-benzylidene-α-D-glucopyranoside 2,3-thionocarbonate (**2**) has been investigated. In each reaction the products have been characterized and the ratio of 2-*O*- to 3-*O*-substituted isomers formed has been determined.

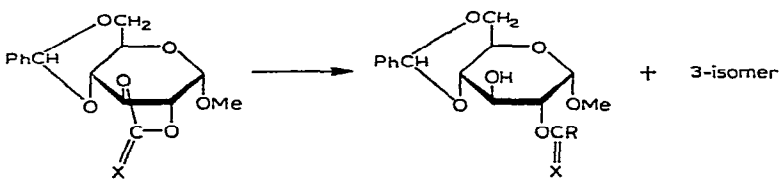
DISCUSSION

Previously[1], we employed ethyl chloroformate and triethylamine to convert methyl 4,6-*O*-benzylidene-α-D-glucopyranoside into **1** in 89% yield. We now report that a 98% yield is achieved by replacing ethyl chloroformate with phosgene.

*This is a laboratory of the Northern Utilization Research and Development Division, Agricultural Research Service, U. S. Department of Agriculture.

Treatment of **1** and **2** with alcohols, thiols, and amines gave the corresponding carbonyl and thiocarbonyl adducts. The position of substitution (C-2 or C-3) was assigned from n.m.r. data. The strong deshielding effect that the carbonyl and thiocarbonyl groups have on the hydrogen atom, attached to the same ring-carbon atom that bears the substituent, results in low-field signals, which are readily identified. Substitution at C-2 gave 1-proton quartets centered at τ 4.6–4.8 for various carbonyl derivatives and at τ 3.9–4.2 for various thiocarbonyl derivatives. Substitution at C-3 gave 1-proton, apparent triplets centered at τ 4.1–4.3 and τ 3.1–3.5 for the carbonyl and thiocarbonyl products, respectively. Complete spectral analysis for each product, as well as for **1** and **2**, and for the epimeric *cis*-fused derivatives, will be reported elsewhere.

Alcohols. — Ring opening of the carbonate group of **1** by alcohols occurs readily in the presence of triethylamine. Conversion of **1** into a mixture of the corresponding 2- and 3-*O*-methoxycarbonyl derivatives was quantitative. Densitometric



1 X=O
2 X=S

R=OMe, OCH_2Ph, SCH_2Ph
NH_2, NC_5H_{10}, $NHCH_2CO_2Me$

measurements of the mixture indicated 2.2 parts of the 2-isomer to 1 part of the 3-isomer. When parallel experiments were conducted with a *cis*-fused carbonate, methyl 4,6-*O*-benzylidene-α-D-mannopyranoside 2,3-carbonate, the rate of ring opening (based on i.r. spectroscopy) was about one-half that observed with **1**. This result might be expected since the *cis*-fused derivative is less strained. With an exocyclic carbonate group, as in 1,2-*O*-isopropylidene-α-D-glucofuranose 5,6-carbonate, no ring opening was observed.

Methyl 4,6-*O*-benzylidene-α-D-glucopyranoside 2,3-thionocarbonate (**2**) underwent reaction with methanol similar to that observed with **1**. A 96% conversion of **2** into a mixture of the 2- and 3-*O*-methoxythiocarbonyl isomers was obtained in 1.5 h at 25°. Analysis of the mixture revealed 2.6 parts of the 2-isomer to 1 part of the 3-isomer. When **2** was refluxed with methanol in the absence of triethylamine, ring opening was complete within 2 h and afforded the 2- and 3-isomers in a ratio of 0.8 to 1.

Ring opening of the carbonate group of **1** with benzyl alcohol in the presence of triethylamine gave a quantitative yield of the 2- and 3-*O*-benzyloxycarbonyl derivatives. The mixture obtained contained the 2- and 3-isomers in a 1.8 to 1 ratio.

α-Toluenethiol. — Compounds **1** and **2** reacted with α-toluenethiol and triethylamine at ambient temperature to afford the corresponding (benzylthio)carbonyl and (benzylthio)thiocarbonyl derivatives in 86 and 95% yields, respectively. The

ratio of isomers formed from the thiol was essentially the same as that from benzyl alcohol. Identities of the four components were established by their n.m.r. spectra and those of the 2-*O*-(benzylthiol)carbonyl and 2- and 3-*O*-(benzylthiol)thiocarbonyl derivatives, by comparison with authentic samples.

Ammonia and amines. — Reaction of **1** and **2** with ammonia and amines proceeds readily to afford the corresponding carbamoyl and thiocarbamoyl derivatives. Treatment with liquid ammonia gave immediate and quantitative conversion into the 2- and 3-carbamates and 2- and 3-thiocarbamates. In each mixture, the ratio of the 2- to 3-isomer was about 1.5 to 1. The carbamates showed characteristic absorption for the carbamoyl group near 1675–1700 cm^{-1}, whereas the thiocarbonyl derivatives exhibited strong absorption near 1610–1620 cm^{-1} for the thiocarbamate group. The latter derivatives also exhibited characteristic absorption in the u.v. at 244 nm (ε 11,500).

Dissolution of **1** and **2** in piperidine at 25° also gave immediate and quantitative conversion to the corresponding piperidino derivatives. The mixture from **1** favored the 2-isomer by 1.8 to 1, whereas with **2** the ratio was 3.6 to 1. The i.r. spectra of the 2- and 3-*O*-piperidinocarbonyl derivatives showed strong carbonyl absorption near 1690 cm^{-1}, but the 2- and 3-*O*-piperidinothiocarbonyl products had characteristic absorption near 1475–1510 cm^{-1}. The u.v. spectra of the thiocarbonyl derivatives showed strong absorption at 253 nm (ε 16,500).

The reaction of **1** and **2** with glycine was accomplished in a water–acetone system in the presence of triethylamine. T.l.c. indicated that reaction was complete within 1 min at ambient temperature. When the triethylamine was omitted, no reaction was observed. The yield of product from **2** and its sulfur content suggested that the derivatives were present as the triethylammonium salts. The i.r. spectrum also showed that the carboxyl group was present in salt form (1620 cm^{-1}). Difficulty encountered in attempts to resolve the mixture into the 2- and 3-isomers was attributed to the salt form and was overcome when the products were converted into the methyl esters. Conversion into the methyl esters resulted in carbonyl absorption near 1700–1750 cm^{-1}. The glycine methyl ester derivatives of **1** and **2** were resolved by preparative t.l.c. and yielded crystalline isomers. In each mixture, the 2-isomer was preponderant.

EXPERIMENTAL

Melting points were determined in a Thomas–Kofler* melting point apparatus and are uncorrected. Optical rotations were measured with a Rudolph polarimeter with 1-dm tubes. I.r. and u.v. spectra were measured with Perkin–Elmer Models 137 and 202 spectrophotometers, respectively. N.m.r. measurements were made in pyridine-d_5 by means of a Varian HA-100 n.m.r. spectrometer with tetramethyl-

*The mention of firm names or trade products does not imply that they are endorsed or recommended by the Department of Agriculture over other firms or similar products not mentioned.

silane (τ 10.0) as the internal reference standard. Silica Gel G was the adsorbent for t.l.c. Densitometry was performed on charred t.l.c. plates by means of a Photovolt Model 530 densitometer.

Methyl 4,6-O-benzylidene-α-D-glucopyranoside 2,3-carbonate (**1**). — To a solution of methyl 4,6-*O*-benzylidene-α-D-glucopyranoside (30 g) in *p*-dioxane (200 ml) was added benzene (400 ml) and triethylamine (64 ml). The solution was stirred while phosgene (20 g) in benzene (150 ml) was added dropwise during 1.5 h. The contents of the flask were filtered, and the filter cake was washed with three 100-ml portions of benzene. The benzene extracts were combined with the original filtrate, and the whole was extracted several times with water. The organic layer deposited crystals upon refrigeration; yield, 32 g (98%). Comparison with authentic **1** confirmed the identity of the product.

Reaction of **1** *with methanol.* — To a solution of **1** (0.30 g) in chloroform (1 ml) was added methanol (0.5 ml) and triethylamine (0.2 ml). The solution was kept at room temperature, and samples were withdrawn periodically to obtain an i.r. spectrum. Initially, there was carbonyl absorption only for cyclic carbonate (near 1830 cm^{-1}), and after 40 min, only for acyclic carbonate (near 1760 cm^{-1}). T.l.c. (3:1 chloroform–acetone) confirmed the absence of **1** and revealed two components having R_F values less than that of **1**. Evaporation of the solution gave a solid (0.33 g, 100%) which was separated by preparative t.l.c. into the known[5] 2- and 3-*O*-methoxycarbonyl derivatives.

During previous studies[4], we observed that methoxycarbonyl derivatives were formed when solutions of **1** in methanol were heated under reflux. In attempts to repeat our work, we noted variable results, which appeared to reflect the purity of the **1** preparation. With preparations of **1** recrystallized two or three times, no ring opening occurred during several h at reflux in methanol. When trace amounts of triethylamine were added to the refluxing solutions, then ring opening proceeded rapidly. Since triethylamine was employed in the synthesis of **1**, presumably in those preparations where ring opening occurred without addition of the amine, traces of the amine may have been present as a residue from preparation of **1**.

Reaction of methyl 4,6-O-benzylidene-α-D-mannopyranoside 2,3-carbonate with methanol. — The title compound[6] (0.150 g) in chloroform (0.5 ml) was treated with methanol (0.25 ml) and triethylamine (0.1 ml) at ambient temperature. Samples were withdrawn periodically and an i.r. spectrum obtained. Initially, there was carbonyl absorption only for cyclic carbonate (near 1820 cm^{-1}) and after 50 min the intensities for cyclic and acyclic were nearly equal. A physical mixture containing equimolar amounts of the cyclic and acyclic carbonates exhibited carbonyl stretching-frequencies of equal intensities.

Treatment of 1,2-O-isopropylidene-α-D-glucofuranose 5,6-carbonate with methanol. — A solution of the title compound[7] (50 mg) in chloroform (1 ml) and *p*-dioxane (1 ml) was treated with methanol (1 ml) and triethylamine (0.4 ml) at 60°. After treatment for 30 min the compound was recovered unchanged.

Reaction of **2** *with methanol.* — (*a*) A solution of **2** (0.50 g) in chloroform (5 ml)

was treated with triethylamine (1 ml) and methanol (1 ml). T.l.c. revealed that reaction was complete in 1.5 h when the solution was kept at 25°. Evaporation under diminished pressure afforded a syrup (0.53 g, 96%), which was resolved into two components by preparative t.l.c. (chloroform). The component of higher R_F (2-isomer) had m.p. 167–168° (benzene–hexane), $[\alpha]_D^{24}$ +75° (*c* 0.8, chloroform); $\lambda_{max}^{ethanol}$ 229 nm (ε 8,870); n.m.r. data: τ 4.22 1-proton quartet.

Anal. Calc. for $C_{16}H_{20}O_7S$: C, 54.3; H, 5.6; S, 9.0. Found: C, 54.0; H, 5.6; S, 8.8.

The 3-*O*-methoxythiocarbonyl isomer had m.p. 125–126° (benzene–hexane), $[\alpha]_D^{24}$ +129° (*c* 0.8, chloroform); $\lambda_{max}^{ethanol}$ 230 nm (ε 8600); n.m.r. data: τ 3.55 1-proton, apparent triplet).

Found: C, 54.0; H, 5.6; S, 8.7.

(*b*) A solution of **2** (0.50 g) in methanol (20 ml) was heated under reflux, and the progress of the reaction was monitored by t.l.c. (chloroform). Conversion into the 2- and 3-isomers was complete in 2 h. During this treatment there was some decomposition of **2** to the free diol.

Reaction of **1** *with benzyl alcohol.* — After compound **1** (1.0 g) was dissolved in a mixture of triethylamine (5 ml) and benzyl alcohol (5 ml) in a closed vessel, it was placed in an oven at 75° and heated for 25 min. Almost all of the amine was removed under diminished pressure, and then the solution was added to water (250 ml) to remove excess benzyl alcohol. The semisolid mass that separated was dissolved in acetone (25 ml) and again added to water (200 ml). The precipitate was dissolved in chloroform (25 ml), and the solution was dried (sodium sulfate). Evaporation of the chloroform gave a syrup (1.36 g, 100%), which showed two components on t.l.c. (19:1 chloroform–acetone). An i.r. spectrum had carbonyl absorption only for acyclic carbonate. Refrigeration of a solution of the syrup in ether (50 ml) gave crystals of the compound of lower R_F (3-isomer), m.p. 154–155° (chloroform–hexane), $[\alpha]_D^{24}$ +68° (*c* 1, chloroform); n.m.r. data: τ 4.22 (1-proton, apparent triplet).

Anal. Calc. for $C_{22}H_{24}O_8$: C, 63.5; H, 5.8. Found: C, 63.2; H, 5.6.

The compound having the higher R_F value (2-isomer) was isolated by preparative t.l.c. and gave m.p. 105–107° (chloroform–hexane), $[\alpha]_D^{24}$ +90° (*c* 1, chloroform); n.m.r. data: τ 4.8 (1-proton quartet).

Found: C, 63.5; H, 5.9.

Reaction of **2** *with benzyl alcohol.* — When **2** was treated with benzyl alcohol under conditions similar to those used for **1**, considerable decomposition of **2** occurred to give the free diol. The 2- and 3-*O*-benzyloxythiocarbonyl derivatives were obtained quantitatively when a solution of **2** (0.50 g) in chloroform (1 ml) was treated with triethylamine (0.5 ml) and benzyl alcohol (0.5 ml) for 50 min at 25°. The two products were separated by preparative t.l.c. (2:1 ether–hexane). The 3-isomer (lower R_F) gave m.p. 148–150° (chloroform–hexane), $[\alpha]_D^{24}$ +55° (*c* 0.9, chloroform); $\lambda_{max}^{ethanol}$ 235 nm (ε 8,550); n.m.r. data: τ 3.48 (1-proton triplet).

Anal. Calc. for $C_{22}H_{24}O_7S$: C, 61.1; H, 5.6; S, 7.4. Found: C, 61.1; H, 5.6; S, 7.3.

The 2-isomer (higher R_F) had m.p. 127–130° (chloroform–hexane), $[\alpha]_D^{24}$ +68° (*c* 1.4, chloroform); $\lambda_{max}^{ethanol}$ 235 nm (ε 8,600); n.m.r. data: τ 4.2 (1-proton quartet).

Found: C, 61.0; H, 5.4; S, 7.3.

Reaction of **1** *with α-toluenethiol.* — α-Toluenethiol (0.5 ml) and triethylamine (0.3 ml) were added to a solution of **1** (700 mg) in chloroform (1.5 ml). After the mixture had been kept 0.5 h, an i.r. spectrum of a portion of the solution showed no absorption for cyclic carbonate. The amine and chloroform were removed under diminished pressure, and the residue was washed with water and with hexane (three 1-ml portions). The residue was dissolved in chloroform and dried (sodium sulfate); the chloroform was then evaporated to yield a syrup (840 mg, 86%). An ether solution of the residue to which hexane had been added produced crystals of the higher R_F 2-isomer. When crystallized either from hexane, ether–hexane, or ethyl acetate–hexane, the needles obtained had m.p. 109–110°. When the melt was allowed to solidify and was then reheated, a m.p. of 122–123° was recorded. Elemental analysis of the original needles suggested that the compound had occluded 0.5 mole per mole of hexane. The product of m.p. 122–123° gave $[\alpha]_D^{24}$ +89.5° (*c* 1.5, chloroform); n.m.r. data: τ 4.8 (1-proton quartet).

Anal. Calc. for $C_{22}H_{24}O_7S$: C, 61.1; H, 5.6; S, 7.4. Found: C, 61.0; H, 5.7; S, 7.1.

The 3-isomer recovered by preparative t.l.c. had m.p. 158–160° (methanol–water), $[\alpha]_D^{24}$ +67.5° (*c* 1.2, chloroform); n.m.r. data: τ 4.01 (1-proton apparent triplet).

Reaction of **2** *with α-toluenethiol.* — A solution of **2** (700 mg) in chloroform (1.5 ml) was treated with α-toluenethiol (0.3 ml) and triethylamine (0.2 ml). After this mixture had been kept for 0.5 h, the amine was removed under diminished pressure, and the residue was decanted into water (100 ml). The syrup that separated was washed first with water and then with a small volume of hexane. Finally, the syrup (920 mg, 95%) was dissolved in chloroform, dried (sodium sulfate), and resolved into the 2- and 3-(*S*-benzyl dithiocarbonates) by preparative t.l.c. (1:1 ether–hexane). The identity of the 2-(higher R_F) and 3-isomers was established by comparison with authentic samples[8] and by n.m.r. spectroscopy (1-proton quartet at τ 3.91 for the 2-isomer and 1-proton triplet at τ 3.09 for the 3-isomer).

Reaction of **1** *with ammonia.* — Compound **1** (1.00 g) was charged into a sidearm test-tube immersed in a solid carbon dioxide–acetone bath. The sidearm was fitted with a length of tubing to prevent entrance of carbon dioxide from the bath. The mouth of the test tube was fitted with a stopper containing an inlet tube through which ammonia was introduced. After about 2 ml of ammonia had been condensed, the tube was removed from the bath and excess ammonia was removed under diminished pressure to yield a solid (1.04 g, 99%). T.l.c. (2:1 ether–acetone) of a portion of the solid residue revealed only two components. Extraction of the solid with acetone removed the compound of higher R_F. The residue showed only the compound of lower R_F, which was identified as the 3-carbamate isomer, m.p. 235–237° (acetone), $[\alpha]_D^{24}$ +96° (*c* 0.4, methanol); n.m.r. data: τ 4.1, (1-proton triplet).

Anal. Calc. for $C_{15}H_{19}NO_7$: C, 55.5; H, 5.8; N, 4.3. Found: C, 55.4; H, 5.9; N, 4.3.

The acetone extract afforded the 2-carbamate upon addition of hexane, m.p. 203–206°, $[\alpha]_D^{24}$ +110° (*c* 0.6, acetone); n.m.r. data: τ 4.65 (1-proton quartet).

Found: C, 55.4; H, 5.9; N, 4.3.

Reaction of **2** *with ammonia.* — The reaction of **2** with ammonia was conducted as for that with **1**. Evaporation of excess ammonia gave a solid (1.1 g, 99%) which was separated by preparative t.l.c. (ether) into the 2- and 3-thiocarbamates. The component of lower R_F (3-isomer) gave m.p. 202–203° (acetone–hexane), $[\alpha]_D^{24}$ +70° (*c* 1, acetone); $\lambda_{max}^{ethanol}$ 244 nm (ε 11,600); n.m.r. data: τ 3.2 (1-proton triplet).

Anal. Calc. for $C_{15}H_{19}NO_6S$: C, 52.8; H, 5.6; N, 4.1; S, 9.4. Found: C, 52.7; H, 5.8; N, 4.1; S, 9.2.

The 2-isomer gave m.p. 105–107° (acetone–hexane), $[\alpha]_D^{24}$ +84° (*c* 1, acetone); $\lambda_{max}^{ethanol}$ 244 nm (ε 11,400); n.m.r. data: τ 3.92 (1-proton quartet).

Reaction of **1** *with piperidine.* — Compound **1** (250 mg) was dissolved in piperidine (1 ml). Conversion of **1** to the two carbamates was immediate as indicated by t.l.c. (1:1 ethyl acetate–hexane). Evaporation of excess piperidine afforded a semisolid mass (320 mg, 100%), which produced crystals from an ether–hexane solution. Several crops of crystals were collected, all of which were homogeneous with respect to the component of higher R_F (2-isomer), m.p. 148–150° (ethyl acetate–hexane), $[\alpha]_D^{24}$ +79° (*c* 0.9, chloroform); n.m.r. data; τ 4.73 (1-proton quartet).

Anal. Calc. for $C_{20}H_{27}NO_7$: C, 61.1; H, 6.9; N, 3.6. Found: C, 61.3; H, 7.1; N, 3.5.

Evaporation of the solution after removal of the last crop of crystals of the 2-isomer gave a syrup that produced crystals of the 3-isomer on dissolution in ethyl acetate and addition of hexane to the point of cloudiness. Recrystallization (ethyl acetate–hexane) gave product having m.p. 108–110°, $[\alpha]_D^{24}$ +23° (*c* 0.4, chloroform); n.m.r. data: τ 4.19 (1-proton, apparent triplet).

Found: C, 61.3; H, 7.0; N, 3.5.

Reaction of **2** *with piperidine.* — Dissolution of **2** (2.0 g) in piperidine (5 ml) gave the 2- and 3-*O*-piperidinothiocarbonyl derivatives. Although t.l.c. in 19:1 chloroform–acetone revealed only a single component (having an R_F value lower than that of **2**), two components were observed with 1:1 ether–hexane as solvent. Evaporation of excess piperidine gave a semisolid residue (2.50 g, 99%). Crystals of the compound of higher R_F (2-isomer) were afforded when the residue was dissolved in ether and hexane was added to the point of cloudiness. Recrystallization from chloroform–hexane gave m.p. 179–182°, $[\alpha]_D^{24}$ +53° (*c* 0.5, chloroform); $\lambda_{max}^{ethanol}$ 253 nm (ε 16,500); n.m.r. data: τ 3.98 (1-proton quartet).

Anal. Calc. for $C_{20}H_{27}NO_6S$: C, 58.7; H, 6.6; N, 3.4; S, 7.8. Found: C, 58.6; H, 6.7; N, 3.4; S, 8.0.

Additional crops of crystals were mixtures of the two isomers. These were combined with the residue that resulted on evaporation of the ether–hexane solution. Further separation of the two isomers was accomplished by preparative t.l.c. The

isomer of lower R_F gave m.p. 159–161° (ether–hexane), $[\alpha]_D^{24}$ −53° (*c* 0.5, chloroform); $\lambda_{max}^{ethanol}$ 253 nm (ε 16,500); n.m.r. data: τ 3.2 (1-proton, apparent triplet).

Found: C, 58.5; H, 6.5; N, 3.4; S, 8.0.

Reaction of **2** *with glycine.* — A solution of glycine (1.0 g) in water (20 ml) was treated with triethylamine (2 ml) and a solution of **2** (1.0 g) in acetone (25 ml). Initially, the reaction mixture was cloudy but within several seconds became a clear yellow. After stirring for 1 min, t.l.c. (19:1 chloroform–acetone) showed no **2**. Acetone (100 ml) was added to precipitate almost all of the unreacted glycine, which was removed by filtration. The filtrate was evaporated and the residue was extracted with acetone and filtered. The yield (1.53 g) of solid recovered on evaporation of the acetone filtrate and an i.r. spectrum (carbonyl at 1620 cm^{-1}) suggested that the glycine adduct was recovered as the triethylammonium salt. The sulfur content (6.4%) was consistent with the proposed structure (theory for the salt $[C_{23}H_{36}N_2O_8S]$ is 6.4% and for the free acid $[C_{17}H_{21}NO_8S]$ is 8.0%). The difficulty encountered during attempts to separate the 2- and 3-isomers was overcome when the components were converted into their corresponding methyl esters. The esters were formed by treating the product in acetone with Rexyn RG-51 (H^+) until carbonyl absorption of the product was approximately 1700–1750 cm^{-1} and then, after filtration and evaporation, by treating the solid with a benzene solution of diazomethane. The diazomethane was added in small increments until a yellow color persisted. Although insoluble initially, the solid became soluble in benzene as reaction with diazomethane occurred. The two isomers were separated and isolated by preparative t.l.c. (ether). The 2-isomer (higher R_F) gave m.p. 146–148° (isopropyl alcohol–hexane), $[\alpha]_D^{24}$ +70° (*c* 0.8, chloroform); $\lambda_{max}^{ethanol}$ 244 nm (ε 11,300); n.m.r. data: τ 3.92 (1-proton quartet).

Anal. Calc. for $C_{18}H_{23}NO_8S$: C, 52.4; H, 5.6; S, 7.7. Found: C, 52.2; H, 5.6; S, 7.9.

The 3-isomer (lower R_F) gave m.p. 152–154° (isopropyl alcohol–hexane), $[\alpha]_D^{24}$ +33° (*c* 0.6, chloroform), $\lambda_{max}^{ethanol}$ 244 nm (ε 11,000); n.m.r. data: τ 3.22 (1-proton, apparent triplet).

Found: C, 52.1; H, 5.6; S, 7.9.

Reaction of **1** *with glycine.* — This reaction was conducted similarly to that used for **2** except that the ammonium salt was converted into the methyl ester without treatment with resin. When the benzene solution was kept at 25°, crystals of the 3-isomer were deposited, which had m.p. 177–178° (ethanol), $[\alpha]_D^{24}$ +77° (*c* 1, chloroform); n.m.r. data: τ 4.12 (1-proton triplet).

Anal. Calc. for $C_{18}H_{23}NO_9$: C, 54.5; H, 5.8. Found: C, 54.5; H, 5.8.

Crystals of the 2-isomer were obtained when the mother liquor was evaporated to a syrup and the syrup was dissolved in ethanol; m.p. 184–186° (ethanol), $[\alpha]_D^{24}$ +58° (*c* 1, chloroform); n.m.r. data: τ 4.12 (1-proton quartet).

Found: C, 54.4; H, 6.0.

ACKNOWLEDGMENTS

We thank Dr. D. Weisleder for n.m.r. measurements and C. E. McGrew, B. R. Heaton, and K. A. Jones for microanalyses.

REFERENCES

1 W. M. DOANE, B. S. SHASHA, E. I. STOUT, C. R. RUSSELL, AND C. E. RIST, *Carbohyd. Res.*, 4 (1967) 445.
2 E. I. STOUT, W. M. DOANE, B. S. SHASHA, C. R. RUSSELL, AND C. E. RIST, *Carbohyd. Res.*, 3 (1967) 354.
3 W. M. DOANE, B. S. SHASHA, E. I. STOUT, C. R. RUSSELL, AND C. E. RIST, *Carbohyd. Res.*, 8 (1968) 266.
4 E. I. STOUT, W. M. DOANE, B. S. SHASHA, C. R. RUSSELL, AND C. E. RIST, *Tetrahedron Lett.*, (1967) 4481.
5 E. J. DUFEK AND W. J. DEJARLAIS, *J. Amer. Oil Chem. Soc.*, 42 (1965) 1104.
6 L. HOUGH AND J. E. PRIDDLE, *Chem. Ind.* (London), (1959) 1600.
7 W. M. DOANE, B. S. SHASHA, C. R. RUSSELL, AND C. E. RIST, *J. Org. Chem.*, 30 (1965) 162.
8 D. TRIMNELL, W. M. DOANE, C. R. RUSSELL, AND C. E. RIST, *Carbohyd. Res.*, 5 (1967) 166.

Carbohydrate Research
Elsevier Publishing Company, Amsterdam
Printed in Belgium

NUCLEOPHILIC DISPLACEMENT REACTIONS IN CARBOHYDRATES PART XII[1]. THE REACTION OF 6-DEOXY-2,3-*O*-ISOPROPYLIDENE-4-*O*-METHANESULPHONYL-α-L-TALOPYRANOSE WITH SODIUM METHOXIDE

J. S. BRIMACOMBE*, F. HUNEDY, AND A. K. AL-RADHI
Chemistry Department, The University, P.O. Box 363, Birmingham 15 (Great Britain)
(Received April 11th, 1969)

ABSTRACT

Oxidation of benzyl 6-deoxy-2,3-*O*-isopropylidene-α-L-mannopyranoside (**3**) with ruthenium tetroxide in carbon tetrachloride gave benzyl 6-deoxy-2,3-*O*-isopropylidene-α-L-*lyxo*-hexopyranosid-4-ulose (**4**) in excellent yield. Ketone **4** was reduced stereospecifically, with sodium borohydride in methanol, to yield benzyl 6-deoxy-2,3-*O*-isopropylidene-α-L-talopyranoside (**5**), which was converted into the crystalline 4-methanesulphonate **6**. Catalytic debenzylation of methanesulphonate **6** gave 6-deoxy-2,3-*O*-isopropylidene-4-*O*-methanesulphonyl-α-L-talopyranose (**7**), which, on solvolysis with sodium methoxide in methanol at room temperature, was converted into 1,4-anhydro-6-deoxy-2,3-*O*-isopropylidene-α-L-mannopyranose (1,5-anhydro-6-deoxy-2,3-*O*-isopropylidene-β-L-mannofuranose) (**9**, 58%), methyl 6-deoxy-2,3-*O*-isopropylidene-α-L-talofuranoside (**12**, 26%), and methyl 6-deoxy-2,3-*O*-isopropylidene-α-L-mannofuranoside (**14**, 12%). The mechanisms of formation of these products are discussed.

INTRODUCTION

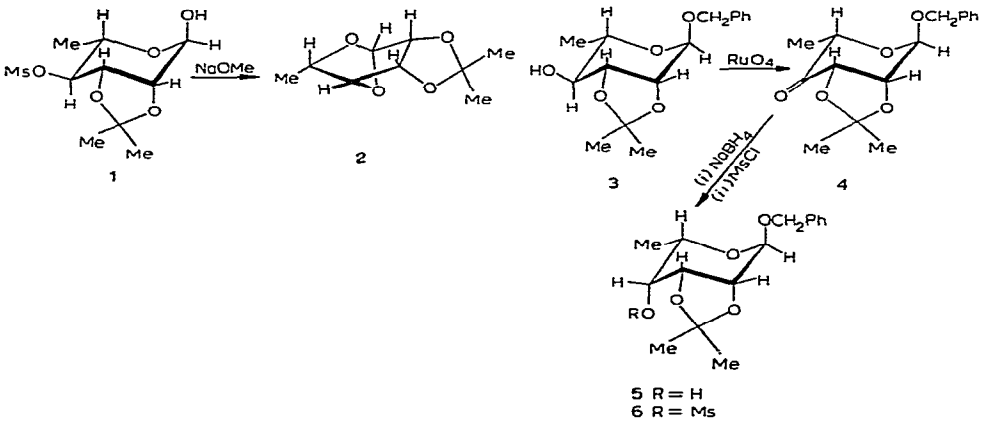
In a previous paper[2], we reported that 6-deoxy-2,3-*O*-isopropylidene-4-*O*-methanesulphonyl-α-L-mannopyranose (**1**) was converted smoothly into 1,4-anhydro-6-deoxy-2,3-*O*-isopropylidene-β-L-talopyranose (**2**) on treatment with sodium methoxide in methanol at room temperature. Although details of the mechanism of this intramolecular displacement were not ascertained, it was suggested[2] that formation of the 1,4-anhydro ring-system was favoured by the fact that the C-Me group and the 1,3-dioxolane ring adopted an *exo*-configuration in the transition state with respect to the dioxabicyclo[2.2.1]heptane ring-system under formation. In a continuation of these studies, we have examined the products resulting from similar treatment of 6-deoxy-2,3-*O*-isopropylidene-4-*O*-methanesulphonyl-α-L-talopyranose (**7**). This reaction is particularly interesting, since, although the C-1 alkoxide ion has free access to the methanesulphonate group in the boat conformation **8**, both the C-Me group and the 1,3-dioxolane ring are required to assume an *endo*-configuration with respect to the bicyclic ring-system under formation. It was of interest to establish

*Present address: Chemistry Department, The University, Dundee DD1 4HN, Great Britain.

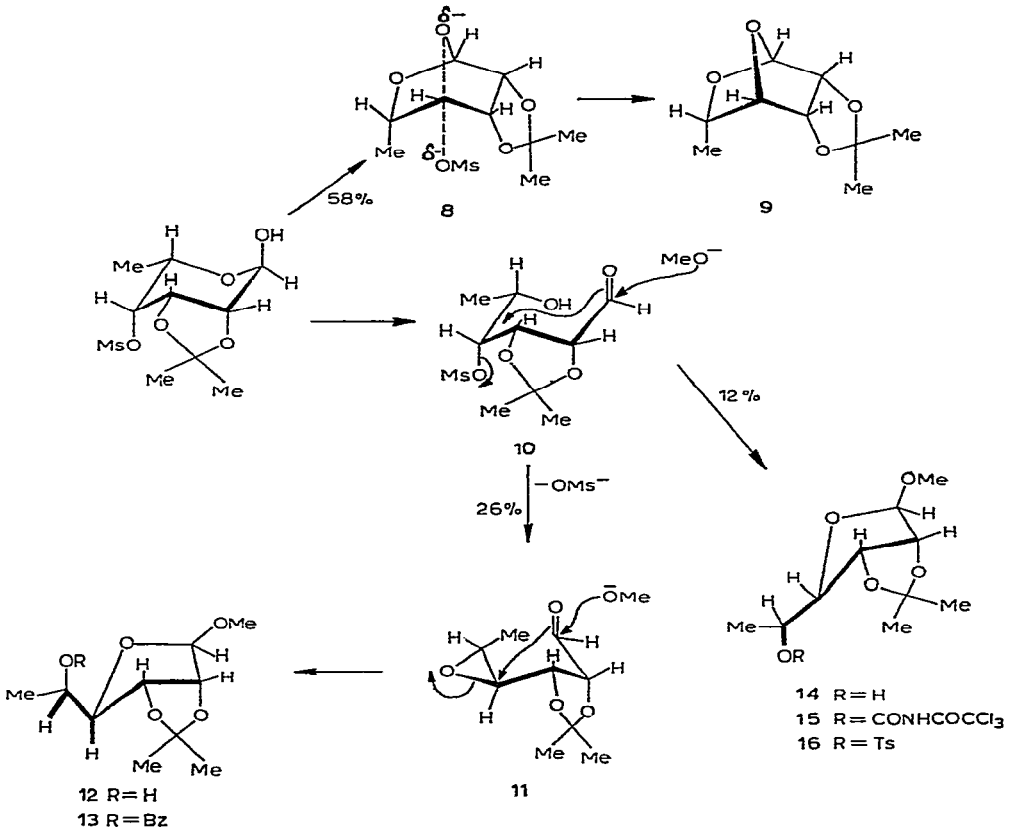
whether the non-bonded interactions developed in the transition state between the *endo*-substituents were of sufficient magnitude to divert the reaction to alternative pathways involving the *aldehydo*-form **10**. This information is important to a fuller understanding of the solvolysis of related sulphonates with sodium methoxide in methanol.

DISCUSSION

Oxidation of benzyl 6-deoxy-2,3-*O*-isopropylidene-α-L-manno pyranoside[2] (**3**) with ruthenium tetroxide[3] gave the syrupy ketone **4** in nearly quantitative yield. Reduction of ketone **4** with sodium borohydride in methanol proceeded stereospecifically to give a crystalline alcohol that was readily distinguishable from compound **3** (by g.l.c., physical constants, etc.) and which is, therefore, benzyl 6-deoxy-2,3-*O*-isopropylidene-α-L-talopyranoside (**5**). The stereochemical course of this reduction was anticipated, since metal hydride reductions of methyl 6-deoxy-2,3-*O*-isopropylidene-α-L-*lyxo*-hexopyranosid-4-ulose and its oxime are highly stereoselective in favour of the L-*talo* epimer[4-7], and Collins and Overend[4] have pointed out the preference for attacking nucleophiles to approach the carbonyl group from the direction remote from the axial substituent at C-2. Treatment of **5** with methanesulphonyl chloride in pyridine afforded benzyl 6-deoxy-2,3-*O*-isopropylidene-4-*O*-methanesulphonyl-α-L-talopyranoside (**6**), which, on catalytic debenzylation, afforded crystalline 6-deoxy-2,3-*O*-isopropylidene-4-*O*-methanesulphonyl-α-L-talopyranose (**7**).



The reaction of methanesulphonate **7** with sodium methoxide in methanol at room temperature yielded a mixture which was shown by g.l.c. (Fig. 1) and t.l.c. to contain one major component, together with at least three minor components. Although these products could not be resolved completely by either preparative g.l.c. or chromatography on silica gel, it was possible, nevertheless, by a combination of these methods to obtain sufficient quantities of three of the components to effect their identification. The major product (58%, estimated by g.l.c.) was separated



from the mixture by preparative g.l.c. and was purified by sublimation. Its molecular formula was determined as $C_9H_{14}O_4$ by elemental analysis and by accurate mass measurement of the top peak[8] at *m/e* 171 (M−15) in its mass spectrum. Absorptions attributable to either C = C, OH, or sulphonic ester groups were absent from its infrared spectrum, and, on acid hydrolysis, it gave a single, reducing sugar which was indistinguishable from 6-deoxy-L-mannose on paper chromatograms. These data are compatible with the structure 1,4-anhydro-6-deoxy-2,3-*O*-isopropylidene-α-L-mannopyranose (1,5-anhydro-6-deoxy-2,3-*O*-isopropylidene-β-L-mannofuranose) (**9**), and this assignment of structure was supported by n.m.r. spectroscopy. In addition to verifying the presence of the isopropylidene and C-Me groups, the n.m.r. spectrum showed a narrow doublet (*J* 2Hz) at low field which was ascribed to the bridgehead proton H-1. The size of the coupling between the *exo*-proton at C-2 and the vicinal, bridgehead proton is the same as that found[9] between similar protons in 1,4-anhydro-2,3-*O*-isopropylidene-α-D-lyxopyranose, although it is less than that (3.2–6.0 Hz) found[10,11] in bicyclo[2.2.1]heptane derivatives. Although the coupling between these protons may be small, it is evidently indicative of the *exo*-configuration of the non-bridgehead proton, since the coupling of *endo*-protons with vicinal, bridgehead

protons is zero in bornane[10], norbornane[11], and structurally related carbohydrate derivatives[2,12].

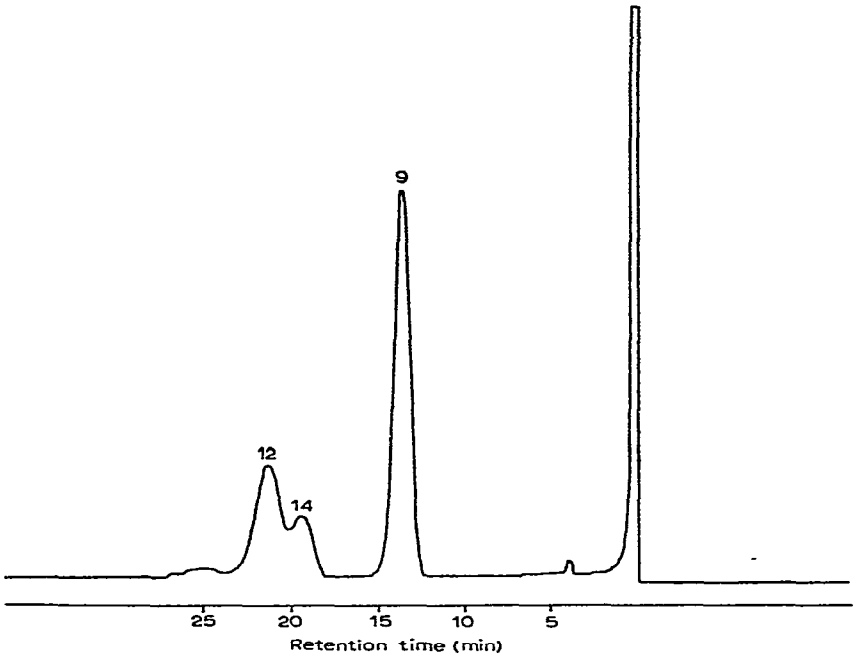


Fig. 1. Gas–liquid chromatogram of the solvolysis products from 6-deoxy-2,3-*O*-isopropylidene-4-*O*-methanesulphonyl-α-L-talopyranose (**7**). The percentages of the products formed were estimated as **9** (58%), **12** (26%), **14** (12%), and unidentified components (4%).

One of the minor components (*ca.* 26%) was obtained as a chromatographically homogeneous syrup, and it liberated 6-deoxy-L-talose (chromatographic identification) on acid hydrolysis. A clear indication of its structure was provided by the presence of a resonance attributable to a methoxyl group at τ 6.52 in its n.m.r. spectrum. Mechanistic considerations (see below) suggest that this compound is probably methyl 6-deoxy-2,3-*O*-isopropylidene-α-L-talofuranoside (**12**); confirmation of this assignment was provided by comparison of its chromatographic and spectroscopic properties with those of an authentic sample[13] and by formation of the known, crystalline 5-benzoate[13] **13**. An essentially similar approach was used to characterise another minor component (12%) which was shown to give 6-deoxy-L-mannose (chromatographic identification) on acid hydrolysis. Accurate mass measurement of the top mass peak[8] at *m/e* 203 (M−15) showed that the compound is isomeric with **12**. Again, a salient feature of the n.m.r. spectrum (Fig. 2) was the presence of a methoxyl resonance at τ 6.70, and the general structure of this component was deduced from its n.m.r. spectrum and that of the derived trichloroacetylcarbamate (see inset, Fig. 2). The latter derivative was formed rapidly *in situ* by the addition of trichloroacetyl isocyanate to a solution of the compound in deuteriochloroform; complete reaction was discerned by the appearance of a resonance, corresponding to one proton, at

low field (τ *ca.* 1.80) due to the presence of the NH proton. The multiplet centre at τ 5.95 (Fig. 2) is shifted well downfield (τ 4.78) in the derived carbamate (see inset, Fig. 2), and, on this evidence, it can be assigned to the proton residing on the same carbon atom as the hydroxyl group[14]. It is also clear from the spectrum that this proton is coupled to the C-Me group and is, therefore, located at C-5. This required the compound to be a methyl 6-deoxyhexofuranoside and, furthermore, the appearance of the anomeric proton as a singlet (at τ 5.09) signified[15] a *trans*-relationship of H-1 and H-2. The combined chemical and spectroscopic evidence indicate that this compound is methyl 6-deoxy-2,3-*O*-isopropylidene-α-L-mannofuranoside (**14**), and this assignment was established by comparison of the n.m.r. spectrum with that of an authentic sample, prepared by gas–liquid chromatographic separation of the anomeric furanosides obtained by acid-catalysed acetonation of 6-deoxy-L-mannose in the presence of methanol[16a], and by formation of the known[16b] 5-toluene-*p*-sulphonate **16**. The other minor component(s) (*ca.* 4%) were not isolated in a sufficiently pure form to permit meaningful characterisation.

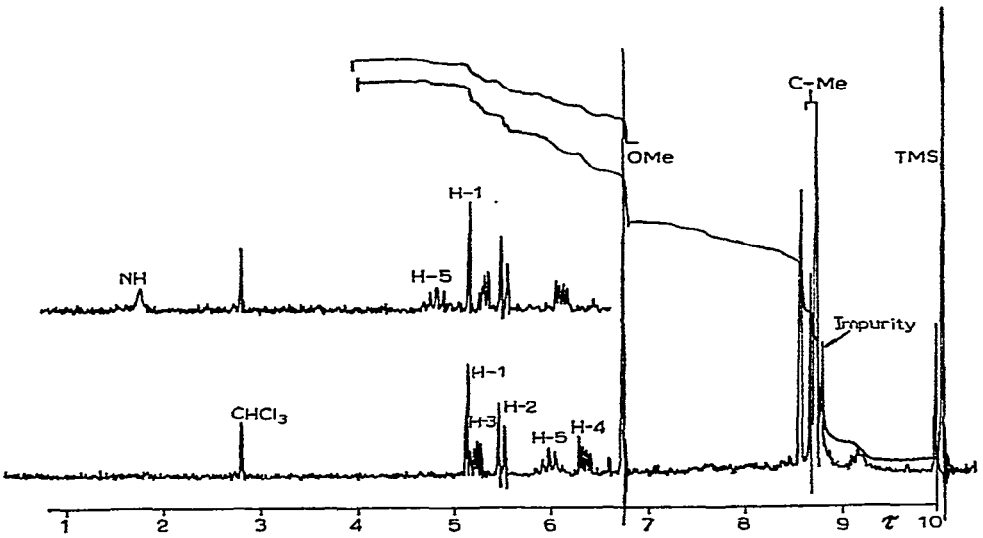
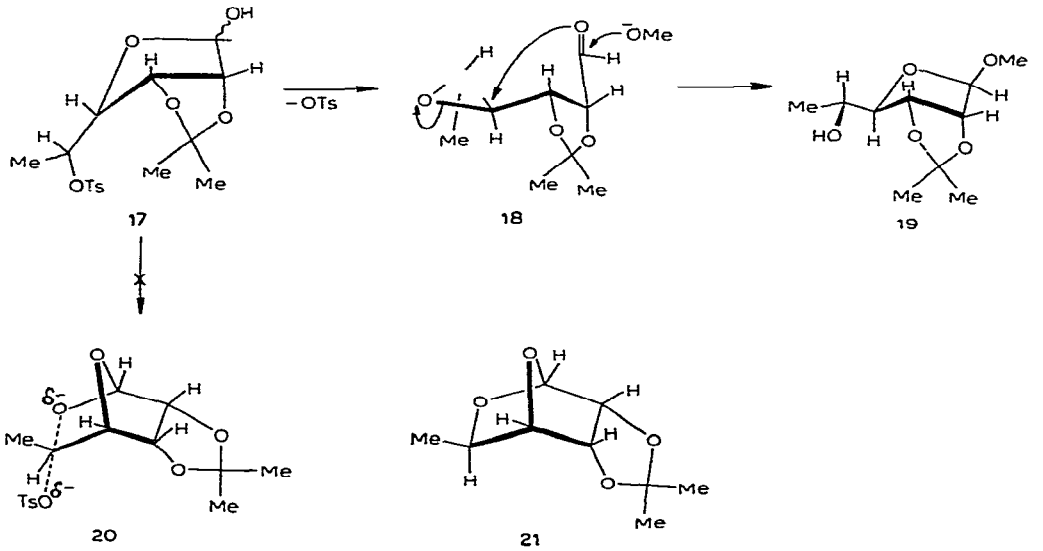


Fig. 2. N.m.r. spectrum ($CDCl_3$) at 100 MHz of methyl 6-deoxy-2,3-*O*-isopropylidene-α-L-mannofuranoside (**14**) and the lower region of the spectrum of the derived trichloroacetylcarbamate **15**. The following coupling constants were derived for **15** by first-order methods and by ignoring the slight second-order perturbation shown by H-3 and H-2: $J_{1,2} \sim 0.5$, $J_{2,3}$ 6, $J_{3,4}$ 3.7, $J_{4,5}$ 7.5, and $J_{5,6}$ 6.7 Hz.

It is clear from the preponderance of anhydro sugar **9** among the products of solvolysis that non-bonded interactions engendered in the transition state between the *endo*-substituents, while undoubtedly having an influence on the course of the reaction, are not sufficiently severe to divert the reaction completely to other routes. This result has a bearing on previous observations[2] regarding the rearrangement of 6-deoxy-2,3-*O*-isopropylidene-5-*O*-toluene-*p*-sulphonyl-L-mannofuranose (**17**) with

sodium methoxide. This reaction yields[9,17] principally methyl 6-deoxy-2,3-*O*-isopropylidene-β-D-allofuranoside (**19**) *via* the acyclic epoxide **18**, but the anhydro sugar **21** is not observed as a product of the solvolysis. Its absence was attributed[2] either to steric and/or electronic interactions preventing the approach of the C-1 alkoxide ion from within the *V*-shape formed by the trioxabicyclo[3.3.0]octane ring-system, or to adverse steric interactions, which would be introduced in the transition state **20**, between the *endo*-hydrogen atom and the dioxolane ring. The geometry of the transition state **20**, which would result in the formation of anhydro sugar **21**, closely resembles that which gives rise to anhydro sugar **9** and which also contains the more unfavourable interactions between *endo*-substituents, due to the *endo*-configuration of the C-Me group. Hence, it is reasonable to assume that the formation of anhydro sugar **21** from the rhamnose sulphonate **17** is prevented more by factors affecting the nucleophile's approach to the rearside of the sulphonate group than to unfavourable interactions between the *endo*-substituents in the transition state of the displacement reaction.



It is significant that both of the minor products contain a glycosidic methoxyl group which must have been introduced by way of the acyclic form **10**. In the formation of taloside **12**, loss of the methanesulphonyloxy group occurs with retention of configuration and ring contraction. There are several analogies[9,12,17,18] to indicate that this compound arises by aldehyde-group participation in the opening of an intermediate epoxide **11**, in the manner shown. Formation of the rhamnoside **14**, on the other hand, involves both a ring contraction and an inversion of configuration at C-4. This can be achieved by neighbouring-group participation by the aldehyde group in displacing the methanesulphonyloxy group (*i.e.*, **10**→**14**). An analogous mechanism rationalises[19] the ready solvolysis of 2,3,5-tri-*O*-benzyl-4-*O*-toluene-*p*-sulphonyl-*aldehydo*-D-ribose, in sodium methoxide–methanol, to give a mixture of

the anomeric methyl 2,3,5-tri-*O*-benzyl-L-lyxofuranosides. In our case, the presence of the isopropylidene ring probably accounts for the preferential formation of the α-glycoside **14**.

EXPERIMENTAL

Thin-layer chromatography (t.l.c.) was performed on silica gel, and detection was effected with vanillin–sulphuric acid[20]. Paper chromatography was performed by downward irrigation with ethyl acetate–pyridine–water (8:2:1), and the components were detected with aniline hydrogen phthalate[21]. N.m.r. spectra were measured in deuteriochloroform (tetramethylsilane as internal reference) at 60 and 100 MHz with either a Varian A-60 or Perkin–Elmer R-14 spectrometer, respectively; infrared spectra were recorded for Nujol mulls with a Perkin–Elmer 257 spectrometer. Analytical gas–liquid chromatography (g.l.c.) was carried out on a Pye 104 instrument at a column temperature of 130°, and preparative g.l.c. on a Pye 105 instrument with flame-ionisation detection at a column temperature of 160°; a column packing of 10% silicon ester-30 on Celite was used in both cases. Molecular weights were measured on an A.E.I. MS-9 mass spectrometer by using a direct-insertion technique.

Benzyl 6-deoxy-2,3-O-isopropylidene-α-L-lyxo-hexopyranosid-4-ulose (**4**). — Ruthenium dioxide dihydrate[3] (4.3 g) was added to a solution of sodium metaperiodate (60 g) in water (600 ml), and the mixture was shaken vigorously until conversion into the tetroxide was complete. The aqueous solution was extracted with carbon tetrachloride (4 × 200 ml), and the combined extracts were added gradually to a stirred solution[2] of **3** (7 g) in carbon tetrachloride (100 ml). On complete addition, the solution was stirred for 1 h, whereupon t.l.c. (acetone–toluene, 3:7) showed that all of the starting material had reacted, and isopropyl alcohol (140 ml) was then added. Solid material was filtered off after 30 min, and the solvents were removed to yield ketone **4** (6.8 g), $[\alpha]_D$ −113° (*c* 1, chloroform), ν_{max} 1740 cm^{-1}, as a chromatographically homogeneous syrup (Found: C, 65.2; H, 6.6. $C_{16}H_{20}O_5$ calc.: C, 65.7; H, 6.9%).

Benzyl 6-deoxy-2,3-O-isopropylidene-α-L-talopyranoside (**5**). — To a solution of ketone **4** (6.7 g) in methanol (140 ml) sodium borohydride (1.35 g) was gradually added, and, on complete addition, the solution was stirred for 30 min at room temperature. Ethyl acetate (40 ml) was then added, the solvents were removed, and the residue was partitioned between water (250 ml) and ether (500 ml). The aqueous layer was extracted further with ether (2 × 100 ml), and the combined organic layers were dried ($MgSO_4$) and filtered. Removal of the solvent and distillation of the residue gave taloside **5** (6.6 g), b.p. 135–140°/0.1–0.2 mmHg, which crystallised on standing and, on recrystallisation from light petroleum (b.p. 80–100°), had m.p. 45–47°, $[\alpha]_D$ −74° (*c* 1, chloroform) (Found: C, 65.6; H, 7.4. $C_{16}H_{22}O_5$ calc.: C, 65.3; H, 7.5%). Compound **5** was readily distinguished from the mannoside **3** by g.l.c. (column temperature, 200°), the retention times of the components being 10.4 and 9.5 min, respectively; g.l.c. also demonstrated the absence of **3** in the original

reduction. N.m.r. data: τ 2.70 (5 aromatic protons); 4.90 (1-proton singlet, H-1); 5.38 (AB quartet, J 12 Hz, benzyl methylene protons); 8.44, 8.65 (3-proton singlets, CMe_2); 8.68 (3-proton doublet, $J_{5,6}$ 6 Hz, CMe).

Hydrolysis of **5** (50 mg) with N sulphuric acid (2 ml) on a boiling water-bath for 2 h, with paper chromatography of the neutralised hydrolysate, revealed that 6-deoxy-L-talose[4] was the only reducing sugar formed.

Benzyl 6-deoxy-2,3-O-isopropylidene-4-O-methanesulphonyl-α-L-talopyranoside (**6**).—A solution of compound **5** (6.6 g) in pyridine (150 ml) was treated with methanesulphonyl chloride (10.8 ml) for 24 h at room temperature, and methane sulphonate **6** (6.8 g), m.p. 138–139° [from ethyl acetate–light petroleum (b.p. 40–60°)], $[\alpha]_D$ −46° (*c* 1, methanol), was then isolated in the usual way (Found: C, 54.6; H, 6.4; S, 8.8. $C_{17}H_{24}O_7S$ calc.: C, 54.8; H, 6.5; S, 8.6%). N.m.r. data: τ 2.70 (5 aromatic protons); 4.93 (1-proton singlet, H-1); 5.38 (AB quartet, J 12 Hz, benzyl methylene protons); 6.94 (3-proton singlet, OMs); 8.43, 8.65 (3-proton singlets, CMe_2); 8.64 (3-proton doublet, $J_{5,6}$ 6 Hz, CMe).

6-Deoxy-2,3-O-isopropylidene-4-O-methanesulphonyl-α-L-talopyranose (**7**). — A solution of the glycoside **6** (4.2 g) in methanol (400 ml) containing 10% palladium–calcium carbonate (8.4 g) was shaken in the presence of a slight overpressure of hydrogen for 24 h at room temperature, and t.l.c. (acetone–toluene, 3:7) then showed that all of the starting material had reacted. The catalyst and solvent were removed, and the residue was extracted with chloroform (100 ml). Evaporation of the solvent, with recrystallisation of the residue from chloroform–light petroleum (b.p. 40–60°), gave compound **7** (2.3 g), m.p. 124–125°, $[\alpha]_D$ +13° (*c* 1, chloroform), ν_{max} 3400 cm^{-1} (OH) (Found: C, 43.0; H, 6.5; S, 11.3. $C_{10}H_{18}O_7S$ calc.: C, 42.6; H, 6.4; S, 11.3%). N.m.r. data: τ 4.63 (broad, 1-proton singlet, H-1, α-D anomer); 6.88 (3-proton singlet, OMs); 8.42, 8.60 (3-proton singlets, CMe_2); 8.63 (3-proton doublet, $J_{5,6}$ 6 Hz, CMe).

Treatment of 6-deoxy-2,3-O-isopropylidene-4-O-methanesulphonyl-α-L-talopyranose (**7**) *with sodium methoxide in methanol.* — A solution of methanesulphonate **7** (2.5 g) in N sodium methoxide in methanol (50 ml) was set aside at room temperature for 4 h, during which time complete reaction had occurred. The solution was neutralised with carbon dioxide, the solvent was removed, and the residue was extracted with ether (50 ml) which was dried ($MgSO_4$). Concentration of the filtered extract afforded a syrup (1.72 g) which g.l.c. (Fig. 1) showed to contain at least four components; the amounts of the products formed were estimated from the gas–liquid chromatogram as **9** (58%), **12** (26%), **14** (12%), and unidentified product(s) (4%). These compounds had retention times of 13.8, 21.7, 19.4, and 25.1 min, respectively.

A portion (0.4 g) of the product mixture was subjected to preparative g.l.c., and this gave 1,4-anhydro-6-deoxy-2,3-*O*-isopropylidene-α-L-mannopyranose (**9**) (0.15 g) which was sublimed at 75°/15 mmHg to give the pure compound, m.p. 39–41°, $[\alpha]_D$ +128.5° (*c* 1, in chloroform) (Found: C, 58.1; H, 7.7. $C_9H_{14}O_4$ calc.: C, 58.05; H, 7.5%). The mass spectrum of **9** contained a top mass peak at *m/e* 171 (M−15) (ref. 8) which was shown by accurate mass measurement to correspond to the

molecular formula $C_8H_{11}O_4$ (Found: 171.067551; Calc.: 171.065728). N.m.r. data: τ 4.57 (1-proton doublet, $J_{1,2}$ 2 Hz, H-1); 5.20–6.00 (H-2, H-3, H-4, and H-5); 8.36 (3-proton doublet, $J_{5,6}$ 7 Hz, CMe); 8.37, 8.66 (3-proton singlets, CMe_2). Hydrolysis of **9** (50 mg) in *p*-dioxane (0.3 ml) and 2N sulphuric acid (0.3 ml) on a boiling water-bath for 2 h gave a reducing sugar which was indistinguishable from 6-deoxy-L-mannose on paper chromatograms.

A second portion (0.4 g) of the product mixture was chromatographed over silica gel (acetone–toluene, 3:7) to give, *inter alia*, a fraction containing anhydro sugar **9** and a small proportion of compound **12**. Preparative g.l.c. afforded a pure sample (20 mg), $[\alpha]_D$ −50° (*c* 1, methanol), of the minor component which was identified as methyl 6-deoxy-2,3-*O*-isopropylidene-α-L-talofuranoside (**12**) by comparison of its n.m.r. and i.r. spectra with those of an authentic sample[13]; n.m.r. data: τ 4.99 (1-proton singlet, H-1); 5.76 (1-proton doublet, $J_{2,3}$ 3.5 Hz, H-2); 6.52 (3-proton singlet, OMe); 8.52, 8.68 (3-proton singlets, CMe_2); 8.78 (3-proton doublet, $J_{5,6}$ 6 Hz, CMe). Benzoylation[13] of **12** (50 mg) gave methyl 5-*O*-benzoyl-6-deoxy-2,3-*O*-isopropylidene-α-L-talofuranoside (**13**) (25 mg), m.p. and mixed m.p. 93–95° (lit.[13] m.p. 93.5–95°); the i.r. spectrum of the benzoate was indistinguishable from that of an authentic sample.

The foregoing separation on silica gel also yielded a fraction containing only the glycosides **12** and **14**, which was subjected to preparative g.l.c. This procedure gave a small amount of methyl 6-deoxy-2,3-*O*-isopropylidene-α-L-mannofuranoside (**14**) (8 mg) as a chromatographically homogeneous syrup. Accurate mass measurement of the top mass peak at *m/e* 203 (M−15) (ref. 8) in the mass spectrum gave a molecular formula of $C_9H_{15}O_5$ for this ion (Found: 203.089021; calc.: 203.091941) which signified the molecular formula $C_{10}H_{18}O_5$ for compound **14**. The n.m.r. spectrum of compound **14** is shown in Fig. 2, and it was identical with that of an authentic sample separated (by g.l.c.) from a mixture of methyl 6-deoxy-2,3-*O*-isopropylidene-α- and β-L-mannofuranosides[16a].

Acid hydrolysis of compound **14**, as previously described, afforded a reducing sugar which was indistinguishable from 6-deoxy-L-mannose on paper chromatograms. Toluene-*p*-sulphonate **16**, prepared from **14** in the usual way, had m.p. 83–84° which was not depressed on admixture with an authentic sample (lit[16b] m.p. 83–84°).

ACKNOWLEDGMENTS

The authors thank Professor M. Stacey, C.B.E., F.R.S., for his interest, Dr. E. F. Mooney and his staff for measuring the n.m.r. spectra, and Dr. J. R. Majer for mass measurements. The Libyan and Iraqi Governments are acknowledged for financial support (to F.H. and A.K.A-R., respectively).

REFERENCES

1 Part XI: J. S. BRIMACOMBE, O. A. CHING, AND M. STACEY, *J. Chem. Soc.* (*C*), (1969) 1270.
2 J. S. BRIMACOMBE AND L. C. N. TUCKER, *Carbohyd. Res.*, 5 (1967) 36.

L. M. BERKOWITZ AND P. N. RYLANDER, *J. Amer. Chem. Soc.*, 80 (1958) 6682; P. J. BEYNON, P. M. COLLINS, D. GARDINER, AND W. G. OVEREND, *Carbohyd. Res.*, 6 (1968) 431; J. S. BRIMACOMBE, A. HUSAIN, F. HUNEDY, AND M. STACEY, *Advan. Chem. Ser.*, 74 (1968) 56.
4 P. M. COLLINS AND W. G. OVEREND, *J. Chem. Soc.*, (1965) 1912.
5 S. W. GUNNER, W. G. OVEREND, AND N. R. WILLIAMS, *Carbohyd. Res.*, 4 (1967) 498.
6 C. L. STEVENS, R. P. GLINSKI, AND K. G. TAYLOR, *J. Org. Chem.*, 33 (1968) 1586.
7 J. S. BRIMACOMBE, O. A. CHING, AND M. STACEY, *Carbohyd. Res.*, 8 (1968) 498.
8 D. C. DEJONGH AND K. BIEMANN, *J. Amer. Chem. Soc.*, 86 (1964) 67.
9 J. S. BRIMACOMBE AND F. HUNEDY, *J. Chem. Soc. (C)*, (1968) 2701.
10 J. I. MUSHER, *Mol. Phys.*, 6 (1963) 93.
11 P. LASZLO AND P. VON R. SCHLEYER, *J. Amer. Chem. Soc.*, 86 (1964) 1171; H. Z. SABLE AND H. KATCHIAN, *Carbohyd. Res.*, 5 (1967) 109.
12 J. S. BRIMACOMBE AND L. C. N. TUCKER, *J. Chem. Soc. (C)*, (1968) 562.
13 E. J. REIST, L. GOODMAN, AND B. R. BAKER, *J. Amer. Chem. Soc.*, 80 (1958) 5775.
14 See, for example, J. S. BRIMACOMBE, F. HUNEDY, AND A. HUSAIN, *Carbohyd. Res.*, 10 (1969) 141; J. M. WILLIAMS AND A. C. RICHARDSON, *Tetrahedron*, 23 (1967) 1369, 1641; A. C. RICHARDSON, *Carbohyd. Res.*, 4 (1967) 415; A. D. BARFORD AND A. C. RICHARDSON, *ibid.*, 4 (1967) 408.
15 R. U. LEMIEUX AND R. NAGARAJAN, *Can. J. Chem.*, 42 (1964) 1270; T. D. INCH AND P. RICH, *J. Chem. Soc. (C)*, (1968) 1784.
16 (*a*) P. A. LEVENE AND I. E. MUSKAT, *J. Biol. Chem.*, 106 (1934) 761; (*b*) P. A. LEVENE AND J. COMPTON, *ibid.*, 116 (1936) 169.
17 E. J. REIST, L. GOODMAN, R. R. SPENCER, AND B. R. BAKER, *J. Amer. Chem. Soc.*, 80 (1958) 3962.
18 J. S. BRIMACOMBE, F. HUNEDY, AND L. C. N. TUCKER, *J. Chem. Soc. (C)*, (1968) 1381; J. BADDILEY, W. FRANK, N. A. HUGHES, AND J. WIECZORKOWSKI, *J. Chem. Soc.*, (1962) 1999.
19 N. A. HUGHES AND P. R. N. SPEAKMAN, *J. Chem. Soc. (C)*, (1967) 1186.
20 *Chromatography*, E. Merck AG, Darmstadt, 2nd edn., p. 30.
21 S. M. PARTRIDGE, *Nature*, 164 (1949) 443.

Carbohydrate Research
Elsevier Publishing Company, Amsterdam
Printed in Belgium

PRIMEVERULOSE (6-*O*-β-D-XYLOPYRANOSYL-D-FRUCTOSE) AND SOME DERIVATIVES THEREOF

DAVID RUTHERFORD* AND NELSON K. RICHTMYER

National Institute of Arthritis and Metabolic Diseases, National Institutes of Health, Bethesda, Maryland 20014 (U. S. A.)

(Received April 12th, 1969)

ABSTRACT

Primeverulose (6-*O*-β-D-xylopyranosyl-D-fructose) has been prepared by the partial rearrangement of primeverose (6-*O*-β-D-xylopyranosyl-D-glucose) with aqueous ammonia followed by separation of the two disaccharides on a cellulose column. The syrupy primeverulose had $[\alpha]_D^{20}$ −27.5° in methanol, and was characterized by its crystalline (2,5-dichlorophenyl)hydrazone. Some derivatives that are common to both primeverose and primeverulose, namely, the phenylosotriazole, phenylosotriazole hexaacetate, and (2,5-dichlorophenyl)osazone, are also described.

INTRODUCTION

Primeverose (6-*O*-β-D-xylopyranosyl-D-glucose) was discovered[1] as a constituent of the phenolic glycosides primeverin and primulaverin in 1912, and later in a number of other glycosides from plant sources. Wallenfels and Lehmann[2] isolated primeverose itself from an aqueous extract of ripe carob beans (St. John's bread; *Ceratonia siliqua* L.), and Begbie and Richtmyer[3] isolated primeverose from an aqueous extract of the dried roots of *Primula officinalis* Jacq. In these last two cases, the possibility of an enzymic hydrolysis of a primeveroside to yield primeverose cannot be excluded.

Inasmuch as D-glucose and D-fructose often occur together in plants, it seemed possible that their 6-*O*-β-D-xylopyranosyl derivatives might also occur together in some plants. To aid in the search for primeverulose (6-*O*-β-D-xylopyranosyl-D-fructose) in plant extracts, we rearranged primeverose to that ketose by mild treatment with aqueous ammonia. The mixture of primeverose and primeverulose was readily separated on a cellulose column, and the primeverulose was obtained in a yield of 12.5% as a chromatographically homogeneous, colorless syrup having $[\alpha]_D^{20}$ −27.5° (in methanol). The sugar was identified as a ketose by its characteristic color-reaction on a paper chromatogram sprayed with the orcinol–hydrochloric acid reagent, and the phenylosazone prepared from it was identical with the phenylosazone prepared

*Associate in the Visiting Program of the National Institutes of Health, March 1966 to April 1967. Present address: Fisons Pharmaceuticals Limited, Loughborough, Leicestershire, England.

from primeverose. It was further characterized by its crystalline (2,5-dichlorophenyl)-hydrazone. Also prepared were the phenylosotriazole, the phenylosotriazole hexa-acetate, and the (2,5-dichlorophenyl)osazone, common to both primeverose and primeverulose.

EXPERIMENTAL

Optical rotations were measured with a Perkin–Elmer 141 polarimeter. Melting points were observed in capillary tubes. Paper chromatography was conducted with Whatman No. 1 filter paper by the descending method at room temperature, with 6:4:3 butyl alcohol–pyridine–water.

Primeverulose (6-O-β-D-xylopyranosyl-D-fructose) from primeverose (6-O-β-D-xylopyranosyl-D-glucose). — Primeverose β-heptaacetate was synthesized directly from 1,2,3,4-tetra-*O*-acetyl-6-*O*-trityl-β-D-glucose (35.44 g, 60 mmoles) and tri-*O*-acetyl-α-D-xylopyranosyl bromide (20.35 g, 60 mmoles) with silver perchlorate (12.50 g, 60 mmoles) and Drierite (7.5 g) in nitromethane (100 ml), according to the general procedure of Bredereck *et al.*[4]. The yield of product, crystallized from ethyl alcohol, was 19.12 g (48%). The melting point was low (200–204°), but was raised by one recrystallization from chloroform–methanol[5] to 213–215°, alone and also when mixed with authentic primeverose heptaacetate*.

Prior to the rearrangement to primeverulose, 20 g of the recrystallized β-hepta-acetate was deacetylated with methanolic sodium methoxide for 3 days at 5°. The resulting solution was made neutral with a little carbon dioxide, and evaporated *in vacuo* to remove methanol and methyl acetate. The syrup, containing about 10.3 g of primeverose, was dissolved in distilled water (200 ml), and the solution was diluted with concentrated aqueous ammonia (50 ml), kept for 20 h at 37°, and evaporated *in vacuo* to a syrup. A solution of the syrup in water was passed down a column of Amberlite MB-3 ion-exchange resin, and the eluate and washings were evaporated to a thick syrup that weighed 9 g. Paper chromatography showed several spots besides those for unchanged primeverose and for the expected primeverulose.

The 9 g of syrup thus obtained was dissolved in a small volume of methanol, and 5.54 g of primeverose was recovered by crystallization. The mother liquor was evaporated to a syrup (2.95 g) that was put on top of a cellulose column (92 × 4 cm) and eluted with aqueous butyl alcohol of successively increased concentration, 15-ml portions of eluate being collected. Butyl alcohol that was one-quarter saturated with water removed substances that had the same mobilities on paper chromatograms as D-xylose (fractions 251–290) and D-glucose (fractions 411–510); half-saturated butyl alcohol eluted the primeverulose (fractions 751–1010); and saturated butyl alcohol eluted the primeverose (fractions 1491–1690).

The primeverulose fractions were combined, and evaporated to give 1.29 g of a colorless syrup (12.5% from primeverose β-heptaacetate), $[\alpha]_D^{20}$ −27.5° (*c* 2.6,

*Kindly supplied by Dr. George H. Coleman (see ref. 5).

methanol). The material gave a single spot on paper chromatograms sprayed with ammoniacal silver nitrate or with orcinol–hydrochloric acid; with the latter spray (2% of orcinol and 3% of concentrated hydrochloric acid in butyl alcohol; heated 3 min at 100–110°), the color developed was orange changing to green, the same as that given by D-fructose alone. The mobility of primeverulose under these conditions was 0.79 relative to D-fructose, 0.91 relative to D-glucose, and 1.52 relative to primeverose; the mobility of primeverose was 0.59 relative to D-glucose.

Gas–liquid chromatography (performed with an F & M Model 5750 Research Gas Chromatograph equipped with a flame-ionization detector; copper column (200 × 0.5 cm) packed with 15% by weight of 2,2-dimethyl-1,3-propanediol (neopentyl glycol) succinate polyester on Gas-Chrom A, 60–80 mesh; column temperature, 200°; flow rate, 120 ml of helium/min) showed only one peak for the per(trimethylsilyl) (TMS) ether of primeverulose. The retention times of the TMS ethers of primeverulose and primeverose, relative to that of α-D-glucose, were 15.2 and 27.9, respectively. The retention times relative to sucrose were 1.59 and 2.94, respectively.

6-O-β-D-Xylopyranosyl-D-arabino-hexulose phenylosazone from primeverulose. — A mixture of 59 mg (189 μmoles) of primeverulose, 1 ml of water, 65 μl (660 μmoles) of phenylhydrazine, and 38 μl (664 μmoles) of glacial acetic acid was heated for 2 h on a steam bath; yellow needles began to separate within 15 min. After being kept overnight at room temperature, the mixture was filtered, and the solid product was washed successively with several portions each of 10% aqueous acetic acid, water, cold ethyl alcohol, and ethyl ether. The phenylosazone weighed 57 mg (62%), and its i.r. spectrum (KBr disk) was identical with that of the phenylosazone prepared similarly from primeverose. The "instantaneous melting point" (the lowest temperature at which the compound, in a capillary tube, decomposed within 5 sec when the tube was plunged into a bath at a preset temperature) was estimated to be about 228°; Goris *et al.*[1] reported the "instantaneous m.p." as 224–226° (Maquenne block). The specific rotation of the phenylosazone prepared from primeverulose was $[\alpha]_D^{20}$ −98.9 (15 min)→ −53.6° (1 week, constant; *c* 1, pyridine); the rotation of the phenylosazone prepared from primeverose was $[\alpha]_D^{20}$ −101.3 (6 min)→ −54.2° (1 week, constant; *c* 1, pyridine). Helferich and Rauch[6] reported that the phenylosazone from primeverose had $[\alpha]_D^{20}$ −109.7° (pyridine).

6-O-β-D-Xylopyranosyl-D-arabino-hexulose phenylosotriazole. — A suspension of 0.93 g (1.9 mmoles) of the phenylosazone prepared from primeverose in a solution of 0.52 g (2.1 mmoles) of copper(II) sulfate pentahydrate in 100 ml of water was boiled for 30 min. The light-brown and bright-red precipitates were filtered off, and the yellowish solution was stirred with 2 g of barium carbonate for 1 h. The mixture was filtered and the solution was then deionized with Amberlite IR-120 and Duolite A-4 ion-exchange resins, and decolorized with a small amount of Darco X carbon. The suspension was filtered, and the filtrate was evaporated *in vacuo* to a foamy syrup (0.57 g, 76%). Crystallization was induced by rubbing the syrup with ethyl acetate. Two recrystallizations from ethyl alcohol yielded 0.34 g

of the phenylosotriazole as clusters of fine needles, m.p. 165–166°, $[\alpha]_D^{20}$ −49.0° (*c* 1, water).

Anal. Calc. for $C_{17}H_{23}N_3O_8$: C, 51.38; H, 5.83; N, 10.57. Found: C, 51.23; H, 5.79; N, 10.74.

6-O-β-D-Xylopyranosyl-D-arabino-*hexulose phenylosotriazole hexaacetate.* — Acetylation of 0.1 g of the phenylosotriazole with 3.5 ml each of acetic anhydride and pyridine for 3 days at room temperature yielded the hexaacetate as a syrup in quantitative yield. Crystals were obtained after several weeks, and these were recrystallized four times from aqueous ethyl alcohol. The final product (fine needles, 86 mg, 53%) had m.p. 91–92° and $[\alpha]_D^{20}$ −50.4° (*c* 1.2, chloroform).

Anal. Calc. for $C_{29}H_{35}N_3O_{14}$: C, 53.62; H, 5.43; N, 6.47. Found: C, 53.74; H, 5.42; N, 6.37.

6-O-β-D-Xylopyranosyl-D-arabino-*hexulose (2,5-dichlorophenyl)hydrazone.* — A solution of 0.25 g (800 μmoles) of primeverulose and 0.35 g (2.0 mmoles) of (2,5-dichlorophenyl)hydrazine in 5 ml of methanol was evaporated on a steam bath until crystallization occurred (30 min). Methanol was added, the solution was evaporated, and the process was repeated (total time, 1.5 h). The dry, crystalline residue was broken up with a spatula, extracted several times with ethyl ether by decantation, and then filtered off and washed further with ethyl ether. The air-dried product (yellow needles) weighed 0.37 g (98%) and melted at ~180° (dec.). The elemental analysis was obtained first on this material.

Anal. Calc. for $C_{17}H_{24}Cl_2N_2O_9$: C, 43.32; H, 5.13; N, 5.94; Cl, 15.05. Found: C, 43.50; H, 5.00; N, 5.88; Cl, 14.83.

It was later found that the product could be recrystallized by dissolving it in warm methanol and adding five volumes of petroleum ether. The m.p. was then ~195° (dec.).

Anal. Calc. for $C_{17}H_{24}Cl_2N_2O_9$: C, 43.32; H, 5.13; Cl, 15.05; N, 5.94. Found: C, 43.47; H, 5.13; Cl, 14.80; N, 6.09.

As the i.r. spectra (Nujol mulls) of the unrecrystallized and the recrystallized samples were identical, and yet the m.p. of each sample remained unchanged after several months, it seems probable that the unrecrystallized sample contained a trace of impurity that accelerated its decomposition. The specific rotation of the recrystallized sample was $[\alpha]_D^{20}$ −34.3 (6 min)→−16.3° (4 weeks, constant; *c* 1, pyridine).

6-O-β-D-Xylopyranosyl-D-arabino-*hexulose (2,5-dichlorophenyl)osazone.* — A mixture of 0.15 g (481 μmoles) of primeverose, 0.34 g (1.92 mmoles) of (2,5-dichlorophenyl)hydrazine, 0.11 ml (1.92 mmoles) of glacial acetic acid, and 10 ml of water was heated for 10 h under a reflux condenser on a steam bath. The mixture was kept overnight at 5°, and then filtered; the solid product was washed several times each with 10% acetic acid, water, cold ethyl alcohol (which removed some dark material), and ethyl ether. The yellow needles of the (2,5-dichlorophenyl)osazone weighed 0.16 g (53%) and melted at 222° (dec.). The rotation was $[\alpha]_D^{20}$ −49.9 (7 min)→−22.4° (2 weeks, constant; *c* 1, pyridine).

Anal. Calc. for $C_{23}H_{26}Cl_4N_4O_8$: C, 43.97; H, 4.17; Cl, 22.57; N, 8.92. Found: C, 43.99; H, 4.29; Cl, 22.20; N, 9.02.

ACKNOWLEDGMENTS

The authors thank Dr. William C. Alford and his associates of the Section on Microanalytical Services and Instrumentation of this Laboratory of Chemistry for obtaining the elemental analyses and i.r. spectra; and Dr. Ingvar Johansson, also of this Laboratory, for performing the gas–liquid chromatography.

REFERENCES

1 A. GORIS, M. MASCRÉ, AND C. VISCHNIAC, *Bull. Sci. Pharmacol.*, 19 (1912) 577, 648.
2 K. WALLENFELS AND J. LEHMANN, *Chem. Ber.*, 90 (1957) 1000.
3 R. BEGBIE AND N. K. RICHTMYER, *Carbohyd. Res.*, 2 (1966) 272.
4 H. BREDERECK, A. WAGNER, G. FABER, H. OTT, AND J. RAUTHER, *Chem. Ber.*, 92 (1959) 1135; H. BREDERECK, A. WAGNER, H. KUHN, AND H. OTT, *ibid.*, 93 (1960) 1201; H. BREDERECK, A. WAGNER, D. GEISSEL, P. GROSS, U. HUTTEN, AND H. OTT, *ibid.*, 95 (1962) 3056; H. BREDERECK, A. WAGNER, D. GEISSEL, AND H. OTT, *ibid.*, 95 (1962) 3064.
5 C. M. MCCLOSKEY AND G. H. COLEMAN, *J. Amer. Chem. Soc.*, 65 (1943) 1778.
6 B. HELFERICH AND H. RAUCH, *Ann.*, 455 (1927) 168.

Carbohydrate Research
Elsevier Publishing Company, Amsterdam
Printed in Belgium

ISOMERIZATION OF D-GALACTURONIC ACID IN NEUTRAL, AQUEOUS SOLUTION

BENGT CARLSSON AND OLOF SAMUELSON
Department of Engineering Chemistry, Chalmers Tekniska Högskola, Göteborg (Sweden)
(Received April 15th, 1969)

ABSTRACT

An aqueous solution of D-galacturonic acid at pH 7 was heated for 5 h at 110°, and the isomerization products were separated by anion-exchange chromatography. The main product was identified as D-*arabino*-5-hexulosonic acid, and the others as D-taluronic, L-*xylo*-5-hexulosonic, L-*xylo*-4-hexulosonic, D-guluronic, D-iduronic, and D-*arabino*-4-hexulosonic acids.

INTRODUCTION

Upon isomerization of D-galacturonic acid in calcium hydroxide solution, Ehrlich and Guttmann[1] observed that D-*arabino*-5-hexulosonic acid was formed. Siddiqui and Purves[2] isolated D-taluronic acid from the reaction in sodium hydroxide, whereas Fisher and Schmidt[3] reported the formation of L-altruronic acid after heating D-galacturonic acid at pH 7.

In earlier papers[4,5], it was shown that D-glucuronic acid, when heated in a neutral, aqueous solution at 110°, gave rise to several hexuronic and hexulosonic acids. The appearance of these acids could be explained by Lobry de Bruyn–Alberda van Ekenstein transformations[6]. Similar experiments made with D-galacturonic acid are described in the present paper.

EXPERIMENTAL

An aqueous solution of the sodium salt of D-galacturonic acid (6 g of D-galacturonic acid per 60 ml; pH 7), prepared in the same way as in the earlier experiments with D-glucuronic acid, was kept for 5 h at 110°.

The resulting organic acids were separated by anion-exchange chromatography in acetic acid on a column (20 × 870 mm) of Dowex 1-X8 (25–32 μm) resin, and the isolated fractions were studied by anion-exchange chromatography combined with a three-channel analyzer[7], in which the eluate was analyzed automatically by chromic acid oxidation, the carbazole reaction, and periodate oxidation with subsequent determination of formaldehyde. Two of the fractions were rechromatographed on a preparative scale, as described below, on the followingcolumn s: Dowex 1-X8 (acetate, 10 × 920 mm) and Dowex 1-X8; (borate, 15 × 800 mm).

Carbohyd. Res., 11 (1969) 347–354

TABLE I

DISTRIBUTION COEFFICIENTS, AND PERIODATE–FORMALDEHYDE AND CARBAZOLE RESPONSES[a] FOR CHROMATOGRAPHIC BANDS (*s*) AND REFERENCE SUBSTANCES (*r*)

Band	*Reference acid*	*Distribution coefficients*, D_v				*Periodate–formaldehyde response*		*Carbazole response*	
		M *HOAc*		*0.08M NaOAc*					
		s	r	s	r	s	r	s	r
S110		7.2		6.6		+++		+	
S1	Galacturonic	11.1	11.1	9.4	9.4	tr	tr	++	++
S2:A1		13.3		10.6		++		+++	
S2:A2	Guluronic	12.4	12.3	11.8	11.8	tr	tr	++	++
S3		14.4		12.9		tr		++	
S4:B1	*xylo*-5-Hexulosonic	19.1	19.0	15.2	15.2	++	++	+++	+++
S4:B2	Iduronic	19.1	19.3	15.4	15.3	tr	tr	++	++
S4:B3		19.7		16.2		+++		+	
S5		22.7		14.8		+++		+	

[a] +++ = large; ++ = medium; + = small; tr = trace only.

The isolated acids were trimethylsilylated, and studied by g.l.c. (QF-1) and by g.l.c.–mass spectrometry[4]. The acids were also reduced with sodium borohydride as described by Perry and Hulyalkar[8]. The identity of the aldonic acids formed was established by anion-exchange chromatography using the three-channel analyzer, by g.l.c. of their fully trimethylsilyl substituted 1,4-lactones on a QF-1 column[9,10], and by mass spectrometry[11]. The identifications by g.l.c.–mass spectrometry were carried out by Göran Petersson.

SEPARATION AND IDENTIFICATION

A chromatogram from an isomerization experiment at 110° is given in Fig. 1. It is seen that five significant elution bands (S1–S5) and one trace compound (S110) were recorded. The bands were isolated from a run on a preparative scale in M acetic acid and rechromatographed in acetic acid, 0.08M sodium acetate, and 0.12M sodium tetraborate on analytical columns. Bands S1, S3, and S5 gave single bands in all eluents, whereas band S2 was resolved into two bands both in sodium acetate and in tetraborate. Band S4 gave two seriously overlapping bands in sodium acetate and three well-separated bands in tetraborate. The individual acids (S2:A1 and S2:A2) contained in band S2 were isolated on a preparative scale by rechromatography in 0.05M sodium acetate. Those contained in band S4 were obtained in a preparative run in 0.12M sodium tetraborate (S4:B1; S4:B2; and S4:B3).

A tentative identification of four of the nine isolated acids was made from their volume distribution coefficients (D_v) calculated from the peak elution volumes[12]

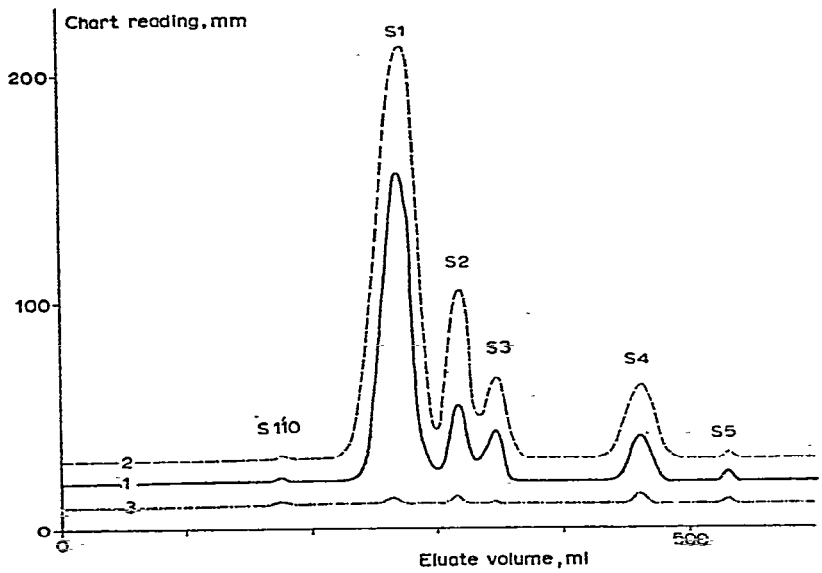


Fig. 1. Separation of isomerization products formed after 5 h at 110°: column (6 × 670 mm) of Dowex 1-x8 (24–27 μm) resin eluted with M acetic acid at 4.4 ml.min^{-1}.cm^{-2}. (1) Chromic acid oxidation, (2) carbazole method, and (3) periodate–formaldehyde method.

and from the response indices[7,13] in the carbazole and periodate–formaldehyde channels (Table I). Additional identification was obtained from g.l.c. and mass spectrometry by comparison with authentic samples, and by determination and identification of the aldonic acids formed after reduction with sodium borohydride (Table II). The acid contained in band S1 had $[\alpha]_D^{25}$ +50.5° (*c* 0.7, water) which is in agreement with that for D-galacturonic acid[14].

TABLE II

DISTRIBUTION COEFFICIENTS OF REFERENCE SUBSTANCES (*r*) AND OF ALDONIC ACIDS FORMED AFTER REDUCTION OF ISOMERIZATION PRODUCTS (*s*)

Reduced acid	*Reference acid*	*Distribution coefficient*, D_e			
		0.5M HOAc		*0.08M NaOAc*	
		s	r	s	r
S1	Galactonic	12.2	12.1	8.3	8.5
S2:A1	Galactonic	12.1	12.1	8.3	8.5
	Altronic	12.6	12.6		8.3
S2:A2	Gluconic	13.2	13.3	8.1	8.0
S3	Altronic	12.7	12.6	8.2	8.3
S4:B1	Gluconic	13.4	13.3	8.1	8.0
	Idonic		13.4	7.5	7.5
S4:B2	Idonic	13.5	13.4	7.6	7.5
S4:B3	Altronic	12.7	12.6	8.3	8.3
	Idonic	13.5	13.4	7.6	7.5
S5	Galactonic	12.1	12.1	8.3	8.5
	Gluconic	13.4	13.3		8.0

One of the other acids (S3) gave color reactions and gas–liquid chromatograms typical of uronic acids. The elution behaviour of the acid differed from that of uronic acids studied previously by ion-exchange chromatography. Upon reduction, a single aldonic acid, identified as altronic acid, was obtained. The results show that the isolated acid was taluronic acid. The mass spectrum confirmed that it was a hexuronic acid. The optical rotation, $[\alpha]_D^{25}$ −2° (*c* 0.6, water), was within the range expected from the rule given by Bose and Chatterjee[15].

The color responses of the acid contained in band S2:A1 were similar to those recorded with 5-hexulosonic acids. A comparison of the mass spectra and gas–liquid chromatograms of this acid with those obtained with *xylo*-5-hexulosonic acid (S4:B1) indicated that the acids were diastereomers. Altronic and galactonic acids were obtained in about equal amounts after reduction, which shows that band S2:A1 consisted of *arabino*-5-hexulosonic acid.

The acids contained in bands S4:B3 and S5 gave periodate–formaldehyde response indices intermediate between those of 5-hexulosonic and aldonic acids, and carbazole reactions weaker than those of 5-hexulosonic and hexuronic acids. Mass spectrometry showed that the acids did not belong to any of these groups. Upon reduction, two aldonic acids were obtained, in approximately equal amounts, from each of the bands (Table II). The results conclusively show that the acid contained in band S4:B3 is *arabino*-4-hexulosonic acid, and that band S5 contained *xylo*-4-hexulosonic acid which has not been reported previously. Both products were obtained as amorphous powders.

Since no isomerization products were found having inverted configuration at C-4 or C-5 relative to galacturonic acid, it can be concluded that the absolute configurations of the acids are those listed in Fig. 2.

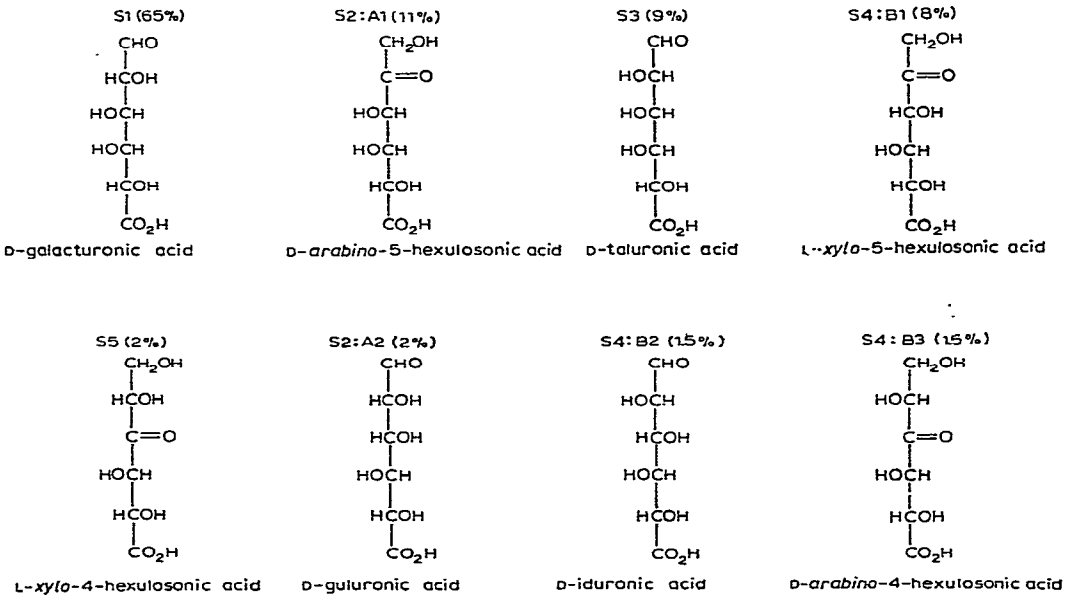


Fig. 2. Acids identified after isomerization at 110°. The relative amounts are given in parenthesis.

The trace compound S110 exhibited properties differing widely from those observed with hexuronic and hexulosonic acids. The amount was too small for identification, and this compound, which could not be detected when the isomerizations were carried out at 100°, will not be discussed further.

DISCUSSION

The appearance of all of the identified acids (Fig. 2) can be explained by Lobry de Bruyn–Alberda van Ekenstein transformations. As expected, and also observed in the experiments with D-glucuronic acid, the 5-hexulosonic acids are the preponderant ketoacids. Among these, D-*arabino*-5-hexulosonic acid is formed directly from

the starting material, and, as can be seen from Fig. 3, appreciable proportions were present after one hour at 100°. The formation of the *xylo* form requires epimerization at C-3, and, as expected, this compound was formed more slowly. Both possible forms of the isomeric 4-hexulosonic acids were formed, but detectable amounts were not recorded until the heating had proceeded for about 5 h at 100°. With glucuronic acid, only L-*ribo*-4-hexulosonic acid was formed in detectable amounts[5].

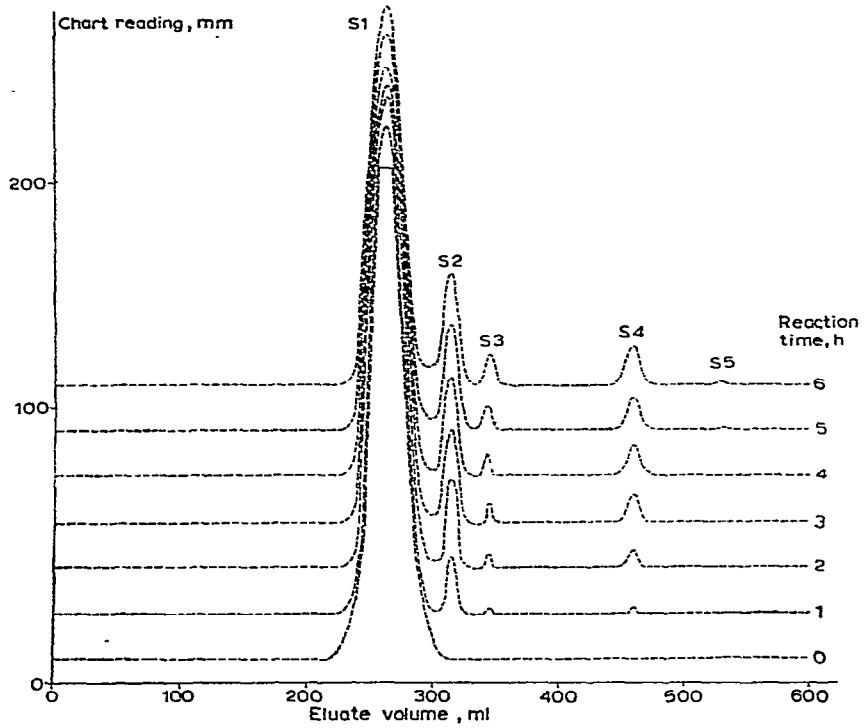


Fig. 3. Separation of isomerization products formed at 100° after various reaction times. D-Galacturonic (S1), D-*arabino*-5-hexulosonic and D-guluronic (S2), D-taluronic (S3), L-*xylo*-5-hexulosonic, D-iduronic and D-*arabino*-4-hexulosonic (S4), and L-*xylo*-4-hexulosonic acids (S5). The curves refer to the reaction with carbazole. Conditions: See Fig. 1.

The three possible uronic acids having inverted configurations at C-2 and C-3 were formed. The same reaction pattern was observed with D-glucuronic acid[4]. In both investigations, the C-2 epimer was the major product.

The total amount of uronic and hexulosonic acids produced from D-glucuronic acid at pH 7 was larger than that obtained with D-galacturonic acid under identical conditions. In addition to the isomerization products, small proportions of other types of compounds are formed[16], but it is outside the scope of this paper to discuss these products. A comparison between the remaining amounts of unchanged glucuronic and galacturonic acids may be of interest, however. The results given in Fig. 4, together with those obtained in parallel experiments with glucose, galactose,

and 4-*O*-methyl-D-glucuronic acid, show that the overall rate of reaction increases in the order D-galactose < D-glucose < D-galacturonic acid < D-glucuronic acid < 4-*O*-methyl-D-glucuronic acid. It is evident that the presence of a carboxyl group at C-6 favors the transformation, and that a methoxyl group at C-4 has an accelerating effect.

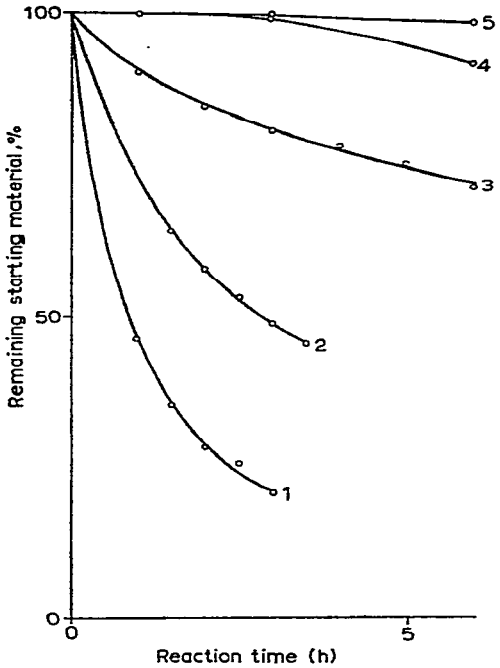


Fig. 4. Isomerization experiments at 100° and pH 7. Remaining starting material as a function of reaction time: (1) 4-*O*-methylglucuronic acid, (2) glucuronic acid, (3) galacturonic acid, (4) glucose, and (5) galactose.

ACKNOWLEDGMENT

The authors express their thanks to the Swedish Council for Applied Research for financial support.

REFERENCES

1 F. EHRLICH AND R. GUTTMANN, *Ber.*, 67 (1934) 573.
2 I. R. SIDDIQUI AND C. B. PURVES, *Can. J. Chem.*, 41 (1963) 382.
3 F. G. FISCHER AND H. SCHMIDT, *Ber.*, 92 (1959) 2184.
4 B. CARLSSON, O. SAMUELSON, T. POPOFF, AND O. THEANDER, *Acta Chem. Scand.*, 23 (1969) 261.
5 B. CARLSSON AND O. SAMUELSON, *Acta Chem. Scand.*, 23 (1969) 318.
6 C. A. LOBRY DE BRUYN AND W. ALBERDA VAN EKENSTEIN, *Rec. Trav. Chim.*, 14 (1895) 203; 16 (1897) 262.
7 B. CARLSSON, T. ISAKSSON, AND O. SAMUELSON, *Anal. Chim. Acta*, 43 (1968) 47.
8 M. B. PERRY AND R. K. HULYALKAR, *Can. J. Biochem.*, 43 (1965) 573.
9 O. SAMUELSON AND L. THEDE, *J. Chromatog.*, 30 (1967) 556.

10 G. PETERSSON, H. RIEDL, AND O. SAMUELSON, *Svensk Papperstid.*, 70 (1967) 371.
11 G. PETERSSON, O. SAMUELSON, K. ANJOU, AND E. VON SYDOW, *Acta Chem. Scand.*, 21 (1967) 1251.
12 O. SAMUELSON, *Ion Exchange Separations in Analytical Chemistry*, Almqvist and Wiksell, Stockholm; Wiley, New York; 1963.
13 S. JOHNSON AND O. SAMUELSON, *Anal. Chim. Acta*, 36 (1966) 1.
14 R. M. MCCREADY, *Methods Carbohyd. Chem.*, 2 (1963) 27.
15 A. K. BOSE AND B. G. CHATTERJEE, *J. Org. Chem.*, 23 (1958) 1425.
16 O. THEANDER, to be published.

Carbohyd. Res., 11 (1969) 347–354

Carbohydrate Research
Elsevier Publishing Company, Amsterdam
Printed in Belgium

AUTOMATED, CONTINUOUS ANALYSIS FOR CONCENTRATION OF TOTAL CARBOHYDRATE AND REDUCING END-GROUP IN THE FRACTIONATION OF DEXTRAN ON POROUS SILICA BEADS

S. A. BARKER, B. W. HATT, AND P. J. SOMERS
Department of Chemistry, The University, Birmingham 15 (Great Britain)
(Received April 17th, 1969)

ABSTRACT

The use of automated, continuous analyses for the concentration of total carbohydrate and reducing end-group to monitor the eluate in the fractionation of samples of dextran on porous silica beads is described. The two elution curves obtained were used to plot a calibration graph for the fractionation of that particular sample, and hence enabled us to make a calculation of the molecular weight distribution. Significant deviations from the S-shaped curve, used previously as the calibration graph, were noted. The mutual interaction of the molecules in the polysaccharide sample is suggested to account for these effects.

INTRODUCTION

The fractionation of polydisperse homopolymers by molecular weight may be achieved with molecular-sieve materials (gel-permeation chromatography). Molecular-sieve columns are usually calibrated by the elution of samples of known molecular weight, the eluate being monitored by an assay giving the weight of material. The distribution of the unknown sample may be assessed from its elution pattern and the calibration curve.

It would be convenient to monitor the fractionation by assays giving both the concentration of sample and the number of molecules. This would obviate the need for previous calibration, as the two assays would give the molecular weight of the species eluted at any point. The fractionation of dextran on porous silica beads has been described previously[1]. In the present investigation, the same separation system has been employed, using an automatic, continuous, reducing end-group assay, in addition to a cysteine–sulphuric acid assay[2] for total hexose concentration.

EXPERIMENTAL

Reducing end-group assay. — In the manual assay[3], an aqueous solution of sodium carbonate and potassium cyanide (solution 1; 0.53% of Na_2CO_3, 0.065% of KCN; 1.0 ml) and an aqueous solution of potassium ferricyanide (solution 2; 0.05% of $K_3Fe(CN)_6$; 1.0 ml) were added to an aqueous solution of a reducing

carbohydrate (5–50 μM; 1.0 ml) in a stoppered test-tube. The solution was heated for 15 min at 100°, and then cooled, and an acidic solution of ferric ammonium sulphate and sodium lauryl sulphate (solution 3a; 0.15% of $FeNH_4(SO_4)_2$, 0.1% of sodium lauryl sulphate, 0.05N H_2SO_4; 5.0 ml) was added. Alternatively, a solution having five times the concentration of solution 3a (solution 3b; 0.75% of $FeNH_4(SO_4)_2$, 0.5% of sodium lauryl sulphate, 0.25N H_2SO_4; 1.0 ml) was added. The solution was shaken, and, after 15 min, the absorbance was determined at 700 nm. The assay was calibrated with standard solutions of D-glucose, using both solutions 3a and 3b. The development of colour with time, at various concentrations of D-glucose, was also measured.

Calibration of reducing end-group assay with isomaltose and isomaltotriose. — Samples of isomaltose and isomaltotriose were prepared from a dextran hydrolysate by separation on a charcoal column[4] and on a column of AG Dowex-50W x2 (Li^+) ion-exchange resin[5]. The purity of the oligosaccharides was checked by paper chromatography (Whatman No. 1; butyl alcohol–pyridine–water, 6:4:3). Stock solutions of the oligosaccharides were prepared, and their concentrations were accurately measured by the cysteine–sulphuric acid assay[6], using a standard solution of D-glucose for calibration. The reducing end-group assay was calibrated with solutions of isomaltose and isomaltotriose (2–13 μg/ml) by using solution 3a.

Effect of a constant amount of dextran on the reducing end-group assay. — The isomaltotriose calibration was repeated by adding to each sample a constant amount of an aqueous solution of dextran (3.6 mg/ml, 200 μl; $\bar{M}_w$ 153,000).

Automation of the reducing end-group assay. — The automated assay used identical reagents to those employed in the manual assay. Technicon AutoAnalyzer modular equipment was used throughout, and a schematic representation is shown in Fig. 1. The column eluate was sampled continuously and mixed with solution 1 and with solution 2. The reaction stream was heated for 15 min at 95°, cooled, and debubbled. The stream was recycled and added to solution 3b, and, after mixing, its absorbance was measured at 660 nm.

The automated cysteine–sulphuric acid assay[2] for total hexose content is also shown in Fig. 1. The column eluate was sampled and mixed with water. After debubbling, the sample solution was recycled and added to the cysteine–sulphuric acid reagent. The reaction stream was heated for 3 min at 95°, cooled, and debubbled, and the absorbance was measured at 420 nm. Care was taken to keep the waste from the two assays separate, in order to avoid the production of hydrogen cyanide gas.

Fractionation of dextran samples on porous silica beads. — Porasil-D (75–100 mesh) from Waters Associates (Instruments) Ltd. (Stockport, Cheshire, Great Britain) was packed as a slurry in deaerated water into a column which was fitted at the bottom with a No. 3 sintered-glass disc. The column was vibrated manually until the bed had reached a minimum volume (76.5 ml; 1.084 × 82.8 cm). Aqueous solutions of dextran fractions (Pharmacia) (4–10 mg; 200 μl), having narrow distributions of molecular weight, were loaded onto the column and eluted at 10 ml/h/cm^2

by using the Technicon peristaltic pump. The eluate was continuously monitored for both total carbohydrate and end-group content, using the automatic cysteine–sulphuric acid and ferricyanide assays. These assays were frequently calibrated with standard solutions of D-glucose.

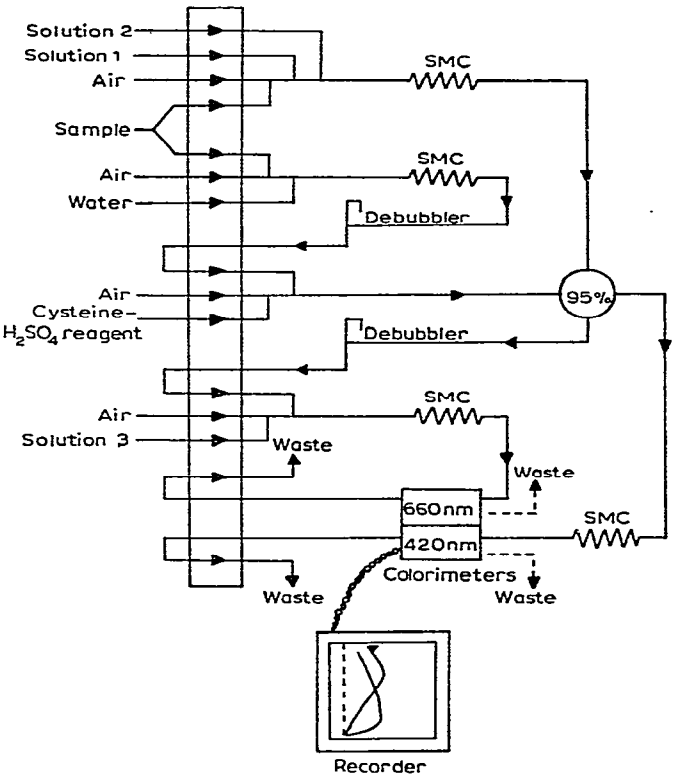


Fig. 1. Schematic representation of automated system for determination of concentration of total hexose and reducing end-group in column eluates. Reagent composition and flow rates: (*a*) total hexose determination; air (0.32 ml/min), sample (0.03 ml/min), water (1.06 ml/min); recycled sample (0.10 ml/min), air (0.32 ml/min), L-cysteine hydrochloride (0.07% w/v) in sulphuric acid (86% v/v, 0.53 ml/min); (*b*) reducing end-group concentration; reagent concentrations as in text; air (0.16 ml/min), sample (0.10 ml/min), solution 1 (0.10 ml/min), solution 2 (0.10 ml/min); recycled reaction stream (0.23 ml/min), air (0.16 ml/min), solution 3 (0.10 ml/min).

RESULTS

Calibration of the ferricyanide assay with D-glucose gave a linear plot of absorbance against D-glucose concentration, using both solution 3a and 3b. The development of colour in both cases was complete in less than 15 min. The calibration of the assay with isomaltose and isomaltotriose also gave linear graphs of absorbance against concentration, and the gradients of these lines were used to calculate the reducing powers of oligosaccharides, relative to D-glucose. The addition of a constant weight of dextran to the calibration samples of isomaltotriose gave a linear graph,

having the same gradient as the previous calibration, but with a different intercept on the ordinate.

Isbell and co-workers[7] have shown that dextrans have higher reducing powers than expected in comparison with the corresponding monosaccharide when assayed with alkaline copper reagents, and they suggest that this may be due to alkaline degradation of the partially oxidized product. The reducing powers of D-glucose, isomaltose, and isomaltotriose were measured with the ferricyanide assay, as these sugars may be regarded as the monomer, dimer, and trimer of the dextran polysaccharide.

If it is assumed that the reducing power of the α-D-(1-6)-linked oligosaccharides increases geometrically with increasing chain length in the assay used, the reducing power of dextran could be represented by the sum to infinity of a geometric progression:

$$(1+r+r^2+r^3+\ldots) = \frac{1}{1-r}.$$

Relative to D-glucose, isomaltose has a reducing power of 1.495, and isomaltotriose a reducing power of 1.78, times greater than theoretical, giving a mean value of $r = 0.505 \pm 0.01$. Thus, dextran could be expected to have a reducing power, relative to D-glucose, of 2.02 times greater than theoretical.

Previously[1], calibration curves for columns of molecular-sieve materials have been constructed by plotting the retention factor (elution volume/total bed volume) for the elution of 50% by weight of the dextran sample against the logarithm of the molecular weight at 50% on the cumulative weight–distribution curve for that sample, supplied by Pharmacia. Fig. 2 shows the curve obtained by applying this treatment to the present results. Also shown is the graph of the 50% retention

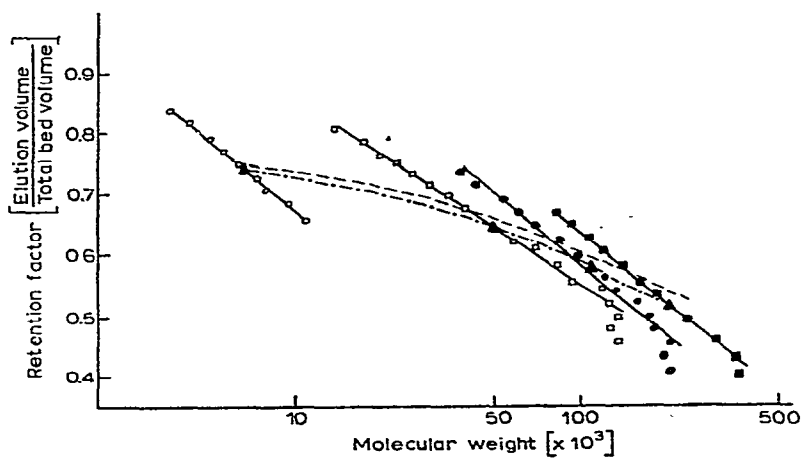


Fig. 2. Calibration graphs for fractionation of dextran on Porasil-D. —○— Dextran D-10 ($\bar{M}_w$ 9,400, 0.9 mg), —□— Dextran D-80 ($\bar{M}_w$ 73,000, 4.0 mg), —●— Dextran D-150 ($\bar{M}_w$ 153,000, 3.8 mg), —■— Dextran D-500 ($\bar{M}_w$ 370,000, 9.6 mg), —·▲—·— $\bar{M}_n$ for sample from absorbance ratio at 50% elution by weight, — — — $\bar{M}_w$ at 50% from Pharmacia data for cumulative weight distribution plotted against 50% elution by weight of the sample.

factor plotted against the molecular weight ($\bar{M}_n$) at that point, calculated from the absorbance ratio of the two assays.

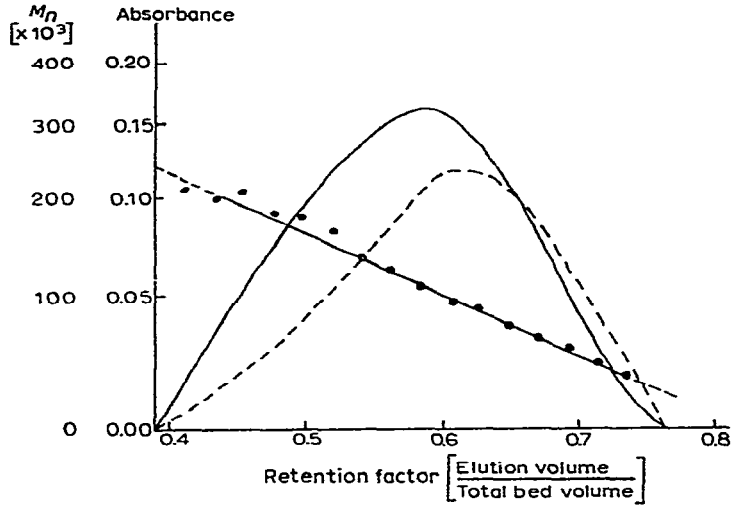


Fig. 3. Fractionation of Dextran D-150 ($\bar{M}_w$ 153,000, 3.8 mg) on Porasil-D: — — — reducing end-group assay; ——— total hexose assay; —●— calibration line constructed from $\bar{M}_n$ values calculated from absorbance ratio of the two assays.

The two curves from the assays obtained by fractionation of the samples (*e.g.*, Fig. 3) were used to calculate the molecular weight at the various retention factors, and these are shown in Fig. 2. The ratio of the areas under the two curves for a sample were used to calculate the number-average molecular weight ($\bar{M}_n$), and these values are given in Table I, together with the Pharmacia $\bar{M}_n$ values.

TABLE I

Sample	$\bar{M}_w$ *(from Pharmacia)*	$\bar{M}_n$ *at 50% elution from absorbance ratio*	*Mol.Wt. at 50% by weight of sample*[a]	$\bar{M}_n$ *from area ratio*	$\bar{M}_n$ *(from Pharmacia)*
D-10	9,400	6,460	7,500	6,120	6,200
D-80	73,000	49,800	60,000	45,500	46,000
D-150	153,000	108,000	136,000	96,200	95,000
D-500	370,000	208,000	280,000	182,000	185,000

[a]Calculated from the distribution curve for cumulative molecular weight, supplied by Pharmacia.

DISCUSSION

Molecular-sieve columns may be calibrated by the elution of samples having a narrow range of molecular weights, the plot of elution volume against the logarithm of the molecular weight giving an S-shaped curve. An assessment of the molecular

weight distribution of an unknown sample may be made by its elution from a column, previously calibrated with the appropriate samples. The assumption is made that the distribution of a sample does not affect the elution position of any particular polymer species in that sample. This assumption, while probably valid to a first approximation for dilute samples, is doubtful with samples of higher concentration.

The present work continues the investigation of the fractionation of dextran samples on porous silica beads. In order to obviate the necessity for prior calibration of the column, a method was developed for the direct determination of a molecular weight parameter of the dextran molecules in the column eluate. The elution of a sample was monitored by two assays, one giving a response proportional to the number of molecules, and the other giving a response proportional to the weight of sample, thereby enabling values of $\bar{M}_n$ to be calculated. Thus, the two elution curves could be used to calibrate the column for that particular sample (Fig. 3). A molecular weight distribution for the sample may be directly obtained from this calibration graph and the elution curves, the molecular-sieve material being used solely as a fractionation medium.

Table I shows the comparison of the $\bar{M}_n$ values given by Pharmacia with the $\bar{M}_n$ values calculated from the ratio of the areas under the two elution curves. The comparison also shows the consistency of the assumption that dextran has a reducing power that is 2.02 times greater than the value theoretically calculated from that of D-glucose, in the assay used. $\bar{M}_n$ values for the whole sample may also be obtained conveniently by direct sampling. Previously, the molecular weight of a species eluted at the volume corresponding to half the sample by weight was equated with the molecular weight at 50% on the Pharmacia plot of cumulative molecular weight distribution, in order to avoid the assumption that the molecular weight of the species eluted at the peak could be equated with $\bar{M}_w$. The molecular weights at 50% from the Pharmacia cumulative graphs, and the values of $\bar{M}_n$ obtained from the elution profiles at 50% by weight, are also shown in Table I.

Comparison of the S-shaped curve, obtained in the usual way, with the graphs of the molecular weights obtained by monitoring the column eluate with the two assays suggests that the elution volume of any particular molecular weight species is dependent on the distribution in the rest of the sample. For example, Fig. 2 shows that dextran molecules having a molecular weight of 100,000 are eluted at three widely different positions in the three samples fractionated (Dextran D-80, D-150, and D-500).

Laurent[8] has shown that solutions of polymers of high molecular weight, such as dextran and hyaluronic acid, have molecular exclusion properties similar to solid-phase molecular sieves. Also, Hellsing[9] has demonstrated that the elution volume of human serum albumin, chromatographed on Sephadex G-200, is increased as the concentration of a neutral polymer (such as dextran) in the eluent is increased. Further, he has confirmed Laurent's earlier conclusion that a solution of a polymer of high molecular weight has molecular exclusion properties that are identical with those of a gel of the same polymer, having a low degree of cross-linking as in Sephadex

G-200. Thus, elution of a sample on a solid-phase molecular sieve with an eluent containing a polymer of high molecular weight, which is itself wholly or partly excluded from the stationary phase, is equivalent to fractionating the sample by distribution between two molecular sieves. If the polymer is partially included in the stationary phase, its exclusion properties must be considered in addition to those of the stationary phase.

As a sample of a dextran polymer is eluted on a column of porous silica beads, it is fractionated primarily on the solid-phase molecular sieve, on a basis of molecular size. Superimposed on this process will be, presumably, the effect of the mutual interaction of the polymer molecules. A species of any particular molecular weight will not occur solely at one point in the elution curve; instead, longitudinal diffusion and other effects will cause molecules of the same weight to be distributed over a certain volume, and hence those molecules will be eluted down the column in the presence of molecules of greater and lesser molecular weight. Previously, it was assumed that the mutual interaction of the species in the sample was minimal, and that the elution curve for the whole sample could be represented theoretically by the addition of the elution curves of each molecular weight species eluted alone. The present results, obtained by using samples that are approximately ten times as concentrated as those used in earlier work[1], show that this assumption is not valid for such samples. Relatively high sample concentrations were necessary for the reducing end-group assay.

During fractionation, a species having a particular molecular weight will be affected both by molecules of greater and lesser molecular weight. Molecules of lower molecular weight have a higher concentration in the stationary phase than in the mobile phase, and the activity of the particular species in the stationary phase is increased, *i.e.*, it is excluded to a greater extent. Molecules of greater molecular weight have a higher concentration in the mobile phase, and this increases the activity of the particular species in that phase, *i.e.*, it is included to a greater extent in the stationary phase. These opposing effects would tend to cancel in the middle range of the sample, where the concentrations of the molecules of higher and lower molecular weight are approximately equal. But, at the lower side of the distribution, the total effect will be to retard the elution of the small species, and, on the higher side, the larger molecules will be accelerated. Thus, the plot of the logarithm of the molecular weight against elution volume, instead of following the appropriate part of the calibration curve, would be expected to give a graph having an increased gradient, as has indeed been found in the present study. The S-shaped curve, constructed from the calibration points in the usual way, as in Fig. 2, is valid for the analysis of elution curves only when the assumption can be made that the mutual effect of molecules being eluted is zero. If their interaction with each other is significantly large, the calibration curve for the sample on that column must be calculated from the determinations of molecular weight on the eluate.

ACKNOWLEDGMENTS

The authors thank Professor M. Stacey, C.B.E., F.R.S., for his interest in this work, and Mr. R. R. Woodbury for technical assistance with automated techniques. Financial support from the Science Research Council and the Lord Rank Organization (to B.W.H.) is gratefully acknowledged.

REFERENCES

1 S. A. BARKER, B. W. HATT, J. B. MARSTERS, AND P. J. SOMERS, *Carbohyd. Res.*, 9 (1969) 373.
2 S. A. BARKER, R. G. JONES, A. R. LAW, AND P. J. SOMERS, unpublished results.
3 Z. DISCHE, *Methods Carbohyd. Chem.*, 1 (1962) 513.
4 R. L. WHISTLER AND D. F. DURSO, *J. Amer. Chem. Soc.*, 72 (1950) 677.
5 S. A. BARKER, B. W. HATT, J. F. KENNEDY, AND P. J. SOMERS, *Carbohyd. Res.*, 9 (1969) 327.
6 Z. DISCHE, L. B. SHETTLES, AND M. OSMOS, *Arch. Biochem.*, 22 (1949) 169.
7 H. S. ISBELL, C. F. SNYDER, N. B. HOLT, AND M. R. DRYDEN, *J. Res. Nat. Bur. Stand.*, 50 (1953) 81.
8 T. C. LAURENT, *Acta Chem. Scand.*, 17 (1963) 2664; *Biochem. J.*, 89 (1963) 253.
9 K. HELLSING, *J. Chromatog.*, 36 (1968) 170.

Carbohydrate Research
Elsevier Publishing Company, Amsterdam
Printed in Belgium

ARYL GLYCOSIDES OF OLIGOSACCHARIDES

PART I. *p*-BROMOPHENYL β-GLYCOSIDES OF CELLOBIOSE, LACTOSE, AND MALTOSE

I. C. M. DEA

Department of Chemistry, University of Stirling, Stirling (Great Britain)

(Received March 25th, 1969; in revised form, April 24th, 1969)

ABSTRACT

The *p*-bromophenyl β-glycosides of cellobiose, lactose, and maltose have been prepared *via* their hepta-*O*-acetyl derivatives, by an adaptation of Griebel and Helferich's method[1] for the preparation of phenyl β-lactoside. In preliminary experiments, it was found that this version of the Koenigs–Knorr reaction gave better results in the synthesis of aryl glycosides of disaccharides than the method outlined by Rosenmund and Güssow[2] for the synthesis of aryl glycosides of monosaccharides.

INTRODUCTION

The preferred conformations of the monosaccharide six-membered ring are fairly well understood[3]. However, an understanding of the preferred conformations of polysaccharide molecules awaits detailed knowledge of the molecular geometry of the glycosidic linkages between sugar residues. X-ray diffraction studies of oligosaccharides have already given some information about the geometry around the glycosidic oxygen-atom[4–12]. The interpretation of X-ray diffraction data is simplified if a heavy atom can be incorporated in the structure. It is best if the ratio of the square of the atomic number of the heavy atom to the sum of the squares of the atomic numbers of the remaining atoms is about unity[13], although ratios as low as *ca.* 0.2 have been successful[14].

In 1959, Corbett and Kidd[15] attempted to make heavy-atom derivatives of cellobiose and lactose for subsequent study by X-ray diffraction. The 2-chloro, 2-bromo, and 2-iodoethyl β-glycosides of cellobiose and lactose were made *via* their hepta-*O*-acetyl derivatives. Unfortunately, the best compound for study, 2-bromoethyl β-cellobioside, was hygroscopic and unsuitable for X-ray work. The derived, quaternary ammonium bromide of 2-bromoethyl β-cellobioside, (2-β-cellobiosyloxyethyl)trimethylammonium bromide, was also hygroscopic. Corbett and Kidd also made β-cellobiosyltrimethylammonium bromide by treating the aceto-bromo derivative of cellobiose with trimethylamine, followed by deacetylation. X-ray structural analyses of the (*p*-bromophenyl)hydrazone of L-arabinose[16] and D-glucose[17] have been carried out; in each case, the sugar residue is in the pyranoid form. However, a study of D-ribose (*p*-bromophenyl)hydrazone showed that the

sugar residue exists in the acyclic form[18]. The aim of the present investigation is to obtain, by X-ray structural analysis, a knowledge of the molecular geometry of the glycosidic linkage between sugar residues in the pyranoid or furanoid form, for which *p*-bromophenyl β-glycosides of disaccharides are suitable compounds.

It has recently been reported[19] that the dichloro derivatives of 3,4-di-*O*-acetyl-D-arabinal, when treated with sodium phenoxide in benzene, gave a mixture of phenyl 2-chloro-2-deoxyglycosides in addition to 3,4-di-*O*-acetyl-2-chloro-D-arabinal. Although it is possible that this reaction could be adapted to the synthesis of *p*-bromophenyl glycosides of disaccharides, the production of a mixture of glycosides is a drawback, and therefore only the methods of Griebel and Helferich[1], and Rosenmund and Güssow[2] were explored.

RESULTS AND DISCUSSION

Rosenmund and Güssow[2] have reported a two-stage synthesis of *p*-bromophenyl tetra-*O*-acetyl-β-D-glucopyranoside *via* penta-*O*-acetyl-β-D-glucopyranose. A similar method has been used for the preparation of *o*- and *p*-chloro- and bromo-phenyl β-glycosides of D-glucuronic acid[20]. An attempt was made to adapt this method to a three-stage synthesis of aryl β-glycosides of disaccharides. Octa-*O*-acetyl-β-lactose was heated at 140° with phenol and etched aluminium foil to give phenyl hepta-*O*-acetyl-β-lactoside (18%); *cf.* the yield of 54% obtained from the reaction of the acetobromo derivative of lactose with phenol in alkaline, 50% aqueous acetone[21]. The synthesis of *p*-bromophenyl β-glycosides of cellobiose, lactose, and maltose was therefore attempted by this adaptation of Griebel and Helferich's extension of the Koenigs–Knorr reaction.

The acetobromo derivatives of cellobiose, lactose, and maltose were treated with *p*-bromophenol and potassium hydroxide in 50% aqueous acetone. The resulting *p*-bromophenyl hepta-*O*-acetyl-β-glycosides were deacetylated with boiling 0.1N methanolic sodium methoxide to give the *p*-bromophenyl β-glycosides of cellobiose and lactose as spherulite crystals, and *p*-bromophenyl β-maltoside as an amorphous solid.

An interesting fact about these reactions is the comparatively low yield of aryl β-glycoside. The hepta-*O*-acetyl derivatives of *p*-bromophenyl β-cellobioside and *p*-bromophenyl β-lactoside were formed in 60 and 52% yields, respectively. An even lower yield of 42% was obtained for *p*-bromophenyl hepta-*O*-acetyl-β-maltoside. Yields in glycosidations could be diminished by elimination side-reactions; thus, it was recently reported[22] that on treatment of the acetoiodo derivative of methyl D-glucuronate with various phenols in alkaline, aqueous acetone, a *ca.* 50% yield of the acetylated "2-hydroxylglucuronal" was obtained. A similar eliminative was observed when the acetobromo derivative (1) of cellobiose was treated with dimethylamine[23]. Moreover, when 1 was treated with trimethylamine to give (hepta-*O*-acetyl-β-cellobiosyl)trimethylamine bromide, a small amount of elimination to the 2-substituted cellobial was also shown to occur[15].

EXPERIMENTAL

Melting points are uncorrected. Rotary evaporations were carried out at or below 40°. Specific rotations were determined at 589 nm with a Perkin–Elmer 141 polarimeter at 23°. N.m.r. spectra were measured on a Perkin–Elmer R-10 spectrometer; tetramethylsilane was used as internal standard for carbon tetrachloride, and sodium 3-(trimethylsilyl)propanesulphonate for deuterium oxide. The acetobromo derivatives of the disaccharides were obtained by the literature methods[24,25].

Phenyl hepta-O-acetyl-β-lactoside. — (*a*) Phenol (5 g) was melted and treated with etched aluminium foil (0.1 g). After the aluminium foil had completely dissolved, octa-*O*-acetyl-β-lactoside (2 g) was added, and the mixture was heated for 2 h at 140°. The cooled mixture was dissolved in chloroform, and this solution was extracted with 2N sodium hydroxide (4 × 50 ml), washed with water, dried (Na_2SO_4), and evaporated. Crystallisation of the residue from ethanol gave phenyl hepta-*O*-acetyl-β-lactoside (350 mg, 18%), m.p. 160–161°, $[\alpha]_D$ −22.5° (*c* 1.62, chloroform), lit.[1,26], m.p. 162°, $[\alpha]_D$ −23°.

(*b*) A solution of phenol (1 g) and potassium hydroxide (0.6 g) in water (6 ml) was mixed with hepta-*O*-acetyl-α-lactosyl bromide (2 g) in acetone (6 ml), and left for 24 h at room temperature. The mixture was evaporated, the residue was taken up in benzene (30 ml), and the solution was washed with 2N sodium hydroxide (4 × 50 ml) and water (3 × 50 ml), dried ($CaCl_2$), and evaporated. Recrystallisation of the residue gave phenyl hepta-*O*-acetyl-β-lactoside (1.1 g, 54%), m.p. and mixed m.p. 160–161°, $[\alpha]_D$ −22.7°.

p-Bromophenyl hepta-O-acetyl-β-glycosides of disaccharides. — A solution of *p*-bromophenol (4.8 g) and potassium hydroxide (1.5 g) in water (15 ml) was mixed with hepta-*O*-acetyl-α-cellobiosyl bromide (5 g) in acetone (15 ml), and left for 24 h at room temperature. After evaporation of the acetone, a solution of the residue in benzene (100 ml) was washed with 2N sodium hydroxide (8 × 50 ml) and water (6 × 50 ml), dried ($CaCl_2$), and evaporated. Recrystallisation of the residue from ethanol gave *p*-bromophenyl hepta-*O*-acetyl-β-cellobioside (3.55 g, 60%), m.p. 235–237°, $[\alpha]_D$ −26.3° (*c* 1.40, chloroform); n.m.r. data (CCl_4): τ 2.52–3.22 (quartet due to *para*-substituted phenyl group), 4.65–6.25 (sugar ring protons), 7.90–8.04 (acetyl groups); integration gave a ratio of 1:3.65:5.3 (theoretical ratio, 1:3.50:5.25) (Found: C, 48.61; H, 5.08. $C_{32}H_{39}BrO_{18}$ calc.: C, 48.53; H, 4.95%).

In like manner, *p*-bromophenyl hepta-*O*-acetyl-β-lactoside (52%) was obtained having m.p. 161–162°, $[\alpha]_D$ −22.0° (*c* 1.61, chloroform); n.m.r. data (CCl_4): τ 2.52–3.22 (quartet due to *para*-substituted phenyl group), 4.60–6.30 (sugar ring protons), 7.85–8.03 (acetyl groups); integration gave a ratio of 1:3.50:5.20 (theoretical ratio, 1:3.50:5.25) (Found: C, 48.37; H, 4.93%).

p-Bromophenyl hepta-*O*-acetyl-β-maltoside (42%) was similarly obtained having m.p. 172.5–173.5°, $[\alpha]_D$ +44.4° (*c* 1.60, chloroform); n.m.r. data (CCl_4): τ 2.56–3.26 (quartet due to *para*-substituted phenyl group), 4.60–6.20 (sugar ring

protons), 7.90–8.02 (acetyl groups); integration gave a ratio of 1:3.60:5.30 (theoretical ratio, 1:3.50:5.25) (Found: C, 48.56, H, 4.86%).

p-Bromophenyl β-glycosides of disaccharides. — *p*-Bromophenyl hepta-*O*-acetyl-β-cellobioside (3.4 g) was deacetylated by refluxing with 0.01N methanolic sodium methoxide (12 ml); anhydrous conditions were maintained by addition of 2,2-dimethoxypropane (0.5 ml) to methanol (20 ml), which was used to dilute a stock solution of 0.1N methanolic sodium methoxide to the required strength. After cooling, the precipitate was collected, and recrystallised from ethanol to give *p*-bromophenyl β-cellobioside (1.65 g, 77%), m.p. 221–224°, $[\alpha]_D$ −48.6° (*c* 1.73, water); n.m.r. data for *O*-deuterated compound (D_2O): τ 2.43–3.07 (quartet due to *para*-substituted phenyl group), 4.89–6.59 (sugar ring protons) (Found: C, 43.28; H, 5.18. $C_{18}H_{25}BrO_{11}$ calc.: C, 43.45; H, 5.07%).

In a similar manner, *p*-bromophenyl β-lactoside (79.5%) was obtained having m.p. 252–254° (from ethanol), $[\alpha]_D$ −37.3° (*c* 0.74, water); n.m.r. data for *O*-deuterated compound (D_2O): τ 2.45–3.08 (quartet due to *para*-substituted phenyl group), 4.85–6.57 (sugar ring protons) (Found: C, 43.40; H, 4.97%).

p-Bromophenyl β-maltoside, prepared by the above method, was precipitated from ethanol as an amorphous solid (73%) having $[\alpha]_D$ +34.5° (*c* 1.43, water); n.m.r. data for *O*-deuterated compound (D_2O): τ 2.47–3.10 (quartet due to *para*-substituted phenyl group), 4.88–6.60 (sugar ring protons) (Found: C, 43.20; H, 5.14%).

ACKNOWLEDGMENTS

The author thanks Dr. D. A. Rees for many helpful discussions, and the Analytical Section of the Department of Chemistry, University of Glasgow, for performing the microanalyses.

REFERENCES

1 B. Griebel and R. Helferich, *Ann.*, 544 (1940) 191.
2 K. W. Rosenmund and E. Güssow, *Arch. Pharm.*, (1954) 38.
3 E. L. Eliel, N. L. Allinger, S. J. Angyal, and G. A. Morrison, *Conformation Analysis*, Interscience, New York, 1965.
4 C. A. Beevers, T. R. R. McDonald, J. A. Robertson, and F. Stern, *Acta Cryst.*, 5 (1952) 689.
5 C. A. Beevers and W. Cochran, *Proc. Roy. Soc.* (London), *Ser. A*, 190 (1947) 257.
6 G. M. Brown and H. A. Levy, *Science*, 141 (1963) 921.
7 R. A. Jacobson, J. A. Wunderlich, and W. N. Lipscomb, *Acta Cryst.*, 14 (1961) 598.
8 A. Hybl, R. E. Rundle, and D. E. Williams, *J. Amer. Chem. Soc.*, 87 (1965) 2779.
9 S. S. C. Chu and G. A. Jeffrey, *Acta Cryst.*, *B24* (1968) 830.
10 C. J. Brown, *J. Chem. Soc.* (*A*), (1966) 927.
11 S. S. C. Chu and G. A. Jeffrey, *Acta Cryst.*, 23 (1967) 1038.
12 B. J. Poppleton and A. McL. Mathieson, *Nature*, 219 (1968) 1046.
13 H. Lipson and W. Cochran, *The Crystalline State*, Vol. III, *The Determination of Crystal Structure*, Bell, London, 1953, p. 267.
14 D. C. Hodgkin, J. Kamper, T. Lindsey, M. Mackay, J. Rickworth, J. A. Robertson, C. B. Shoemaker, J. G. White, R. J. Prosen, and K. N. Trueblood, *Proc. Roy. Soc.* (London), *Ser. A*, 242 (1957) 228.
15 W. M. Corbett and J. Kidd, *J. Chem. Soc.*, (1959) 1594.

16 S. FURBERG AND C. S. PETERSEN, *Acta Chem. Scand.*, 16 (1962) 1539.
17 K. BJAMER, S. DAHM, S. FURBERG, AND C. S. PETERSEN, *Acta Chem. Scand.*, 17 (1963) 559.
18 K. BJAMER, S. FURBERG, AND C. S. PETERSEN, *Acta Chem. Scand.*, 18 (1964) 587.
19 L. VARGHA AND J. KUSZMANN, *Ber.*, 96 (1963) 411.
20 G. N. BOLLENBACK, J. W. LONG, D. G. BENJAMIN, AND J. A. LINDQUIST, *J. Amer. Chem. Soc.*, 77 (1955) 3310.
21 E. GLASER AND W. WULWEK, *Biochem. Z.*, 145 (1924) 514.
22 F. B. ANDERSON AND D. H. LEABACK, *Chem. Ind.* (London), (1960) 967.
23 W. M. CORBETT, J. KIDD, AND A. M. LIDDLE, *J. Chem. Soc.*, (1960) 616.
24 C. S. HUDSON AND J. M. JOHNSON, *J. Amer. Chem. Soc.*, 37 (1915) 2748.
25 W. T. HASKINS, R. M. HANN, AND C. S. HUDSON, *J. Amer. Chem. Soc.*, 64 (1942) 1289.
26 E. M. MONTGOMERY, N. K. RICHTMYER, AND C. S. HUDSON, *J. Amer. Chem. Soc.*, 65 (1943) 1848.

Carbohydrate Research
Elsevier Publishing Company, Amsterdam
Printed in Belgium

NUCLEOSIDES

PART LXII. SYNTHETIC STUDIES ON NUCLEOSIDE ANTIBIOTICS. 2. SYNTHESES OF METHYL 4-AMINO-4-DEOXY-D-GLUCOSIDURONIC ACID DERIVATIVES RELATED TO THE CARBOHYDRATE MOIETY OF GOUGEROTIN*

M. P. KOTICK, R. S. KLEIN, K. A. WATANABE, AND J. J. FOX

Division of Biological Chemistry, Sloan-Kettering Institute For Cancer Research, Sloan-Kettering Division of Cornell University Medical College, New York, N. Y. 10021 (U.S.A.)

(Received April 24th, 1969)

ABSTRACT

The first synthesis of 4-amino-4-deoxy-D-hexuronic acid has been achieved. Tritylation of methyl 4-azido-4-deoxy-α-D-glucopyranoside (**1**) gave the 6-trityl ether (**2**) which was converted into its 2,3-dibenzoate (**3**) and deritylated to methyl 4-azido-2,3-di-*O*-benzoyl-α-D-glucopyranoside (**5**). Oxidation of **5** afforded the glucuronic acid derivative **6** which upon esterification to **7**, followed by reduction and benzoylation yielded methyl (methyl 4-benzamido-2,3-di-*O*-benzoyl-4-deoxy-α-D-glucopyranosid)uronate (**8**), the structure and conformation of which were firmly established by n.m.r. analysis. De-benzoylation of methyl (methyl 4-azido-2,3-di-*O*-benzoyl-4-deoxy-α-D-glucopyranosid)uronate (**7**) with sodium methylate to **9**, followed by de-esterification and subsequent hydrogenation afforded crystalline methyl 4-amino-4-deoxy-α-D-glucopyranosiduronic acid (**11**), the structure of which was established by esterification and benzoylation to **8**. *N*-Acetylation of **11** yielded methyl 4-acetamido-4-deoxy-α-D-glucopyranosiduronic acid (**14**) which was esterified and peracetylated to the methyl ester **16**. Derivative **16** was also obtained by hydrogenation and peracetylation of **9**. Epimerization at C-5 was not observed in the conversion of 7→11, which suggests that a total synthesis of the gougerotin-derived C-substance from the 4-amino-4-deoxy-hexuronic acid derivatives reported herein is feasible.

INTRODUCTION

The nucleoside antibiotics[1], gougerotin[2] and blasticidin S[3] contain 4-amino-4-deoxy-D-hexopyranuronic acid moieties. The structures of these antibiotics have recently been firmly established[4]. As part of our program[5] designed toward the total syntheses of these antibiotics, we have investigated the chemistry of aminodeoxyhexuronic acid derivatives. Although interesting biological properties have been reported for aminodeoxyhexuronic acid-containing polysaccharides[6-8], literature on the chemistry of the monomers is sparse. To our best knowledge, only

*This investigation was supported in part by funds from the National Cancer Institute, National Institutes of Health, U. S. Public Health Service (Grant No. CA-08748).

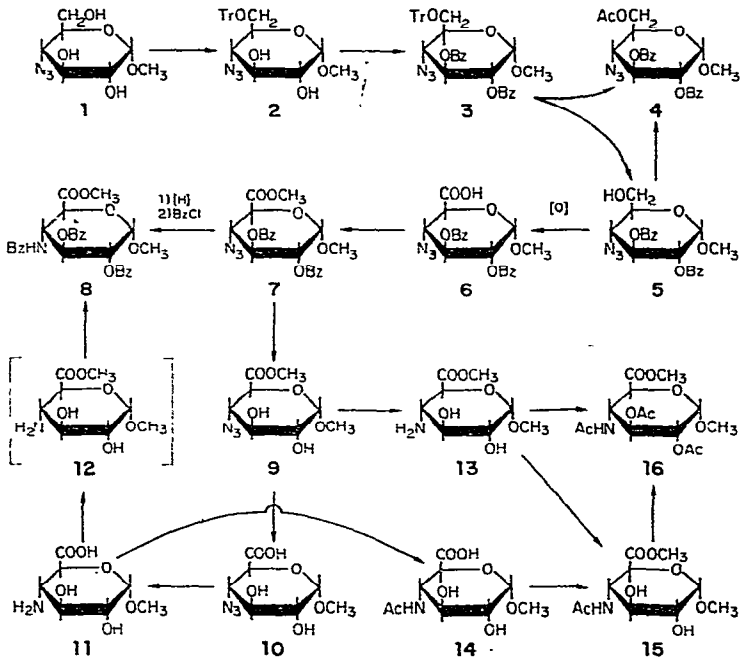
three examples of aminodeoxyhexuronic acids had been reported, namely, the 2-amino-2-deoxyuronic acid derivatives of the *gluco*[6], *galacto*[7], and *manno*[8] configuration. Of these, the former two were prepared[9,10] by catalytic oxidation of their corresponding hexosamine derivatives prior to their discovery in Nature. More recently, a 5-amino-5-deoxy-D-allofuranuronic acid has been discovered as a component of the nucleoside antibiotics, the polyoxins[11a], and 3-amino-3-deoxy-D-allouronic acid[11b] was prepared chemically. No other aminodeoxyhexuronic acid has either been synthesized chemically or (except for the above-mentioned nucleoside antibiotics) been found in Nature. This paper deals with the first chemical synthesis of a 4-amino-4-deoxyhexuronic acid, namely, 4-amino-4-deoxy-D-glucuronic acid from methyl α-D-galactopyranoside.

Conversion of methyl α-D-galactopyranoside[12] to methyl 4-azido-4-deoxy-α-D-glucopyranoside (**1**) was achieved in 4 steps, in improved yields, using a modification[5] of the procedure of Reist *et al.*[13] in which hexamethylphosphoric triamide was employed as solvent for nucleophilic displacement of the 4-mesyloxy function by an azide group. Attempts to oxidize compound **1** with either platinum-on-carbon[14] or platinum black[9,10] were unsuccessful.

Tritylation of **1** gave a yield of about 70% of the crystalline 6-trityl ether **2** which was benzoylated in high yield to **3**. On a preparative scale, **3** was isolated in 70% overall yield from **1** without isolation of the intermediate **2**. Detritylation of **3** in 80% acetic acid afforded **5** and a by-product (6%). This by-product was characterized as the 6-acetate **4** by the identity of its n.m.r. and i.r. spectra with those of the product obtained by direct acetylation of **5**. All attempts to crystallize chromatographically-homogeneous **5** or its 6-acetate **4** were unsuccessful. Oxidation of **5** with potassium permanganate in a mixture of acetic acid and acetone[15], followed by esterification of sirupy **6** with diazomethane gave the methyl ester **7** as a chromatographically-pure syrup in 55–60% overall yield from **5**.

Hydrogenation of ester **7** in the presence of palladium-on-charcoal in methanol afforded two products in approximately equal amounts, as judged by t.l.c. Benzoylation of this mixture gave only a single crystalline product (**8**) and trace amounts of other products which were not further characterized. These observations are best explained by partial $O \rightarrow N$ benzoyl migration during the reduction of **7**. Addition of triethylamine to the reaction mixture prior to the hydrogenation of **7** prevented this transbenzoylation. The n.m.r. spectrum (in chloroform-*d*) of **8** showed two methoxy resonances at δ 3.48 (aglycon) and δ 3.79 (ester). The aromatic proton signals (between δ 7.2 and 8.2) integrated for 15 protons corresponding to three benzoyl groups. On addition of deuterium oxide, the lower field NH doublet (δ 6.78; $J_{4,NH} \cong 9.5$ Hz) disappeared and the higher field H-4 quartet (δ 4.87) collapsed to a triplet ($J_{3,4} \cong 9.5$). Signals for H-2, H-3, and H-5 were observed at δ 5.48 (quartet, $J_{1,2} \cong 3.5$; $J_{2,3} \cong 9.5$), δ 6.03 (triplet, $J_{2,3} \cong J_{3,4} \cong 9.5$), and δ 4.47 (doublet, $J_{4,5} \cong 10.0$), respectively. These data establish the axial orientation for H-2, H-3, H-4, and H-5, and the equatorial orientation for the anomeric proton consistent with the D-*gluco* configuration in the *C1* conformation for **8**.

Methyl 4-amino-4-deoxy-α-D-glucopyranosiduronic acid (**11**) was synthesized from **7** in good overall yield. Treatment of **7** with sodium methoxide in methanol afforded the crystalline, debenzoylated product (**9**) which was de-esterified with aqueous alkali to give **10** in high yield. Catalytic reduction of the azido derivative **10** gave methyl 4-amino-4-deoxy-α-D-glucopyranosiduronic acid (**11**), as the crystalline monohydrate, in 83% overall yield from **9**. The structure of **11** was established by esterification of the latter with diazomethane to give **12** (not isolated), followed by benzoylation to a crystalline product which was identical with **8**.



Catalytic hydrogenation of the azido derivative **9** in presence of palladium-on-charcoal gave the amino sugar **13** which, without isolation, was *N*-acetylated to give the 4-acetamido derivative **15**. Acetylation of **15** gave the peracetylated derivative **16** which was isolated in crystalline form only after passage through a Silica Gel G column. Compound **16** was also obtained by direct acetylation of **13**.

Treatment of the 4-amino-4-deoxyuronic acid derivative **11** with aqueous alkali and acetic anhydride yielded methyl 4-acetamido-4-deoxy-α-D-glucopyranosiduronic acid (**14**) in crystalline form. Esterification of **14** with diazomethane yielded **15**, identical with that obtained by N-acetylation of **13**.

It should be noted, that compounds **9** and **11** were the *sole* products obtained, even though strongly basic conditions were employed during these reactions, that is, no isomerization at C-5 to the β-L-*ido* configuration was observed. Such an epimerization might have been expected on the basis of a reported[16] epimerization of alduronic acids at C-5 which occurred when the acids were heated in aqueous solution. Perry and Hulyalkar[17] later failed to observe this epimerization by g.l.c. A simple

explanation for the apparent absence of C-5 epimerization in our case may be rationalized on the basis that the α-D-*gluco* configuration for the uronic acid derivatives **9**→**11** is thermodynamically more stable than the corresponding β-L-*ido* configuration.

The data herein suggest that it should be possible to synthesize the gougerotin-derived C-substance [1-(4-amino-4-deoxy-β-D-glucopyranosyluronic acid)cytosine] from some of the glucuronic acid derivatives described in this report. Studies directed toward the total synthesis of gougerotin are in progress.

EXPERIMENTAL

Melting points were determined with a Hoover-Thomas capillary apparatus and are corrected. Thin-layer chromatography (t.l.c.) was performed on microscope slides coated with Silica Gel GF 254 (Merck), and preparative layer-chromatography (p.l.c.) on 2 mm layer plates of Silica Gel PF 254 (Merck) in the indicated solvent systems. Compounds were detected with u.v. light and/or by spraying with 20% (v/v) H_2SO_4 in ethanol, followed by heating to 130°. All evaporations were carried out *in vacuo*.

N.m.r. spectra were obtained on a Varian A-60 instrument, using tetramethylsilane as an internal standard: Chemical shifts are reported in p.p.m. (δ), and signals are quoted as s (singlet), d (doublet), t (triplet), q (quartet), or m (complex multiplet). Coupling constants are first order. I.r. spectra were determined on a Perkin-Elmer model 221 spectrophotometer. Elemental analyses were performed by Spang Microanalytical Laboratory, Ann Arbor, Michigan.

Methyl 4-azido-4-deoxy-α-D-glucopyranoside (**1**). — Methyl 2,3,6-tri-*O*-benzoyl-4-*O*-mesyl-α-D-galactopyranoside[13] (104 g, 0.178 mole) was dissolved in hexamethylphosphoric triamide (250 ml) and treated with sodium azide (42 g, 0.645 mole) at 80° with stirring. After 16 h, the reaction was diluted with an ice–water mixture (4 l). The precipitate was filtered off, washed with water, and dissolved in methylene chloride, and the solution was dried with magnesium sulfate. The solvent was evaporated, and traces of methylene chloride were removed by azeotropic distillation with methanol. The colorless residue was dissolved in methanol (700 ml), M methanolic sodium methoxide (20 ml) was added, and the mixture was kept overnight at room temperature.

Dowex 50-W (H^+, previously washed with methanol) was added to neutralize the reaction. The resin was filtered off, and the filtrate was evaporated to dryness. The residue was partitioned between water and ether. The aqueous layer was separated and evaporated to a syrup which was then dissolved in hot acetonitrile (*ca* 100 ml). The crystals were filtered off (29.9 g, m.p. 63–64°), and dried over phosphorus pentoxide, for 24 h at 40°, under vacuum to yield anhydrous material (27.7 g, m.p. 107.5–110°; lit.[13], m.p. 108–109°). From the mother liquor, an additional amount of crystalline **1** (6.8 g) was obtained for an 80% overall yield of **1**.

Methyl 4-azido-4-deoxy-6-O-trityl-α-D-glucopyranoside (**2**). — A solution of **1** (8.8 g) and chlorotriphenylmethane (14.0 g) in dry pyridine (35 ml) was heated on the

steam bath for 1 h, and then stirred overnight at room temperature. The solution was diluted with chloroform and washed twice with water, sodium hydrogen carbonate solution, and water. After drying (magnesium sulfate), the chloroform solution was evaporated to a thick syrup, which was crystallized from benzene to give **2** in three crops (12.7 g, 69%), sintering at 100°, m.p. 102–104°. Recrystallization from benzene–petroleum ether (30–60°) gave an analytical sample, sintering at 100°, m.p. 102–105°, $[\alpha]_D^{26}$ +87° (*c* 0.9, chloroform); λ_{max}^{KBr} 2.7 (OH), 4.7 (N_3), and 9.6 μm (C–O–C); n.m.r. in chloroform-*d*: δ 2.75 (OH, s, 2), δ 3.46 (OMe, s, 3), δ 3.24–3.81 (ring and CH_2 protons, 6), δ 4.91 (H-1, d, 1, $J_{1,2}$ ~3 c.p.s.), and δ 7.27–7.68 (trityl, 15).

Anal. Calc. for $C_{26}H_{27}N_3O_5$: C, 67.66; H, 5.90; N, 9.10. Found: C, 67.59; H, 5.93; N, 9.06.

*Methyl 4-azido-2,3-di-*O*-benzoyl-4-deoxy-6-*O*-trityl-α-*D*-glucopyranoside* (**3**). *From* **2**. — A solution of **2** (5.00 g) in pyridine (25 ml) was cooled in an ice-bath. Benzoyl chloride (4.5 ml) was added dropwise, and, after stirring for 2 h at room temperature, the pyridine solution was diluted with chloroform and treated as in the preparation of **2**. Concentration of the chloroform solution and removal of the residual pyridine by azeotrope distillation with water gave a syrup which crystallized on addition of ethanol. The crystalline residue was recrystallized from ethanol to give 4.74 g of **3**, m.p. 163–165°. Concentration of the mother liquor yielded an additional 1.15 g of **3** for an overall yield of 81%. A sample for analysis was recrystallized from ethanol, to give cube-like crystals, m.p. 164–165°, $[\alpha]_D^{26}$ +114° (*c* 1.0, chloroform); λ_{max}^{KBr} 4.7 (N_3), and 5.76 μm (ester carbonyl).

Anal. Calc. for $C_{40}H_{35}N_3O_7$: C, 71.74; H, 5.27; N, 6.27. Found: C, 71.67; H, 5.31; N, 6.29.

From **1**. — Compound **1** (14.5 g, 0.066 mole) and chlorotriphenylmethane (23.6 g, 0.088 mole) were dissolved in dry pyridine (100 ml). The mixture was heated on a steam-bath for 3 h, then stirred overnight at room temperature. To the reaction mixture was added benzoyl chloride (25 ml), and, after 2 h, the mixture was poured into a stirred ice–water mixture. The precipitate was dissolved in chloroform, and washed with water, aqueous sodium hydrogen carbonate, and water. Evaporation of the chloroform extract, followed by azeotropic removal of pyridine with water and then with ethanol gave a crystalline residue. Recrystallization of the residue from acetone–ethanol gave **3** (32.2 g, 74%, m.p. 160–162°) of sufficient purity for the next step.

*Methyl 4-azido-2,3-di-*O*-benzoyl-4-deoxy-α-*D*-glucopyranoside* (**5**). — A suspension of **3** (3.35 g) in 80% aqueous acetic acid (30 ml) was heated at reflux for 30 min, and the resultant clear solution was kept in an ice-bath for 1 h. After removal of the triphenylcarbinol by filtration, the filtrate was evaporated to a syrup which was chromatographed[18] on Silica Gel G (150 g) with 5:1 benzene–ethyl acetate as the eluent. Eluted first from the column was the 6-*O*-acetyl derivative **4** (0.15 g, 6%) which was obtained as a clear syrup, $[\alpha]_D^{26}$ +158° (*c* 1.0, chloroform); λ_{max}^{film} 4.7 (N_3), 5.75 (ester carbonyl), and 6.9 μm (aromatic); n.m.r. in chloroform-*d*: δ 2.16 (Ac, s, 3) δ 3.42 (OMe, s, 3), δ 3.82–4.00 (H-4, H-5, m, 2), δ 4.46 (H-6, H-6, d, 2), δ 5.19 (H-1, d, 1), δ 5.25 (H-2, q, 1), δ 6.00 (H-3, t, 1), and δ 7.12–8.12 (aromatic, 10).

Continued elution of the column with the same solvent pair yielded the major product **5** (1.9 g, 91%) as a clear syrup which resisted all attempts at crystallization, $[\alpha]_D^{26}$ +182° (*c* 1.7, chloroform), λ_{max}^{film} 2.45 (OH), 4.7 (N_3), and 5.75 μm (ester carbonyl); n.m.r. in chloroform-*d*: δ 2.50 (OH, s, 1 proton exchangeable with D_2O), δ 3.40 (OMe, s, 3), δ 3.80–4.03 (H-4, H-5, m, 2), δ 5.12 (H-2, q, 1), δ 5.20 (H-1, t, 1), δ 5.99 (H-3, t, 1), and δ 7.17–8.10 (aromatic, 10).

Acetylation of **5** with acetic anhydride in pyridine gave the 6-*O*-acetyl derivative **4** which was identical in all respects (t.l.c., i.r., and n.m.r.) with the material previously described.

*Methyl (methyl 4-azido-2,3-di-*O*-benzoyl-4-deoxy-α-*D*-glucopyranosid)uronate* (**7**). — Compound **5** (1.90 g) was dissolved in a mixture of acetic acid–acetone (1:1, 40 ml) and powdered potassium permanganate (1.00 g) was added with stirring over a period of 10 min. After stirring of the reaction mixture for 1 h at room temperature, additional potassium permanganate (0.50 g) was added, followed by another charge (0.50 g) after 1 h. The dark suspension was stirred overnight at room temperature, after which the excess permanganate was decomposed with sulfur dioxide. The resultant gel was evaporated to a volume of *ca* 10 ml to which was added water (150 ml). The aqueous suspension was extracted thrice with methylene chloride (100 ml), and the combined methylene chloride extracts were washed four times with water (100 ml). After drying (magnesium sulfate), the methylene chloride solution was evaporated to a clear syrup which was dissolved in methanol (25 ml), cooled, and treated with an excess of diazomethane. The solution was evaporated to dryness and chromatographed[18] on Silica Gel G (100 g) with benzene–ethyl acetate (15:1) as the eluent.

Fractions eluted just prior to the methyl ester **7** were contaminated by a substance moving slightly faster on t.l.c., which was not apparent on thin-layer plates and tended to elute with the major product from the column, thereby lowering the yield of chromatographically-homogeneous ester **7** to about 55–60%. Fractions contaminated with the faster-moving component were partially separated in another experiment. The minor product did not show an azide band in the i.r. spectrum, and was not investigated further. Homogeneous **7** was obtained as a clear syrup, $[\alpha]_D^{26}$ +152° (*c* 1.0, chloroform); λ_{max}^{film} 4.7 (N_3), 5.75 (ester carbonyl), and 7.9 μm (ester C–O–C).

*Methyl (methyl 4-benzamido-2,3-di-*O*-benzoyl-4-deoxy-α-*D*-glucopyranosid)-uronate* (**8**). — A solution of **7** (0.583 g) in methanol (25 ml) was hydrogenated at atmospheric pressure and room temperature in the presence of 10% palladium-on-carbon (75 mg). T.l.c. (9:1 benzene–ethyl acetate) at 1.5 h indicated the presence of starting material, in addition to two slower-moving components in approximately equal amounts. Hydrogenation was continued for an additional 45 min, at which time no more starting material could be detected by t.l.c. Filtration of the catalyst, followed by evaporation of the filtrate gave a syrup to which pyridine was added, and then benzoyl chloride (1 ml). After being stirred for 2 h at room temperature, the solution was poured into ice–water and extracted with chloroform, and the chloroform solution was washed with water, sodium hydrogen carbonate solution, and

water, and dried (magnesium sulfate). After filtration of the salts, the chloroform solution was evaporated to dryness, and the pyridine was removed by azeotropic distillation with water, and then with ethanol to give 560 mg of a partly crystalline residue. T.l.c. (5:1 benzene–ethyl acetate) indicated the presence of a single major spot, along with traces of faster-migrating impurities. Crystallization of this residue from ethanol gave 363 mg (55%), of crystalline tribenzoate **8**, m.p. 216–217°. A sample for analysis was prepared by recrystallization of **8** from ethanol to afford needles, m.p. 217–218°; $[\alpha]_D^{26}$ +101° (*c* 1.1, chloroform).

Anal. Calc. for $C_{29}H_{27}NO_9$: C, 65.28; H, 5.10; N, 2.63. Found: C, 65.18; H, 5.17; N, 2.54.

When the hydrogenation of **7** was conducted in the presence of triethylamine, the time required for reduction was reduced to ~1.5 h, as indicated by t.l.c. In addition, only a single spot was noted prior to benzoylation. T.l.c. of the benzoylation mixture prior to isolation of crystalline **8** indicated the presence of a single spot, unaccompanied by traces of impurities.

Methyl (methyl 4-azido-4-deoxy-α-D-glucopyranosid)uronate (**9**). — To **7** (6.90 g) in methanol (75 ml) was added a solution of sodium (45 mg) in methanol (20 ml), and the mixture was stirred for 3 h at room temperature. Dowex 50-W resin (H^+, previously washed with methanol) was added, the neutral solution was filtered, and the resin washed with methanol. The combined filtrates were evaporated to a thick syrup which was partitioned between water and ether. The aqueous solution was evaporated to a clear syrup which was dried by several azeotropic distillations with benzene, and finally crystallized from benzene–petroleum ether (30–60°) to give 3.16 g of **9** (85%), sintering at 79°, m.p. 80–81°. A sample for analysis was recrystallized from the same solvent pair to give fine needles, sintering at 80°, m.p. 82–83°, $[\alpha]_D^{26}$ +166° (*c* 1.0, chloroform).

Anal. Calc. for $C_8H_{13}N_3O_6$: C, 38.87; H, 5.30; N, 17.00. Found: C, 39.71; H, 5.14; N, 16.81.

Methyl 4-amino-4-deoxy-α-D-glucopyranosiduronic acid (**11**). — To a solution of **9** (1.00 g) in water (5 ml) was added dropwise M sodium hydroxide (4 ml). Dowex 50-W (H^+) was added and the acidic solution filtered from the resin. The resin was washed with water (3 × 5 ml), the combined filtrates were treated with triethylamine (6 drops), and the mixture was hydrogenated in presence of 10% palladium-on-carbon (95 mg) for 1.5 h. After removal of the catalyst by filtration, the solution was evaporated to a crystalline residue which was recrystallized from water–ethanol to give 0.76 g of **11** (83%), as the monohydrate which did not melt below 250°. Recrystallization from water–ethanol, followed by drying in presence of phosphorus pentoxide for 18 h at 100° gave an analytical sample of the monohydrate, $[\alpha]_D^{26}$ +95° (*c* 1.1, water).

Anal. Calc. for $C_7H_{13}NO_6 \cdot H_2O$: C, 37.35; H, 6.72; N, 6.22. Found: C, 37.46; H, 6.73; N, 6.28.

A solution of **11** (200 mg) in methanol (25 ml) was treated with an excess of

diazomethane, and subsequently benzoylated in pyridine in the usual manner to give crystalline **8**, identical in all respects to the material obtained previously.

Methyl 4-acetamido-4-deoxy-α-D-glucopyranosiduronic acid (**14**). — To a solution of **11** (414 mg) in water (5 ml), cooled in an ice-bath, was added M sodium hydroxide (3 ml) followed by acetic anhydride (0.3 ml). Rapid stirring and cooling were continued for 15 min, Dowex 50-W (H^+) was added, and the stirring continued for 5 min. After filtration, the solution was evaporated to give a crystalline residue which was recrystallized from ethanol to give 382 mg of **14** (71%) as the monoethanolate as determined by the n.m.r. spectrum in D_2O, sintering at ~125°, m.p. 133–137° (with effervescence), $[\alpha]_D^{26}$ +117° (*c* 0.5, water). A sample for analysis was dried *in vacuo* for 24 h at 100°.

Anal. Calc. for $C_9H_{15}NO_7$: C, 43.35; H, 6.07; N, 5.62. Found: C, 43.55; H, 6.40; N, 5.26.

Methyl (methyl 4-acetamido-4-deoxy-α-D-glucopyranosid)uronic acid (**15**). — A solution of **9** (500 mg) in methanol (25 ml) containing triethylamine (5 drops) was hydrogenated in the presence of 10% palladium-on-carbon (60 mg) for 1 h. After removal of the catalyst by filtration, acetic anhydride (0.5 ml) was added to the filtrate, and the clear solution was stirred for 1 h at room temperature. Evaporation gave **15** which was purified by p.l.c. with 6:1 chloroform–methanol as the developing phase to yield a foam, $[\alpha]_D^{26}$ +92° (*c* 0.79, chloroform), which crystallized on prolonged standing at room temperature. Recrystallization from ethanol–petroleum ether gave fine needles, m.p. 159–160°.

Anal. Calc. for $C_{10}H_{17}NO_7$: C, 45.64; H, 6.45; N, 5.27. Found: C, 45.70; H, 6.47; N, 5.24.

Methyl (methyl 4-acetamido-2,3-di-O-acetyl-4-deoxy-α-D-glucopyranosid)uronate (**16**). — A solution of **14** (200 mg) dissolved in methanol (15 ml) was treated with an excess of diazomethane at 0°. After evaporation of the solution to dryness, the residual syrup was dissolved in pyridine, and acetic anhydride (1 ml) was added. The solution was stirred overnight at room temperature, and then it was poured into water and extracted with chloroform. After the chloroform extract had been washed in the usual manner and evaporated, compound **14** (193 mg) was obtained as a foam in 82% yield. A sample for analysis was purified by p.l.c. with 4:1 ethyl acetate–acetone as the developing solvent; $[\alpha]_D^{26}$ +107° (*c* 1.3, chloroform); n.m.r. in pyridine-d_5: δ 1.94, 2.02, 2.05 (Ac), δ 3.26 (aglycon OMe, s, 3), δ 3.82 (ester OMe, s, 3), δ 4.40–5.50 (H-4, H-5, m, 2), δ 5.20 (H-1, d, 1), δ 5.33 (H-2, q, 1, $J_{1,2}$ ~3.5, $J_{2,3}$ ~9.5 c.p.s.), δ 5.98 (H-3, t, 1, $J_{3,4}$ ~10 c.p.s.), and δ 9.15 (NH, d, 1, $J_{4,NH}$ ~9.5 c.p.s.).

Anal. Calc. for $C_{14}H_{21}NO_9$: C, 48.41; H, 6.10; N, 4.03. Found: C, 48.29; H, 6.06; N, 3.84.

The n.m.r. spectrum (in pyridine-d_5) and t.l.c. of this compound were identical to those of the compound obtained by reduction of **9** in methanol containing triethylamine, followed by acetylation in pyridine. After the reaction mixture had been processed in the usual manner, the product was chromatographed on silica gel G

with 4:1 ethyl acetate–acetone as the eluting solvent. In this case, complete removal of the solvents gave **16** in a crystalline form, but it could not be recrystallized.

REFERENCES

1 J. J. Fox, K. A. Watanabe, and A. Bloch, *Progr. Nucleic Acid Res. Mol. Biol.*, 5 (1966) 251.
2 T. Kanzaki, E. Higashide, H. Yamamoto, M. Shibata, K. Nakazawa, H. Iwasaki, T. Takewaka, and A. Miyake, *J. Antibiot.* (Tokyo), *Ser. A*, 15 (1962) 93.
3 S. Takeuchi, K. Hirayama, K. Ueda, H. Sakai, and H. Yonehara, *J. Antibiot.* (Tokyo), *Ser. A*, 11 (1958) 1.
4 J. J. Fox, Y. Kuwada, K. A. Watanabe, T. Ueda, and E. B. Whipple, *Antimicrob. Agents Chemother.*, (1964) 518; J. J. Fox, Y. Kuwada, and K. A. Watanabe, *Tetrahedron Lett.*, (1968) 6029; N. Otake, S. Takeuchi, T. Endo, and H. Yonehara, *Tetrahedron Lett.*, (1965) 1404 and 1411; J. J. Fox and K. A. Watanabe, *Tetrahedron Lett.*, (1966) 897; H. Yonehara and N. Otake, *Tetrahedron Lett.*, (1966) 3785.
5 K. A. Watanabe, M. P. Kotick and J. J. Fox, *Chem. Pharm. Bull.* (Tokyo), 17 (1969) 416.
6 A. R. Williamson and S. Zammenhof, *J. Biol. Chem.*, 238 (1963) 2255; S. Hanessian and T. H. Haskell, *J. Biol. Chem.*, 239 (1964) 2758, and references therein.
7 K. Heyns, G. Kiessling, W. Lindenberg, H. Paulsen, and M. E. Webster, *Chem. Ber.*, 92 (1959) 2435.
8 H. R. Perkins, *Biochem. J.*, 86 (1963) 475.
9 K. Heyns and H. Paulsen, *Chem. Ber.*, 88 (1955) 188.
10 K. Heyns and M. Beck, *Chem. Ber.*, 90 (1957) 2443.
11 (*a*) K. Isono and S. Suzuki, *Tetrahedron Lett.*, (1968) 203; (*b*) A. Tsuji, T. Kinoshita, and M. Maeda, *Chem. Pharm. Bull.* (Tokyo), 16 (1968) 539.
12 J. L. Frahn and J. A. Mills, *Aust. J. Chem.*, 18 (1965) 1303.
13 E. J. Reist, R. R. Spencer, D. F. Calkins, B. R. Baker, and L. Goodman, *J. Org. Chem.*, 30 (1965) 2321.
14 C. L. Mehltretter, B. H. Alexander, R. L. Mellies, and C. E. Rist, *J. Amer. Chem. Soc.*, 73 (1951) 2424.
15 M. Stacey, *J. Chem. Soc.*, (1939) 1529.
16 F. G. Fischer and H. Schmidt, *Chem. Ber.*, 92 (1959) 2184.
17 M. B. Perry and R. K. Hulyalkar, *Can. J. Biochem.*, 43 (1965) 573.
18 B. J. Hunt and W. Rigby, *Chem. Ind.* (London), (1967) 1868.

Carbohydrate Research
Elsevier Publishing Company, Amsterdam
Printed in Belgium

THE CONVERSION OF BENZYL 3-*O*-BENZOYL-4,6-*O*-BENZYLIDENE-β-D-GALACTOPYRANOSIDE INTO THE 2-BENZOATE BY ACYL MIGRATION

G. J. F. CHITTENDEN AND J. G. BUCHANAN

Microbiological Chemistry Research Laboratory, Department of Organic Chemistry, The University, Newcastle upon Tyne NE1 7RU (Great Britain)

(Received April 25th, 1969)

ABSTRACT

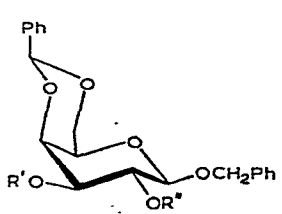
Benzoylation of benzyl 4,6-*O*-benzylidene-β-D-galactopyranoside (**1**) with one equivalent of benzoyl chloride in pyridine–dichloromethane solution yields preferentially the 3-benzoate **3**. Under alkaline conditions, benzoate **3** can be converted, in high yield, into the 2-benzoate **5**, thus providing an example of acyl migration towards C-1.

INTRODUCTION

The 2- and 3-benzoates of benzyl 4,6-*O*-benzylidene-β-D-galactopyranoside were required as potential intermediates in the synthesis of D-galactose 2-phosphate and D-galactose 3-phosphate[1]. Although the phosphates were finally synthesized by an alternative route[1], we now report some points of interest arising from the benzoates themselves.

RESULTS

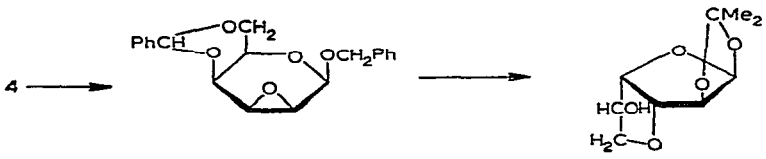
Treatment of benzyl 4,6-*O*-benzylidene-β-D-galactopyranoside[2,3] (**1**) with one equivalent of benzoyl chloride, in pyridine–dichloromethane solution at 0°, gave a crystalline monobenzoate, m.p. 179–180°, as the main product. Thin-layer chromatography (t.l.c.) of the crude product showed the presence of unreacted diol **1**, dibenzoate **2**, and a monobenzoate with R_F value lower than that of the major product. The diol was easily recovered from the mixture, and the yield, allowing for this, was 64–78%.



1 R′ = R″ = H
2 R′ = R″ = Bz
3 R′ = Bz, R″ = H
4 R′ = Bz, R″ = Ts
5 R′ = H, R″ = Bz
6 R′ = Ts, R″ = Bz

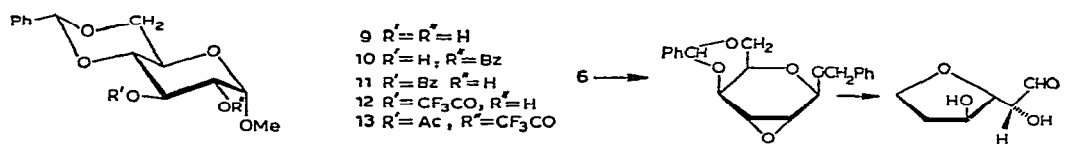
The crystalline monobenzoate was assigned structure **3**, based on the following evidence. Toluene-*p*-sulphonylation yielded a sulphonate **4**, m.p. 171–172°, which

was converted into the epoxide **7** by means of sodium methoxide in methanol. It has been established[4] that 3,6-anhydrohexoses are major reaction products when methyl 2,3-anhydrohexopyranosides are hydrolyzed in dilute sulphuric acid. Treatment of epoxide **7** under these conditions led to exactly the same products as were formed by hydrolysis of methyl 2,3-anhydro-β-D-talopyranoside. The two hydrolysates showed identical paper-chromatographic behaviour, 3,6-anhydroidose being the major product, together with traces of idose and galactose. On a larger scale, 3,6-anhydro-1,2-*O*-isopropylidene-β-D-idofuranose[4] (**8**) was isolated after treatment of the hydrolysis products with acetone and sulphuric acid. This proved that the epoxide had the *talo* configuration (**7**) and that the benzoate is the 3-benzoate **3**. The possibility of benzoyl group migration prior to sulphonylation[5] was precluded by showing that the monobenzoate formed no other products (t.l.c.) when allowed to stand in dry pyridine solution for 7 days. Further work on the isomeric monobenzoate (see below) confirms the assignment.



7 8

Bourne *et al.*[6] found that treatment of methyl 4,6-*O*-benzylidene-α-D-glucopyranoside 2-benzoate (**10**) with alkali gave the isomeric 3-benzoate (**11**) in high yield. Under similar conditions, the 3-benzoate **3** gave an isomeric compound, m.p. 188–189°, in high yield, which was shown to be the 2-benzoate **5**. Toluene-*p*-sulphonylation of benzoate **5** gave the sulphonate **6** which, with sodium methoxide in methanol, yielded the anhydroguloside **14**. Paper chromatograms of the dilute acid hydrolysates of compound **14** and of authentic methyl 2,3-anhydro-4,6-*O*-benzylidene-α-D-gulopyranoside were indistinguishable[4]. The formation of 3,6-anhydro-D-galactose (**15**) was substantiated by the isolation of 3,6-anhydro-D-*lyxo*-hexulose phenylosazone after treatment of the hydrolysate of **14** with phenylhydrazine[4].



14 15

With one equivalent of benzoic anhydride, glycoside **1** yielded a mixture of the dibenzoate **2**, the two monobenzoates, **3** and **5**, and the unreacted diol. No single component preponderated (t.l.c.), and attempted fractional crystallization was ineffective. In view of the comparative ease of obtaining the two esters by the earlier methods, no attempt was made to separate this mixture chromatographically.

DISCUSSION

Differences in the reactivities of the secondary hydroxyl groups of carbohydrates towards acylating agents are well recognized[7,8]. The alkyl 4,6-*O*-benzylidene-aldohexopyranosides, in which only the C-2 and C-3 hydroxyl groups are available for reaction, have shown interesting variations in reactivity order on acylation[9-17]. Methyl 4,6-*O*-benzylidene-α-D-glucopyranoside (**9**), for example, undergoes preferential acylation of the C-2 hydroxyl group by benzoyl chloride or toluene-*p*-sulphonyl chloride in pyridine[9-11]. Reichstein and his co-workers found, however, that 3-esters were the major products when methyl 4,6-*O*-benzylidene-α (or β)-D-galactopyranoside was treated with one equivalent of an acid chloride in pyridine[14-17], and the present results are in agreement with this.

Lemieux and McInnes[18] showed that, in 1,4:3,6-dianhydro-D-glucitol, it is the sterically hindered *endo*-5-hydroxyl group which reacts preferentially with toluene-*p*-sulphonyl chloride in pyridine. Only this hydroxyl group is capable of intramolecular hydrogen bonding[18], and subsequent work by Foster and his colleagues[19,20] has shown that this effect is important in the selectivity of the reaction. Hughes and Speakman[21] have invoked a similar hydrogen-bonding effect to explain the results of toluene-*p*-sulphonylation of 1,2-*O*-isopropylidene-α-D-ribofuranose compared with the *xylo* isomer. Schwarz[22] has suggested that the reactivity of the 2-hydroxyl group in methyl α-D-glucopyranosides, towards acyl chlorides in pyridine[8,11,23], is due to formation of an intramolecular hydrogen bond with the axial C-1 methoxyl group. The formation of the 3-benzoate **3**, from glycoside **1**, would then be due to hydrogen bonding of the equatorial 3-hydroxyl group to the axial oxygen atom on C-4. It may be significant that Reichstein[14-17], with the methyl 4,6-*O*-benzylidenegalactosides, found, in most cases, a higher proportion of 3-ester in the β- than in the α-series in which both 2- and 3-hydroxyl groups may form a hydrogen bond. It is interesting, also, that toluene-*p*-sulphonylation of the 3-benzoate **3** required a longer period (8–10 days) than the 2-benzoate **5** (2 days). The difference in selectivity of benzoyl chloride and benzoic anhydride, in pyridine, towards glycoside **1** is not surprising in view of earlier work[11,20].

The generalization has been made[24] that acyl migrations in carbohydrate esters takes place in a direction away from C-1, but exceptions exist[25], and there seems to be no general theoretical basis for this[26,27]. The conversion of **3** into **5** is a case of migration from O-3 to O-2 in a pyranoside. Another case was the reported migration of a trifluoroacetyl group during conversion of **12** into **13** by means of acetic anhydride in pyridine[28,29], but the evidence is less direct. Lemieux[26] and Angyal[27] have emphasized the reversibility of acyl migration and the influence of external factors on the apparent equilibrium. The conversion of **3** into **5** was carried out under non-equilibrium conditions, in that **5**, the less soluble isomer, crystallized during the reaction. The high yield of **5** was not necessarily a consequence of the greater stability of **5** compared to **3**. We therefore examined the interconversion of **3** and **5** in aqueous acetone under homogeneous conditions[27]. Examination of the

neutralised reaction mixture by t.l.c. showed that the 2-benzoate **5** was the more stable, but only to a slight extent. When the interconversion of **10** and **11** was examined in the same way, there was no strong preference for the 3-benzoate **11**, despite the high yield of **11** which may be obtained from **10** under crystallizing conditions[6].

EXPERIMENTAL

I.r. spectra were determined for KBr discs. Paper chromatography was carried out on Whatman No. 1 paper in butyl alcohol–pyridine–water (3:1:1, v/v). Reducing sugars were detected by aniline hydrogen phthalate[30]. Silica gel G (Merck) was used for t.l.c. with benzene–ether (4:1, v/v) as the developing solvent; compounds were detected by charring with sulphuric acid or by exposure to iodine vapour. Evaporations were carried out at 40°, *in vacuo*. All melting points are uncorrected.

*Benzyl 2,3-di-*O*-benzoyl-4,6-*O*-benzylidene-β-*D*-galactopyranoside* (**2**). — A solution of the diol[2,3] **1** in pyridine was treated with an excess of benzoyl chloride for 4 days at 0° in the usual manner. The crude product was recrystallized from ethanol to give the dibenzoate **2** (69%), m.p. 177–178°, $[\alpha]_D^{21}$ +118° (*c* 1.2, chloroform) (Found: C, 71.7; H, 5.5; $C_{34}H_{30}O_8$ calc.: C, 72.1; H, 5.3%).

*Benzyl 3-*O*-benzoyl-4,6-*O*-benzylidene-β-*D*-galactopyranoside* (**3**). — A solution of the diol **1** (12.06 g) in anhydrous pyridine (35 ml) and dichloromethane (65 ml) was treated dropwise, with stirring at −5°, with a solution of benzoyl chloride (4.68 g, 0.99 mol.) in dry dichloromethane (60 ml) during 1.5 h. The mixture was kept overnight at 0° and then for 2 days at room temperature. It was then diluted with dichloromethane (100 ml) and extracted successively with 3N sulphuric acid (2 × 250 ml), saturated, aqueous sodium hydrogen carbonate (200 ml), and water. Evaporation of the dried (sodium sulphate) extract gave a colourless residue which was treated with boiling benzene, and the solution was allowed to cool overnight to room temperature. A colourless crystalline solid was removed by filtration, washed with benzene, and recrystallized from methanol to yield the unreacted diol **1** (4.2–4.8 g), m.p. 207–209°.

The combined benzene filtrate and washings were evaporated to dryness, and the residue was recrystallized from propan-2-ol to give the 3-benzoate **3** (6.0–7.3 g, 64–78% based on reacted diol), m.p. 179–180°, $[\alpha]_D^{22}$ +64.6° (*c* 1.6 chloroform), ν_{max} 1695 cm^{-1} (hydrogen bonded COPh) (Found: C, 69.8; H, 5.7. $C_{27}H_{26}O_7$ calc.: C, 70.1; H, 5.7%).

*Benzyl 3-*O*-benzoyl-4,6-*O*-benzylidene-2-*O*-toluene-*p*-sulphonyl-β-*D*-galacto-pyranoside* (**4**). — A solution of the 3-benzoate **3** (1.1 g) in dry pyridine (10 ml) was treated with toluene-*p*-sulphonyl chloride (1.0 g) for 8–10 days at room temperature and then poured into ice–water (120 ml). The resulting buff-coloured precipitate was recrystallized from propan-2-ol–acetone and then from ethanol to give **4** (1.2 g, 81.5%), m.p. 171–172°, $[\alpha]_D^{21}$ +89.5° (*c* 0.95, chloroform) (Found: C, 66.5; H, 5.1. $C_{34}H_{32}O_{10}S$ calc.: C, 66.2; H, 5.2%).

Benzyl 2,3-anhydro-4,6-O-benzylidene-β-D-talopyranoside (**7**). — A solution[2] of the sulphonate **4** (1.4 g) in methanol (35 ml) was treated with 2.7N sodium methoxide (9 ml) and then heated under reflux for 75 min. During the course of the reaction, a precipitate was formed. At the end of this time, water (25 ml) was added, and the mixture was kept for 1 h at 0°. The resulting, white solid was recrystallized from ethanol–acetone to give **7** (0.7 g, 93%), m.p. 207–209°, $[\alpha]_D^{22}$ −88° (*c* 1.23, *N,N*-dimethylformamide) (Found: C, 70.4; H, 5.7. $C_{20}H_{20}O_5$ calc.: C, 70.6; H, 5.9%).

Hydrolysis of **7**. — (*a*) The epoxide **7** (10 mg) in *p*-dioxane (2 ml) was heated in a sealed tube with 0.1N sulphuric acid (2 ml) for 3 h at 100°. The cooled hydrolysate was neutralized ($BaCO_3$) and centrifuged, and the supernatant was evaporated to dryness. The residue was dissolved in water (0.5 ml) and examined by paper chromatography. The main products were 3,6-anhydroidose and idose, together with a trace of galactose, and were identical with those from the hydrolysis of methyl 2,3-anhydro-β-D-taloside[4].

(*b*) The epoxide **7** (0.8 g) in *p*-dioxane (10 ml) and 0.1N sulphuric acid (12 ml) was heated on a water bath for 3 h at 100°. The solution was neutralized ($BaCO_3$), filtered, and evaporated to a syrup, which was dehydrated by re-evaporation with several portions of benzene and finally by storage *in vacuo* over P_4O_{10}. The dry syrup was shaken with acetone (220 ml) containing conc. sulphuric acid (1.1 ml) for 30 min. The solution was neutralized with solid, anhydrous sodium carbonate (22 g) overnight. The filtered solution was evaporated, and the residue was recrystallized several times from benzene to give 3,6-anhydro-1,2-*O*-isopropylidene-β-D-idofuranose (**8**) (0.26 g, 56%), m.p. and mixed m.p. 102–105°, $[\alpha]_D^{22}$ −23.8° (*c* 1.3, water). The i.r. spectrum was identical with that of an authentic sample[4].

Benzyl 2-O-benzoyl-4,6-O-benzylidene-β-D-galactopyranoside (**5**). — A solution of the 3-benzoate **3** (1.0 g) in acetone (50 ml) was treated with 0.05N sodium hydroxide (50 ml). A precipitate formed immediately, and the mixture was allowed to stand for a further 1 min at room temperature, whereupon ice–water (40 ml) was added. The precipitated product was collected by filtration, washed well with water, and dried *in vacuo*. Recrystallization from ethanol gave the benzoate **5** (0.81 g, 81%), m.p. 188–189°, $[\alpha]_D^{21}$ +24° (*c* 1.0, chloroform), ν_{max} 1733 cm^{-1} (COPh) (Found: C, 69.8; H, 5.8. $C_{27}H_{26}O_7$ calc.: C, 70.1; H, 5.7%).

Benzyl-2-O-benzoyl-4,6-O-benzylidene-3-O-toluene-p-sulphonyl-β-D-galactopyranoside (**6**). — A solution of the 2-benzoate **5** (0.4 g) in dry pyridine (10 ml) was cooled to −10° and treated with toluene-*p*-sulphonyl chloride (0.8 g). The mixture was kept overnight at 0° and then for 2 days at room temperature. It was then poured into ice–water (60 ml), and the resulting, pink precipitate was recrystallized from propan-2-ol to give **6** (0.48 g, 89.5%), m.p. 184–185°, $[\alpha]_D^{22}$ +63° (*c* 2.0, chloroform) (Found: C, 66.5; H, 5.0. $C_{34}H_{32}O_{10}S$ calc.: C, 66.2; H, 5.2%).

Benzyl 2,3-anhydro-4,6-O-benzylidene-β-D-gulopyranoside (**14**). — A solution of the sulphonate **6** (0.33 g) in dry methanol (7 ml) was treated with 2.7N sodium methoxide (2 ml) and then heated under reflux for 90 min. The mixture became pale yellow during the course of the reaction. At the end of this time, water (10 ml) was added,

and the methanol was removed *in vacuo*. The aqueous residue was extracted with chloroform (5 × 50 ml), and the combined extracts were washed well with water. Evaporation of the dried (sodium sulphate) extracts gave a cream residue that was recrystallized from propan-2-ol to give **14** as needles, (0.13 g, 73%), m.p. 151–152° (sublimed), $[\alpha]_D^{21}$ −124° (*c* 0.55, chloroform) (Found: C, 71.1; H, 6.2. $C_{20}H_{20}O_5$ calc.: C, 70.6; H, 5.9%).

Hydrolysis of **14**. — (*a*) The epoxide **14** (10 mg) was hydrolyzed with 0.1N sulphuric acid (1.1 ml) in a sealed tube for 1 h at 100°. After neutralization with barium carbonate, the product was examined by paper chromatography. The main product was 3,6-anhydrogalactose, together with traces of galactose and idose, and the chromatogram was indistinguishable from one of a similar hydrolysate of methyl 2,3-anhydro-4,6-*O*-benzylidene-α-D-gulopyranoside[4].

(*b*) The epoxide **14** (0.5 g) and 0.1N sulphuric acid (10 ml) were heated for 1.5 h at 100°. After cooling, the mixture was extracted with ether, and to the aqueous fraction was added acetic acid (1.5 ml), anhydrous sodium acetate (0.7 g), and phenylhydrazine hydrochloride (0.7 g). After 30 min at 100°, the mixture was cooled and then left at 0° overnight. The precipitate was collected, washed with water, 2N acetic acid, and finally ice-cold methanol. The yellow crystals (0.054 g) had m.p. 212–215° and an i.r. spectrum identical with that of authentic 3,6-anhydro-D-*lyxo*-hexulose phenylosazone[4], m.p. 213–215°; a mixed m.p. was not depressed.

Treatment of benzyl 4,6-O-benzylidene-β-D-galactopyranoside (**1**) *with benzoic anhydride*. — A solution of the diol **1** (2.0 g) in dry pyridine (15 ml) was treated portionwise at 0° with benzoic anhydride (1.26 g, 1 mol.) during 1 h. The mixture was kept overnight at 0° and then for 2 days at 37°. It was then poured into ice–water (200 ml), and the resulting, white precipitate was collected by filtration and dissolved in chloroform (100 ml), and the extract was washed successively with 3N sulphuric acid (2 × 100 ml), saturated, aqueous sodium hydrogen carbonate (2 × 100 ml), and water. Concentration of the dried extract gave a white residue. Treatment with benzene, followed by recrystallization from propan-2-ol as described above for the 3-benzoate **3**, gave a crystalline product. T.l.c. indicated that the material was an intimate mixture of the two monobenzoates **3** and **5** and the dibenzoate **2**.

Treatment of benzyl 4,6-O-benzylidene-β-D-galactopyranoside monobenzoates with dilute alkali under homogeneous conditions. — A solution of the benzoate (**3** or **5**, 200 mg) in acetone (60 ml) was treated with 0.05N sodium hydroxide (20 ml) for 1 min at room temperature. The mixture was neutralized with acetic acid in acetone and evaporated to dryness. The product was dissolved in dichloromethane (25 ml), washed with water (2 × 10 ml), dried (sodium sulphate), and evaporated to give a white residue. Examination by t.l.c. showed that equilibrium between **3** and **5** had been established with a slight (*ca.* 10%) preponderance of **5**.

A similar experiment was carried out with the monobenzoates[11,16] **10** and **11**. At equilibrium, there appeared to be about equal amounts of **10** and **11**.

ACKNOWLEDGMENTS

We thank Dr. N. A. Hughes for helpful discussions, and Professor J. Baddiley, F.R.S., for his interest.

REFERENCES

1 G. J. F. CHITTENDEN, W. K. ROBERTS, J. G. BUCHANAN, AND J. BADDILEY, *Biochem. J.*, 109 (1968) 597.
2 E. FISCHER AND B. HELFERICH, *Ann.*, 383 (1911) 68.
3 J. R. TURVEY AND T. P. WILLIAMS, *J. Chem. Soc.*, (1962) 2119.
4 J. G. BUCHANAN AND J. CONN, *J. Chem. Soc.*, (1965) 201.
5 S. J. ANGYAL, P. T. GILHAM, AND G. J. H. MELROSE, *J. Chem. Soc.*, (1965) 5252.
6 E. J. BOURNE, A. J. HUGGARD, AND J. C. TATLOW, *J. Chem. Soc.*, (1953) 735.
7 J. M. SUGIHARA, *Advan. Carbohyd. Chem.*, 8 (1953) 1.
8 J. M. WILLIAMS AND A. C. RICHARDSON, *Tetrahedron*, 23 (1967) 1369.
9 G. J. ROBERTSON AND C. F. GRIFFITH, *J. Chem. Soc.*, (1935) 1193.
10 H. R. BOLLIGER AND D. A. PRINS, *Helv. Chim. Acta*, 28 (1945) 465.
11 R. W. JEANLOZ AND D. A. JEANLOZ, *J. Amer. Chem. Soc.*, 79 (1957) 2579.
12 C. P. J. GLAUDEMANS AND H. G. FLETCHER, JR., *Carbohyd. Res.*, 7 (1968) 480.
13 J. G. BUCHANAN AND J. C. P. SCHWARZ, *J. Chem. Soc.*, (1962) 4770.
14 E. SORKIN AND T. REICHSTEIN, *Helv. Chim. Acta*, 28 (1945) 1.
15 M. GYR AND T. REICHSTEIN, *Helv.. Chim. Acta*, 28 (1945) 226.
16 R. REBER AND T. REICHSTEIN, *Helv. Chim. Acta*, 28 (1945) 1164.
17 A. C. MAEHLY AND T. REICHSTEIN, *Helv. Chim. Acta*, 30 (1947) 496.
18 R. U. LEMIEUX AND A. G. MCINNES, *Can. J. Chem.*, 38 (1960) 136.
19 K. W. BUCK, A. B. FOSTER, A. R. PERRY, AND J. M. WEBBER, *J. Chem. Soc.*, (1963) 4171.
20 K. W. BUCK, J. M. DUXBURY, A. B. FOSTER, A. R. PERRY, AND J. M. WEBBER, *Carbohyd. Res.*, 2 (1966) 122.
21 N. A. HUGHES AND P. R. H. SPEAKMAN, *Carbohyd. Res.*, 1 (1966) 341.
22 J. C. P. SCHWARZ, Personal communication.
23 R. C. CHALK, D. H. BALL, AND L. LONG, *J. Org. Chem.*, 31 (1966) 1509.
24 F. BROWN, L. HOUGH, AND J. K. N. JONES, *J. Chem. Soc.*, (1950) 1125.
25 W. A. BONNER, *J. Org. Chem.*, 24 (1959) 1388.
26 R. U. LEMIEUX, in P. DE MAYO (Ed.), Molecular Rearrangements, Part 2, Interscience, New York, 1964, pp. 763–768.
27 S. J. ANGYAL AND G. J. H. MELROSE, *J. Chem. Soc.*, (1965) 6494.
28 E. J. BOURNE, M. STACEY, C. E. M. TATLOW, AND J. C. TATLOW, *J. Chem. Soc.*, (1951) 826.
29 D. HORTON AND J. H. LAUTERBACK, *J. Org. Chem.*, 34 (1969) 86.
30 S. M. PARTRIDGE, *Nature*, 164 (1949) 443.

Carbohydrate Research
Elsevier Publishing Company, Amsterdam
Printed in Belgium

ENZYMIC DEGRADATION OF YEAST CELL-WALL MANNANS AND GALACTOMANNANS TO POLYMERIC FRAGMENTS CONTAINING α-(1→6)-LINKED D-MANNOPYRANOSE RESIDUES*

P. A. J. GORIN, J. F. T. SPENCER, AND D. E. EVELEIGH
National Research Council of Canada, Prairie Regional Laboratory, Saskatoon, Saskatchewan (Canada)

(Received, May 6th, 1969)

ABSTRACT

The cell-wall D-mannans of *Candida parapsilosis*, *Endomycopsis fibuliger*, *Saccharomyces rouxii*, *Torulopsis apicola* (Hajsig strain), and *Torulopsis bombi* were degraded with an exo α-D-mannosidase from *Arthrobacter* GJM-1 to their α-(1→6)-linked D-mannopyranose main-chains, as demonstrated by p.m.r. spectroscopy. D-Galacto-D-mannans from *Candida lipolytica*, *Torulopsis gropengiesseri*, *Torulopsis lactis-condensi*, *Torulopsis magnoliae*, *and Trichosporon fermentans* could be degraded to polysaccharides containing mainly 6-*O*-linked α-D-mannopyranosyl residues following preferential removal of their enzyme-resistant, D-galactopyranosyl non-reducing end-units with acid. The D-mannans of *Saccharomyces lodderi*, *Citeromyces matritensis*, and *Pichia pastoris* could also be enzymically degraded to polysaccharides containing predominantly α-(1→6)-linked D-mannopyranosyl residues after hydrolysis of most of the β-D-linked residues in their side chains with acid. The exo α-D-mannosidase, as would be expected, produced β-D-mannose on splitting of an α-(1→2)-linked D-mannopyranose tetramer. It is, however, very selective in its action since it did not cleave α-D-Man*p*-(1→2)-β-D-Man*p*-(1→2)-β-D-Man*p*-(1→2)-α-D-Man*p*-(1→2)-D-Man. Apparently a D-mannopyranose non-reducing end-unit and two consecutive α-D-mannopyranose residues are required by the enzyme for cleavage of a substrate to take place.

INTRODUCTION

Recently, work has been carried out on the identification and classification of yeasts by the chemical structures of their cell-wall polysaccharides. A D-mannan[1-4], D-galacto-D-mannan[4,5], or a D-mannose-containing heteropolymer[6,7] may be isolated from nearly all species following extraction of the cell-wall with hot aqueous alkali. From all of the yeasts investigated, the polysaccharides contain a main chain consisting of D-mannopyranose residues, which can be linked α-D-(1→3), α-D-(1→6) or can contain alternating β-D-(1→3) and β-D-(1→4) linkages. Two of the three

*Issued as NRCC No. 10976.

main-chain types, the α-(1→3)-D-mannopyranose[6,7] and the alternating β-(1→3) and β-(1→4)-D-mannopyranose[1] are readily characterized by chemical techniques. The former type of main chain can be isolated from a branched polysaccharide by removal of the side chains by one or more Smith degradations[8], the number required depending on the chemical structure of the side chains. The latter type of β-D-linked chain has only been found as a linear polysaccharide[1]. On the other hand, α-(1→6)-D-mannopyranose main chains have been unambiguously demonstrated in only a few D-mannans [3,4] and D-galacto-D-mannans [4,5]. The standard techniques used in structural studies on the polysaccharides give, in most cases, equivocal results. For example, the D-mannan of *Saccharomyces rouxii*, on methylation followed by hydrolysis, gives 2,3,4,6-tetra-*O*-, 3,4,6-tri-*O*-, and 3,4-di-*O*-methyl-D-mannose in an approximately 1:1:1 molar ratio. Coupled with the isolation of an α-(1→2)-linked D-mannopyranose trisaccharide on partial acetolysis, structures of the D-mannan can be proposed varying from I, having an α-D-(1→6)-linked main chain to II having a main chain containing α-D-(1→2) and α-D-(1→6) linkages[9,10]. Gorin and associates have recently investigated the structures of several other D-mannans and D-galacto-D-mannans and have shown, by similar methylation–fragmentation and partial acetolysis techniques, that they contain D-mannopyranose residues which exist in the 2-*O*-, 6-*O*-, and 2,6-di-*O*-substituted forms and as non-reducing end-units, and contain successive α-D-(1→2) linkages[2-5].

A technique has now been provided by G. H. Jones and C. E. Ballou that can lead to the isolation of an α-D-(1→6)-linked mannan chain from D-mannans following enzymolysis[11,12]. *Arthrobacter* GJM-1, a soil bacterium, when grown on a medium containing the D-mannan of baker's yeast (*Saccharomyces cerevisiae*), produces an inducible, exocellular α-D-mannosidase that can preferentially remove individual D-mannose substituents of the side chains of the yeast D-mannan. Similar degradations were carried out on D-mannans from *Candida stellatoidea* and *Kloeckera brevis*. The present publication describes (*A*) the properties of crude exo α-D-mannosidase (which differ somewhat from those of the purified enzyme used by Jones and Ballou), (*B*) the degradation of several D-mannans and D-galacto-D-mannans (previously partly characterized) to fragments containing predominantly α-D-(1→6)-linked D-mannopyranosyl residues, and (*C*) the characterization of β-D-mannose as the hexose anomer formed on enzymolysis of an α-(1→2)-linked, D-mannopyranose tetrasaccharide.

RESULTS AND DISCUSSION

Properties of the crude exo α-D-mannosidase from Arthrobacter GJM-*1*. — Jones and Ballou[11,12] degraded D-mannans with a purified exocellular exo α-D-mannosidase preparation from *Arthrobacter GJM*-1. Since the crude enzyme has the required property of removing side-chain residues from D-mannans, a purification step was not carried out for the present investigations. However, some differences in properties between the two preparations were observed. It was found that the crude α-D-mannosidase degraded the α-D-(1→6)-linked mannan main chains of *Schizo-*

saccharomyces octosporus D-galacto-D-mannan and *S. rouxii* D-mannan, whereas the purified preparation of Jones and Ballou only removes D-mannose side chains[12]. Their enzyme preparation can attack α-D-(1→6)-linked mannose oligosaccharides, but not the main chain of *S. cerevisiae* D-mannan. Initially our degradations of the D-mannans of *S. octosporus* and *S. rouxii* proceeded at rates that were somewhat less than that for the D-mannan of *S. cerevisiae*, as evidenced by the production of D-mannose (Fig. 1). Only limited degradations of each of the two main chains took

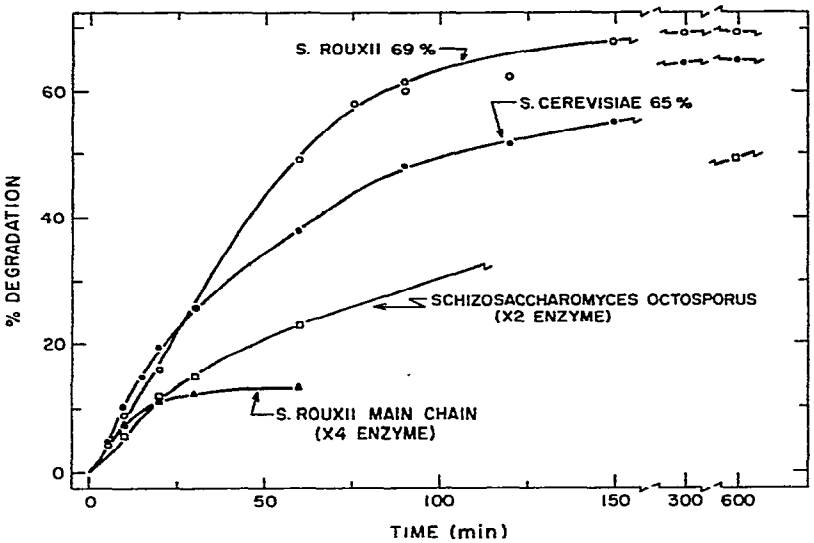


Fig. 1. Degradation of yeast D-mannans with α-D-mannosidase. The relative initial degradation rates were: yeast D-mannan (*S. cerevisiae*), 1.0; *S. rouxii* D-mannan, 0.9; *S. octosporus* main chain, 0.285; *S. rouxii* main chain, 0.18.

place, presumably because the exo enzyme reached resistant units in the chains. Since the main chains were partly vulnerable to attack, a limited degradation time of 10 h was used (activity of crude enzyme in 1% solution, 0.169 units/ml; specific activity, 0.422). This is sufficient to degrade the 7-unit, α-(1→2)-linked, D-mannopyranosyl side chain of *T. bombi* mannan completely. The corresponding α-D-(1→2)-linked octasaccharide isolated following partial acetolysis undergoes complete degradation to D-mannose in 6 h.

The crude preparation contains enzymes other than α-D-mannosidase. Jones and Ballou, when using a purified α-D-mannosidase lacking phosphatase and protease activity[11], noted that the extent to which several D-mannans from *Candida* spp. were hydrolyzed was inversely proportional to their phosphorus content[12]. (*Kloeckera brevis* D-mannan, which is heavily phosphorylated, proved an exception). Disc-electrophoretic examination of our crude enzyme at pH 8.3 showed six major and seven minor protein components. One of these components was an active alkaline phosphatase [a 1% solution of the crude lyophilized enzyme had 3.78 units/ml at pH 7.0 (4.12 units/ml at pH 8.5), specific activity 9.45].

B. *Enzymolysis of* D-*mannans and acid-degraded* D-*galacto*-D-*mannans and* D-*mannans.* — Some of the D-mannans, for which some structural information is available, could be degraded with the crude exo α-D-mannosidase to their α-(1→6)-linked D-mannopyranose main chains. These are from *Endomycopsis fibuliger*[4], *Saccharomyces rouxii*[9,10], *Torulopsis apicola* (Hajsig strain), *Torulopsis bombi*[4], and *Candida parapsilosis*[13]. The end-products were characterized, following their isolation by deionization followed by ethanol precipitation, by the H-1 proton magnetic resonance (p.m.r.) signal at τ 4.57 (*J* 1 Hz). This signal has the same chemical shift as that of H-1 of α-D-(1→6)-linked mannans formed by partial acid hydrolysis of the D-galacto-D-mannan of *Schizosaccharomyces octosporus*[3], and by partial enzymolysis of the D-mannan of *S. cerevisiae*[12]. Addition of sodium borate shifts the signal downfield by approximately 10 Hz because of the formation of a 2,3-substituted borate complex[2]. These properties distinguish the H-1 signal from those of α-D-(1→2)-linked (τ 4.20) and α-D-(1→3)-linked (τ 4.35) polymers, which do not exhibit such a large downfield shift on the addition of borate[2].

The above direct enzymolysis technique could not be used on D-galacto-D-mannans having D-galactosyl side chains and D-mannans containing β-linkages in the side chains. D-Galacto-D-mannans containing D-galactopyranose non-reducing end-units isolated from *Trichosporon fermentans*[5], *Torulopsis magnoliae*, *Torulopsis gropengiesseri*, *Candida lipolytica*, and *Torulopsis lactis-condensi*[4] were resistant to the action of the crude α-D-mannosidase. Mannans from *Saccharomyces lodderi*, *Citeromyces matritensis*, and *Pichia pastoris*[3], which contain β-linkages in the side chains were similarly resistant. The D-mannan of *Trichosporon aculeatum*[2] appears to have a minor proportion of β-D-linked residues, as evidenced[3] by a small signal (13%) at a relatively high field of τ 4.62. It was only partially degraded by the α-D-mannosidase because of the lack of accessibility of the α-D-linked units. In order to render each of these polysaccharides vulnerable to attack by α-D-mannosidase, it was first necessary to remove the D-galactopyranosyl residues or β-D-linked residues. This was accomplished by partial acid hydrolysis with 0.33N sulfuric acid at 100°.

The α-D-galactopyranosyl end-units in *T. fermentans*, *C. lipolytica*, and *T. lactis-condensi* D-galacto-D-mannans were mostly removed by partial hydrolysis, only traces of D-galactose residues remaining in the degraded polysaccharides. β-D-Galactopyranosyl end-units in the D-galacto-D-mannans of *T. gropengiesseri* and *T. magnoliae* were indicated by methylation–fragmentation analyses and the D-galacto-D-mannans appear to be β-D-linked since acid degradation gives polymers having higher specific rotations than the starting materials (Table I). β-Linked D-mannopyranosyl residues should be absent since H-1 signals at higher field than τ 4.53 were not detected in the parent D-galacto-D-mannan. On partial hydrolysis the β-D-galactopyranosyl non-reducing end-units were completely removed from *T. gropengiesseri* and *T. magnoliae* D-galacto-D-mannans, but the D-mannan from the latter appeared to be contaminated with a trace of a D-glucan.

The β-linked D-mannopyranosyl side-chain residues in the D-mannans of *S. lodderi*, *C. matritensis*, and *P. pastoris* were mostly removed, since the acid-

TABLE I

YIELD AND SPECIFIC ROTATIONS OF ACID-DEGRADED, CELL-WALL POLYSACCHARIDES AND THE OVERALL YIELDS OF ENZYME-DEGRADED POLYSACCHARIDES

Polysaccharide[a] *(reference)*	*Acid-degraded* D-*mannan* *Yield* (%)	$[\alpha]_D$, *degrees* (*c*, 0.5%)	*Enzyme-degraded* D-*mannan* *Yield* (%)
D-Galacto-D-mannans			
Candida lipolytica CBS 599 (4)	45	+86	17
Torulopsis gropengiesseri NRRL-Y1445 (4)	22	+74[b]	8
Torulopsis lactis-condensi CBS 52 (4)	29	+77	7
Torulopsis magnoliae CBS 166 (4)	16	+69[c]	3
Trichosporon fermentans PRL 2263 (5)	31	+92	27
Mannans having β-D-linkages			
Citeromyces matritensis CBS 2764 (3)	19	+44	11
Pichia pastoris PRL 63-208 (3)	33	+57	22
Saccharomyces lodderi JPV 193 (3)	47	+69	17
Trichosporon aculeatum IGC 3551 (4)	40	+71	30
α-D-linked mannans			
Candida parapsilosis BMCI (3, 13)			30
Endomycopsis fibuliger NCYC 13 (4)			38
Saccharomyces rouxii PRL 411-64 (9, 10)			22
Torulopsis apicola (Hajsig strain) CBS 2868 (4)			36
Torulopsis bombi 319-67 (4)			11

[a]CBS, Centraalbureau voor Schimmelcultures, Yeast Division, Delft, The Netherlands; PRL (also BMC), Prairie Regional Laboratory, National Research Council, Saskatoon, Saskatchewan, Canada; NCYC, National Collection of Yeast Cultures, Brewing Industry Research Foundation, Nutfield, Surrey, England; JPV, Collection of J. P. van der Walt, Pretoria, South Africa; IGC, Gulbenkian Institute of Science, Oerias, Portugal; NRRL, Northern Regional Research Laboratory, USDA, Peoria, Illinois, USA. [b]Undegraded polysaccharide[4] has $[\alpha]_D$ +46°. [c]Undegraded polysaccharide[4] has $[\alpha]_D$ +42°.

degraded D-mannans had specific rotations close to the value of +88° reported for an α-D-linked mannan[14]. In addition, the H-1 p.m.r. signals of the degraded D-mannans contain greatly decreased proportions of the high-field signals that are characteristic of β-D-mannopyranoside linkages (Fig. 2). The yields of acid-degraded polysaccharides are presented in Table I.

Most of the above acid-degraded polysaccharides could then be degraded by the α-D-mannosidase to polysaccharides giving predominantly H-1 signals at approximately τ 4.57, which were shifted downfield by addition of sodium borate. The lack of complete degradation to pure α-(1→6)-linked D-mannopyranose main chains is probably due to the incomplete removal of α-D-mannosidase-resistant, side-chain residues. The H-1 p.m.r. spectra of the polysaccharide substrates, the degraded polysaccharides formed on partial acid hydrolysis, and the products formed on enzymolysis are presented in Fig. 2.

The isolation of fragments consisting predominantly of α-(1→6)-linked D-mannopyranose units from D-mannans of *E. fibuliger*[4], *P. pastoris*, and *C. matri-*

tensis[3] and the D-galacto-D-mannan of *T. fermentans*[5] is useful, since it means that the predominating structures, previously postulated on the basis of chemical evidence,

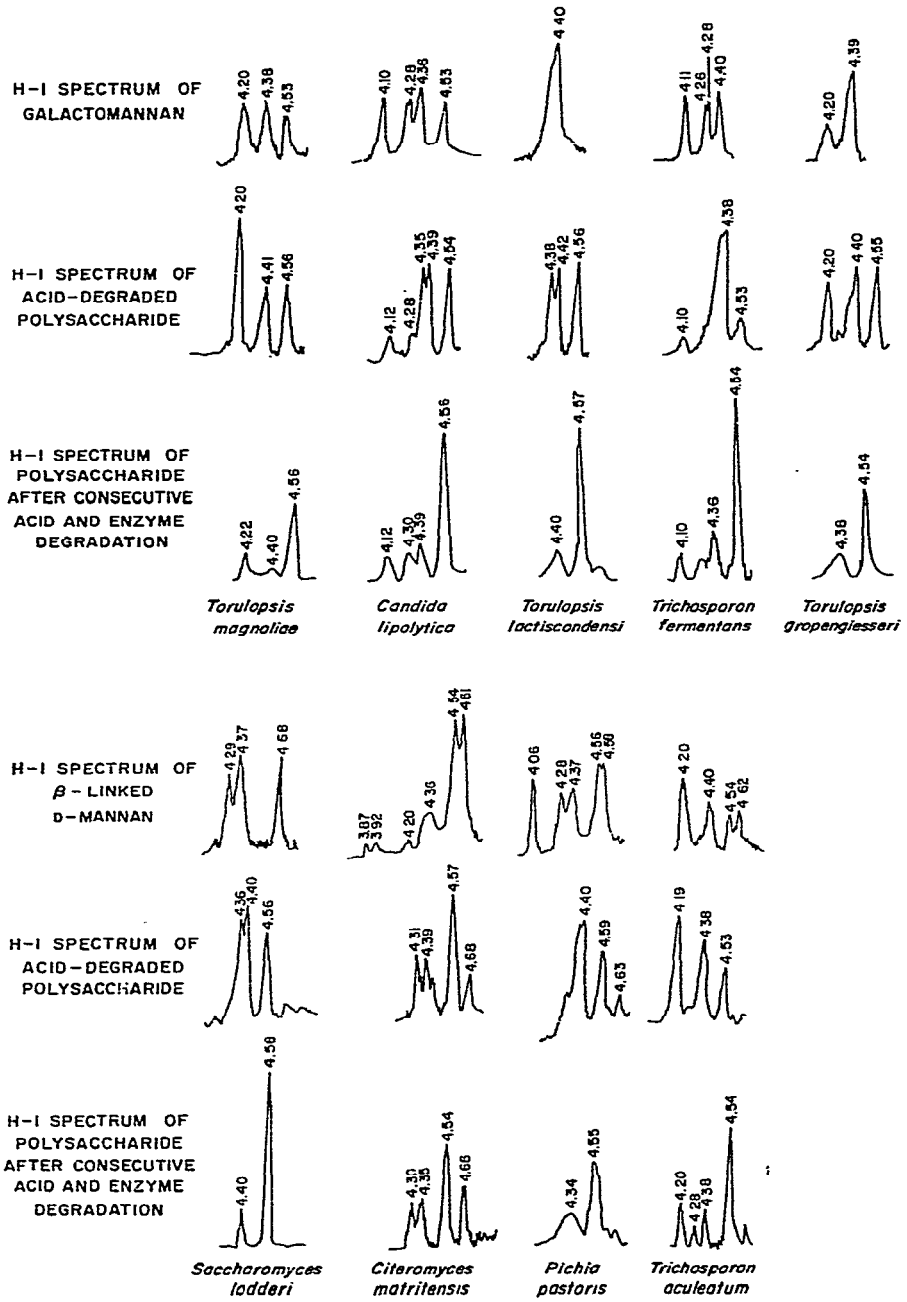


Fig. 2. P.m.r. spectra of D-galacto-D-mannans, β-linked D-mannans and the polysaccharides formed on successive degradations with acid and D-mannosidase.

TABLE II

NATURE OF SIDE CHAINS IN D-MANNOSE-CONTAINING POLYSACCHARIDES HAVING α-(1→6)-D-MANNOPYRANOSE MAIN CHAINS

Source of D-*mannose-containing polysaccharides*	*Largest side-chain substituents linked* α-D-(1→2) *to* α-(1→6)-D-*mannopyranose main chain*	*Polysaccharide contains* 6-O-*substituted* α-D-*mannopyranose residues according to* τ 4.57 *signal*
D-Galacto-D-mannans		
Candida lipolytica[4]	α-D-Gal*p*-(1→2)-D-Man*p*	+
Torulopsis gropengiesseri[4]	β-D-Gal*p*-(1→?)-α-D-Man*p*-(1→2)-D-Man*p*	−
Torulopsis lactis-condensi[4]	α-D-Gal*p*-(1→6)-α-D-Man*p*-(1→2)-D-Man*p*	−
Torulopsis magnoliae[4]	α-(1→2)-linked, 9-unit D-Man*p* side chain terminated by Gal*p* non-reducing end-unit with β-D-linkage	+
Trichosporon fermentans[5]	α-D-Gal*p*-(1→2)-D-Man*p*	trace
D-Mannans having β-D-linkages		
Citeromyces matritensis[3]	β-D-Man*p*-(1→2)-β-D-Man*p*-(1→2)-D-Man*p* (linked β-D-(1→2)-to main chain)	?
Pichia pastoris[3]	α-D-Man*p*-(1→2)-β-D-Man*p*-(1→2)-β-D-Man*p*-(1→2)-D-Man*p*	?
Saccharomyces lodderi[3]	—	
Trichosporon aculeatum[2]	α-D-Man*p*-(1→2)-α-D-Man*p*-(1→2)-α-D-Man*p*-(1→2)-α-D-Man*p*-(1→2)-D-Man*p*	+
α-Linked D-mannans		
Candida parapsilosis[3,13]	Not known	+
Endomycopsis fibuliger[3]	α-D-Man*p*-(1→3)-D-Man*p* and-D-Man*p*	−
Saccharomyces rouxii[9,10]	α-D-Man*p*-(1→2)-D-Man*p*	−
Torulopsis apicola[4]	α-D-Man*p*-(1→2)-α-D-Man*p*-(1→2)-α-D-Man*p*-(1→2)-α-D-Man*p*-(1→2)-D-Man*p*	+
Torulopsis bombi[4]	α-(1→2)-Linked, 7-unit D-Man*p* side chain.	+

are now confirmed. It should be emphasized, however, that on the basis of present data, the possibility of 6-*O*-substituted branch-points in the side chains is not eliminated and our postulated structures may be oversimplified.

The isolation of main-chain fragments from D-mannose-containing polysaccharides is useful when considered in the light of partial acetolysis data previously published (references presented in Table II). The nature of the oligosaccharides isolated from each polysaccharide show the structures of the side chains that are attached by α-D-(1→2)-links to the α-D-(1→6)-linked mannopyranose main chain (Table II). Some of these polysaccharides contain very long side chains, up to 9 units in length. *Since many yeast* D-*mannans and* D-*galacto*-D-*mannans contain* α-(1→6)-D-*mannopyranose main chains, it is evident that the wide diversity of H-1 p.m.r. spectra from polysaccharides having this backbone is caused by differences in side chain structure.*

Two D-mannans from *Hansenula subpelliculosa* and *Candida catenulata* were only partly degraded by the α-D-mannosidase, with only small increases in the H-1 p.m.r. signal at τ 4.57, characteristic of the α-(1→6)-linked D-mannopyranose chain. In cases where a polysaccharide gives an H-1 p.m.r. signal at τ 4.57, this is taken as evidence of unsubstituted 6-*O*-linked, α-D-mannopyranose main-chain residues. This is of particular interest since the specific rotations of the undegraded D-mannans, +71° and +110°, respectively[4], indicate a preponderance of α-D-mannosidic linkages and few, if any, enzyme-resistant β-D-linkages. It therefore appears that not all α-linked D-mannopyranose residues can be cleaved with the exo α-D-mannosidase.

C. *Mechanism of enzymolysis of* α-(1→2)-*linked* D-*mannopyranose tetramer.* — Oligosaccharide fragments obtained by partial acetolysis of the above polysaccharides, and which contain either β-D-mannopyranose or D-galactopyranosyl residues, were not attacked by the crude α-D-mannosidase. This contrasts with the breakdown of oligosaccharides containing only α-(1→2), or mixed α-(1→2) and α-(1→3) D-mannopyranose residues (refs. 11, 12, and Table III). The resistance of 2-*O*-linked tetra-

TABLE III

SPECIFICITY OF ACTION OF CRUDE EXO α-D-MANNOSIDASE OF OLIGOSACCHARIDES CONTAINING D-MANNOSE RESIDUES

Oligosaccharides degraded by crude α-D-*mannosidase*

1 α-D-Man*p*-(1→2)-D-Man[9]
2 α-D-Man*p*-(1→2)-α-D-Man*p*-(1→2)-α-D-Man*p*-(1→2)-D-Man[3]
3 α-D-Man*p*-(1→2)-α-D-Man*p*-(1→2)-α-D-Man*p*-(1→2)-α-D-Man*p*-(1→2)-α-D-Man*p*-(1→2)-α-D-Man*p*-(1→2)-α-D-Man*p*-(1→2)-D-Man[4]
4 α-D-Man*p*-(1→3)-α-D-Man*p*-(1→2)-D-Man[4]
5 α-D-Man*p*-(1→3)-α-D-Man*p*-(1→2)-α-D-Man*p*-(1→2)-α-D-Man*p*-(1→2)-D-Man[4]
6 α-(1→6)-Linked D-mannopyranose main chains from *S. octosporus*[3] and *S. rouxii*.

Oligosaccharides resistant to the crude α-D-*mannosidase*

7 α-D-Man*p*-(1→2)-β-D-Man*p*-(1→2)-β-D-Man*p*-(1→2)-D-Man[3]
8 α-D-Man*p*-(1→2)-β-D-Man*p*-(1→2)-β-D-Man*p*-(1→2)-α-D-Man*p*-(1→2)-D-Man[3]
9 β-D-Man*p*-(1→2)-β-D-Man*p*-(1→2)-D-Man[3]
10 β-D-Man*p*-(1→2)-β-D-Man*p*-(1→2)-β-D-Man*p*-(1→2)-D-Man[3]
11 α-D-Gal*p*-(1→2)-D-Man[3]
12 α-D-Gal*p*-(1→2)-α-D-Man*p*-(1→2)-D-Man[5]
13 α-D-Man*p*-(1→6)-D-G[19]

saccharide **7** and pentasaccharide **8**, which contain α- and β-linkages (Table III) to cleavage by an exo-enzyme at the non-reducing, α-D-linked end demonstrates a high degree of specificity for the enzyme. Since the bacterial enzyme attacks *p*-nitrophenyl α-D-mannopyranoside slowly and methyl α-D-mannopyranoside not at all[11] it appears that the enzyme requires an α-D-mannopyranose non-reducing end and two consecutive α-D-mannopyranosyl residues. The enzyme resembles an exo-amylase from microorganisms that can cleave amylose, but not methyl α-D-glucopyranoside[14,15].

Jones and Ballou[12] have shown that degradation of branched-chain oligosaccharides (from the D-mannan of *S. cerevisiae*) with the exo α-D-mannosidase proceeds in a stepwise fashion starting from the non-reducing ends. Further studies of the reaction mechanism have now been made by using the p.m.r. technique of Eveleigh and Perlin[16]. An α-(1→2)-linked, D-mannopyranose tetrasaccharide was used as substrate rather than a polysaccharide since it gave an H-1 p.m.r. spectrum that is better defined. The α-D-mannosidase had been concentrated for the p.m.r. spectrum by precipitation with ammonium sulfate. Enzymolysis gave β-D-mannose (showing an H-1 signal at τ 4.65, *J* 1 Hz) and a high proportion of trisaccharide (paper chromatogram) in the early stages (4 min). After β-D-mannose had been formed in 14% yield, α-D-mannose (τ 4.40, *J* 1 Hz, H-1), resulting from mutarotation, could be detected (Fig. 3). The occurrence of anomeric inversion at C-1 is similar

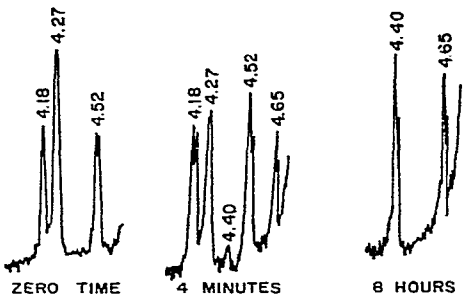
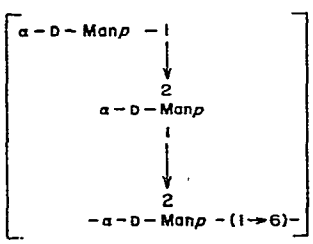
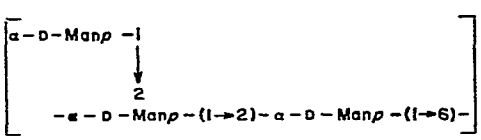


Fig. 3. Course of D-mannosidase enzymolysis of α-(1→2)-linked D-mannopyranose tetrasaccharide, as followed by p.m.r. spectroscopy of the H-1 region.

to the findings for polysaccharases by Eveleigh and Perlin[16]. They stated that, in general, D-glucanases of the exo type, which attack from the non-reducing chain-end,



I



II

cause inversion of anomeric configuration, whereas D-glucosidases and D-glucanases of the endo type, which attack the chain randomly, give products in which the anomeric configuration of the substrate is retained. One apparent exception to these generalizations is an endo-dextranase, which gives products in which the configuration of the product differs from the substrate[17]. Although the enzyme of Jones and Ballou was originally called an α-D-mannosidase[11], it does not degrade glycosides of D-mannopyranose, but can degrade α-(1→6)-linked D-mannopyranose main chains (see above). It therefore has some of the properties of an exo α-D-mannanase and the crude enzyme may possibly be named an α-1→(2, 3 or 6)-D-mannanase preparation (according to a private communication from E.T. Reese).

EXPERIMENTAL

Measurement of p.m.r. spectra. — The H-1 spectra of polysaccharides were obtained from D_2O solutions at 70° by using a Varian 100-MHz spectrometer and a sweep width of 1000 Hz. The concentration of polysaccharide used was 10%. Tetramethylsilane was used as an external standard and was contained in a capillary tube mounted coaxially inside the p.m.r. tube. Chemical shifts are expressed on the τ scale.

Partial acid hydrolysis of D-mannose-containing polysaccharides. — In a typical experiment the polysaccharide (0.60 g) was treated[5,18] for 18 h in 0.33N sulfuric acid (6 ml) at 100°. The degraded polysaccharide was precipitated by addition of ethanol (50 ml). The precipitate was centrifuged and washed successively once with ethanol and twice with acetone. The yields, specific rotations, and sugar compositions of the products are recorded in Table I. The sugar compositions of acid-degraded polysaccharides and enzyme-degraded polysaccharides were determined by hydrolysis with N H_2SO_4 for 18 h at 100° followed by paper chromatography with butyl alcohol–ethanol–water (40:11:19 v/v) as solvent and *p*-anisidine hydrochloride as spray reagent.

Preparation of exo α-D-mannosidase of Arthrobacter GJM-*1.* — The enzyme was obtained by growth of the microorganism on a medium of baker's yeast D-mannan according to the method of Jones and Ballou[11]. For enzymolysis of D-mannans, a crude preparation was used consisting of exocellular medium that had been lyophilized following dialysis against phosphate buffer. A 20% solution of enzyme in D_2O was examined by p.m.r. spectroscopy and was shown to be carbohydrate-free. Some of the enzyme was purified by fractional precipitation by ammonium sulfate up to a concentration of 55%, and was utilized in the enzymolysis of an α-(1→2)-linked D-mannose tetrasaccharide to β-D-mannose (see below).

Enzyme assays. — A minor modification of the α-D-mannosidase assay procedure of Jones and Ballou was used[11]. The assay mixture consisted of yeast D-mannan (400 μg), calcium chloride (0.1 μM) in 0.1M potassium phosphate buffer (0.50 ml, pH 6.8) and enzyme, which was diluted to 1.0 ml with water. After incubation for 10 min at 30°, alkaline Somogyi–Nelson[20] copper reagent (0.5 ml) was added.

The reaction mixture was heated for 30 min at 100° and reducing groups were estimated by using the arsenomolybdate reagent[20]. An approximately linear relationship between enzyme concentration and product was found up to 45% substrate degradation (Fig. 1). Repeated assays showed that the initial reaction rate was slightly lower than the rate attained after 0.5% degradation of the substrate. A unit (U) of activity is defined as that amount of enzyme which causes the release of 1 μmole of D-mannose (or its equivalent) per min under these conditions. One U equals 85.8 units of Jones and Ballou[11]. Specific activity is defined as unit of activity of enzyme per mg of protein[21]. Alkaline phosphatase was assayed by using *p*-nitrophenyl phosphate as a substrate[22]. A unit is defined as the release of 1 μmole of *p*-nitrophenol/min; the molar absorbancy index for *p*-nitrophenol in M Tris buffer (pH 8.0) equals 1.62×10^4.

Enzymolysis of polysaccharides. — The substrate (50 mg) was dissolved in water (2 ml) and treated with crude enzyme having an activity (in 1% solution) of 0.169 U/ml; specific activity 0.422. After the required time the enzyme was deactivated for 10 min at 100° and salts were then removed by adding mixed resins consisting of Amberlite IR-120 (H^+) form and Dowex-1 (hydrogen carbonate form). The filtered solution was then evaporated to approximately 0.3 ml and could be applied directly to the paper chromatograms. The polysaccharide residue was precipitated by addition of excess ethanol and the residue, after centrifugation, was washed with ethanol and then with acetone. The yields of products, based on the original polysaccharides, are presented in Table I. The specific rotations of the enzyme-degraded polysaccharides in H_2O are $+80°$ ($\pm 15°$).

Enzymolysis of α-linked D-*mannopyranose tetrasaccharide.* — Enzyme (4.9 U) that had been precipitated by ammonium sulfate (30–55%) was added to a solution of the tetramer (30 mg) in D_2O (0.7 ml) at 30° and the enzymolysis was followed by the change of the H-1 signals in the p.m.r. spectrum. Initially β-D-mannose (H-1, τ 4.65) was formed, but anomerization to α-D-mannose (H-1, τ 4.40) was detected after 4 min. The H-1 signals of the two anomers were distinguishable from those of the starting material (τ 4.19, 4.25, and 4.52), and other degradation products having similar chemical shifts, namely 2-*O*-α-D-mannopyranosyl-D-mannose and a trisaccharide having α-(1→2)-linked D-mannopyranose residues (Fig. 3). The trisaccharide and D-mannose were detected on paper chromatograms [solvent: 2:1:1 (v/v) butyl alcohol–ethanol–water; spray: ammoniacal silver nitrate] after 6 min.

ACKNOWLEDGMENTS

The authors are indebted to Dr. C. E. Ballou for a culture of *Arthrobacter* GJM-1. Thanks are due to Mr. M. Mazurek for determining p.m.r. spectra and to Mr. R. J. Magus, Mr. A. Sieben, and Mr. N. R. Gardner for technical assistance.

REFERENCES

1 P. A. J. Gorin, K. Horitsu, and J. F. T. Spencer, *Can. J. Chem.*, 43 (1965) 950.
2 P. A. J. Gorin, M. Mazurek, and J. F. T. Spencer, *Can. J. Chem.*, 46 (1968) 2305.

3 P. A. J. GORIN, J. F. T. SPENCER, AND S. S. BHATTACHARJEE, *Can. J. Chem.*, 47 (1969) 1499.
4 P. A. J. GORIN, R. J. MAGUS, AND J. F. T. SPENCER, *Can. J. Chem.*, in press.
5 P. A. J. GORIN AND J. F. T. SPENCER, *Can. J. Chem.*, 46 (1968) 2299.
6 P. A. J. GORIN AND J. F. T. SPENCER, *Can. J. Chem.*, 45 (1967) 1543.
7 P. A. J. GORIN AND J. F. T. SPENCER, *Can. J. Chem.*, 46 (1968) 3407.
8 I. J. GOLDSTEIN, G. W. HAY, B. A. LEWIS, AND F. SMITH, *Abstracts Papers Amer. Chem. Soc. Meeting*, 135 (1959) 3D.
9 P. A. J. GORIN AND A. S. PERLIN, *Can. J. Chem.*, 34 (1956) 1796.
10 P. A. J. GORIN AND A. S. PERLIN, *Can. J. Chem.*, 35 (1957) 262.
11 G. H. JONES AND C. E. BALLOU, *J. Biol. Chem.*, 244 (1968) 1043.
12 G. H. JONES AND C. E. BALLOU, *J. Biol. Chem.*, 244 (1968) 1052.
13 R. J. YU, C. T. BISHOP, F. P. COOPER, J. HASENCLEVER, AND F. BLANK, *Can. J. Chem.*, 45 (1967) 2205.
14 S. A. BARKER, E. J. BOURNE, AND J. G. FLEETWOOD, *J. Chem. Soc.*, (1957) 4865.
15 J. H. PAZUR AND K. KLEPPE, *J. Biol. Chem.*, 237 (1962) 1002.
16 D. E. EVELEIGH AND A. S. PERLIN, *Carbohyd. Res.*, 10 (1969) 87.
17 T. KOBAYASHI, *J. Agr. Chem. Soc. Japan*, 28 (1954) 352; *Chem. Abstr.*, 48 (1954) 12194.
18 S. PEAT, W. J. WHELAN, AND T. E. EDWARDS, *J. Chem. Soc.*, (1961) 29.
19 E. A. TALLEY, D. D. REYNOLDS, AND W. L. EVANS, *J. Amer. Chem. Soc.*, 65 (1943) 575.
20 N. NELSON, *J. Biol. Chem.*, 153 (1944) 375.
21 O. H. LOWRY, N. J. ROSEBROUGH, A. L. FARR, AND R. J. RANDALL, *J. Biol. Chem.*, 193 (1951) 265.
22 A. GAREN AND C. LEVINTHAL, *Biochim. Biophys. Acta*, 38 (1960) 470.

Carbohydrate Research
Elsevier Publishing Company, Amsterdam
Printed in Belgium

LIGHT-SCATTERING STUDIES ON AQUEOUS SOLUTIONS OF AMYLOSE AND AMYLOPECTIN*

W. BANKS, C. T. GREENWOOD, AND J. SLOSS
Department of Chemistry, The University of Edinburgh (Great Britain)
(Received May 8th, 1969)

ABSTRACT

Light-scattering studies have been made on amylose and amylopectin in water. True scattering intensities of the solvent were obtained by degrading the polysaccharide solutions with alpha-amylase in the light-scattering cell. This technique enables the molecular parameters of size and shape to be obtained more speedily and accurately than hitherto. In addition, information regarding the contribution of dust to the scattering intensity of a polymer solution was obtained.

INTRODUCTION

In view of the reported differences in the hydrodynamic behaviour of amylose in aqueous potassium chloride[1] and in pure water[2], we have recently extended our light-scattering studies to the latter solvent. However, we then found much greater difficulties in obtaining linear graphs of c/R_θ against $\sin^2 \theta/2$, and could not decide whether the observed curvature was real or was an artefact caused by dust in solution. To distinguish between these possibilities, we applied a technique similar to that introduced by Stainsby[3].

Experiencing similar difficulties whilst working with collagen in aqueous solution, Stainsby[3] obtained the true solvent-scatter by adding a minute amount of the enzyme trypsin to the protein solutions. The peptides resulting from treatment with trypsin were too small to scatter light, and so the difference between the apparent solvent(water)-scatter and that remaining after enzymic digestion represented the contribution of dust in the collagen solution.

Our variation of this elegant technique is to use alpha-amylase to degrade aqueous solutions of amylose and amylopectin to maltodextrins that are too small to scatter light to any appreciable extent.

EXPERIMENTAL

Amylose and amylopectin fractions were isolated from potato and cereal starches, as previously described[4,5]. Bacterial (*A. oryzae*) alpha-amylase was obtained from the Sigma Chemical Co., St. Louis, Missouri, U. S. A.

*This is Part 48 in the Series "*Physicochemical Studies on Starches*"; Part 47: *European Polymer J.*, in the press.

Preparation of solutions. — Amylose solutions were prepared by dissolving the hard-packed butan-1-ol complex in boiling water, boiling being continued for 30 min whilst the solution was flushed with nitrogen to remove butan-1-ol. The final concentration of amylose was *ca.* 0.35%.

Amylopectin solutions were obtained directly from the starch dispersion, after removal of the amylose as the thymol complex[4]. Residual thymol was removed by shaking the aqueous solution with chloroform, and the chloroform in turn was removed by passing nitrogen through the solution. The concentration of amylopectin was *ca.* 0.4%.

Enzyme solution was prepared by dissolving the solid in water to give a 0.1% solution, having an activity of 20 units per ml (the unit of activity was defined as the amount of enzyme which will release 1 mg of maltose from a solution of AnalaR soluble-starch (0.5%) in 3 min at pH 6.9 and 20°).

Clarification of solvent and solution. — Water was clarified by distillation from an all-glass still. Water distilled directly into the light-scattering cell gave a scattering envelope as good as water which was distilled, and subsequently filtered through sintered glass (G5).

Solutions of amylose and amylopectin were clarified by ultracentrifugation (the force fields used are detailed in the next section) for 1 h, and subsequent filtration through a G4 sintered-glass filter. In the case of amylopectin, the solution was diluted five-fold prior to filtration.

Alpha-amylase solutions were clarified by ultracentrifugation at 20,000 *g* for 75 min. Aliquots were withdrawn from the centrifuge tube by syringe, the needle of which did not penetrate more than 0.5 cm below the surface of the solution.

Light-scattering. — The apparatus was built by the Phoenix Precision Instrument Co. to the design of Brice, Halwer, and Speiser[6]. Cylindrical cells were employed in conjunction with the narrow-slit system. Angular measurements were made between 30 and 135°, and corrections were applied for backward reflections[7]. The green mercury line (λ_0 = 5461 Å) was used throughout.

The refractive index increment (*dn/dc*) was measured with a Brice–Phoenix differential refractometer; *dn/dc* was 0.150 ml per g for both amylose and amylopectin in water at 5461 Å.

The light-scattering technique used was as follows. The scattering envelope of the solvent (25–35 ml) was measured at 5°-intervals from 30 to 50°, then at 10°-intervals to 130°, and also at 135°. An aliquot (3 ml) of the clarified amylose, or amylopectin, solution was added to the solvent, mixing was achieved by gentle shaking, and the measurements were repeated. A small drop of alpha-amylase solution (formed at the tip of a syringe needle) was then touched onto the surface of the solution in the light-scattering cell. After mixing, the cell and its contents were incubated for 30 min at 20° before the readings were repeated. Control experiments showed that the angular scatter reached a constant limiting-value after about 10 min, and that this limit was not affected by further addition of enzyme.

Treatment of the data. — The scattering due to the polymer is the excess of the

solution-scatter over the solvent-scatter. In these experiments, there are two distinct values for scattering due to solvent: (*a*) the scatter of the water distilled into the cell (apparent solvent-scatter), and (*b*) the scatter of the solution resulting from alpha-amylase treatment (true solvent-scatter). Plots of c/R_θ, where R_θ is the Rayleigh ratio at angle θ, are shown on the basis of both apparent and true solvent-scatter.

Values of c/R_θ were extrapolated to $\theta = 0$, and $\overline{M}_w$ obtained from

$$\overline{M}_w^{-1} = Kc/R_\theta,$$

where K contains various optical constants. The root-mean-square radius of gyration $(\overline{\rho_g^2})^{\frac{1}{2}}$ was obtained from the equation

$$(\overline{\rho_g^2})^{\frac{1}{2}} = (S/I)^{\frac{1}{2}} \times 565,$$

where S is the limiting slope of the plot of c/R_θ against $\sin^2 \theta/2$ as $\theta \rightarrow 0$, and I is the intercept at $c = 0$.

RESULTS AND DISCUSSION

Fig. 1*a* shows a graph of c/R_θ against $\sin^2 \theta/2$ for oat amylose, calculated on

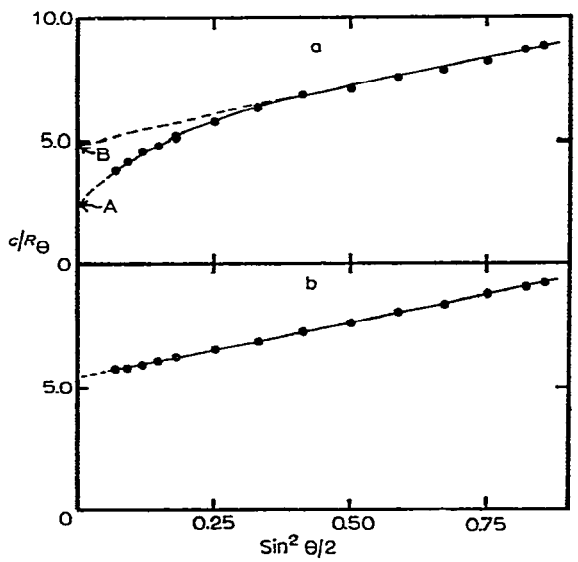


Fig. 1. Graph of c/R_θ as a function of $\sin^2 \theta/2$ for oat amylose in water on the basis of (*a*) apparent solvent-scatter, and (*b*) true solvent-scatter. (The parent amylose solution was centrifuged at 20,000 *g*.)

the basis of the apparent solvent-scatter. At angles below 80°, a pronounced downward curvature occurs. Three explanations may account for this effect:

(*1*) The presence of microgel, or aggregates, of amylose molecules.

(*2*) The curvature is a measure of the breadth of the molecular weight distribution. For a monodisperse system of Gaussian coils of degree-of-polymerization N,

the angular distribution of the scattered light is given by[8]

$$P(\theta) = (2/N^2u^2)\,[Nu-1+\exp(-Nu)],$$

where $u = \mu^2b^2/6$, b being the length of the statistical element, $\mu = (4\pi/\lambda)\sin(\theta/2)$, and λ is the wavelength of light in the medium.

If $P^{-1}(\theta)$ is graphed as a function of Nu, there is an asymptote y defined by $y = Nu/2+\frac{1}{2}$. However, this asymptote is normally observed only with very large macromolecules, *i.e.*, those having radii of gyration of the order of 850 Å. Thus, curvature may be used as a measure of the width of the distribution [9]. Similarly, curvature will result from monodisperse coils which are branched. Thus, it is possible to obtain useful information from the curvature in Fig. 1*a* only if it results from either polydispersity, or branching.

(*3*) The downward curvature at low angles results directly from the presence of dust which has been introduced with the amylose. An attempt may then be made to obtain molecular parameters by extrapolation of the linear portion of the curve, *i.e.*, at angles greater than 70°.

Molecular parameters obtained on the basis of each of these explanations are given in Table I. If the curvature in Fig. 1*a* is real, $\overline{M}_w$ is defined by the intercept A,

TABLE I

MOLECULAR PARAMETERS OBTAINED FROM FIG. 1*a* ON THE BASIS OF VARIOUS ASSUMPTIONS

Assumption	$\overline{M}_w \times 10^{-6}$	$(\overline{\rho_g^2})^{\frac{1}{2}}$, Å	$\overline{M}_n \times 10^{-6}$
Curvature real, due to			
(1) Association	2.86	1770	—
(2) Breadth of distribution[9]	2.86	1770	0.69
Curvature an artefact, due to dust	1.38	535	—

and, in the specific case of being due to a broad distribution, $\overline{M}_n$ may be obtained from intercept B. On the other hand, if the curvature is an artefact, the intercept B may be taken as a measure of $\overline{M}_w$. Thus $\overline{M}_w$ may be 1.38×10^6 or 2.86×10^6, depending on the assumption. A sample of this amylose had earlier been converted into the triacetate under conditions of minimal degradation, and its molecular weight determined by light scattering in nitromethane solution[5]. The value obtained corresponded to $\overline{M}_w = 1.23\times10^6$ for the native amylose. This is much smaller than the values obtained from intercept A in Fig. 1*a*, but is in reasonable agreement with that from intercept B, suggesting the curvature is an artefact.

Fig. 1*b* shows the graph of c/R_θ against $\sin^2\theta/2$ for the same amylose solution as in Fig. 1*a*, but now on the basis of true solvent-scatter. The curvature at low angles has disappeared, and the graph is linear over the entire angular range. The intercept corresponds to $\overline{M}_w = 1.26\times10^6$, in excellent agreement with the value of 1.23×10^6 derived from the acetate, and the root-mean-square radius of gyration is 500 Å.

If the curvature shown in Fig. 1*a* had been real, it should have been repeated in Fig. 1*b*. Downward curvature is due to the photomultiplier "seeing" large intensities of light at low angles. Any high intensities due to molecular aggregates, or to very large molecules, will disappear on adding alpha-amylase. Fig. 2 shows the experimental data in the form of the intensity of the scattered light as a function of

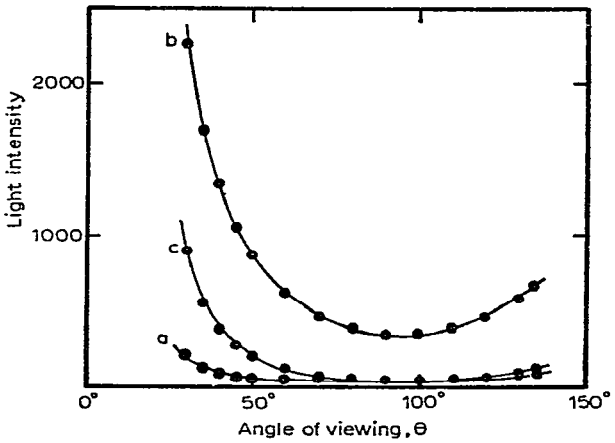


Fig. 2. Graph of light intensity (arbitrary units) as a function of angle for (*a*) water, (*b*) water+amylose, and (*c*) water+amylose+alpha-amylase.

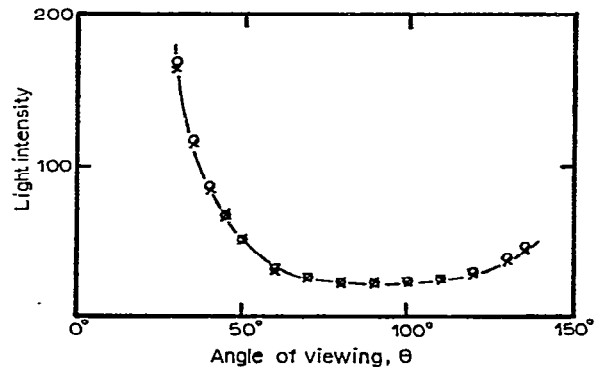


Fig. 3. Graph of light intensity (arbitrary units) as a function of angle for water (×) and water+alpha-amylase (○).

angle for (*a*) water, (*b*) water+amylose, and (*c*) water+amylose+alpha-amylase. Vastly more light is scattered at low angles in (*c*) than in (*a*), and so scattering material is present which is not attacked by alpha-amylase, and may therefore be regarded as non-amylaceous, *i.e.*, it is contaminating dirt.

In this technique, it is of course essential that the excess scattering due to the added alpha-amylase can be ignored. Fig. 3 shows a graph of the scattering intensity as a function of the angle of viewing for water, and for water plus alpha-amylase. Even at low angles, the difference between the two is so small that the addition of the enzyme does not contribute to the observed scattering intensity.

We have shown that $\overline{M}_w$ (1.38×10^6) obtained from Fig. 1*a* by neglecting the data for low angles is comparable to that (1.26×10^6) obtained from Fig. 1*b*, in which all of the angles are included. To investigate whether this observation is generally valid, we have used a fraction of amylose of low molecular weight, and also an amylopectin of high molecular weight. The results for the amylose are shown

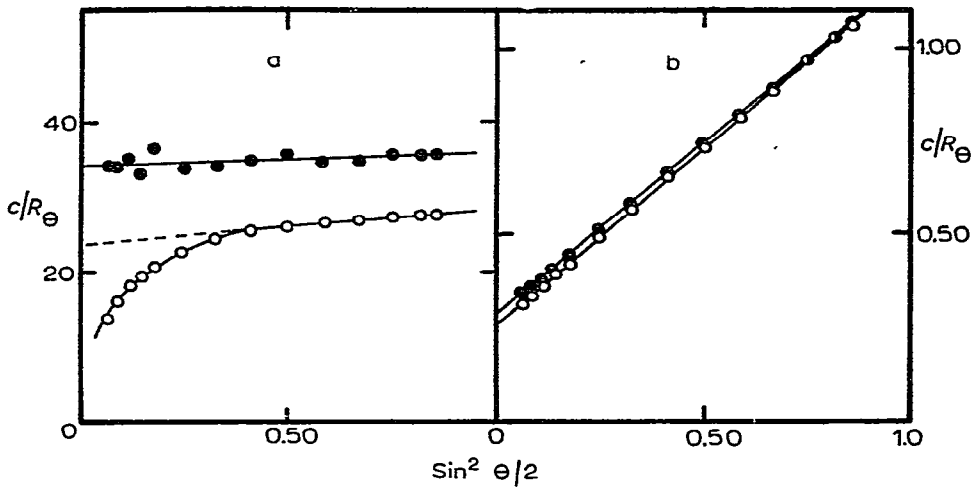


Fig. 4. Graph of c/R_θ against $\sin^2 \theta/2$ for amylose (*4a*) and amylopectin (*4b*). Points have been). calculated on the basis of apparent solvent-scatter (○), and by using true solvent intensity (●).

in Fig. 4*a*, and those for the amylopectin in Fig. 4*b*, and the corresponding molecular parameters are given in Table II.

TABLE II

MOLECULAR PARAMETERS FOR AMYLOSE AND AMYLOPECTIN

Component	$\overline{M}_w \times 10^{-6}$	$(\overline{\rho_g^2})^{\frac{1}{2}}$ Å
Amylose		
Apparent solvent	0.292	260
True solvent	0.202	135
Amylopectin		
Apparent solvent	27.4	1095
True solvent	24.9	1035

A very high error (~50%) is associated with the approximation involved in extrapolating the linear portion of the graph based on apparent solvent-scatter in Fig. 4*a*. The present technique is being used to its limit to give the graph based on true solvent intensity, and this explains the scatter of points about the line in Fig. 4*a*. (At 30°, the true solvent-scatter is approximately 70% of the solution-scatter.)

In the case of the amylopectin, contaminating material contributes very little to the total intensity of light scattered, even at the low angles. Hence, the values based on apparent solvent intensity and true solvent intensity differ by less than 10%.

TABLE III

VALUES OF c/R_θ AS A FUNCTION OF ANGLE FOR 3 CONCENTRATIONS OF AMYLOSE IN WATER. PARENT AMYLOSE SOLUTION CENTRIFUGED AT 54,000 *g* PRIOR TO TREATMENT WITH ALPHA-AMYLASE

Value of c/R_θ *at*	*30°*	*35°*	*40°*	*45°*	*50°*	*60°*	*70°*	*80°*	*90°*	*100°*	*110°*	*120°*	*130°*	*135°*
$c = 0.334 \times 10^{-3}$ g/ml	6.60	6.59	6.73	6.86	7.01	7.48	7.84	8.15	8.55	8.95	9.29	9.66	10.1	10.2
$c = 0.560 \times 10^{-3}$ g/ml	6.55	6.64	6.80	6.94	7.06	7.44	7.75	8.17	8.63	8.95	9.37	9.71	10.00	10.2
$c = 0.736 \times 10^{-3}$ g/ml	6.57	6.59	6.78	6.95	7.07	7.48	7.88	8.25	8.60	9.00	9.35	9.67	10.1	10.2

Our calculations are based on a zero value for the second virial coefficient (A_2) for both amylose and amylopectin in water. This is known to be correct for amylopectin in water[10], and for amylose in neutral, aqueous potassium chloride[1], but finite values of A_2 have been reported for amylose in water[2]. Table III shows c/R_θ as a function of angle for 3 different values of c. In each case, the data refer to true solvent-scatter. Within experimental error, the values are identical, confirming that A_2 is zero for amylose in water.

We conclude that this extension of Stainsby's technique[3] provides an accurate method of determining the size and shape of the starch components in neutral, aqueous solutions. Further, our studies have shown that ignoring the downward curvature at low angles, and extrapolating the linear portion of the c/R_θ against $\sin^2 \theta/2$ graph, may lead to errors as high as 50% for molecular weights of the order of 10^5.

ACKNOWLEDGMENTS

This work was supported in part by a grant made by the United States Department of Agriculture under P.L. 480.

REFERENCES

1 W. BANKS AND C. T. GREENWOOD, *Makromol. Chem.*, 67 (1963) 49.
2 W. BURCHARD, *Makromol. Chem.*, 64 (1963) 110.
3 G. STAINSBY, *Nature*, 177 (1956) 745.
4 W. BANKS, C. T. GREENWOOD, AND J. THOMSON, *Makromol. Chem.*, 31 (1959) 197.
5 W. BANKS AND C. T. GREENWOOD, *Stärke*, 19 (1967) 394.
6 B. A. BRICE, M. HALWER, AND R. SPEISER, *J. Opt. Soc. Amer.*, 40 (1950) 768.
7 H. SCHEFFER AND J. C. HYDE, *Can. J. Chem.*, 30 (1952) 817.
8 P. DEBYE, *J. Phys. Coll. Chem.*, 51 (1947) 18.
9 H. BENOIT, *J. Polymer Sci.*, 11 (1953) 507.
10 I. G. JONES, Ph. D. Thesis, Edinburgh, 1959.

Carbohydrate Research
Elsevier Publishing Company, Amsterdam
Printed in Belgium

GLYCOSYL ESTERS OF AMINO ACIDS

PART I. SYNTHESIS OF 1-*O*-(2-ACETAMIDOACYL)-2,3,4,6-TETRA-*O*-ACETYL-β-D-GLUCOPYRANOSES

A. KORNHAUSER AND D. KEGLEVIĆ
Tracer Laboratory, Institute "Ruder Bošković", Zagreb (Yugoslavia)
(Received May 9th, 1969)

ABSTRACT

The synthesis of crystalline 1-*O*-(2-acetamidoacyl)-2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranoses by the silver salt and dicyclohexylcarbodiimide method is described. The structure of the esters was assigned on the basis of elemental analyses, optical rotations, and i.r. and n.m.r. spectra. On alkaline hydrolysis, the C-1 ester bond of the glucosyl esters is cleaved before complete deacetylation of the molecule takes place.

INTRODUCTION

Recent progress in glycoprotein chemistry has stimulated studies of the synthesis of simple model compounds corresponding to portions of these biopolymers. There are, in general, three possible types of sugar–protein linkage: (*a*) the "*N*-acylglycosylamine" linkage (the amide bond) is well established in glycoproteins, and it has been extensively studied[1] in a number of model compounds; (*b*) the "*O*-glycosidic" linkage has also been detected in glycoproteins[2], and model compounds of this type, involving an ether linkage between a sugar and the hydroxyl group of serine[3,4] and threonine[5], have been prepared; (*c*) there is no evidence so far for the natural occurrence of the third possible type, the "glycosyl ester" linkage[6], involving the C-1 hydroxyl group of the sugar moiety and the carboxyl group of the amino acid, and no model compounds have been described in detail. Although acetylated glucosyl esters of *N*-acetylglycine and DL-alanine, prepared by the silver salt and dicyclohexylcarbodiimide (DCC) method, have been reported[7], no experimental data have been published.

We now report on the synthesis of some 1-*O*-(2-acetamidoacyl)-2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranoses.

RESULTS AND DISCUSSION

Glucosyl esters of *N*-acetylamino acids (**1–5**) were synthesized in two ways, by using (*a*) the silver salt method, and (*b*) the DCC method. Method (*a*) was carried out by treating tetra-*O*-acetyl-α-D-glucopyranosyl bromide with the appropriate silver

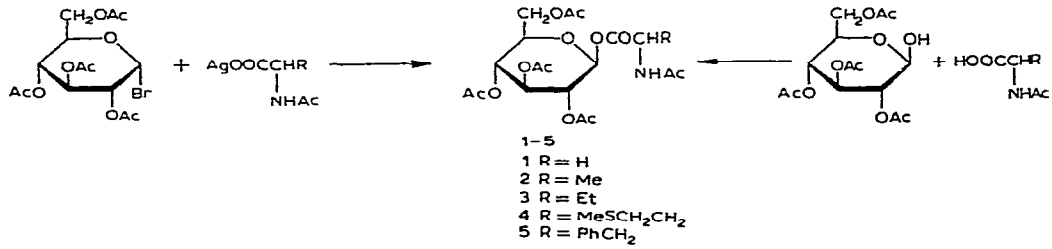
TABLE I

YIELDS AND PROPERTIES OF 1-*O*-(2-ACETAMIDOACYL)-2,3,4,6-TETRA-*O*-ACETYL-β-D-GLUCOPYRANOSES

Compound	*Aminoacyl group*	*Yield, %* *Method* (a)	(b)	*M.p.* *(degrees)*	$[\alpha]_D$ (c, *1–2*)[a] *(degrees)*	*Mol. wt.* *Mol. formula*	*Analyses* *% C*	*% H*	*Calc.* *Found* *% N*	*N.m.r.[c] data, τ (J)* *H-1*	*N-H[d]*	*Ac[e]*
1	Glycyl	42	48	166–168	+10.6	446.40 $C_{18}H_{24}NO_{12}$	48.43 48.21	5.42 5.41	3.14 3.36	4.66 d (7.5)		8.50–8.70
2	Alanyl	35	40	137–139	+8.5	461.44 $C_{19}H_{27}NO_{12}$	49.46 49.33	5.90 6.11	3.04 2.97	4.21 d (7.0)	4.21 d (7.0)	7.90–8.00
3	α-Aminobutyryl	32	—	oil	+18.0	475.46 $C_{20}H_{29}NO_{12}$	50.52 50.81	6.15 6.15	2.95 3.20	4.22 d (7.0)	3.60 d (7.0)	7.80–7.85
4	Methionyl	28	30	100–102	+3.0	521.56 $C_{21}H_{31}NO_{12}S$	48.36 48.13	5.99 6.06	2.69[b] 2.83	4.20 d (7.0)	3.65 d (7.0)	7.88–7.94[f]
5	Phenylalanyl	26	33	120–122	+15.4	537.54 $C_{25}H_{31}NO_{12}$	53.86 53.57	5.81 5.75	2.61 2.88	4.18 d (7.3)	3.80 d (7.0)	7.88–8.00

[a] Determined in acetone for **1**; in chloroform for **2–5**. [b] S calc.: 6.15; found: 6.42%. [c] Coupling constants (*J*) in Hz; in acetone-d_6 for **1**; in chloroform-*d* for **2–5**; d, doublet. [d] The signals disappeared on deuteration. [e] The integration curve gave 15 H (5 Ac groups) in all cases. [f] S-Me, τ 7.80, singlet.

salt of an *N*-acetylated amino acid in refluxing dry benzene; when the reaction was conducted at room temperature, distinctly lower yields of glucosyl esters were obtained. The reaction failed when tetra-*O*-acetyl-α-D-glucopyranosyl chloride was used as the sugar component. In method (*b*), the condensation of 2,3,4,6-tetra-*O*-acetyl-D-glucopyranose and the corresponding *N*-acetylamino acid was performed in the presence of DCC with triethylamine as the catalyst; with pyridine or without any catalyst, no reaction took place.



1–5
1 R = H
2 R = Me
3 R = Et
4 R = $MeSCH_2CH_2$
5 R = $PhCH_2$

With the exception of **3**, the products (**1–5**) were obtained in crystalline form (Table I), each of which showed a low, positive rotation in chloroform. The yields obtained by method (*b*) were slightly superior to those from (*a*). The i.r. spectra revealed absorptions characteristic of amide (3400, 1660 and 1540 cm^{-1}, NH, amide I and II) and ester carbonyl (1770 and 1740 cm^{-1}, *O*-acetyl and C-1 ester linkage) functions. The n.m.r. spectra of the crystalline products in Table I contained a doublet (7 Hz) centered at τ 4.18–4.22 characteristic of the *trans*-diaxial arrangement of H-1 and H-2, thus indicating the β-D configuration of the compounds.

The mother liquors obtained after crystallization of the β-D anomers contained small proportions of oily products which showed chromatographic mobilities identical to those of the corresponding crystalline esters in all of the solvents tried. The elemental analyses and i.r. spectra were consistent with the structure of the relevant glucosyl ester, but the optical rotations were distinctly more dextrorotatory than those of the β-D anomers. N.m.r. data revealed the anomeric composition of these oils, since, in addition to the doublet (7 Hz) at τ 4.2 attributed to H-1 of the β-D anomer, there appeared, after deuterium exchange of the broad amino N-H signal, a well-defined doublet (3 Hz) at τ 3.60–3.80, as would be expected for an α-D anomer. From the n.m.r. spectra, it could be estimated that the total yields of the α-D anomers formed by methods (*a*) and (*b*) were 5–8% and 8–12%, respectively.

The finding that method (*a*) gave amino acid glucosyl esters predominantly having the β-D configuration accords with the generally accepted mechanism for the silver salt method[8]. On the other hand, from the few available data dealing with the DCC syntheses of glucosyl[9] and glucosyluronic acid esters[10], the formation of anomeric mixtures, as well as of pure β-D anomers, could be expected; the formation of the latter was presumed to be a consequence of the steric hindrance associated with a bulky aglycone group. In the present investigation, one has also to take into account that the reaction was performed with tetra-*O*-acetyl-D-glucose in which the β-D anomer was preponderant.

The acetylated amino acid glucosyl esters (**1**, **2**, **4**, and **5**) were stable in the crystalline state as well as in neutral and slightly acidic solutions. However, as expected[11], in alkaline media, even under very mild conditions, the cleavage of the C-1 ester bond took place. T.l.c. examination of various alkaline solutions of the glucosyl ester **2** revealed a sequence of reactions involving initial, rapid hydrolysis of the C-1 ester bond (liberating *N*-acetylalanine), followed by slower and successive cleavage of the remaining glucose *O*-acetyl groups. The acetylated glucosyl esters of *N*-acetylamino acids cannot, therefore, be converted by alkaline hydrolysis into the free glucosyl esters.

EXPERIMENTAL

General. — Melting points are uncorrected. Solvent evaporation was performed in a rotary evaporator *in vacuo* at <40°. Column chromatography was conducted on silica gel (0.2–0.5 mm, E. Merck). Thin-layer chromatography (t.l.c.) was performed on silica gel G (E. Merck). The following solvent systems were used: ether–acetone–light petroleum (5:1:1) (*A*); benzene–methanol (5:1) (*B*); isopropyl alcohol–light petroleum–water (5:2.7:1) (*C*); butyl alcohol–acetic acid–water (65:25:15) (*D*). Detection was effected by spraying with a 10% (v/v) solution of sulphuric acid in water and subsequent heating, with ninhydrin, or with Bromocresol green (0.1% in ethanol + 1 drop of morpholine). I.r. spectra were determined on a Perkin–Elmer Model 137 spectrometer; n.m.r. spectra were obtained with a Varian A-60A spectrometer, using tetramethylsilane as the internal standard. Tetra-*O*-acetyl-α-D-glucopyranosyl chloride was obtained by the $TiCl_4$ method[12]. Silver salts of *N*-acetylamino acids were obtained by the general procedure[13] for substituted silver benzoates, using 5 mmoles of the appropriate *N*-acetylamino acid. After one recrystallization (ethanol–water, 1:1), the silver salts were analytically pure.

Preparation of 1-O-(2-acetamidoacyl)-2,3,4,6-tetra-O-acetyl-D-glucopyranoses. — (*a*) *Silver salt method.* In a three-necked flask equipped with a stirrer, reflux condenser ($CaCl_2$), and a dropping funnel, 2 mmoles of the appropriate silver salt of the *N*-acetylamino acid were added in 25 ml of dry benzene. The suspension was refluxed, and a solution of an equimolar amount of tetra-*O*-acetyl-α-D-glucopyranosyl bromide in 10 ml of dry benzene was added with stirring. The reaction, which was monitored by t.l.c. (in solvent *A*, the glucosyl ester appeared as the slowest moving spot, $R_F \sim 0.3$), was complete after *ca.* 2 h. The reaction mixture was filtered, and evaporated *in vacuo*, and the residual oil was eluted from a column (30 g) of silica gel by using solvent *A*. After displacement of the unchanged bromide, followed by tetra-*O*-acetyl-D-glucose, the glucosyl ester emerged. The first fractions of glucosyl ester, still contaminated with some tetra-*O*-acetyl-D-glucose, were purified by fractionation on a second column. The fractions containing chromatographically homogeneous glucosyl esters were combined and evaporated, and the oily residue was crystallized from dry ether–acetone (5:1) by the addition of light petroleum.

(*b*) *Carbodiimide (DCC) method.* — A solution of *N*-acetylamino acid (2 mmoles)

in 5 ml of *N,N*-dimethylformamide was added gradually to a solution of tetra-*O*-acetyl-D-glucose {2 mmoles, $[\alpha]_D$ +10° (*c* 1, chloroform)}, DCC (2 mmoles), and 0.1 ml of dry triethylamine in 5 ml of dichloromethane, and kept for 12–24 h at room temperature. The *N,N'*-dicyclohexylurea (75–90%) was filtered off, and the solvent was evaporated. The oily residue was dried over sulphuric acid and then submitted to chromatography on silica gel, as described under (*a*).

Hydrolysis of 1-O-(2-acetamidoacyl)-2,3,4,6-tetra-O-acetyl-β-D-glucopyranose (**2**) — (*a*) A solution of 230 mg (0.5 mmole) of ester **2** in 10 ml of hydrochloric acid (1:1) was refluxed for 1 h, and then evaporated *in vacuo*. The residue, dissolved in 10 ml of hot water, was treated with charcoal and filtered. The solution was passed through a column of Dowex-50 x8 (H^+), and the amino acid was eluted with 2N ammonium hydroxide; on evaporation, 70% of chromatographically homogeneous alanine was recovered.

(*b*) Batches of 0.01 mmole of glucosyl ester **2** were treated with 0.5, 1.0, and 2.0 equivalents of either 0.01M sodium methoxide in dry methanol or 0.1N sodium hydroxide in acetone at 25°. The reaction progress was followed by t.l.c. in solvents (*A*) and (*D*). With 2.0, 1.0, and 0.5 equivalents of the reagent, the splitting of the C-1 ester bond was practically complete after 5, 20, and 60 min, respectively, regardless of the base used.

ACKNOWLEDGMENTS

We are indebted to Dr. O. Hadžija for microanalyses, and to Mrs. D. Orlić for valuable technical assistance.

REFERENCES

1 A. GOTTSCHALK, *Glycoproteins*, Elsevier, Amsterdam, 1966, p. 275.
2 Ref. 1, p. 282.
3 K. KUM AND S. ROSEMAN, *Biochemistry*, 5 (1966) 3061.
4 E. RÜDE AND M. MEYER-DELIUS, *Carbohyd. Res.*, 8 (1968) 219.
5 V. A. DEREVITSKAYA, E. M. KLIMOV, AND N. K. KOCHETKOV, *Carbohyd. Res.*, 7 (1968) 7.
6 A. GOTTSCHALK AND W. KÖNIG, *Biochim. Biophys. Acta*, 158 (1968) 358.
7 L. KISFALUDY AND M. LOW, *Peptides Proc. European Symp.*, 5th, Oxford 1962, pp. 239–240; *Chem. Abstr.*, 62 (1965) 7857e.
8 I. CONCHIE, G. A. LEVVY, AND C. A. MARSH, *Advan. Carbohyd. Chem.*, 12 (1957) 157.
9 J. DEGUTIS AND D. DZJUVENE, *Zh. Obshch. Khim.*, 32 (1962) 1253.
10 N. PRAVDIĆ AND D. KEGLEVIĆ, *J. Chem. Soc.*, (1964) 4633.
11 D. KEGLEVIĆ, N. PRAVDIĆ, AND J. TOMAŠIĆ, *J. Chem. Soc.*, (1968) 511.
12 R. U. LEMIEUX, *Methods Carbohyd. Chem.*, 2 (1963) 223.
13 C. V. WILSON, *Org. Reactions*, 9 (1957) 355.

Carbohydrate Research
Elsevier Publishing Company, Amsterdam
Printed in Belgium

2-DEOXY SUGARS

PART XVII. PYRIMIDINE NUCLEOSIDES DERIVED FROM 2-DEOXY-β-D-*lyxo*-HEXOPYRANOSE*

W. WERNER ZORBACH, SILVANO L. DE BERNARDO, AND K. VENKATRAMANA BHAT

Department of Bio-Organic Chemistry, Gulf South Research Institute, New Iberia, Louisiana 70560 (U. S. A.)

(Received May 20th, 1969)

ABSTRACT

A new, crystalline *O*-acylglycosyl halide of 2-deoxy-D-*lyxo*-hexose has been prepared by the following sequence of reactions: methyl 2-deoxy-α-D-*lyxo*-hexopyranoside→methyl 2-deoxy-6-*O*-trityl-α-D-*lyxo*-hexopyranoside→methyl 3,4-*O*-carbonyl-2-deoxy-6-*O*-trityl-α-D-*lyxo*-hexoside→methyl 3,4-*O*-carbonyl-2-deoxy-α-D-*lyxo*-hexopyranoside→methyl 3,4-*O*-carbonyl-2-deoxy-6-*O*-*p*-nitrobenzoyl-α-D-*lyxo*-hexoside→3,4-*O*-carbonyl-2-deoxy-6-*O*-*p*-nitrobenzoyl-α-D-*lyxo*-hexosyl bromide. The new halide was treated with 2,4-dimethoxypyrimidine by the Hilbert–Johnson procedure to afford 1-(3,4-*O*-carbonyl-2-deoxy-6-*O*-*p*-nitrobenzoyl-β-D-*lyxo*-hexosyl)-4-methoxy-2(1*H*)-pyrimidinone, which underwent ammonolysis to yield 1-(2-deoxy-β-D-*lyxo*-hexopyranosyl)cytosine. Demethylation of the pyrimidinone, followed by deacylation, gave the corresponding uracil nucleoside. 1-(2-Deoxy-β-D-*lyxo*-hexopyranosyl)thymine, the C-4′ epimer of 1-(2-deoxy-β-D-*arabino*-hexopyranosyl)thymine (a powerful and specific inhibitor of a pyrimidine phosphorylase obtained from Ehrlich ascites tumor cells) was prepared in a manner similar to that for the uracil nucleoside.

INTRODUCTION

1-(2-Deoxy-β-D-*arabino*-hexopyranosyl)thymine ["2-deoxy-D-glucosylthymine"][1] is a powerful and specific inhibitor of a non-specific pyrimidine nucleoside phosphorylase obtained from Ehrlich ascites cells[2]. It also enhances incorporation of 2′-deoxy-5-iodouridine into the 2′-deoxy-D-ribonucleic acid (DNA) of cat tissues *in vivo*, by inhibition of a "uridine-deoxyuridine" phosphorylase present therein[3]. In terms of the carbohydrate component of the synthetic nucleoside, the structural requirements for inhibition of the enzyme(s) appear to be highly specific. Although "2-deoxy-D-glucosylthymine" is a powerful inhibitor, the corresponding β-D-glucopyranosyl nucleoside is without effect[2a]. Also ineffective as an inhibitor is 1-(2-deoxy-β-D-*ribo*-hexopyranosyl)thymine ["2-deoxy-D-allosylthymine"][2b,4], the structure of which differs from that of "2-deoxy-D-glucosylthymine" only with respect to a reversal

*This work was largely supported by the Louisiana State Science Foundation, Grant No. GSRI-NS257. Exploratory work, on the conversion of 2 into 7, was performed at Georgetown University, Washington, D. C., under support of U. S. Public Health Service Grant No. CA5180 from the National Cancer Institute.

of configuration at C-3 of the carbohydrate component. On the basis of the latter situation, it is logical to predict that 1-(2-deoxy-β-D-*xylo*-hexopyranosyl)thymine (having, therefore, the same configuration at C-3 of the sugar residue) would likewise be ineffective; consequently, this nucleoside has been eliminated as a candidate for phosphorylase inhibition studies. However, the effect brought about by a reversal of configuration at C-4 of the carbohydrate fragment of "2-deoxy-D-glucosylthymine" has not yet been determined, and it is for this reason that we undertook the preparation of a thymine nucleoside that contains a 2-deoxy-β-D-*lyxo*-hexopyranose (C-4 epimer of 2-deoxy-β-D-*arabino*-hexopyranose) residue. In addition to describing the thymine nucleoside, we now report the preparation of two additional 2-deoxy-β-D-*lyxo*-hexopyranosyl ("2-deoxy-β-D-galactopyranosyl") pyrimidine nucleosides; each of the three was synthesized *via* a uniquely constituted, crystalline *O*-acyl-2-deoxyglycosyl halide, the preparation of which has been accomplished in six steps, starting with the commercially available "2-deoxy-D-galactose".

DISCUSSION AND RESULTS

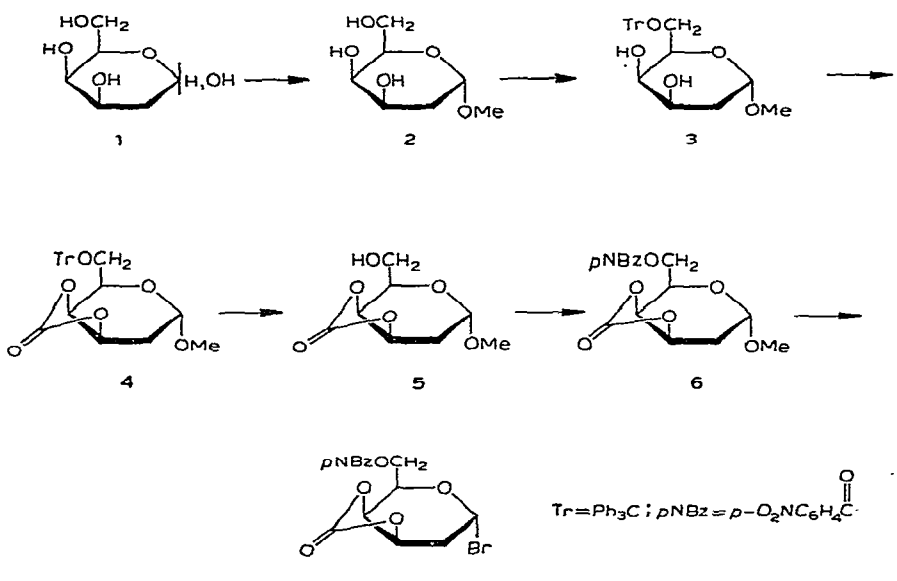
We have shown[5] that *p*-nitrobenzoic esters of 2-deoxy sugars yield stable, *crystalline* *O*-acyl-2-deoxyglycosyl halides; these may be prepared by the simple expedient of stirring the *p*-nitrobenzoic ester in dichloromethane presaturated with anhydrous hydrogen halide. The *p*-nitrobenzoyloxy group at C-1 is rapidly replaced by halogen, and, because of its very low solubility in dichloromethane, the liberated *p*-nitrobenzoic acid separates in almost quantitative yield. Filtration of the suspension and evaporation of the filtrate affords the desired halide as a solid residue which may readily be obtained in crystalline form.

By this method, we were successful in preparing crystalline halides of acylated digitoxose (2,6-dideoxy-D-*ribo*-hexose)[5], 2-deoxy-D-*arabino*-hexose[6], and 2-deoxy-D-*ribo*-hexose[7], each of which was employed successfully in a modified Koenigs–Knorr synthesis of cardiac glycosides[6–8] containing the respective sugar residues. The utility of the three halides was further demonstrated in the synthesis of some 2-deoxy-aldohexopyranosyl nucleosides[9]; for the pyrimidine nucleosides thus obtained, the Hilbert–Johnson procedure[10] was the method of choice, because basic conditions, which tend to eliminate the elements of hydrogen halide from the *p*-nitrobenzoylated glycosyl halide, are avoided[1]. Also, the three halides are of sufficient reactivity to condense readily at room temperature with either 2,4-diethoxy- or 2,4-dimethoxy-pyrimidines, and, in each case studied, the reaction was highly stereoselective, affording the β-D anomers. Although it is unlikely that at least small proportions of the other anomers were not formed, these were not observed under the conditions by which the reaction products were processed. In one instance, involving the condensation of 2-deoxy-3,4,6-tri-*O*-*p*-nitrobenzoyl-α-D-*arabino*-hexosyl bromide with 2,4-diethoxypyrimidine[11], removal of unreacted pyrimidine by extraction left a crystalline residue that was almost pure β-D nucleoside (protected), not requiring further purification.

Because of the aforementioned success in the preparation of such pyrimidine

nucleosides, it appeared desirable to perform, in an analogous manner, the synthesis of some pyrimidine nucleosides containing "2-deoxy-D-galactose" residues. 2-Deoxy-D-*lyxo*-hexose ("2-deoxy-D-galactose", **1**) was readily converted into an anomerically pure tetrakis-*p*-nitrobenzoic ester, which underwent reaction with hydrogen bromide in dichloromethane to afford crystalline 2-deoxy-3,4,6-tri-*O*-*p*-nitrobenzoyl-α-D-*lyxo*-hexosyl bromide that reacted readily with methanol in the presence of silver carbonate to give, by inversion, methyl 2-deoxy-3,4,6-tri-*O*-*p*-nitrobenzoyl-β-D-*lyxo*-hexoside[9]. In sharp contrast, the new halide failed to condense with 2,4-diethoxypyrimidine, even on heating the mixture at 75°, and in no experiment could even trace amounts of material identifiable as protected nucleoside be recovered from the reaction mixture. We have explained this failure[9] on the grounds that the approach of the dialkoxypyrimidine to C-1 of the halide is hindered by the axially oriented *p*-nitrobenzoyloxy group on C-4, and that the same group in the corresponding halides prepared from digitoxose, "2-deoxy-D-glucose", and "2-deoxy-D-allose" is an equatorial substituent; consequently, with the latter, C-1 is unhindered, and the condensations proceed in the normal way.

Because of the failure with 2-deoxy-3,4,6-tri-*O*-*p*-nitrobenzoyl-α-D-*lyxo*-hexosyl bromide in the Hilbert–Johnson synthesis[10], we strove to prepare an acylated glycosyl halide of 2-deoxy-D-*lyxo*-hexopyranose in which the substituent at C-4 would have

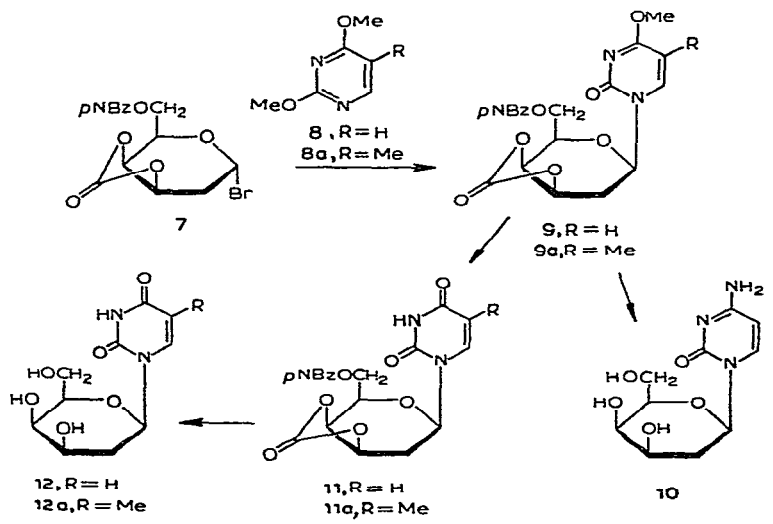


the smallest possible bulk, thus overcoming the steric resistance displayed by the *p*-nitrobenzoylated bromide. 2-Deoxy-D-*lyxo*-hexose (**1**) was converted into the known methyl 2-deoxy-α-D-*lyxo*-hexopyranoside[12] (**2**) in improved yield[13], and unimolar tritylation of **2** afforded, in good yield, the 6-trityl ether (**3**), accompanied by a small proportion (about 5%) of another product, the composition of which agreed with that of a ditrityl ether of **2**. The second trityl group is, most probably, located on

O-3 (equatorial), tritylation on O-4 being precluded because of the axial orientation of the hydroxyl group at this position. Furthermore, it is unlikely that a second trityl group would enter at O-4, because of the proximity of the 4- and 6-positions, which would cause severe crowding.

Treatment of **3** with carbonyl chloride gave the expected methyl 3,4-*O*-carbonyl-6-*O*-trityl-α-D-*lyxo*-hexoside (**4**) as a crystalline product. Quantitative detritylation of **4** in glacial acetic acid with one molar equivalent of hydrogen bromide[14] was almost instantaneous, resulting in **5** as a syrupy product, which was, nevertheless, homogeneous on thin-layer chromatograms. Compound **5** reacted with *p*-nitrobenzoyl chloride in pyridine to yield the 6-*p*-nitrobenzoate (**6**). In the conversion of **6** into 3,4-*O*-carbonyl-6-*O*-*p*-nitrobenzoyl-α-D-*lyxo*-hexosyl bromide (**7**), the concentration of hydrogen bromide is critical. With dichloromethane presaturated with hydrogen bromide[5], only ~60% conversion of **6** into **7** was observed (t.l.c.) after 24 hours. When a 1:1 solution of 30% hydrogen bromide–acetic acid and dichloromethane was employed, for example, gross decomposition of the product occurred. However, when the conversion was performed in dichloromethane under conditions in which both the concentration and the time of reaction were carefully regulated, excellent yields of crystalline **7** were obtained. Provided that the new halide was recrystallized, it proved to be extremely stable, and could be stored under anhydrous conditions for relatively long periods of time.

From past experience in this Laboratory, it has been shown that, when the Hilbert–Johnson synthesis[10] is conducted on a large scale, the yield of protected nucleoside becomes very low. It is, therefore, more efficient to scale down the synthesis and to repeat the reaction on the same basis as many times as is necessary to consume the total amount of halide to be employed. Accordingly, several small-scale reactions between **7** and 2,4-dimethoxypyrimidine (**8**) were performed, in which the two compounds reacted readily at room temperature (the reaction being judged complete in



30 min) to afford the acylated pyrimidinone* (**9**) in yields of 55–60%. Ammonolysis of **9** was readily effected in methanol presaturated with ammonia, to yield 1-(2-deoxy-β-D-*lyxo*-hexopyranosyl)cytosine (**10**). Demethylation of **9** afforded the acylated uracil nucleoside (**11**), which, on deacylation in methanol with methoxide ion, gave 1-(2-deoxy-β-D-*lyxo*-hexopyranosyl)uracil (**12**). Because 2,4-dimethoxy-5-methylpyrimidine (**8a**) is a solid at room temperature, it was necessary to heat a mixture of **7** and **8a** to effect intimate mixing; as with **8**, the reaction was complete in about 30 min, giving comparable yields of the pyrimidinone* (**9a**). Demethylation of **9a**, followed by removal of the protecting groups of the acylated intermediate (**11a**), afforded 1-(2-deoxy-β-D-*lyxo*-hexopyranosyl)thymine (**12a**).

The β-D configuration for the thymine nucleoside (**12a**) has been assigned on the basis of its n.m.r. spectrum, which showed, for the anomeric proton, a quadruplet (two doublets) centered at τ 4.4 ($J_{a,a}$ 9 and $J_{a,e}$ 3.5 Hz). An analysis of the n.m.r. spectrum of the cytosine nucleoside (**10**) was somewhat complicated, owing to the fact that one of the doublets of the quartet (centered at τ 4.38) for the anomeric proton was obscured by the peak for the C-5 proton of the pyrimidine ring (τ 4.25). However, a careful examination of the peaks and the integration curve indicated that the doublet was obscured by one-half of the doublet for the C-5 proton, giving $J_{a,a}$ ~9 and $J_{a,e}$ ~3.5 Hz, indicative of a β-D-nucleoside. This fact, taken together with the rotational data, compared with those for both the protected and unsubstituted thymine nucleosides (**9a** and **12a**), confirm the β-D configuration for **10**, from which, that of the uracil nucleoside (**12**) must follow.

Tests with "uridine–deoxyuridine" phosphorylase disclosed that the thymine nucleoside (**12a**) was completely inactive as an inhibitor**, and these results are in agreement with the structure–activity relationships for the enzyme, as set forth by Etzold *et al.*[15].

EXPERIMENTAL

All melting points were determined with a Kofler hot-stage, optical rotations were measured with a Rudolph Model 80 polarimeter, i.r. spectra were recorded with a Perkin–Elmer Model 457 spectrophotometer, and n.m.r. spectra were recorded on a Varian Model A-60 spectrometer, with methyl sulfoxide-d_6 as the solvent.

T.l.c. was performed on 250-μm, silica gel (Camag DF-5) plates, and the following solvents were employed: *A*, 1:1 ethyl acetate–cyclohexane; *B*, 1:4 ethyl acetate–cyclohexane; *C*, 1:4 cyclohexane–ethyl acetate; *D*, 1:1:8 butyl alcohol–2,2,4-trimethylpentane–ethyl acetate, and *E*, upper layer of 3:5:6:10 2,2,4-trimethylpentane–water–ethyl alcohol–ethyl acetate. The spots were visibilized either with u.v. light, or by spraying with 80% aqueous sulfuric acid and charring at 110° for 5 min.

*As disclosed by t.l.c., the crude reaction product was contaminated with about 5% of what was probably the anomer.

** The authors are indepted to Dr. P. Langen, Institut für Biochemie, Deutsche Akademie der Wissenschaften zu Berlin, for performing the inhibition tests.

Methyl 2-deoxy-6-O-trityl-α-D-lyxo-hexopyranoside (**3**). — To a solution of 10 g (56.5 mmoles) of methyl 2-deoxy-α-D-*lyxo*-hexopyranoside[13] (**2**) (m.p. 115.5–117°) in 220 ml of anhydrous pyridine was added 19.7 g (70.5 mmoles) of freshly prepared chlorotriphenylmethane. The mixture was stirred under exclusion of moisture until it became homogeneous, and it was then kept in the dark for 4 days, during which time the reaction was monitored periodically by t.l.c. (solvents *A* and *B*). The mixture was slowly poured, with vigorous stirring, into 1.9 l of ice–water, and the amorphous precipitate that separated was filtered off, and washed thoroughly with water. The solid was dissolved in 1 liter of dichloromethane, and the solution was washed with two 300-ml portions of water and dried with sodium sulfate. The solvent was evaporated off at 35° under diminished pressure, and the residual pyridine was removed by co-evaporation with three 100-ml portions of toluene. The residue was dissolved in the minimal volume of dichloromethane, and pentane was added, in small portions, until a gel-like precipitate had formed; on standing, the gel became crystalline, and pentane was added until its total volume was 1.5 times that of the dichloromethane solution. The resulting crystals were collected by filtration and dissolved in hot ethyl acetate, followed by the addition of a small volume of cyclohexane and sufficient pentane to double the volume of the solution, giving 14.9 g of pure **3**, m.p. 111–112°. By carefully processing the mother liquors, there was obtained an additional 4.01 g of product, m.p. 109.5–111.5°, bringing the total yield to 80%.

An analytical sample was prepared by chromatographing 1.25 g of **3** on a column (4 × 50 cm) of 250 g of Silica Gel (E. Merck AG, Darmstadt; 0.05–0.2 mm); elution was performed with 1:8 cyclohexane–ethyl acetate, 6-ml fractions being collected from the time of appearance of triphenylmethanol. The chromatography was monitored continuously by t.l.c. with solvent *C* (R_F of **3**, 0.44), and, from fractions 30–55, 1.05 g of chromatographically homogeneous product was obtained; $[\alpha]_D^{23}$ +53° (*c* 1.00, dichloromethane).

Anal. Calc. for $C_{26}H_{28}O_5$: C, 74.26; H, 6.71. Found: C, 74.11; H, 6.81.

Methyl 2-deoxy-3,6-di-O-trityl-α-D-lyxo-hexopyranoside. — The liquors remaining from the preceding experiment were combined and evaporated to dryness, leaving a residue which was digested with 160 ml of boiling 1:15 tetrahydrofuran–ethyl alcohol. The solid was filtered off, to give 3 g (8%) of the ditrityl derivative, m.p. 232–235°. An analytical sample was prepared by dissolving the material in the minimal volume of warm tetrahydrofuran, followed by the addition of ethyl alcohol, to afford pure product, m.p. 235–237°, $[\alpha]_D^{23}$ +46.1° (*c* 1.0, chloroform), $\nu_{max}^{CHCl_3}$ 3590 cm^{-1} (CHOH); t.l.c. with solvent *B*, R_F 0.36.

Anal. Calc. for $C_{45}H_{42}O_5$: C, 81.54; H, 6.38. Found: C, 81.78; H, 6.84.

Methyl 3,4-O-carbonyl-2-deoxy-6-O-trityl-α-D-lyxo-hexoside (**4**). — To a solution of 18.9 g (45 mmoles) of **3** in 300 ml of dry pyridine in a 500-ml flask (fitted with a dropping funnel) and precooled to −18° (ice–salt bath), was added dropwise, during 1 h (vigorous magnetic stirring), 75 ml of a 19% (w/w) solution of carbonyl chloride in toluene. The mixture was stirred for 2 h at −18°, and was then poured into a stirred suspension of 25 g of freshly prepared barium carbonate in 2 l of ice–water,

stirring being maintained until the ice had melted. The liquid was decanted from the oily residue that collected on the bottom of the beaker, and was extracted with three 800-ml portions of dichloromethane. The residue in the beaker was extracted with three 200-ml portions of dichloromethane, and all extracts were combined, thoroughly washed with water, dried (sodium sulfate), and filtered through a bed of Celite 545; the filtrate was evaporated under diminished pressure at 35°. Residual pyridine was removed by co-evaporation with three 50-ml portions of toluene, the resulting syrup was dissolved in ether–tetrahydrofuran, and the solution was treated with Darco G-60 decolorizing carbon. The suspension was filtered on a bed of Celite 545, the filtrate was evaporated to dryness under diminished pressure at 35°, and the syrup was dissolved in 100 ml of ether. Crystallization was effected by the portionwise addition of small volumes of pentane during 3 days (with refrigeration), yielding 16.85 g (83.5%) of product (**4**), m.p. 132–134.5°, sufficiently pure for the following conversion.

An analytical sample was prepared by chromatographing 600 mg of the product on a column (4 × 50 cm) of 220 g of Silica Gel (E. Merck AG, Darmstadt; 0.05–0.2 mm). Elution was performed with solvent *A*, and the effluent was monitored continuously by t.l.c. (solvents *A* and *C*). Collection (6-ml fractions) was begun with the first appearance of product in the eluate, and the pure compound (**4**) was collected in fractions 1–25. Evaporation of the solvent and recrystallization of the residue from ether–pentane gave 480 mg of **4**, m.p.* 135–136°, $[\alpha]_D^{23}$ −25.0° (*c* 1.05, dichloromethane).

Anal. Calc. for $C_{27}H_{26}O_6$: C, 72.63; H, 5.87. Found: C, 72.44; H, 5.94.

*Methyl 3,4-O-carbonyl-2-deoxy-α-*D*-lyxo-hexopyranoside* (**5**). — To a solution of 12.35 g (27.7 mmoles) of **4** (m.p. 132–134°) in 50 ml of glacial acetic acid precooled to 0°, was added, with vigorous stirring, 5.0 ml (28 mmoles) of a freshly prepared, 35% (w/w) solution of hydrogen bromide in glacial acetic acid. The mixture was filtered after 10 sec, and the crystalline bromotriphenylmethane was washed with 10 ml of acetic acid. The filtrate was poured, with stirring, into a solution of 8 g of sodium hydrogen carbonate in 600 ml of water, and solid sodium hydrogen carbonate was added, in small portions with stirring, until the solution had pH 5. The mixture was filtered through a bed of Celite 545, the Celite was washed with 100 ml of water, and the filtrate was extracted with twelve 500-ml portions of 1:3 ethyl alcohol–chloroform. The extracts were combined and dried (sodium sulfate), the solvent was evaporated off under diminished pressure at 35°, and the residue was co-evaporated with three 100-ml portions of toluene and then with three 100-ml portions of absolute ethyl alcohol. The syrupy product (**5**) weighed 5.08 g (90%), and was virtually homogeneous by t.l.c. (solvents *C* and *D*).

It was further purified by chromatographing 500 mg on a column (4 × 55 cm) of 200 g of silicic acid (Mallinckrodt), elution being conducted (collection of 5-ml fractions) with solvent *D*. From fractions 150–280, there was obtained 450 mg of

*On occasion, the compound melts at 95–105°, crystallizes again at 105–110°, and remelts at 135–136°.

pure **5** (t.l.c., solvent *D*), which resisted all efforts to crystallize it; $[\alpha]_D^{23}$ +54.6° (*c* 1.03, chloroform), $\nu_{max}^{CHCl_3}$ 3630 (CH_2OH) and 1815 cm^{-1} (C=O).

*Methyl 3,4-O-carbonyl-2-deoxy-6-O-p-nitrobenzoyl-α-*D-lyxo-*hexoside* (**6**). — To a solution of 5.1 g (25 mmoles) of **5** in 120 ml of dry pyridine, precooled to 0°, was added 5.76 g (31.2 mmoles) of *p*-nitrobenzoyl chloride, and the mixture was stirred for 30 min at 0° and then at room temperature to effect complete dissolution. After being kept in a refrigerator for 24 h, the mixture was slowly poured, with stirring, into 500 ml of 5% aqueous sodium hydrogen carbonate, and the resulting suspension was stirred for 15 min and then diluted with ice-water. After the ice had melted, the solid that formed was collected by filtration, washed well with water, and then dried in a vacuum desiccator (phosphorus pentaoxide). The crude product was dissolved in 120 ml of dichloromethane, the solution was treated with Darco G-60 decolorizing carbon, and the suspension was filtered through a bed of Celite 545, followed by washing with a small volume of dichloromethane. The filtrate was concentrated to about 120 ml and, on portionwise addition of about 200 ml of pentane, there was obtained 6.9 g (78.5%) of product (**6**), melting at 150–151.5° and homogeneous by t.l.c. (R_F 0.50) with solvent *C*. An analytical sample was prepared by recrystallization from dichloromethane–pentane; m.p. 151.5–152.5°, $[\alpha]_D^{23}$ +26.5° (*c* 1.09, dichloromethane).

Anal. Calc. for $C_{15}H_{15}NO_9$: C, 50.99; H, 4.28; N, 3.96. Found: C, 51.19; H, 4.24; N, 3.86.

*3,4-O-Carbonyl-2-deoxy-6-O-p-nitrobenzoyl-α-*D-lyxo-*hexosyl bromide* (**7**). — To a solution of 2.0 g (5.7 mmoles) of **6** in 20 ml of dry dichloromethane was added 2.5 ml of a freshly prepared, 36% (w/w) solution of hydrogen bromide in glacial acetic acid. Separation of crystalline product (**7**) was observed in ~45 min and, after 1 h, examination of the reaction mixture by t.l.c. (solvent *C*) showed complete disappearance of the starting compound (**6**) (R_F 0.51) and presence of the bromide (R_F 0.40) as the sole carbohydrate component. Dry ether (30 ml) was added, followed by 30 ml of dry pentane after 15 min. After the mixture had been kept for an additional 20 min, the separated halide (**7**) was collected by filtration and washed with five 10-ml portions of dry ether; it had m.p. 120–122° and was of sufficient purity for the nucleoside syntheses that follow*. Recrystallization was effected by dissolving it in 75 ml of warm dichloromethane and adding 30 ml of dry ether. After 15 min, 50 ml of pentane was added, to afford 1.72 g (75.4%) of **7**, m.p. 120–122°, $[\alpha]_D^{23}$ +64.5° (*c* 0.53, dichloromethane). (In small-scale preparations, with 75–150 mg of **6**, the yield of **7** was 90–94%.)

*1-(3,4-O-Carbonyl-2-deoxy-6-O-p-nitrobenzoyl-β-*D-lyxo-*hexosyl)-4-methoxy-2(1H)-pyrimidinone* (**9**). — The bromide (**7**) (750 mg, 1.85 mmoles) was divided into three equal portions, and each was added, with mixing, to 1 g of 2,4-dimethoxypirimidine[16] (**8**), in a small, stoppered test-tube. The bromide dissolved rapidly, and the pale-yellow solution solidified completely within 20–30 min. The mixtures were transferred

*The product must, however, be scrupulously free from hydrogen bromide.

to a 125-ml Erlenmeyer flask by rinsing with ether, additional ether was added to bring the volume to 100 ml, and the mixture was stirred to effect complete dissolution of unreacted **8**. The resulting, amorphous precipitate was collected by filtration, washed with ether, and recrystallized twice from a small volume of dichloromethane–ether–pentane; yield of **9**, 460 mg (55%), m.p. 222–225°, $[\alpha]_D^{23}$ +106.8° (*c* 1.01, dichloromethane); homogeneous by t.l.c. [R_F 0.29 (solvent *D*) and R_F 0.68 (solvent *E*)]. An analytical sample, m.p., 223–225.5°, was obtained by an additional recrystallization from dichloromethane–ether.

Anal. Calc. for $C_{19}H_{17}N_3O_{10}$: C, 51.01; H, 3.83; N, 9.39. Found: C, 50.89; H, 3.82; N, 9.23.

*1-(2-Deoxy-β-*D-lyxo-*hexopyranosyl)cytosine* (**10**). — A solution of 300 mg (670 μmoles) of **9** in 25 ml of anhydrous methanol presaturated with ammonia was heated in a pressure flask for 12 h at 85–90°. The solvent was removed under diminished pressure at 30°, the residue was partitioned between 15 ml of water and 15 ml of chloroform, and the chloroform layer was discarded. The aqueous layer was extracted (to remove the *p*-nitrobenzamide) with two 15-ml portions of chloroform and three 15-ml portions of ether, and stirred with a small amount of decolorizing carbon, and the suspension was filtered through a bed of Celite 545, followed by washing with a small volume of 1:1 ethyl alcohol–water. The filtrate was evaporated to dryness under diminished pressure, and the residue was dissolved in 1 ml of water, followed by addition of 4 ml of absolute ethyl alcohol; crystallization was completed by the portionwise addition of 20 ml of ether during 1 day; yield of **10**, 153 mg (89%), m.p. 246–250° (dec.), $[\alpha]_D^{23}$ +55.6° (*c* 0.55, water); homogeneous by t.l.c. (R_F 0.04, solvent *E*). Two additional recrystallizations from water (1.5 ml)–ethyl alcohol (5 ml)–ether (20 ml) gave analytically pure **10**, m.p. 251–253°.

Anal. Calc. for $C_{10}H_{15}N_3O_5$: C, 46.69; H, 5.88; N, 16.33. Found: C, 46.51; H, 5.91; N, 16.18.

*1-(3,4-*O*-Carbonyl-2-deoxy-6-*O*-*p*-nitrobenzoyl-β-*D*-lyxo-hexosyl)uracil* (**11**). — To a solution of 279 mg (624 μmoles) of **9** in 15 ml of dichloromethane was added 5.2 ml of a 39% (w/w) solution of hydrogen chloride in absolute ethyl alcohol, the demethylation being complete in 2 h, as disclosed by t.l.c. with solvent *D*. The solution was concentrated to a small volume by evaporation *in vacuo* at <20°, diluted with dichloromethane, and reconcentrated by evaporation. This procedure was repeated several times and the solution was then evaporated to dryness, leaving a residue which was suspended in 10 ml of dichloromethane and dissolved by addition of methanol. The solution was treated with decolorizing carbon, the suspension was filtered through a bed of Celite 545, and the crude product (**11**) was precipitated from the filtrate by addition of ether. After being filtered off, the solid was suspended in dichloromethane (10 ml) and dissolved by addition of methanol (1 ml); portionwise addition of ether gave 152 mg (57%) of **11**, m.p. 234–235.5° (dec.), $[\alpha]_D^{23}$ +80.9° (*c* 0.503, 1:9 methanol–dichloromethane).

Anal. Calc. for $C_{18}H_{15}N_3O_{10}$: C, 49.89; H, 3.49; N, 9.70. Found: C, 49.79; H, 3.47; N, 9.69.

*1-(2-Deoxy-β-*D*-lyxo-hexopyranosyl)uracil* (**12**). — To a stirred suspension of 142 mg (330 μmoles) of compound **11** in 15 ml of dry methanol was added 200 μl of M methanolic sodium methoxide. After 2 h, the mixture was stirred for 10 min with 1 g of Dowex-50W X8 (H^+) ion-exchange resin, and filtered, and the resin was washed with methanol. The filtrate was evaporated to dryness under diminished pressure at 30°, the residue was partitioned between 15 ml of water and 15 ml of ether, and the ether layer was discarded. The aqueous layer was washed with three 15-ml portions of ether, and stirred with a small amount of decolorizing carbon, and the suspension was filtered through a bed of Celite 545. The filtrate was evaporated to dryness, and the residue was co-evaporated with absolute ethyl alcohol to afford 50 mg (59%) of crude, hygroscopic nucleoside (**12**), which was chromatographically homogeneous by t.l.c. (R_F 0.16) with solvent *E*. The compound was dried by co-evaporating it three times with absolute ethyl alcohol; it was then dissolved in 2 ml of absolute alcohol, and ether was added to incipient turbidity, affording crystalline **12**, m.p. 217.5–220°, $[\alpha]_D^{23}$ +15.1° (*c* 0.10, water); similar recrystallization gave an analytically pure sample.

Anal. Calc. for $C_{10}H_{14}N_2O_6$: C, 46.51; H, 5.46; N, 10.85. Found: C, 46.73; H, 5.41; N, 11.09.

*1-(3,4-*O*-Carbonyl-2-deoxy-6-*O*-p-nitrobenzoyl-β-*D*-lyxo-hexosyl)-4-methoxy-5-methyl-2(1*H*)-pyrimidinone* (**9a**). — The bromide (**7**) (850 mg, 2.11 mmoles) was divided into three equal portions, and each was added, with mixing, to 850 mg of premelted 2,4-dimethoxy-5-methylpyrimidine[16] (**8a**) in a small flask, which was then evacuated for 4 min at 60°. The vacuum was disconnected, each flask was stoppered and kept for 30 min at 60°, and the resulting solid (from each of the experiments) was transferred to a 125-ml Erlenmeyer flask with the aid of ether. Sufficient ether was added to bring the total volume to 100 ml, the suspension was stirred until complete dissolution of unreacted **8a** was effected, and the remaining solid was filtered off and washed with ether. Two recrystallizations from dichloromethane–ether–pentane gave 575 mg (59%) of chromatographically pure (t.l.c., solvent *E*) **9a**, m.p. 213–216°, $[\alpha]_D^{23}$ +101.4° (*c* 1.38, dichloromethane). An additional recrystallization from dichloromethane–ether afforded an analytical sample, m.p. 215–218°.

Anal. Calc. for $C_{20}H_{19}N_3O_{10}$: C, 52.06; H, 4.15; N, 9.11. Found: C, 52.00; H, 4.01; N, 8.91.

*1-(3,4-*O*-Carbonyl-2-deoxy-6-*O*-p-nitrobenzoyl-β-*D*-lyxo-hexosyl)thymine* (**11a**). — To a solution of 500 mg (1.08 mmoles) of the pyrimidinone **9a** in 12 ml of dry dichloromethane was added 5 ml of a 39% (w/w) solution of hydrogen chloride in ethyl alcohol, and the mixture was stirred for 6 h at room temperature. The product (250 mg) that separated (fraction *A*)* was collected by filtration, and the filtrate was evaporated to dryness at 40° under diminished pressure. The resulting residue was dissolved in 10 ml of 1:9 methanol–dichloromethane, and sufficient ether was added

*This fraction is sufficiently pure (m.p. 255–260°) for conversion into the unsubstituted nucleoside **12a**.

to precipitate virtually all of the product (140 mg) in the solution; this was combined with fraction *A*, and dissolved (with heating) in 75 ml of 1:4 methanol–dichloromethane. The volume of the solution was diminished to about one-third by boiling, yielding 338 mg (70%) of **11a**, m.p. 260–265°, $[\alpha]_D^{23}$ +86.2° (*c* 0.21, 1:4 methanol–dichloromethane).

Anal. Calc. for $C_{19}H_{17}N_3O_{10}$: C, 51.01; H, 3.83; N, 9.39. Found: C, 51.65; H, 3.67; N. 9.20.

*1-(2-Deoxy-β-*D*-lyxo-hexopyranosyl)thymine* (**12a**). — A suspension of 336 mg (750 μmoles) of compound **11a** in 40 ml of 30 mM methanolic sodium methoxide was stirred for 4 h at room temperature. To the resulting solution was added 2 g of Dowex-50W X8 (H^+) ion-exchange resin (100–200 mesh), the suspension was stirred for 10 min and filtered, and the filtrate was evaporated to dryness at 45° under diminished pressure. The residue was rinsed into a separatory funnel with 15 ml of water, and the solution was extracted with 4 × 15 ml of ether, treated with a little Darco G-60 decolorizing carbon, and the suspension filtered. The filtrate was evaporated to dryness at 50° under diminished pressure, leaving a crystalline residue which was dissolved in 1 ml of water; 10 ml of absolute ethyl alcohol and sufficient ether to produce turbidity were then added. The solution was kept overnight in a refrigerator, and the crystals that formed were collected by filtration, to give 173 mg (82%) of pure **12a**, m.p. 252–254°, $[\alpha]_D^{23}$ +38.7° (*c* 0.95, water).

Anal. Calc. for $C_{11}H_{16}N_2O_6$: C, 48.53; H, 5.92; N, 10.29. Found: C, 48.34; H, 5.97; N, 10.12.

ACKNOWLEDGMENTS

The authors are indebted to Messrs. V. W. Tripp and G. J. Boudreaux, Southern Regional Research Service, U. S. Department of Agriculture, New Orleans, Louisiana, for recording the n.m.r. spectra.

REFERENCES

1 W. W. Zorbach and G. J. Durr, *J. Org. Chem.*, 27 (1962) 1474.
2 (*a*) P. Langen and G. Etzold, *Biochem. Z.*, 339 (1963) 190; (*b*) M. Zimmerman, *Biochem. Biophys. Res. Commun.*, 16 (1964) 600.
3 P. Langen and G. Etzold, *Mol. Pharmacol.*, 2 (1966) 89.
4 W. W. Zorbach and S. Saeki, *J. Org. Chem.*, 29 (1964) 2018.
5 W. W. Zorbach and T. A. Payne, Jr., *J. Amer. Chem. Soc.*, 80 (1958) 5564.
6 W. W. Zorbach and G. Pietsch, *Ann.*, 655 (1962) 26.
7 W. W. Zorbach and W. Bühler, *Ann.*, 670 (1963) 116.
8 W. W. Zorbach and T. A. Payne, Jr., *J. Amer. Chem. Soc.*, 81 (1959) 1519; 82 (1960) 4979.
9 W. W. Zorbach, C. C. Bhat, and K. V. Bhat, *Advan. Chem. Ser.*, 74 (1968) 1.
10 G. E. Hilbert and T. B. Johnson, *J. Amer. Chem. Soc.*, 52 (1930) 4489.
11 W. W. Zorbach, H. R. Munson, and K. V. Bhat, *J. Org. Chem.*, 30 (1965) 3955.
12 W. G. Overend, F. Shafizadeh, and M. Stacey, *J. Chem. Soc.*, (1950) 671.
13 C. C. Bhat, K. V. Bhat, and W. W. Zorbach, *Carbohyd. Res.*, 10 (1969) 197.
14 B. Helferich, H. Bredereck, and A. Schneidmüller, *Ann.*, 458 (1927) 111.
15 G. Etzold, B. Preussel, R. Hintsche, and P. Langen, *Acta Biol. Med. Ger.*, 20 (1968) 437.
16 C. C. Bhat and H. R. Munson, in W. W. Zorbach and R. S. Tipson (Eds.), *Synthetic Procedures in Nucleic Acid Chemistry*, Vol. 1, Interscience Publishers, Inc., New York, N. Y., 1968, pp. 83–85.

Carbohydrate Research
Elsevier Publishing Company, Amsterdam
Printed in Belgium

Note

Treatment of 1,3,4,6-tetra-*O*-acetyl-α-D-galactopyranose and -α-D-glucopyranose with methyl sulphoxide–acetic anhydride; formation of kojic acid diacetate

G. J. F. CHITTENDEN

Microbiological Chemistry Research Laboratory, Department of Organic Chemistry, The University, Newcastle upon Tyne NE1 7RU (Great Britain)

(Received April 18th, 1969)

During the oxidation of some D-galactopyranose derivatives with methyl sulphoxide–acetic anhydride[1,2], an attempt was made to prepare 1,3,4,6-tetra-*O*-acetyl-D-*lyxo*-hexopyranosulose (**1**) from 1,3,4,6-tetra-*O*-acetyl-α-D-galactopyranose (**2**). The required product was not formed, instead a high yield of 5-hydroxy-2-(hydroxymethyl)pyran-4-one diacetate (kojic acid diacetate) (**3**) was given. The same product was formed by similar treatment of 1,3,4,6-tetra-*O*-acetyl-α-D-glucopyranose (**4**). The formation of kojic acid dibenzoate (**5**) from 1-*O*-acetyl-3,4,6-tri-*O*-benzoyl-D-*arabino*-hexopyranosulose (**6**) was also investigated, in an attempt to clarify the mechanism of formation of kojic acid derivatives from monosaccharides.

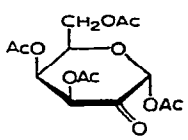
Although the true biochemical significance of kojic acid (**7**) has not been fully elucidated, its formation represents the simplest conversion of sugars into γ-pyrones. These are of interest, since many complex pyrones *e.g.*, flavones, flavanols, and furochromones, are widely distributed in the plant kingdom. The chemical synthesis of kojic acid derivatives from sugars has been described a number of times[3]. Since the γ-pyrone structure does not contain any asymmetric carbon atoms, its formation is independent of the configuration of the original sugar. This is demonstrated below, and had earlier been shown by chemical synthesis from D-glucose[4] and D-galactose[5].

Treatment of the tetra-acetate **2** with methyl sulphoxide–acetic anhydride at room temperature gave kojic acid diacetate (**3**) in 84% yield. The infrared spectrum of the product exhibited three carbonyl bands, and that occurring at 1666 cm^{-1} (pyrone carbonyl group) is characteristic of α,β,α′,β′ unsaturated, six-membered, ring ketones[6]. Those at 1752 and 1772 cm^{-1} are attributed to a normal saturated ester group and to a vinylic ester, respectively. It is known that vinylic esters show a marked enhancement of the carbonyl frequency, regardless of whether the double bond is normal or part of an aromatic ring system[7]. Definite phenolic character has been attributed to the C-5 hydroxyl group in kojic acid [8].

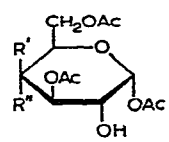
Deacetylation of compound **3** in methanolic ammonia at 0° gave kojic acid (**7**), further characterized as the known[10] phenylosazone.

Treatment of 1,3,4,6-tetra-*O*-acetyl-α-D-glucopyranose (**4**) under the same

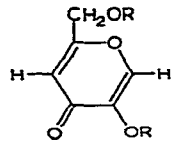
conditions described for the galactose compound **2** gave kojic acid diacetate (**3**) in 81% yield.



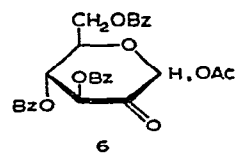
1



2 R′=OAc, R″=H
4 R′=H, R″=OAc



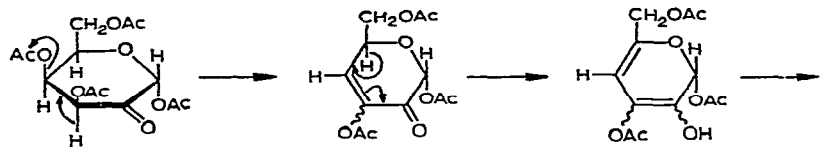
3 R = Ac
5 R = Bz
7 R = H



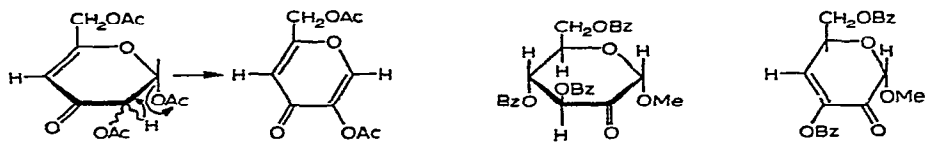
6

Assuming that the ketone **1** is initially produced by oxidation of the C-2 hydroxyl group in the acetate **2**, the formation of kojic acid diacetate (**3**) may be explained as follows. β-Elimination of the acetate group on C-4 would lead to the enone **8**, which can enolise to give the conjugated diene-diol monoacetate **9**. Intramolecular migration of an acetyl group from C-3 to C-2, to give the isomer **10**, would then facilitate a second β-elimination, giving rise to the product **3**, stabilized by resonance. The migration of acyl groups in partially acylated sugars[11–13] and similar compounds[14] is well established. The formation of diacetate **3** from the tetra-acetate **4** would proceed in the same way.

It has also been noted[15] that certain glycopyranosiduloses undergo facile eliminations to give stable enones. Attempted purification of the hexopyranosidulose **11** (obtained by the oxidation of the corresponding glycoside with ruthenium tetroxide) on silica gel led to the enone **12**, which is structurally very similar to the proposed enone intermediate **8**, and was considered to be formed from the ketone **11** by an elimination reaction.



1 **8** **9**



10 **3** **11** **12**

The above route agrees with an earlier suggestion[16]. It can also explain an alternative mechanism[9] which assumed that a proton acceptor is the essential factor in the conversion of osone hydrates into kojic acid derivatives. The formation[16,17] of tetra-acetoxybenzene from inosone pentacetates could be explained in an analogous manner.

Previously[18], it had been shown that treatment of 1-*O*-acetyl-3,4,6-tri-*O*-benzoyl-D-*arabino*-hexopyranosulose (**6**) with pyridine failed to yield kojic acid dibenzoate (**5**). Treatment of **6** with methyl sulphoxide–acetic anhydride has now given the dibenzoate **5** in 64% yield. Its formation under these conditions, with the appearance of a benzoyl group at C-5 in the γ-pyrone ring, adds support for the intramolecular migration step in the proposed mechanism. The same dibenzoate **5** was formed when the ketone **6** was treated with acetic anhydride in pyridine. It is thought probable that acetate ions may be effective in promoting the eliminations described above. The formation of acetate ions has been proposed from the reaction of methyl sulphoxide with acetic anhydride[1,2].

The reactions described provide an easy route to kojic acid derivatives from hexoses. Since free tautomeric equilibrium between the groups on C-2 and C-3 is possible in ketone **1**, the results indicate that methyl sulphoxide–acetic anhydride may be unsuitable for the preparation of hexopyranos-3-uloses from 1,2,4,6-tetra-*O*-acylhexopyranoses.

EXPERIMENTAL

Methyl sulphoxide was redistilled from calcium hydride under diminished pressure. T.l.c. was performed on silica gel (Kieselgel G); detection was by iodine vapour. I.r. spectra were determined for KBr discs.

*Treatment of 1,3,4,6-tetra-*O*-acetyl-α-*D*-galactopyranose* (**2**) *with methyl sulphoxide–acetic anhydride.* — The tetra-acetate[19] (6.96 g) in methyl sulphoxide (60 ml) was treated with acetic anhydride (40 ml) and kept for 48 h at 20°. Removal of excess reagents and by-products at 70–75°/0.5 mm gave an oil, which crystallized spontaneously on cooling. Recrystallization from isopropyl ether–dichloromethane gave 5-hydroxy-2-(hydroxymethyl)pyran-4-one diacetate (**3**, 3.8 g, 84%), m.p. 101–102°; lit.[9] m.p. 103°; λ_{max}^{MeOH} 255 μm, ε_{max} 11,000 (*cf.* data in ref. 9) [Found: mol. wt. m/e (M^+) 226·0481. $C_{10}H_{10}O_6$ calc.: 226·0478]. The m.p. was not depressed on admixture with the diacetate described below, and the i.r. spectra of the compounds were identical.

*Treatment of 1,3,4,6-tetra-*O*-acetyl-α-*D*-glucopyranose with methyl sulphoxide–acetic anhydride.* — Treatment of the tetra-acetate[19] (6.96 g) in the same way as described above gave the diacetate **3** (3.66 g, 81%), m.p. 100–102°.

Acetylation of kojic acid (**7**). Kojic acid (2 g) suspended in dry pyridine (5 ml) was treated with acetic anhydride (6 ml) and kept for 2 days at room temperature. Diisopropyl ether (25 ml) was added to give a crystalline precipitate which was kept at 0° overnight. The product was collected by filtration, washed well with ice-cold ether, and recrystallized from diisopropyl ether–dichloromethane to give kojic acid diacetate (**3**, 2.3 g, 73%), m.p. 101–103°.

Deacetylation of kojic acid diacetate (**3**). — The diacetate **3** (2 g, prepared from **2**) was treated for 18 h at 0° with methanol (15 ml) previously saturated with anhydrous ammonia. The excess ammonia was removed by aeration, and the solution was concentrated. Recrystallization of the residue from ethyl acetate–methanol gave kojic acid (**7**) as needles (61%), m.p. 150–152°; lit.[20] m.p. 152–153°.

Treatment of a portion of the product with phenylhydrazine in aqueous acetic acid gave the known phenylosazone, m.p. 169–170° (from aqueous ethanol); lit.[10] m.p. 169.5–171°.

Treatment of 1-O-acetyl-3,4,6-tri-O-benzoyl-D-arabino-hexopyranosulose[18] (6). — (*a*) *With methyl sulphoxide–acetic anhydride.* Compound **6** (1.5 g) in methyl sulphoxide (10 ml) was treated with acetic anhydride (7 ml) as described above. Recrystallization of the product from diisopropyl ether–dichloromethane gave kojic acid dibenzoate (**5**, 0.63 g, 64%), m.p. 134–136°; lit.[4] m.p. 136°.

(*b*) *With acetic anhydride–pyridine.* Compound **6** (1 g) in acetic anhydride (10 ml) and pyridine (10 ml) was kept for 14 h at 60°. Evaporation *in vacuo* at 45° gave the dibenzoate (**5**, 0.7 g, 61%), m.p. 135–136°.

ACKNOWLEDGMENTS

The author thanks Drs. J. G. Buchanan and N. A. Hughes for helpful discussion.

REFERENCES

1 J. D. Albright and L. Goldman, *J. Amer. Chem. Soc.*, 87 (1965) 4214.
2 J. D. Albright and L. Goldman, *J. Amer. Chem. Soc.*, 89 (1967) 2416.
3 A. Beélik, *Advan. Carbohyd. Chem.*, 11 (1956) 145, for complete review.
4 K. Maurer, *Ber.*, 63 (1930) 25.
5 K. Maurer and A. Müller, *Ber.*, 63 (1930) 2069.
6 R. N. Jones, P. Humphries, and K. Dobriner, *J. Amer. Chem. Soc.*, 72 (1950) 956.
7 E. J. Hartwell, R. E. Richards, and H. W. Thompson, *J. Chem. Soc.*, (1948) 1436.
8 T. Soda, T. Katura, and O. Yoda, *J. Chem. Soc. Japan*, 61 (1940) 1227.
9 M. Stacey and L. M. Turton, *J. Chem. Soc.*, (1946) 661.
10 W. M. Corbett, *J. Chem. Soc.*, (1959) 3213.
11 E. Fischer, *Ber.*, 53 (1920) 1621.
12 J. M. Sugihara, *Advan. Carbohyd. Chem.*, 8 (1953) 1.
13 E. J. Bourne, A. J. Huggard, and J. C. Tatlow, *J. Chem. Soc.*, (1953) 735.
14 S. J. Angyal, P. T. Gilham, and G. J. H. Melrose, *J. Chem. Soc.*, (1965) 5252.
15 P. J. Beynon, P. M. Collins, P. T. Doganges, and W. G. Overend, *J. Chem. Soc.* (*C*), (1966) 1131.
16 H. S. Isbell, *J. Res. Nat. Bur. Stand.*, 32 (1944) 45; *cf.* A. J. Fatiadi, *Chem. Commun.* (1967) 441.
17 T. Posternak, *Helv. Chim. Acta*, 24 (1941) 1045.
18 K. Maurer and W. Petsch, *Ber.*, 66 (1933) 995.
19 B. Helferich and J. Zirner, *Ber.*, 95 (1962) 2604.
20 D. Barnard and F. Challenger, *J. Chem. Soc.*, (1949) 110.

Carbohydrate Research
Elsevier Publishing Company, Amsterdam
Printed in Belgium

Note

Eine einfache Darstellung von 2-Desoxy-L-*glycero*-L-*gulo*-octarsäure-1,4:8,5-dilacton

I. DIJONG
Organisch-Chemisches Institut der Westf. Wilhelms-Universität, 44 Münster, Westf. (Deutschland)
(Eingegangen den 31. März, 1969)

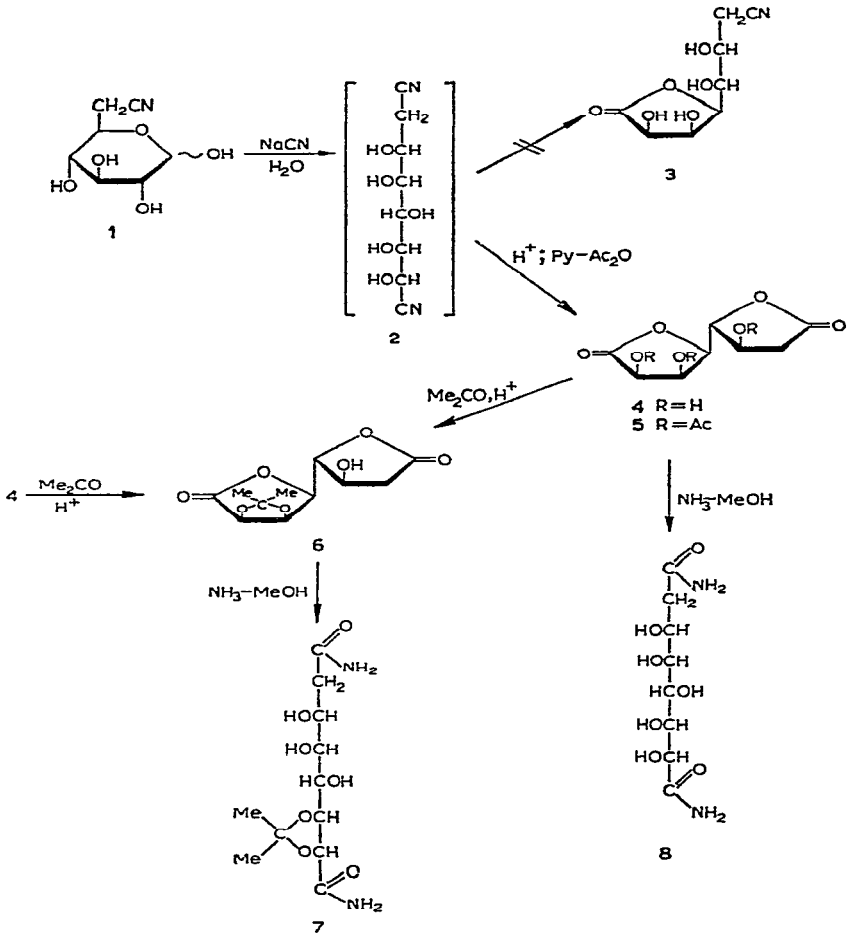
Die Lactone längerkettiger 2-Desoxyaldonsäuren sind bisher nur wenig untersucht worden. So konnten z. B. Wolfrom und Mitarbeiter[1] 2,3,4,5,6-Penta-*O*-acetyl-D-gluconsäure-chlorid mit Diazomethan in das entsprechende Diazoketon überführen, aus dem durch Wolff-Umlagerung mit Silberoxid in wäßriger Lösung 2-Desoxy-3,4,6,7-tetra-*O*-acetyl-D-*gluco*-heptonsäure-1,5-lacton entstand.

Die einfache Synthese des 2-Desoxydilactons **4** mit einer Kette von 8 C-Atomen gelingt, wenn man 6-Cyan-6-desoxy-α- oder β-D-glucose[2] (**1**) unter den üblichen Bedingungen in wäßriger Lösung mit einem geringen Überschuß an Natriumcyanid zur Reaktion bringt. Durch Acetylierung erhält man aus **4** leicht das Tri-*O*-acetyl-Derivat **5**. Das Dilacton **4** ist insofern von Interesse, als seine offenkettigen Derivate mit reaktiven Endgruppen und einer aktivierten Methylengruppe (an C-2) Verwendung finden können für die Synthese hydroxylhaltiger Carbocyclen mit bekannter Konfiguration an den Asymmetrie-Zentren.

Bei der Darstellung der Titelverbindung **4** wird eine Zwischenstufe **2** durchlaufen, die sowohl eine Cyanhydrin-Gruppierung als auch eine Nitril-Funktion im Molekül enthält. Es ist überraschend, daß unter den Bedingungen der Cyanhydrin-Synthese auch die Nitrilgruppe verseift wird, obwohl die Hydrolyse von Nitrilen im allgemeinen schärfere Reaktionsbedingungen erfordert als die Verseifung von Cyanhydrinen.

Aus der Mutterlauge von **4** konnte das als Zwischenprodukt vermutete Monolacton **3** nicht isoliert werden und auch IR-spektroskopisch ließ sich **3** nicht nachweisen. Falls das 7-Cyan-7-desoxy-D-*glycero*-D-*gulo*-heptonsäure-1,4-lacton (**3**) in nennenswerter Menge vorliegt, sollte im IR-Spektrum eine Bande bei 2250 cm^{-1} auftreten, die bei allen früher dargestellten und untersuchten Derivaten der anomeren Methylglykoside der 6-Cyan-6-desoxy-D-glucopyranose und -D-glucofuranose[3] beobachtet wurde. Es ist also nicht erforderlich, besondere Hydrolysebedingungen bei der Synthese des gesuchten Dilactons **4** anzuwenden. Die Struktur von **4** ergibt sich aus folgenden spektroskopischen Daten und aus seinem chemischen Verhalten: im IR-Spektrum beobachtet man 2 CO-Valenzschwingungsbanden bei 1760 und 1780 cm^{-1}. Durch Untersuchungen von Bourne und Mitarbeitern[4] an zahlreichen Aldonsäure-1,4- und -1,5-lactonen ist bekannt, daß 1,4-Lactone zwischen 1765 und

1790 cm^{-1} und die 1,5-Isomeren zwischen 1726 und 1760 cm^{-1} absorbieren. Die Verbindung **4** ist in wäßriger Lösung stabil und zeigt keine Änderung des Drehwertes. Die stark negative Drehung von **4** ($[\alpha]_D^{25}$ −89°) und der positive Drehwert des Diamids **8** ($[\alpha]_D^{24}$ +18°) sprechen nach Hudson[5] ebenfalls für die Struktur eines 1,4:8,5-dilactons. Das NMR-Spektrum des Tri-*O*-acetyl-Derivates **5** bei 60 MHz



in Deuteroaceton zeigt einen starken π-Beitrag zur geminalen Kopplung der H-2-Methylenprotonen ($J_{2,2'}$ 19 Hz), der charakteristisch ist für Fünfring-Ketone (H-2,2': τ 6,75–7,93 ppm; $J_{2,3}$ 7,0 Hz, $J_{2',3}$ 3,2 Hz). Die Glykolspaltung zwischen C-6 und C-7 mit Perjodat erfolgt momentan. Hierbei entsteht eine unbeständige Verbindung, die mit fuchsinschwefliger Säure reagiert. Fehlingsche Lösung wird reduziert. Mit Schwefelsäure als Katalysator bildet sich in Aceton die Mono-*O*-isopropyliden-Verbindung **6**. Hierdurch ist bewiesen, daß die Hydroxylgruppen an C-6 und C-7 im Dilacton **4** *cis*-orientiert sind, d.h. **4** besitzt L-*glycero*-L-*gulo*-Konfiguration. Die Ketalisierung zur Monoaceton-Verbindung **6** verläuft überraschend

langsam. Selbst nach 14 Stdn. läßt sich die Ausgangsverbindung **4** noch nachweisen. Sie ist durch Umkristallisieren von **6** nur schwer zu entfernen (vgl. den gefundenen C-Gehalt von **6**).

Sowohl **4** als auch das Mono-*O*-isopropyliden-Derivat **6** bilden mit Ammoniak in Methanol schon unter milden Bedingungen (Raumtemperatur) sehr schnell die Diamide **7** und **8**. Signifikante Unterschiede in der Stabilität des Lacton- und des 2-Desoxylacton-Ringes in **4** und **6** sind unter diesen aminolytischen Bedingungen also nicht zu beobachten. Dies ist insofern ungewöhnlich, als systematische Untersuchungen an verschiedenen 2-Desoxyaldonsäure-1,4- und 1,5-lactonen und ihren *O*-Methyl-äthern gezeigt haben, daß sich 2-Desoxyaldonsäure-lactone allgemein von normalen Lactonen durch eine besondere Stabilität gegenüber Ringöffnungsreaktionen (z. B. Hydrolyse) unterscheiden[6–8]. Mit der Leichtigkeit, mit der sich beide Lactonringe des 2-Desoxy-L-*glycero*-L-*gulo*-octarsäure-1,4:8,5-dilactons (**4**) öffnen lassen, ist eine wesentliche Voraussetzung für die Synthese von substituierten Carbocyclen erfüllt.

EXPERIMENTELLER TEIL

2-Desoxy-L-glycero-L-gulo-*octarsäure-1,4:8,5-dilacton* (**4**). — 6-Cyan-6-desoxy-α,β-D-glucose (13,6 g) wird in Wasser (120 ml) gelöst und mit Natriumcyanid (3,72 g, 1,1 Äquiv.) versetzt. Nach 20 Stdn. bei Raumtemperatur wird die schwach gelb gefärbte Lösung mit Amberlite (IR-120; H^+) behandelt und das Eluat im Vakuum eingedampft. Der teilweise kristalline Rückstand wird mit wasserfreiem Äthanol verrieben, abgesaugt und gut mit Äthanol gewaschen (6,26 g, 40%). Es wird aus Äthanol unter Zusatz von wenig Wasser umkristallisiert (5,45 g); Schmp. 219° (Bräunung ab 192°), $[\alpha]_D^{25}$ −89,3° (*c* 1,415, Wasser).

Ber. für $C_8H_{10}O_7$: C, 44,04; H, 4,62. Gef.: C, 44,30; H, 4,81.

3,6,7-Tri-O-*acetyl-2-desoxy*-L-glycero-L-gulo-*octarsäure-1,4:8,5-dilacton* (**5**). — Das Octarsäure-1,4:8,5-dilacton **4** (0,3 g) wird in wasserfreiem Pyridin (4 ml) gelöst. Die gekühlte Lösung wird mit Essigsäureanhydrid (2 ml) versetzt und über Nacht bei Raumtemperatur aufbewahrt. Das Acetylierungsgemisch wird im Vakuum entfernt und der Rückstand aus Äthanol umkristallisiert (0,36 g, 76,3%); Schmp. 183–185° (Bräunung ab 174°), $[\alpha]_D^{25}$ −71,9° (*c* 0,21, Pyridin).

Ber. für $C_{14}H_{16}O_{10}$: C, 48,84; H, 4,68. Gef.: C, 48,87; H, 4,65.

2-Desoxy-6,7-O-*isopropyliden*-L-glycero-L-gulo-*octarsäure-1,4:8,5-dilacton* (**6**). — Das Octarsäure-1,4:8,5-dilacton **4** (5 g) wird in Aceton (400 ml) und konz. Schwefelsäure (8 ml) 14 Stdn. bei Raumtemperatur gerührt. Die rotbraune Lösung wird mit Calciumhydroxid (80 g) geschüttelt (ca. 1 Stde.). Das hellgelbe neutrale Filtrat wird eingedampft und der Rückstand in Äthanol gelöst. Beim Abkühlen erfolgt bald Kristallisation, die durch vorsichtige Zugabe von Petroläther vermehrt werden kann. Es wird abgesaugt und gut mit Petroläther gewaschen (1,9 g, 32,1%). Nach mehrfachem Umkristallisieren aus Äthanol–Petroläther: Schmp. 137°, $[\alpha]_D^{26}$ −89,7° (*c* 1,346, Pyridin).

Ber. für $C_{11}H_{14}O_7$: C, 51,16; H, 5,47. Gef.: C, 50,44; H, 5,62.

*2-Desoxy-6,7-O-isopropyliden-*L-glycero-L-gulo-*octarsäure-diamid* (**7**). — Das 6,7-*O*-Isopropyliden-1,4:8,5-dilacton **6** (1,3 g) wird in ammoniak-gesättigtem Methanol (30 ml, wasserfrei) gelöst und über Nacht bei Raumtemperatur aufbewahrt. Hierbei bilden sich wenige gut ausgebildete Kristalle. Die Kristallisation wird durch Anreiben und Kühlung beschleunigt. Nach 10 Stdn. wird abgesaugt (1,1 g). Aus der Mutterlauge läßt sich durch Zugabe von Äther weiteres **7** gewinnen. Gesamtausb. 1,25 g (84,8%); Schmp. 129°, $[\alpha]_D^{22}$ +40,4° (*c* 1,22, Pyridin).

Ber. für $C_{11}H_{20}N_2O_7$: C, 44,02; H, 6,90; N, 9,57. Gef. C, 44,08; H, 6,90; N, 9,26.

*2-Desoxy-*L-glycero-L-gulo-*octarsäure-diamid* (**8**). — Das Octarsäure-1,4:8,5-dilacton **4** (1 g) wird in ammoniak-gesättigtem Methanol (10 ml, wasserfrei) gelöst. Nach ca. 1 Stde. beginnt die Abscheidung von **8**. Nach Kühlung über Nacht: Ausb. 1,15 g (100%). Es wird aus Äthanol–Wasser umkristallisiert; Schmp. 159–161° (Bräunung ab 155°), $[\alpha]_D^{24}$ +18,5° (*c* 1,025, Wasser).

Ber. für $C_8H_{16}N_2O_7$: C, 38,09; H, 6,39; N, 11,11. Gef.: C, 38,02; H, 6,70; N, 10,76.

DANK

Der Deutschen Forschungsgemeinschaft bin ich für eine Sachbeihilfe sehr zu Dank verpflichtet.

LITERATUR

1 M. L. WOLFROM, S. W. WAISBROT UND R. L. BROWN, *J. Amer. Chem. Soc.*, 64 (1942) 1701.
2 R. GREWE UND G. ROCKSTROH, *Chem. Ber.*, 86 (1953) 536.
3 I. DIJONG UND U. WITTKÖTTER, *Chem. Ber.*, 101 (1968) 1848.
4 S. A. BARKER, E. J. BOURNE, R. M. PINKARD UND D. H. WHIFFEN, *Chem. Ind.* (London), (1958) 658.
5 C. S. HUDSON, *J. Amer. Chem. Soc.*, 32 (1910) 338, 61 (1939) 1525, 39 (1917) 462, 40 (1918) 813, 41 (1919) 1141.
6 R. E. DERIAZ, W. G. OVEREND UND M. STACEY, *J. Chem. Soc.*, (1949) 2836.
7 I. W. HUGHES, W. G. OVEREND UND M. STACEY, *J. Chem. Soc.*, (1949) 2846.
8 W. G. OVEREND, F. SHAFIZADEH UND M. STACEY, *J. Chem. Soc.*, (1950) 671.

Carbohydrate Research
Elsevier Publishing Company, Amsterdam
Printed in Belgium

Note

The condensation of 2-amino-2-deoxy-D-mannose with 2,4-pentanedione. N.m.r. analysis of the reaction product*

NORMAN S. BHACCA

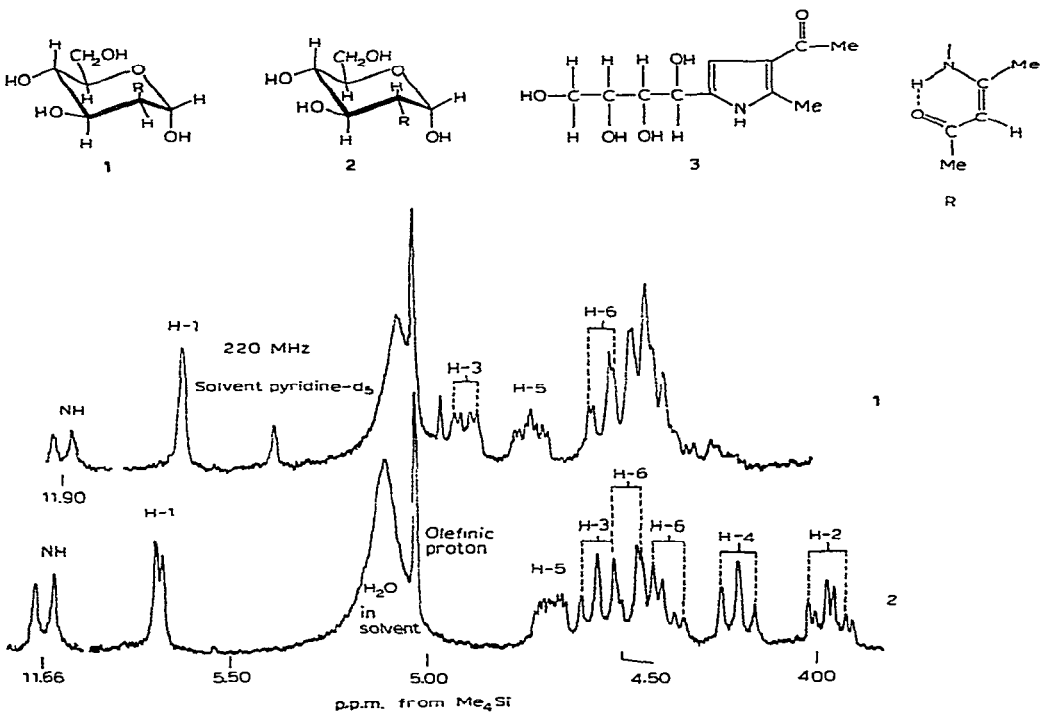
Department of Chemistry, College of Chemistry and Physics, Louisiana Sate University, Baton Rouge, Louisiana 70803 (U.S.A)

AND JULIO J. LUDOWIEG

Department of Orthopaedic Surgery, University of California, San Francisco Medical Center, School of Medicine, San Francisco, California 94122 (U.S.A.)

(Received January 30th, 1969; in revised form April 28th, 1969)

The enamine derivatives **1** and **2** are condensation products formed from 2-amino-2-deoxy-D-mannose and 2-amino-2-deoxy-D-glucose, respectively, in an alkaline solution of 2,4-pentanedione[1-3]. These compounds, in neutral or basic solutions (pH 9.5), undergo cyclization to produce substituted pyrrole derivatives, one of which is 3-acetyl-2-methyl-5-(D-*arabino*-tetrahydroxybutyl)pyrrole[2] (**3**).



*This work was supported in part by U. S. Public Health Service Grant AM-10126.

Structures for **2** and **3** have been proposed and established by n.m.r. spectroscopy[4]. The structure of **1** has been, however, inferred from chemical composition and i.r. analysis. This report is to complement a previous study on these compounds[3] by presenting proof of the structure of **1** by n.m.r. analysis.

N.m.r. analysis. — The structure of the enamine derivative of 2-amino-2-deoxy-D-mannose (**1**) was determined by comparing its 220-MHz n.m.r. spectrum with that of **2**. The doublet at $\delta = 5.64$ in the spectrum of **2** is assigned to H-1. The splitting of 3.5 Hz observed in this signal is also seen in the resonance at $\delta = 3.96$, which corresponds to H-2. The two large spin-couplings of 9 Hz in the H-2 absorption are due to the two equal spin-interactions of the two protons whose resonances occur at $\delta = 4.55$ and 11.66. The signal at $\delta = 4.55$, which is assigned to H-3, appears as a triplet ($J_{2,3} = J_{3,4} = 9$ Hz) because of the equal spin-interactions of H-3 with those of H-2 and H-4. The low-field resonance at $\delta = 11.66$ corresponds to the intramolecularly hydrogen-bonded amino proton, whose signal appears as a doublet because of coupling with H-2. Another triplet having a coupling constant of 9 Hz ($J_{3,4} = J_{4,5} = 9$ Hz) is attributed to H-4. The eight-line pattern at $\delta = 4.67$ corresponds to H-5, which is spin-coupled to H-4 with a coupling of 9 Hz and also to the C-6 protons with couplings of 2.5 and 5.5 Hz. Signals of the C-6 protons occur at $\delta = 4.47$ and 4.35 as two pairs of doublets. From the n.m.r. spectral data of the ring protons in **2**, it is clear that the bonds to H-2, H-3, and H-4 are all axial, whereas the one to H-1 is equatorial. Therefore, the pyranoid ring of the enamine derivative **2** of 2-amino-2-deoxy-D-glucose has the α-D-configuration and *Cl* (D) conformation.

In the spectrum of **1** the signal caused by H-1 appears as a doublet (splitting ~1 Hz) at $\delta = 5.60$. The resonance patterns and the chemical shifts of the H-1 resonances in the two compounds are very similar, indicating that both **1** and **2** have H-1 equatorial. The triplet of doublets representing the axially oriented H-2, which was so evident in the spectrum of **2**, does not appear to be present in the spectrum of **1**. The NH doublet that was observed for **2** is also present with **1**, although at somewhat lower field. This indicates that H-2 in **1**, although adjacent to the amino hydrogen as in **2**, is disposed equatorially rather than axially, and its n.m.r. signal, being more lowfield, must be located in the undecipherable region near 4.45 p.p.m. where the resonances caused by H-4 and H-6 also occur. The pair of doublets at $\delta = 4.90$ is assigned to the axially oriented H-3. The large coupling of 9 Hz is caused by the spin interaction of H-3 with that of the axially situated H-4, and the smaller coupling of 3.5 Hz is attributed to spin-coupling between H-3 and the equatorially oriented H-2. The multiplet resonance pattern at $\delta = 4.73$ is once again assigned to H-5, as in the spectrum of **2**. The broad signals at 5.09 and 5.06 p.p.m., in the n.m.r. traces of **1** and **2**, respectively, are assigned to water present as an impurity in the pyridine-d_5. The sharp signals near 5.02, 2.14, and 2.05 p.p.m. in the spectra of **1** and **2** represent the olefinic, allylic methyl, and acetyl-group protons, respectively. The proximity of the chemical shifts of these protons in the two compounds conclusively shows that the protons in question have almost identical chemical environments.

REFERENCES

1 C. CESSI AND F. SERAFINI-CESSI, *Biochem. J.*, 88 (1963) 132.
2 F. GARCÍA GONZÁLEZ, A. GÓMEZ SÁNCHEZ, AND M. I. GOÑI DE REY, *Carbohyd. Res.*, 1 (1965) 261.
3 J. J. LUDOWIEG AND J. D. BENMAMAN, *Carbohyd. Res.*, 8 (1968) 185.
4 A. GÓMEZ SÁNCHEZ, M. GÓMEZ GUILLÉN, AND U. SCHEIDEGGER, *Carbohyd. Res.*, 3 (1967) 486.

Carbohyd. Res., 11 (1969) 432–434

Carbohydrate Research
Elsevier Publishing Company, Amsterdam
Printed in Belgium

Book review

The Metabolic Roles of Citrate, Biochemical Society Symposium Number 27: edited by T. W. GOODWIN, Academic Press, London and New York, 1968, ix +144 pp., 40*s*, $ 4.80.

Carbohydrate chemists will already be familiar with the central and important role of the Tricarboxylic Acid Cycle in the function of aerobic, cellular metabolism. The cycle, as first enunciated by Krebs and Johnson in 1937, was based on their demonstration that citric acid was formed from oxaloacetate and pyruvic acid; this condensation, now known to be mediated by acetyl-CoA, made a sequence of individual reaction-steps into a cycle, and provided the important link between the catabolism of carbohydrates to pyruvate and the terminal oxidation of this substrate to carbon dioxide and water. This experimentally verifiable explanation of the total combustion of carbohydrate, and the nature of the cyclic process associated with it, has had such a profound impact on subsequent developments in biochemistry that the Biochemical Society decided to arrange a Symposium specifically to pay tribute to Sir Hans Krebs. This volume is a permanent record of this tribute and consists of a collection of papers by distinguished biochemists, all of whom are actively engaged in the study of the many ramifications of the TCA cycle.

After the opening remarks by the Chairman, Professor F. Dickens, an informative and interesting bibliographical sketch is given by H. L. Kornberg. This is followed by: "Studies on Purified Citrate Enzymes", by Paul A. Srere, in which the author describes work carried out in his own laboratories on the structure and mechanism of citritase synthase, citritase, and citrate cleavage enzyme, the three enzymes that catalyse the aldol cleavage of citrate, thereby providing an alternative role for citrate as an acetyl group generator. "Citrate and the Citrate Cycle in the Regulation of Energy Metabolism" by D. E. Atkinson outlines some of the principles of metabolic control and, in particular, the importance of adenylate/ATP levels in the regulation of the balance of the energy-yielding and energy-requiring processes of the TCA cycle. Further aspects of control are dealt with in "Control of Citrate Synthesis in Mitochondria", by P. B. Garland, in which the interrelationship between fatty acid oxidation and the TCA cycle in isolated mitochondria is discussed with particular reference to the controls regulating the synthesis of citrate or acetoacetate *via* the common intermediary acetyl-CoA. Another important role of this latter substrate is dealt with in "Citrate and the Conversion of Carbohydrate to Fat" by J. M. Lowenstein. In a lucidly presented paper, Lowenstein discusses the various control mechanisms that determine whether foodstuffs are combusted or converted into fat *via* acetyl-CoA. The role of citrate as a metabolic regulator is reviewed and discussed in "Citrate as a Metabolic Regulator in Muscle and Adipose Tissue"

by P. J. Randle, R. M. Denton, and P. J. England. The activity of phosphofructokinase and acetyl-CoA carboxylase are known to be influenced by citrate, and the authors describe their recent studies of the regulation of phosphofructokinase and glycolysis by citrate in the rat heart, using perfusion techniques to determine substrate level concentrations. The problem of relating extra- and intra-mitochondrial metabolism is examined in "Metabolic Control in Mitochondria by Adenine Nucleotide Translocation" by M. Klingenberg and E. Pfaff. These authors elaborate further on their scheme for the translocation of adenine nucleotides between the inner mitochondrial membrane or matrix and the outer mitochondrial membrane, which is considered to be mediated by an adenylate translocase. The properties of this system provide the basis for the exchange and concentration of substrates between the intramitochondrial and cytoplasmic compartments. Finally, the transport of substrates across the mitochondrial membrane is further examined in "Penetration of the Mitochondrial Membrane by Tricarboxylic Acid Anions", by J. B. Chappell and B. H. Robinson, in which the elegant techniques developed for following the penetration of mitochondria by substrate anions are presented, and a mechanism for the transfer of citrate from the cytoplasm to inside the mitochondrion is proposed.

Each article, which serves as an excellent, up-to-date review as well as lucidly illustrating recent metabolic methodology, will be welcomed by research workers in the field, final-year biochemistry students, and those involved with the teaching of intermediary metabolism. Molecular cybernetics would aptly describe the general theme of the Symposium, which can be unreservedly recommended to those with a general background in biochemistry and an interest in the factors that regulate and control chemical events in the living cell. The Biochemical Society and the organiser of the Symposium, Professor T. W. Goodwin, are to be congratulated on bringing together a worthy tribute to one of the world's outstanding biochemists. The book, which also contains an author and subject index, is modestly priced at 40*s*.

Bath University N. F. TAYLOR

Carbohyd. Res., 11 (1969) 435–436

Carbohydrate Research
Elsevier Publishing Company, Amsterdam
Printed in Belgium

P M R. SPECTRA AND CONFORMATION OF ACYCLIC CARBOHYDRATES ISOMERIC 3,4,5,6-TETRA-ACETOXY-*trans*-1-NITRO-1-HEXENES

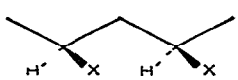
J M WILLIAMS
Department of Chemistry, University College, Swansea (Great Britain)
(Received April 29th, 1969)

ABSTRACT

The vicinal coupling constants derived from the p m.r. spectra of the title compounds are consistent with the planar, zigzag conformation of the carbon skeleton (C-2–C-6) for the D-*arabino* isomer but not for the D-*xylo* and D-*ribo* isomers The latter possess an unfavourable, non-bonded interaction between acetoxyl groups on alternate carbon atoms in the zigzag conformation Some of the p m r spectra provide examples of deceptively simple ABX type systems

INTRODUCTION

It has often been assumed[1,2] that the most stable conformation of acyclic carbohydrates is that in which the carbon skeleton adopts a planar, zigzag conformation, but the evidence cited in support of this assumption has been unsatisfactory Simple alkanes and substituted ethanes have been cited[1] as model compounds, but this has ignored the non-bonded interaction between substituents attached to alternate carbon atoms of the same configuration (**1**) Such non-bonded interactions (hereafter referred to as eclipsed β-interactions) in a perfectly planar, zigzag conformation are



1

$$O_2NCH^{1}=CH^{2}-CH^{3}(OAc)-CH^{4}(OAc)-CH^{5}(OAc)-CH_2^{6,6'}OAc$$

2

entirely analogous to 1,3-diaxial interactions in the chair form of cyclohexane, and the energy of such interactions is available from work on cyclohexane[3] (and pyranose[4]) systems. The importance of such eclipsed interactions in the conformations of polymer chains and such model compounds as 2,4-disubstituted pentanes, 2,4,6-trisubstituted heptanes, and substituted propanes and butanes is well recognised[5]

In 1965, when this work was started, the conformation of only one acyclic carbohydrate derivative [2-(*arabino*-tetrahydroxybutyl)quinoxaline] in solution had been studied[6] The vicinal coupling constants derived from the p m r. spectrum of this compound were consistent with the planar, zigzag conformation, which contains no unfavourable eclipsed β-interaction between hydroxyl groups More recently,

Lyle and Piazza[7] have reported that p m r. spectroscopy indicated that the planar zigzag is the preferred conformation for the acetic esters of the acyclic phenylosotriazole derivatives of carbohydrates, and El Khadem *et al* [8a] have reached the same conclusion for the unesterified phenylosotriazoles (in methyl sulphoxide solution), except for those isomers having an eclipsed β-interaction of hydroxyl groups in the planar zigzag This last conclusion was based essentially on one coupling constant in a spectrum of the *xylo* isomer Some of the parameters of the p m.r spectra of the acetic esters of quinoxaline derivatives of carbohydrates have recently been reported by Chilton and Krahn [8b], who also refer to the importance of β-eclipsed interactions in their discussion of the conformation of the polyacetoxyalkyl chain

The present paper reports the application of p m r. spectroscopy to the conformational analysis of three isomers (D-*arabino*, D-*ribo*, and D-*xylo*) of 3,4,5,6-tetra-acetoxy-1-nitro-1-hexene (**2**).

EXPERIMENTAL

D-*arabino*-3,4,5,6-Tetra-acetoxy-1-nitro-1-hexene, prepared according to the literature[9], had m.p 111–112 5° (corr) The D-*xylo* and D-*ribo* isomers were donated by Professor L Hough and Dr. A. Farrington, and had m p 115–116° and 86 5–87.5°, respectively.

P m r. spectra were measured at 60 MHz on Perkin–Elmer R10 and Varian A-60 spectrometers, at 100 MHz on Varian HA-100 spectrometers, and at 220 MHz on the Varian instrument at the I.C I Petrochemicals and Polymer Laboratory, Runcorn Spectra were measured for $CDCl_3$ solutions (solute concentration between 16% and 21% w/v) unless otherwise stated Tetramethylsilane was used as internal standard

RESULTS

General features of the spectra — The olefinic protons, H-1 and H-2, resonate at lowest field in the region of τ 3, and the allylic proton, H-3, resonates at the next lowest field; these three protons form an ABX subsystem The methylene protons, H-6 and H-6′, resonate at highest field*, and, together with the H-5 proton, form another ABX type subsystem.

The D-arabino *isomer* — The 60 Hz spectrum of a solution in deuterochloroform is shown in Fig. 1. The signals for H-3, H-4, and H-5 were clearly resolved Of the 8 theoretically possible AB resonance signals for the olefinic protons (H-1 and H-2), only four were visible When acetone was used as solvent (Fig 2), only two olefinic proton signals were observed, and the H-3 absorption was now a quintet The remainder of the spectrum was similar in the two solvents H-1, H-2, and H-3 provide an example of deceptively simple ABX systems which were first discussed by Abraham and Bernstein[10] The appearance of deceptively simple (nearly degener-

*Excluding the acetoxyl resonances

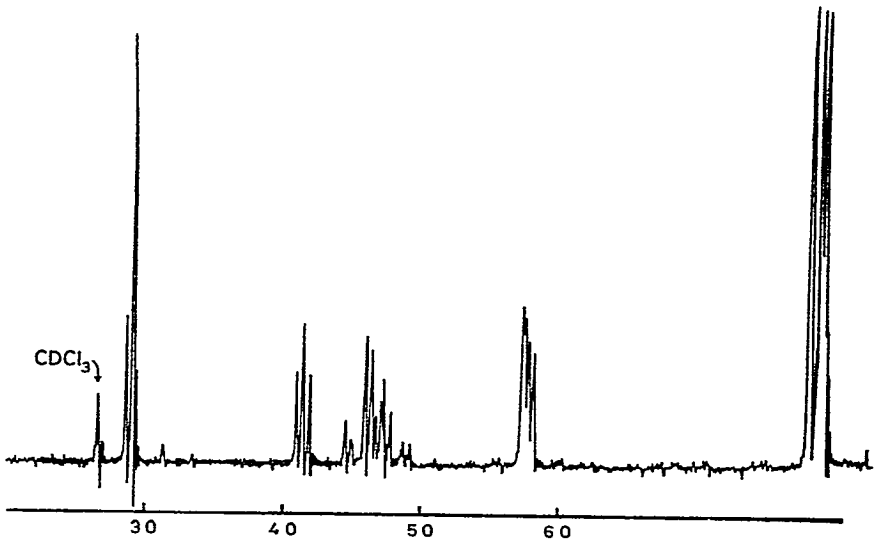


Fig 1 N m r spectrum of the *arabino* isomer of **2** in $CDCl_3$ at 60 MHz.

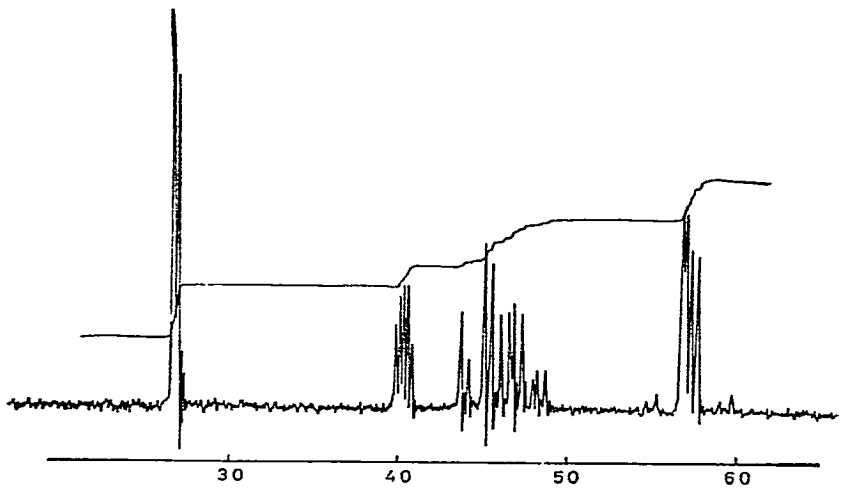


Fig 2 N m r spectrum (excluding acetoxyl resonances) of *arabino* isomer of **2** in acetone

ate) spectra is very sensitive to small changes in δ (the chemical shift between A and B). Small changes in δ can be effected by varying the concentration or solvent[11], and nearly degenerate ABX spectra have been classified on the basis of appearance[11]. The acetone spectrum above corresponds to the extreme case of a deceptively simple ABX spectrum (for H-1, H-2, and H-3) for which

$$\delta + \tfrac{1}{2}\left|J_{AX} - J_{BX}\right| \ll J_{AB}$$

In this system[10], there are two AB signals and three X signals (intensity ratio 1 2 1); the observed quintet for the X (H-3) proton is due to the coupling of H-3 with H-4

In the deuterochloroform spectrum (Fig. 1), one of the AB (olefinic) quartets is degenerate, and its signal overlaps with one of the signals of the other quartet ABX calculations show that coupling of H-3 with H-1 and H-2 gives a doublet*, the observed triplet for H-3 being due to further coupling with H-4 The 100 MHz spectrum (of a deuterochloroform solution) contained six olefinic AB signals, and ABX calculations gave the parameters reported.

ABX calculations were also used for the H-5, H-6, and H-6′ signals, the remaining parameters being obtained by first-order analysis That the parameters so obtained are very close to the true values is indicated by non-iterative calculations for the protons 4, 5, 6, and 6′, obtained by using computer programme LAME of Mr C W. Haigh Small adjustments (<1 Hz) to the chemical shifts of H-4 and H-5 gave computed values for the line positions which differed by $\leqslant 0.3$ Hz from the observed values. Chemical shifts and coupling constants are given in Tables I and II

The D-ribo *isomer.* — The 60 MHz spectrum of this compound contained six olefinic AB signals, but the signals for H-4 and H-5 overlapped and could not be analysed on a first-order basis The signals for H-4 and H-5 were partially resolved at 100 MHz and fully resolved at 220 MHz (Fig 3, concentration 8%), and first-order analysis was possible†. The coupling constants obtained for the H-1, H-2, H-3, and H-5, H-6, H-6′ subsystems by ABX calculations differed by $\leqslant 0.25$ Hz from the first-order values at 220 MHz Calculated values are given in Tables I and II

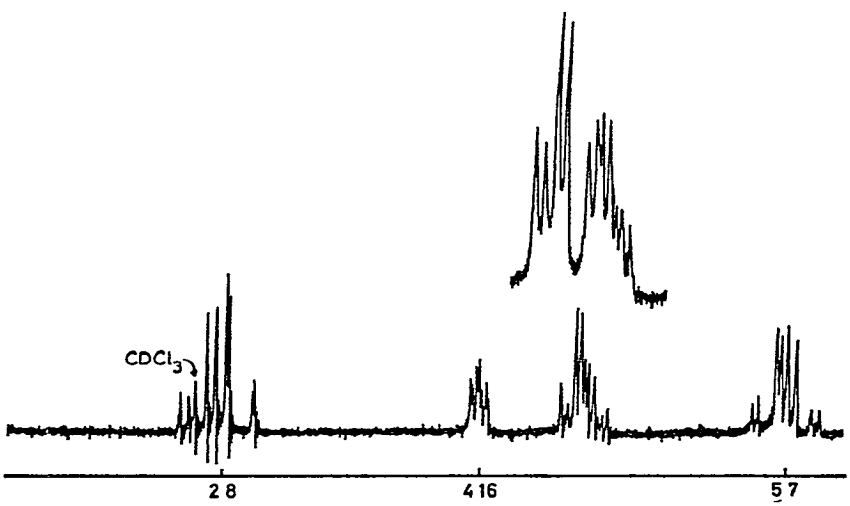


Fig 3 N m r spectrum (excluding acetoxyl resonances) of the *ribo* isomer of 2 in $CDCl_3$ at 100 MHz Upper trace is the H-4–H-5 multiplet at 220 MHz

*This is discussed further in the appendix

†Measurements at 220 MHz, in order to obtain first-order spectra, have recently been reported for several carbohydrates[12a].

The D-xylo *isomer.* — First-order analysis of the overlapping signals for H-4 and H-5 was not possible at 60, 100, and even 220 MHz, and the spectrum was analysed by computation[12b]. Computed values are given in Tables I and II.

TABLE I

CHEMICAL SHIFTS[a] (τ-VALUES) FOR COMPOUNDS **2**

Isomer	*H-1*	*H-2*	*H-3*	*H-4*	*H-5*	*H-6*	*H-6'*
D-*arabino*	2 98	2 88	4 16	4 59	4 80	5 72	5 83
D-*ribo*	3 00	2 66	4 16	4 64	4 74	5 59	5 89
D-*xylo*	2 97	2 79	4 27	4 65	4 69	5 70	6 00

[a]Acetoxyl resonances were as follows for the *arabino* isomer, τ 7 84, 7 91, 7 93, 7 94, for the *ribo* isomer, τ 7 87, 7 92, 7 94, 7 95, for the *xylo* isomer, τ 7 83, 7 87, 7 95, 7 96

TABLE II

COUPLING CONSTANTS (Hz) FOR COMPOUNDS **2**

Isomer	$J_{1\,2}$	$J_{2\,3}$	$J_{1\,3}$	$J_{3\,4}$	$J_{4\,5}$	$J_{5\,6}$	$J_{5\,6'}$	$J_{6\,6'}$
D-*arabino*	13 5	4 9	−1 3	2 8	8.7	2 3	4 6	−12 4
D-*ribo*	13 4	5 2	−1 4	3 4	7 2	2 9	4 9	−12 4
D-*xylo*	13 4	4 9	−1 8	5 43	4 22	4 83	6 16	−11 91

DISCUSSION

That the configuration of the olefins (**2**) is *trans* is indicated by the large values of $J_{1,2}$ for all three isomers. The *trans* coupling constants for nitroethylene[13] and 1-nitro-1-propene[14] are 15 0 and 14 5 Hz, the *cis* coupling constant for the former compound being smaller (7 6 Hz) The *trans* configuration is also supported by the C–H out-of-plane bending vibrations which give strong absorption at 965 cm^{-1} in the infrared spectrum

It is possible to draw some conclusions concerning conformation from the observed vicinal coupling constants (J) which are related to the dihedral angle (ϕ) by the Karplus equation[15] However, there are a number of limitations to this method, since J depends[15,16] on other factors besides ϕ. One of these factors is the electronegativity of substituents[17], an increase in substituent electronegativity decreases J. The electronegativity effect has also been shown to have a stereochemical dependence; the substituent exerts the maximal effect on J when it is *trans* and coplanar (antiperiplanar) to one of the coupling protons[18]. However, it has not proved possible to rationalise vicinal coupling constants observed in cyclic carbohydrate derivatives in terms of a stereochemically dependent substituent-electronegativity effect[19]. Accordingly, for the present work, it is assumed that the coupling (J_G) between gauche-related vicinal protons (dihedral angle, *ca.* 60°) is in the range 2–4 Hz and coupling (J_T) between *trans*-related vicinal protons (dihedral angle,

ca. 180°) is in the range 8–10 Hz for the system C–CH(OAc)–CH(OAc)–C These generalisations are based on literature data[20a] for cyclic compounds

At any given time, molecules will populate several different conformations, depending on their relative energies, and the coupling constants observed in the n m.r. method represent an average for all the conformations If the planar, zigzag conformation of **2** is highly populated, the observed coupling constants should be close to the values corresponding to the dihedral angles in that conformation

The conformation (**2a**) in which the carbon skeleton (C-2–C-6 inclusive) forms a planar zigzag is shown for the D-*arabino* isomer* The $J_{3,4}$ and $J_{4,5}$ coupling constants are consistent with this zigzag conformation which contains no eclipsed β-interaction between acetoxyl groups It should be noted that the n m r. data do not *prove* the planar, zigzag conformation for the following reasons Firstly, solution of the Karplus equation gives two possible values for the dihedral angle, namely ϕ and approximately $(180-\phi)$ For example, solution of the Karplus equation for $J \sim 2.5$ Hz gives $\phi = ca$ 60° or *ca* 120°, however, the latter value corresponds to a relatively unfavourable, eclipsed conformation Secondly, there is often more than one possible conformation for a given dihedral angle. For example, H-3 and H-4 are gauche-related in the planar zigzag conformation and also in the conformation in which the C-3–H bond bisects the projected H–C-4–O angle However, ambiguities can often be eliminated on steric grounds, for example, the latter conformation contains an unfavourable, eclipsed β-interaction between acetoxyl groups at C-3 and C-5

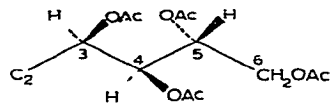
Barker *et al*[21,22], in discussing the conformations of alditols, referred to the interaction between eclipsed β-hydroxyl groups, but they considered[22] that the most probable conformation of an alditol was one in which adjacent hydroxyl groups were gauche-related† Such a conformation for the *arabino* isomer of **2** is excluded by the large $J_{4,5}$ coupling constant which demonstrates that the acetoxyl groups at C-4 and C-5 are *trans* related as in **2a**

The planar, zigzag conformation of the D-*ribo* isomer is shown in Formula **2b.** The observed values of $J_{3,4}$ (3 4 Hz) and $J_{4,5}$ (7 2 Hz) demonstrate that the predominant conformer is not the planar zigzag, but one in which H-3 and H-4 are gauche-related. The planar zigzag contains an unfavourable, eclipsed β-interaction between acetoxyl groups at C-3 and C-5. It is interesting to note that X-ray crystallography has shown[23] that, in crystalline riboflavin hydrobromide monohydrate, there is, in the ribitol moiety, a gauche relationship between H-2′ and H-3′ (corresponding to H-3 and H-4 in the nitrohexene)

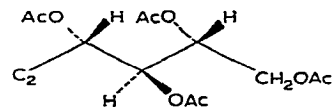
---

*It was not possible to deduce, unambiguously, the preferred orientation of the C=C bond relative to C-2–C-3–C-4 from $J_{1,3}$ and $J_{2,3}$ by using the data of Garbish[20b] and Barfield[20c] The observed values of $J_{1,3}$ and $J_{2,3}$, together with the published data[20d] on simple alkenes and substituted propenes, suggest that there is no marked preference for one orientation of the C=C bond in 2 In the following discussion, the planar, zigzag conformation refers to the carbon skeleton excluding C-1

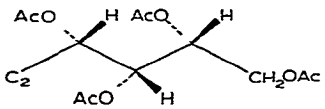
†Barker *et al* are unjustified in ignoring interactions other than between hydroxyl groups



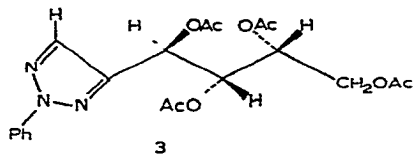
2a



2b



2c



3

The planar, zigzag conformation of the D-*xylo* isomer is shown in Formula **2c** The observed value of $J_{3\,4}$ (5.4 Hz) is not consistent with the planar, zigzag conformation in which there is an eclipsed β-interaction between acetoxyl groups at C-3 and C-5*. Similar values for $J_{3,4}$ (5 6† and 6 5 Hz) have been recently reported for L-*xylo*-hexulose phenylosotriazole[8a] and the acetate of the corresponding quinoxaline derivative[8b].

The *lyxo* isomer of **2** was not available, but it was anticipated that the predominant conformation would be the planar zigzag, since the latter contained no eclipsed β-interactions between acetoxyl groups This is supported by the coupling constants recently reported[7] ($J_{3\,4}$ 7 5, $J_{4\,5}$ 3 5 Hz) for the acetate of D-*lyxo*-hexulose phenylosotriazole (**3**) Similar coupling constants have been reported for the corresponding quinoxaline derivative[8b].

The preferred orientation of the acetoxyl group attached to position 6 cannot be deduced from the values of $J_{5,6}$ and $J_{5\,6'}$ without making certain assumptions Snyder[24], in the conformational analysis of compounds of the type XCH_2CHYZ, made the assumption that the conformation with X gauche to both Y and Z was energetically highly unfavourable However, such an assumption cannot be made for larger molecules, because of the importance of β-eclipsed interactions Variable temperature n m r. studies are in progress in order to identify the preferred orientation of the acetoxyl group at C-6

Because of the possibility of intramolecular hydrogen-bonding, the conformations of unesterified acyclic carbohydrate derivatives can be expected to be very dependent on solvent Intramolecular hydrogen-bonding of the type $-OH\ \cdots N\lessdot$ has been observed[7 8a] in the phenylosotriazole derivatives, and rotatory dispersion results for quinoxaline derivatives and their acetic esters have suggested that intramolecular hydrogen-bonding is involved in the former[8b]. The importance of intramolecular hydrogen-bonding between hydroxyl groups in such hydrogen-bonding solvents as pyridine, methyl sulphoxide, and water, which are normally used to dissolve polyhydroxylic compounds, is uncertain. That intramolecular hydrogen-bonding between β-eclipsed hydroxyl groups in *meso*-2,4-pentanediol (in D_2O) does

*Since there are three acetoxyl groups *trans* and coplanar to H-3 and H-4, $J_{3,4}$ might be expected[18] to be very small (*e g* <2) in the zigzag **2c**

†Obtained by first-order analysis of one signal, the remaining four aliphatic protons being unresolved

not stabilise the planar, zigzag conformation is indicated by analysis of its p m r. spectrum[25].

The foregoing results clearly demonstrate that the predominant conformation of acyclic carbohydrates of type **2** is not the planar zigzag *when* this conformation possesses substituents attached to alternate carbon atoms having the same configuration Non-bonded interactions between such substituents destabilise the planar, zigzag conformation Earlier generalisations[1,2] concerning the planar, zigzag conformation of acyclic carbohydrates are thus invalidated for acetic esters in solution

APPENDIX

The appearance of the H-3 multiplet in the spectra of the *arabino* isomer is worthy of comment In an ABX system, there are six X transitions two of which (14 and 15) are often of low intensity In the spectrum of the acetone solution at 60 MHz, H-1, H-2, and H-3 form a deceptively simple ABX subsystem, in which coupling of H-3 (X) with H-1 (A) and H-2 (B) gives a triplet which is further split by coupling with H-4 to give a quintet However, in the spectrum of the deuterochloroform solution, coupling of H-3 with H-1 and H-2 gives only two lines (the observed triplet being due to further coupling with H-4) This is due to the coalescence of lines 10 and 12 and also lines 11 and 9 The condition for such line coincidence is:

$$J_{AX}+J_{BX} = [(\delta+|J_{AX}-J_{BX}|)^2+J_{AB}^2]^{0.5} - [(\delta-\tfrac{1}{2}|J_{AX}-J_{BX}|)^2+J_{AB}^2]^{0.5}$$

The right-hand side of this equation represents the separation of lines 10 and 11. This separation increases with δ, and when $\delta-\frac{1}{2}J_{AX}-J_{BX}| \gg J_{AB}$ it becomes approximately $|J_{AX}-J_{BX}|$ Thus, no coalescence of lines 10 and 12 can occur if $|J_{AX}+J_{BX}| > |J_{AX}-J_{BX}|$. Therefore, the condition for coalescence of lines 10 and 12 (also 9 and 11) is that J_{AX} and J_{BX} have opposite signs The variation of the X line positions

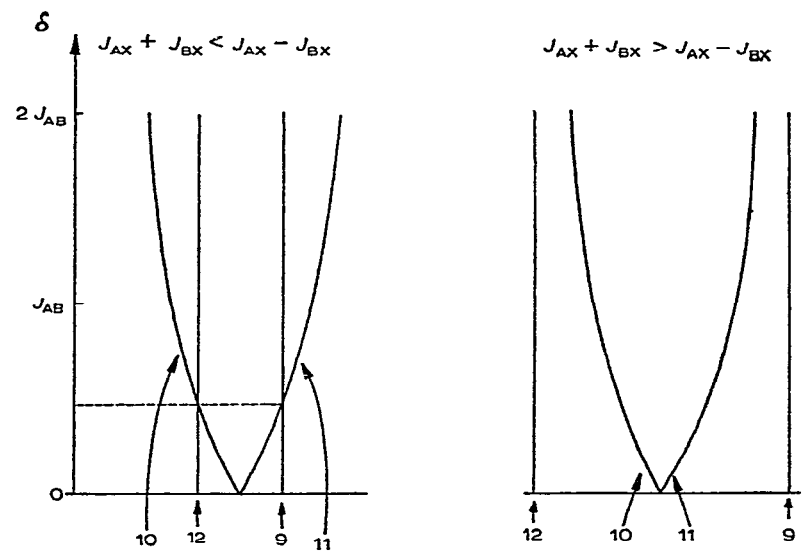


Fig 4 Variation of X-line positions with δ for ABX systems

with δ (in units of J_{AB}) is plotted in Fig 4 for ABX systems in which J_{AX} and J_{BX} have the same and opposite signs. The value of δ for which line coalescence occurs is indicated by the dotted line. The X signal is a doublet when, in addition, lines 14 and 15 have negligible intensity.

ACKNOWLEDGMENTS

The author is indebted to Professor L Hough and Dr A Farrington for the donation of samples, to Drs C Cone and J Feeney for measuring 100 MHz spectra, to Dr. Becconsall of I C.I. Limited, and the Science Research Council for 220 MHz spectra, and to Mr. C. W. Haigh for helpful discussion and the use of his computer programme LAME

REFERENCES

1 S A Barker, E J Bourne, and D H Whiffen, *J Chem Soc*, (1952) 3865.
2 E L Eliel, N L Allinger, S J Angyal, and G A Morrison, *Conformational Analysis*, Wiley, London 1965, p 391
3 Ref 2, p 52.
4 S J Angyal, V A Pickles, and R Ahluwahlia, *Carbohyd Res*, 1 (1966) 365
5 F A Bovey, *Pure Appl Chem*, 12 (1966) 525, and references cited therein, A B Dempster, K Price, and N Sheppard, *Chem Commun*, (1968) 1457, D C Best and C A Kingsbury, *J Org Chem*, 33 (1968) 3252
6 D Horton and M J Miller, *J Org Chem*, 30 (1965) 2457
7 G G Lyle and M J Piazza, *J Org Chem*, 33 (1968) 2478
8 (*a*) H S El Khadem, D Horton, and J F Page, Jr, *J Org Chem*, 33 (1968) 734, (*b*) W S Chilton and R C Krahn, *J Amer Chem Soc*, 90 (1968) 1318
9 J C Sowden and H O L Fischer, *J Amer Chem Soc*, 69 (1947) 1048
10 R J Abraham and H J Bernstein, *Can J Chem*, 39 (1961) 216
11 C W Haigh, M H Palmer, and B Semple, *J Chem Soc*, (1965) 6004
12 (*a*) N S Bhacca and D Horton, *Chem Commun*, (1967) 867, C V Holland, D Horton, M J Miller, and N S Bhacca, *J Org Chem*, 32 (1967) 3077, N S Bhacca and D Horton, *J Amer Chem. Soc*, 89 (1967) 5993, N S Bhacca, D Horton, and H Paulsen, *J Org Chem*, 33 (1968) 2484
12 (*b*) C W Haigh and J. M Williams, *J Mol Spec*, in press
13 W Brugel, Th Ankel, and K Kruckeberg, *Z Elektrochem*, 64 (1960) 1121
14 Y V Baskov, T. Urbanski, M Witanowski, and L Stefaniak, *Tetrahedron*, 20 (1964) 1519
15 M J Karplus, *J Chem Phys*, 30 (1959) 11
16 M J. Karplus, *J Amer Chem Soc*, 85 (1963) 2870
17 R J Abraham and K G. R Pachler, *Mol Phys*, 7 (1964) 165, K L Williamson, *J Amer Chem Soc*, 85 (1963) 516
18 N S Bhacca and D. H Williams, *J Amer Chem Soc*, 86 (1964) 2742, H Booth, *Tetrahedron Lett*, (1965) 411
19 See, for example. B Coxon, *Tetrahedron*, 21 (1965) 3481
20 (*a*) See, for example A A Bothner-By, *Advan Magn Resonance*, 1 (1965) 195, (*b*) E W Grabish, Jr, *J. Amer Chem. Soc*, 86 (1964) 5561, (*c*) M Barfield, *J Chem Phys*, 41 (1964) 3825, (*d*) A A Bothner-By, C Naar-Colin, and H Gunther, *J Amer Chem Soc*, 84 (1962) 2748, A A Bothner-By, S Castellano, S J Ebersole, and H Günther, *ibid*, 88 (1966) 2466
21 C R Gray, F C Hartman, and R Barker, *J Org Chem*, 30 (1965) 2020
22 F C Hartman and R Barker, *Biochemistry*, 4 (1965) 1068
23 N Tanaka, T Ashida, Y Sasada, and M Kakudo, *Bull Chem Soc Japan*, 40 (1967) 1737
24 E I Snyder, *J Amer Chem Soc*, 88 (1966) 1165
25 P E McMahon and W C Tincher, *J Mol Spec*, 15 (1965) 180

Carbohydrate Research
Elsevier Publishing Company, Amsterdam
Printed in Belgium

THE FORMATION OF AN UNSATURATED HEPTONIC ACID DURING THE WOLFF REARRANGEMENT OF 1-DEOXY-1-DIAZO-D-*gluco*-HEPTULOSE PENTAACETATE

DANIEL CHARON
Institut de Biochimie, Faculté des Sciences, Orsay (France)

(Received February 28th, 1969, in revised form, May 22nd, 1969)

ABSTRACT

Application of the Wolff rearrangement to an acetylated α-diazoketone (3,4,5,6,7-penta-*O*-acetyl-1-deoxy-1-diazo-D-*gluco*-heptulose), using silver oxide as catalyst, gave an α,β-unsaturated acid (*trans*-4,5,6,7-tetra-*O*-acetyl-2,3-dideoxy-D-*arabino*-hept-2-enonic acid) in good yield, instead of the expected, saturated β-acetoxy acid. The structure of this new compound, established by spectroscopic data, was confirmed by degradation studies

INTRODUCTION AND DISCUSSION

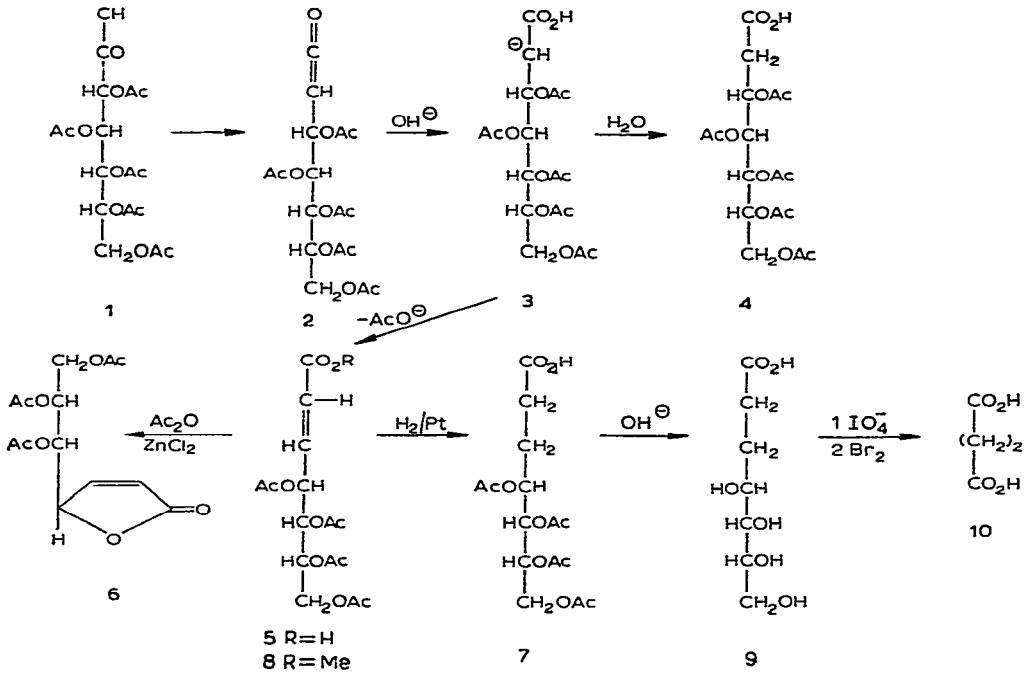
A general method, based on the Wolff rearrangement[1] of diazoketones, for lengthening the carbon chain of carboxylic acids has been described by Arndt and Eistert[2] In the carbohydrate field, this reaction apparently has been applied only in the syntheses of di-*O*-acetyl-4-deoxy-L-*threo*-pentaric acid dimethyl ester[3] and of a compound believed[4] to be the δ-lactone of tetra-*O*-acetyl-2-deoxy-D-*gluco*-heptonic acid.

In connection with other work, 2-deoxy-D-*gluco*-heptonic acid was required, and repetition of the synthesis described by Wolfrom *et al.*[4] gave, without difficulty, the reported product. However, the structure of this compound has now been established as *trans*-4,5,6,7-tetra-*O*-acetyl-2,3-dideoxy-D-*arabino*-hept-2-enonic acid (**5**) on the basis of the following evidence

The i.r. spectrum of compound **5** shows bands (1690 and 3200 cm^{-1}) indicative of a free carboxyl group. Treatment with diazomethane affords a crystalline methyl ester **8**, the i.r. spectrum of which shows no band due to hydroxyl groups Hydrogenation over Adams' catalyst resulted in the uptake of 1 mol of hydrogen by compound **5** to give a crystalline acid **7**, with concomitant loss of the i.r. band at 1630 cm^{-1} assignable[6] to a C=C bond. There was also a shift in the carbonyl band (1690→1710 cm^{-1}) consistent with the loss of α,β-unsaturation. Deacetylation of compound **5** gave a product having a u.v. absorption (λ_{max} 201 nm, ε 12,800), characteristic[7] of an α,β-unsaturated carboxylic acid. This absorption disappeared upon hydrogenation.

Deacetylation of compound **7** and treatment of the product (**9**) with periodate

at room temperature resulted in the uptake of 3 mol of oxidant and the release of 2 mol of formic acid and no carbon dioxide Further oxidation with bromine gave succinic acid. These data are consistent with structure **9**



The n m r. spectrum of compound **5** contains signals attributable to an acidic proton and to *trans* olefinic protons (δ 6 90 and 5 92, J 15 5 Hz)

Treatment of compound **5** with zinc chloride and acetic anhydride gave an acetylated lactone **6**, elemental analysis of which was consistent with the formula $C_{13}H_{16}O_8$ The n m r. spectrum of compound **6** indicated the absence of an acid proton and the presence of *cis* (J 6 Hz) ethylenic protons, the chemical shifts (δ 7 4 and 6 2) of which indicated[8] that the double bond was α,β to a carbonyl group The i r. spectrum of compound **6** contained, in addition to an acetate carbonyl band (1750 cm^{-1}), a strong carbonyl band at 1785 cm^{-1} assignable[6] to an α,β-unsaturated γ-lactone In the mass spectrum of compound **6**, ions m/e 83 and 217 of high intensity were observed, arising by cleavage of the C-4–C-5 bond All of these data are consistent with structure **6**

It is generally considered that, in the Wolff rearrangement, the key intermediate is a ketene, derived from the initial diazoketone *via* a carbene[9], this ketene, upon reaction with water or alcohol, then yields the corresponding deoxy acid or ester. However, the ketene **2**, derived from the carbene **1**, contains a good leaving group situated at the β position to the carbonyl function, the loss of an acetate ion from the intermediate carbanion **3** will therefore lead to the formation of the olefinic acid **5**. The mechanism proposed[10] to explain the formation of cinnamic and crotonic acid

derivatives from the corresponding diazoketones is probably not a major pathway when silver oxide is used to initiate the rearrangement, since free olefinic acids were not formed when the catalytic rearrangement was carried out in methanol, free acids as well as esters can be expected to appear as a result of random attack of the nucleophile on the suggested intermediate

Since the nature of the product in the Wolff rearrangement is largely determined by the relative rates of competitive reactions, it is possible that conditions may be found which will yield 2-deoxyaldonic acids.

EXPERIMENTAL

Unless otherwise stated, all solvents were removed *in vacuo*. I r. spectra were recorded with a Leitz spectrometer (KBr pellets) N m r spectra were obtained with a JEOL spectrometer at 60 MHz for *ca* 20% solutions in deuteriochloroform, with tetramethylsilane as internal reference. The mass spectra were measured with an Atlas (CH-4) spectrometer. Optical rotations were determined at 25±2° with a Perkin–Elmer 141 polarimeter. All named products gave single spots on t l c (Kieselgel G; ethyl acetate–benzene, 30 70, methanol–benzene, 2 98, detection with 30% sulphuric acid at 120°)

4,5,6,7-Tetra-O-*acetyl-2,3-dideoxy*-D-arabino-*hept-2-enonic acid* (**5**). — Freshly prepared silver oxide (8 g) was added to a suspension of penta-*O*-acetyl-1-deoxy-1-diazo-D-*gluco*-heptulose[11] (45 g) in water (1600 ml), and the mixture was heated to 60°. More silver oxide (8 g) was added, in small portions with shaking, during 15 min After a further 30 min at 60°, the cooled mixture was filtered, and the filtrate was decationised with Amberlite IR-120 resin (H^+) and kept for 2 days at 2° The product **5** (26.5 g) was filtered off, washed with cold water, and dried (P_2O_5) Acid **5** had m p 130° (raised to 132° after one recrystallisation from toluene), $[\alpha]_D^{24}$ +39° (*c* 2, chloroform), lit [4], m p 129–130°, $[\alpha]_D$ +40° (Found C, 50 11, H, 5 63; saponification value, 5 equivalents $C_{15}H_{20}O_{10}$ calc C, 50 00; H, 5.60, saponification value, 5 equivalents). I r. data ν_{max}^{KBr} 1625 (C=C conjugated), 1690 (C=O α,β-unsaturated carboxylic acid), 1740 (acetyl C=O), 3200 cm^{-1} (carboxyl OH). N.m.r. data: δ 10.8 (H-1); 6.9 (1-proton quartet, $J_{2,3}$ 15 5, $J_{3,4}$ 5 Hz, H-3); 5.92 (1-proton quartet $J_{2,3}$ 15 5, $J_{2,4}$ 1 6 Hz, H-2), 5.7–5 (complex multiplets, H-4, H-5, H-6), 4 2 (2-proton multiplet, H-7, H-7'); 2 15, 2 10 (singlets, 12 protons, 4 CH_3).

5,6,7-Tri-O-*acetyl-2,3-dideoxy*-D-arabino-*hept-2-enonolactone* (**6**) — The acid **5** (100 mg) was added to acetic anhydride (2 ml) containing freshly fused zinc chloride (50 mg). The mixture was kept for 68 h at room temperature and then washed with water (10 ml) The aqueous layer was extracted with chloroform (3×5 ml), and the combined extracts were dried (Na_2SO_4) and evaporated. Toluene was distilled several times from the residue which was then crystallised from the same solvent at 2° to give compound **6**, m p. 135–140° (Found C, 51.55, H, 5 33, O, 42 60. $C_{13}H_{16}O_8$ calc · C, 51 94; H, 5 32, O, 42 62%) I r data $\nu_{max}^{CCl_4}$ 1750 (acetyl C=O) and 1785 cm^{-1} (C=O α,β-unsaturated-γ-lactone) N m r data δ 7 42 (1-proton quartet, $J_{1,2}$ 6,

$J_{2,3}$ 1.5 Hz, H-2), 6.2 (1-proton quartet, $J_{1,2}$ 6, $J_{1,3}$ 2.0 Hz, H-1); 5.5–5.3 (complex 3-proton multiplet, H-3, H-4, H-5); 4.5–4.3 (2-proton multiplet, H-6, H-6'); 2.15, 2.075, 2.037 (singlets, 9 protons, 3 CH_3). The mass spectrum showed main peaks at *m/e* 83 and 217. The peak at 217 gives rise to a peak at 175 by loss of a ketene fragment, as proved by the presence of a metastable peak at 141.1. By loss of one molecule of acetic acid, the peak at 175 gives rise to a peak at 115 (metastable peak at 75.5).

trans-*4,5,6,7-Tetra*-O-*acetyl-2,3-dideoxy*-D-arabino-*hept-2-enonic acid methyl ester* (**8**) — Treatment of acid **5** with diazomethane gave ester **8** (in almost quantitative yield), m.p. 118–119°, $[\alpha]_D^{25}$ +37° (*c* 3.5, chloroform) (Found: C, 51.30; H, 5.99. $C_{16}H_{21}O_{10}$ calc. C, 51.32, H, 5.88%). The n.m.r. and infrared spectra were in accord with the structure assigned.

4,5,6,7-Tetra-O-*acetyl*-2,3-*dideoxy*-D-arabino-*heptonic acid* (**7**). — The acid **5** (4 g) in ethanol (100 ml) was hydrogenated in the presence of Adam's platinum catalyst (250 mg). The hydrogenation (1 mol uptake) was complete after 70 min. The filtered mixture was concentrated, and the residue was dissolved in carbon tetrachloride (25 ml) and kept for 3 days at −20° to give the tetra-acetate **7** (3 g), m.p. 69° (from carbon tetrachloride), $[\alpha]_D^{24}$ +27.96° (*c* 1.5, chloroform) (Found: C, 49.59, H, 6.05, O, 43.77; saponification number, 5. $C_{15}H_{22}O_{10}$ calc.: C, 49.68, H, 6.07; O, 44.16%, saponification number, 5). N.m.r. data: δ 9.05 (1-proton singlet, H-1); 5.4–5 (complex 3-proton multiplet, H-4, H-5, H-6); 4.3–4.1 (2-proton multiplet, H-7 and H-7'), 2.4, 2.28 (two peaks, H-2, H-2'), 2.12, 2.04 (2 singlets, 4 CH_3); 1.9, 1.8 (two peaks, H-3, H-3').

trans-*2,3-Dideoxy*-D-arabino-*hept-2-enonic acid* — The acid **5** (5 g) was treated with barium hydroxide (10.95 g, octahydrate) in carbon dioxide-free water (110 ml). After 1 h, the pH of the solution was adjusted to 9 by the addition of baryta. The solution was stored for another hour, and was then decationised with Amberlite IR-120 (H^+) resin, concentrated until crystallisation commenced, and stored overnight at 2°. The compound had m.p. 174° (lit.[4] 170°, for product designated as 2-deoxy-δ-glucoheptonolactone), $[\alpha]_D^{24}$ +19° (*c* 2, water) (Found: C, 43.79; H, 6.19; O, 49.69. $C_7H_{12}O_6$ calc.: C, 43.68, H, 6.24; O, 49.92%). U.v. data: λ_{max} 201 nm (ε 12,800).

2,3-Dideoxy-D-arabino-*heptonic acid* (**9**). — The acetate **7** (1.5 g) was treated for 6 h with barium hydroxide [3.3 g of the octahydrate in CO_2-free water (100 ml)]. The solution was decationised with Amberlite IR-120 (H^+) resin and concentrated, and the residue was crystallised from methanol–ether at −20° to give the heptonic acid **9** (350 mg), m.p. 113–115°, $[\alpha]_D^{24}$ −56.9° (*c* 2, water) (Found: C, 43.26; H, 7.31; O, 48.23. $C_7H_{14}O_6$ calc.: C, 43.29, H, 7.26, O, 49.43%).

Degradation of acid **5** *to succinic acid* (**10**) — A solution of the acid **5** (10 g) in methanol (100 ml) was hydrogenated in the presence of Adam's catalyst (700 mg). When the hydrogen uptake had ceased (*ca.* 1 h), the filtered solution was diluted to 1 litre with water, and barium hydroxide (22 g, octahydrate) was added. After storage for 2 days at room temperature, the solution was decationised with Amberlite IR-120 (H^+) resin and concentrated (to *ca.* 500 ml). Sodium metaperiodate (18.5 g), dissolved in the minimal quantity of water, was added dropwise, the pH of the solution being

maintained at 7 with aqueous sodium hydroxide. After 3 h, bromine (3ml) and sufficient aqueous sodium hydroxide to bring the pH to 7 were added The solution was kept overnight, concentrated (to 50 ml), and then kept overnight at 2° Barium bromide (3 3 g, dihydrate) was added to the filtered solution, which was then kept for 24 h at room temperature. Barium succinate (2 g), slightly contaminated with iodate, was filtered off. A further quantity of barium succinate (1 g), free from iodate, was obtained by addition of further barium bromide (3 3 g) to the filtrate which was kept overnight at room temperature, heated to boiling point, and filtered A portion of this salt (250 mg) was suspended in water (25 ml) and decationised with Amberlite IR-120 (H^+) resin The filtered solution was concentrated to dryness, and the residue was crystallised from a few drops of water at 2° to give succinic acid, m p and mixed m p. 185–186°, the i r. spectrum was identical with that of an authentic sample

REFERENCES

1 L WOLFF, *Ann*, 394 (1912) 23
2 F ARNDT AND B EISTERT, *Ber*, 68 (1935) 200
3 H J BESTMANN AND R SCHMIECHEN, *Ber*, 94 (1961) 751.
4 M L WOLFROM, S W WAISBROT, AND R L BROWN, *J Amer. Chem Soc*, 64 (1942) 1701
5 R T MAJOR AND E W COOK, *J Amer Chem Soc*, 58 (1936) 2474
6 C N R RAO, *Chemical Applications of Infrared Spectroscopy*, Academic Press, New York, 1964, pp 147 and 232
7 R M SILVERSTEIN AND G C BASSLER, *Spectrometric Identification of Organic Compounds*, 2nd edn, Wiley, New York, p 162
8 Ref 7, p 138
9 PETER A S SMITH, in P DE MAYO (Ed), *Molecular Rearrangements*, Interscience, 1967, p 528
10 F W BACHELOR AND G A MIANA, *Tetrahedron Lett*, (1967) 4333
11 M L WOLFROM, D I WEISBLAT, W H ZOPHY, AND S W WAISBROT, *J Amer Chem Soc*, 63 (1941) 201

Carbohydrate Research
Elsevier Publishing Company Amsterdam
Printed in Belgium

THE CHEMICAL SYNTHESIS OF POLYSACCHARIDES

PART I. DEVELOPMENT OF THE METHOD AND SYNTHESIS OF GENTIODEXTRINS

D McGRATH*, E. E LEE, AND P S O'COLLA
Department of Chemistry, University College, Galway (Ireland)

(Received July 18th, 1968, in revised form, May 23rd, 1969)

ABSTRACT

Methods have been developed for the specific synthesis of oligosaccharides by using partially acetylated derivatives of D-glucose When 1,2,3,4-tetra-*O*-acetyl-β-D-glucopyranose was fused with a catalytic amount of toluene-*p*-sulphonic acid, 1,6-anhydro-β-D-glucopyranose, gentiobiose, gentiotriose, and gentiotetraose were the major sugars recovered following de-acetylation of the reaction mixture. With zinc chloride as catalyst under more forcing conditions, larger proportions of gentiodextrins, some of which were of the polysaccharide type, were formed. Two polysaccharide preparations have been examined and shown to be mainly composed of β-(1→6)-linked D-glucopyranose residues

INTRODUCTION

The unequivocal chemical synthesis of oligo- and poly-saccharides owes its slow development, most probably, to the difficulty in obtaining suitable intermediates[1,2] However, oligosaccharides containing only one type of linkage have been synthesised from a single reactant in a Koenigs–Knorr type reaction[3], and stereospecific syntheses of polysaccharides have recently been reported[4,5] A new method for the synthesis of polymers containing only one type of linkage, by the melt-polymerisation of partially acetylated glucose derivatives in the presence of suitable catalysts, has been reported[6] We now report on the development of this method and its application to the preparation of two (1→6)-linked D-glucans and a homologous series of β-D-(1→6)-linked oligosaccharides from 1,2,3,4-tetra-*O*-acetyl-β-D-glucopyranose

DISCUSSION

Initially, paper-chromatographic analysis showed that zinc chloride and toluene-*p*-sulphonic acid were both effective catalysts in the formation of oligosaccharides by the polymerisation of 1,2,3,4-tetra-*O*-acetyl-β-D-glucopyranose. Experiments were then carried out to determine the relative action of the two catalysts on

*Present address An Foras Taluntais, Soils Division, Johnstown Castle, Wexford (Ireland)

1,2,3,4-tetra-*O*-acetyl-β-D-glucopyranose, 1,2,3,4-tetra-*O*-acetyl-α-D-glucopyranose, 1,2,3,-tri-*O*-acetyl-β-D-glucopyranose, and methyl 2,3-di-*O*-acetyl-β-D-glucopyranoside To ensure an intimate mixture, acetate and catalyst were dissolved or dispersed in acetone which was then removed under diminished pressure at room temperature When the residue was heated at 100° or above, it fused and liberated acetic acid which was removed to prevent charring. This was done by allowing a stream of air to pass through the melt while the reaction flask was being continuously exhausted by means of a water pump. To prevent discoloration and charring of the melt, the proportion of catalyst, temperature, and heating period had to be carefully controlled.

Some generalisations may be made concerning the factors which influenced the course of the reaction. Firstly, the β-D-tetra-acetate was, as expected, much more reactive than its α-D anomer, and the primary hydroxyl group was more reactive than the secondary hydroxyl group, as was shown by the similarity of the products obtained when 1,2,3,4-tetra-*O*- and 1,2,3-tri-*O*-acetyl-β-D-glucopyranose were each heated in the presence of zinc chloride or toluene-*p*-sulphonic acid Secondly, catalyst amounts in excess of 5% of zinc chloride and 0 25% of toluene-*p*-sulphonic acid caused charring Polymerisation did not occur in the absence of catalyst Thirdly, reaction temperatures of 100° and above were required in order to bring about the necessary fusion of the acetate. Within limits, the higher the temperature the more rapid was the formation of oligosaccharides and the greater was the tendency towards the production of dextrins of higher molecular weight

Zinc chloride and toluene-*p*-sulphonic acid were chosen initially on the basis of their catalytic effect on the condensation of D-glucose penta-acetates with phenol in the preparation of the phenyl α- and β-D-glucosides[7] The amount of catalyst used in these preparations was about five times that used in this polymerisation technique, and it was found that, whereas zinc chloride gave the α-D-glucoside mainly, toluene-*p*-sulphonic acid gave the β-D-glucoside almost entirely Bredereck and Hoschele have shown[8] that condensation (by elimination of water), acetyl migration (C-4→C-6), anomerisation (β→α), and formation of anhydro sugar occur when some D-glucose tetra-acetates are heated with zinc chloride and other catalysts Under our experimental conditions, formation of anhydro sugar and, almost certainly, anomerisation[9] occurred

In a typical experiment, the β-D-tetra-acetate (14 8 g) was heated with 0 25% of toluene-*p*-sulphonic acid for 1 5 h at 100°/20 mmHg, cooled, and de-acetylated[10] Chromatographic examination of the de-acetylated mixture indicated that it contained glucose and at least seven oligosaccharides, the R_M values[3] of which indicated that they were gentiodextrins. When this mixture was fractionated on charcoal–Celite[11], 2 11 g of glucose, 0 55 g of 1,6-anhydro-β-D-glucopyranose, 2 30 g of gentiobiose (6-*O*-β-D-glucopyranosyl-D-glucose), 0 50 g of gentiotriose, 90 mg of gentiotetraose, and two sugar mixtures containing gentiobiose and one or both of two sugars (*X* and *Y*) of unestablished constitution were obtained. The total recovery of sugar was *ca.* 80%. The two unknown sugars were not investigated in detail; both gave glucose only on hydrolysis The major component (*Y*), having a negative

optical rotation, was possibly 5-*O*-β-D-glucopyranosyl-D-glucose[12]. No trace of isomaltose or cellobiose was detected among the reaction products; all of the experimental evidence indicated that no α-D-linkages were formed, and that acetyl migration did not occur Small proportions of artifacts were formed, and the reactant was inactivated, probably by conversion into the α-D-anomer and into 1,6-anhydro-β-D-glucopyranose tri-acetate

Better yields of oligomers were obtained by using zinc chloride as catalyst. Chromatography indicated that, in the melt polymerisation of 1,2,3,4-tetra-*O*-acetyl-β-D-glucose with 5% of zinc chloride, oligo- and poly-saccharides were formed within ten minutes Two polysaccharides (*P1* and *P2*) were synthesised by this technique, the relevant details are as follows

Product	*Time of heating (min)*	*Temp (degrees)*	*Yield (%)*	$[\alpha]_D$ *in water (degrees)*
P1	30	125	9	+4 8
P2	10	140–145	11 3	−2 9

In each case, the polysaccharide was isolated after deacetylation and dialysis

Both *P1* and *P2* were ash-free, contained no chromatographically mobile sugars, and yielded glucose on acid hydrolysis. Comparison of the specific rotations with the values of −46° for pustulan, the β-(1→6)-linked D-glucan, and +215° for a typical dextran composed largely of α-(1→6)-linked D-glucose residues indicated a preponderance of linkages having a β-D configuration. Synthetic glucans prepared by non-specific procedures are highly branched and have rotations of +60° to +110°. Hypoiodite oxidation[13] gave *DP* (degree of polymerisation) values of *ca* 9 for *P1* and *P2*, but this must be taken as a minimum, since overoxidation gives low values[14], and what was apparently the nonaose was detected in the dialysate One mole of *P1* consumed 2 08 moles of periodic acid and liberated 0 99 mole of formic acid, whereas *P2* consumed 2 02 moles and liberated 0 98 mole. Further evidence that the polymers incorporated mainly (1→6)-linked D-glucopyranose residues was obtained by standard methylation and hydrolysis procedures The only trimethyl ether detected was 2,3,4-tri-*O*-methyl-D-glucose, which constituted 75% of the *P1* and 86% of the *P2* methyl ethers. Small proportions of a di-*O*-methylglucose, probably the 3,4-di-*O*-methyl derivative, were obtained from both the *P1* and *P2* methyl ethers during fractionation on cellulose, this may have been due to incomplete methylation, or, more likely, to demethylation The ratio of tri- to tetra-methyl ether indicated a *DP* of *ca* 10 for both polymers

EXPERIMENTAL AND RESULTS

Materials — 1,2,3,4-tetra-*O*-acetyl-β-D-glucopyranose[15] (m p 127°), 1,2,3,4-tetra-*O*-acetyl-α-D-glucopyranose[16] (m p 99–100°), 1,2,3-tri-*O*-acetyl-β-D-glucopyranose[17] (syrup), and methyl 2,3-di-*O*-acetyl-β-D-glucopyranoside[18] (syrup) were prepared as described in the literature

General methods — Solutions of sugars were concentrated *in vacuo* at temper-

atures below 40°. Optical rotations were determined in water for sugars, and in chloroform for their acetates The following solvent systems (v/v) were used with Whatman No. 1 and 3MM papers· (*A*) butyl alcohol–pyridine–water (6 4·3); (*B*) butyl alcohol–pyridine–water–benzene (5 3 3·1); (*C*) propyl alcohol–ethyl acetate–water (6 1·3); (*D*) butyl alcohol–acetic acid–water (4 1 5, upper layer), (*E*) butyl alcohol–ethanol–water (5 1 4, upper layer), (*F*) butyl alcohol–ethanol–water–ammonia (40 10 49 1, upper layer). Homologous series of sugars were resolved in solvent systems *A* and *C* by a multiple development technique[19]. Reducing sugars were detected with aniline hydrogen phthalate and non-reducing sugars with the potassium permanganate–periodate reagent[20]. Paper electrophoresis was carried out in borate buffer (pH 10) at 400 volts Sugar acetate mixtures were de-acetylated with sodium methoxide in methanol–chloroform Sugars were fractionated on charcoal–Celite (1·1) by stepwise elution with water and aqueous ethanol Periodate oxidations of polysaccharides were carried out in M HIO_4, periodate consumption was measured by titration with thiosulphate, and formic acid release by titration with sodium hydroxide.

Polymerisation procedure — (*a*) A weighed amount of anhydrous zinc chloride or the calculated amount of toluene-*p*-sulphonic acid in acetone was added to the acetate (200 mg) in a test tube fitted with a side arm for attachment to a water pump The volume was made up to *ca.* 2 ml with acetone, the test tube was shaken and then stoppered with a rubber bung through which passed a fine capillary just reaching to the bottom of the tube Solvent was evaporated off under diminished pressure at room temperature The capillary was sealed, and the residue was heated at a previously selected temperature and under the maximum pressure attainable with the water pump The heating time was decided largely by the colour acquired by the melt. The cooled reaction product was taken up in chloroform (1 ml), transferred to a stoppered test-tube, and treated with excess of sodium methoxide in methanol (0 5M, 1 ml) After 1 h at room temperature, the product was extracted into water (3 ml) The extract was acidified with a slight excess of acetic acid, de-ionised with Biodeminrolit (Permutit, London), and concentrated to a syrup which was examined in solvent systems *A*, *B*, and *D*.

(*b*) *Catalysis by toluene*-p-*sulphonic acid* When 1,2,3,4-tetra-*O*-acetyl-*β*-D-glucopyranose was heated on the boiling water-bath with 0 25, 0 5, 1 0, and 2 5% of catalyst, D-glucose, 1,6-anhydro-D-glucose, gentiobiose, gentiodextrins, and an unidentified disaccharide were detected following de-acetylation and examination of the product in solvent systems *A* and *B* Gentiodextrin formation could be intensified by increasing the heating period, or, better, by raising the temperature (to 150°), but there was no advantage to be gained by using more than 0 25% of catalyst. High concentration of catalyst caused more rapid discoloration of the melt, with 1% of catalyst at temperatures of 130° and above, the melt darkened rapidly, and only a trace of gentiobiose was recovered together with much glucose

Trial experiments with 1,2,3-tri-*O*-acetyl-*β*-D-glucopyranose gave results similar to those obtained with the *β*-D-tetra-acetate, without increase in formation of the

unidentified disaccharide. However, similar experiments with 1,2,3,4-tetra-*O*-acetyl-α-D-glucopyranose and methyl 2,3-di-*O*-acetyl-β-D-glucopyranoside gave no indication of polymerisation.

(*c*) *Catalysis by zinc chloride* When β-D-glucose tetra-acetate was heated on a boiling water-bath with 5, 10, or 20% of zinc chloride, oligosaccharide formation occurred Thus, the acetate obtained with 5% of zinc chloride for 30 min gave D-glucose, 1,6-anhydro-D-glucose, the unidentified disaccharide (less than with toluene-*p*-sulphonic acid), gentio-biose, -triose, and -tetraose (the coloration of these with aniline hydrogen phthalate was more pronounced than that of glucose), and other gentiodextrins Zinc chloride caused less degradation than toluene-*p*-sulphonic acid, no excessive discoloration occurring when the acetate was heated with 10% of catalyst for up to 30 min at 150° With 20% of catalyst, the melt darkened rapidly above 120°

β-D-Glucose 1,2,3-tri-acetate gave results similar to those obtained with the β-D-tetra-acetate The α-D-tetra-acetate, with 5 or 10% of catalyst, gave D-glucose and 1,6-anhydro-D-glucose, together with gentiobiose (and/or isomaltose) and oligosaccharides, but in much smaller proportions than did the β-D-tetra-acetate Methyl 2,3-di-*O*-acetyl-β-D-glucopyranoside gave no oligosaccharides

(*d*) *Catalysis by mercuric chloride.* 1,2,3,4-Tetra-*O*-acetyl-β-D-glucopyranose (but not its α-D anomer) with 5 or 10% of mercuric chloride for 1 h at 120° yielded only a trace of gentiobiose

Action of heat on acetates — When the α- and β-D-glucose 1,2,3,4-tetra-acetates, β-D-glucose 1,2,3-tri-acetate, or methyl 2,3-di-*O*-acetyl-β-D-glucopyranoside were separately heated at temperatures up to 150° for long periods, no oligosaccharides were formed

Large-scale polymerisation of β-D-glucopyranose tetra-acetate — The tetra-acetate (14 8 g), in acetone containing toluene-*p*-sulphonic acid (0 1%, 37 ml), was placed in a 250-ml flask and washed down with acetone (13 ml) The flask was attached to a water pump by means of an adapter fitted with a side arm, a fine, glass capillary (reaching to the bottom of the flask) was placed in position, and the solvent was evaporated under diminished pressure at room temperature. The residue was heated on a boiling-water bath for 90 min at a pressure of 20 mmHg The light-yellow, translucent melt was taken up in chloroform (30 ml) and treated with methanolic sodium methoxide (0 5M; 30 ml) After 1 h at room temperature, the free sugars were taken up in water (100 ml), and the aqueous extract was acidified with acetic acid and concentrated. A portion (0 50 g) of the syrupy product was de-ionised with Biodeminrolit and examined in solvent systems *A* and *C* by the multiple-development technique In both solvent systems, there was a linear relationship between presumed *DP* (2–7) and R_M ($R_M = \log 1/R_F - 1$) of the main sugars resolved.

The residual syrup was placed on a column (50 × 750 mm) of charcoal–Celite Eluate fractions (500 ml) were collected, concentrated to a syrup, and examined in solvent systems *A* and *B*

Fractions 3 and 4, eluted with water, contained salts

Fractions 6–10, eluted with water, contained D-glucose (2 11 g), characterised by conversion into its β-D-penta-acetate, m p and mixed m p 131°, $[\alpha]_D$ +4 0°

Fractions 12–18, eluted with water, gave 1,6-anhydro-D-glucose (550 mg), $[\alpha]_D$ −64°, lit. −66 4°; which was characterised as the tri-acetate, m p and mixed m p 108–109°, $[\alpha]_D$ −59°, lit [21], m p 110°, $[\alpha]_D$ −62 5°

Fractions 20–25, eluted with 5% aqueous ethanol, gave gentiobiose (2 30 g), $[\alpha]_D$ +10.1° (lit [3] +9 9°), characterised as the octa-acetate, m p and mixed m p 194–195°, $[\alpha]_D$ −5 6° (lit [3] −5 3°)

Fractions 27–29, eluted with 5% aqueous ethanol, contained a mixture (80 mg) of a reducing substance (*X*) and gentiobiose *X* had chromatographic mobility similar to that of cellobiose in solvent systems *A* and *B*, but its M_G value (0 90) was different (cellobiose 0 24) On hydrolysis with N H_2SO_4, the mixture gave glucose only

Fractions 30–37, eluted with 5% aqueous alcohol, gave a mixture (160 mg) of gentiobiose, *X*, and a second, unidentified, reducing substance (*Y*) with chromatographic mobility slightly greater than that of cellobiose, and with M_G 0 39 The mixture had $[\alpha]_D$ −10 6° and, on hydrolysis, gave glucose only

Fractions 43–61, eluted with 15% aqueous alcohol, gave gentiotriose (501 mg), $[\alpha]_D$ −11° (lit.[3] −10 3°), which was characterised as the acetate, m p. 214–215°, $[\alpha]_D$ −9 3°; lit [3], m p 214–215°, $[\alpha]_D$ −9 4°.

Fractions 63–75, eluted with 25% aqueous alcohol, gave gentiotetraose, contaminated with gentiotriose (100 mg) Fractionation on Whatman No 1 paper, in solvent system *A*, gave the tetraose (88 mg), $[\alpha]_D$ −14°, lit [3] −18 4° It yielded an impure acetate, m p. 120–124°, lit [3] 134–135°

Preparation and examination of polysaccharide P1 — β-D-Glucose 1,2,3,4-tetra-acetate (14.9 g) was treated with zinc chloride (745 mg) and heated *in vacuo* for 30 min at 125°. The melt was de-acetylated, and the water-soluble material was obtained as a syrup that was redissolved in water (150 ml). The solution was filtered, and dialysed against frequent changes of distilled water during 36 h The dialysate (4 92 g) contained glucose and gentiodextrins (*DP* 2–9) The polymer solution was further dialysed in running tap-water for 36 h, concentrated to a small volume (15 ml), and treated with a six-fold volume of ethanol The polysaccharide (401 mg) was removed on a sintered-glass funnel, and the filtrate was treated with acetone (50 ml). This gave a further quantity (303 mg) of polysaccharide, the final filtrate contained gentiopentaose and higher gentiodextrins Both polysaccharide preparations were non-mobile on development for 48 h in solvent systems *A* and *B*, and had ash contents of <1% Hypoiodite oxidation[13] gave *DP* values of 9 0 and 8 85 (averages of two determinations) for the alcohol-precipitated and acetone-precipitated materials, respectively. Since the products appeared to be essentially similar, they were combined to give polysaccharide *P1* It had $[\alpha]_D$ +4 8° (*c* 2 5, water), and, on hydrolysis, gave glucose only On oxidation for 25 h with periodate, the polysaccharide consumed 2 08 moles of periodate, and liberated 0.99 mole of formic acid per 162 g; for a

polymer incorporating (1→6)-linked D-glucopyranose residues only, the calculated values are 2.00 and 1.00, respectively

Methylation analysis of polysaccharide P1. — Polysaccharide *P1* (400 mg) was successively methylated by the Haworth method (twice) and by the Purdie method (8 times). The product (130 mg) had $[\alpha]_D$ +14.5° (*c* 3.04, chloroform) and was insoluble in water (Found. OMe, 41.9%). Following methanolysis of a portion (91 mg) in boiling, methanolic HCl (4%, 10 ml) for 4 h, with subsequent hydrolysis of the glycosides in aqueous N HCl (10 ml) for 6 h on a boiling-water bath, methyl ethers (77 mg) were recovered. A portion (20 mg) was fractionated on Whatman No. 1 paper, and resolved methyl ethers were quantitatively estimated by the hypoiodite procedure[22], giving di-, tri-, and tetra-*O*-methylglucose in the ratio 1.0:7.7:1.3. Fractionation of a second portion (35 mg) on a cellulose column gave 1,2,3,4-tetra-*O*-methylglucose (2.0 mg), R_G 1.00, 2,3,4-tri-*O*-methyl-D-glucose (19.1 mg) which was characterised as the "anilide", m.p. 140–141° (lit.[23] 145°), and a di-*O*-methylglucose (4.0 mg), R_G 0.54 (lit.[22] 0.52 for the 3,4-dimethyl ether) and M_G 0.28 (lit.[24] 0.31).

Preparation and examination of polysaccharide P2. — β-D-Glucose tetra-acetate (14.0 g) was heated with zinc chloride (700 mg) *in vacuo* for 10 min at 140–145°. The recovered product was dialysed against tap water for 48 h, and polysaccharide *P2* (820 mg) was obtained as described for *P1*. It was non-mobile on development in solvents *A* and *B* for 48 h, and had $[\alpha]_D$ −2.9° (*c* 3.4, water). Hypoiodite oxidation gave a *DP* value of 8.25, whereas periodate oxidation resulted in consumption of 2.02 moles of periodate and release of 0.98 mole of formic acid per glucose residue.

P2 was methylated by the Haworth method (twice) and the Purdie method (9 times), giving a product (200 mg) having $[\alpha]_D$ +8.1° (*c* 4.7, chloroform) (Found: OMe, 42.7%). Hydrolysis of a portion (160 mg) in formic acid (98%, 20 ml) on a boiling-water bath for 9 h, followed by treatment in hot water, gave methyl ethers (148 mg). These were resolved on cellulose, giving 2,3,4,6-tetra-*O*-methyl-D-glucose (11 mg), $[\alpha]_D$ +80° (lit.[23] +82°), characterised as the "anilide", m.p. and mixed m.p. 131–133° (lit.[23] 135–136°), 2,3,4-tri-*O*-methyl-D-glucose (91 mg), $[\alpha]_D$ +62.7° (lit.[23] +60°), characterised as the "anilide", m.p. 142–144°; and a di-*O*-methyl-D-glucose (3 mg), R_G 0.54 and M_G 0.28.

REFERENCES

1 R. W. Bailey, *Oligosaccharides*, Pergamon, Oxford, 1965, p. 12
2 I. J. Goldstein and T. L. Hullar, *Advan. Carbohyd. Chem.*, 21 (1966) 431
3 S. Haq and W. J. Whelan, *J. Chem. Soc.*, (1956) 4543
4 E. Husemann and G. T. M. Muller, *Makromol. Chem.*, 91 (1966) 212; E. R. Ruckel and C. Schuerch, *J. Amer. Chem. Soc.*, 88 (1966) 2605
5 N. K. Kochetkov, A. F. Bochkov, and I. G. Yazlovetsky, *Carbohyd. Res.*, 5 (1967) 243
6 P. S. O'Colla, E. E. Lee, and D. McGrath, *Chem. Ind.* (London), (1962) 178
7 B. Helferich and E. Schmitz-Hillebrecht, *Ber.*, 66 (1933) 378
8 H. Bredereck and G. Hoschele, *Ber.*, 86 (1953) 1286
9 P. S. O'Colla, E. E. Lee, and D. McGrath, *Proc. Roy. Dublin Soc.*, *A*, 1, No. 13 (1963) 337
10 G. Zemplen, *Ber.*, 60 (1927) 1555

11 R L WHISTLER AND D F DURSO, *J Amer. Chem Soc*, 72 (1950) 677
12 P S O'COLLA, E E LEE, AND D MCGRATH, *J Chem Soc*, (1962) 2730
13 S CHANDA, E L HIRST, J K. N JONES, AND E G V PERCIVAL, *J Chem. Soc*, (1950) 1289
14 H F LAUNER AND Y TOMIMATSU, *Anal Chem*, 33 (1961) 79
15 B HELFERICH AND W KLEIN, *Ann*, 450 (1926) 219
16 H A LARDY, *J Amer Chem Soc*, 69 (1947) 518
17 A L RAYMOND, *J Biol Chem*, 113 (1936) 378
18 D S MATHERS AND G J ROBERTSON, *J Chem Soc*, (1933) 696
19 A JEANES, C. S WISE, AND R J DIMLER, *Anal Chem*, 23 (1951) 415
20 R U LEMIEUX AND H F. BAUER, *Anal Chem*, 26 (1954) 920
21 A THOMPSON, K ANNO, M L WOLFROM, AND M INATOME, *J Amer Chem Soc*, 76 (1954) 1309
22 E L HIRST, L HOUGH, AND J. K N JONES, *J Chem Soc*, (1949) 928
23 S PEAT, E SCHLUCHTERER, AND M STACEY, *J Chem Soc*, (1939) 581
24 A B FOSTER AND M STACEY, *J Appl Chem*, 3 (1953) 19

Carbohydrate Research
Elsevier Publishing Company, Amsterdam
Printed in Belgium

THE CHEMICAL SYNTHESIS OF POLYSACCHARIDES
PART II (1→2)-, (1→3)-, AND (1→4)-LINKED GLUCANS

D. McGRATH, E E LEE, AND P S O'COLLA
Department of Chemistry, University College, Galway (Ireland)
(Received July 18th, 1968, in revised form, May 23rd, 1969)

ABSTRACT

When the tetra-*O*-acetyl derivatives of D-glucopyranose having a free hydroxyl group at C-2, C-3, or C-4 were separately fused with a catalytic amount of toluene-*p*-sulphonic acid, or zinc chloride, oligosaccharides incorporating (1→2)-, (1→3)-, or (1→4)-linkages, respectively, were formed By using toluene-*p*-sulphonic acid, a homologous series of oligosaccharides incorporating β-D-linkages only was detected, whereas zinc chloride gave a product with an overall 2 1 or 3.1 ratio of β- to α-D-linkage configurations Sophorose, laminaribiose, maltose, and cellobiose were characterised as their crystalline acetates, and kojibiose, laminaritriose, and cellotriose were identified by their chromatographic mobilities and optical rotations

INTRODUCTION

The ability of toluene-*p*-sulphonic acid and zinc chloride to catalyse the formation of oligosaccharides incorporating β-(1→6)-linked D-glucopyranose residues from 1,2,3,4-tetra-*O*-acetyl-β-D-glucopyranose was described in the previous paper[1] Since D-glucose tetra-acetates are readily available, additional experiments were carried out with a view to opening up a general route for the synthesis of oligosaccharides containing only one type of linkage. Such products would be of value for comparative purposes in the structural examination of polysaccharides We now report on the specificity of the method and its applicability to the synthesis of glucans.

DISCUSSION

Preliminary experiments indicated that di- and oligo-saccharides were readily formed when 1,3,4,6-tetra-*O*-acetyl-α-D-glucopyranose[2], or 1,2,4,6-[3], or 1,2,3,6-tetra-*O*-acetyl-β-D-glucopyranose[4] was heated with zinc chloride; toluene-*p*-sulphonic acid, although not as effective as zinc chloride, also catalysed the formation of oligosaccharides from the β-D-tetra-acetates

In large-scale experiments (3–4 g of material), a mixture of each acetate and catalyst was heated *in vacuo* for 10–20 min at 120°. The deacetylated product mixture was fractionated on charcoal–Celite, and the results were as follows 1,3,4,6-Tetra-*O*-

acetyl-α-D-glucopyranose (4 2 g) (zinc chloride, 15 min) gave D-glucose (400 mg), sophorose (2-*O*-β-D-glucopyranosyl-D-glucose) and kojibiose (2-*O*-α-D-glucopyranosyl-D-glucose) in a ratio of *ca* 2 1, at least three trisaccharides, and some tetrasaccharide All oligosaccharides, characteristically, failed to react with triphenyltetrazolium chloride and were detected with aniline hydrogen phthalate No oligosaccharide larger than the tetraose was detected There was no trace of any anomalous product When 3,4,6-tri-*O*-acetyl-β-D-glucopyranosyl chloride was used in place of the α-D-tetra-acetate, some disaccharide was formed, but the chloride charred rapidly, and recoveries were low

β-D-Glucose 1,2,4,6-tetra-acetate (3 0 g) (toluene-*p*-sulphonic acid, 20 min) gave D-glucose (890 mg), laminaribiose (3-*O*-β-D-glucopyranosyl-D-glucose) (100 mg), laminaritriose (30 mg), and laminaridextrins (*DP* 3–7) having chromatographic mobilities similar to those of the components of a laminarin hydrolysate A minute amount of anomalous material, which was not nigerose, was also formed The relatively small proportions of oligosaccharides, together with the large quantity of glucose recovered, confirmed the lack of reactivity of the C-3 hydroxyl group compared with the primary hydroxyl group

1,2,3,6-Tetra-*O*-acetyl-β-D-glucopyranose (3 0 g) (zinc chloride, 15 min) gave D-glucose (510 mg), cellobiose and maltose in a ratio of *ca* 3 1, trisaccharides including cellotriose, and higher oligosaccharides (up to *DP* 6)

It is probable that the polymerisation reactions proceed *via* either (*a*) a C-1 carbonium ion or (*b*) a 1,2-acetoxonium ion intermediate The reaction catalysed by toluene-*p*-sulphonic acid must involve (*b*) alone, since only β-D-glucose tetra-acetates, with a 1,2-*trans*-arrangement, are reactive, and the β-D configuration is retained in the oligosaccharide product Zinc chloride, however, may act through the formation of (*a*) alone, through (*a*) and (*b*) simultaneously, or through (*b*) with subsequent anomerisation of some of the interglucosidic linkages The first course would appear most reasonable for the α-D-glucose tetra-acetates and the second for the β-D isomers The third possibility cannot be discounted, since β→α anomerisations of this type have previously been effected for (1→6)-linked disaccharides with titanium tetrachloride in chloroform[5] For this reason, we are currently examining the effect, if any, of zinc chloride on the interglycosidic linkages of peracetylated disaccharides

The melt-polymerisation has opened up a new route towards the synthesis of specific D-glucose oligosaccharides incorporating β- or both β- and α-linkages, the technique should be applicable to sugars other than glucose where suitable reactants are available. With refinements and using forcing conditions, it may prove possible to apply the method to the synthesis of polysaccharides having specific structures and linkage configurations

EXPERIMENTAL

1,3,4,6-Tetra-*O*-acetyl-α-D-glucopyranose[2] (m p. 95–96°), 1,2,4,6-tetra-*O*-acetyl-β-D-glucopyranose[3] (m p 126–127°), 1,2,3,6-tetra-*O*-acetyl-β-D-glucopyranose[4] (m p

131–132°), and 3,4,6-tri-*O*-acetyl-β-D-glucopyranosyl chloride[6] (m p 152–154°) were prepared as described in the literature

The polymerisation technique and methods of examination of sugars are described in the previous paper[1].

Polymerisation of 1,3,4,6-tetra-O-*acetyl-α-D-glucopyranose* — (*a*) The tetra-acetate with 5% of zinc chloride, when kept for 10, 20, or 30 min at 120°/~12 mmHg, gave D-glucose (much) and traces (to aniline hydrogen phthalate) of di- and tri-saccharides, with no apparent difference between the 10- and 30-min polymerisations Both oligosaccharides remained undetected with alkaline triphenyltetrazolium chloride (TTC) In a similar experiment, 3,4,6-tri-*O*-acetyl-β-D-glucopyranosyl chloride with 5% of zinc chloride for 2 min at 120° gave D-glucose and di- and tri-saccharides showing with aniline hydrogen phthalate but not with TTC The reactant charred considerably over the heating period It decomposes above its m p (152–154°)

(*b*) The tetra-acetate (4 2 g) with zinc chloride (210 mg) was heated for 15 min at 120°/~12 mmHg After de-acetylation, the sugars were fractionated on a column (50 × 220 mm) of charcoal–Celite Fractions (250 ml) were collected and examined

Fractions 2–4, eluted with water, contained salts.

Fractions 6–10, eluted with water, gave D-glucose (400 mg)

Fractions 15–18, eluted with 5% aqueous alcohol, gave kojibiose (174 mg), $[\alpha]_D$ + 130°; lit[7] + 133°. It yielded an impure acetate, in low yield, with m p 96–98°, lit.[8], m p 118°

Fractions 19–20, eluted with 5% aqueous alcohol, contained a mixture (62 mg, $[\alpha]_D$ + 81°) of kojibiose and sophorose

Fractions 21–28, eluted with 5% aqueous alcohol, gave sophorose (378 mg), $[\alpha]_D$ +20°; lit[9] +19 1° It yielded an acetate having m p 188–189°, $[\alpha]_D$ −3°, lit[9], m p 193–194°, $[\alpha]_D$ −3 2°.

Fractions 29–32, eluted with 5% aqueous alcohol, contained a small amount of di- and tri-saccharides

Fractions 33–44, eluted with 15% aqueous alcohol, gave a mixture (595 mg) of what were apparently trisaccharides, on paper-chromatographic examination in butyl alcohol–pyridine–water (6 4 3), the mixture was resolved into three fractions, all of which failed to react with TTC

Fractions 45–60, eluted with 50% aqueous alcohol, gave a mixture (191 mg) of trisaccharide and presumed tetrasaccharide.

No further material was eluted with 50% aqueous alcohol

Polymerisation of 1,2,4,6-tetra-O-*acetyl-β-D-glucopyranose* — (*a*) The tetra-acetate (100 mg) was separately heated with 5% of zinc chloride and 0 25% of toluene-*p*-sulphonic acid for 20 min at 120°/~12 mmHg D-Glucose (much) and a series of oligosaccharides were shown, these had chromatographic mobilities similar to the laminaridextrins obtained by partial hydrolysis (N H_2SO_4 for 30 min at 100°) of insoluble laminarin. The intensity of spots on a chromatogram indicated that oligosaccharide formation was much more rapid with zinc chloride as catalyst than with toluene-*p*-sulphonic acid

(*b*) The tetra-acetate (3 0 g) was treated for 20 min at 120°/~12 mmHg with 0 25% of toluene-*p*-sulphonic acid Following de-acetylation, the sugars were eluted from a column (50 × 200 mm) of charcoal–Celite. Fractions (500 ml) were collected and examined

Fractions 2–4, eluted with water, contained salts

Fractions 6–12, eluted with water, contained D-glucose (890 mg) No material was eluted with 5% aqueous alcohol.

Fractions 22–26, eluted with 10% aqueous alcohol, contained a mixture (35 mg, $[\alpha]_D$ +50°) of laminaribiose and a second sugar (M_G 0 25) which was neither nigerose nor 5-*O*-*β*-D-glucopyranosyl-D-glucose[1].

Fractions 27–40, eluted with 10% aqueous alcohol, gave laminaribiose (100 mg), $[\alpha]_D$ +21°; lit [10] + 20.4°. It yielded a *β*-acetate having m p 160 5–161 5°, $[\alpha]_D$ −29°, lit [10], m p 160–161°, $[\alpha]_D$ −28 6°.

Fractions 50–56, eluted with 15% aqueous alcohol, gave laminaritriose (30 mg), $[\alpha]_D$ +3°, lit [10] +2 4°

Fractions 57–66, eluted with 50% aqueous alcohol, gave a mixture (189 mg) of triose and higher oligosaccharides (*DP* 4–7) whose mobilities were chromatographically identical with the main components of the laminarin hydrolysate

*Polymerisation of 1,2,3,6-tetra-*O*-acetyl-β-*D*-glucopyranose* — (*a*) The tetra-acetate (100 mg quantities), when heated for 30 min at 100° or 120°/~12 mmHg with 0 25% of toluene-*p*-sulphonic acid, gave D-glucose, cellobiose and/or maltose, and a series of oligosaccharides The more pronounced of these (*DP* 3–6) had chromatographic mobilities similar to those shown by cellodextrins obtained by acetolysis of cellulose[11]

(*b*) The tetra-acetate (3 0 g) with zinc chloride (150 mg) was heated for 15 min at 120°/~12 mmHg The product was deacetylated with methanolic sodium methoxide and placed on a column (50 × 300 mm) of charcoal–Celite Fractions (500 ml) were collected following stepwise elution with water and aqueous alcohol

Fractions 3–5, eluted with water, contained salts

Fractions 7–13, eluted with water, contained D-glucose (510 mg) characterised as the penta-acetate, m.p 130–131°.

Fractions 22–37, eluted with 5% aqueous alcohol, contained a mixture (83 mg, $[\alpha]_D$ +16°) of maltose and substance *Y* (probably[1] 5-*O*-*β*-D-glucopyranosyl-D-glucose)

Fractions 38–44, eluted with 5% aqueous alcohol, gave maltose (68 mg) It yielded a *β*-acetate, m p. and mixed m.p. 158°, $[\alpha]_D$ +60°, lit.[12], m p 159–161°, $[\alpha]_D$ +60 5°.

Fractions 46–50, eluted with 5% aqueous alcohol, contained a mixture (97 mg, $[\alpha]_D$ +50°) of maltose and cellobiose, as shown by ionophoretic examination.

Fractions 51–65, eluted with 5% aqueous alcohol, gave cellobiose (225 mg), $[\alpha]_D$ +34°, lit.[13] +35° It formed a *β*-acetate, m p 195–197°, $[\alpha]_D$ −14°, lit [12], m p 197–199°, $[\alpha]_D$ −15 7°.

Fractions 66–84, eluted with 15% aqueous alcohol, gave a mixture of at least two trisaccharides, with some cellobiose, and was not further examined

Fractions 85–98, eluted with 15% aqueous alcohol, gave a chromatographically pure trisaccharide (108 mg), $[\alpha]_D$ +22°, lit.[14] +23.2° for cellotriose

REFERENCES

1 D McGrath, E E Lee, and P S O'Colla, *Carbohyd Res*, 11 (1969) 453-460
2 R U Lemieux and G Huber, *Can J Chem*, 33 (1955) 128
3 K Freudenberg and E Plankenhorn, *Ann*, 536 (1938) 257
4 B Helferich and W Klein, *Ann*, 450 (1926) 219
5 B Lindberg, *Nature*, 164 (1949) 706
6 W J Hickinbottom, *J Chem Soc*, (1929) 1676
7 S Haq and W J Whelan, *Nature*, 178 (1956) 1222
8 K Matsuda, *Nature*, 180 (1957) 985
9 M J Clancy, *J Chem Soc*, (1960) 4213
10 S Peat, W J Whelan, and H G Lawley, *J Chem Soc*, (1958) 724
11 E Heuser, *The Chemistry of Cellulose*, Wiley, New York, 1944, p 513
12 A Thompson, K Anno, M L Wolfrom, and M Inatome, *J Amer Chem Soc*, 76 (1954) 1309
13 W T Haskins, R M Hann, and C S Hudson, *J Amer Chem Soc*, 64 (1942) 1289
14 L Zechmeister and G Toth, *Ber*, 64 (1931) 854

Carbohydrate Research
Elsevier Publishing Company Amsterdam
Printed in Belgium

PARTIAL METHYLATION OF METHYL α-D-MANNOPYRANOSIDE. PREPARATION AND DISTRIBUTION OF MONO-, DI-, AND TRI-METHYL ETHERS OF D-MANNOSE*

NOBUHIKO HANDA† AND REX MONTGOMERY‡
Department of Biochemistry, University of Iowa, Iowa City, Iowa 52240 (U S A)
(Received May 23rd, 1969)

ABSTRACT

The partial methylation of methyl α-D-mannopyranoside produced a mixture of methyl ethers that was fractionated by extraction with chloroform, followed by t l c. on a column of hydrocellulose. Ethers that are difficult to separate by this procedure were converted into their *O*-trityl or *O*-isopropylidene derivatives, or one component was removed by oxidation with periodate to aid the isolation of pure material By combinations of these procedures, it has been possible to isolate the 2,3,4,6-tetra-, 3,4,6-, 2,4,6-, 2,3,6-, and 2,3,4-tri-; 2,6-, 3,6-, and 3,4-di-; and 2-, 3-, and 6-methyl ethers of D-mannose The partial methylation of methyl α-D-mannopyranoside by the procedures of either Haworth, Kuhn, or Hakomori gave the same methyl ethers but the degree of substitution at each position differed markedly. Methyl sulfate and sodium hydroxide gave a relative substitution in the order 6-OH>2-OH>3-OH>4-OH, whereas methyl iodide and silver oxide in *N,N*-dimethylformamide gave the order 2-OH>3-OH>4-OH≥6-OH and methyl iodide and methylsulfinyl carbanion in methyl sulfoxide gave 2-OH>6-OH>4-OH≥3-OH The reactions were carried out with limiting amounts of methylating reagents and were not time controlled

INTRODUCTION

The application of methylation techniques to the structural elucidation of oligosaccharides and polysaccharides has been applied widely in spite of its limitations In early work, the isolation of unknown methyl ethers as cleavage fragments from the hydrolysis of methylated polysaccharides frequently gave enough material to establish the positions of the methoxyl groups by chemical means, so that the proof of structure of these ethers by synthesis was not critical With the introduction of more delicate microanalytical procedures, such as g l.c , for the analysis of such

*This work was supported by research grants HE 06717 and GM 14013 from the National Institutes of Health
†Present address Water Research Laboratory, Faculty of Science, Nagoya University, Nagoya, Japan
‡To whom correspondence should be addressed

mixtures, it has become necessary to prepare known compounds in order to standardize the method both qualitatively and quantitatively.

D-Mannose is commonly found in the carbohydrate groups of glycoproteins, glycolipids, and many polysaccharides Reference samples of the methyl ethers required for structural studies may be synthesized unambiguously by the methylation of known derivatives of D-mannose, from which the protecting groups can be removed finally to give the corresponding free methyl ethers. Certain methyl ethers are difficult to prepare by this route because derivatives having proper protecting groups cannot be prepared easily[1] It seemed reasonable therefore to prepare a mixture of the methyl ethers of D-mannose, such as might be obtained from a methylated highly branched D-mannan, by partial methylation of methyl α-D-mannopyranoside and to apply chromatographic procedures for their separation

Of the several methods of methylation in the field of carbohydrate chemistry, probably those most generally used involve methyl sulfate and sodium hydroxide (Haworth method)[2], methyl iodide and silver oxide in *N,N*-dimethylformamide (Kuhn method)[3], and the more recent method of Hakomori with methyl iodide and methylsulfinyl carbanion in methyl sulfoxide[4]. In these methods, it is usual to use a large excess of the reagents to ensure as complete methylation as possible in each reaction The present study approached the question of the partial methylation of methyl α-D-mannopyranoside by using less of the methylating reagents than usual and, in some cases, an amount insufficient to complete the etherification of the four hydroxyl groups in the glycoside For clarity the results of the work are presented in two parts, first, the application of partial methylation to the preparation of methyl ethers of D-mannose and second, the relative distribution of these ethers in the reaction mixtures as it reflects the reactivity of the hydroxyl groups in methyl α-D-mannopyranoside

PREPARATION OF METHYL ETHERS OF D-MANNOSE

A mixture of methyl ethers of methyl α-D-mannopyranoside, prepared in this case by the Haworth method, was fractionated first by the batch extraction of an aqueous solution with chloroform to give a concentration of the tetra- and trimethyl ethers in the organic phase. A more extensive fractionation could have been achieved by the countercurrent extraction procedure of Bell[5] or by chromatography on a column of hydrocellulose[6], but the loading of sample on these columns does not permit a convenient separation of large quantities It is true, nevertheless, that some of the finer separations, such as that of 2,4,6- from 2,3,6-tri-*O*-methyl-D-mannose, can be most expeditiously achieved by t l c in spite of these limitations However, the attempt was made in this study to avoid such limitations wherever possible and to separate the components by preparing specific chemical derivatives, such as the 6-trityl ethers or the 1,2-*O*-isopropylidene derivatives, or by oxidizing away some of the products with periodate Depending upon the particular methyl ether of D-mannose required, so the procedures described here were modified slightly.

TABLE I

RELATIVE RETENTION TIME OF PARTIALLY METHYLATED MANNOSES AS TMS DERIVATIVES

Methyl ethers of methyl α-D-mannoside	*Relative retention times*[a]	
	Column B	*Column* C
2,3,4,6-tetra-	0 654	0 665
3,4,6-tri-	0 691	0 700
2,3,4-tri-	0 729	0 731
2,4,6-tri-	0 743	0 757
2,3,6-tri-	0 748	0 769
3,4-di-	0 767	0 790
3,6-di-	0 798	0 814
2 4-di-	0 817	0 828
2,6-di-	0 870	0 877
4-mono-	0 859[b]	0 859[b]
3-mono-	0 869	0 883
6-mono-	0 909	0 919
2-mono-	0 902	0 934
methyl α-D-mannoside	1 000	1 000

[a]Relative to the TMS derivative of methyl α-D-mannopyranoside [b]Determined by shoulder of the peak

The sugars extracted by chloroform, CE-F*, separated on t l c into methyl 2,3,4,6-tetra-*O*-, methyl 2,4,6-tri-*O*-, and a mixture of the other methyl tri-*O*-methyl-α-D-mannosides Alternatively, hydrolysis of CE-F to the reducing sugars and treatment with acetone–sulfuric acid gave specifically 1,2-*O*-isopropylidene-3,4,6-tri-*O*-methyl-D-mannose, which was extracted from an aqueous solution of the mixture by ether From the remaining trimethyl ethers, reconverted into the methyl glycosides, the 2,3,4-isomer was separated as the 6-trityl ether, and the unreacted sugars were separated by t l c to give methyl 2,3,4,6-tetra-, methyl 2,3,6-tri-, and methyl 2,4,6-tri-*O*-methyl-α-D-mannoside It was noted occasionally that the first acetalation step to isolate the 3,4,6-trimethyl ether was not quantitative, so this isomer, remaining in the mixture after tritylation, was removed by periodate oxidation of the reducing sugar forms.

A similar preliminary separation was achieved with the methyl ether fraction (CN-F*) not extracted by chloroform from the original total mixture of methyl ethers The CN-F was treated with chlorotriphenylmethane to give the 6-trityl ethers of methyl 2-*O*-, 3-*O*-, 3,4-di-*O*-, and 2,4-di-*O*-methyl-α-D-mannoside, which after hydrolysis to remove the trityl and methyl glycosidic groups, were separated by chromatography

*CE-F refers to the fractions of methyl ethers that are extracted by chloroform from an aqueous solution, which then contains the residual non-extractable (CN-F) methyl ethers

on hydrocellulose to give all except the 2-methyl ether as pure components (Table II) 2-*O*-Methyl-D-mannose was obtained by treating the monomethyl ether fraction with benzaldehyde and oxidizing the crude mixture of 4,6-*O*-benzylidene derivatives of the 2- and 3-methyl ethers with periodate, thus degrading the 3-methyl ether to the corresponding D-arabinose derivative After removal of the benzylidene group, the methylated sugars were chromatographed on hydrocellulose to give pure 2-*O*-methyl-D-mannose 3-*O*-Methyl-D-mannose was also isolated from the methyl mono-*O*-methyl mannosides after periodate oxidation and rechromatography

TABLE II

CHROMATOGRAPHIC FRACTIONATION OF FRACTION CN-F(T)

Fractions	*Tube*[a]	*Weight (mg)*	*Analysis by g l c.*
1	19–25	15	3,4-di-*O*-methyl-D-mannose
2	26–42	71	mixture of two di-*O*-methyl-D-mannoses
3	43–57	23	2,4-di-*O*-methyl-D-mannose
4	67–84	21	3-*O*-methyl-D-mannose
5	85–99	99	mixture of mono-*O*-methyl-D-mannoses
6	100–169	224	mixture of mono-*O*-methyl-D-mannoses
7	170[b]	647	D-mannose
	Total	1100 mg[c]	

[a]Volume of each fraction was 17 6 ml [b]After tube No 169, 1850 ml of the eluate were collected as one fraction (No. 170) [c]Recovery of methyl D-mannoside ethers was 91%

6-*O*-, 2,6-Di-*O*-, and 3,6-di-*O*-methyl-D-mannose were obtained pure after chromatography on hydrocellulose of those ethers in CN-F that did not form an insoluble 6-trityl ether (Table III).

TABLE III

CHROMATOGRAPHIC FRACTIONATION OF FRACTION CN-F(NT)

Fractions	*Tube*	*Weight (mg)*	*Analysis by g l c.*
1	26–50	290	3,6-di-*O*-methyl-D-mannose
2	51–65	250	3,6- and 2,6-di-*O*-methyl-D-mannose
3	66–75	150	2,6-di-*O*-methyl-D-mannose
4	76–80	60	2,6-di-*O*-methyl-D-mannose and 6-*O*-methyl-D-mannose
5	81–105	730	6-*O*-methyl-D-mannose
	Total	1480[a]	

[a]The recovery was 98 0%.

Many alternative schemes were tried to effect a simple separation of the products from the partial methylation of methyl α-D-mannopyranoside by methods that could

be applied to larger scale operations. Those outlined above require relatively few steps to produce gram quantities of all of the methyl ethers (except the 4-, 2,3-, and 4,6-isomers) all of which are well characterized compounds and for which authentic samples are available[1-7].

DISTRIBUTION OF THE METHOXYL GROUPS FROM PARTIAL METHYLATION

The three methods of methylation studied differ from each other in several ways The Haworth procedure is effected in an aqueous medium, whereas the other two are carried out in non-aqueous, polar solvents Because of the reaction of excess base with the methyl sulfate, the reagents are usually added in aliquot fractions, and in this study aliquot eighths were used Since the total amounts of alkali added were close to the theoretical amount for complete alkoxide formation (see Table IV), it was expected that the hydroxyl groups would be etherified in the order of their relative reactivities and, with the exception of the preponderance of the 2,4,6-trimethyl ether over the 2,3,6-trimethyl ether, this is found to be the case (Table V)

Although when striving for complete methylation by the Purdie or Kuhn procedures, with silver oxide, it is best to add the base in portions over a period of time, the purpose of the present study was best served by a single addition at the start of the reaction, which is heterogeneous from the standpoint of the alkoxide formation Alkoxide formation and alkylation take place concomitantly, which complicates an explanation of the relative ratios of the products (Table VI) and the overall preponderance of substitution at each position on the sugar ring (Table VII) If each hydroxyl group reacts independently, then from the results in Table VII, one might expect the preponderating monomethyl ether to be 2-methyl, and the di- and trimethyl ethers to be 2,3- and 2,3,6- or 2,3,4- As in the case of the products from the Haworth reaction, substitution at position 4 is present in the principal trimethyl ether (2,4,6-), and for the Kuhn method, the 2,4-dimethyl fraction was also not predicted.

The Hakomori procedure is different in several respects from the two discussed above Alkoxide formation is driven to equilibrium before any alkylating reagent is added Since the conditions of the present reaction involved only about 90% of the theoretical amount of base, the least reactive hydroxyl group(s) would appear after alkylation in the lowest amount, with the others in proportion to the rate of reaction. This was generally the case (see Tables VI and VII)

The different proportions of the reagents used are summarized in Table IV, where it will be noted that the largest excess over that required for complete methylation was 2 4 times (Kuhn, Expt 2) and that several ratios down to equimolar amounts (Haworth, Expt 1) were analyzed It is clear that the amount of more highly substituted ethers (CE-F) (Tables IV and V) increased as the proportion of the methylating reagents was raised but that even in the experiments where more than theoretical amounts were employed, the CE-F fraction does not predominate.

Regardless of the relative amounts of base and alkylating reagent, the distribution of the methyl ethers in each method remained essentially the same. The

Carbohyd Res, 11 (1969) 467–484

TABLE IV

REACTION CONDITION AND YIELD OF METHYLATED PRODUCTS IN HAWORTH, KUHN, AND HAKOMORI METHODS

Haworth method	*Methyl α-D-mannoside, mmoles*	*Me_2SO_4 mmoles*	*NaOH (30%) mmoles*	*CE-F g*	*CN-F g*	*Total g*	*Yield[a] %*
Expt. 1	51.5	51 5	187.5	0 35	8.92	9.27	92.7
2	20 0	40.0	62 6	0 53	2.64	3 17	94 0
3	51 5	154.5	187 5	2 17	7 10	9 27	92.7
4	51 5	309 0	237.5	4 75	5 10	9 85	98.5
Kuhn method	*Methyl α-D-mannoside mmoles*	*MeI mmoles*	*Ag_2O g*	*CE-F g*	*CN-F g*	*Total g*	*Yield %*
Expt. 1	18 8	94	18	0 32	3 00	3 32	90.5
2	18.8	184	22	0.72	2 85	3 57	97.0
Hakomori method	*Methyl α-D-mannoside mmoles*	*MeI mmoles*	*Methylsulfinyl carbanion mmoles*	*CE-F g*	*CN-F g*	*Total g*	*Yield %*
Expt. 1	5 2	17.7	18	0.14	0.78	0.92	92 0
2	5 2	35.4	18	0 36	0 58	0 94	94.0

[a]See text.

TABLE V

YIELDS AND RELATIVE RATIO OF METHYL α-D-MANNOSIDE AND ITS MONO-, DI-, TRI-, AND TETRA-SUBSTITUTED FRACTIONS

Methyl substituted methyl α-D-*mannoside*	*Haworth method*								*Kuhn method*				*Hakomori method*			
	Expt. 1		*Expt. 2*		*Expt. 3*		*Expt. 4*		*Expt. 1*		*Expt. 2*		*Expt. 1*		*Expt 2*	
	g	%	*g*	%	*g*	%	*g*	%	*g*	%	*g*	%	*g*	%	*g*	%
Mono-	3 12	33.7	1.23	38 4	3 39	36 5	1 24	12 6	1.27	38 4	0.86	24.3	0.26	28 2	0.20	21.0
Di-	1.56	16.8	0.46	14 5	2.19	23 6	2.74	27 8	1 25	37 8	1 31	37.0	0.17	18 5	0 23	24.0
Tri-	0.28	3.0	0.41	12.8	1 12	12 1	2.59	26 3	0 28	8.5	0.51	14 4	0.10	11 0	0 20	21.6
Tetra-	0 06	0 7	0 12	3 8	1.05	11.3	2 16	22 0	0 04	1 1	0.21	5.9	0 04	3.4	0.16	16.7
Methyl α-D-mannoside	4.24	45 9	0 96	30 4	1 53	16 3	1 14	11 5	0 45	13 6	0.65	18 4	0 35	38 0	0.15	16.4
Total recovery	9 26		3 18		9 27		9 87		3.31		3 54		0 92		0.94	
Average degree of substitution[a]	0 79	1.21		1 65		2.35		1.44		1.65		1 12		2 00		

[a]The percentage of each fraction was multiplied by the degree of substitution for that fraction and the four values were added together For complete methylation the value would be 4 00

TABLE VI

RELATIVE RATIO OF ISOMERS IN MONO-, DI- AND TRI-METHYL ETHERS

	Haworth method			*Kuhn method*		*Hakomori method*	
	Expt 1 %	*Expt 3* %	*Expt 4* %	*Expt 1* %	*Expt 2* %	*Expt 1* %	*Expt 2* %
Mono-							
2-	29 4	28 4	30 6	31 4	31.3	35 4	35 0
3-[a]	20 5	18 7	17 7	26 0	26 2	22 2	21 2
4-[a]	4 1	3 7	3 5	26 9	27 1	7 7	7 3
6-	46 2	49 0	47 9	15 5	15 3	34 7	36 5
Di-							
2,4-	24 8	27 7	27 6	43 6	45 6	55 4	55 5
2,6-	35 5	36 5	37 0	18 3	21 0	21 4	22 2
3,4-	6 6	6 3	4 0	9 9	8 7	1 9	3 3
3,6-	33 5	31 1	31 1	28 0	28 8	21 4	18 9
Tri-							
2,3,4-	22 3	20 0	21 4	26 4	26 3	36 2	34 2
2,3,6-[b]	26 3	27 0	27 4	9 7	9 0	32 7	34 0
2,4,6-[b]	30 5	31.1	32 2	35 4	36 2	25 8	25 0
3,4,6-	20 9	21 7	18 8	28 2	28 5	5 0	6 6

[a]These ratios were calculated from the results obtained from periodate oxidation of a mixture of 3- and 4-isomers (see Table IV) [b]These ratios were obtained by g l c for the Kuhn and Hakomori method and by t l c for the Haworth method

absence of certain isomers, such as 2,3- or 4,6-di-*O*-methyl-D-mannose, although carefully sought in the reaction products, may indicate these groups were never introduced in this combination, or that once formed, the subsequent alkylation to a trimethyl ether was very rapid The choice between these types of alternatives will best be made by studying the alkylation of partially methylated methyl α-D-mannosides or by rate studies, which are quite feasible with the analytical facilities now at hand.

The differences in reactivity between the hydroxyl groups in monosaccharides and polysaccharides has been studied principally for D-glucose[8] and D-glucans[9–11], where the reaction rates are in the order 6-OH > 2-OH > 4-OH > 3-OH for the Haworth method[8]. No similar study has been reported for D-mannose or by other methylation procedures. The differences in reactivity will depend upon such factors as electronic and steric effects, but the distribution of the individual methyl ethers in the reaction mixture will reflect other conditions, including (*1*) the extent to which each of the hydroxyl groups is converted into the alkoxide by reaction with the base, (*2*) the relative reactivity of each alkoxide group with the alkylating agent, (*3*) the excess of free base that can compete with the alkoxide for the alkylating agent, (*4*) the total amount of alkylating reagent added, (*5*) the change in reactivity of the hydroxyl and alkoxide groups as methoxy groups are introduced into the molecule, and (*6*) the nature and order of addition of the methylating reagents.

To the first order of approximation, the relative reactivities of the four hydroxyl groups in methyl α-D-mannopyranoside will be proportionate to their overall relative substitution, considering all of the methyl ethers. Such an analysis of the position of methylation for each method and condition in the present study is summarized in Table VII, with the method of calculation given in Table VIII for one experiment.

TABLE VII

OVERALL RELATIVE SUBSTITUTION OF HYDROXYL GROUPS AT C-2, C-3, C-4, AND C-6

Methylation method	*2-OH*	*3-OH*	*4-OH*	*6-OH*
Haworth				
Expt 1	1 00	0.72	0 40	1 32
Expt 3	1 00	0 78	0 64	1 23
Expt 4	1 00	0 79	0 73	1 05
Kuhn				
Expt 1	1 00	0 94	0 74	0 74
Expt 2	1 00	0 87	0 79	0 67
Hakomori				
Expt 1	1 00	0 59	0 62	0 75
Expt 2	1 00	0 67	0 70	0 79

Methyl sulfate and sodium hydroxide gave a relative substitution in the order 6-OH > 2-OH > 3-OH > 4-OH, whereas methyl iodide and silver oxide in *N,N*-dimethylformamide gave the corresponding order 2-OH > 3-OH > 4-OH ≥ 6-OH, and methyl iodide and methylsulfinyl carbanion in methyl sulfoxide gave 2-OH > 6-OH > 4-OH ≥ 3-OH. The reactions were effected with limiting amounts of methylating reagents and were not time controlled. These orders of substitution were also those present in the monomethyl ethers for the corresponding methylation methods and, particularly where the di- and tri-methyl fractions were least, such as Haworth experiment 1, the initial reaction rate-constants for each hydroxyl group were reflected by the proportions of these monomethyl ethers. The system is complicated, however, by the effect that one substitution or a combination of substitutions has upon the reactivity of another hydroxyl group. Spurlin has calculated the relative rate-constants for etherification from the substitution pattern in homogeneous reactions[12]. A more precise derivation would require 32 rate constants for each method of methylation, but it is probable that some of the eight constants for the overall conversion of hydroxyl to methoxyl groups for each position would differ only slightly within a single procedure Some exceptions were noted in the present study. Whereas the relative amount of the 4-methyl ether in the Haworth method is low, 25% of the dimethyl fraction is the 2,4-isomer and the 2,4,6-trimethyl ether is the principal isomer in that fraction Similar exceptions are noted for the Hakomori method. The 2- and 3-monomethyl ethers amount to about 50% of that fraction in the Haworth and Hakomori methods, yet the 2,3-dimethyl ether was not detected, but the 2,3,4-

TABLE VIII

METHOD OF CALCULATION OF RELATIVE SUBSTITUTION OF HYDROXYL GROUPS (HAWORTH METHOD, EXPT 1)

Isomer (% of fraction)		*mmoles*	*mMoles of methoxyl residue for each isomer* ($\times 10^2$)			
			2-OH	*3-OH*	*4-OH*	*6-OH*
Mono-*O*-methyl fraction (3 12 g, 15 mmoles)						
2-	29 4	4 4	4 4			
3-	20 5	3 1		3 1		
4-	4 1	0 6			0 6	
6-	46 2	6 9				6 9
Di-*O*-methyl fraction (1 56 g, 7 mmoles)						
2,4-	24 8	1 7	1 7		1 7	
2,6-	35 5	2 4	2 5			2 5
3,4-	6 6	0 5		0 5	0 5	
3,6-	33 5	2 4		2 4		2 4
Tri-*O*-methyl fraction (0 28 g, 1 1 mmoles)						
2,3,6-	26 3	0 3	0 3	0 3		0 3
2,4,6-	30 5	0 5	0 4		0 4	0 4
2,3,4-	22 3	0 3	0 3	0 3	0 3	
3,4,6-	20 9	0 2		0 2	0 2	0 2
Tetra-*O*-methyl fraction (0 06 g, 0 2 mmoles)						
2,3,4,6-		0 2	0 2	0 2	0 2	0 2
Total substitution			9 8	7 0	3 9	12 6
Relative substitution	6-OH (12 6)> 2-OH (9 8)> 3-OH (7 0)> 4-OH (3 9)					
or	6-OH (13 2)> 2-OH (10 0)> 3-OH (7 2)> 4-OH (4 0)					

and 2,3,6-isomers were present in significant amounts One explanation would be that the reactivity of the 4-OH group is influenced by substitution at the 2-OH position, and that the activity of the 3-OH group is enhanced by substitution at positions 2 and 4, or 2 and 6 The most reactive group in the Kuhn and Hakomori procedures is the 2-OH, and the Kuhn methylation has the lowest degree of substitution at the 6-position, quite the opposite of the Haworth method. The explanation of these results call for a more detailed study of actual rates of methylation of not only the methyl α-D-mannopyranoside but also the various methyl ethers From a practical standpoint, however, it is seen that some hydroxyl groups are more difficult to methylate than others and that these groups are not the same for the different methylation methods or for the various sugars A combination of methylation procedures has been commonly employed to achieve complete methylation, without which the structural studies of carbohydrates by this approach is misleading

EXPERIMENTAL

General. — Reagents were used without further purification Solutions were concentrated *in vacuo* with bath temperatures no higher than 50° Melting points (Fisher–Johns apparatus) are uncorrected. T l c was effected on plates (20 × 20 cm) of Silica gel G (E Merck, Darmstadt, Germany) with the organic layer of the mixture benzene–ethanol–water–ammonia (200 47 15 1, v/v) as developing solvent Multiple ascents were used, twice for CE-F and five times for CN-F. The zones were detected by exposure of the plate to iodine vapor, the mobilities of the mono-, di-, tri-, and tetra-methyl ethers relative to methyl α-D-mannopyranoside being 1 5 5 10 18, respectively. Each zone was carefully scraped from the plate and the sugar extracted three times with methanol (20 ml). The combined methanol extracts were evaporated *in vacuo* The residue was weighed and dissolved in water, and the sugar content determined by the phenol–sulfuric acid procedure. Colorimetric determinations by the phenol–sulfuric acid procedure were standardized by using D-mannose or the methyl ethers of methyl α-D-mannopyranoside The absorbancies for 10 μg of methyl α-D-mannopyranoside, and its mono-, di-, tri-, and tetra-methyl ethers were 0.153, 0.153, 0 086, 0 080, and 0 067, respectively. G l c was carried out on an F and M model 1609 instrument with a hydrogen flame detector Helium was used as the carrier gas Three types of column and chromatographic procedures were used *A*, neopentylglycol succinate (1/4″ × 7 5′), 10% liquid phase on Anakrom at 160°, *B*, SE-52 (Applied Science Laboratories) stainless steel column (1/8″ × 5′), 3% liquid phase on 80–100 mesh dichlorodimethylsilane-treated Chromosorb W, with a linear temperature program from 60 to 150° at 1° per min; *C*, SE-52, similar to *B* but with 4% liquid phase and programing from 60 to 165°. The retention times, relative to methyl α-D-mannopyranoside, of the trimethylsilyl (TMS)-derivatives are summarized in Table I for columns *B* and *C*

Periodate oxidation — The sugar or mixture was dissolved in 0 2M periodic acid at room temperature At appropriate intervals of time the absorbancy at 222 5 nm of an aliquot, diluted 250 times, was determined When the oxidation was complete, as indicated by no further change in absorbancy, the excess periodic acid was reduced with an equivalent of ethylene glycol The solution was neutralized with barium hydroxide and filtered, and the filtrate evaporated to a syrup, from which the methyl ethers were extracted twice with absolute ethanol The combined extracts were evaporated to a syrup, and the procedure was repeated until the final syrup was free from inorganic salts

Methylation procedures — A Methylation with sodium hydroxide and methyl sulfate A solution of methyl α-D-mannoside in water (5 ml) was maintained at 50° and calculated amounts of methyl sulfate and sodium hydroxide (30%) were added dropwise with vigorous stirring in 1/8 portions every 10 min After addition of the reagents, the reaction mixture was maintained for 1 h at 50–60°, and the reaction completed by heating for 30 min at 100°. During all these procedures, the reaction mixture was stirred vigorously The reaction mixture was cooled and neutralized to

Methyl Red indicator with 10% sulfuric acid, and an equal volume of ethanol (95%) was added. The salt that precipitated was removed and the filtrate was evaporated to a syrup.

B. Methylation with methyl iodide and silver oxide in N,N-*dimethylformamide.* Dry methyl α-D-mannoside in dry *N,N*-dimethylformamide (70 ml) was stirred magnetically overnight at room temperature to dissolve the sugar and calculated amounts of silver oxide and methyl iodide were added successively with cooling in an ice bath. The mixture was stirred for 48 h at room temperature after which time the insoluble material was removed by centrifugation, and water (50 ml) was added to the supernatant, which was extracted directly with chloroform (25 ml × 3) as the first step in the analysis

C. Methylation with methyl iodide and methylsulfinyl carbanion in methyl sulfoxide. Sodium hydride in a three-necked round-bottomed flask was washed three times by stirring magnetically with *n*-pentane (30 ml) After the final wash, the residual was removed *in vacuo* and dry methyl sulfoxide (30 ml) was added The suspension of sodium hydride in methyl sulfoxide was stirred under nitrogen at 50° until the solution became clear and evolution of hydrogen gas ceased The concentration of methylsulfinyl carbanion was determined by titration with 0 1M hydrochloric acid in aqueous solution

For the preparation of methyl α-D-mannoside alkoxide, dry methyl α-D-mannoside (1 g) was stirred under nitrogen at 60° with methyl sulfoxide (25 ml) until it was dissolved (about 20 min) After cooling to room temperature, a solution of methylsulfinyl carbanion was added and the reaction mixture was stirred for 4 h at room temperature For the methylation reaction, the mixture was maintained first at 20° in a cold bath and methyl iodide was added dropwise with continued stirring The reaction mixture was kept for 15 min at room temperature To the solution was then added iced water (50 ml) and extraction with chloroform (50 ml × 3) removed the most volatile ethers The combined chloroform extracts were washed with water (50 ml × 3) and evaporated to a syrup under diminished pressure. The water phase was combined with washings of chloroform extracts and evaporated to a syrup These two syrups were each dissolved in 25 ml of water and combined The combined solutions were extracted with chloroform (25 ml × 3) to commence the analyses

Analytical procedures — A. Chloroform extraction. The products of each methylation reaction were fractionated by batchwise extraction of an aqueous solution with chloroform (50 ml × 3) The combined chloroform extracts were washed with water (25 ml × 3) and evaporated to a syrup (CE-F) under diminished pressure The water phase and the washings were combined and deionized with Amberlite IR-120 (H^+) and Duolite A-4 (OH^-) resins The resulting solution was evaporated to a syrup (CN-F) under diminished pressure

B Determination of relative ratios of isomers in the methyl substituted fractions CE-F and CN-F were separated by t l.c. into methyl α-D-mannoside and its mono-, di-, tri-, and tetramethyl ethers, which were determined by the phenol–sulfuric acid method. Each fraction (*ca* 10 mg), dissolved in 1 ml of dry pyridine, was treated

with hexamethyldisilazane (0.2 ml) (Eastman Organic Chemicals Co) and chlorotrimethylsilane (0.1 ml) (Aldrich Chemicals Co), the reaction mixture being shaken vigorously for 30 sec and then kept for 15 min at room temperature[13]. An aliquot of the reaction mixture (usually 10 μl) was injected into the gas chromatograph with trimethylsilylated methyl α-D-mannoside as an internal standard. Methyl 2,3,4,6-tetra-*O*-methyl-α-D-mannoside also was used as an additional standard. Areas of the peaks in g.l.c. were measured by planimeter and the relative amounts of each sugar calculated.

C. Relative amounts of methyl 3-O- and methyl 4-O-methyl-α-D-mannoside. The ratio of 3- and 4-methyl ethers of methyl α-D-mannoside, which were not separated by g.l.c., was determined by periodate oxidation. The monomethyl ether fractions, isolated by t.l.c. as described previously, were weighed and each mixed with methyl 2,3,4,6-tetra-*O*-methyl-α-D-mannoside (9.20 mg). Each mixture was submitted to periodate oxidation for 48 h as described above and the resulting sugar mixture was re-isolated. The sugar mixtures before and after oxidation were analyzed by g.l.c. and the peak areas corresponding to methyl 2,3,4,6-tetra-*O*-methyl-α-D-mannoside and methyl 3-*O*-methyl-α-D-mannoside were measured. The difference in peak area of the latter compound and the mixture before and after oxidation was due to methyl 4-*O*-methyl-α-D-mannoside. All of the peak areas of methyl 3-*O*- and methyl 4-*O*-methyl-α-D-mannosides were related to the internal standard of methyl 2,3,4,6-tetra-*O*-methyl-α-D-mannoside. The results are summarized in Table IX.

TABLE IX

RELATIVE RATIO OF 3- AND 4-METHYL ETHERS

Methylation method	*Before IO_4^- oxidation*		*After IO_4^- oxidation*		*Calculated ratio*		*Percentage*	
	Tetra-[a]	*3- and 4-*[b]	*Tetra-*	*3-*	*3-*	*4-*	*3-*	*4-*
Haworth (Expt. 4)	1.00	1.50	1.00	1.27	1.27	0.23	83.3	16.6
Kuhn (Expt. 2)	1.00	6.12	1.00	3.00	3.00	3.12	49.1	50.9
Hakomori (Expt. 2)	1.00	0.77	1.00	0.56	0.56	0.21	74.3	25.6

[a]Methyl 2,3,4,6-tetra-*O*-methyl-α-D-mannoside. [b]Mixture of methyl 3-*O*- and 4-*O*-methyl-α-D-mannosides.

D. Relative amounts of 2,3,6- and 2,4,6-tri-O-methyl-D-mannose. The relative ratio of 2,3,6- and 2,4,6-trimethyl ethers was determined in the trimethyl ether fraction by treating first with chlorotriphenylmethane in the usual way to remove methyl 2,3,4-tri-*O*-methyl-α-D-mannoside. After hydrolysis of the remaining glycosides with 0.5M sulfuric acid, the sugar mixture was oxidized with periodate to remove 3,4,6-tri-*O*-methyl-D-mannose, and the final mixture of trimethyl ethers was separated by g.l.c. of the methyl glycosides. The methyl 2,3,6- and methyl 2,4,6-tri-*O*-methyl-α-D-mannosides thus separated were determined by the phenol–sulfuric acid procedure

(see Table X) Alternatively, the sugars could be analyzed by g l c on column *C* which, unlike column *B*, effected a separation of the two components (see Table VI) The t l c. and g l c. methods agree closely (see Tables VI and X).

TABLE X

RELATIVE RATIO OF 2,4,6- AND 2,3,6-METHYL ETHERS BY T L C ANALYSIS

Method	*Position of substitution*			
	2,4,6- *mg*	*2,3,6-* *mg*	*2,4,6-* *%*	*2,3,6-* *%*
Haworth (Expt 4)	25 6	20 8	53 3	46 7
Kuhn (Expt 2)	19 2	5 1	79 0	21 1
Hakomori (Expt 2)	10 5	14 1	42 7	57.3

E. Yield of methylated products. Fractions CN-F and CE-F were weighed after deionizing with ion-exchange resins and the total recovery of methyl ethers was taken as the combined weights of CN-F and CE-F The weight of the products expressed in terms of the weight of methyl α-D-mannoside ranged from 90 5 to 98 5% (Table IV)

Fractionation of methyl ethers of D-*mannose* — The experiments described for the separation and identification of the methyl ethers of D-mannose refer to the product from the partial methylation by the Haworth method, with reagents in the proportions given in experiment 2 (Table IV) Where larger quantities of material were fractioned, the preparation still followed the conditions used in experiment 2

*2,3,4,6-Tetra-*O*-methyl-α-*D*-mannose* Methyl 2,3,4,6-tetra-*O*-methyl-α-D-mannoside was isolated from CE-F by t l c After hydrolysis of the glycoside with 0 5M sulfuric acid as usual, *O*-methyl-D-mannose (4 22 mmoles) was dissolved in ethanol (5 ml), to which was added aniline (43 mg, 0 46 mmoles) The resulting solution was heated for 3 h at 95° and the alcohol was evaporated off *in vacuo* The residue was triturated with petroleum ether and the 2,3,4,6-tetra-*O*-methyl-*N*-phenyl-D-mannosylamine crystallized, m p 135–138°, not depressed upon admixture with an authentic sample; $[\alpha]_D^{26}$ −82 2° → −6.7° (*c* 0 9, methanol, after 12 h)

*Tri-*O*-methyl-*D*-mannoses* The methyl tri-*O*-methyl-α-D-mannoside fraction, isolated from CE-F by t l c , showed three peaks on g l c. by column *A* More complete separation of the components was achieved by means of various chemical derivatives with the single exception of 2,4,6-tri-*O*-methyl D-mannose As each component was isolated, it was identified by g l c comparison with an authentic sample

*A 2,4,6-Tri-*O*-methyl-*D*-mannose* The methyl tri-*O*-methyl-α-D-mannosides were separated further by t l c to yield methyl 2,4,6-tri-*O*-methyl-α-D-mannoside as a single faster-moving band and a second band contained the remaining sugars After hydrolysis, 2,4,6-tri-*O*-methyl-D-mannose (28.6 mg, 0 13 mmole) was treated with aniline (20 mg, 0 21 mmole) in ethanol (1 ml) for 5 h at 100° The ethanol was removed by evaporation and the *N*-phenylglycosylamine was crystallized and recrys-

tallized from ether; yield 25.6 mg, m p. 123–125°, $[\alpha]_D^{27.8}$ −145 → +2 1° (*c* 2 56, methanol), $[\alpha]_D^{29}$ −152 5 → +6 2° (*c* 1.01, methanol) [lit.[14], m.p. 134°, $[\alpha]_D$ −150 → +8° (methanol)]

B 2,3,4-Tri-O-methyl-D-mannose The methyl tri-*O*-methyl-α-D-mannoside fraction (505 6 mg) was dissolved in pyridine (2 ml) and chlorotriphenylmethane (500 mg) was added After shaking until the reagents had dissolved, the reaction mixture was kept for 48 h at room temperature and water was added until the solution became turbid The mixture was stirred for 1 h and was then poured into 100 ml of water and stirred for a further 2 h The trityl derivatives were extracted with ethyl ether (50 ml × 5); the non-tritylated methyl ethers were isolated from the aqueous solution (see *C*) The combined ether extracts were washed with saturated sodium hydrogen sulfate (20 ml × 3), sodium hydrogen carbonate (20 ml × 3), and water (30 ml × 3) The extracts were dried (sodium sulfate) and evaporated to a syrup (50 mg) The syrup was dissolved in glacial acetic acid (2 ml) and 50% HBr (0 2 ml) was added After a few min, the precipitate that formed was filtered off and the filtrate was poured into about 100 ml of iced water containing sodium hydrogen carbonate The methylated sugar was extracted from the solution with chloroform (30 ml × 3) and the combined extracts were washed with sodium hydrogen carbonate solution and water, and evaporated to syrup. The syrup was dissolved in ethanol (1 ml), water (5 ml) added, the precipitate was removed by filtration, and the filtrate was evaporated to a syrup. This procedure was repeated until the ethanol solution was not turbid after the addition of water The final syrup was subjected to t l c to give 22 mg of methyl 2,3,4-tri-*O*-methyl-D-mannoside, $[\alpha]_D^{26}$ +44.3° (*c* 4 42, water) [lit.[15], $[\alpha]_D$ +47° (water)]

C 3,4,6-Tri-O-methyl-D-mannose The residual methyl tri-*O*-methyl-α-D-mannosides (389 mg) from (*B*) above were hydrolyzed for 15 h at 100° with 0 5M sulfuric acid (20 ml) in the usual way. The free sugars were dissolved in acetone (10 ml) containing concentrated sulfuric acid (0 25 ml) and the reaction mixture was stirred for 4 h at room temperature, after which time it was neutralized with anhydrous sodium carbonate The precipitate was removed and the filtrate was refluxed for 1 h in the presence of anhydrous sodium carbonate. The acetone solution was then evaporated to a syrup, which was purified by t l c to yield 1,2-*O*-isopropylidene-3,4,6-tri-*O*-methyl-D-mannose as a syrup (46 mg). The isopropylidene group was removed at 100° with 10 ml of 1% sulfuric acid and the resultant 3,4,6-tri-*O*-methyl-D-mannose (30 mg) showed $[\alpha]_D^{28.5}$ +10 1° (*c* 2 96, water), [lit[14], $[\alpha]_D$ +21 → +8.1° (water)]

The syrupy 3,4,6-tri-*O*-methyl-D-mannose was heated for 12 h under reflux with 3.75% methanolic hydrogen chloride After cooling, the solution was neutralized with silver carbonate, the suspension was filtered, and the filtrate was evaporated *in vacuo* to a syrup (10 mg) This was analyzed by gas chromatography and identified as methyl 3,4,6-tri-*O*-methyl-α-D-mannoside

D 2,3,6-Tri-O-methyl D-mannose Commencing with CE-F (4 5 g), the glycosides were hydrolyzed with 0 5M sulfuric acid, the free sugars in the mixture were treated

with acetone (120 ml) and concentrated sulfuric acid (2 8 ml) as usual, and the product was isolated and dissolved in water (100 ml), from which solution the isopropylidene acetals were extracted with ethyl ether The sugars remaining in the aqueous solution were converted into the methyl glycosides (3 14 g), which were treated with chlorotriphenylmethane (3 5 g) in pyridine (12 ml) as described under (*B*). The precipitated crude methyl 6-*O*-trityl-2,3,4-tri-*O*-methyl-α-D-mannoside was filtered off and the filtrate was evaporated to a syrup (2 45 g) from which the methyl 2,3,4,6-tetra-*O*-methyl-α-D-mannoside was removed by t l c. and residual methyl tri-*O*-methyl-α-D-mannosides were isolated as a syrup (0 92 g) The syrup still contained some of the 3,4,6-trimethyl ether, which was removed by periodate oxidation after hydrolysis of the glycosides with 0.5M sulfuric acid for 16 h at 100°. The resulting syrup (0 43 g) was reconverted into the methyl glycosides and chromatographed by t l c to give two components, methyl 2,4,6-tri-*O*-methyl-α-D-mannoside (0 16 g), $[\alpha]_D^{29}$ +5 0° (*c* 2 83, methanol), and the slower moving methyl 2,3,6-tri-*O*-methyl-α-D-mannoside (0 14 g), $[\alpha]_D^{29}$ +5 0° (*c* 2 83, methanol).

The 2,4,6-trimethyl ether was hydrolyzed to the free sugar (0.14 g), $[\alpha]_D^{29}$ +14.3° (*c* 4.18, water)[14], which was converted into the *N*-phenylglycosylamine as described under (*A*), m p. 128–131°, $[\alpha]_D^{29}$ −149 → +4 2° (*c* 1 25, methanol)

The 2,3,6-trimethyl ether was similarly hydrolyzed to the free sugar, a syrup (0 11 g), $[\alpha]_D^{29}$ +7.4° (*c* 2 16, water), a portion (30 mg) of which was refluxed with aniline (43 mg) in ethanol (1 ml) for 4 h to give 2,3,6-tri-*O*-methyl-*N*-phenyl-D-mannosylamine, m p. 126–128°, $[\alpha]_D^{29}$ −158 → −31° (*c* 1.10, methanol, after 24 h) [lit [16] m p. 127–128°, $[\alpha]_D$ −155 → −39° (in methanol)]

Fractionation of CN-F fraction. — Tritylation To CN-F (10 g) in dry pyridine (20 ml) was added chlorotriphenylmethane (10 g). After 48 h at room temperature, the solution was cooled to 0° and water added with stirring until a turbidity was produced The turbid solution was stirred for 1 h and poured into ice–water (500 ml), and the syrupy precipitate was washed with water to remove the residual pyridine. During this time the syrup crystallized and the crude trityl ethers were isolated by filtration and dried over phosphorus pentaoxide; yield 13.5 g. The aqueous mother liquors and washings were concentrated together to about 100 ml, extracted with chloroform to remove the triphenylcarbinol, and evaporated *in vacuo* to a syrup (2.9 g), designated CN-F(NT).

The tritylated methyl mannoside ethers (13 g) were dissolved in glacial acetic acid (25 ml) by gentle warming To the solution, previously cooled to room temperature, was added HBr (50%, 4 ml) and after 3 min the precipitate formed was filtered off The filtrate was poured into iced water (50 ml) containing a slight excess of sodium hydrogen carbonate and passed through Amberlite IR-120 (H^+) followed by Duolite A-4 (OH^-), to remove sodium acetate. The eluate was concentrated under diminished pressure to a syrup (5.8 g), designated CN-F(T).

Separation of CN-F(T) by column chromatography on hydrocellulose — Fraction CN-F(T) was hydrolyzed with 0.5M sulfuric acid as usual to give the mixture of free sugars as a syrup (5.56), which was dissolved in water (10 ml) A portion (2 2 ml,

equivalent to 1 21 g of methyl sugars) was applied to a column of hydrocellulose[6] at 30.5° and methylated sugars were developed with butanone–water azeotrope The fractions were examined by g l c. and combined as shown in Table II or were collected as dimethyl and monomethyl ethers from a knowledge of the chromatographic behavior of the column

3,4-Di-O-methyl-D-mannose Fraction 1, Table III, $[\alpha]_D^{27.3}$ +1.1° (*c* 4 32, water) [lit [14], $[\alpha]_D^{27.3}$ +3° (water)] was converted into the methyl glycoside, $[\alpha]_D^{27.3}$ +66 9° (*c* 3 8, water) [lit [14], $[\alpha]_D$ +67° (water)], which co-chromatographed with an authentic sample of methyl 3,4-di-*O*-methyl-α-D-mannoside on g l c

2,4-Di-O-methyl-D-mannose. Fraction 3, Table III, $[\alpha]_D^{30.5}$ +11.0° (*c* 2 52, water) [lit [7], $[\alpha]_D^{25}$ +13 5° (*c* 0 6, water)] did not crystallize It was converted into the methyl glycoside, $[\alpha]_D^{29}$ +41.7° (*c* 1.26, chloroform), $[\alpha]_D^{27}$ +32 0° (*c* 1 01, methanol) [lit.[7], $[\alpha]_D^{25}$ +47.6° (*c* 1.0, ethanol)]

3-O-Methyl-D-mannose. A combined monomethyl ether fraction (160 mg) from a column chromatogram was converted into the methyl glycosides (134 mg), which were oxidized with periodic acid as described above. The product was hydrolyzed with 0 5M sulfuric acid as usual and rechromatographed on a column of cellulose to give 3-*O*-methyl-D-mannose as a syrup, $[\alpha]_D^{25}$ +2 7° (*c* 2 50, water) [lit [17], $[\alpha]_D^{19}$ +3° (*c* 0 60, water)]

2-O-Methyl-D-mannose. A combined fraction of monomethyl ethers (0.7 g) was stirred with freshly distilled benzaldehyde (20 ml) in the presence of powdered zinc chloride (2 g) for 4 h at room temperature The mixture was then cooled to 0° and diluted with water (50 ml), and after 30 min, the solid precipitate was filtered and washed successively with cold water (10 ml × 2) and cold *n*-pentane (10 ml × 2) The dried mixture of benzylidene derivatives (200 mg) was dissolved in aqueous *p*-dioxane and oxidized with 0 2M ammonium periodate for 15 days at room temperature The oxidation was terminated by adding ethylene glycol and the solution was evaporated to dryness The residue was extracted with ethanol (10 ml × 2) and the extract was evaporated to a syrup, which was deionized by passing an aqueous solution through Amberlite IR-120 (H^+) and Duolite A-4 (OH^-). The resulting solution was brought to 0 1M with respect to sulfuric acid and kept for 1 h at 60° Neutralization with barium hydroxide, and filtration and evaporation of the filtrate gave a syrup that was re-chromatographed on a column of hydrocellulose to give 2-*O*-methyl-D-mannose (68 mg), $[\alpha]_D^{25}$ +4.2° (*c* 1.21, water) [lit.[17], $[\alpha]_D^{19}$ +5° (*c* 1 3, water)]

The 2-methyl ether (50 mg) in water (1 ml) containing phenylhydrazine hydrochloride (0 1 g) and sodium acetate (0 2 g) was refluxed for 1 h D-*arabino*-Hexulose phenylosazone crystallized, m p. 198–200°, not depressed upon admixture with an authentic sample, $[\alpha]_D^{27.5}$ −55 → −35° [*c* 1.01, 6 4 (v/v) ethanol–pyridine] [lit [18], m p. 204–206°, $[\alpha]_D$ −65 → −34° (in ethanol–pyridine)]

Separation of CN-F(NT) by cellulose column chromatography — Fraction CN-F(NT) (1 76 g) was hydrolyzed with 0 5M sulfuric acid as usual to give the free sugars (1 52 g), which were separated on a column of hydrocellulose[6]. The results are summarized in Table III

3,6-Di-O-methyl-D-mannose Fraction 1, Table III, was chromatographically pure and the 3,6-dimethyl ether in fraction 2 could be isolated from the 2,6-dimethyl isomer by periodate oxidation of the methyl glycosides followed by rechromatography of the free sugars. 3,6-Di-*O*-methyl-D-mannose was isolated from fraction 2 in this way as a syrup (59 mg), $[\alpha]_D^{29.5}$ +22.4° (*c* 1.82, water); $[\alpha]_D^{29.5}$ +32.4° (*c* 1.86, methanol).

2,6-Di-O-methyl-D-mannose Fraction 3, Table III, gave a methyl glycoside $[\alpha]_D^{27.5}$ +50.3° (*c* 0.64, methanol). A sample (70.6 mg) was converted into 6-*O*-methyl-D-*arabino*-hexulose phenylosazone m.p. 169–172°, not depressed upon admixture with an authentic sample, $[\alpha]_D^{26}$ −70.5 → −39.6° (*c* 1.10, ethanol, after 15 h), [lit.[19], m.p. 172 $[\alpha]_D$ −68.6 → −48.0° (ethanol)]

6-O-Methyl-D-mannose Fraction 5 gave a syrup, $[\alpha]_D^{28}$ +14.0° (*c* 2.25, chloroform) [lit.[19], $[\alpha]_D$ +15.3° (chloroform)] which was converted in part into the methyl glycoside, $[\alpha]_D^{27.5}$ +83.5° (*c* 0.58, methanol) and is part into the 6-*O*-methyl-D-*arabino*-hexulose phenylosazone m.p. 172–175°, not depressed upon admixture with an authentic sample, $[\alpha]_D^{27.5}$ −65.2 → −45.0° (*c* 1.25, ethanol, after 15 h)

ACKNOWLEDGMENTS

The authors gratefully acknowledge the receipt of samples from Drs. C. T. Bishop, A. S. Perlin, P. A. J. Gorin, and M. B. Perry

REFERENCES

1 G. O. Aspinall, *Advan. Carbohydrate Chem.*, 8 (1953) 217
2 W. N. Haworth and H. Machemer, *J. Chem. Soc.*, (1932) 2270
3 R. Kuhn and H. Trischmann, *Ber.*, 96 (1963) 284
4 S. Hakomori, *J. Biochem.* (Tokyo), 55 (1964) 205
5 D. J. Bell, *Biochem. J.*, 29 (1935) 2031
6 J. D. Geerdes, B. A. Lewis, R. Montgomery, and F. Smith, *Anal. Chem.*, 26 (1954) 264
7 S. S. Bhattacharjee and P. A. J. Gorin, *Can. J. Chem.*, 47 (1969) 1207
8 B. Norrman, *Acta Chem. Scand.*, 22 (1968) 1623
9 I. Croon, *Svensk Papperstid.*, 63 (1960) 247
10 I. Croon, *Acta Chem. Scand.*, 13 (1959) 1235
11 B. Norrman, *Acta Chem. Scand.*, 22 (1968) 1381
12 H. M. Spurlin, in E. Ott, H. M. Spurlin, and M. W. Grafflin (Eds.), *Cellulose and Cellulose Derivatives, II High Polymers*, Vol. V, Interscience, New York, 1954
13 C. C. Sweeley, R. Bentley, M. Makita, and W. W. Wells, *J. Amer. Chem. Soc.*, 85 (1963) 2497.
14 W. N. Haworth, R. L. Heath, and S. Peat, *J. Chem. Soc.*, (1941) 833
15 F. Smith, M. Stacey, and P. I. Wilson, *J. Chem. Soc.*, (1944) 131
16 W. N. Haworth, E. L. Hirst, and M. M. T. Plant, *J. Chem. Soc.*, (1931) 1354
17 G. O. Aspinall and G. Zweifel, *J. Chem. Soc.*, (1957) 2271
18 E. Pacsu and S. M. Trister, *J. Amer. Chem. Soc.*, 63 (1941) 925
19 A. J. Watters, R. C. Hockett, and C. S. Hudson, *J. Amer. Chem. Soc.*, 61 (1939) 1528

Carbohydrate Research
Elsevier Publishing Company, Amsterdam
Printed in Belgium

A SYNTHESIS OF 2,3,4,6-TETRADEOXY-4-(DIMETHYLAMINO)-D-*erythro*-HEXOSE (FOROSAMINE) AND ITS D-*threo* EPIMER*

ESTER L ALBANO† AND D HORTON‡
Department of Chemistry, The Ohio State University, Columbus, Ohio 43210 (U S A)
(Received May 24th, 1969)

ABSTRACT

Oxidation of methyl 2,3,6-trideoxy-α-D-*erythro*-hexopyranoside (methyl α-amicetoside, **1**) gave the 4-ketone **2**, which was converted into the oxime **3** and (*p*-nitrophenyl)hydrazone Hydrogenation of **3** in ethanolic hydrochloric acid over platinum gave a 2 3 mixture of methyl 4-amino-2,3,4,6-tetradeoxy-α-D-*erythro*-hexopyranoside hydrochloride and the D-*threo* isomer which, on *N*-dimethylation gave methyl 2,3,4,6-tetradeoxy-4-(dimethylamino)-α-D-*erythro*-hexopyranoside (**8**) and the D-*threo* isomer (**11**) Hydrolysis of **8** gave crystalline 2,3,4,6-tetradeoxy-4-(dimethylamino)-D-*erythro*-hexose (forosamine), and hydrolysis of **11** gave 2,3,4,6-tetradeoxy-4-(dimethylamino)-D-*threo*-hexose Reduction of the ketone **2** with lithium aluminum hydride regenerated the starting alcohol **1** stereospecifically. Base-catalyzed deuterium-exchange converted ketone **2** into its 3,3-dideuterio derivative. Treatment of **2** with base, and subsequently with (*p*-nitrophenyl)hydrazine, in the presence of deuterium oxide, gave a 3,3,5-trideuterio (*p*-nitrophenyl)hydrazone derivative

INTRODUCTION

Forosamine[1,2] is one of three sugars found in the spiramycins (A, B, and C) isolated from *Streptomyces ambofaciens*[3] The gross structure of forosamine was established by degradation[1], and its stereochemistry was proved by synthesis[4]. Ethyl 2,3,6-trideoxy-4-*O*-(methylsulfonyl)-α-D-*erythro*-hexopyranoside, obtainable[5] in 2% overall yield in 8 steps from D-glucose, was converted through two successive inversion reactions at C-4 into the 4-azido-4-deoxy analog and thence, in ~0 1% overall yield from D-glucose, into 2,3,4,6-tetradeoxy-4-(dimethylamino)-D-*erythro*-hexose (forosamine)

A previous paper from this laboratory[6] recorded a convenient preparation of methyl 2,3,6-trideoxy-α-D-*erythro*-hexopyranoside (methyl α-amicetoside, **1**) from

*Supported, in part, by the National Institutes of Health, Public Health Service, Department of Health, Education, and Welfare, Bethesda, Md 20014, Grant No GM-11976-05 (The Ohio State University Research Foundation Project 1820)
†Stauffer Chemical Co , Fellow, October, 1967–June, 1968
‡To whom inquiries should be addressed

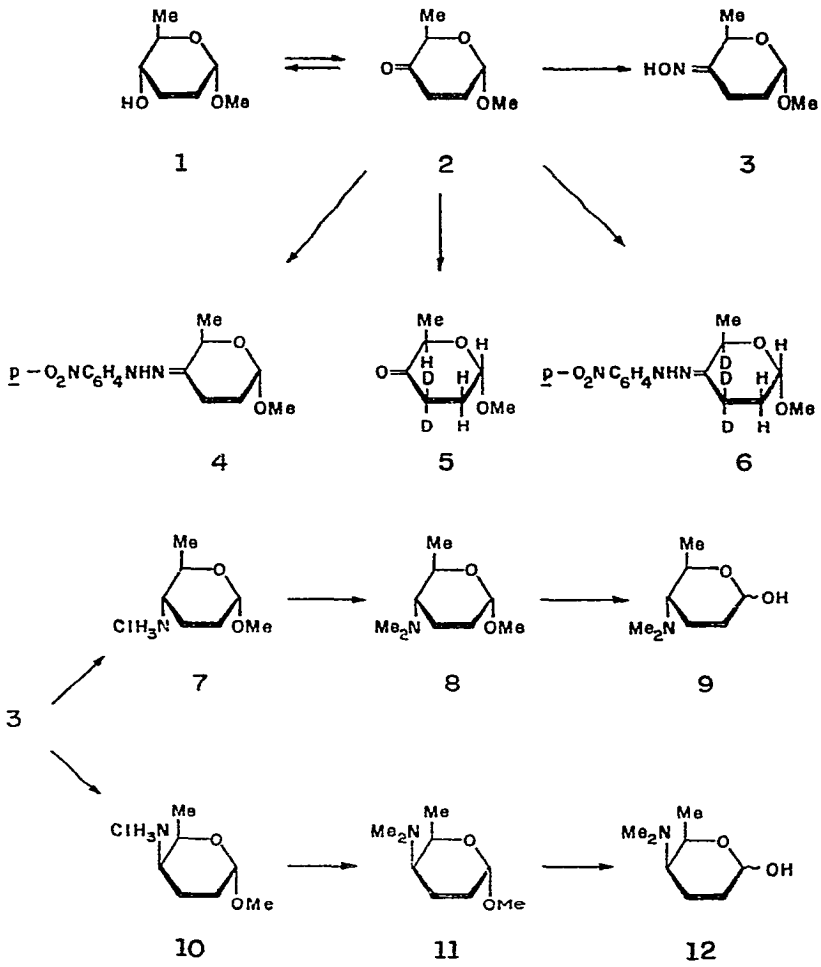
D-glucose. The present article describes the amination of this compound at C-4 by way of the oxime (3) of the derived 4-ketone (2) The method gives forosamine (9) in about 1% overall yield from D-glucose, and simultaneously provides the 4-epimer (12) of 9 in rather greater yield

DISCUSSION

Oxidation of methyl 2,3,6-trideoxy-α-D-*erythro*-hexopyranoside[6] (1) with ruthenium tetraoxide in carbon tetrachloride[7-9] gave the 4-ketone 2, in 82% yield, as a chromatographically homogeneous syrup that showed carbonyl absorption in its i r spectrum The 100-MHz n m r spectrum of 2 in chloroform-*d* (see Tables I–III) showed a triplet signal for H-1, the anticipated[6] high-field multiplets for the C-2 and C-3 methylene groups, and a high-field doublet for the C-6 methyl group The proton at C-5 gave a sharp quartet (τ 5.72) through coupling with the C-6 protons ($J_{5\,6}$ 6 8 Hz), and the absence of additional splitting of the H-5 signal confirmed the location of the carbonyl group at C-4. The ketone 2 was quite unstable and could not be kept, even at 0°, for more than a few days On t l c plates, the ketone 2 immediately gave a black spot in the cold when the plate was sprayed with sulfuric acid The crystalline (*p*-nitrophenyl)hydrazone (4) of ketone 2 was prepared, its i r. spectrum showed N–H and C=N absorptions, and its n m.r. spectrum (see Tables I–III) showed, in addition to signals closely similar to those of the parent ketone 2, additional signals for an NH group and the aryl protons These data accord with the structure 4, and not with conceivable, isomeric formulations

Treatment of the ketone 2 with hydroxylamine hydrochloride in pyridine–methanol gave, in 81% yield, an oxime 3 as a mixture of two isomers that migrated at different rates on t l c The major isomer was obtained, in 66% yield, as an analytically pure, distilled syrup, its n m r spectrum (see Tables I–III) was very similar to that of the parent ketone 2, except for the presence of an additional signal for the OH proton. The i r. spectrum of 3 showed a band at 3.00 μm (OH), but no C=N absorption band was observed. The absence of infrared absorption for the C=N group has been noted with certain other oximes of sugars[8-10], possibly, the inductive effects of the substituents at each end of the C=N bond coincidentally give rise to little net polarization of the bond, so that absorption in the infrared region is weak or absent The minor, isomeric oxime was not obtained free from the major isomer, but the unseparated mixture of isomers could be used in the next step of the synthesis.

Hydrogenation of the oxime 3 over platinum, in ethanol containing one molar equivalent of hydrochloric acid, gave a mixture of amine hydrochlorides (7 and 10) that migrated at different rates by t l c on microcrystalline cellulose. These products were not separated at this stage, but were reductively dimethylated[11] with formaldehyde and hydrogen over a Raney nickel catalyst to give, after neutralization with ammonium hydroxide, a mixture of methyl 2,3,4,6-tetradeoxy-4-(dimethylamino)-α-D-*erythro*-hexopyranoside (8) and the D-*threo* isomer (11). Column-chromatographic



resolution of the mixture on silica gel gave pure **8** in 18% yield (based on the oxime **3**) and **11** in 26% yield, both products were obtained as distilled liquids giving correct elemental analyses The n m r. spectra of **8** and **11** (see Tables I–III) showed the sharp signals anticipated for the three kinds of methyl groups present, and the four protons at C-2 and C-3 gave rise to a multiplet at high field Signals for H-1, H-4, and H-5 could be assigned, but the extensive second-order effects observed in the signals made analysis of spin-couplings unrewarding Differentiation of **8** from **11** was based on the fact that the more dextrorotatory isomer ($[\alpha]_D$ +174° in chloroform), which also migrated the faster on t l c , could be identified as the D-*erythro* isomer **8**, because, on hydrolysis, it gave the known amino sugar **9**

The glycoside **8** was hydrolyzed under mild conditions (M sulfuric acid for 12 h at room temperature) to give a product, purified by distillation, that was chromatographically homogeneous and analytically pure, and had m.p. 58–60°, $[\alpha]_D$ +90°

TABLE I

CHEMICAL SHIFTS OF RING PROTONS[a]

Compound	*Chemical shifts (τ) from 100-MHz spectra*					
	H-1	*H-2,2'*	*H-3,3'*	*H-4*	*H-5*	*H-6*
2	5 08 t	7.65–8 15 m	7 38–7.60 m		5 72 q	8 69 d
3	5 25 t	8 02–8 25 m	6 99–7.81 m		5 60 q	8 67 d
4	5 26 t	7 80–8 28 m	7 58 m		5 55 q	8 59 d
6[b]	5 26 t	7 79–8 36 m				8 59 s
8[b]	5 30–5 43 m	7 95–8 50 m		~7 74 m	5 89–6 42 m	8.76 d
9[c]	4 81 t[d], 5 29 q[e]	7.95–8 60 m		7 65–7.80 m	5 62 o, 6 44 o	8 72 d, 8 80 d
11[b]	5 22–5 40	8 08–8 41 m		~7.65 m	5 60–5 98 m	8.73 d
12[c]	4 95 m, 5 08 m	8 05–8.60 m		7 65–7 85 m	5 70 m, 6 17 m	8 68 d, 8 78 d

[a]The solvent was chloroform-*d*, and first-order values are given. Peak multiplicities d, doublet, m, multiplet; o, octet, q, quartet, s, singlet, t, triplet. [b]Measured at 60 MHz with a Varian A-60 n m r spectrometer [c]Mixture of anomers [d]H-1 of α-D anomer [e]H-1 of β-D anomer.

TABLE II

CHEMICAL SHIFTS OF ARYL, OMe, NMe_2, OH, AND NH PROTONS[a]

Compound	*Aryl H-3, H-5*	*Aryl H-2, H-6*	*OMe*	*NMe_2*	*OH*	*NH*
2			6 53 s			
3			6 60 s		1 38[b]	
4	1 95 d	3 04 d	6 62 s			2 39 s[b]
6[c]	1 95 d	3 04 d	6 62 s			2 30 s[b]
8[c]			6 64 s	7 74 s		
9[d]				7 76 s, 7.79 s	6 45[e]	
11[c]			6 57 s	7 65 s		
12[d]				7 69 s, 7 78 s	5 80[e]	

[a]The solvent was chloroform-*d* Peak multiplicities d, doublet, s, singlet [b]Broadened [c]Measured at 60 MHz [d]Mixture of anomers [e]Disappeared on deuteration

TABLE III

FIRST-ORDER COUPLING-CONSTANTS FOR RING PROTONS AND ARYL PROTONS[a]

Compound	*Coupling constants (Hz) from 100-MHz spectra*				
	$J_{1,2}$	$J_{1,2'}$	$J_{4,5}$	$J_{5,6}$	J_{ortho}
2	4 5	4 5		6 8	
3	4 0	4 0		6 5	
4	4 5	4 5		6 0	9 0
6[b]	4 5	4 5			9 0
8[b]	[c]	[c]		6 5	
9[d]	<1, 2	2[e], 9[f]	9 0	6 5	
11[b]	[c]	[c]		6 8	
12[b]	[c]	[c]	[c]	7 0	

[a]The solvent was chloroform-*d* [b]Measured at 60 MHz [c]Not measured because of second-order effects [d]Mixture of anomers [e]α-D Anomer [f]β-D Anomer

(in methanol). This compound was identified as 2,3,4,6-tetradeoxy-4-(dimethylamino)-D-*erythro*-hexose (forosamine, **9**), for which the values of m p 60° and $[\alpha]_D$ +88° (in methanol) have been reported[4]. Hydrolysis of the isomeric glycoside (**11**) under similar conditions gave, after distillation, 2,3,4,6-tetradeoxy-4-(dimethylamino)-D-*threo*-hexose (**12**) as a chromatographically homogeneous, analytically pure liquid, $[\alpha]_D$ −2° in methanol (reported[4] value, $[\alpha]_D$ −2 5° in methanol)

The n m r spectrum of forosamine (**9**) in chloroform-*d* (see Tables I–III) showed H-1 signals for the two anomers of the pyranose form A broadened triplet at τ 4.81 was assigned to H-1 of the α-D form, and a broadened quartet at τ 5 29, of almost equal intensity, was assigned to H-1 of the β-D form Separate signals for the NMe_2 groups and the C-6 methyl groups could be observed for the two anomers The 4-epimer (**12**) of forosamine, in chloroform-*d*, also gave n m.r. data indicating a mixture of anomers, although the H-1 signals were not specifically differentiated

Reduction of the ketone **2** with lithium aluminum hydride in anhydrous ether gave methyl 2,3,6-trideoxy-α-D-*erythro*-hexopyranoside (**1**) exclusively, there was no evidence, by t l c or g l c, for the α-D-*threo* isomer The reduction product **1** was isolated pure in 78% yield, and was further characterized as the crystalline 4-(3,5-dinitrobenzoate)[6] The approach of a nucleophile to either side of the carbonyl group in **2** does not appear to present the possibility of substantial steric hindrance The stereospecificity observed in the reduction of **2** may be caused by coordination of the aluminum atom to the ring-oxygen atom of **2**, leading to attack by hydrogen from the "top" side of the molecule

A solution of the ketone **2** in chloroform-*d* was treated at room temperature with sodium deuteriooxide in deuterium oxide The n m r spectrum after 12 h indicated complete exchange of the two protons on C-3, but the proton on C-5 was not exchanged The quartet signal for H-5 and the doublet for H-6 remained as observed before deuteration, and the signals of H-2 and H-2′ appeared as the AB portion of an ABX system These results show that base-catalyzed exchange of H-5 is considerably more difficult than exchange of H-3 An enolate anion formed by abstraction of the C-5 proton can be expected to be less stable than one formed by abstraction of a C-3 proton. In the former case, there would be an unfavorable build-up of negative charge on a carbon atom bonded to O-5 Furthermore, C-5 has three substituents, whereas C-3 has only two, so that the anion formed by abstraction of a proton at C-3 should, even in the absence of electronic effects, be the more stable one

Treatment of the ketone **2** with sodium deuteriooxide in deuterium oxide–acetone-d_6 for 12 h at room temperature, and subsequently with (*p*-nitrophenyl)-hydrazine in deuterium oxide and pyridine, gave a crystalline product that was identical by X-ray powder diffraction pattern with the (*p*-nitrophenyl)hydrazone **4** of **2**, but whose n m r spectrum indicated that it was a trideuterio derivative **6** having both of the C-3 protons and the C-5 proton exchanged by deuterium The spectrum of **6** resembled that of the non-deuterated analog **4**, except that the H-5 quartet was absent, the C-6 methyl group gave a single peak, and the multiplet at τ 7.58 assigned to the C-3 protons in **4** was absent from the spectrum of **6** These data indicate that, as anticipated, exchange of H-5 is possible, but that it does not take place so readily as exchange at C-3. This situation is in contrast to that occurring in bridged molecules, where exchange of certain bridgehead protons may be inhibited completely[12]. The exchange of H-5 in the conversion of **2** into **6** should result in equilibration of the C-5 epimers in the product Only the α-D-*erythro* product was isolated, but the yield was low, and it is entirely possible that the β-L-*threo* isomer of **6** was also formed

In addition to providing a convenient, preparative route to forosamine and its 4-epimer, the present synthesis offers a method for preparing specifically deuterated (or tritiated) derivatives of these and related sugars that are of potential value for biochemical and mass-spectrometric studies

EXPERIMENTAL

General. — Unless otherwise stated, solutions were evaporated below 50° under diminished pressure Melting points were determined with a Thomas–Hoover "Unimelt" apparatus (Arthur H Thomas Co , Philadelphia, Pa). Specific rotations were determined at at least two different concentrations in a 2-dm, narrow-bore, polarimeter tube I r spectra were recorded with a Perkin–Elmer Model 137 "Infracord" i r. spectrophotometer N m r spectra were recorded at 100 MHz with a Varian HA-100 n m r spectrometer Chemical shifts are given on the τ scale, with tetramethylsilane (τ = 10 00) as the internal standard that also provided a lock signal Signal assignments were verified by spin decoupling Deuteration was performed by adding 1 drop of deuterium oxide to the prepared sample Microanalyses were made by W N. Rond. X-ray powder diffraction data give interplanar spacings, Å, for CuKα radiation, the camera diameter was 114 59 mm, relative intensities were estimated visually, m, moderate, s, strong, v, very, w, weak The strongest lines are numbered (1, strongest), double numbers indicate approximately equal intensities T l c was performed with 0 25-mm layers of Silica Gel G (E Merck, Darmstadt, Germany), activated at 120°, as the adsorbent, and sulfuric acid as the indicator Column chromatography was performed with Silica Gel (7734, Merck) as the adsorbent, with 1 g of mixture to be separated per 30 g of adsorbent Free sugars were chromatographed on microcrystalline cellulose[13] (Avicel, technical grade, American Viscose Division of Food Machinery Corp , Marcus Hook, Pa) with 4 1 5 butyl alcohol–ethanol–water (upper phase) as the mobile phase and silver nitrate–sodium hydroxide as the indicator G l c was effected with a Wilkens Autoprep Model 705 gas chromatograph (Varian Aerograph, Walnut Creek, California), equipped with a flame-ionization detector, and a stainless-steel column (10 ft × 0 25 in) with 5% Carbowax on Chromosorb W as the liquid phase at a temperature of 140°

*Methyl 2,3,6-trideoxy-α-*D-glycero-*hexopyranosid-4-ulose* (**2**) — Ruthenium tetraoxide was prepared[9] by stirring ruthenium dioxide (Engelhard Industries, Newark, N. J., 56%, 9 60 g, 52 9 mmoles) in carbon tetrachloride (500 ml) with 10% aqueous sodium metaperiodate (200 ml) for 0 5 h at 0° The layers were separated, and the aqueous layer was washed with two 300-ml portions of carbon tetrachloride The combined organic extracts were added, without being dried, to a solution of methyl 2,3,6-trideoxy-α-D-*erythro*-hexopyranoside (**1**, 5 24 g, 36 0 mmoles) in carbon tetrachloride (10 ml) The resultant mixture, which immediately turned black, was stirred for 2 h at 0° Isopropyl alcohol (5 ml) was added to decompose the excess of oxidant, and the mixture was filtered Evaporation of the filtrate gave **2** as a chromatographically homogeneous syrup, yield 4 24 g (82%), R_F 0 70 (1 1 dichloromethane–ether), $[\alpha]_D^{22}$ +310 ±2° (*c*, 1 3, chloroform), λ_{max}^{film} 5 58, 5.75 μm (C=O) On t l c , the ketone immediately gave a black spot when the plate was sprayed with cold sulfuric acid Compound **2** was unstable, and had decomposed after 3–4 days, even at 0°

*Methyl 2,3,6-trideoxy-α-*D-glycero-*hexopyranosid-4-ulose* (p-*nitrophenyl*)*hydraz-*

one (4) — A solution of (*p*-nitrophenyl)hydrazine hydrochloride (660 mg, 3.48 mmoles) in water (5 ml) and pyridine (5 ml) was mixed with a solution of the ketone **2** (419 mg, 2 91 mmoles) in methanol (25 ml) The resulting mixture was kept for 24 h at room temperature, and then evaporated The residue was dissolved in benzene (50 ml), washed with two 20-ml portions of water, dried (magnesium sulfate), and evaporated The residue was recrystallized from ethanol–water; yield 501 mg (62%) A second recrystallization from ethanol–water gave pure **4** as yellow needles, m p 158–159°, $[\alpha]_D^{20}$ +347 ±2° (*c* 0 6, chloroform), λ_{max}^{KBr} 3 05 (NH), 6 30 (C=N), 13 3, 14.5 μm (phenyl), X-ray powder diffraction data· 9 90 m, 7 09 m, 6 32 vs (1), 5 05 w, 4 72 s (3,3), 4 44 w, 4 09 m, 3 81 s (2), 3 43 s (3,3), 3 12 w, and 3 01 w.

Anal Calc. for $C_{13}H_{17}N_3O_4$ C, 55 90, H, 6 13, N, 15 04 Found C, 56 17, H, 6 19, N, 14 92.

*Methyl 2,3,6-trideoxy-α-*D-glycero-*hexopyranosid-4-ulose oxime* (**3**) — A mixture of the ketone **2** (4 24 g, 29 1 mmoles) and hydroxylamine hydrochloride (4 20 g, 60 mmoles) in pyridine (5 ml) and methanol (25 ml) was refluxed for 1 5 h, and the solution was evaporated to dryness at room temperature Addition of cold water (20 ml) to the residue caused an oil to separate The mixture was extracted with three 20-ml portions of dichloromethane, and the extract was dried (magnesium sulfate), and evaporated to a syrup The syrup was dissolved in the minimal volume of dichloromethane, and the solution was passed through a column (3 × 30 cm) of silica gel Ether–dichloromethane (1 10) was used as the eluant to remove a fast moving impurity, and then 1 5 ether–dichloromethane was used to elute the oxime **3**; yield 3 10 g (66%). A small amount of sample was distilled at 95–98° (bath)/0.1 mmHg, to give pure **3**, $[\alpha]_D^{20}$ +223 ±2° (*c* 0 5, chloroform), R_F 0 64 (3 10 ether–dichloromethane), λ_{max}^{film} 3 00 (OH), 7 30 μm (C-Me)

Anal Calc for $C_7H_{13}NO_3$· C, 52 81, H, 8 23, N, 8 80 Found C, 52 87, H, 8 41; N, 8 77

A small proportion (less than 10%) of another isomer, R_F 0 49 (3 10 ether–dichloromethane) was present, but all attempts to isolate it pure from the other isomer failed A fraction containing a mixture of the two isomers was isolated, yield 0 665 g (15%).

*Methyl 4-amino-2,3,4,6-tetradeoxy-α-*D-erythro-*hexopyranoside hydrochloride* (**7**) *and methyl 4-amino-2,3,4,6-tetradeoxy-α-*D-threo-*hexopyranoside hydrochloride* (**10**) — A mixture of the oxime **3** (2 33 g, 14 7 mmoles), 12M hydrochloric acid (1 23 ml, 14 7 mmoles), and platinum oxide (450 mg) in ethanol (60 ml) was hydrogenated for 16 h at room temperature and a pressure of 40 lb in^{-2}. The catalyst was filtered off, the solvent was evaporated, and codistillation with propyl alcohol removed any excess acid The resultant syrup gave two ninhydrin-positive spots on microcrystalline cellulose, R_F 0 89 and 0 67.

*Methyl 2,3,4,6-tetradeoxy-4-(dimethylamino)-α-*D-erythro-*hexopyranoside* (**8**) *and methyl 2,3,4,6-tetradeoxy-4-(dimethylamino)-α-*D-threo-*hexopyranoside* (**11**) — The mixture of amine hydrochlorides **7** and **10** (14 7 mmoles, from 2 33 g of the oxime **3**) was dissolved in ethanol (60 ml), and to the solution were added 37 2%

aqueous formaldehyde (4 66 ml, 58 mmoles), anhydrous sodium acetate (160 mg), and Raney nickel (4 g) The mixture was hydrogenated for 16 h at room temperature at a pressure of 50 lb in^{-2}, the catalyst was filtered off, and the solvent was evaporated The residue was dissolved in 2% aqueous ammonium hydroxide (100 ml), the solution was extracted with two 50-ml portions of dichloromethane, and the extracts were combined, dried (magnesium sulfate), and evaporated A solution of the resultant syrup in dichloromethane was passed through a column (3 × 30 cm) of silica gel, with 10 l ether–dichloromethane as the eluant The faster-moving isomer, methyl 2,3,4,6-tetradeoxy-4-(dimethylamino)-α-D-*erythro*-hexopyranoside (**8**), R_F 0 31 (ether), was obtained as a chromatographically homogeneous liquid, yield 460 mg (18%, from the oxime) It distilled at 30–35° (bath)/0 5 mmHg to give pure **8**, $[\alpha]_D^{22}$ +174 ±2° (*c* 1.5, chloroform), λ_{max}^{film} 3 60 (N-Me), 7 30 μm (C-Me).

Anal Calc for $C_9H_{19}NO_2$ C, 62 38, H, 11 05, N, 8 08 Found C, 62 39, H, 11 01, N, 8 03

The slower-moving isomer, methyl 2,3,4,6-tetradeoxy-4-(dimethylamino)-α-D-*threo*-hexopyranoside (**11**), was also obtained, yield 650 mg (26%, from the oxime) R_F 0 17 (ether), $[\alpha]_D^{25}$ +55 2 ±1° (*c* 0 72, chloroform); λ_{max}^{film} 3 50 (N-Me), 7 30 μm (C-Me) For analysis, the compound was distilled at 40–45° (bath)/0 5 mmHg

Anal Calc for $C_9H_{19}NO_2$ C, 62 38, H, 11 05, N, 8 08 Found C, 62 26, H, 10 95, N, 7 82

2,3,4,6-Tetradeoxy-4-(dimethylamino)-D-erythro-*hexose* (*Forosamine*) (**9**) — Methyl 2,3,4,6-tetradeoxy-4-(dimethylamino)-α-D-*erythro*-hexopyranoside (**8**, 312 mg) was hydrolyzed in M sulfuric acid (10 ml) for 12 h at room temperature The solution was made neutral with barium carbonate, the solids were filtered off, and the filtrate was evaporated to a syrup Distillation at 50–55° (bath)/0 5 mmHg gave pure **9**, yield 138 mg (49%), m p 58–60°, $[\alpha]_D^{25}$ +90 0 ±1° (*c* 1, methanol) [lit [4] m p. 60°, $[\alpha]_D^{27}$ +88° (*c* 1 1, methanol)], R_F 0 17 (on microcrystalline cellulose), λ_{max}^{KBr} 3 00 (OH), 3 40 (N-Me), 7 30 μm (C-Me), X-ray powder diffraction data· 8 04 m, 6 36 w, 5 91 vs (1,1), 5 39 vs (1,1), 5 08 m, 4 72 s (2), 4 17 vw, 3 97 s (3), 3 67 w, 3 49 w, 2 99 w, and 2 77 m.

Anal Calc for $C_8H_{17}NO_2$ C, 60 37, H, 10 77, N, 8 80 Found C, 60 12, H, 10 49, N, 8.74

2,3,4,6-Tetradeoxy-4-(dimethylamino)-D-threo-*hexose* (**12**) — Methyl 2,3,4,6-tetradeoxy-4-(dimethylamino)-α-D-*threo*-hexopyranoside (**11**, 440 mg) was hydrolyzed in M sulfuric acid (10 ml) for 12 h at room temperature The solution was made neutral with barium carbonate, the solids were filtered off, and the filtrate was evaporated to a syrup that was distilled at 100–110° (bath)/0 5 mmHg, yield 248 mg (57%), $[\alpha]_D^{20}$ −2 12 ±0 5° (*c* 1 3, methanol) [lit [4] $[\alpha]_D^{26}$ −2 46° (*c* 1.1, methanol)], R_F 0 22 (on microcrystalline cellulose), λ_{max}^{film} 3 00 (OH), 3 60 (N-Me), 7 30 μm (C-Me)

Anal Calc for $C_8H_{17}NO_2$· C, 60 37, H, 10 77, N, 8 80 Found C, 60 19, H, 10 57, N, 8 75

Reduction of ketone **2** *with lithium aluminum hydride* — To a solution of **2** (542 mg, 3 78 mmoles) in anhydrous ether (30 ml) was added an excess of lithium

aluminum hydride (250 mg, 6.6 mmoles) After 2 h at room temperature, the excess reductant was decomposed by the dropwise addition of ethyl acetate (5 ml) The solution was washed successively with 0 01M hydrochloric acid (10 ml) and saturated, aqueous sodium hydrogen carbonate (50 ml). The basic, aqueous layer was washed with three 50-ml portions of dichloromethane, and the extracts were combined, dried (magnesium sulfate), and evaporated to a chromatographically homogeneous syrup, yield 430 mg (78%), $[\alpha]_D^{22}$ +145 ±1° (*c* 1 1, water) Examination of the product by g l c and by t l c (1·1 dichloromethane–ether or dichloromethane) showed that only one component, corresponding to methyl 2,3,6-trideoxy-α-D-*erythro*-hexopyranoside (methyl α-amicetoside, **1**) was present Its i r. and n m r. spectra were superposable on those of authentic **1**.

Methyl 2,3,6-trideoxy-4-O-(3,5-dinitrobenzoyl)-α-D-erythro-hexopyranoside — A mixture of the foregoing reduction product (150 mg, 1.03 mmoles) and 3,5-dinitrobenzoyl chloride (285 mg, 1 23 mmoles) in pyridine (10 ml) was stirred for 18 h at room temperature The mixture was poured over ice–water (50 ml), and the resulting crystalline product was filtered off, washed several times with water, and dried, yield 206 mg (59%) The product was recrystallized twice from methanol to give analytically pure material, m p 99–100°, $[\alpha]_D^{25}$ +132 ±1° (*c* 0 25, chloroform) The product was identical by mixed m p , i r spectrum, and X-ray powder diffraction pattern with authentic methyl 2,3,6-trideoxy-4-*O*-(3,5-dinitrobenzoyl)-α-D-*erythro*-hexopyranoside obtained[6] from methyl α-amicetoside X-ray powder diffraction data 7.84 s (1), 5 95 m, 5 74 m, 5 14 w, 4 76 m, 4.51 m, 4.31 s (2), 3 93 m, 3 71 s (3), 3 49 vw, 3 31 vw, 3 15 w, and 2 96 w

Methyl 2,3,6-trideoxy-3,3-dideuterio-α-D-glycero-hexopyranosid-4-ulose (**5**) — A solution of ketone **2** (40 mg, 0 28 mmole) in chloroform-*d* (0 4 ml) was shaken for 12 h at room temperature with 0 25 M sodium deuteriooxide in deuterium oxide (~0 05 ml) The n m r. spectrum of the resultant product indicated that it was the 3,3-dideuterio derivative **5**, n.m r data τ 5 08 (1-proton triplet, $J_{1,2} = J_{1,2} =$ 4 5 Hz), τ 5 73 (1-proton quartet, H-5), τ 6 52 (3-proton singlet, OMe), τ 7 69, 7.99 (1-proton broadened quartets, $J_{2\,2'}$ 15 Hz, H-2,2'), τ 8 68 (3-proton doublet, $J_{5\,6}$ 6 8 Hz, H-6)

Methyl 2,3,6-trideoxy-3,3,5-trideuterio-α-D-glycero-hexopyranosid-4-ulose (p-*nitrophenyl*)*hydrazone* (**6**). — To a solution of the ketone **2** (190 mg, 1 32 mmoles) in acetone-d_6 (0 5 ml) was added a 0 25M solution of sodium deuteriooxide in deuterium oxide (1 ml). The mixture was kept for 12 h at room temperature, the acetone-d_6 was evaporated off, and the mixture was extracted with two 10-ml portions of carbon tetrachloride. The extracts were combined, washed with two 2-ml portions of deuterium oxide, dried (magnesium sulfate), and evaporated. To the resultant syrup was added (*p*-nitrophenyl)hydrazine hydrochloride (296 mg, 1 56 mmoles), deuterium oxide (5 ml), pyridine (3 ml), and methanol (3 ml) The mixture was stirred for 24 h at room temperature, and then evaporated to dryness The residue was dissolved in benzene (50 ml), washed with two 20-ml portions of water, dried (magnesium sulfate), and evaporated The residue was crystallized from ethanol–

water to give yellow crystals of **6**, yield 180 mg (46%), m.p. 157–157.5°, λ_{max}^{KBr} 3.00 (NH), 6.30 (C=N), 13.3, 14.4 μm (phenyl). The X-ray powder diffraction pattern was superposable on that of **4**.

ACKNOWLEDGMENTS

The authors thank Mr. J. D. Wander for n.m.r. spectral measurements, and Mr. J. K. Thomson for assistance with the g.l.c. analyses.

REFERENCES

1 R. PAUL AND S. TCHELITCHEFF, *Bull. Soc. Chim. Fr.*, (1957) 443, 734
2 R. PAUL AND S. TCHELITCHEFF, *Bull. Soc. Chim. Fr.*, (1965) 1059
3 S. PINNERT-SINDICO, L. NINET, J. PREUD'HOMME, AND C. COSAR, *Antibiotics Ann.*, (1954) 724; R. CORBAZ, L. ETTLINGER, E. GAUMANN, W. KELLER-SCHEIRLEIN, F. KRADOLFER, E. KYBURZ, L. NEIPP, V. PRELOG, W. WETTSTEIN, AND H. ZAHNER, *Helv. Chim. Acta*, 39 (1956) 304, M. E. KUEHNE AND B. W. BENSON, *J. Amer. Chem. Soc.*, 87 (1965) 4660
4 C. L. STEVENS, G. GUTOWSKI, K. G. TAYLOR, AND C. P. BRYANT, *Tetrahedron Lett.*, (1966) 5717
5 A. B. FOSTER, R. HARRISON, J. LEHMANN, AND J. M. WEBBER, *J. Chem. Soc.*, (1963) 4471
6 ESTER L. ALBANO AND D. HORTON, *J. Org. Chem.*, 34 (1969) 3519
7 P. M. COLLINS, D. GARDINER, AND W. G. OVEREND, *Carbohyd. Res.*, 6 (1968) 431
8 D. HORTON AND J. S. JEWELL, *Carbohyd. Res.*, 2 (1966) 251
9 D. HORTON AND E. K. JUST, *Carbohyd. Res.*, 9 (1969) 129
10 D. HORTON AND E. K. JUST, unpublished data
11 E. H. WOODRUFF, J. P. LAMBOOY, AND W. E. BURT, *J. Amer. Chem. Soc.*, 62 (1940) 922
12 D. HORTON AND J. S. JEWELL, *Carbohyd. Res.*, 3 (1966) 255
13 M. L. WOLFROM, R. M. DE LEDERKREMER, AND D. L. PATIN, *J. Chromatogr.*, 17 (1965) 488

Carbohydrate Research
Elsevier Publishing Company, Amsterdam
Printed in Belgium

MIGRATION OF THIOLTHIOCARBONYL GROUPS IN SELECTIVELY PROTECTED METHYL α-D-GLUCOPYRANOSIDE XANTHATES*

D TRIMNELL, W. M DOANE, C R RUSSELL, AND C E RIST
*Northern Regional Research Laboratory**, *Peoria, Illinois 61604 (U S. A)*
(Received April 3rd, 1969, in revised form May 25th, 1969)

ABSTRACT

A series of benzylxanthate esters of methyl α-D-glucopyranoside selectively methylated at various hydroxyl groups was synthesized. The esters were converted into the corresponding xanthate salts, and their behavior was investigated under alkaline conditions typical of xanthation media. Results reveal that migration of thiolthiocarbonyl groups from the 2- or 3-position to the 6-position proceeds via the hydroxyl group at C-4 Direct migration across the pyranose ring from the 2- or 3-position to the 6-position was negligible The (benzylthio)thiocarbonyl position for each ester was identified by n.m r. spectroscopy. Primary substitution caused the signals for the two protons at C-6 to be displaced downfield by about 1 0 p p m, and secondary substitution caused a 2.4-p p m. downfield shift of the signals for protons at C-2, C-3, and C-4.

INTRODUCTION

An earlier report[1] showed that the course of xanthation of methyl α-D-glucopyranoside and starch was similar. In each system, migration of thiolthiocarbonyl groups from the secondary positions to the primary position was rapid†. Migration of the thiolthiocarbonyl substituent from C-2 to C-6 in the monosaccharide proceeded via C-3. Only traces of the C-4 thiolthiocarbonate (xanthate) were found during transposition from C-2 to C-6, which suggests that the migration proceeded either rapidly through C-4 to C-6 or directly from C-3 to C-6.

We now report studies undertaken to elucidate fully the pathway of migration of thiolthiocarbonyl groups in methyl α-D-glucopyranoside xanthates For this purpose we prepared the following selectively substituted *S*-benzylxanthates: methyl 3-*O*-[(benzylthio)thiocarbonyl]-2-*O*-methyl-α-D-glucopyranoside (**1a**), methyl 3-*O*-[(benzylthio)thiocarbonyl]-2,4-di-*O*-methyl-α-D-glucopyranoside (**2a**), methyl 2-*O*- and 3-*O*-[(benzylthio)thiocarbonyl]-4-*O*-methyl-α-D-glucopyranosides (**3a** and **3b**), and methyl

*To be presented at 158th Meeting of the American Chemical Society, New York, N Y, September 7–12, 1969
**This is a laboratory of the Northern Utilization Research and Development Division, Agricultural Research Service, U S Department of Agriculture
†Migration refers to groups attached to oxygen atoms at the carbon positions specified

4-*O*-[(benzylthio)thiocarbonyl]-2,3-di-*O*-methyl-α-D-glucopyranoside (**4a**) Each of these was converted into the corresponding sodium xanthate, which was kept in 18% sodium hydroxide for 2 h at 20° and then *S*-benzylated The mixture of isomers formed was resolved by t l c. and the quantity of each determined

The corresponding primary (C-6) substituted xanthate of each *O*-methylated methyl α-D-glucopyranoside was also prepared The position of substitution for each benzylxanthate ester was confirmed by n m r. spectra.

RESULTS AND DISCUSSION

Compound **1a**, prepared via methyl 4,6-*O*-benzylidene-2-*O*-methyl-α-D-glucopyranoside, was converted into the 3-(sodium xanthate), and subjected to 18% sodium hydroxide. Considerable decomposition occurred, as evidenced by formation of trithiocarbonate and methyl 2-*O*-methyl-α-D-glucopyranoside Benzylation of the reaction mixture gave methyl 6-*O*-[(benzylthio)thiocarbonyl]-2-*O*-methyl-α-D-glucopyranoside (**1b**) as the only recoverable ester, in 37% yield The identity of **1b** was confirmed by its independent synthesis via selective xanthation and *S*-benzylation of methyl 2-*O*-methyl-α-D-glucopyranoside.

To determine wheter C-3 to C-6 migration can occur without participation by a hydroxyl group at C-4, compound **2a**, prepared by two independent routes, was employed. Conversion of **2a** into the corresponding 3-(sodium xanthate) was slow, and 30% of unreacted **2a** was recovered after treatment with sodium hydrosulfide for 1 h at 40°. With other xanthate esters the conversion into sodium xanthate required only a few min at room temperature The sodium xanthate derivative of **2a** was isolated and subjected to 18% sodium hydroxide, after 2 h it was treated with benzyl bromide to form the xanthate ester Comparative t l c . in parallel with authentic **2a** and methyl 6-*O*-[(benzylthio)thiocarbonyl]-2,4-di-*O*-methyl α-D-glucopyranoside (**2b**), showed that the major component of the reaction mixture was **2a**, with only a trace of **2b**.

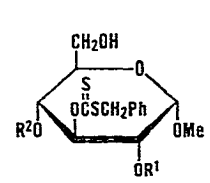
Studies conducted with xanthates of methyl 4-*O*-methyl-α-D-glucopyranoside confirm the observation made with **2a** that direct migration from C-3 to C-6 does not occur Aqueous xanthation of the 4-methyl ether followed by *S*-benzylation gave a monosubstituted xanthate fraction containing the 2-, 3-, and 6-xanthate isomers in a ratio of 1 1 3 3 3, respectively Xanthation in methyl sulfoxide provided a ratio of about 10 1 1

When **3a** was converted into the sodium xanthate and kept in aqueous sodium hydroxide, redistribution occurred to give almost equal amounts of the C-2 and C-3 xanthates No C-6 xanthate was detected by t l c A similar mixture of the C-2 and C-3 xanthates was obtained when **3b** was converted into the sodium xanthate and treated with alkali, a minor amount of the 6-xanthate was also formed Some redistribution of xanthate groups also occurred when methyl 6-*O*-[(benzylthio)thiocarbonyl]-4-*O*-methyl-α-D-glucopyranoside (**3c**) was treated in a manner similar to that for the 2- and 3-isomers After alkaline treatment of the 6-xanthate, *S*-benzylation produced mostly **3c** with only minor amounts of **3a** and **3b**

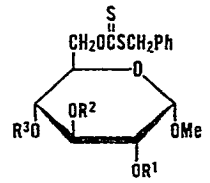
The results with **2a**, **3a**, **3b**, and **3c** indicate that no significant amount of intramolecular migration takes place directly between C-3 and C-6 and that migrations to C-6 proceed via C-4

It remained to be shown that migration of thiolthiocarbonyl groups could proceed from C-4 to C-6 For this purpose, **4a** was prepared Conversion of **4a** into the sodium xanthate, followed by alkaline treatment and *S*-benzylation, formed a mixture of 96% of methyl 6-*O*-[(benzylthio)thiocarbonyl]-2,3-di-*O*-methyl-α-D-glucopyranoside (**4b**) and 4% of **4a** Compound **4b** was prepared alternatively by xanthation and *S*-benzylation of methyl 2,3-di-*O*-methyl α-D-glucopyranoside When **4b** was converted into the sodium xanthate, subjected to alkali, and *S*-benzylated, it afforded a mixture similar to that obtained from **4a**.

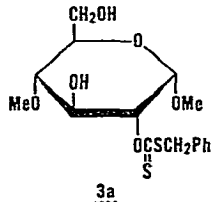
These studies show that migration of the thiolthiocarbonyl group in methyl α-D-glucopyranoside xanthates follows a pathway from the 2- or 3-position to the 6-position via C-4



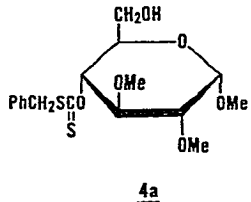
1a R^1 = Me, R^2 = H
2a R^1 = R^2 = Me
3b R^1 = H R^2 = Me



1b R^1 = Me, R^2 = R^3 = H
2b R^1 = R^3 = Me R^2 = H
3c R^1 = R^2 = H R^3 = Me
4b R^1 = R^2 = Me R^3 = H



3a



4a

N m r spectra — Positions of the various xanthate groups were identified through n m r. spectra Table I shows that substitution of a (benzylthio)thiocarbonyl group at the primary (C-6) position causes a downfield displacement of about 1.0 p p m , in agreement with Forsén and coworkers[2] In addition, Table I shows that substitution of a (benzylthio)thiocarbonyl group at any secondary position causes downfield displacements of about 2 4 p p m

Fig 1 shows n m r spectra of representative 2-, 3-, 4-, and 6-substituted xanthates Chemical shifts characteristic of 2-substitution were τ 4.4–4 5 for H-2, 3-substitution, τ 3 6–3 8 for H-3, 4-substitution, τ 4 2 for H-4; 6-substitution, τ 5 2 for H-6 and H-6′.

Infrared and ultraviolet spectra — I r. spectroscopy was useful in monitoring the preparations of the xanthate compounds Any series involving variations in

TABLE I

EFFECT OF *O*-(BENZYLTHIO)THIOCARBONYL SUBSTITUTION ON PROTON CHEMICAL SHIFTS[a]

Compound	*Position of substitution*	*Chemical shift, τ*	*Displacement of shift relative to −OH, p p m*
1b	C-6 (H-6, H-6')	5 22	−0 93
2b		5 22	−1 00
3c		5 25	−1 02
4b		5 19	−1 14
3a	C-2 (H-2)	4 55	−2 30
1a	C-3 (H-3)	3 78	−2 41
2a		3 65	−2 44
3b		3 81	−2 41
4a	C-4 (H-4)	4 25	−2 35

[a]Details of spectral measurements are given in the text

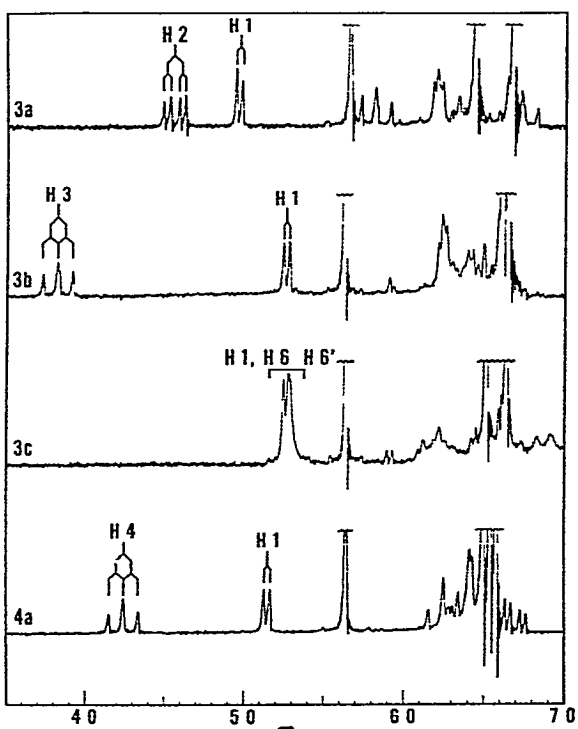


Fig 1 Portions of the n m r. spectra of xanthates of methyl ethers of methyl α-D-glucopyranosides, showing proton shifts due to (benzylthio)thiocarbonyl substitution, measured in chloroform-*d* at 100 MHz. Methyl 2-*O*-[(benzylthio)thiocarbonyl]-4-*O*-methyl-α-D-glucopyranoside (3a), methyl 3-*O*-[(benzylthio)thiocarbonyl]-4-*O*-methyl-α-D-glucopyranoside (3b), methyl 6-*O*-[(benzylthio)thiocarbonyl]-4-*O*-methyl-α-D-glucopyranoside (3c), and methyl 4-*O*-[(benzylthio)thiocarbonyl]-2,3-di-*O*-methyl-α-D-glucopyranoside (4a)

position of xanthate ester substitution showed characteristic pattern differences in the 9–11 μm region All of the esters showed characteristic intense C–O–C stretching bands near 8 1–8.3 μm and C=S stretching[3] near 9.4–9 6 μm The most intense bands were at 9.4–9 5 μm in the 2- and 6-substituted esters, and at 9 5–9 6 μm in the 3- and 4-substituted esters

U v. spectroscopy was used primarily for monitoring chromatographic fractions to detect xanthate compounds Absorption maxima were observed for these compounds at 283–287 nm (ε 10,500–14,800) and 355–365 nm (ε 60–90).

EXPERIMENTAL

N m r spectra were obtained with a Varian* HA-100 spectrometer Spectra were determined in chloroform-*d* with tetramethylsilane (τ 10 00) as internal standard I r spectra were recorded with a Perkin–Elmer Model 137 spectrophotometer between 2 5–15 0 μm of thin films cast from chloroform solution onto silver chloride plates. Wavelengths were calibrated by polystyrene film U.v. spectra were determined with a Perkin–Elmer Model 202 spectrophotometer between 245–390 nm, 1-cm silica cuvettes and chloroform as solvent were used Melting points were determined in sealed capillaries in an oil bath and are uncorrected Optical rotations were measured with a Rudolph polarimeter T l c was performed with Silica Gel G (E Merck, Germany) Qualitative separations were made on microscope slides and the components detected by charring with 5% sulfuric acid in methanol Preparative t l c was carried out with 1–2 mm layers on glass plates or aluminum trays and with Rhodamine 6-G as a fluorescent indicator. Larger scale separations were made on a chromatographic column packed with 100-mesh silicic acid (Mallinckrodt, U S. A)

Methyl 4,6-O-benzylidene-2-O-methyl-α-D-glucopyranoside. — Methyl 4,6-*O*-benzylidene-α-D-glucopyranoside (49 5 g) was methylated[4] with methyl iodide (35 ml) and barium oxide (28 g) in *N,N*-dimethylformamide (70 ml) The solution was filtered to remove barium oxide, evacuated to remove *N,N*-dimethylformamide, treated with chloroform and shaken with dilute acetic acid to neutrality, shaken with sodium hydrogen sulfite solution to remove free iodine, and dried over sodium sulfate. Evaporation of the chloroform, followed by three extractions with warm hexane left a solid (26 4 g) which was recrystallized from hexane to give methyl 4,6-*O*-benzylidene-2-*O*-methyl-α-D-glucopyranoside, m p 167–169°, $[\alpha]_D^{23}$ +78 6° (*c* 1 07, ethanol), lit [5] m p 168°, $[\alpha]_D^{17}$ +78 9° (*c* 1.19, ethanol)

Methyl 3-O-[(benzylthio)thiocarbonyl]-2-O-methyl-α-D-glucopyranoside (**1a**) — Methyl 4,6-*O*-benzylidene-2-*O*-methyl-α-D-glucopyranoside (20 g) in methyl sulfoxide (50 ml) was stirred with 13 5M sodium hydroxide (5 ml) and carbon disulfide (10 ml) for 15 min at 25°, the mixture was cooled in an ice bath, neutralized with acetic acid, and treated with benzyl bromide (8 ml) After 10 min chloroform (500 ml) was added and the solution washed ten times with 500 ml of ice–water to remove methyl sulfoxide.

*The mention of firm names or trade products does not imply that they are endorsed or recommended by the Department of Agriculture over other firms or similar products not mentioned

The chloroform solution was dried and then evaporated to a syrup that was transformed into a solid (26 g) by trituration with hexane T l c showed the solid to be a mixture of starting material and a product of higher R_F Selective desorption of the higher R_F component from silicic acid with hexane–chloroform gave methyl 4,6-*O*-benzylidene-3-*O*-[(benzylthio)thiocarbonyl]-2-*O*-methyl-α-D-glucopyranoside, yield 17.6 g (56%), m p 122–124° (from hexane), $[\alpha]_D^{23.5}$ +24.0° (*c* 1.12, chloroform)

Anal. Calc for $C_{23}H_{26}O_6S_2$· C, 59.7; H, 5.7; S, 13.9. Found. C, 59.3, H, 5.9, S, 13 7.

This derivative was also prepared by methylation of methyl 4,6-*O*-benzylidene-3-*O*-[(benzylthio) thiocarbonyl]-α-D-glucopyranoside (0 84 g) with diazomethane and boron trifluoride catalysis. Yield 0 60 g (46%), m p. 120–122° (from hexane), $[\alpha]_D^{23.5}$ +26.0° (*c* 1.00, chloroform)

Anal. Found C, 59.7; H, 5.8; S, 13.6.

The mixed m.p. of the components prepared by these two routes showed no depression. I r. and u.v. spectra of these products confirmed their identity. Treatment of this compound (20 g) with 0.03M hydrochloric acid in methanol (500 ml)[1] gave **1a** as a syrup; yield 15.8 g (98%), which below 0° was obtainable as a solid from ether–hexane, $[\alpha]_D^{24}$ +169.8° (*c* 0 96, chloroform)

Anal Calc. for $C_{16}H_{22}O_6S_2$· C, 51 3; H, 5 9; S, 17.1. Found C, 51 0; H, 6 0; S, 17.4.

Methyl 6-O-[(benzylthio)thiocarbonyl]-2-O-methyl-α-D-glucopyranoside (**1b**) — The benzylidene group was removed from methyl 4,6-*O*-benzylidene-2-*O*-methyl-α-D-glucopyranoside (4.41 g) with 0 03M hydrochloric acid in methanol[1] and the resulting product crystallized from ethyl acetate to give methyl 2-*O*-methyl-α-D-glucopyranoside, 1 57 g (51%), m p. 146–148°, $[\alpha]_D^{21}$ +158 9° (*c* 1 03, water), lit [6] m p 147–148°, $[\alpha]_D^{19}$ +155° (*c* 0.7, water) This compound (1.57 g) was stirred overnight at room temperature with 18% sodium hydroxide (1.7 ml) and carbon disulfide (0.6 ml) The mixture was cooled, neutralized with acetic acid, stirred with benzyl bromide (0 9 ml) overnight, and then extracted with chloroform. Evaporation of the chloroform gave a syrup; yield 0 84 g (30%), $[\alpha]_D^{24}$ +70 5° (*c* 1.05, chloroform) T.l.c (ethyl acetate) showed a single component, distinguishable from the 3-ester

Anal Found· C, 51.3; H, 6 0; S, 17 2.

Migration studies with **1a**. — Compound **1a** (1 20 g) was converted into the corresponding xanthate salt by adding sodium hydrosulfide in methyl sulfoxide[1] Conversion into the sodium xanthate was rapid, but extensive decomposition to trithiocarbonate (identified by u v.) and methyl 2-*O*-methyl-α-D-glucopyranoside (identified by t.l.c.) occurred. This decomposition lowered the overall yield of xanthate recovered from the alkaline treatment and rebenzylation to 0 45 g T l c, i r, u.v, and n m.r. showed that the recovered product was identical with **1b** obtained from the direct xanthation and benzylation of methyl 2-*O*-methyl-α-D-glucopyranoside

Methyl 2,4-di-O-methyl-6-O-trityl-α-D-glucopyranoside. — Methyl 4,6-*O*-benzylidene-2-*O*-methyl-α-D-glucopyranoside (5.87 g) was acetylated in pyridine[7] to give the 3-acetate (6.69 g), m p. 141–146° (lit. 145°)[5], treated with hydrochloric acid in

methanol to remove the benzylidene group, tritylated[8] to give methyl 3-*O*-acetyl-2-*O*-methyl-6-*O*-trityl-α-D-glucopyranoside (5.11 g), m.p. 60–70°, and then treated with diazomethane and boron trifluoride[1] to methylate the C-4 hydroxyl group. The products (4 01 g) had m p 136° (from hexane), $[\alpha]_D^{24}$ +102.5° (*c* 1 65, chloroform), n m r data τ 4 68 (chloroform-*d*, triplet, H-3), confirming the position of the acetyl group at O-3

Anal Calc for $C_{30}H_{34}O_7$ C, 71 1, H, 6 8 Found C, 71.1; H, 7.0.

Deacetylation with sodium methoxide in methanol[9] gave methyl 2,4-di-*O*-methyl-6-*O*-trityl-α-D-glucopyranoside (2 46 g), m p. 152–154° after recrystallization from hexane, $[\alpha]_D^{24}$ +91 3° (*c* 1 60, chloroform)

Anal Calc for $C_{28}H_{32}O_6$ C, 72 4, H, 6 9 Found C, 72 4, H, 7.1.

Methyl 3-O-[(benzylthio)thiocarbonyl]-2,4-di-O-methyl-α-D-glucopyranoside (**2a**) — Methyl 2,4-di-*O*-methyl-6-*O*-trityl-α-D-glucopyranoside (0 30 g) was xanthated and *S*-benzylated by a procedure similar to that described in the preceding paragraph The product was purified by adsorption onto silicic acid from chloroform solution followed by desorption with hexane to remove impurities and then desorption with 4 1 hexane–chloroform to give methyl 3-*O*-[(benzylthio)thiocarbonyl]-2,4-di-*O*-methyl-6-*O*-trityl-α-D-glucopyranoside (0 15 g), m p. 162–164°

Anal. Calc for $C_{36}H_{38}O_6S_2$· C, 68.5; H, 6 1, S, 10 2 Found C, 68 6; H, 6 2, S, 9 9.

A portion (1 00 g) was detritylated with hydrochloric acid in methanol[10] to **2a**, which was purified by desorption from silicic acid (9 1 dichloromethane–ethyl acetate). The product was a syrup, yield 0 46 g (74%), $[\alpha]_D^{23}$ +97 5° (*c* 1 02, chloroform)

Anal Calc for $C_{17}H_{24}O_6S_2$ C, 52 6; H, 6 2, S, 16 5 Found C, 52 5; H, 6 4; S, 16 6

An alternative route to **2a** was as follows Tritylation of **1a** (15 8 g) yielded methyl 3-*O*-[(benzylthio)thiocarbonyl]-2-*O*-methyl-6-*O*-trityl-α-D-glucopyranoside, 19 4 g, m p 139–140°, $[\alpha]_D^{25}$ +113 3° (*c* 2 63, chloroform)

Anal Calc for $C_{35}H_{36}O_6S_2$ C, 68 2, H, 5 9, S, 10 4. Found C, 68 2, H, 6 2; S, 10 0.

This compound (5 0 g) was methylated with a tenfold excess of diazomethane in the presence of boron trifluoride, and the resulting mixture was separated by column chromatography on silicic acid (13 5 2 hexane–carbon disulfide–ethyl acetate) The product of highest R_F value was obtained as crystals from hexane, 0 21 g (4%), m p 162–163°, $[\alpha]_D^{25}$ +92 6° (*c* 2 31, chloroform) Unchanged starting compound (2 0 g) and detritylated components were also recovered The crystals were shown to be methyl 3-*O*-[(benzylthio)thiocarbonyl]-2,4-di-*O*-methyl-6-*O*-trityl-α-D-glucopyranoside by i r. spectra and mixed m p Detritylation of this intermediate again produced **2a**

Methyl 6-O-[(benzylthio)thiocarbonyl]-2,4-di-O-methyl-α-D-glucopyranoside (**2b**). — Detritylation of methyl 2,4-di-*O*-methyl-6-*O*-trityl-α-D-glucopyranoside (0 50 g) on silica gel[11] (Davison Grade 12) gave methyl 2,4-di-*O*-methyl-α-D-glucopyranoside (0 094 g) which, on crystallization from ethyl acetate–hexane, had m p

79–81°, $[\alpha]_D^{23}$ +180 2° (*c* 1 32, acetone); lit.[12] m p 79–80°, $[\alpha]_D^{23}$ +186° (*c* 1.0, acetone) This compound (0 090 g) was dissolved in methyl sulfoxide (0 5 ml) and stirred for 30 min at 25° with 5M sodium hydroxide (0 1 ml) and carbon disulfide (0 2 ml). The mixture was neutralized with carbon dioxide, cooled, and stirred for 30 min with benzyl bromide (0 06 ml) The mixture was treated with ice–water and filtered, and the filter cake was dissolved in chloroform and purified by preparative t l c (1 4 ethyl acetate–chloroform) The middle zone was sectioned, eluted with acetone, evaporated, and triturated with hexane to give the title compound (0 065 g). m p 70–71°, $[\alpha]_D^{23}$ +96.8° (*c* 0 95, chloroform).

Anal Calc. for $C_{17}H_{24}O_6S_2$ C, 52 6, H, 6 2; S, 16 5. Found C, 52 8, H, 6 3, S, 16 7.

Migration studies with **2a**. — Compound **2a** (750 mg) in methyl sulfoxide (7 ml) was stirred with sodium hydrosulfide (112 mg) for 1 h at 40° under nitrogen Extraction of the methyl sulfoxide solution with ether removed 207 mg (28%) of uncleaved **2a**. The residue, which contained xanthate salt and some occluded methyl sulfoxide, was kept in 18% sodium hydroxide (5 ml) for 2 h at 20° Decomposition of some xanthate was indicated by absorption for trithiocarbonate in the u v. spectrum and the evolution of a small amount of carbon disulfide, detected in an amine–alcohol[1] trap after flushing with nitrogen The reaction solution was cooled, neutralized with dilute acetic acid, and agitated rapidly while benzyl bromide (0 24 ml) was added Nitrogen flushing into the amine–alcohol solution at this stage showed a larger amount of carbon disulfide, possibly from trithiocarbonate formed earlier The mixture was refrigerated overnight, extracted with chloroform, and resolved by preparative t l c (8 1 1 dichloromethane–ethyl acetate–benzene) The xanthate ester zone was eluted to give 0 22 g (29%) of syrup, shown by t l c , i.r , and n m r to be identical with **2a** Only a trace of **2b** was found Byproduct carbon disulfide and trithiocarbonate ester accounted for 31% of **2a** Dexanthation was confirmed by the finding of methyl 2,4-di-*O*-methyl-α-D-glucopyranoside in the aqueous portion of the rebenzylation mixture.

Methyl 4-O-methyl-α-D glucopyranoside — The title compound was prepared by a modification* of a previously attempted route[13] as follows Methyl 4,6-*O*-benzylidene-α-D-glucopyranoside (50 g) was acetylated in pyridine[7] to methyl 2,3-di-*O*-acetyl-4,6-*O*-benzylidene-α-D-glucopyranoside (63.5 g), m p ,106–109°. The benzylidene group was removed, without appreciable deacetylation, by dissolving the compound (30 g) in 1 liter of ethanol and 800 ml of 0 5M hydrochloric acid at 45°, stirring for 15 min, cooling to 5°, neutralizing with barium carbonate, filtering, and concentrating to 900 ml After extraction with hexane to remove benzaldehyde, the remaining product was extracted into chloroform and the chloroform removed by evaporation to give methyl 2,3-di-*O*-acetyl-α-D-glucopyranoside (22 9 g) Tritylation gave methyl 2,3-di-*O*-acetyl-6-*O*-trityl-α-D-glucopyranoside (32 5 g), which was methylated with

*Unpublished results of E I Stout of this Laboratory

diazomethane and boron trifluoride[1] to methyl 2,3-di-*O*-acetyl-4-*O*-methyl-6-*O*-trityl-α-D-glucopyranoside (24 3 g), m p 140–152° This compound was purified by preparative t l c (1 9 ethyl acetate–chloroform) and had m p 153–156°, $[\alpha]_D^{26}$ +92 2° (*c* 1 18, chloroform)

Anal Calc for $C_{31}H_{34}O_8$ C, 69 7, H, 6 4 Found C, 69.5, H, 6 3

Deacetylation of the diacetate (10 0 g) with sodium methoxide in methanol[9] gave methyl 4-*O*-methyl-6-*O*-trityl-α-D-glucopyranoside as a syrup, which was detritylated with hydrochloric acid in methanol[10] The title compound was obtained as a syrup (4 0 g), which was crystallized from ethyl acetate to yield 3 14 g, m p 85–90° Purification by preparative t l c (4 1 ethyl acetate–methanol) gave m p. 93–95°, $[\alpha]_D^{21}$ +164 0° (*c* 0 94, water), lit [14] m p 94–95°, $[\alpha]_D^{25}$ +167° (*c* 1 17, water)

*Methyl 2-*O*-, 3-*O*-, and 6-*O*-[(benzylthio)thiocarbonyl]-4-*O*-methyl-α-D-glucopyranosides* (**3a**, **3b**, *and* **3c**) — (*a*) *Xanthation under aqueous conditions* Methyl 4-*O*-methyl-α-D-glucopyranoside (1 00 g) was stirred with 18% sodium hydroxide (1 07 ml) and carbon disulfide (0 20 ml) for 2 5 h at 25° The solution was cooled, diluted with ice water, neutralized with acetic acid, stirred with benzyl bromide (0 40 ml), and refrigerated overnight The mixture was extracted with chloroform, the extract was dried and evaporated to yield 0 72 g Preparative t l c (7 3 ethyl acetate–carbon disulfide) showed a mixture of the 2-*O*- (18%), 3-*O*- (23%), and 6-*O*-[(benzylthio)-thiocarbonyl] (59%) derivatives

(*b*) *Xanthation in methyl sulfoxide.* Methyl 4-*O*-methyl-α-D-glucopyranoside (1 00 g) in methyl sulfoxide (5 ml) was stirred with 18% sodium hydroxide (1 07 ml) and carbon disulfide (0 30 ml) for 30 min at 25° The mixture was cooled and stirred with benzyl bromide (0 59 ml) for 10 min and then extracted with 50 ml of chloroform The chloroform extract was washed with 0 05M hydrochloric acid and water, then dried, and evaporated to a syrup Preparative t l c gave 0 97 g of monoester fraction containing the 2-, 3-, and 6-isomers (84%, 8%, and 8%, respectively).

(*c*) *Xanthation and S-benzylation of methyl 4-O-methyl-6-O-trityl-α-D-glucopyranoside* The title compound (2 88 g) was xanthated and *S*-benzylated in methyl sulfoxide as described in the preceding section. Separation of the major component by preparative t l c (3% ethyl acetate in chloroform) gave methyl 2-*O*-[(benzylthio)-thiocarbonyl[-4-*O*-methyl-6-*O*-trityl-α-D-glucopyranoside (2 77 g), m p 65–70° (amorphous from hexane), $[\alpha]_D^{22}$ +71.0° (*c* 1 38, chloroform)

Anal Calc for $C_{35}H_{36}O_6S_2$ C, 68 2, H, 5 9, S, 10 4 Found C, 68 2, H, 6 0, S, 9 8 Detritylation of this compound (2 44 g) with hydrochloric acid in methanol gave **3a**, 1.40 g (95%).

Isomers **3a**, **3b**, and **3c** had the following properties

3a M p 103–105°, $[\alpha]_D^{24}$ +118 4° (*c* 1 01, chloroform) *Anal* Calc for $C_{16}H_{22}O_6S_2$ C, 51 3; H, 5 9, S, 17.1. Found C, 51 4, H, 6 0, S, 17.1

3b M p 96–97°, $[\alpha]_D^{24}$ +99 5° (*c* 0 97, chloroform). *Anal* Found C, 51.3; H, 5 9; S, 17 4

3c Syrup, $[\alpha]_D^{22}$ +95.5° (*c* 1 01, chloroform) *Anal* Found C, 51 0, H, 5.7, S, 17.4

These isomers were clearly distinguishable by their i.r. and n m r. spectra and R_F values

Migration studies with **3a**, **3b**, *and* **3c**. — Compounds **3a**, **3b**, and **3c** were subjected to the same series of reactions as with **1a** and **2a**, and the products were separated by preparative t.l c. From **3a** (0 50 g) was recovered 0 26 g (52%) on rebenzylation The mixture contained **3a** (50%), **3b** (50%), but not **3c** From **3b** (79 mg) was recovered 34 mg (43%) on rebenzylation. The mixture contained **3a** (37%), **3b** (55%), and **3c** (8%) From **3c** (30 mg) was recovered 22 mg (73%) on rebenzylation. T.l.c. showed primarily **3c** and traces of **3a** and **3b** In each series, the ester losses were accounted for as carbon disulfide and trithiocarbonate

Methyl 2,3-di-O-methyl-α-D-glucopyranoside — The hexane-soluble fraction recovered from methylation of methyl 4,6-*O*-benzylidene-α-D-glucopyranoside (see first section of Experimental) consisted mainly of methyl 4,6-*O*-benzylidene-2,3-di-*O*-methyl-α-D-glucopyranoside Adsorption of this fraction onto silicic acid from chloroform solution followed by desorption with hexane selectively removed this component (14 1 g), m p. 121–123°, $[\alpha]_D^{23}$ +96 2° (*c* 0 45, acetone), lit [5] m p 122°, $[\alpha]_D^{18}$ +98° (*c* 0 43, acetone). Mild, acid hydrolysis of this derivative (12 1 g) gave the title compound (8 23 g), m p. 81–84°, $[\alpha]_D^{25}$ +143 3° (*c* 1 29, water); lit [15] m p 83–85°, $[\alpha]_D$ +142 6° (water)

Methyl 4-O-[(benzylthio)thiocarbonyl]-2,3-di-O-methyl-α-D-glucopyranoside (**4a**) — Methyl 2,3-di-*O*-methyl-α-D-glucopyranoside (5 00 g) was tritylated to give methyl 2,3-di-*O*-methyl-6-*O*-trityl-α-D-glucopyranoside (8 7 g), recrystallization from ethanol gave product, m.p. 172–174°, $[\alpha]_D^{24}$ +66 6° (*c* 1 11, chloroform); lit [16] m.p. 169–170°, $[\alpha]_D$ +66.4° (chloroform) This product (3.00 g) was xanthated and *S*-benzylated in methyl sulfoxide and purified by preparative t l c with 4% ethyl acetate in chloroform to give methyl 4-*O*-[(benzylthio)thiocarbonyl]-2,3-di-*O*-methyl-6-*O*-trityl-α-D-glucopyranoside (2 52 g), m p 132–135°, $[\alpha]_D^{26}$ +70 3° (*c* 1.65, chloroform).

Anal Calc. for $C_{36}H_{38}O_6S_2$ C, 68 5; H, 6 1; S, 10 2 Found C, 68 7; H, 6 2; S, 10 0. Detritylation of this derivative with hydrochloric acid in methanol[10] gave **4a** (0 89 g), m p. 77–80°, $[\alpha]_D^{25}$ +16 7° (*c* 1 20, chloroform)

Anal. Calc. for $C_{17}H_{24}O_6S_2$ C, 52 6; H, 6 2, S, 16 5 Found C, 52 7, H, 6 3, S, 16.5.

Methyl 6-O-[(benzylthio)thiocarbonyl]-2,3-di-O-methyl-α-D-glucopyranoside (**4b**) — Methyl 2,3-di-*O*-methyl-α-D-glucopyranoside (1.00 g) was xanthated and *S*-benzylated in methyl sulfoxide and then partially purified with silicic acid as described for **2a** Preparative t l c (2 3 ethyl acetate–carbon disulfide) afforded the title compound as a syrup; yield 0 56 g (32%), $[\alpha]_D^{25}$ +67 4° (*c* 1.46, chloroform)

Anal. Found. C, 52 3; H, 6 3; S, 16 4

Migration studies with **4a** *and* **4b** — Compounds **4a** and **4b** were subjected to the same series of reactions as **1a**, **2a**, and **3a–3c**, and the products were separated by preparative t l c. From **4a** (124 mg) was recovered 77 mg (62%) of ester mixture on rebenzylation The ester mixture contained **4a** (4%) and **4b** (96%) From **4b** (97 mg) was recovered 78 mg (80%) of ester mixture on rebenzylation The ester mixture con-

tained **4a** (3%) and **4b** (97%) Losses of ester in each case were accounted for as carbon disulfide and trithiocarbonate.

ACKNOWLEDGMENTS

Sincere appreciation is extended to Walter Boyd for the n m r. data and to Mrs Clara McGrew and Mrs Bonita Heaton for the microanalyses.

REFERENCES

1 D TRIMNELL, W M DOANE, C R RUSSELL, AND C E RIST, *Carbohyd Res*, 5 (1967) 166
2 S FORSEN, P J GAREGG, B LINDBERG, AND E PETTERSSON, *Acta Chem Scand*, 20 (1966) 2763
3 L H LITTLE, G W POLING, AND J LEJA, *Can J Chem*, 39 (1961) 745
4 R KUHN, H H BAER, AND A SEELIGER, *Ann*, 611 (1958) 236
5 E J BOURNE, M STACEY, C E M TATLOW, AND J C TATLOW, *J Chem Soc*, (1951) 826
6 W N HAWORTH, E L HIRST, AND E G TEECE, *J Chem Soc*, (1931) 2858
7 J J WILLARD AND E PACSU, *J Amer Chem Soc*, 82 (1960) 4347
8 B HELFERICH AND J BECKER, *Ann*, 440 (1924) 7
9 G ZEMPLÉN AND A KUNZ, *Ber*, 56 (1923) 1705
10 A ROBERTSON AND R B WATERS, *J Chem Soc*, (1931) 1709
11 J LEHRFELD, *J Org Chem*, 32 (1967) 2544
12 R E REEVES AND W. F GOEBEL, *J Biol Chem*, 139 (1941) 511
13 R L WHISTLER AND S J KAZENIAC, *J Amer Chem Soc*, 76 (1954) 3044
14 R L WHISTLER, E G LINKE, AND S J KAZENIAC, *J Amer Chem Soc*, 78 (1956) 4704
15 H BREDERECK, *Ber*, 68 (1935) 781
16 G J ROBERTSON, *J Chem Soc*, (1933) 737

Carbohydrate Research
Elsevier Publishing Company, Amsterdam
Printed in Belgium

THE INTERACTION OF POLYSACCHARIDES WITH IODINE

PART I. INVESTIGATION OF THE GENERAL NATURE OF THE REACTION

BLANCHE D E GAILLARD*, NORMAN S THOMPSON, AND ALFRED J MORAK
Carbohydrate Chemistry Group, The Institute of Paper Chemistry, Appleton, Wisconsin 54911 (U S A)
(Received February 19th, 1969, in revised form, May 28th, 1969)

ABSTRACT

A comparison of the interaction of a commercially available xylan, a D-galactose-deficient galactoglucomannan from Engelmann spruce holocellulose, a highly branched "amyloid" from tamarind seed, and a commercial amylose preparation from potato starch, in concentrated aqueous calcium chloride solution showed that all four of these polymers reacted with iodine–potassium iodide solution to give a blue product that was soluble at low concentrations of reagents In agreement with data in the literature, other highly branched polysaccharides such as cherry gum and D-galactose-rich galactoglucomannans did not react with iodine under these conditions Qualitative tests showed that iodine and calcium ion, as well as polysaccharide, were present in the isolated complexes, and spectrophotometric measurements showed the dependence of complex formation on the concentration of iodine and polysaccharide as well as on the time and temperature of reaction Although the polysaccharides reacted to give a dark blue, starch-like coloration with iodine, potentiometric titration showed that only the xylan was complexed with iodine in a manner similar to that of amylose, while galactoglucomannan and "amyloid" bound iodine in a looser fashion typical of poly(vinyl alcohol)–iodine complexes The iodine content of the complexes from xylan, "amyloid", and galactoglucomannan, unlike the iodine content of the amylose complex, was found to vary with the concentration of the reactants, indicating the absence of a preferred stoichiometry.

INTRODUCTION

Many linear polysaccharides have been shown to give a blue coloration with iodine when they are dissolved in concentrated aqueous calcium chloride solution, although they will not do so when dissolved in salt-free aqueous solutions[1] Research has shown that highly branched polysaccharides do not react with iodine under these circumstances, but a limited degree of branching does not prevent the formation of a colored product. It has also been shown that other multivalent ions may be

*Present address. Department of Animal Physiology, Agricultural University, Wageningen, The Netherlands

substituted for calcium ion, and that bromine may be used instead of iodine in some instances[2]. More-recent research has shown that a primary requirement for the formation of a blue product is the presence of a sequence of at least three (1→4)-linked D-glucose, D-xylose, or D-mannose residues in the polysaccharide[3] The role of the various components involved in the formation of the blue color is complicated, and explanations based on these qualitative observations have been offered by Gaillard and Bailey[3].

Other polysaccharides, such as amylose and various naturally occurring "amyloids", react in aqueous solution with iodine–potassium iodide to give blue to black complexes[4,5] Bates and coworkers[6] showed that iodine was bound in the amylose complex within a helical structure of D-glucose residues, and that the amount of bound iodine could be determined by potentiometric titration On the other hand, very little is known about the "amyloid" complexes except they may be differentiated from the amylose complexes by qualitative tests[7]. Colored complexes known to exist between iodine and other organic polymers have been investigated by many researchers Tebelev and coworkers[8], for example, found spectrophotometrically that the iodine was loosely sorbed or bound in some undefined manner onto the poly(vinyl alcohol) molecule.

It appears, therefore, that iodine may react with a great variety of organic substances to yield colored complexes whose characteristics in many instances have not been precisely defined The purpose of the present research was to investigate the nature of the interaction of polysaccharides with calcium chloride and iodine in aqueous solution This report will compare the reaction products of various hemicellulose-like polymers with those of amylose, while subsequent reports will describe in greater quantitative detail the effect of different functional groups and degrees of branching of specific polysaccharides on their ability to form complexes.

RESULTS AND DISCUSSION

Introductory experiments — A commercially available xylan (Pfanstiehl Laboratories) was chosen as a representative D-xylan having a moderate degree of branching Although the source and the structure of this xylan are not known, it was assumed that it was typical of naturally occurring xylans from monocotyledons and that it consisted of a D-xylan backbone to which were attached single terminal branches of L-arabinose and 4-*O*-methyl-D-glucuronic acid residues Subsequent research (to be described in another publication of this series) with xylans of accurately known structures, compositions, and sources similar to those speculated for this commercial xylan, showed similar results in reactions with iodine A D-galactose-deficient D-galacto-D-gluco-D-mannan isolated from Engelmann spruce holocellulose[9] was chosen as a model for slightly branched hexoglycans, while an "amyloid" isolated from tamarind seed[7], and D-galactose-rich D-galacto-D-gluco-D-mannans isolated from black spruce and Parana pine were additional examples of highly branched hexoglycans[10]. Examples of linear polysaccharides employed in this

investigation were a hydrocellulose and a commercial amylose (Superlose, Stein Hall, Inc.)

Most of these polysaccharides, in concentrated aqueous calcium chloride solution (specific gravity 1 3), reacted with iodine to yield a blue product In agreement with the literature, the highly branched D-galactose-rich galactoglucomannan from black spruce and Parana pine did not react, whereas the equally highly branched "amyloid" did react to give a blue color These results, summarized in Table I, show that only amylose and "amyloid" react with iodine in aqueous solutions free from calcium chloride, and the intensity of the blue color was increased when sodium sulfate was added. The addition of calcium chloride to these aqueous solutions brought about no change in the intensity of the amylose–iodine complex but did result in a marked deepening of color when added to the iodine–"amyloid" complex Other qualitative observations showed that the addition of a drop of stock iodine solution to amylose solutions caused a blue color to appear immediately, whereas larger quantities of stock iodine had to be added to all other polysaccharides studied here before the blue color appeared When the blue complexes of the latter were diluted with water, or heated to temperatures below 100°, the color disappeared and a yellowish solution characteristic of aqueous iodine–potassium iodide appeared The decomposed iodine–"amyloid" complex reappeared when the diluted solution was cooled to 0° or the heated solution was cooled to room temperature

TABLE I

QUALITATIVE COMPOSITION OF CERTAIN POLYSACCHARIDE–CALCIUM CHLORIDE–IODINE COMPLEXES

Complexes	*Reacts in aqueous solution*	*Reacts in concentrated* $CaCl_2$ *solution*	*Composition of complex* Ca^{2+}	I	*Forms methanol-soluble calcium complex*[a]
Hydrocellulose	−	+	+	+	−
Commercial amylose (Superlose)	+	+	+	+	−
"Amyloid"	+	+	[b]	+	+
Galactose-deficient galactoglucomannan	−	+	+	+	−
Galactose-rich galactoglucomannan	−	−	−	−	−
Commercial xylan	−	+	+	+	−
Cherry gum	−	−	−	−	+

[a]The polysaccharides in concentrated aqueous calcium chloride indicated by (+) are not precipitated or incompletely precipitated (~20%) by the addition of three volumes of methanol saturated with calcium chloride [b]Not analyzed

At higher polysaccharide and iodine concentrations than those employed for spectrophotometric determinations, the iodine complexes became insoluble and could be separated from the soluble components by centrifugation. Storage of the isolated complex from xylan, hydrocellulose, and amylose, in the air for six weeks,

brought about no apparent change in the intense dark color Prolonged storage of the galactoglucomannan complex resulted in a gradual fading of the color of the complex, and the "amyloid" complex decomposed within hours of isolation After washing the complexes with aqueous calcium chloride to remove excess iodine, they were freed of excess calcium chloride by spreading them on a piece of unglazed porcelain in a desiccator Analysis of the blue-black, greasy products by X-ray diffraction techniques showed no crystalline calcium chloride to be present, although an X-ray powder pattern typical of $CaCl_2\ 2H_2O$ could be obtained from the dry complex that had not been purified on the porcelain plate Since the blue color could be removed by reducing agents capable of reducing iodine (with a destruction of the complex), and since qualitative tests for iodine[11] could be obtained from the purified complexes, iodine is assumed to be present in the complex Since calcium ion (but no crystalline calcium chloride) was found by flame spectrophotometry to be in excess of that required by the carboxyl contents of the polymers, the possibility exists that calcium ion may contribute to the stability of the isolated complex Quantitative experiments describing the relationships of these components to precisely defined polysaccharides will be the subject of later publications[12]

When the blue precipitates were washed with pure methanol, the complexes of all polysaccharides were destroyed, the iodine was liberated, and the polysaccharides themselves were isolated as insoluble residues Quantitative sugar analysis showed no significant differences in composition between the polysaccharides investigated here and these insoluble residues If methanol saturated with calcium chloride was employed, the polysaccharide–iodine complexes were destroyed and alcohol-insoluble fractions were isolated from the amylose, xylan, and galactoglucomannan Although the "amyloid"–iodine complex was also destroyed by this treatment, the "amyloid" component itself was found to be soluble in the presence of methanol saturated with calcium chloride under these circumstances Besides "amyloid", additional tests showed that cherry gum and black spruce glucuronoarabinogalactan (both highly branched polysaccharides unable to form a blue iodine complex in aqueous $CaCl_2$ solution) cannot be completely precipitated from an aqueous solution containing calcium chloride with methanol saturated with calcium chloride, although all three may be precipitated from these solutions with pure methanol

These results suggest that many polysaccharides can form complexes with iodine in concentrated aqueous calcium chloride solution and that the complexes may be destroyed by adding methanol Although certain polysaccharides may react with aqueous calcium chloride in such a way that they cannot be precipitated from solution by the addition of methanol saturated with calcium chloride, this characteristic did not ensure the formation of a blue complex when iodine was added to the aqueous solution Conversely, the limited data obtained here suggest, but do not prove, that calcium ion is associated with the dark blue complexes when they are formed from concentrated aqueous calcium chloride

Potentiometric determinations — The potentiometric titrations employed in this investigation differed in some respects from those described in the literature

for the titration of starch[13] with iodine solutions. Not only were the titrations carried out in the presence of calcium chloride (although Colburn and Schoch have demonstrated that this can be done in starch titrations[14]), but greater iodine concentrations had to be used to ensure reaction of iodine with the polysaccharides In agreement with the researches of Adkins and Greenwood[15], more satisfactory results were obtained when titrations were conducted at the temperature of an ice bath By plotting the amounts of bound iodine against free iodine, as described by Anderson and Greenwood[13], for example, it was found that the xylan bound about 9 mg of iodine per 100 mg of polymer, which is about half the amount normally found for undegraded amylose under slightly different conditions of titration. The galactoglucomannan and the tamarind "amyloid" did not bind iodine in a manner that could be detected by potentiometric titration. These results, which are similar to those of the poly(vinyl alcohol)–iodine system, suggest that the latter two polysaccharides may react with iodine but that the iodine is not firmly held in these complexes This observation is supported by the fact that the isolated complexes of these two polymers gradually lose iodine on storage The firmly bonded starch–iodine, xylan–iodine, or xylan–bromine complexes may be stored for long periods of time without showing signs of decomposition

Spectrophotometric determinations — The development of the blue color with time and temperature after the addition of iodine was followed in dilute solutions (0 004% of polymer and 0 04% of iodine) in order to avoid the complications caused by the formation of an insoluble component The results shown in Fig 1 demonstrate that the xylan under these conditions gives a maximum extinction within 90 min, provided that the temperature of the solution was cooled in an ice bath before the addition of the iodine–potassium iodide solution The increase of the extinction coefficient for the reaction mixture at 30° was more gradual and never reached the same magnitude as that of the cooled solution The galactoglucomannan (Fig 1) was much less sensitive to the differences in initial temperature but it required a higher concentration (0 04%) of polymer to develop a measurable, but less intense color The reaction of the "amyloid" with iodine under these circumstances is more complex and is shown in Fig 2 The extinction increased very slowly when the reaction was carried out at 30° and was still increasing after reaction for 7 h. The addition of sodium sulfate to the "amyloid" resulted in a much faster initial rate of reaction at 30°, but the maximum value was no higher than that at 30° in calcium chloride solution When the reaction mixture was cooled in an ice bath, a maximum development of color was achieved in 90 min, which was greater than the maximum after 7 h at 30° The results obtained here may be rationalized by assuming that a complex relationship exists between the components of the system These components may include free polymer, possible calcium complexes of the polymer, chloride ion, calcium ion, solubility characteristics of the complexes, temperature, and time of reaction In all likelihood, iodide ion is necessary for the formation of these complexes in a manner analogous to their influence on the formation of starch–iodine complexes[16].

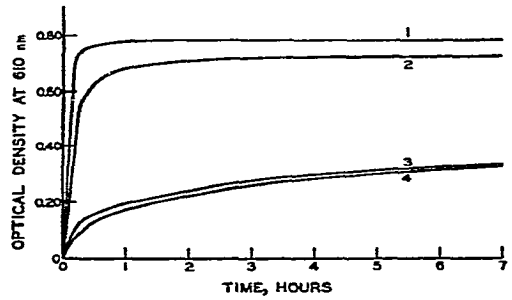


Fig 1 The variation with time of the optical density at 610 nm of the colored complex formed by the reaction of polysaccharide in concentrated aqueous calcium chloride solution (specific gravity 1.3) with 0 04% I_2 and 0 16% KI (*1*) Commercial xylan (0 004%) at 4°, (*2*) Commercial xylan (0 004%) at 30°, (*3*) Coniferous galactoglucomannan (0 04%) at 4°, (*4*) Coniferous galactoglucomannan (0 04%) at 30°.

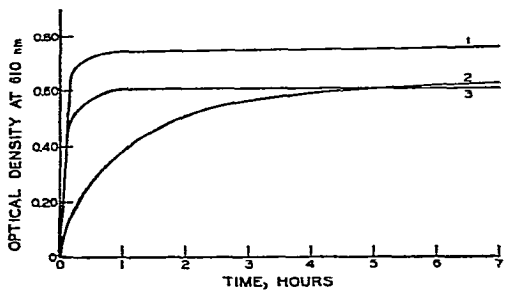


Fig 2 The variation with time of the optical density at 610 nm of the colored complex formed by the reaction of tamarind "amyloid" with I_2 under different conditions (*1*) 0 004% "amyloid", 0 04% I_2 and 0 16% KI in aqueous calcium chloride (specific gravity 1 3) at 4°, (*2*) 0 004% "amyloid", 0 04% I_2 and 0 16% KI in aqueous calcium chloride (specific gravity 1 3) at 30°, (*3*) 0 004% "amyloid" with 0 04% I_2 and 0 16% KI in 15% Na_2SO_4

The plots in Fig 3 demonstrate that the blue colors developed in solutions of increasing polysaccharide content at constant iodine content is a straight line and could be used for quantitative estimation of the hemicellulose content of the solutions. The plots for amylose, xylan, and "amyloid" are only slightly different, whereas the smaller slope of the galactoglucomannan curve is indicative of the less intense color of the galactoglucomannan–iodine complex

If the iodine in a polysaccharide–iodine complex is firmly bound in that complex, the composition may be determined by plotting the relative percentage of iodine in the reaction (at constant total composition) against the extinction coefficient of the solution for the particular wavelength of light that is absorbed by the complex. The content of iodine that indicates the greatest amount of absorption is a measure of the iodine found in the complex, and if for different reactant ratios of iodine to polysaccharide the ratio of iodine in the complex is always the same, it can be concluded that the complex has a constant ratio of components This has been found to be the case for the iodine–amylose complex[6], and the results shown

in Table II show that it is likely to be the case for the iodine complex of amylose formed in the presence of aqueous calcium chloride. The data given in Table II

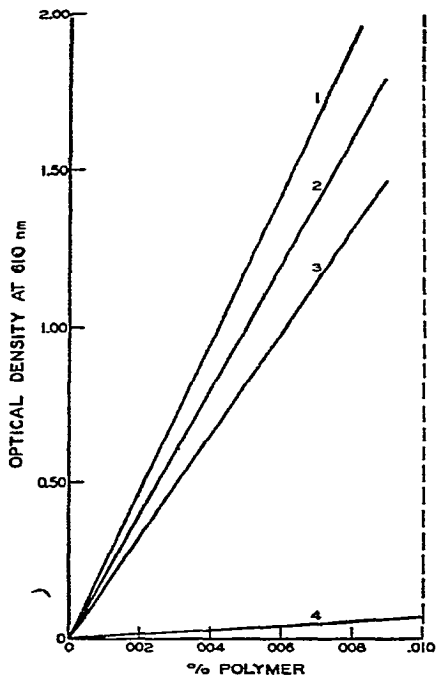


Fig 3 The variation of optical density at 610 nm of colored complexes with increasing concentration of polysaccharide in concentrated aqueous $CaCl_2$ solution (specific gravity 1 3) and 0 05% I_2 (*1*) Commercial amylose, (*2*) Commercial xylan, (*3*) Tamarind "amyloid", (*4*) Coniferous galactoglucomannan

show that the iodine complexes of the other polysaccharides studied here did not react in this manner but reacted in a manner suggesting that the iodine content of their complexes was a function of the concentration of the reactants in solution. The plots from which the data for the ratio of xylan to iodine at different total xylan-plus-iodine concentrations were obtained are shown in Fig 4 and are typical of the plots used to obtain data for the other polysaccharides listed in the table.

These results confirm the observations in the literature that certain linear polysaccharides will react with iodine in concentrated aqueous calcium chloride solution to give a blue complex, although they will not do so in a purely aqueous solution The present research also demonstrates that the branched polysaccharide "amyloid" is unlike other branched polysaccharides and is able to form a complex under the two conditions of reaction described above. At the present time, it is assumed that the blue color of these iodine complexes is due to the ability of the polysaccharide, in the divalent salt solution, to trap a linear array of iodine atoms in a manner analogous to the behavior of starch and poly(vinyl alcohol) It remains to be proven whether the polysaccharides achieve this by forming a helical structure

TABLE II

THE COMPOSITION OF POLYSACCHARIDE AND IODINE AT WHICH MAXIMUM EXTINCTION COEFFICIENT OCCURS AT DIFFERENT TOTAL COMPOSITIONS OF POLYSACCHARIDE AND IODINE

Percentage (polymer and iodine) of solution	*Relative percentage of iodine at which maximum extinction occurs*				
	4-O-methyl-glucurono-arabinoxylan	*Galacto-glucomannan*	*Amylose*	*"Amyloid" (in $CaCl_2$)*	*"Amyloid" (in Na_2SO_4)*
0 01	60	50	20		80
0 02		40	20	95	50
0 03	50			70	40

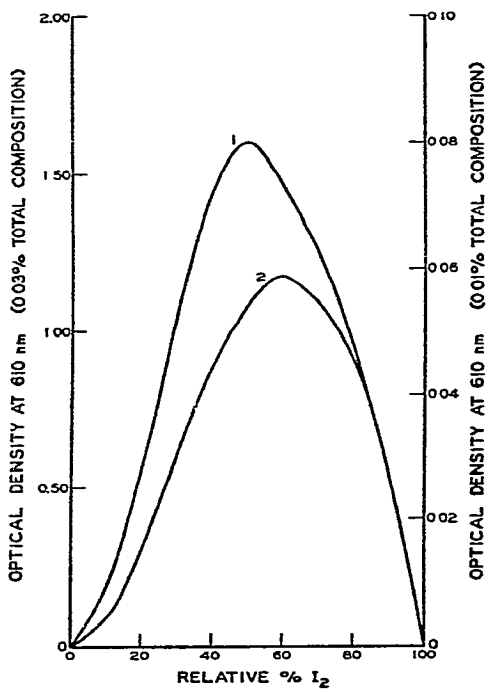


Fig 4 The variation of optical density at 610 nm of the colored complex of commercial xylan with relative percent of I_2 at constant total (xylan $+I_2$) composition (*1*) Concentration of xylan $+I_2$, 0 03%, (*2*) Concentration of xylan $+I_2$, 0 01%.

similar to starch, as was suggested by Gaillard and Bailey[3], or whether some other linear arrangement is responsible A complex, dipole interaction can be conceived to exist between the hydroxyl groups, iodine, iodide ion, and possibly even calcium ion if the β-D-(1→4)-linked polysaccharides are arranged in a helical configuration. Although it is difficult to imagine an exactly similar mechanism between parallel sections of polysaccharide, it is possible that complexing between divalent ions and hydroxyl groups of several molecules may cause the formation of a tubular structure

capable of containing strings of iodine atoms The weak chemical stability of these complexes is consistent with either conjecture

EXPERIMENTAL

Preparation of samples — The amylose used was commercial material (Superlose, Stein Hall Inc.) prepared from potato starch A commercial xylan (Pfanstiehl Laboratories) was also investigated The source of this xylan was not known and quantitative analysis showed it to contain 73.6% xylose, 12 2% arabinose, 3 9% uronic acid, 0 2% rhamnose, 0 3% mannose, 2 9% galactose, 2 4% glucose, and 4.5% of an acid-insoluble, lignin-like material.

The "amyloid" used was extracted from tamarind seed with hot water by the technique described by Rao[7] Quantitative analysis after hydrolysis indicated 16.9% galactose, 47 6% glucose, 1 3% arabinose, and 34 1% xylose The D-galactose-deficient galactoglucomannan was isolated from Engelmann spruce holocellulose for use in another study[9] and quantitative analysis showed it to be composed of 7 4% galactose, 21 1% glucose, 64% mannose, 3 5% xylose, and 4% arabinose The galactose-rich galactoglucomannans were isolated from black spruce and Parana pine holocelluloses by conventional techniques[10] and had compositions approximated by 20% galactose, 20% glucose, and 60% mannose The hydrocellulose used was prepared by dissolving a suitable cellulose preparation, such as Whatman filter paper or Solka Floc (a purified, commercial, wood cellulose), in 85% phosphoric acid and recovering the hydrolyzed cellulose according to the procedure outlined by Smith and coworkers[17] That fraction of the hydrocellulose which dissolved in stock calcium chloride solution was used

Reagents and analytical procedures — The calcium chloride solutions used were prepared by dissolving 412 1 g of analytical-grade reagent in 1 liter of water to give a specific gravity of 1 3. The stock iodine solutions were prepared by dissolving 2 000 g of iodine and 8 3 g of potassium iodide in 100 ml of water, and dilutions were made to required concentrations with stock calcium chloride solution The stock solutions of polysaccharide were prepared by dissolving 100 to 500 mg of polymer in 5 ml of M NaOH, neutralizing with HCl, and adding sufficient stock calcium chloride solution to make 100 ml. The solutions were slightly viscous, but remained clear for months after the completion of this study without evidence of precipitation or bacterial decomposition.

The hydrolysis and the quantitative analysis of the polysaccharide were accomplished by the chromatographic method of Saeman *et al*[18], and the uronic acid contents of polymers were estimated by the technique of Whistler, Martin, and Harris[19]

The presence or absence of crystalline material in the polysaccharide–iodine complexes was determined by conventional powder techniques

Samples for calcium analysis were mixed with 50 mg of Li_2CO_3 and with 180 mg of graphite containing a known amount of tin as internal standard. A portion of this mixture was arced by using an a c power source, and the spectrum was

recorded with a Bausch and Lomb 1.5-meter grating spectrograph The line intensities of the elements were measured on a Jarrel Ash microphotometer and the concentrations of elements were derived from the line intensities

Potentiometric titration of bound iodine. — A stock solution containing 40 to 50 mg of polysaccharide was added to a solution composed of 4.15 g of potassium iodide in 5 ml of water The solution was made up to 50 ml with stock calcium chloride solution A blank solution was prepared by dissolving 4 15 g of potassium iodide in 5 ml water and diluting to 50 ml with stock calcium chloride solution The two solutions were stored in a refrigerator until used. Titrations were conducted in an ice bath with undiluted stock iodine solution, with a Leeds and Northrup Student type of potentiometer in combination with a sensitive galvanometer and bright platinum and normal calomel electrodes.

Spectrophotometric determinations — All extinctions were measured in a Beckman DU spectrophotometer at 610 nm with conventional 1-cm cells The increase in extinction with time was followed by using polysaccharide solutions containing 5 mg of polymer in 10 ml of standard calcium chloride solution at 4° and at 30°. After storage overnight, the stock iodine solution, was diluted with a tenfold excess of stock calcium chloride solution An aliquot (2 5 ml) of this solution at the required temperature was added to the polymer solution, and the extinctions were measured in the spectrophotometer at 15, 30, and 60-min intervals

The effect of sodium sulfate on the increase in extinction coefficient of the aqueous solution of "amyloid" and iodine with time was followed by measuring the changes caused by adding 5 ml of sodium sulfate solution (20 g/100 ml) to a solution prepared by mixing 0 5 ml of iodine solution (3 ml of stock iodine solution diluted with water to 10 ml) with 1 ml of solution containing 0 26% of polymer

The comparison of the intensities of colors developed at low iodine concentrations was accomplished by preparing a series of solutions in which the polymer concentration ranged from 0 001 to 0 010% The solvents were prepared by diluting 2 5 ml of cooled (4°) stock iodine solution with 25 ml of cooled (4°) stock calcium chloride solution, and the extinctions of the complexes were measured at periodic intervals until they had reached their maximum values

Stock solutions of combined polysaccharide and iodine were diluted with stock calcium chloride solution to give combined concentrations of polymer and iodine ranging from 0 01 to 0 04% (the concentration employed depending upon the intensities of the colors produced by the polysaccharide) The percentage of iodine relative to the combined weight of polymer and iodine ranged from 10 to 90%. The development of color was measured in the Beckman DU at the point of maximum development with time

ACKNOWLEDGMENTS

The authors acknowledge the assistance provided by Miss M. Hollenback for sugar analysis, L G. Borchardt for calcium analysis, and Dr G Richards for X-ray powder diffraction patterns

REFERENCES

1 B D E GAILLARD, *Nature*, 191 (1961) 1295
2 A. J MORAK AND N S THOMPSON, *Nature*, 205 (1965) 69
3 B D E GAILLARD AND R W. BAILEY, *Nature*, 212 (1966) 202
4 T H VOGEL AND M J SCHLEIDEN, *Pogg Ann Phys Chem*, 45 (1839) 327
5 P KOOIMAN, *Nature*, 179 (1957) 107
6 F L BATES, D FRENCH, AND R E RUNDLE *J Amer Chem Soc*, 65 (1943) 142
7 P S RAO, in R L WHISTLER (Ed), *Industrial Gums*, Academic Press, New York, 1959, "Tamarind", Chap 19
8 L G. TEBELEV, G F NIKUL'SKII, E P KORCHAGINA, AND S A GLIKMAN, *Vysokomol Soed*, 7 (1965) 123
9 J K ROGERS AND N S THOMPSON, *Carbohyd Res*, 7 (1968) 66, *Svensk Papperstid*, 72 (1969) 61
10 N S THOMPSON AND O A KAUSTINEN, *Paperi ja Puu*, 47 (1964) 637
11 C L WILSON AND D W WILSON, *Comprehensive Analytical Chemistry 1A*, Elsevier, New York 1959
12 B D E GAILLARD, N S THOMPSON, AND A J MORAK, to be published (1969)
13 D M W ANDERSON AND C T GREENWOOD, *J Chem Soc*, (1955) 3016
14 C R COLBURN AND T J SCHOCH, *Methods Carbohyd Chem*, 4 (1964) 161
15 G K ADKINS AND C T GREENWOOD, *Carbohyd Res*, 3 (1966) 152
16 J A THOMA AND D FRENCH, *J Amer Chem Soc*, 80 (1958) 6142, 82 (1960) 4144
17 J D GEERDES, B A LEWIS, R MONTGOMERY, AND F SMITH, *Anal Chem*, 26 (1954) 264
18 J F SAEMAN, W E MOORE, R L MITCHELL, AND M A MILLETT, *Tappi*, 37 (1954) 336
19 R L WHISTLER, A R MARTIN, AND M HARRIS, *J Res Nat Bur Stand*, 24 (1940) 13

Carbohydrate Research
Elsevier Publishing Company, Amsterdam
Printed in Belgium

RELATIVE STABILITY OF THE INTERGLYCOSIDE BONDS OF SUCROSE TO ACID HYDROLYSIS IN $H_2{}^{18}O$

FRANK EISENBERG, JR.

National Institute of Arthritis and Metabolic Diseases, National Institutes of Health, Public Health Service, United States Department of Health, Education, and Welfare, Bethesda, Maryland 20014 (U S A)

(Received February 19th, 1969; in revised form, June 6th, 1969)

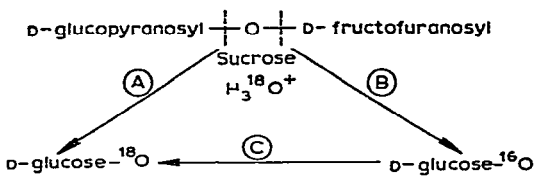
ABSTRACT

Sucrose, a disaccharide with two interglycoside bonds, was hydrolyzed with acid in $H_2{}^{18}O$ Through a kinetic study of the labeling of D-glucose with ^{18}O, an estimate was made of the relative stabilities of the two bonds It was found in agreement with the known greater lability of fructofuranosides compared to glucopyranosides that for every glucopyranosyl–oxygen bond split three fructofuranosyl–oxygen bonds were hydrolyzed.

INTRODUCTION

Glycosides, in general, undergo acid hydrolysis at the glycosyl–oxygen bond[1]. Sucrose is an unusual disaccharide in that both constituent sugars are linked through their respective anomeric-carbon atoms, a circumstance which raises the question of the relative susceptibilities of the two glycosyl–oxygen bonds to acid hydrolysis. Studies on the formation of dextran and levan by the transferases dextransucrase and levansucrase have shown that sucrose can donate either D-glucosyl or D-fructosyl residues depending on the enzyme involved[2] From structural considerations, however, the D-fructofuranosyl–oxygen linkage should be the more labile and kinetic studies on acid hydrolysis of sucrose suggest that this is the weaker bond[3] In a more direct approach to this problem, described in this report, hydrolysis in $H_2{}^{18}O$ was examined, the extent of incorporation of ^{18}O into D-glucose serving as a measure of glucosyl cleavage

Complicating this simple approach is the fact that incorporation of ^{18}O into D-glucose can also occur by D-fructosyl cleavage The time required, however, for D-glucose to acquire label from the medium is a basis for differentiating these two processes which are outlined in the following diagram; D-fructose has been omitted, since it was not isolated for ^{18}O-measurement in these studies



D-Glucose produced by reaction A is instantaneously labeled at the equilibrium water abundance of ^{18}O, while glucose arising by reaction B is labeled only after sufficient time has elapsed for exchange with the solvent to occur by reaction C. Since the observed ^{18}O abundance in D-glucose took finite time to reach a maximum, exclusive D-glucosyl cleavage was eliminated as the hydrolytic mechanism. Which mode of hydrolysis predominated was determined by a kinetic study of the overall hydrolytic reaction ($A+B$) and the exchange reaction (C). The results show that sucrose is hydrolyzed in acid in the ratio of three D-fructosyl–oxygen bonds split for every D-glucosyl–oxygen.

MATHEMATICAL DERIVATION

An equation governing the rate of incorporation of ^{18}O into D-glucose as a result of consecutive reactions B and C can be derived in terms of the initial sucrose concentration S, the overall hydrolytic rate constant H, the hydrolytic rate constant F of the D-fructosyl residue, and the exchange rate constant E, as follows. Let $s = [\text{Sucrose}]_t$, $G = [\text{Glucose-}^{16}O]_t$, $G^* = [\text{Glucose-}^{18}O]_t$, $M_0 =$ mole % Glucose-^{18}O at zero time, $M =$ mole % Glucose-^{18}O at time $t = 100G^*/(G+G^*)$, and $f = F/H =$ fraction of sucrose molecules hydrolyzed at the fructosyl–oxygen, then the first-order equation for the overall hydrolytic reaction is

$$s = Se^{-Ht} \tag{1}$$

The quantity of glucose-^{16}O at time t is the resultant of its formation by reaction B and its elimination by reaction C. If these reactions are both of first order and of nearly equal velocity it can be shown[4] that

$$G = \frac{SF}{E-F}(e^{-Ft}-e^{-Et}) \tag{2}$$

At any time t: $S = s+G+G^*$ (3)

then from (1), (2), and (3)

$$G^* = S-s-G = S-Se^{-Ht}-\frac{SF}{E-F}(e^{-Ft}-e^{-Et}) \tag{4}$$

from (1) and (3)

$$G+G^* = S-Se^{-Ht} \tag{5}$$

and from (4) and (5)

$$M = \frac{100\left[S-Se^{-Ht}-\dfrac{SF}{(E-F)}(e^{-Ft}-e^{-Et})\right]}{S-Se^{-Ht}}$$

$$M = 100[1-\frac{F(e^{-Ft}-e^{-Et})}{(1-e^{-Ht})(E-F)}] \quad (6)$$

$$= 100[1-\frac{Fe^{-Ft}(1-e^{-(E-F)t})}{(1-e^{-Ht})(E-F)}] \quad (6a)$$

Equation (6a) can be simplified by application of the e^x power series where terms of higher power than x can be made negligibly small and

$$\lim_{x\to 0} e^x = 1+x$$

thus

$$\lim_{t\to 0} e^{-(E-F)t} = 1-(E-F)t \text{ and } \lim_{t\to 0} e^{-Ht} = 1-Ht \quad (7)$$

then

$$1-e^{-(E-F)t} = (E-F)t \text{ and } 1-e^{-Ht} = Ht \quad (8)$$

and from (6a) and (8)

$$M = 100[1-\frac{Fe^{-Ft}t(E-F)}{Ht(E-F)}] = 100(1-\frac{Fe^{-Ft}}{H}) \quad (9)$$

Since the rate of the overall hydrolytic reaction is the sum of the rates of the D-fructosyl and D-glucosyl cleavage reactions, F/H represents the sought fraction, f, of sucrose hydrolyzed at the fructosyl–oxygen bond

Then

$$M = 100(1-fe^{-Ft}) \text{ and } M_0 = 100(1-f) \quad (10), (11)$$

Alternatively, equation (11) can be derived from equation (6) by differentiation of numerator and denominator with respect to t and final substitution of $t = 0$

Equation (10), containing a transcendental function for which there is no general mathematical solution, can be solved for f graphically by assigning to F arbitrary values from 0 ($f = 0$) to H ($f = 1$) Various values of M can thus be computed by equation (9) over a range of time to give a family of curves showing the expected incorporation of ^{18}O into D-glucose for any fraction, f, of sucrose split by D-fructosyl cleavage Comparison of the curve of observed values with that of the calculated values permits a selection of the actual value of f.

EXPERIMENTAL

Hydrolytic and exchange reactions. — Three measurements were made in this study, all at 100°, except as noted in the legend to Fig 7 (*1*) the rate of hydrolysis of sucrose, (*2*) the rate of uptake of ^{18}O by D-glucose during hydrolysis; and (*3*) the rate of exchange of D-glucose with $H_2{}^{18}O$. All experiments were performed with the apparatus shown in Fig 1 In view of the short time-intervals used in these studies,

it was essential that time be measured from an exact zero point To achieve this, one of the solid components of the reaction mixture was placed at the top of the filter funnel in a hollow cone made from a thin sheet of Wood's metal, steam admitted to the funnel jacket throughout the course of the reaction maintained the temperature of the reaction mixture at 100° and melted the Wood's metal, thus dropping the required solid component into the liquid and providing an accurate zero-time point

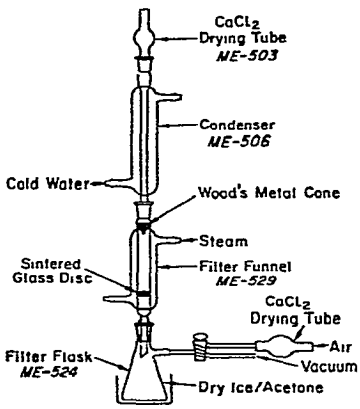


Fig 1 Experimental apparatus Numbers refer to the catalog of Metro Scientific, Inc , 2121 Broad Hollow Road, Farmingdale, N Y

The reaction mixture was held in the funnel and agitated by an air stream directed from below. In experiments (*1*) and (*2*), dry resin IR-120 (H^+)[5] was placed in the cone, and an equal weight of sucrose with enough water or $H_2{}^{18}O$, resp , to make a 20% solution in the funnel In experiment (*3*), equal weights of D-glucose and D-fructose were placed in the cone, and twice the weight of resin and enough $H_2{}^{18}O$ to make a 20% combined sugar solution in the funnel After a given time, the air flow was reversed by vacuum, quickly draining the entire liquid contents, free of resin, into the flask below. Samples from experiment (*1*) were transferred to polarimeter tubes preparatory to measurement of the optical rotation in a Rudolph polarimeter In experiments (*2*) and (*3*), the receiving flask was chilled in dry ice-acetone, instantaneously freezing the filtrates, which were then lyophilized to dryness Water sublimates and residues from lyophilization were prepared for ^{18}O assay as described in the next two paragraphs

Conversion of water into benzoic acid — Samples of the thawed sublimates were diluted with water to about 0 5 atom-%-excess of ^{18}O and were shaken at room temperature with about 50 μl of benzoyl chloride until crystals of benzoic acid appeared. The suspensions were chilled, and the crystals were collected by filtration, washed with cold water, and dried *in vacuo.*

Isolation of D-*glucose.* — The residues of lyophilization were further dried by evaporation after addition of pyridine, and were converted into β-D-glucose by heating at reflux for 5 min in pyridine solution[6] After concentration and chilling of the solution, crystallization was initiated by the addition of a minute seed-crystal of

β-D-glucose The crystals were freed of adhering syrup by absorption on a chilled, porous clay-plate, from which they were then scraped and washed by centrifugation with cold pyridine When the samples of D-glucose were obtained from sucrose at short time-intervals, and therefore were heavily contaminated with sucrose, it was necessary to wash the precipitates several times with cold petroleum-ether in order to produce a dry powder. The product was recrystallized from pyridine

Determination of ^{18}O. — Samples of benzoic acid (10 mg) and D-glucose (5 mg) were analyzed for ^{18}O as $CO^{18}O$ by the method of Rittenberg and Ponticorvo[7] with a mass spectrometer (Consolidated Engineering Model 21-401) The atom percentage of ^{18}O was calculated from the formula $100R_2(1+R_1)/[2+R_2(1+R_1)]$ where R_1 and R_2 are the ratios of masses 45/44 and 46/(45+44), resp The atom-%-excess of ^{18}O was computed by subtraction of the normal abundance of ^{18}O determined on tank carbon dioxide.

Qualitative test of purity of D-*glucose-*^{18}O — D-Glucose samples from both hydrolysis and exchange were acetylated and tested for the presence of contaminating sugar acetates by t l c. on 125-μ layers of silica gel G developed in 3.97 methanol–benzene The sugar (2 mg) was treated for 1 h in a solution of pyridine (50 μl) and acetic anhydride (50 μl), aliquots (3 μl, 60 μg) were spotted on t l c. plates which, after development and drying, were sprayed with 10% conc. sulfuric acid in methanol and heated for 5 min at 150° to visualize the sugars.

Quantitative estimate of purity of D-*glucose-*^{18}O — By visualization of the acetates alternatively as ferric hydroxamates, an estimate was made of the extent of contamination of D-glucose-^{18}O by sucrose, permitting correction of the observed ^{18}O abundance As standards, samples of sucrose octaacetate (0 03 to 0 09 μmole) and of D-glucose pentaacetate, (0 15 to 0 5 μmole) were chromatographed as described above and sprayed with the alkaline hydroxylamine–acid ferric nitrate reagent

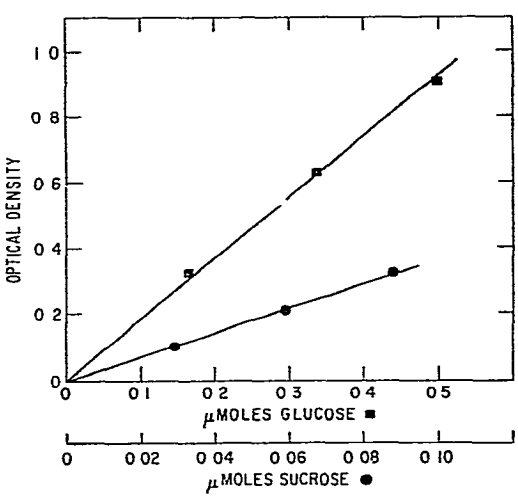


Fig 2 Standard curves for determination of D-glucose pentaacetate and sucrose octaacetate as ferric hydroxamates scraped from thin-layer silica gel plates

described by Tate and Bishop[8], modified by replacement of water by methanol. The deep purple spots appearing on a yellow background were scraped from the plate and eluted with *aqueous* ferric nitrate reagent[8] (1 ml), and the suspension was centrifuged to remove the silica gel The optical density of the supernatants, determined at 540 nm, showed a linear relationship with the sugar acetate concentration (Fig 2)

RESULTS

Purity of glucose-^{18}O. — Qualitative t l c of the acetates showed the absence of D-fructose and sucrose from all samples except from one of the 0 5-min hydrolytic samples, which was found to be contaminated with sucrose (Fig 3, Row 8) By quantitative t l c , it was estimated that this sample contained only 71 8% of D-glucose Since the isotope abundance observed for this D-glucose was 0 738 atom-%-excess of ^{18}O and 2 71% for the solvent, the enrichment relative to the solvent ^{18}O, corrected for sucrose contamination, was $(0\ 738 \times 100)/(2\ 71 \times 0\ 718) = 37\ 9\%$ (Fig 6, 30 sec, ■)

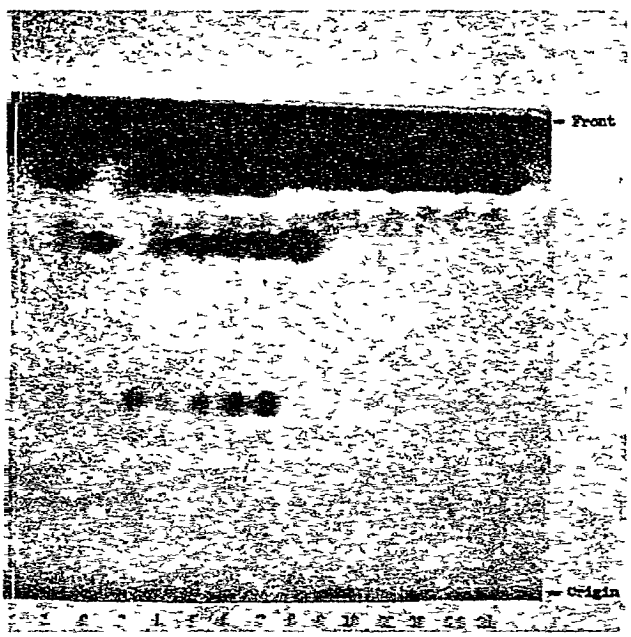


Fig 3 Qualitative t l c of D-glucose pentaacetate for detection of sugar contaminants (1) D-glucose, 54 μg, (2) sucrose, 3 μg, (3) D-fructose, 3 μg, (4–7) mixtures of D-glucose, sucrose, and D-fructose in ratio of 95 2 5 3 5, 90 5 5, 85 7 5 7 5, and 80 10 10, respectively, (8–10) D-glucose-^{18}O from 0 5, 1, and 2-min hydrolyzate, (11–14) D-glucose-^{18}O from 0 5, 1, 2, and 4-min exchange reaction Ascending chromatography on silica gel G (125-μ layer) in 3 97 methanol–benzene, sprayed with 10% methanolic sulfuric acid, and heated for 5 min at 150° Only one of two sets of samples obtained is shown in (8–14), all samples in the other set were free of contaminants Driprint[9] reproduction of the original chromatogram

Determination of rate constants, E *and* H — To establish the applicability of equation (2), and therefore of equation (10), it was necessary to show that reactions

B and *C* are of first order and like speed But reaction *B* obviously cannot be isolated for separate study On the assumption that the mode of hydrolysis is primarily that of the D-fructosyl residue, the rates and orders of reactions *C* (exchange) and *A*+*B* (overall hydrolysis) were compared instead.

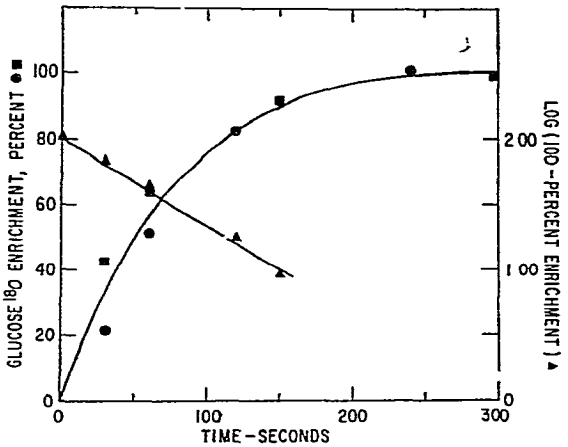


Fig 4 Left ordinate kinetics of exchange of glucose with $H_2{}^{18}O$ Right ordinate log plot test for first order The symbols indicate two sets of observations

In Fig 4 (left ordinate), the percent enrichment of ^{18}O of D-glucose relative to the solvent is plotted against time If the percent enrichment is a measure of G^*, then (100 − % enrichment) is a measure of *G* at any time, t For first-order kinetics, log (100 − % enrichment) must be a linear function of time [equation (12)] As seen from Fig 4 (right ordinate), this requirement is satisfied By substitution of the graph value for the log term at 150 sec, *E* is calculated from the equation

$$E = \frac{2\,303}{t} \log \frac{100}{(100 - \%\ \text{enrichment})} = \frac{2\,303}{150}(2\,00 - 1\,00) = 0\,0154\ \text{sec}^{-1} \qquad (12)$$

Similarly, in the inversion of sucrose (Fig 5, left ordinate) for first-order kinetics, $\log(\alpha_t - \alpha_\infty)$ must vary linearly with time [equation (13)] As shown in Fig 5, right ordinate, this condition is met By substitution of the graph value for the log term at 300 sec, *H* is calculated from the equation

$$H = \frac{2\,303}{t} \log \frac{(\alpha_0 - \alpha_\infty)}{(\alpha_t - \alpha_\infty)} = \frac{2\,303}{300} \log \frac{31\,6}{1\,59} = \frac{2\,303}{300}(1\,500 - 0\,200) = 0\,0100\ \text{sec}^{-1} \qquad (13)$$

Thus equations (2) and (10) apply, since reactions *B* (by inference from *A*+*B*) and *C* are of similar speeds and both first order.

Calculation of M — *H* having been determined, various values of *F* between 0 0030 ($f = 0\,3$) and 0 0100 ($f = 1$) were substituted into equation (9) to give, over a range of 180 sec, a family of theoretical curves (Fig 6, solid lines) showing the

incorporation of ^{18}O into D-glucose for any percentage of D-fructosyl residue cleavage of sucrose from 30% ($M_0 = 70$) to 100% ($M_0 = 0$) [equation (11)]

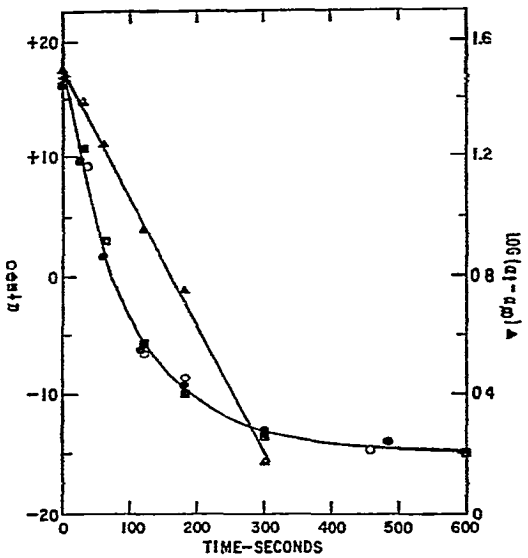


Fig 5 Left ordinate kinetics of acid hydrolysis of sucrose Right ordinate Log plot test for first order The symbols indicate three sets of observations

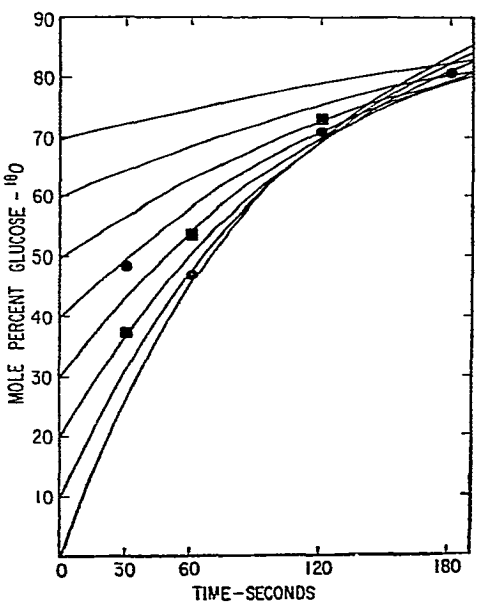


Fig 6 Family of theoretical curves showing the kinetics of labeling of D-glucose by $H_2{}^{18}O$ for various fractions of D-fructosyl cleavage of sucrose from 30 to 100% (70 to 0% D-glucosyl cleavage measured as mole percent of D-glucose-^{18}O) The symbols represent two sets of values observed for D-glucose-^{18}O abundances falling on the 25 75 D-glucosyl to D-fructosyl curve

Evaluation of f. — The percent enrichment of ^{18}O, observed for the D-glucose isolated from the sucrose hydrolyzate, relative to solvent ^{18}O, was plotted on the same graph (Fig 6, ●■) It is seen that, on the average, these points define a curve beginning at 25% D-glucose-^{18}O, corresponding to $f = 0.75$.

DISCUSSION

In their study of sucrose inversion by cation-exchange resins, Bodamer and Kunin[5] found that it was necessary to include a small quantity of anion-exchange resin to remove the soluble acid, which led to homogeneous catalysis They measured the energy of activation of hydrolysis A with and without the addition of anion exchanger, and found the lower energy (18,300 cal per mole) in its presence compared to 27,600 cal per mole in presence of IR-120 resin alone. In the present study with IR-120 resin, well washed before use and without anion exchanger, the reaction mixture was never more acid than pH 5, in contrast to pH 3 observed by Bodamer and Kunin under the same conditions Consistent with this absence of soluble acid was the low energy of activation of 16,700 cal per mole calculated from the slope (Fig. 7) of the equation

$$\log H = \frac{A}{2.303RT} + \text{constant}$$

$$A = 2.303 \times R \times \text{Slope} = 16{,}700 \text{ cal per mole}$$

The hydrolytic rate constant, H, was measured at 38, 60, and 100°

It can be seen from Fig 6 that, with increasing time, the evaluation of f by comparison of the values observed for D-glucose enrichments with those calculated

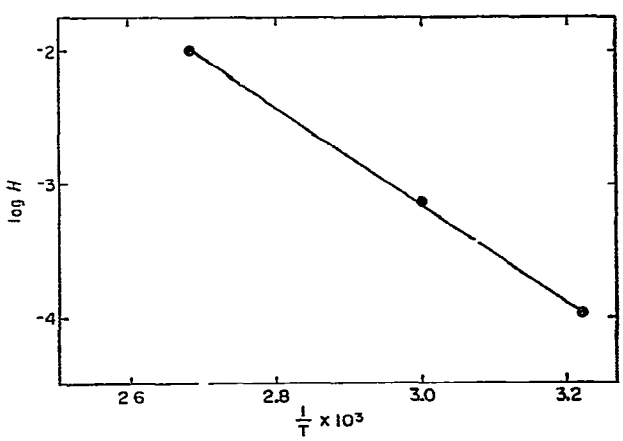


Fig 7 Energy of activation of hydrolysis of sucrose by the cation-exchange resin Amberlite IR-120 The log of the hydrolytic rate-constant H is plotted against 10^3 times the reciprocal of the absolute temperature (T) For measurement of H at 38° and 60°, water at the requisite temperature was circulated through the funnel jacket, the condenser and Wood's metal cone were omitted The resin was dropped from a spatula into the reaction mixture, at zero time

becomes increasingly ambiguous, until at 180 sec no choice is possible On the other hand, although the choice becomes clearer with decreasing time as seen from the increased spread of points, the data become increasingly less reliable Two factors account for these errors inaccuracy in timing the reaction reproducibly at shorter time-intervals, and less accurate measurement of ^{18}O abundance resulting from the lower incorporation of isotope and greater contamination of D-glucose by sucrose Most of these errors have been minimized by the use of a special apparatus (Fig 1) for timing the reaction, and by chromatographic examination of the labeled D-glucose for impurities, estimated quantitatively where present

In a study of the stability of simple glycosides to acid hydrolysis, Haworth and Hirst[10] found that furanosides are 100 times as labile as pyranosides. Sucrose was hydrolyzed at a rate comparable to that of the furanosides, suggesting the greater lability of the fructosyl–oxygen bond The present studies make a direct attack on this problem, and lead to the same conclusion That the ratio of fructosyl to glucosyl bonds cleaved is 3 to 1 in these studies, and not 100 to 1 as suggested by the earlier studies, may be the result of an effect of the coexisting pyranosyl-oxygen bond obviously absent from the simple glycosides Alternatively, the time for exchange of ^{18}O into nascent D-glucose by reaction *C* may be considerably shorter than indicated by the study of the isolated exchange-reaction, in which case the family of theoretical curves would be in error and lead to an erroneously high estimate of D-glucosyl cleavage

ACKNOWLEDGMENTS

The author is indebted to Mr Arthur H. Bolden and Mr. Paul K Goldsmith for expert technical assistance, and to Mr William E Comstock for mass-spectrometric analyses

REFERENCES

1 C A BUNTON, T A LEWIS, D R LLEWELLYN, AND C A VERNON, *J Chem Soc*, (1955) 4419
2 F EISENBERG, JR AND S HESTRIN, *Bull Res Counc Israel Sect A*, 11 (1963) 269
3 J N BEMILLER, *Advan Carbohyd Chem*, 22 (1967) 25
4 S GLASSTONE, *Textbook of Physical Chemistry*, Van Nostrand, New York, 1946, p 1075
5 G BODAMER AND R KUNIN, *Ind Eng Chem*, 32 (1951) 1082
6 A W MANGAM AND S F ACREE, *J Amer Chem Soc*, 39 (1917) 965
7 D RITTENBERG AND L PONTICORVO, *Int J Appl Radiat Isotopes*, 1 (1956) 208
8 M E TATE AND C T BISHOP, *Can J Chem*, 40 (1962) 1043
9 F EISENBERG, JR, *J Chromatogr*, 9 (1962) 390
10 W. N. HAWORTH AND E L HIRST, *J Chem Soc*, (1930) 2615

Carbohydrate Research
Elsevier Publishing Company Amsterdam
Printed in Belgium

THE SYNTHESIS AND REACTIONS OF UNSATURATED SUGARS

PART II INTRODUCTION OF ENDOCYCLIC DOUBLE BONDS INTO PENTOSES AND HEXOSES

S DIMITRIJEVICH AND N F TAYLOR

Biochemistry Group, School of Biological Sciences, Bath University, Bath (Great Britain)

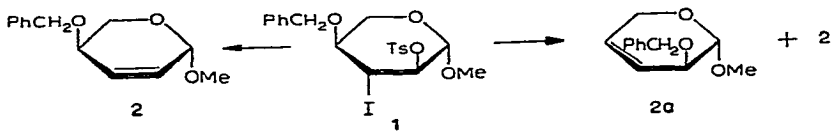
(Received March 10th, 1969, in revised form, June 12th, 1969)

ABSTRACT

The action of sodium iodide in acetone on methyl 4-*O*-benzyl-3-deoxy-3-iodo-2-*O*-tosyl-β-L-xylopyranoside (**1**) is described At room temperature, methyl 4-*O*-benzyl-2,3-dideoxy-β-L-*glycero*-pent-2-enoside (**2**) and methyl 2-*O*-benzyl-3,4-dideoxy-β-L-*glycero*-pent-3-enoside (**2a**) were formed. At lower temperatures (0–5°), the action of sodium iodide in acetone on **1** produces only the expected 2,3-alkene **2**, and a mechanism, based on conformational analysis, is suggested to account for this fact The action of sodium iodide in acetone on methyl 4-*O*-benzyl-3-deoxy-3-iodo-2-*O*-tosyl-6-*O*-trityl-α-D-glucopyranoside [**12**, made from the iodohydrin **9** obtained by the action of methylmagnesium iodide on methyl 2,3-anhydro-4-*O*-benzyl-6-*O*-trityl-α-D-allopyranoside (**8**)] is also described The structure of the iodohydrin **9** was established by mild, acid hydrolysis, followed by sequential hydrogenolysis, to yield methyl 3-deoxy-α-D-*ribo*-hexopyranoside Treatment of **12** with sodium iodide in refluxing acetone yielded a crystalline alkene, which has an n m r spectrum consistent with the structure of methyl 4-*O*-benzyl-2,3-dideoxy-6-*O*-trityl-α-D-*erythro*-hex-2-enoside (**13**) This structure was further confirmed by *cis* hydroxylation of **13** with neutral potassium permanganate, followed by catalytic hydrogenolysis, to give methyl α-D-mannopyranoside

INTRODUCTION

A number of methods are now available for the introduction of a double bond into carbohydrates Thus, based on the general method of Corey and Winter[1], selected *cis* and terminal vicinal diols may be converted, *via* their thionocarbonates, into unsaturated sugars[2,3]. Selected *trans* vicinal diols may also be converted into unsaturated sugars by the action of potassium ethylxanthate on their disulphonic esters or epoxides[2] Alternatively, elimination of *trans* vicinal tosylated iodohydrins offer a route for the introduction of endocyclic double bonds into sugars[5,6] We now report on the synthesis and rearrangement of methyl 4-*O*-benzyl-2,3-dideoxy-β-L-*glycero*-pent-2-enoside (**2**) and the synthesis of methyl 4-*O*-benzyl 2,3-dideoxy-6-*O*-trityl-α-D-*erythro*-hex-2-enoside (**13**) from *trans* vicinal tosylated iodohydrins.



4 R=R'=H
5 R=R=Ac
6 R=R=Ms
7 R=H, R'=Tr
8 R=CH_2Ph, R'=Tr

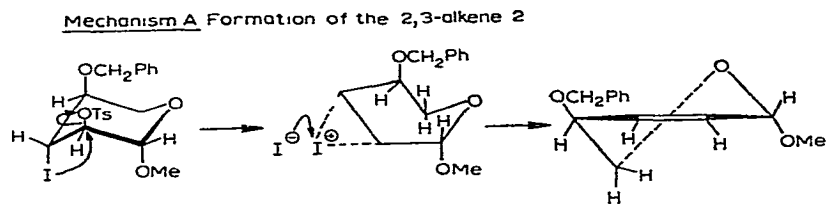
9 R=I, R=Tr, R''=H
10 R=I, R=R''=H
11 R=R=R''=H
12 R=I, R=Tr, R''=Ts

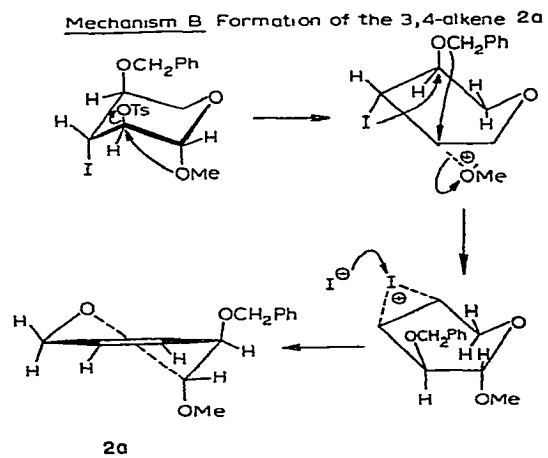
13

14 R=CH_2Ph, R'=Tr
15 R=R'=H

RESULTS AND DISCUSSION

We previously reported[6] the synthesis of methyl 4-*O*-benzyl-2,3-dideoxy-β-L-*glycero*-pent-2-enoside (**2**) by treatment of methyl 4-*O*-benzyl-3-deoxy-3-iodo-2-*O*-tosyl-β-L-xylopyranoside (**1**) with sodium iodide in acetone at room temperature. The *trans* elimination of the iodine atom and the tosyloxy group from **1** resulted in the formation of an unsaturated product which distilled at a constant temperature and gave the correct empirical analysis for the expected pentenoside **2** Thin-layer chromatography of this compound, however, revealed two well-defined components





Carbohyd Res, 11 (1969) 531–539

(R_F 0 4 and 0 6), suggesting the formation of two isomeric alkenes which could have occurred (*a*) by an allylic rearrangement of the benzyl ether group in the 2,3-alkene **2** to give an equilibrium mixture of **2** and the 3,4-alkene **2a**, or (*b*) by elimination of the iodine atom and tosyloxy group from xyloside **1**, involving group participation of the all-*trans*-substituents in the *C1* conformation to form the expected 2,3-alkene **2** (Mechanism A) and the 3,4-alkene **2a** (Mechanism B).

The formation of the two isomeric alkenes from **1** was shown to be temperature dependent, thus treatment with sodium iodide in acetone at 0–5° gave only one crystalline product, the n m r. spectrum (100 MHz, $CDCl_3$) of which was consistent with the structure of the expected 2,3-alkene **2** and contained a signal for the olefinic protons at τ 4 06 This τ value compares favourably with those reported for other 2,3-unsaturated hexoses and pentoses[7,8] The isolation of the other isomer, **2a**, was achieved by treating **1** at room temperature with sodium iodide in acetone, followed by p l c of the distilled mixture of alkenes The n m r spectrum of the faster-moving component was consistent with the structure of the 3,4-alkene **2a** and contained a quartet (τ 4 28) for the olefinic protons, *cf* τ 4 33 for benzyl 2-*O*-benzyl-3,4-dideoxy-α-D-*glycero*-pent-3-enoside[7] Since the crystalline alkene **2** did not rearrange either on heating, or when treated with sodium iodide in acetone at room temperature, we favour the kinetic mechanisms (A) and (B) for the formation of the two alkenes Moreover, the dramatic changes in the specific rotation of the 2,3-alkene (−76 5°, $CHCl_3$) on rearrangement to the 3,4-alkene (+2 5°, $CHCl_3$) is consistent with the idea that substituents at C-4 in hex-2-enopyranoses makes an important rotatory contribution[9] The low, positive rotation of the proposed methyl 2-*O*-benzyl-3,4-dideoxy-β-L-*glycero*-pent-3-enoside (**2a**) is also consistent with the much higher, positive rotation (+115°, $CHCl_3$) of benzyl 2-*O*-benzyl-3,4-dideoxy-α-D-*glycero*-pent-3-enoside[7] Although 1,2-migration[10] and 1,4-migration[11] of methyl ethers in carbohydrate derivatives have been reported, this is believed to be the first example of an allylic benzyl ether migration in the cyclic carbohydrate series

An extension of the above method of introducing a double bond into cyclic carbohydrates has been made to methyl 4-*O*-benzyl-3-iodo-2-*O*-tosyl-6-*O*-trityl-α-D-glucopyranoside (**12**) Treatment of methyl 2,3-anhydro-4,6-*O*-benzylidene-α-D-allopyranoside[12] (**3**) at room temperature with methanol containing concentrated hydrochloric acid gave methyl 2,3-anhydro-α-D-allopyranoside (**4**), characterised as the 4,6-diacetate (**5**) and 4,6-dimethanesulphonate (**6**) This method of removing the 4,6-*O*-benzylidene group is considered to be better than the methods previously used[13,14] Treatment of epoxide **4** with chlorotriphenylmethane gave the 6-trityl ether **7** which, after being shaken at room temperature with benzyl bromide and silver oxide in *N*,*N*-dimethylformamide, gave methyl 2,3-anhydro-4-*O*-benzyl-6-*O*-trityl-α-D-allopyranoside (**8**) Epoxide scission of **8** with methylmagnesium iodide (in tetrahydropyran at −15°) gave the expected *trans* iodohydrin **9** (*cf.* ref 15) The structure of the iodohydrin **9** was established by hydrolysis with aqueous acetic acid, to yield methyl 4-*O*-benzyl-3-deoxy-3-iodo-α-D-glucopyranoside (**10**), followed by sequential hydrogenolysis to yield methyl 4-*O*-benzyl-3-deoxy-α-D-*ribo*-hexopyranos-

ide (**11**), and methyl 3-deoxy-α-D-*ribo*-hexopyranoside which was characterised as the crystalline 4,6-*O*-benzylidene derivative[16]. The *trans* arrangement of the iodine and hydroxyl group in **9** was finally confirmed by its conversion into the 2,3-anhydro-alloside **8** on treatment with sodium methoxide in chloroform The action of toluene-*p*-sulphonyl chloride on **9** readily gave methyl 4-*O*-benzyl-3-deoxy-3-iodo-2-*O*-tosyl-6-*O*-trityl-α-D-glucopyranoside (**12**) which, on treatment with a refluxing solution of sodium iodide in acetone, lost the iodine atom and the tosyloxy group to give methyl 4-*O*-benzyl-2,3-dideoxy-6-*O*-trityl-α-D-*erythro*-hex-2-enopyranoside (**13**) The n.m.r. spectrum ($CDCl_3$) was consistent with this structure, showing a quartet for the olefinic protons at τ 4.10, comparable to that for the 2,3-alkene **2** and other similar 2,3-alkenes[17]. Further proof for the structure of the unsaturated sugar **13** was provided by its reaction (at 4°) with neutral potassium permanganate to give exclusively one hydroxylated adduct (**14**) which, on catalytic hydrogenolysis to remove the 4-*O*-benzyl and 6-*O*-trityl groups, gave methyl α-D-mannopyranoside[18] (**15**) This *cis* addition of hydroxyl groups to **13**, which occurs *trans* to the substituents at C-1 and C-4, is consistent with the stereoselective hydroxylation reactions of other similar 2,3-alkenes[17,19].

EXPERIMENTAL

All melting points were determined by using an Electrothermal apparatus, and are uncorrected. The specific rotations were measured on a Bellinger and Stanley (Model A) polarimeter and 0 5-dm tube. The n m r spectra were recorded, with spin decoupling, on a Varian HA-100 spectrometer, for deuteriochloroform solutions with tetramethylsilane as the internal standard Thin-layer chromatography (t l c.) was performed on 20 × 20 cm glass plates coated with a 0 25-mm layer of Silica Gel G according to Stahl (Shandon Scientific Co. Ltd) Carbohydrate components were detected with sulphuric acid–ethanol (1 1) and heating for 2–5 min at 120°. Preparative layer chromatography (p l c) was performed on 40 × 20 cm glass plates coated with a 1.3-mm layer of Silica Gel PF_{254} (Shandon Scientific Co. Ltd.) Carbohydrate components were detected as bands of fluorescence or quenching on exposure to u v. radiation (254 nm) The solvent systems employed were *A* light petroleum (b p. 40–60°)–methanol (97.3); *B* ethyl acetate–light petroleum (b p. 40–60°) (1 1); *C* ethyl acetate–ethanol (4·1)

*Methyl 4-O-benzyl-2,3-dideoxy-β-*L-glycero-*pent-2-enoside* (**2**). — To a cold (0°) solution of methyl 4-*O*-benzyl-3-deoxy-3-iodo-2-*O*-toluene-*p*-sulphonyl-β-L-xylo-pyranoside (1 0 g) in dry acetone (20 ml) was added a cold (0°) solution of dry sodium iodide (1 0 g) in dry acetone (10 ml). The reaction mixture was kept for 24 h at 0°, and the filtered solution was evaporated, under diminished pressure and at room temperature, to half the original volume. Water (50 ml) was then added, and the resulting solution was extracted with ether (3 × 50 ml). The combined extracts were washed successively with water (2 × 25 ml), 10% aqueous sodium thiosulphate (3 × 25 ml), and water (2 × 25 ml). The ethereal layer was dried (Na_2SO_4) and evaporated, under diminished pressure and at room temperature, to give compound **2**

(0 35 g, 83 3%), m p 33°, $[\alpha]_D^{20}$ −76 5° (*c* 1 0, chloroform) (Found: C, 70 2; H, 7.2. $C_{13}H_{16}O_3$ calc.· C, 70 7, H, 7 2%), n m r. data τ 6 65 (3-proton singlet, OMe), 6.38 (1-proton multiplet, H-4), 6 15 (2-proton triplet, H-5,5'), 5 48 (2-proton singlet, benzylic H_2), 5 2 (1-proton doublet, H-1), 4.06 (2-proton octet, H-2,3), 2.79 (5-proton singlet, Ph)

Methyl 2-O-*benzyl-3,4-dideoxy-β-*L-glycero-*pent-3-enoside* (**2a**). — Dry sodium iodide (2.0 g) was added to a solution of methyl 4-*O*-benzyl-3-deoxy-3-iodo-2-*O*-toluene-*p*-sulphonyl-β-L-xylopyranoside (2 0 g) in dry acetone (25 ml), whereupon sodium toluene-*p*-sulphonate began to separate almost immediately. After refluxing on a water bath for 15 min, the reaction mixture was filtered, and the combined filtrate and washings were evaporated to dryness under diminished pressure. Water (100 ml) was added to the residue, the resulting solution was extracted with chloroform (3 × 50 ml), and the combined extracts were washed with water (2 × 50 ml), 10% aqueous sodium thiosulphate (3 × 50 ml), and water (3 × 50 ml). After drying (Na_2SO_4), the chloroform solution was evaporated, and the residue was distilled to give a mixture of compounds **2** and **2a** (0 8 g), b p 89°/0 05 mmHg, $[\alpha]_D^{22}$ −81 5° (*c* 1 0, chloroform) (Found· C, 70 6, H, 7.1 $C_{13}H_{16}O_3$ calc. C, 70 7, H, 7.2%).

T l c of the syrup revealed two major components (R_F 0.4 and 0.6 in solvent *A*). P l c in solvent *A* gave methyl 4-*O*-benzyl-2,3-dideoxy-β-L-*glycero*-pent-2-enoside (R_F 0 4, 0.4 g) and the faster-moving component (R_F 0 6, 0 2 g) which is probably methyl 2-*O*-benzyl-3,4-dideoxy-β-L-*glycero*-pent-3-enoside; $[\alpha]_D^{20}$ +2 5° (*c* 0 4, chloroform), n m r data τ 6 62 (3-proton singlet, OMe), 6 25 (2-proton triplet, H-5,5'), 5 98 (1-proton multiplet, H-2), 5.47 (2-proton singlet, benzylic H_2), 5 27 (1-proton broad singlet, H-1), 4 28 (2-proton quartet, H-3,4), 2 75 (5-proton singlet, Ph)

Methyl 2,3-*anhydro-α-*D-*allopyranoside* (**4**). — Methyl 2,3-anhydro-4,6-*O*-benzylidene-α-D-allopyranoside (4 0 g) in anhydrous methanol (200 ml) containing conc hydrochloric acid (0 04 ml) was shaken for 20 h at room temperature The resulting solution was neutralised (Na_2CO_3), filtered, and evaporated to dryness under diminished pressure at 40°. A solution of the residue in the minimal volume of water was washed with ether (3 × 50 ml) and then evaporated to dryness under diminished pressure at 40° The syrupy residue was extracted with ethyl acetate–ethanol (1 1), and the combined extracts were filtered and evaporated to dryness *in vacuo*. Crystallisation of the residue from the minimal volume of acetone gave the product (2 65 g, 99 9%), m p 103°, $[\alpha]_D^{23}$ +151 4° (*c* 2 5, methanol) (Found. C, 47.8, H, 6 8; OCH_3, 17 0. $C_7H_{12}O_5$ calc.· C, 47.7, H, 6 8; OCH_3, 17 6%)

With methanesulphonyl chloride–pyridine, anhydride **4** gave the 4,6-dimethanesulphonate **6** (78 2%), m p 138° (from methanol–acetone), $[\alpha]_D^{23}$ +139 5° (*c* 1 7, chloroform) (Found· C, 32 6; H, 4 9, S, 19.28 $C_9H_{16}O_9S_2$ calc C, 32 5; H, 4.8; S, 19 28%). With acetic anhydride–pyridine, compound **4** gave the 4,6-diacetate **5** (75 5%), b.p. 155°/0 3 mmHg, $[\alpha]_D^{20}$ +160° (*c* 1 0, chloroform) (Found C, 50 2, H, 6 5; OCH_3, 12 4 $C_{11}H_{16}O_7$ calc C, 50.7; H, 6.2; OCH_3, 12 4%)

*Methyl 2,3-anhydro-6-*O-*trityl-α-*D-*allopyranoside* (**7**). — Methyl 2,3-anhydro-α-

D-allopyranoside (1 0 g) was dissolved in anhydrous pyridine (10 ml), and to this solution chlorotriphenylmethane (2 0 g) was added The resulting solution was kept for 24 h at room temperature and then poured into ice-cold water (100 ml) The syrup which separated was extracted with chloroform (3 × 50 ml), the combined extracts were washed successively with 2N hydrochloric acid (3 × 50 ml), saturated aqueous sodium hydrogen carbonate (3 × 50 ml), and water, dried (Na_2SO_4), and evaporated under diminished pressure Crystallisation of the residue from ethanol, with recrystallisation from ethanol–acetone, gave compound **7** (1 5 g, 63 3%), m p 184°, $[\alpha]_D^{20}$ +96 5° (*c* 0 41, chloroform) (Found C, 74 5, H, 6 5 $C_{26}H_{26}O_5$ calc C, 74 7, H, 6 2%)

Methyl 2,3-anhydro-4-O-benzyl-6-O-trityl-α-D-allopyranoside (**8**) — Compound **7** (1 0 g) was dissolved in anhydrous *N,N*-dimethylformamide (5 0 ml), and to this solution silver oxide (1 0 g) and benzyl bromide (1 0 ml) were added The mixture was shaken for 4 days at room temperature, diluted with chloroform (100 ml), and filtered The filtrate was washed with water (5 × 50 ml), and the chloroform layer was dried (Na_2SO_4) and evaporated Addition of ether to the residue, followed by cooling, gave a crystalline solid which was recrystallised from methanol–acetone to give compound **8** (1 2 g, 98 7%), m p 162°, $[\alpha]_D^{18}$ +91 5° (*c* 1 0, chloroform) (Found C, 77 8, H, 6 4 $C_{33}H_{32}O_5$ calc C, 77 9, H, 6 3%)

Methyl 4-O-benzyl-3-deoxy-3-iodo-6-O-trityl-α-D-glucopyranoside (**9**) — The Grignard reagent, prepared in the usual way from methyl iodide (0 55 g) and dry magnesium turnings (0 096 g), in anhydrous tetrahydropyran (10 ml), was cooled to −15°. To this solution was added, dropwise and with stirring, a cold (4°) solution of methyl 2,3-anhydro-4-*O*-benzyl-6-*O*-trityl-α-D-allopyranoside (1.0 g) in anhydrous tetrahydropyran (25 ml) The stirred mixture was allowed to attain room temperature and then kept overnight Powdered ice, followed by 2N hydrochloric acid (10 ml), was then added, the tetrahydropyran layer was separated, and the aqueous layer was extracted with chloroform (3 × 50 ml) The chloroform extracts and the tetrahydropyran layer were combined, and washed with saturated aqueous sodium hydrogen carbonate (2 × 50 ml), 10% aqueous sodium thiosulphate (2 × 50 ml), and water, dried (Na_2SO_4), and evaporated under diminished pressure to give a pale-yellow syrup which, on addition of methanol and cooling, produced a crystalline solid Recrystallisation from methanol gave compound **9** (0 9 g, 72%), m p 172°, $[\alpha]_D^{20}$ +65° (*c* 0 92, chloroform) (Found C, 62 3, H, 5 2, I, 20 1 $C_{33}H_{33}IO_5$ calc C, 62 3, H, 5 2, I, 19 9%)

A solution of compound **9** (0 5 g) in chloroform (20 ml) was cooled to 0° and treated with 2 7N methanolic sodium methoxide (5 0 ml) The resulting solution was allowed to attain room temperature, kept overnight, and then poured into water (50 ml) The chloroform layer was washed with water until neutral, dried (Na_2SO_4), and evaporated under diminished pressure Recrystallisation of the residue from methanol–acetone gave compound **8** (0 3 g, 75 2%), m p 162° alone or in a mixture with an authentic sample of methyl 2,3-anhydro-4-*O*-benzyl-6-*O*-trityl-α-D-allopyranoside (**8**)

With toluene-*p*-sulphonyl chloride in pyridine, in the usual manner, compound **9** gave the 2-toluene-*p*-sulphonate **12** (83 3%), m p 132° (from methanol), $[\alpha]_D^{23}$ +34 9° (*c* 0 92, chloroform) (Found· C, 60 7, H, 5 05, I, 15 75, S, 4 1 $C_{40}H_{39}IO_7S$ calc . C, 60 7, H, 5 0, I, 16 0, S, 4 0%)

Methyl 4-O-benzyl-3-deoxy-3-iodo-α-D-glucopyranoside (**10**) — A solution of compound **9** (1 0 g) in 80% aqueous acetic acid (50 ml) was heated for 15 min under reflux, and then poured into water (100 ml) The syrup which separated was extracted with ether (3 × 50 ml), and the combined extracts were washed with water (3 × 50 ml), saturated aqueous sodium hydrogen carbonate (3 × 50 ml), and water (3 × 50 ml), dried (Na_2SO_4), and evaporated under diminished pressure P l c of the syrup with solvent *B* and isolation of the product having R_F 0 25, followed by crystallisation from ether–light petroleum (b p 40–60°), gave compound **10** (0 5 g, 80 7%), m p 105°, $[\alpha]_D^{25}$ +76 3° (*c* 2 0, chloroform) (Found C, 42.6, H, 5 0, I, 32 1 $C_{14}H_{19}IO_5$ calc C, 42 6, H, 4.9, I, 32 2%)

Methyl 4-O-benzyl-3-deoxy-α-D-ribo-hexopyranoside (**11**) — To a suspension of 10% palladium-on-calcium carbonate (2 5 g) and 5% palladium-on-charcoal (1 0 g) in ethanol (50 ml), was added a solution of methyl 4-*O*-benzyl-3-deoxy-3-iodo-α-D-glucopyranoside (0 6 g) in ethanol (60 ml) The mixture was treated, at room temperature for 24 h, with hydrogen at a pressure of 50 atmospheres The catalyst was then filtered off, and the filtrate was evaporated to give a syrup which crystallised on standing The product was purified by p l c with solvent *C*, and elution of the component having R_F 0 64 from the adsorbent with acetone Recrystallisation from chloroform–light petroleum (b p 40–60°) gave compound **11** (0 3 g, 73 5%), m p 124°, $[\alpha]_D^{23}$ +194 2° (*c* 1 0, chloroform) (Found C, 62 4, H, 7 4 $C_{14}H_{20}O_5$ calc.. C, 62 6, H, 7 5%)

Methyl 3-deoxy-α-D-ribo-hexopyranoside — A suspension of 5% palladium-on-charcoal (1 0 g) in ethanol (25 ml) was treated with hydrogen, at room temperature, until there was no further uptake of hydrogen A solution of compound **11** (0 2 g) in ethanol (25 ml) was then added, and the hydrogenolysis was allowed to proceed overnight The filtered solution was evaporated to give a thick, colourless syrup (0 12 g, 90 3%), $[\alpha]_D^{22}$ +171 7° (*c* 1 4, ethanol), which was identical chromatographically (R_F 0 33, solvent *C*) with an authentic sample of methyl 3-deoxy-α-D-*ribo*-hexopyranoside The product was further characterised as the crystalline 4,6-*O*-benzylidene derivative, m p 190°, $[\alpha]_D^{20}$ +115° (*c* 1 0, chloroform), lit [16], m p 191–192°, $[\alpha]_D^{12}$ +115 8° (*c* 1 2, chloroform)

Methyl 4-O-benzyl-2,3-dideoxy-6-O-trityl-α-D-erythro-hex-2-enoside (**13**) — A solution of methyl 4-*O*-benzyl-3-deoxy-3-iodo-2-*O*-toluene-*p*-sulphonyl-6-*O*-trityl-α-D-glucopyranoside (1 0 g) in anhydrous acetone (30 ml) containing anhydrous sodium iodide (1 0 g) was heated under reflux for 1 h The resulting solution was poured into water, the syrup which separated was extracted with ether, and the combined extracts were washed successively with water (3 × 50 ml), 10% aqueous sodium thiosulphate (2 × 50 ml), and water (3 × 50 ml), dried (Na_2SO_4), and evaporated under diminished pressure The residue was crystallised from methanol and

recrystallised from methanol–acetone to give compound **13** (0.55 g, 88 3%), m p. 130°, $[\alpha]_D^{24}$ +72.3° (*c* 0.82, chloroform) (Found· C, 80.4; H, 6.8. $C_{33}H_{32}O_4$ calc C, 80.5; H, 6 5%); n.m.r. data: τ 6 8 (2-proton quartet, H-6,6'), 6 53 (3-proton singlet, OMe), 6 0 (2-proton multiplet, H-4,5), 5.62 (2-proton quartet, benzylic H_2), 5.1 (1-proton doublet, H-1), 4 10 (2-proton quartet, H-2,3), 2.85 (15-proton multiplet, Ph_3), 2 53 (5-proton multiplet, Ph).

Methyl 4-O-benzyl-6-O-trityl-α-D-mannopyranoside (**14**). — A solution of compound **13** (0 5 g) in acetone (10 ml) was cooled to 4° and treated dropwise (with stirring in an atmosphere of nitrogen) with cold (4°) 0.5% potassium permanganate (24 ml) in 10% aqueous acetone On completion of the addition, the treatment was repeated, and the reaction was allowed to proceed overnight. The filtered solution was then evaporated under diminished pressure to give a colourless syrup, which crystallised on addition of methanol. Recrystallisation from methanol gave compound **14** (0 48 g, 83 8%), m p 127°, $[\alpha]_D^{20}$ +46 9° (*c* 0 68, chloroform) (Found· C, 75 32; H, 6 41. $C_{38}H_{34}O_6$ calc . C, 75 26; H, 6 51%)

Compound **14** (0 3 g) was added to a suspension of 5% palladium-on-charcoal (2.0 g) in ethanol (100 ml), and the resulting mixture was treated at 50° with hydrogen at a pressure of 6 atmospheres for 20 h. The filtered mixture was then evaporated under diminished pressure, the residue was dissolved in water (20 ml), and the resulting solution was washed with ether (3 × 50 ml) The aqueous solution was then evaporated under diminished pressure to give a syrup which, on addition of methanol and standing, crystallised. Recrystallisation from a small volume of methanol gave methyl α-D-mannopyranoside (0 1 g, 90 8%), $[\alpha]_D^{20}$ +82° (*c* 1.5, water), m.p 193° alone or in admixture with an authentic sample[18].

ACKNOWLEDGMENTS

The authors are most grateful to Dr. J. Feeney, Varian Associates Ltd , who kindly performed and interpreted the n m r. spectra One of us (S D) thanks the University for the award of a studentship

REFERENCES

1 E. J COREY AND R. E. WINTER, *J Amer Chem Soc*, 85 (1963) 2667
2 D HORTON AND W. N TURNER, *Tetrahedron Lett*, (1964) 2531
3 A H HAINES, *Chem Ind* (London), (1964) 1991
4 E ALBANO, D HORTON, AND T TSUCHIYA, *Carbohyd Res*, 2 (1966) 349
5 F. H NEWTH, *J Chem Soc*, (1956) 471
6 N F. TAYLOR AND G. M RIGGS, *J Chem Soc*, (1963) 5600
7 A. H. HAINES, *Carbohyd Res*, 1 (1965) 214
8 R. J. FERRIER, *J. Chem Soc*, (1964) 5443
9 J A. MILLS, *J Chem. Soc*, (1952) 4976, A K BOSE AND B G. CHATTERJEE, *J Org. Chem*, 23 (1958) 1425
10 R. U LEMIEUX AND R FRASER-REID, *Can J Chem*, 42 (1964) 539
11 N A HUGHES AND P. P. H SPEAKMAN, *Chem Commun*, (1965) 199
12 N. K RICHTMYER AND C. S HUDSON, *J Amer. Chem Soc*, 63 (1941) 1727
13 G J ROBERTSON AND H G. DUNLOP, *J Chem Soc*, (1938) 472.

14 J G BUCHANAN AND R N SAUNDERS, *J. Chem Soc*, (1964) 1796.
15 F H NEWTH, *Quart Rev* (London), 13 (1959) 10
16 D A PRINS, *Helv Chim Acta*, 29 (1946) 1
17 D M CIMENT AND R. J FERRIER, *J. Chem Soc*, (1966) 441
18 J. E CADOTTE, F SMITH, AND D SPRIESTERBACH, *J Amer Chem Soc*, 74 (1952) 1501
19 R J FERRIER, W G OVEREND, AND R E RYAN, *J Chem Soc*, (1962) 3667

Carbohydrate Research
Elsevier Publishing Company, Amsterdam
Printed in Belgium

AUTOCATALYTIC MUTAROTATION OF D-GLUCOSE IN PYRIDINE

A S HILL AND R S SHALLENBERGER

New York State Agricultural Experiment Station, Cornell University, Geneva, New York 14456 (U S A)

(Received May 7th, 1969, in revised form, June 12th, 1969)

ABSTRACT

The mutarotation of α- and β-D-glucose in dry pyridine was followed gas chromatographically Kinetic treatment of the data for a wide range of concentrations of D-glucose showed the reaction to be first-order in the starting anomer with a unimolecular dependence on the total concentration of D-glucose Thus, the mutarotation of D-glucose in dry pyridine appears to be autocatalytic, and a sterically acceptable mechanism for the reaction is a modification of the mechanism of Swain and Brown Specific rotations of the D-glucopyranose anomers in dry pyridine were calculated to be +152° for α-D-glucopyranose and +11° for β-D-glucopyranose At equilibrium, $[\alpha]_D = +72°$, and the distribution of α- and β-D-glucopyranose is 43% and 57%, respectively.

INTRODUCTION

The mutarotation of D-glucose in water has been extensively investigated[1-5] In contrast, relatively few studies of this reaction in non-aqueous solvents have been published [5-9] It is known to be a reversible, first-order reaction[1,2] in which the rate-determining step has been postulated to be formation of the free aldehyde[3]. The reaction shows general acid and general base catalysis[3,4] and requires the presence of both an acid and a base[3-5] For most studies, mutarotations have been followed polarimetrically. An exception is a recent report in which the results obtained by following the mutarotation reaction of D-glucose in water by gas chromatography of trimethylsilated aliquots were found to be in agreement with polarimetric results[2]

Studies of the mutarotation of D-glucose in non-aqueous solvents have employed mainly the 2,3,4,6-tetra-*O*-acetyl and 2,3,4,6-tetra-*O*-methyl derivatives, which show rate constants comparable to those for free D-glucose The first-order rate constant for the mutarotation of D-glucose in pyridine has been reported[5,6] as 0 0008 min^{-1} and approximately 0 001 min^{-1} More recently, the dependence of the rate on a second-order term has been recognized for the mutarotation of 2,3,4,6-tetra-*O*-acetyl-D-glucose in pyridine[7] and for that of 2,3,4,6-tetra-*O*-methyl-D-glucose in pyridine[8]

EXPERIMENTAL

General. — α- and β-D-glucose (analytical grade) were dried over P_2O_5 under vacuum for at least 1 h at 76°, and then stored over molecular sieve A-4 until used. Pyridine (analytical grade) was distilled from BaO at atmospheric pressure (b p. 113–114°) and kept over molecular sieve A-4 until used. All transfer and storage procedures were carried out in a dry box that contained molecular sieve A-4. The box was swept with dry nitrogen just prior to and during use.

Kinetic procedure — Solution of the sugar samples in pyridine was accomplished by stirring the mixtures with a micromagnet Aliquots were trimethylsilylated[10] (2 1 hexametyl disilazane–chlorotrimethylsilane) immediately after complete solution and thereafter at convenient time intervals The mutarotating samples were stored in vials having Teflon-lined caps The ambient temperature was 24 ±0 5°.

Gas chromatography. — Gas chromatography of the trimethylsilylated samples was carried out with a Research Specialties instrument equipped with a hydrogen flame-ionization detector and an XF-1150 column, 10% on Gas Chrom A. The chromatograms were quantitated by weighing at least two traced, and cut-out, forms of each peak and taking the average of the results

Optical rotations. — Optical rotations were determined with a Rudolph Model 80 polarimeter equipped with a Rudolph Model 200 photoelectric indicator.

RESULTS AND DISCUSSION

The mutarotation of α- and β-D-glucopyranose at various concentrations (13.6–219 mM) in dry pyridine at 24° was followed by gas chromatography of trimethylsilated aliquots. The method used for trimethylsilation was that of Sweeley *et al.*[10], which is known not to alter the tautomer composition of the treated mixtures[10,11]. The gas chromatograms showed only two peaks; they were completely separated and corresponded to α- and β-D-glucopyranose

The tautomer composition at mutarotational equilibrium ($[\alpha]_D^{20}$ +72°) was 43% α-D-glucopyranose and 57% β-D-glucopyranose. To determine $[\alpha]_D$ values for each of the two D-glucose tautomers in dry pyridine, the anomeric composition and the corresponding $[\alpha]_D$ of aliquots of mutarotating solutions of α- and β-D-glucose (119 and 118 mM, respectively) in dry pyridine at 24° were determined at three convenient time intervals (Table I) From these data six equations of the type (%α) ($[\alpha]_D$ of α) + (%β)($[\alpha]_D$ of β) = ($[\alpha]_D$ of α,β mixture) can be written. Solution of these three sets of simultaneous equations yielded an average value of $[\alpha]_D$ +152 2 ±1.2° for α-D-glucopyranose and $[\alpha]_D$ +10.7 ±1.7° for β-D-glucopyranose in dry pyridine. From these values, the calculated $[\alpha]_D^{24}$ for the equilibrium mixture of α- and β-D-glucopyranose is +71.5°

The data obtained for the mutarotation at any one concentration of D-glucose were found to conform to the rate equation for a reversible first-order reaction (representative plots are given in Fig. 1), and not to that for a reversible, second-order

TABLE I

ISOMER COMPOSITION AND SPECIFIC OPTICAL ROTATION OF ALIQUOTS OF MUTAROTATING SOLUTIONS OF α- AND β-D-GLUCOSE IN DRY PYRIDINE

Time, h	$[\alpha]_D^{24}$, *degrees*	*α-D-Glucose*[a]		$[\alpha]_D^{24}$, *degrees*	*β-D-Glucose*[a]	
		%α	%β		%α	%β
0 5	+145 7	96 5	3 5	+18 5	6 3	93 7
3	+136 4	88 2	11 5	+27 1	15.7	84 3
5	+125 8	80 8	19 2	+35 1	25 7	74 3

[a]These are the anomers initially dissolved in pyridine The solutions were 119mM and 118mM in α- and β-D-glucose respectively

reaction[12] Fig 1 shows that the values of the observed first-order rate constants, $k_1''+k_2''$, determined as the slopes, are directly proportional to the total concentration of D-glucose, $[G_t]$ The slopes of the plots shown in Fig 1 (and other data in the series) were calculated by using natural logarithms.

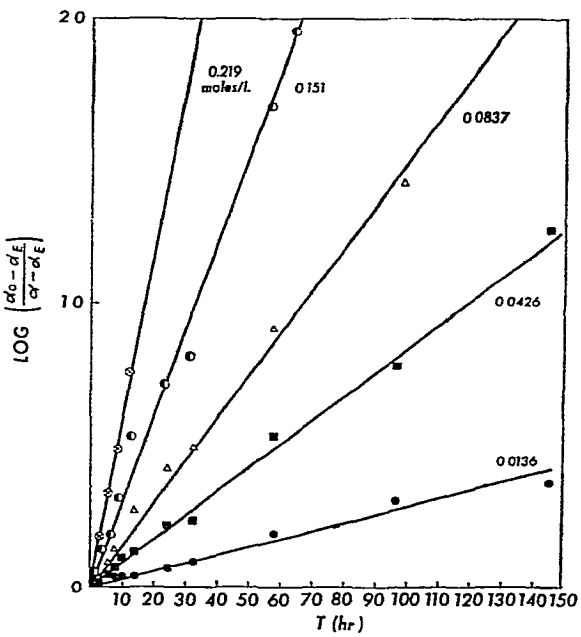


Fig 1. Reversible, first-order reaction plot for the mutarotation of α-D-glucose at various concentrations in dry pyridine

There can be little doubt that the solvent pyridine is also a participant in this reaction, as the mutarotation of D-glucose in dry pyridine is faster than in dry quinoline[9] or in dry benzene[8], which are both weaker bases and less polar than pyridine. A plot of log $(k_1''+k_2'')$ *versus* log $[G_t]$ is linear (Fig. 2) The logarithms used in Fig 2 are to the base 10, without conversion, since the values of the slope and the

antilogarithm of the intercept are independent of the base used for the logarithms The equation for the linear plot in Fig 2 can be derived from the general expression for the mutarotation reaction (Eq *1*) and its rate expression (Eq *2*) In Eq *2* the factor

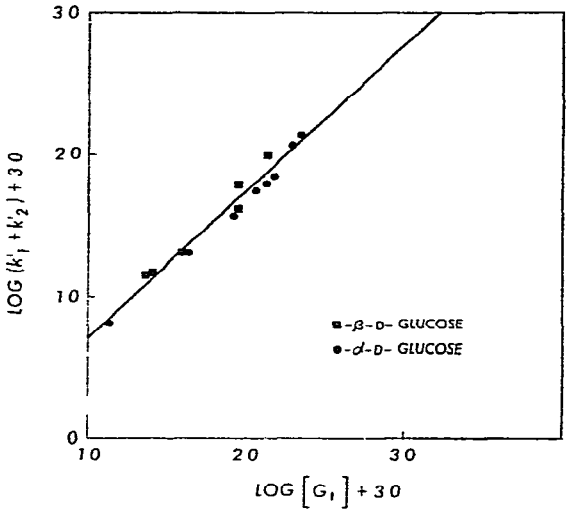


Fig 2 Plot of log ($k_1+k'_1$) *vs* log [G_t] for the mutarotation of D-glucose in dry pyridine

for concentration of pyridine, which is essentially constant in all reactions is included in $k'_1+k'_2$. Assuming that D-glucose (α or β, that is, [G_t]) acts as a catalyst, which is reasonable in view of the data in Fig 1, we arrive at Eq *3*, which is in linear form when expressed logarithmically (Eq *4*) In Eq *4* the slope, n, represents

Eq. *1* $\quad A+nG_t+mP \underset{k_2}{\overset{k_1}{\rightleftharpoons}} B+nG_t+mP \qquad P = \text{pyridine}$

Eq. *2* $\quad -dA/dt = k'_1[A][G_t]^n - k'_2[B][G_t]^n$

Eq *3* $\quad (k''_1+k''_2) = [G_t]^n(k'_1+k'_2)$

Eq *4* $\quad \log(k''_1+k''_2) = n(\log[G_t]) + \log(k'_1+k'_2)$

the molecularity of the catalytic D-glucose in the mutarotation and the intercept at $n(\log[G_t]) = 0$ represents the $\log_{10}$ of the pseudo-bimolecular, reaction rate-constant, $k'_1+k'_2$ These values, calculated by the standard regression of log [G_t] on log ($k''_1+k''_2$), are $n = 1.026$ and $\log(k'_1+k'_2) = -3\,3207$, from which $k'_1+k'_2 = 4\,78\times10^{-4}$ l mole^{-1} h^{-1} The correlation coefficient for this linear plot is 0 983

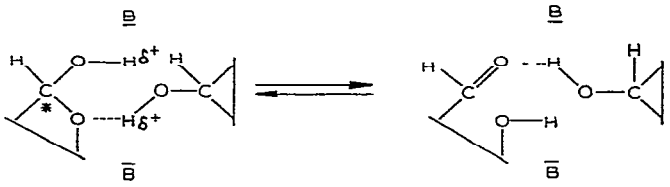
Thus the mutarotation of α- or β-D-glucopyranose in dry pyridine is first-order in that anomer used as the starting material and shows a unimolecular dependence of the rate on total concentration of D-glucose This latter fact indicates that catalytic participation of D-glucose in its own mutarotation in dry pyridine is independent of the steric disposition of the hydroxyl group at the anomeric center of the catalyst molecule

Direct measurement of the effect of pyridine on the rate of mutarotation is

not possible without altering the reaction system and with it, possibly, the manner in which pyridine participates in the mutarotation In benzene, the mutarotation of 2,3,4,6-tetra-*O*-methyl-D-glucose is reported to be first-order in pyridine[8].

The data presented are in agreement with the mechanism for mutarotation postulated by Swain and Brown[8] Here pyridine functions as the base and D-glucose (α or β) functions as the acid as well as the reactant, in a third-order reaction In this respect, the more acidic, anomeric hydroxyl groups would be expected to be more active participants in this reaction than the less acidic, alcoholic hydroxyl groups However, the difference in acid strength between the α and β anomeric hydroxyl groups is too small to be apparent in the data obtained

In the Swain and Brown mechanism the hydroxyl group of D-glucose that participates as an acid catalyst would bear a transitory, full negative charge that could presumably gain little stability from the solvent pyridine We suggest the following modification of the Swain and Brown mechanism in which one hydroxyl group



functions as both the acid and the base In this mechanism, the protons are transferred to oxygen atoms with assistance from the solvent pyridine, in a more or less concerted manner The resulting, six-membered, cyclic transition-state is sterically favored This mechanism suggests that mutarotation can occur, very slowly perhaps, in any solvent so long as the sugar is in some degree soluble in the solvent

ACKNOWLEDGMENTS

We are indebted to Dr T. E Acree, Dr C Y Lee, and Mr R. M. Butts for recording most of the gas chromatograms We thank the National Institutes of Health for financial support

REFERENCES

1 W PIGMAN AND H S ISBELL, *Advan Carbohyd Chem*, 23 (1968) 11
2 R BENTLEY AND N BOTLOCK, *Anal Biochem*, 20 (1967) 312
3 T M LOWRY, *J Chem Soc*, 127 (1925) 1371
4 J N BRONSTED AND E A GUGGENHEIM, *J Amer Chem Soc*, 49 (1927) 2554
5 T M LOWRY AND I J FAULKNER, *J Chem Soc*, 127 (1925) 2883
6 T M LOWRY AND E M RICHARDS, *J Chem Soc*, 127 (1925) 1385
7 A M EASTHAM, E L BLACKALL, AND G A LATREMOUILLE, *J Amer Chem Soc*, 77 (1955) 2182
8 C G SWAIN AND J F BROWN, JR, *J Amer Chem Soc*, 74 (1952) 2534
9 A DE GRANDCHAMP-CHAUDUN, *Compt Rend*, 247 (1958) 1511, *Ann Pharm Franc*, 26 (1968) 115
10 C C SWEELEY, R BENTLEY, M MAKITA, AND W W WELLS, *J Amer Chem Soc*, 85 (1963) 2497
11 C Y LEE, T E ACREE, AND R S SHALLENBERGER, *Carbohyd Res*, 9 (1969) 356, T E ACREE, R S SHALLENBERGER, C Y LEE, AND J W EINSET, *Carbohyd Res*, 10 (1969) 355
12 A A FROST AND R G PEARSON, *Kinetics and Mechanism*, 2nd Ed, John Wiley and Sons, New York, 1961, p 185

Carbohydrate Research
Elsevier Publishing Company, Amsterdam
Printed in Belgium

REACTION OF ALKYL VINYL ETHERS WITH D-GALACTOSE DIETHYL DITHIOACETAL

M. L WOLFROM* AND G G PAREKH
Department of Chemistry, The Ohio State University, Columbus, Ohio 43210 (U S A)
(Received April 9th, 1969, in revised form, June 16th, 1969)

ABSTRACT

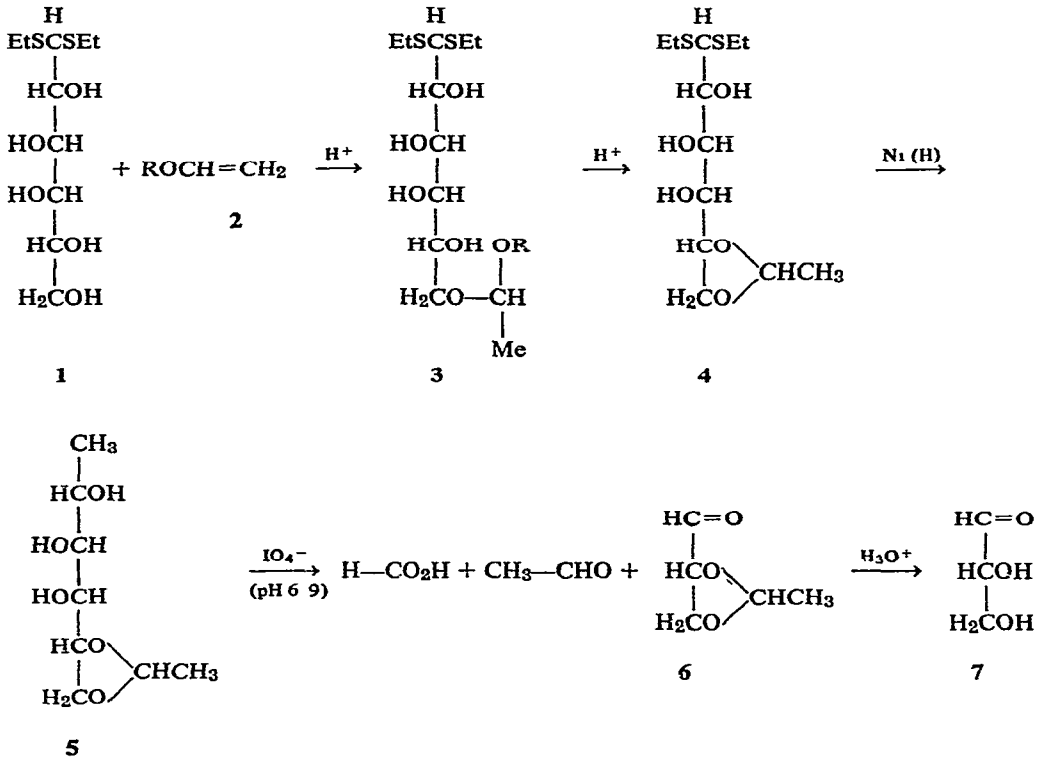
D-Galactose diethyl dithioacetal was brought into reaction, under acid catalysis, with each of several alkyl vinyl ethers in equimolar quantities The major reaction product, 5,6-*O*-ethylidene-D-galactose diethyl dithioacetal, was isolated and its structure was proved by reductive desulfurization with subsequent periodate oxidation This structure was confirmed by mass spectral data. When ethyl vinyl ether was used, the minor reaction-product was 6-*O*-(1-ethoxyethyl)-D-galactose diethyl dithioacetal. A pathway is proposed for this reaction. 5,6-*O*-Ethylidene-D-galactose diethyl acetal was partially demercaptalated to ethyl 5,6-*O*-ethylidene-1-thio-α-D-galactofuranoside, whose structure was confirmed through its synthesis by the reaction of isopropyl vinyl ether with ethyl 1-thio-α-D-galactofuranoside under acid catalysis

INTRODUCTION

Vinyl ethers react with hydroxyl groups, under acid catalysis, to form mixed acetals[1] It has been reported[2] that the acid-catalyzed reaction of methyl vinyl ether with ethylene 1-thioglycol gave 2-methyl-1,3-oxathiolane. Watanabe[3] obtained excellent yields of oxazolidines by the reaction of butoxyethyl vinyl ether with ethanolamines in the presence of mercuric benzoate. Shostakovskiĭ and co-workers[4,5] isolated 1-*O*-(1-ethoxyethyl)-2,3-*O*-ethylideneglycerol and 1,2 3,4-di-*O*-ethylidene-pentaerythritol as the main reaction-products in the acid-catalyzed reaction of ethyl vinyl ether with glycerol and pentaerythritol, respectively. The synthesis has been reported[6] of methyl 4,6-*O*-ethylidene-α-D-glucopyranoside by the reaction of alkyl vinyl ethers with methyl α-D-glucopyranoside under acid catalysis A reaction mechanism for the formation of the ethylidene derivative was proposed.

In the present paper, extension of such a reaction of alkyl vinyl ethers (2) with D-galactose diethyl dithioacetal (1) is described. This work was undertaken to investigate the possibility of protecting the terminal 5- and 6-hydroxyl groups of an acyclic hexose derivative in order to synthesize a furanoid structure The analogous formation of furanoid forms in the pentose series, by suitable protection of the terminal hydroxyl group, is well established, and has been used especially in the

*Deceased, June 20th, 1969.



where R = Et, Me_2CH, or Me_3C·

synthesis of nucleosides[7 8] In the model compound chosen herein, ring closure by partial demercaptalation with mercuric chloride[9] should give a 1-thiofuranoside, possibly in higher yield than is obtained[10 11] when the terminal 5,6 positions are not protected

EXPERIMENTAL

General methods — Melting points were determined with a Thomas–Hoover apparatus Specific rotations were determined in a 2-dm polarimeter tube I r. spectra were recorded with a Perkin–Elmer infrared spectrometer. N m r spectra (with tetramethylsilane as the internal reference-standard) were recorded with a Varian A-60 spectrometer or, by J H Lauterbach, on a Varian HA-100 spectrometer, with assignment of chemical shifts by decoupling Mass spectra were recorded with an MS-9 mass spectrometer by C R. Weisenberger under the direction of R. Dougherty X-Ray powder diffraction data give interplanar spacings, Å, CuKα radiation, λ 1 539 Å, nickel filter, camera diameter 114 6 mm, photographic recording Relative intensities were estimated visually: m, moderate, s, strong, v, very, w, weak, parenthetical numerals indicate the order of the most intense line· 1, most intense, multiple numbers indicate approximately equal intensities Ascending t.l c was performed on

0 25–1 25-mm layers of Silica Gel G (E Merck, Darmstadt, Germany), activated at 110°, with indication by sulfuric acid and 1.1 (v/v) chloroform–acetone as the developer Partition chromatography was effected on silica gel (60–200 mesh, Grace Davison Chemical Co) by using 4 1 (v/v) chloroform–acetone Trimethylsilyl ethers were prepared according to Sweeley and associates[1,2] G l c was conducted with a Beckman GC-5 instrument, the column packing contained 15% SE-52 silicone gum rubber (Wilkens Instrument and Research, Inc) supported on Chromosorb-W (80–100 mesh, Johns–Manville Products Co.) Microanalytical determinations were made by W N Rond Unless otherwise mentioned, solvents were evaporated under diminished pressure below 35° (water aspirator)

Reaction of D-*galactose diethyl dithioacetal* (**1**) *with one molar equivalent of alkyl vinyl ethers, preparation of 5,6-*O*-ethylidene-*D*-galactose diethyl dithioacetal* (**4**) — D-Galactose diethyl dithioacetal (**1**, 10 6 g) was dissolved in dry *N,N*-dimethylformamide (40 ml) One molar equivalent of the alkyl vinyl ether (see Table I) was added, followed by a catalytic amount of *p*-toluenesulfonic acid (100 mg) The

TABLE I

DEPENDENCE OF YIELD OF 5,6-ETHYLIDENE ACETAL OF D-GALACTOSE DIETHYL DITHIOACETAL ON SIZE OF ALKYL GROUP OF REACTANT VINYL ETHER[a]

Alkyl vinyl ether	*Yield (%) of 5,6-O-ethylidene acetal* (**4**)
Ethyl	23
Isopropyl	41
t-Butyl	56

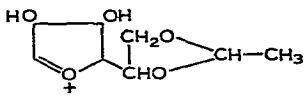
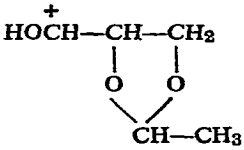
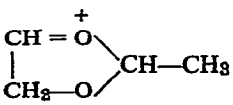
[a]See Experimental section for details

reaction mixture was shaken for 2 5 h at room temperature Sodium carbonate (1 g) was added to the reaction mixture, and the mixture was shaken for 0 5 h The solvent was evaporated and, after addition of chloroform to the residue, the mixture was kept overnight in a refrigerator The small amount of starting material deposited was separated from the chloroform solution by filtration The syrup obtained on removal of the chloroform consisted of two principal components, as indicated by t l c On addition of ether–petroleum ether (b p 30–60°), the major component (**4**) crystallized partially The remaining **4** was isolated from the syrup by partition chromatography The total yields obtained are given in Table I, m p 120°, $[\alpha]_D^{20}$ +72 ±2° (*c* 1 3, chloroform), λ_{max}^{KBr} 2 83 (OH), 3.40 (C–H), 6 91, 7 10, 7 26, 7.36, 7.47, 7 86, 8 05, 8 66, 9 05, 9 25, 9 50, 9 75, 9 90, 10 20, 11 36, 11 65, 11 97, and 13 05 μm; X-ray powder diffraction data 11 62 s (1), 6 85 vw, 5 85 s (3), 4.91 m, 4.69 m, 4.37 s (2), 4 10 m, 3 71 w, 3.58 w, 3 45 w, 3 32 vw, 3 09 m, 3.00 w, 2 91 m, 2 76 m, 2 61 m, 2 57 m, 2 52 m, and 2 22 m, mass spectral data are given in Table II.

Anal Calc for $C_{12}H_{24}O_5S_2$ C, 46 16; H, 7.69; S, 20 51 Found: C, 46 26, H, 7.85, S, 20 43

TABLE II

MASS SPECTRUM OF 5,6-*O*-ETHYLIDENE-D-GALACTOSE DIETHYL DITHIOACETAL (4)

m/e	*Relative intensity*, %	*Postulated structure*
312	13	$[M^+]^+$
233	10-15	$[M^+-(C_2H_5S + H_2O)]^+$
189	10-15	
177	10-15	$[M-CH(SC_2H_5)_2]^+$
171	10-15	189–H_2O
159	10-15	177–H_2O
135	100	$CH(SC_2H_5)_2^+$
117	8	
107	10-15	$CH_3-CH_2-S-\overset{+}{C}H-SH$
105	10-15	$C_2H_5\overset{+}{S}H-CH=CHOH$
87	58	
75	55	$\overset{+}{C}H_2-S-CH_2-CH_3$

Isolation of 6-O-(1-ethoxyethyl)-D-galactose diethyl dithioacetal (3, *R* = *Et*) *by reaction of D-galactose diethyl dithioacetal with ethyl vinyl ether* — D-Galactose diethyl dithioacetal (1) and ethyl vinyl ether (2, R = Et) were brought into reaction as already described. The minor reaction product, R_F 0 65, was isolated by preparative t l c The compound crystallized with difficulty from chloroform–petroleum ether (b.p 30–60°), m p. 113°, yield 300 mg (2%), $[\alpha]_D^{20}$ +64° (*c* 1, chloroform), λ_{max}^{KBr} 2 91 (OH), 3 40 (C–H), 6 95, 7 25, 7 50, 7 90, 8 80, 9 15, 9 40, 9 80, 10 80, 11.30, 13.00, and 13.30 μm, X-ray powder diffraction data 13.67 s (1), 11.39 m, 8.18 vw, 6 83 m, 5 24 w, 4.89 s (2), 4 60 m, 4 26 m, 4 09 m, 3 88 m, 3 70 s (3), 3.50 m, 3 21 m, 3.06 w, and 2 82 w.

Anal. Calc for $C_{14}H_{30}O_6S_2$: C, 46 92, H, 8 37, S, 17 87 Found. C, 46 78; H, 8.09; S, 17.60.

1-Deoxy-5,6-O-ethylidene-D-galactitol (5) — Raney nickel (30 g) was added to a solution of 5,6-*O*-ethylidene-D-galactose diethyl dithioacetal (4, 2 g) in 95% ethanol

(250 ml), and the mixture was refluxed for 5 h The Raney nickel was removed by filtration, and extracted with hot ethanol On evaporation of the solvent from the combined filtrate and extract, a crystalline product (5) was obtained which was recrystallized from ethanol–petroleum ether (b.p 30–60°), yield 870 mg (64%), m p 141–142°, $[\alpha]_D^{20}$ −14° (*c* 1 0, ethanol), X-ray powder diffraction data: 10.97 s, 6 88 vs (3), 5 53 vw, 4.99 m, 4.71 s, 4 44 vs (1), 4 34 vs (2), 4.14 m, 3 63 w, 3.47 m, 3 33 s, 3 21 m, 3 03 m, 2 87 s, 2.63 vw, 2 55 w, 2 46 m, 2.42 w, and 2 30 m.

Anal. Calc for $C_8H_{16}O_5$ C, 50 00, H, 8 33. Found. C, 50.05; H, 8.42, IO_4^- consumption, 2 0 moles/mole.

*Isolative, periodate oxidation of 1-deoxy-5,6-O-ethylidene-*D*-galactitol* (5). — The reduced product 5 (1 92 g, 0.01 mole) was added to a solution of sodium metaperiodate (4 2 g, 0 02 mole) in 100 ml of water. The solution was maintained at pH 6 9 by addition of M sodium hydroxide solution, and kept at room temperature in the dark After 2 h, the solution was distilled under diminished pressure at room temperature, the distillate was collected in a cooled, saturated solution of (2,4-dinitrophenyl)hydrazine in 2M hydrochloric acid The yellow precipitate obtained was filtered off, and recrystallized from ethanol, yield 1 9 g (86%), m p 146°, identical with that of the (2,4-dinitrophenyl)hydrazone of acetaldehyde

The undistilled residue was extracted with chloroform The syrup obtained on removal of the solvent from the dried extract (sodium sulfate) was treated with sulfuric acid (25 mM, 10 ml) and kept overnight at room temperature After removal of sulfate ion with barium ion, the solution was evaporated to dryness The dimedone derivative was obtained from the resultant syrupy residue by the standard method, m p 196°, $[\alpha]_D^{20}$ +207° (*c* 1 2, ethanol), lit [13] m p 194–200°, $[\alpha]_D^{27}$ +208° (ethanol) for the dimedone derivative of D-glyceraldehyde.

*Preparation of 6-*O*-(1-ethoxyethyl)-*D*-galactose diethyl dithioacetal* (3) *from tetra-*O*-acetyl-*D*-galactose diethyl dithioacetal* — 2,3,4,5-Tetra-*O*-acetyl-*aldehydo*-D-galactose diethyl dithioacetal was prepared by the method of Wolfrom and co-workers[11], m p 97°, n m r data (100 MHz, Me_2SO-d_6) τ 4.00 (proton quartet, $J_{1,2}$ 8 Hz, $J_{2,3}$ 2 5 Hz, H-3), τ 4 34 (proton quartet, $J_{3,4}$ 8 Hz, $J_{4,5}$ 2 5 Hz, H-4), τ 4 50 (proton quartet, $J_{1,2}$ 8 Hz, $J_{2,3}$ 2 5 Hz, H-2), τ 4 49–4 70 (2-proton multiplet, H-5 and -OH), τ 5 77 (one-proton doublet, $J_{1,2}$ 8 Hz, H-1), τ 6 28 (2-proton triplet, becoming a doublet in the presence of water, $J_{5,6}$ 5 8 Hz, $J_{6,6}$ 5 8 Hz, H-6), τ 6 90–7.15 (4-proton multiplet, methylene of EtS), τ 7 60–7 65 (12-proton multiplet, acetyl), τ 8 46 (3-proton triplet, *J* 7 Hz, methyl of EtS), and τ 8 48 (3-proton triplet, *J* 7 Hz, methyl of EtS)

To the solution of the tetra-*O*-acetyl derivative (2 g) in chloroform (20 ml) were added ethyl vinyl ether (0 5 g) and *p*-toluenesulfonic acid (10 mg), and the temperature was kept for 15 min below 10° The mixture was washed with aqueous sodium hydrogen carbonate solution, dried (sodium sulfate), and evaporated to a syrup, the i r spectrum of which showed the absence of free hydroxyl group The syrup was dissolved in methanol (10 ml), 0 5 M sodium methoxide solution (1 ml) was added, and the mixture was kept overnight below 5°; it was then made neutral

with Amberlite IR-120 (H^+) resin. After removal of the resin, the solution was evaporated, and the product was recrystallized from chloroform–petroleum ether (b.p 30–60°); yield 400 mg (25%); m p. 113°; i r. spectrum and X-ray powder diffraction pattern identical with those of product 3 already described.

Acid-catalyzed conversion of 6-O-(*1-ethoxyethyl*)-D-*galactose diethyl dithioacetal* (3) *into the 5,6-ethylidene acetal* (4) — To a solution of the 6-ether (3, 50 mg) in dichloromethane (50 ml) was added *p*-toluenesulfonic acid (3 mg) The reaction was monitored by t l c , after 1.5 h, only traces of the 6-ether 3 were detected (R_F 0 65), but two other major compounds were present (R_F 0.75 and R_F 0). By t.l.c.

TABLE III

PARTIAL DEMERCAPTALATION[a] OF 5,6-*O*-ETHYLIDENE-D-GALACTOSE DIETYL DITHIOACETAL (4)

Mercuric oxide[b]	*Mercuric chloride*[b]	*T l c , spot intensity*[c] 4 (R_F 0 74)	9[e] (R_F 0 69)	10[f] (R_F 0 24)	*Approx yield*[d] *of* 9, %
2	0 5	—	m	vs	>20
2	0 5	—	m	s	~25
2 5	0 3	w	s	m	40–50
5	0 3	vw	vs	m–w	70–75
5	0 1	m	s	vw	40
5[g]	0 3	s	m	m	~20
2 5[g]	0 5	m	m–w	m	~20
2 5[g,h]	1 0	w	vw	s	>10

[a]See Experimental section for details, temperature 35 to 40°, solvent was acetone–water, unless otherwise noted [b]Molar ratio to 4 [c]Estimated visually m, medium, s, strong, v, very, w, weak [d]By visual estimation of t l c. spots [e]Ethyl 5,6-*O*-ethylidene-1-thio-α-D-galactofuranoside (9) [f]Considered to be 5,6-*O*-ethylidene-D-galactofuranose (10) [g]*N,N*-Dimethylformamide as solvent [h]Temperature, 55°.

comparison with authentic samples, these two compounds were found to be 4 and 1, respectively.

Partial demercaptalation of 5,6-O-*ethylidene*-D-*galactose diethyl dithioacetal* (4), *preparation of ethyl* 5,6-O-*ethylidene*-α-D-*galactofuranoside* (9) — Following the optimal conditions established by the data given in Table III, 5,6-*O*-ethylidene-D-galactose diethyl dithioacetal (4, 1 40 g, 4.5 mmoles) was dissolved in a solution of acetone (15 ml) in water (20 ml) by warming on a hot-water bath. The temperature was raised to 35°, and freshly prepared, yellow mercuric oxide (4 8 g, 22.5 mmoles) was added To the heterogeneous mixture, a solution of mercuric chloride (406 mg, 1.5 mmoles) in water (10 ml) was added dropwise, at 35°, with stirring, and the mixture was stirred for 24 h at 35°. A very small amount of the starting material then remained in the mixture, as indicated by t l c Pyridine (20 ml) was added, and the mixture was kept for several hours below 10°. The mixture was filtered, and the pyridine–mercuric chloride complex was washed with acetone and water

The filtrate and washings were combined, and evaporated in the presence of sodium carbonate, and the residue was extracted with ethyl acetate The extract was filtered through Celite, and dried (sodium sulfate). The syrup obtained on removal of solvent was a mixture of two components, together with a small amount of unconverted starting-material The major component (R_F 0 69) was separated by partition chromatography, it was recrystallized from ether–petroleum ether (b p. 30–60°), yield 0 81 g (37%), m.p. 80–81°, $[\alpha]_D^{20}$ +69° (*c* 1 26, chloroform), λ_{max}^{KBr} 2 90 (OH), 3 43 (C–H), 6 90, 7 04, 7 90, 8 23, 8 68, 9 08, 9 28, 9 8–10 (broad band), 10 25, 10.74, 11 00, 11.70, 12.93, 13 3, and 13 7 μm; n m r. data ($CDCl_3$) τ 4.50–4 80 (2-proton multiplet, H-6), τ 5 50–6 15 (6-proton multiplet, H-1,2,3,4,5 and methine of >CH-CH_3), τ 5 92 and τ 6 50 (protons of hydroxyl group on C-2 and C-3, signals disappeared in the presence of D_2O), τ 7 25 (2-proton quartet, *J* 7 Hz, methylene of EtS), and 8 47–8.80 (6-proton multiplet, methyl of ethylidene and EtS), X-ray powder diffraction data 10 21 s (3), 7 39 w, 5 15 s, 4.64 vs (2), 3 89 m, 3 65 vs (1), 3 50 m, 3 29 s, 3.13 w, 2 99 w, 2 87 s, 2.60 m, 2 54 m, 2 47 m, 2 33 w, 2 29 w, 2 17 m, 2.05 m, and 1.97 m

Anal Calc. for $C_{10}H_{18}O_5S$ C, 48.00, H, 7 20, S, 12 80 Found C, 47.77, H, 7.39, S, 12.94.

The g l c pattern obtained from the per(trimethylsilyl) derivative indicated the presence of a mixture of diastereoisomeric acetals in the ratio of ~9.1. As the two peaks were not completely resolved, it was not possible to find the exact ratio

Synthesis of ethyl 5,6-O-ethylidene-1-thio-α-D-galactofuranoside (**9**). — Syrupy ethyl 1-thio-α-D-galactofuranoside (**11**, 2 2 g, 0 01 mole), prepared according to the method of Wolfrom and co-workers[11], was dissolved in *N,N*-dimethylformamide (8 ml) To this solution was added isopropyl vinyl ether (0 8 g, 0 01 mole), followed by *p*-toluenesulfonic acid (40 mg), and the mixture was shaken for 2 5 h After neutralization of the acid with sodium carbonate, the solvent was evaporated, giving a syrup that showed one major spot (R_F 0 69) on t l c The major product was isolated in crystalline form by partition chromatography, and was recrystallized from ether–petroleum ether (b p. 30–60°); yield 0 60 g (27%), m p 81°, $[\alpha]_D^{20}$ +77° (*c* 0 84, chloroform) The i r. and n m r. spectral data were identical with those already reported for ethyl 5,6-*O*-ethylidene-α-D-galactofuranoside (**9**).

Anal Calc for $C_{10}H_{18}O_5S$ C, 48 00; H, 7 20, S, 12 80. Found C, 48 26, H, 7 08, S, 12 85

The g l c pattern of the per(trimethylsilyl) derivative indicated a mixture of diastereoisomeric acetals in the ratio of ~13 1. As the two peaks were not completely resolved, it was not possible to find the exact ratio

RESULTS AND DISCUSSION

Although it has been shown that vinyl ethers react preferentially, but not selectively, with primary alcohol groups of sugars[6,14], it should be possible to obtain a high degree of selectivity by proper control of the proportions of the reagents.

Equimolar quantities of D-galactose diethyl dithioacetal (**1**) and each of three alkyl vinyl ethers (**2**) were brought into reaction, under mild conditions, in *N,N*-dimethylformamide in the presence of *p*-toluenesulfonic acid as catalyst. It is noteworthy that the yield of **3** improved with increase in size of the alkyl group of the vinyl ether, in the series ethyl < isopropyl < t-butyl (see Table I)

The syrupy product was found, by t l c, to be a mixture of two principal compounds, both of which were obtained crystalline The major product corresponded in elementary analysis to a monoethylidene acetal of a hexose dithioacetal. On reaction of each of three alkyl vinyl ethers with D-galactose diethyl dithioacetal, each yielded this same, crystalline compound (see Table I), together with the other, minor product (R_F 0.65)

The mono-*O*-ethylidene derivative was proved to be 5,6-*O*-ethylidene-D-galactose diethyl dithioacetal (**4**) by reductive desulfurization[15], and subsequent periodate-oxidation studies Thus, periodate oxidation, at pH 6 9, of the reduction product **5** showed the consumption of 2 moles of oxidant (per mole of **4**) with the production of acetaldehyde and 2,3-*O*-ethylidene-D-glyceraldehyde (**6**, not isolated) The acetaldehyde was isolated, in quantitative yield, as the (2,4-dinitrophenyl)-hydrazone After removal of the ethylidene group by acid hydrolysis, the D-glyceraldehyde (**7**) was identified as its dimedone derivative[13].

The mass spectrum of **4** confirmed these results, as it gave a fragmentation pattern (see Table II) similar to those obtained from hexose dithioacetals[16]. The molecular ion peak at *m/e* 312 had an intensity of 13%, relative to that of the base peak at *m/e* 135. The base peak results from cleavage at the C-1–C-2 bond, with charge retention on C-1, where it is stabilized by two sulfur atoms The peak at *m/e* 87, having the relative intensity of 58%, could be accounted for by cleavage between C-4 and C-5, with charge retention on C-5 to form the fragment shown in Table II.

In order to obtain more information on the reaction pathway for the formation of **4**, the reaction of D-galactose diethyl dithioacetal (**1**) with ethyl vinyl ether was studied in more detail The other product (R_F 0 65) was isolated in crystalline condition by preparative t l c The substance showed elemental analyses in agreement with those required by a mono-(ethoxyethyl)-hexose diethyl dithioacetal (such as **3**, R = Et) The structure of **3** was established by a synthetic route

2,3,4,5-Tetra-*O*-acetyl-*aldehydo*-D-galactose diethyl dithioacetal, described by Wolfrom and co-workers[17], was shown by n m r analysis to have its free hydroxyl group on C-6, in methyl sulfoxide-d_6, on a 100-MHz instrument, the chemical shift of methylene protons on C-6 was a triplet at τ 6 28, $J_{5,6}$ 5 8 Hz, $J_{6,6-OH}$ 5 8 Hz, which became a doublet having a coupling constant of J 5 8 Hz when the spectrum was recorded in methyl sulfoxide-d_6 containing a trace of water On reaction with an equimolar amount of ethyl vinyl ether, at low temperature and under acid catalysis, followed by deacetylation, this tetraacetate yielded the same crystalline product (R_F 0.65) as was isolated from the reaction mixture Therefore, the structure of **3** (R = Et) is established as 6-*O*-(1-ethoxyethyl)-D-galactose diethyl dithioacetal Under

acid catalysis, **3** (R = Et) underwent further reaction, to give the 5,6-ethylidene acetal **4** The progress of the conversion could be followed by t l c.

The above results establish the pathway **1** + **2** → **3** → **4**. The 6-ether (**3**) is first formed, and then may undergo the postulated reactions shown in Fig 1. The pathway *A* indicates the reaction reversal, whereas *B* would lead to formation of the

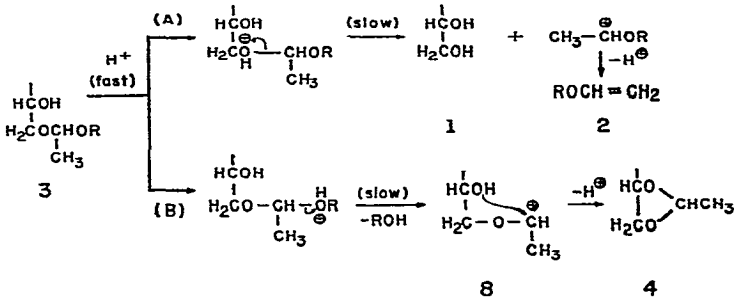


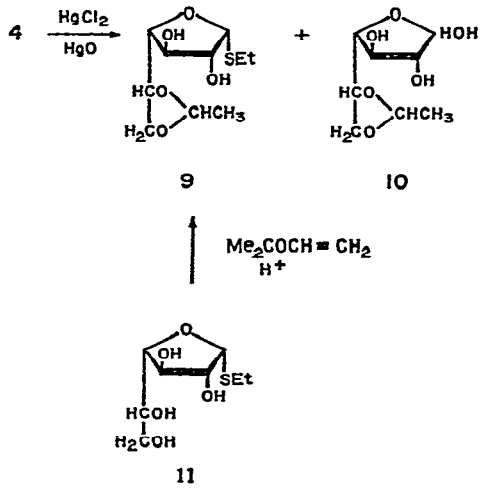
Fig 1 Postulated reaction-scheme for the conversion of the 6-ether **3** into the 5,6-ethylidene acetal **4**

5,6-ethylidene acetal The carbonium ion **8**, formed by the elimination of a molecule of an alcohol (ROH) in a slow reaction, would then react rapidly with the adjacent 5-hydroxyl group to give the 5,6-ethylidene acetal **4** This proposed reaction-course also explains the increasing yield of **4** (see Table I) on reaction of **1** with ethyl to isopropyl to t-butyl vinyl ether. The increasing steric requirement of the *R* group in the 6-ether (**3**) facilitates the formation of the carbonium ion (**8**) owing to release of steric strain in the slow step, and so can account for the increase in yield of **4** That the formation of carbonium ion is the slow step in the hydrolysis of acetals would appear to be well established[18] A similar enhancement of reaction has been reported[19] for the solvolysis of tertiary halides, in which cleavage of the carbon–halogen bond is facilitated by release of steric strain caused by the presence of bulky groups near the reaction site.

The alcohols formed during the reaction with ethyl, isopropyl, and t-butyl vinyl ether are ethyl, isopropyl, and t-butyl alcohol, respectively. The secondary and tertiary alcohols are less reactive than primary alcohols toward vinyl ethers and, thus, for isopropyl vinyl ether and t-butyl vinyl ether, the overall yields from the reaction are successively higher as compared with ethyl vinyl ether

In studies directed toward utilization of the 5,6-protected **4** in synthesis of furanosides, partial demercaptalation with mercuric chloride was employed. 5,6-*O*-Ethylidene-D-galactose diethyl dithioacetal (**4**) was treated with mercuric chloride and mercuric oxide in aqueous acetone, to afford crystalline ethyl 5,6-*O*-ethylidene-1-thio-α-D-galactofuranoside (**9**) The reaction was investigated by using various proportions of mercuric chloride and mercuric oxide, and temperatures ranging from 35 to 55° The reaction was also studied in an aprotic solvent, *N*,*N*-dimethylformamide, in which the reaction was slow, and free sugar (**10**) preponderated. The

progress of the reaction was monitored by t l c , the best results were obtained when only 0 3 molar equivalent of mercuric chloride and 5 molar equivalents of mercuric oxide were used in aqueous acetone The results obtained on using various proportions of mercuric chloride and mercuric oxide at 35–40° are tabulated in Table III.



The α-D anomeric configuration of C-1 in ethyl 5,6-*O*-ethylidene-1-thio-α-D-galactofuranoside (**9**) was established through synthesis of **9** by the reaction of isopropyl vinyl ether with syrupy ethyl 1-thio-α-D-galactofuranoside (**11**), of established structure[10], prepared according to Wolfrom and co-workers[11] The crystalline products obtained by each of these two methods were identical in melting point, i r. and n m r. spectra, and X-ray powder diffraction pattern The specific rotations of the two products were slightly different, $[\alpha]_D^{20}$ +69° (chloroform) for the product from **4**, and $[\alpha]_D^{20}$ +77° (chloroform) for that from ethyl 1-thio-α-D-galactofuranoside (**11**), due to the difference in the proportions of the two diastereoisomers resulting from the creation of the additional asymmetric center at the carbon atom in the 5,6-*O*-ethylidene group The difference in the proportions of the two diastereoisomers was indicated by the g l c pattern of their per(trimethylsilyl) ethers, but the two peaks detectable were insufficiently separated for an exact estimation of their relative proportions.

Although the yield of crude **9** obtained by the partial demercaptalation of **4** was ~70–75% (see Table III), only 37% of the pure crystalline product was obtained by partition chromatography This difference may have been due to the added isomerism provided by the asymmetry of the ethylidene carbon atom in the acetal The yield of crystalline **9** (~40%) is comparable to that (43%, as the crystalline tetraacetate) reported by Wolfrom and co-workers[10] for the partial demercaptalation of D-galactose diethyl dithioacetal (**1**) This reaction has been found to be difficultly

reproducible, but a reasonably reproducible yield of 48.5% was established[11] by partial hydrolysis of the dithioacetal **1** with acid

There is probably a higher proportion of furanoid material present in the partial-demercaptalation reaction-product than is represented by the yield of 1-thiofuranoside, because a portion very probably becomes completely demercaptalated without loss of the terminal, cyclic-acetal group. Such a possible product (**10**) was indicated by chromatographic evidence, but it was not characterized.

ACKNOWLEDGMENTS

This work was supported by the Agricultural Research Service, U. S Department of Agriculture, Grant No 12-14-100-7652(71) (O S U Research Foundation Project 1856), administered by the Northern Regional Research Laboratory, Peoria, Illinois Preliminary work on this problem was performed by S. S Bhattacharjee. We thank H Gausepohl for aid in preparing this work for publication. Purchase of an MS-9 mass spectrometer was made possibly by a grant from the National Science Foundation

REFERENCES

1 R PAUL, *Bull Soc Chim Fr* [5], 1 (1934) 971
2 J W COPENHAVER, British Patent 642,253 (1950), *Chem Abstracts*, 45 (1951) 4746
3 W H WATANABE, U. S Patent 2,752,357 (1956), *Chem Abstracts*, 51 (1957) 4445
4 M F SHOSTAKOVSKIĬ, V V ZHEBROVSKII, AND M A MEDELYANOVSKAYA, *Izv Akad Nauk SSSR Otd Khim Nauk*, (1955) 350, *Bull Acad Sci USSR, Div Chem Sci*, (1955) 313
5 M F SHOSTAKOVSKIĬ, A S ATAVIN, AND V V ZHEBROVSKII, *Izv Akad Nauk SSSR Otd Khim Nauk*, (1955) 539, *Bull Acaa Sci USSR, Div Chem Sci*, (1955) 477
6 M L WOLFROM, A BEATTIE, AND S S BHATTACHARJEE, *J Org Chem*, 33 (1968) 1067
7 J J FOX AND I WEMPEN, *Advan Carbohyd Chem*, 14 (1959) 283
8 J A MONTGOMERY AND H J THOMAS, *Advan Carbohyd Chem*, 17 (1962) 301
9 J W GREEN, *Advan Carbohyd Chem*, 21 (1966) 112
10 M L WOLFROM, Z YOSIZAWA, AND B O JULIANO, *J Org Chem*, 24 (1959) 1529
11 M L WOLFROM, P MCWAIN, R PAGNUCCO, AND A THOMPSON, *J Org Chem*, 29 (1964) 454
12 C L SWEELEY, R BENTLEY, M MAKITA, AND W W WELLS, *J Amer Chem Soc*, 85 (1963) 2497
13 A S PERLIN AND C BRICE, *Can J Chem*, 34 (1956) 85
14 M L WOLFROM, A BEATTIE, S S BHATTACHARJEE, AND G G PAREKH, *J Org Chem*, 33 (1968) 3990.
15 M L WOLFROM AND J V KARABINOS, *J Amer Chem Soc*, 66 (1964) 909
16 D C DEJONGH, *J Org Chem*, 30 (1965) 1563
17 M L WOLFROM, J L QUINN, AND C C CHRISTMAN, *J Amer Chem Soc*, 57 (1935) 713
18 E H CORDES, *Progr Phys Org Chem*, 4 (1967) 1
19 H C. BROWN AND H L BERNEIS, *J. Amer Chem Soc*, 75 (1953) 10

Carbohydrate Research
Elsevier Publishing Company, Amsterdam
Printed in Belgium

Note

The use of the periodate–Schiff spray reagents in the linkage analysis of oligosaccharides

A R ARCHIBALD AND J G BUCHANAN

Microbiological Research Laboratory, Department of Organic Chemistry, The University, Newcastle upon Tyne NE1 7RU (Great Britain)

(Received April 18th, 1969, in revised form, May 19th, 1969)

Barker and his colleagues have recently described a micromethod for the linkage analysis of oligosaccharides, based on thin-layer chromatography of their alkaline-degradation products[1]. In this note, we summarise some of the methods which have been used in this laboratory for linkage analysis, using the periodate–Schiff spray reagents[2] for the examination of oligosaccharides and their degradation products

Characteristic colours are produced when various carbohydrates and their derivatives are examined by the periodate–Schiff reagents[2] By using these reagents, the position of linkage in hexose-containing disaccharides can be established by paper chromatography of the disaccharide and its simple degradation products In some cases, the method may be extended to higher oligosaccharides

Periodate oxidation of (1→2)-linked disaccharides gives a substituted malonaldehyde derivative so that such compounds give an intense yellow colour with the periodate–Schiff reagents, whereas oxidation of the reduced disaccharide does not give malonaldehyde Thus, kojibiose and kojitriose give intense yellow colours[3] within 40 min of being sprayed, whereas the alcohols derived by prior reduction with sodium borohydride give a purple colour immediately, due to the formation of formaldehyde[2], and this colour changes to purple blue within 1 h Similar behaviour is observed with 2-*O*-α-D-glucopyranosyl-D-galactose and 2-*O*-methyl-D-galactose[4] Neither (1→6)-linked disaccharides, nor the alcohols derived from them, give rise to malonaldehyde intermediates, and such compounds give blue and purple-blue colours, respectively, with the spray reagents

Production of malonaldehyde intermediates on oxidation of (1→3)- and (1→4)-linked disaccharides requires prior hydrolysis of the formic ester group formed on C-5 of the reducing terminal sugar by cleavage between C-1 and C-2 This does not occur under the brief oxidation conditions used, so that maltose, maltotriose, cellobiose, and laminaribiose[3] all give blue colours with the spray reagents The derived alcohols, however, produce formaldehyde and malonaldehydes, leading either to purple spots with a yellow "halo" (when larger chromatographic samples are used) or yellow spots when the concentration is low[2] The (1→3)- and (1→4)-linked disaccha-

rides are therefore clearly distinguished from those having (1→2)- and (1→6)-linkages They may be readily distinguished from each other by examination of the saccharinic acids formed by alkaline degradation Isosaccharinic acids give a yellow colour with the spray reagents, which is to be expected from their structure Metasaccharinic acids, on the other hand, give a purple-blue colour This has been used in the identification of the saccharinic acids formed on alkaline degradation of the glycerol teichoic acid[5] from *Staphylococcus lactis* I3, and of the capsular polysaccharide[6] from *Pneumococcus Type* 11A Thus, degradation of 3-*O*-methyl-D-glucose forms a saccharinic acid which gives a purple-blue colour, whereas that produced by degradation of 4-*O*-methyl-D-galactose gives an intense yellow colour. Degradation of glucose and laminaribiose gives a product having similar chromatographic properties to that formed from 3-*O*-methylglucose, whereas degradation of maltose, cellobiose, and chitobiose gives, in addition, an intense yellow spot of similar chromatographic mobility.

The above procedures permit the rapid demonstration of the position of linkage in any hexopyranose disaccharide and may, in suitable cases, be extended to higher oligosaccharides A total of less than 1 mg is required for the complete examination, and the methods described have proved useful in this laboratory for the rapid, preliminary identification of small quantities of oligosaccharides derived from bacterial polymers

EXPERIMENTAL

Methods — Paper chromatography was carried out with Whatman No. 1 paper and propyl alcohol–water–conc ammonia (7 2·1) as solvent Compounds were detected by the periodate–Schiff reagents[2].

Sugars and their borohydride reduction products. — Di- and oligo-saccharides, and the alcohols derived from them by reduction with borohydride, were examined chromatographically, with the results given in the text[2–4].

Alkaline degradation of sugars — The sugar sample (1 0 mg) was dissolved in N sodium hydroxide (0 10 ml) and heated for 3 h at 100° in a sealed glass tube After being cooled, the solution was passed through a small column containing Dowex-50 (NH_4^+) resin (1 ml) which was then washed with water (5 ml) The eluate was evaporated to dryness *in vacuo* and dissolved in water (10–15 μl), and the solution was applied to a paper chromatogram (5 μl per spot)

The saccharinic acid obtained from 4-*O*-methyl-D-galactose gave an intense yellow spot, R_G 0 90, which developed within 5 min after spraying with Schiff's reagent This compound was clearly distinguished from those produced from glucose or (1→3)-linked disaccharides which gave a major spot of R_G 0 88 and a minor spot of R_G 0 96, both purple in colour In addition to, and largely obscuring, these two spots, (1→4)-linked disaccharides gave the strong yellow spot, which clearly distinguishes them from (1→3)-linked disaccharides

ACKNOWLEDGMENTS

We thank Professor J. Baddiley, F R S , for his interest, and Dr. A. R. Edgar for a sample of 4-*O*-methyl-D-galactose

REFERENCES

1 S A BARKER, J M EDWARDS, P J SOMERS, AND A REPAS, *Carbohyd Res* , 6 (1968) 341
2 F E HARDY AND J G BUCHANAN, *J Chem Soc* , (1963) 5881
3 A J WICKEN AND J BADDILEY, *Biochem J* , 87 (1963) 54
4 W K ROBERTS, J. G BUCHANAN, AND J BADDILEY, *Biochem J* , 88 (1963) 1
5 A. R. ARCHIBALD, J BADDILEY, AND D BUTTON, *Biochem J* , 95 (1965) 8C, 110 (1968) 543
6 D A KENNEDY, Ph D Thesis, University of Newcastle upon Tyne, 1964, D A. KENNEDY, J G BUCHANAN, AND J BADDILEY, unpublished results

Carbohydrate Research
Elsevier Publishing Company, Amsterdam
Printed in Belgium

Note

Heterocyclic amino sugar derivatives
Part III*. Epimino and oxazolidinone derivatives of 2-amino-2-deoxy-D-allose

WILLIAM D RHOADS AND PAUL H GROSS
Department of Chemistry, University of the Pacific, Stockton, California 95204 (U S A)
(Received May 1st, 1969)

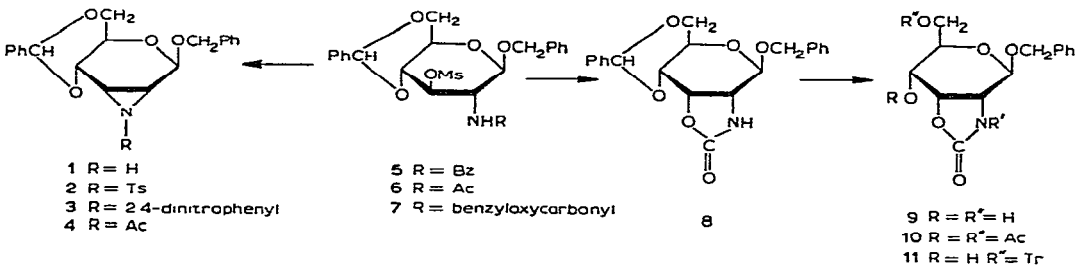
In a recent study of the reactions involving neighboring groups at C-2 and C-3 of 2-amino-2-deoxy-D-glucose and 2-amino-2-deoxy-D-altrose derivatives[1], the steric requirements and reaction conditions for tridentate neighboring groups were established in order to obtain 2,3-epimines or 5-membered heterocycles The diaxial *trans* disposition of the leaving group and of the neighboring group, the axial arrangement of the glycosidic groups, and the high basicity of the reagents are known to favor epimine formation[1]. Goodman has reviewed the influence of the conformation on these competitive reactions[2].

In the elimination of the mesyloxy group of benzyl 2-acylamino-4,6-*O*-benzylidene-2-deoxy-3-*O*-methylsulfonyl-β-D-glucopyranosides, only the basicity of the reagent was found to be of importance Sodium isopropoxide, which is more basic than is the previously used sodium ethoxide[3], was selected as the reagent. It had been used for the preparation of 3,4-epoxides of 2-amino-2-deoxy-D-galactose and -D-allose[3], and later[4] of 2,3-epimines by anchimerically assisted elimination of sulfonyloxy groups Irrespective of the substituent on the nitrogen atom, compounds **5**–**7** gave only benzyl 4,6-*O*-benzylidene-2,3-dideoxy-2,3-epimino-β-D-allopyranoside (**1**), although the leaving and participating groups had a diequatorial disposition, and no axial glycosidic group was hindering[1,5] formation of a 5-membered heterocycle Phenyl oxazoline, which could have resulted from **5**, and oxazolidinone, which could have resulted from **7**, were shown to be absent in the respective reaction mixtures The epimine **1** was characterized by *N*-tolylsulfonyl (**2**), *N*-(2,4-dinitrophenyl) (**3**), and *N*-acetyl (**4**) derivatives Attempts to cleave the epimino ring of the syrupy, but chromatographically pure, *N*-acetyl derivative **4** with ammonia gave only **1**.

When a weakly basic reagent, potassium acetate in aqueous 2-ethoxyethanol, was used, compounds **5**–**7** formed 5-membered heterocycles Whereas a stable phenyl oxazoline had resulted[6] from treatment of the *N*-benzoyl derivative **5**, the methyl

*For the previous paper in this series, see K Miyai and P H Gross, *J Org Chem*, 34 (1969) 1638
From the Ph D thesis, W D Rhoads, University of the Pacific, February, 1968

oxazoline, presumably resulting from the *N*-acetyl derivative **6**, was hydrolyzed under these conditions[6]. The *N*-(benzyloxycarbonyl) derivative **7** gave the oxazolidinone **8**, which was identical with an authentic sample[6] When the benzylidene group of **8** was split off, the resulting **9** could not be obtained in crystalline form The *N*-acetyl-di-*O*-acetyl derivative **10** and the 6-*O*-trityl derivative **11** were well characterized The latter compound is useful for disaccharide syntheses.



EXPERIMENTAL

Melting points were determined on a Thomas-Hoover melting-point apparatus model No 6404 H, and are uncorrected Optical rotations were measured with a Rudolph polarimeter, model No 956 Infrared spectra were recorded with a Perkin-Elmer Spectrophotometer, model 337, on potassium bromide pellets. All compounds were found to be homogeneous and different from their precursors by thin-layer chromatography on Silica Gel GF (Merck) with chloroform containing a sufficient portion of ethanol or hexane to produce R_F-values between 0 2 and 0 7 The spots were visualized by spraying with sulfuric acid (10–15%) in methanol, and heating at 120° The microanalyses were performed by Alfred Bernhardt of the Mikroanalytisches Laboratorium, Max-Planck-Institut fur Kohlenforschung, Muhlheim, Germany.

Benzyl 4,6-O-benzylidene-2,3-dideoxy-2,3-epimino-β-D-allopyranoside (**1**) — Sodium (0 23 g, 10 mmoles) was dissolved in 2-propanol (10 ml) and anhydrous dioxane (30 ml). Benzyl 2-acylamino-4,6-*O*-benzylidene-2-deoxy-3-*O*-methylsulfonyl-β-D-glucopyranoside (4 mmoles, acyl = benzoyl[7], acetyl[8], or benzyloxycarbonyl[9]) was added to this solution, and the mixture was heated for 18 h at reflux, cooled, and filtered The filtrate was evaporated *in vacuo*, and the residue was treated with water The precipitate was filtered off and recrystallized from methanol to give flaky crystals (0.8 g, 62%), m.p. 149°, $[\alpha]_D^{23}$ −5° (*c* 1.0, pyridine), ν_{max}^{KBr}. 3320 (epimine), 740 and 690 (Ph) cm^{-1}

Anal Calc. for $C_{20}H_{21}NO_4$ C, 70 80; H, 6 24; N, 4 15; O, 19.85. Found C, 70 93; H, 6 38; N, 4 20, O, 19 02.

The *N*-acetyl derivative, benzyl 2,3-acetylepimino-4,6-*O*-benzylidene-2,3-dideoxy-β-D-allopyranoside (**4**) was prepared from **1** (0 45 g) with pyridine (5 ml) and acetic anhydride (0 29 g) at 20°. After 3 days, the resulting solution was poured

onto ice, whereupon **4** precipitated as a syrup It was washed with ice-cold water, dried by azeotropic evaporation *in vacuo* with ethanol and toluene, and gave a syrup (0 37 g) which was found to be homogeneous and different from **1** on t l c.; ν_{max}^{KBr} 1710 (*N*-Ac) cm^{-1}. The N-H absorption at 3320 cm^{-1} shown by **1** was absent in the i r. spectrum of **4**

Benzyl 4,6-O-benzylidene-2,3-dideoxy-2,3-(p-tolylsulfonyl)epimino-β-D-allopyranoside (**2**). — A solution of **1** (0.5 g, 1 52 mmole) in anhydrous pyridine (10 ml) was treated with *p*-toluenesulfonyl chloride (0 35 g, 18 3 mmoles) at 10°. After 12 h at 0°, the mixture was poured onto ice, and the resulting precipitate was filtered off and recrystallized from a large volume of 2-propanol to give white needles (0.44 g, 60%), m.p 266–267°, $[\alpha]_D^{26}$ −17 5° (*c* 1.0, pyridine), ν_{max}^{KBr} 1330, 1160 (sulfonamide), 755, 730, and 710 (Ph) cm^{-1}.

Anal Calc. for $C_{27}H_{27}NO_6$ C, 65 70; H, 5 51; N, 2 84; O, 19 45 Found C, 65 58; H, 5.56; N, 2.94, O, 19.68

Benzyl 4,6-O-benzylidene-2,3-dideoxy-2,3-(2,4-dinitrophenyl)epimino-β-D-allopyranoside (**3**) — A mixture of **1** (0 5 g, 1.5 mmole), sodium hydrogen carbonate (1 g), 1-fluoro-2,4-dinitrobenzene (0.3 g, 1 8 mmole), and *N,N*-dimethylformamide (7 5 ml) was stirred for 22 h at 25° Ice–water was added, and the resulting precipitate was filtered off It was recrystallized from 2-propanol to give light-yellow crystals (0 39 g, 51%); m.p 72–74°, $[\alpha]_D^{23}$ −271° (*c* 1.0, pyridine), ν_{max}^{KBr} 1600, 1525, 835 (dinitrophenyl), 1340 (Ph-N), and 700 (Ph) cm^{-1}

Anal. Calc for $C_{26}H_{23}N_3O_8$: C, 61.78, H, 4.59, N, 8.31; O, 25 32 Found C, 61 54, H, 4 95, N, 8 22; O, 25 44

Benzyl 4',6'-O-benzylidene-2'-deoxy-β-D-allopyranosido[2',3' 4,5]-2-oxazolidinone (**8**) — Benzyl 4,6-*O*-benzylidene-2-(benzyloxycarbonyl)amido-2-deoxy-3-*O*-methylsulfonyl-β-D-allopyranoside (**7**) (3 0 g, 54 mmoles) and potassium acetate (3 g) in 2-ethoxy-ethanol (90 ml) containing 5% of water was heated for 5 days under reflux The reaction mixture was kept 24 h at 0° and filtered The filtrate was evaporated *in vacuo*, and the syrupy residue was treated with excess water. The tan crystals which formed were collected, washed with water, dried, and recrystallized twice from absolute ethanol to give 0 9 g (43%) of long, white needles, m p. 205–206°, $[\alpha]_D^{25}$ +13° (*c* 1.0, pyridine), ν_{max}^{KBr} 3300, 1750 (oxazolidinone), no amide II absorption, and 700 (Ph) cm^{-1} The identity with authentic material[6] was established by comparison of the i r spectra (identical), mixed m p. 206°, and identical mobility on t l c

Benzyl 4',6'-di-O-acetyl-2'-deoxy-β-D-allopyranosido[2',3'·4,5]-1-acetyl-2-oxazolidinone (**10**) — To a solution of **8** (0 6 g, 1 6 mmole) in glacial acetic acid (22 ml), water (12 ml) was added dropwise during 50 min at 80°. The solvents were removed *in vacuo* and then by coevaporation with ethanol and toluene The residual syrup, presumably benzyl-2'-deoxy-β-D-allopyranosido[2',3' 4,5]-2-oxazolidinone **9** was found to be different from **10** and homogeneous on t l c It was dissolved in pyridine (5 ml), treated with acetic anhydride (4 3 g, 42 mmoles) for 4 days at 20°, and poured onto ice The syrupy precipitate was treated with water, dried azeotropically with ethanol and toluene, and recrystallized from 2-propanol to give white flakes (0 21 g,

32%), m p. 89–91°, $[\alpha]_D^{23}$ +39° (*c* 1.0, pyridine); ν_{max}^{KBr} 1785 (*O*-Ac), 1740 (oxazolidinone), 1695 (*N*-Ac), and 705 (Ph) cm^{-1}.

Anal. Calc for $C_{20}H_{23}NO_9$ C, 57.00, H, 5.50; N, 3 33; O, 34 17. Found C, 57.09; H, 5 58, N, 3.16, O, 34 53.

Benzyl 2'-deoxy-6-O-triphenylmethyl-β-D-allopyranosido[2',3' 4,5]-2-oxazolidinone (**11**) — A solution of **9**, prepared from **8** (0 6 g) as above, in pyridine (2 ml) was shaken with chlorotriphenylmethane for 24 h at 25°. The mixture was poured into ice–water, and the precipitate was collected, dried, and recrystallized from a mixture of toluene, ether, and hexane to give white crystals (0 2 g, 24%), m p. 215–217°, $[\alpha]_D^{22}$ −9° (*c* 1 0, pyridine); ν_{max}^{KBr} 3400, 1760 (oxazolidinone), and 705 (Ph) cm^{-1}.

Anal. Calc. for $C_{33}H_{31}NO_6$ C, 73 72, H, 5 81, N, 2 61; O, 17 86 Found C, 73 88, H, 5 81, N, 2 80, O, 17 93

ACKNOWLEDGMENTS

Helpful discussions with Dr H K Zimmerman are acknowledged This work was supported, in part, by a grant (GP-4587) from the National Science Foundation

REFERENCES

1 B R Baker and T L Hullar, *J Org. Chem*, 30 (1965) 4038
2 L Goodman, *Advan Carbohyd Chem*, 22 (1967) 175
3 P H Gross, K Brendel, and H K Zimmerman, *Ann Chem*, 680 (1964) 155
4 Y. Ali, A C Richardson, C F Gibbs, and L Hough, *Carbohyd Res*, 7 (1968) 255
5 D H Buss, L Hough, and A C Richardson, *J Chem Soc*, (1963) 5295
6 K. Miyai, K K Zimmerman, and P H Gross, *J Org Chem*, 34 (1969) 1635
7 K Miyai, P H Gross, and H K Zimmerman, *Ann Chem*, 722 (1969) 210
8 P H Gross and R W Jeanloz, *J Org Chem*, 32 (1967) 2759
9 P H Gross and H K Zimmerman, *Ann Chem*, 674 (1964) 211

Carbohydrate Research
Elsevier Publishing Company Amsterdam
Printed in Belgium

Note

Periodate oxidation of methyl 4,6-*O*-benzylidene-α-D-alloside and -β-D-mannoside

C B BARLOW AND R D GUTHRIE
School of Molecular Sciences, University of Sussex, Brighton BN1 9QJ (Great Britain)
(Received May 5th, 1969)

The periodate oxidation of methyl 4,6-*O*-benzylidene-α-D-alloside and -β-D-mannoside in aqueous solution has been studied for comparison with the oxidation of amino sugars[1], and as an extension of the work of Honeyman and Shaw[2]. Second-order rate constants at pH 4.06 and 25° were determined, by the usual procedure (*cf.* ref. 1), and are given in Table I.

TABLE I

SECOND-ORDER RATE CONSTANTS FOR THE PERIODATE OXIDATION OF METHYL 4,6-*O*-BENZYLIDENE-D-GLYCOPYRANOSIDES, AT pH 4.06 AND 25°

Methyl 4,6-O-benzylidene-D-glycoside	$10^3 k_2$ (*l mole*$^{-1}$ *sec*$^{-1}$)	*Ref.*
α-Alloside	190	—
α-Mannoside	15.9	2
β-Mannoside	82	—
α-Glucoside	2.88	2
β-Glucoside	1.13	2
β-Guloside	35.5	2
α-Guloside	319	2
α-Galactoside	3.69	2
β-Galactoside	2.09	2

Honeyman and Shaw[2] found that for compounds having an equatorial C-2 hydroxyl group, such as the methyl 4,6-*O*-benzylidene-D-glycosides of gulose, glucose, and galactose, methyl 4,6-*O*-ethylidene-D-glucoside, and methyl 4-*O*-methyl-D-glucopyranoside, the second-order rate constant for the α-D anomer was greater than that for the β-D anomer. This rate difference between anomers in which the methoxyl group is axial for the α-D form and equatorial for the β-D form was explained[2] as due to a larger "steric interference" to complex formation with periodate when the methoxyl group is equatorial than when it is axial. The exact nature of this steric interference was not discussed.

The rate constant for periodate oxidation of the β anomer of methyl 4,6-*O*-benzylidene-D-mannoside has now been shown to be *greater* than that for the

α anomer. This result is the first comparison between anomeric pyranosides possessing an axial C-2 hydroxyl group, and suggests that interference from the axial aglycone in the α-D anomer must now be greater than that from the equatorial one in the β-D compound This is consistent with the earlier findings, in that the anomer having the *cis*-1,2 arrangement of groups is oxidised more rapidly, but does not allow a detailed explanation of the results.

The oxidation results for methyl 4,6-*O*-benzylidene-α-D-alloside complete a stereochemical series, the *altro*, *gluco*, and *manno* compounds having already been studied[2] Of the rate constants for the four glycosides, that for alloside is the largest This result is not inconsistent with the idea of a cyclic periodate–diol complex, as suggested for the other compounds[2] The greater rate constant of the alloside may be explained by greater ease of complexing with periodate brought about as a means of relieving the 1,3-diaxial interaction between the C-3 hydroxyl and C-1 methoxyl groups The same explanation could account for the much faster oxidation of methyl 4,6-*O*-benzylidene-α-D-guloside with respect to its β anomer[2], since the former compound again has a 1,3-diaxial interaction in the *C1* conformation

The rate constant for the alloside relative to those for the other diols is consistent with the results obtained for the amino sugars[1]. As the amino group present in the two amino-allosides may help to form a more stable periodate–sugar complex than would a hydroxyl group, the 1,3-diaxial interaction favouring the initial complex formation may be masked

In view of the above data, the study of methyl 4,6-*O*-benzylidene-β-D-alloside, and the corresponding α- and β-D-talopyranosides would be of great interest It is predicted that the β-D-alloside will be oxidised at a slower rate than its α-D anomer, since the latter has the *cis*-1,2 grouping.

ACKNOWLEDGMENTS

We thank Dr P. Garegg for a sample of methyl 4,6-*O*-benzylidene-β-D-mannopyranoside, and the S R C. for the award of a research studentship (to C B B)

REFERENCES

1 C B BARLOW AND R D GUTHRIE, *Carbohyd Res*, 11 (1969) 53
2 J HONEYMAN AND C J G SHAW, *J Chem Soc*, (1959) 2454

Carbohydrate Research
Elsevier Publishing Company, Amsterdam
Printed in Belgium

Note

2-Deoxy sugars
Part XVIII. The anomers of 3β-(2-deoxy-D-*lyxo*-hexopyranosyloxy)-14β-hydroxy-5β-card-20(22)-enolide*

W. WERNER ZORBACH, SILVANO L. DEBERNARDO, AND K. VENKATRAMANA BHAT

Department of Bio-Organic Chemistry, Gulf South Research Institute, New Iberia, Louisiana 70560 (U. S. A.)

(Received May 20th, 1969)

The work described constitutes an extension of our earlier studies[1] designed to obtain a series of cardiac glycosides having minimal structural variations in the sugar component in the hope of clarifying the relation between the structure of the carbohydrate residue and the cardiotonic activity of the cardiac glycoside. In particular, we have shown that the 2-deoxy-β-D-*arabino*-hexoside and the 2-deoxy-β-D-*ribo*-hexoside of digitoxigenin [3β,14β-dihydroxy-5β-card-20(22)-enolide, **1**] have approximately the same potency, and it appears, therefore, that a reversal of the configuration at C-3 of the carbohydrate residue has no effect. However, for all of the glycosides of digitoxigenin (**1**) thus far studied (see Table I, Ref. 1), the hydroxyl group on C-4 of the sugar residue is an equatorial substituent, and it is for this reason that we undertook the synthesis of the title glycoside (4-OH axial), differing from the 2-deoxy-β-D-*arabino*-hexoside only with respect to a reversal of configuration at this carbon atom.

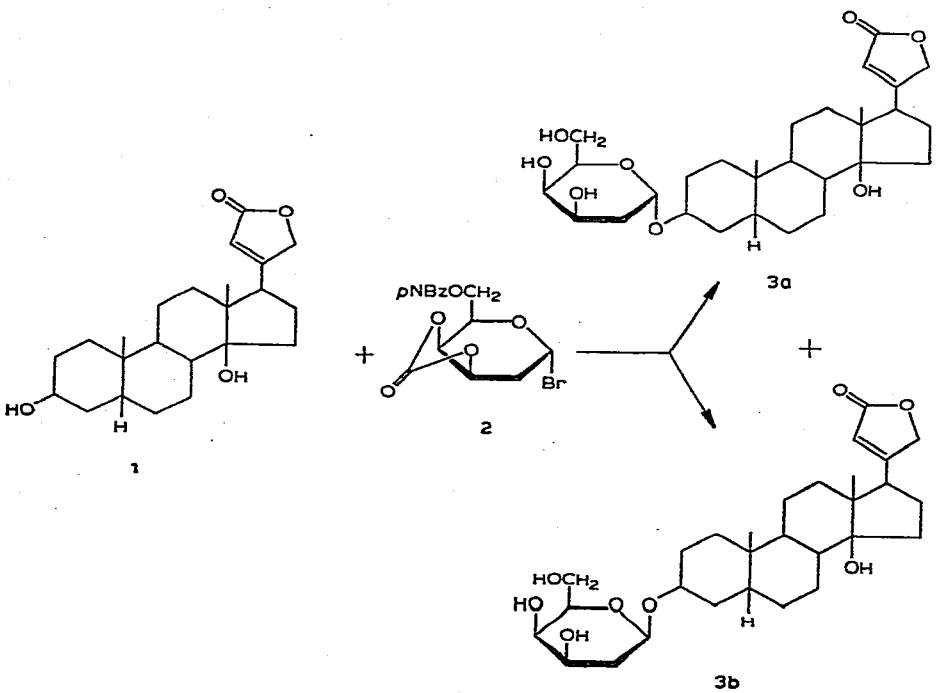
To this end, and also with a view to synthesizing some pyrimidine nucleosides containing 2-deoxy-β-D-*lyxo*-hexopyranose residues, we undertook, and were successful in, the preparation of crystalline 2-deoxy-3,4,6-tri-*O*-*p*-nitrobenzoyl-α-D-*lyxo*-hexosyl bromide[2]. In contrast to similarly constituted halides of other 2-deoxyhexoses[2], the new halide failed to condense with dialkoxypyrimidines, even at elevated temperatures, and, because of this situation, it appeared unlikely that the bromide would have utility in the synthesis of cardiac glycosides.

We have attributed the failure of the *p*-nitrobenzoylated halide to form *N*-glycosyl derivatives to the axially oriented *p*-nitrobenzoyloxy group on C-4, which, apparently, causes C-1 to become a hindered position[2]. Accordingly, we sought to prepare a halide of 2-deoxy-D-*lyxo*-hexose in which the substituent at C-4 would have the smallest possible bulk, and, subsequently, were successful in converting the sugar, in six steps, into crystalline 3,4-*O*-carbonyl-2-deoxy-6-*O*-*p*-nitrobenzoyl-α-D-*lyxo*-hexosyl bromide[3] (**2**). The latter halide readily underwent reaction with dialkoxy-

*This work was supported by Grant No. GSRI-NS257, from the Louisiana State Science Foundation.

pyrimidines at room temperature, and, from these results, it appeared that the halide would have utility in the preparation of some new cardiac glycosides.

Digitoxigenin (**1**) was treated with the new bromide (**2**) under conditions of a modified Koenigs–Knorr synthesis (see Ref. 1, pp. 307–308), affording an anomeric mixture in low yield. The acylated intermediates were not isolated, but were saponified in aqueous methanol with sodium hydrogen carbonate to give the unsubstituted glycosides (**3a** and **3b**). The anomeric configuration of each was determined by the method of molecular rotational additivities[4].



EXPERIMENTAL

Melting points were determined with a Kofler hot-stage, and optical rotations were measured with a Rudolph Model 80 polarimeter. T.l.c. was performed on 250-μm silica gel (Camag DF-5) plates with 1:1:8 butyl alcohol–2,2,4-trimethylpentane–ethyl acetate. The spots were visibilized by spraying either with Kedde reagent[5] or with 80% sulfuric acid, followed by heating the plates at 110° for 5 min.

*3β-(2-Deoxy-α and β-*D*-*lyxo*-hexopyranosyloxy)-14β-hydroxy-5β-card-20(22)-enolide* (**3a** *and* **3b**). — Dry, freshly prepared silver carbonate (1.4 g), 740 mg (2 mmoles) of digitoxigenin (**1**), and 70 ml of dry 1,2-dichloroethane were placed in a 100-ml, 2-necked flask equipped with a dropping funnel and a condenser. The mixture was heated, with magnetic stirring, in an oil bath (105°), and about 20 ml of the solvent was distilled off. A solution of 1.61 g (4 mmoles) of the bromide (**2**) in 100 ml of dry

1,2-dichloroethane (contained in the dropping funnel) was added, with efficient stirring, during 2 h, distillation of the solvent from the reaction flask being maintained at a rate equal to that of the addition of the solution of the bromide. An additional 100 ml of 1,2-dichloroethane was added during 2 h, under the same conditions as those for the addition of the solution of the bromide. To the reaction mixture was added 30 ml of acetone, the mixture was filtered, and the silver salts were thoroughly washed with acetone. The filtrate was evaporated almost to dryness, the residue was dissolved in 50 ml of tetrahydrofuran, and the solution was diluted with 500 ml of methanol, followed by the addition of 200 ml of 1.65% aqueous sodium hydrogen carbonate. The solution was kept for 6 days at room temperature, and then concentrated to about 200 ml by evaporation under diminished pressure at 40°. The mixture was extracted with four 150-ml portions of chloroform, and the aqueous layer (*A*) was preserved. The chloroform extracts were combined, dried with sodium sulfate, and evaporated to dryness under diminished pressure at 40°. The resulting residue was extracted with three 50-ml portions of ether, which were discarded, and the residue was dissolved in 3 ml of absolute ethyl alcohol, followed by the addition of 15 ml of ether. Pentane was added to incipient turbidity, and the mixture was kept in a refrigerator overnight. The crystals that formed were filtered off, giving 85 mg (8.2%, based on **1**) of the α-D-glycoside (**3a**), m.p. 238–248°. Two recrystallizations from ethyl alcohol–ether–pentane afforded 46 mg of pure **3a**, m.p. 248–250°, $[\alpha]_D^{23}$ +73.4° (*c* 0.46, ethyl alcohol), λ_{max}^{EtOH} 217 nm (log ε 4.27), homogeneous by t.l.c. (R_F 0.19). Calc. for [M] (digitoxigenin + methyl 2-deoxy-α-D-*lyxo*-hexopyranoside)[6]: +71° + 292° = +363°. Found for [M] (**3a**): +382°. The glycoside has, therefore, the α-D configuration.

Anal. Calc. for $C_{29}H_{44}O_8$: C, 66.89; H, 8.52. Found: C, 66.74; H, 8.57.

The aqueous layer (*A*) was extracted with five 150-ml portions of 1:4 ethyl alcohol–chloroform and five 150-ml portions of 1:3 ethyl alcohol–chloroform. The extracts were combined, dried with sodium sulfate, and evaporated to dryness under diminished pressure at 40°. The resulting residue was chromatographed on a column (5 × 40 cm) of 220 g of Silica Gel (E. Merck, Darmstadt; 0.05–0.2 mm) premixed with 100 ml of water. Elution was conducted with water-saturated ethyl acetate, and the first 100 ml of eluate was discarded. Collection was made in 6-ml fractions, and, from fractions 64–84, an additional 35 mg of the α-D glycoside was secured, bringing the total yield of **3a** to 11.5%. Fractions 85–145 were combined, and evaporated to dryness under diminished pressure, and the residue was dissolved in 1 ml of absolute ethyl alcohol, followed by the addition of 8 ml of ether and 5 ml of pentane, affording 35 mg (3.4%) of the β-D-glycoside (**3b**), m.p. 195–200° $[\alpha]_D^{23}$ +23.7° (*c* 0.20, ethyl alcohol), λ_{max}^{EtOH} 217 nm (log ε 4.27), homogeneous by t.l.c. (R_F 0.15). Calc. for [M] (digitoxigenin + methyl 2-deoxy-β-D-*lyxo*-hexopyranoside)[6]: +71° −83° = −12°. Found for [M] (**3b**): +123°. The glycoside has, therefore, the β-D configuration.

Anal. Calc. for $C_{29}H_{44}O_8 \cdot H_2O$: C, 64.66; H, 8.61. Found: C, 64.82; H, 8.54.

REFERENCES

1 W. W. ZORBACH AND K. V. BHAT, *Advan. Carbohyd. Chem.*, 21 (1966) 273.
2 W. W. ZORBACH, C. C. BHAT, AND K. V. BHAT, *Advan. Chem. Ser.*, 74 (1968) 1.
3 W. W. ZORBACH, S. L. DEBERNARDO, AND K. V. BHAT, *Carbohyd. Res.*, 11 (1969) 413.
4 W. KLYNE, *Biochem. J.*, 47 (1950) xli.
5 D. L. KEDDE, *Pharm. Weekblad*, 82 (1947) 741.
6 C. C. BHAT, K. V. BHAT, AND W. W. ZORBACH, *Carbohyd. Res.*, 10 (1969) 197.

Carbohydrate Research
Elsevier Publishing Company, Amsterdam
Printed in Belgium

Note

Quantitative determination with periodate of compounds subject to non-Malapradian oxidation
Part III. Cyclohexanehexols

S ROBERT SARFATI AND PATRICIA SZABO
Institut de Biochimie, Faculte des Sciences, 91 Orsay (France)
(Received May 23rd, 1969)

It is well known[1,2] that periodate oxidations of the inositols are "anomalous" under the usual conditions of oxidation, instead of reduction of six molar equivalents of the oxidant and formation of six equivalents of formic acid, about 6 7 equivalents of periodate are reduced and about 5 equivalents of formic acid are formed Carbon dioxide is also a product in these oxidations and is formed, in all probability, from glyoxylic acid, which has been shown to be an intermediate in the periodate oxidation of *myo*-inositol[3,4] and which is known to be oxidised by periodate to carbon dioxide and formic acid[5-8]

We now report that when dilute solutions of inositols in 0 1N sulphuric acid are oxidised with periodate at 0° ("cold acid" method[9]), each inositol reduces exactly six molar equivalents of periodate Glyoxylic acid, which is not oxidised by periodate in these conditions[10], is formed in amounts which vary slightly according to the inositol oxidised (Table I)

TABLE I
PERIODATE OXIDATION OF INOSITOLS

Inositol (cyclonexanehexol)	*Equiv of IO_4^- reduced*	*Equiv of glyoxylic acid formed*
L-*chiro*-(1,2,4/3,5,6)	6 0	0 85
muco-(1,2,4,5/3,6)	5 9	0 8
myo-(1,2,3,5/4,6)	6 0	0 7
epi-(1,2,3,4,5/6)	5 95	0 7
scyllo-(1,3,5/2,4,6)	5 95	0 66

It is generally accepted that the first intermediate in the periodate cleavage of cyclitols is a hexodialdose, which may then be further oxidised either in the straight-chain form or as a cyclic tautomer Possible pathways for the oxidation of inositols *via* either of these forms have been proposed by Schwarz[4]. From the straight-chain form, glyoxylic acid is formed *via* hydroxymalonaldehyde/triose reductone, whereas the cyclic form would yield a formic ester of hydroxymalonaldehyde, which upon hydroxylation, cleavage by periodate, and hydrolysis would also yield glyoxylic

acid. In the conditions of the cold acid method, oxidation of the inositols by any of the mechanisms proposed by Schwarz would entail the reduction of six molar equivalents of periodate, since glyoxylic acid is then not attacked by periodate. The question as to which pathways are actually involved thus remains open However, we have shown[9] that, in these conditions, over-oxidation, *i e*, hydroxylation, of malonaldehyde does not occur. Moreover, we have inferred[10] from these results that the methyl ether of hydroxymalonaldehyde, formed during the periodate oxidation of carbohydrate methyl ethers, also does not undergo a hydroxylation reaction, but rather forms, by enolisation, triose reductone methyl ether. Free triose reductone, formed by hydrolysis of this ether, is then oxidised stoichiometrically to yield formic and glyoxylic acids Similarly, it can be expected that the formic ester of hydroxymalonaldehyde mentioned above will enolise to give triose reductone formic ester. As it is known[11] that enol esters are easily hydrolysed, cleavage of the hydroxymalonaldehyde ester could proceed by this pathway rather than by the hydroxylation reaction.

It is interesting to note that Angyal and McHugh[12], who examined the initial rates of reaction of the inositols with periodate, found that *epi*-inositol reacted extremely rapidly with periodate, whereas, in our conditions, this inositol reacts relatively slowly (Fig. 1) Both inositols (*myo-* and *epi-*) which react more slowly with periodate in our conditions have, if their most favoured conformation is considered, an axial hydroxyl group flanked by two equatorial hydroxyl groups, a feature which has been considered[12] to be unfavorable for rapid reaction with periodate, whereas no such groupings are found in the two inositols (*muco-* and L-*chiro-*) which rapidly reduce periodate

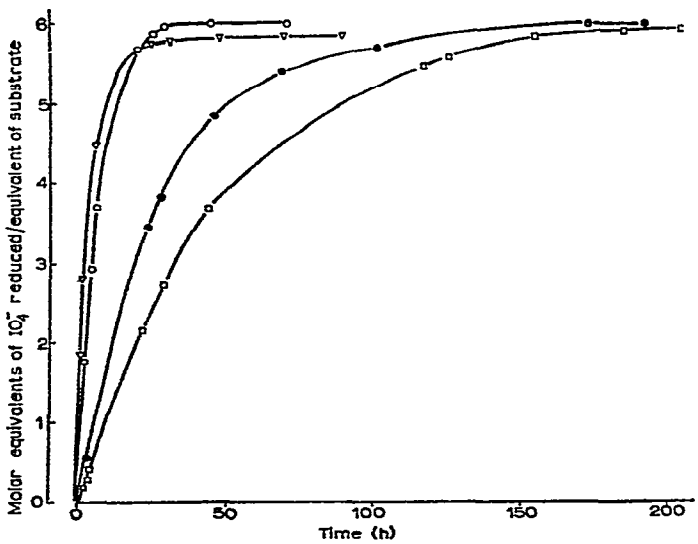


Fig 1 Periodate oxidation of L-*chiro-* (—○—), *muco-* (—▽—), *myo-* (—●—), and *epi-* (—□—) inositols.

EXPERIMENTAL

Periodate oxidations at 0° in 0.1N sulphuric acid (6×10^{-4}M in substrate and 6.6×10^{-3}M in sodium periodate)[9], and glyoxylic acid determinations[10] were carried out as described previously. L-*chiro*-inositol was prepared from (−)-quebrachitol[13].

ACKNOWLEDGMENTS

We thank Professor S. J. Angyal for gifts of *epi*- and *muco*-inositols, Dr. V. Plouvier for the *scyllo*-inositol, and Dr. S. D. Gero for the (−)-quebrachitol. We also thank the Ligue Nationale Française contre le Cancer for a grant (to S. R. S.).

REFERENCES

1 S. J. Angyal and L. Anderson, *Advan. Carbohyd. Chem.*, 14 (1959) 135
2 T. Posternak, *Les Cyclitols*, Hermann, Paris, 1962, pp. 44, 68.
3 P. Fleury and L. LeDizet, *Bull. Soc. Chim. Biol.*, 37 (1955) 1099
4 J. C. P. Schwarz, *Chem. Ind.* (London), (1955) 1388
5 C. F. Huebner, S. R. Ames, and E. C. Bubl, *J. Amer. Chem. Soc.*, 68 (1946) 1621
6 P. Fleury and G. Bon-Bernatets, *J. Pharm. Chem.* [8], 23 (1936) 85
7 D. B. Sprinson and E. Chargaff, *J. Biol. Chem.*, 164 (1946) 433
8 L. Hough, T. J. Taylor, G. H. S. Thomas, and B. M. Woods, *J. Chem. Soc.*, (1958) 1212
9 P. Szabo and L. Szabo, *Carbohyd. Res.*, 4 (1967) 206
10 J. P. Girma, M. T. Rokicka, and P. Szabo, *J. Chem. Soc. (C)*, (1969) 909
11 L. F. Fieser and M. Fieser, *Advanced Organic Chemistry*, Reinhold, New York, 1962, p. 410
12 S. J. Angyal and D. J. McHugh, *J. Chem. Soc.*, (1957) 1423
13 C. Tanret, *Compt. Rend.*, 109 (1889) 908, *cf.* S. J. Angyal and R. M. Hoskinson, *Methods Carbohyd. Chem.*, 2 (1963) 87

Carbohydrate Research
Elsevier Publishing Company, Amsterdam
Printed in Belgium

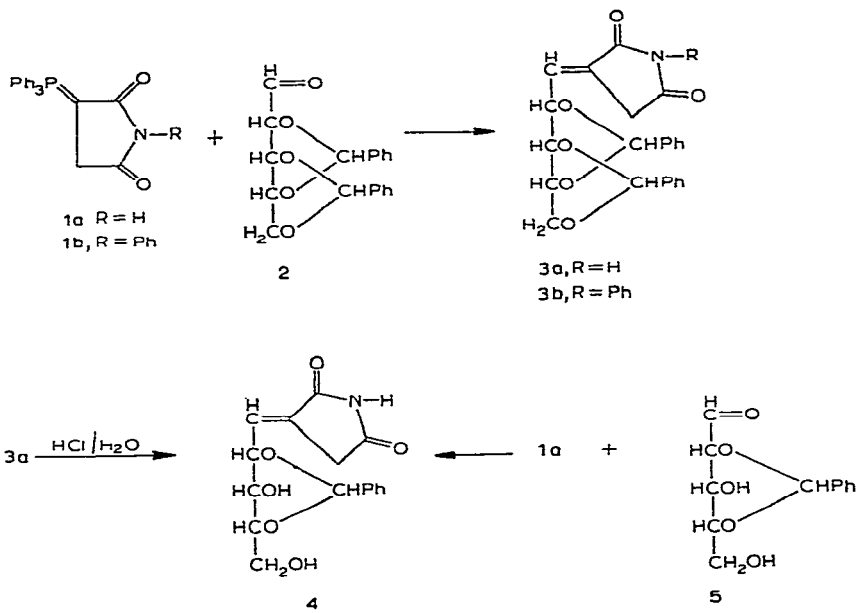
Note

The reaction of ylids of maleimide with derivatives of D-ribose

ROBERT E HARMON, GEORGE WELLMAN, AND S K GUPTA
Department of Chemistry, Western Michigan University, Kalamazoo, Michigan 49001 (U S A)
(Received June 12th, 1969)

Because of the importance of long-chain monosaccharides as intermediates in the synthesis of other carbohydrates, we have investigated the reaction of triphenylphosphoranylidenesuccinimide[1] (**1a**) and its *N*-phenyl derivative **1b** with derivatives of D-ribose The reaction of D-ribose with the ylids **1a** and **1b** was studied in order that intermediates for the total synthesis of the antibiotic Showdomycin[2] might be prepared The reaction of resonance-stabilized ylids with several monosaccharides, as well as with acetylated *aldehydo* monosaccharides, has been reported[3]

Attempts to cause unprotected D-ribose and D-glucose to react directly with the ylids **1a** and **1b** were unsuccessful Similarly, the reaction of acetylated *aldehydo*-D-ribose and *aldehydo*-D-glucose with these ylids did not lead to any isolable product In contrast, reaction occurred between 2,4 3,5-di-*O*-benzylidene-*aldehydo*-D-ribose (**2**) (prepared by the procedure of Potgieter and MacDonald[4]) and the ylids **1a** and **1b**, and respectively afforded **3a** and **3b**, each in ~85% yield When **3a** was treated with



aqueous hydrochloric acid, selective hydrolysis of the 3,5-*O*-benzylidene group occurred, yielding 2,4-*O*-benzylidene-1-deoxy-1-(2,5-dioxo-3-pyrrolidinylidene)-D-ribitol (**4**) Compound **4** could also be prepared by the reaction of 2,4-*O*-benzylidene-*aldehydo*-D-ribose[4] (**5**) with the ylid **1a**

In summary, the reaction of ylids **1a** and **1b** derived from maleimide and *N*-phenylmaleimide with benzylidene-protected *aldehydo*-D-ribose (compounds **2** and **5**) gives the corresponding Wittig reaction-products

EXPERIMENTAL

Melting points were determined with a Thomas–Hoover melting-point apparatus and are corrected The elemental analyses were performed by Galbraith Laboratories, Inc , Knoxville, Tennessee 37921. A Beckman IR-8 spectrophotometer was used for recording the i r spectra The ylids **1a** and **1b** were prepared by the procedure of Heyda and Theodoropulos[1] The method of Potgieter and MacDonald[4] was used for preparing the D-ribose derivatives **2** and **5**

2,4 3,5-Di-O-benzylidene-1-deoxy-1-(2,5-dioxo-3-pyrrolidinylidene)-D-ribitol (**3a**) — To a solution of **2** (1 7 g, 5 mmoles) in ethanol (200 ml) was added the ylid **1a** (1 7 g, 5 mmoles) with stirring. After 1 h, the solution was concentrated under diminished pressure to 75 ml, and cooled to 10° The white precipitate thus obtained was filtered off, dried, and crystallized from absolute ethanol, to yield 1 7 g (86%) of colorless needles, m.p. 194–195°, $[\alpha]_D^{25}$ −94 1° (*c* 0.12, ethanol) ν_{max}^{Nujol} 3180 (NH), 1770, 1730, 1680 cm^{-1} (C = O)

Anal. Calc. for $C_{23}H_{21}NO_6$ C, 67 80, H, 5 16, N, 3 44 Found C, 67 81, H, 5 31, N, 3 40

2,4 3,5-Di-O-benzylidene-1-deoxy-1-(2,5-dioxo-1-phenyl-3-pyrrolidinylidene)-D-ribitol (**3b**) — Reaction of the ylid **1b** with **2** by the foregoing procedure afforded **3b** in 85% yield, m p. 212–213°, $[\alpha]_D^{25}$ −143 2° (*c* 0 38, ethanol), ν_{max}^{Nujol} 1770, 1710, 1680 cm^{-1} (C=O)

Anal Calc for $C_{29}H_{26}NO_6$ C, 72 04, H, 5 17, N, 2 90 Found C, 72 25, H, 5 32, N, 2 91

2,4-O-Benzylidene-1-deoxy-1-(2,5-dioxo-3-pyrrolidinylidene)-D-ribitol (**4**) —

Method A To a solution of **3a** (3 g, 7.3 mmoles) in methanol (300 ml) was added concentrated hydrochloric acid (3 ml) The solution was stirred for 5 h at 30°, and then evaporated under diminished pressure to an oil, this was triturated with cold water, and the resulting precipitate was crystallized from water to yield colorless needles which, after being dried at 110°/0 1 mmHg, afforded 1 6 g (63%) of **4**, m.p. 189–190°, ν_{max}^{Nujol} 3500 (CH), 3380 (NH), 1760, 1720, and 1680 cm^{-1} (C = O).

Anal Calc for $C_{16}H_{17}NO_6$ C, 60 18, H, 5 36, N, 4 38 Found C. 59.93; H, 5 31; N, 4 22

Method B Reaction of **5** with the ylid **1a** by the procedure used for the preparation of **3b** afforded a 51% yield of **4**, m p 189–190° The m p of a mixture of the products from Methods A and B was undepressed, and the i r spectra of the two samples were superposable

ACKNOWLEDGMENT

This work was supported by Grant No CA-06140 from the National Cancer Institute.

REFERENCES

1 E HEYDA AND S THEODOROPULOS, *Tetrahedron*, 24 (1968) 2241
2 Y NAKAGAWA, H KANO, Y TSUKUDA, AND H KAYAMA, *Tetrahedron Lett*, 42 (1967) 4105
3 N K KOCHETKOV AND B A DMITRIEV, *Chem Ind* (London), (1963) 864, *Dokl Akad Nauk SSSR* 151 (1963) 106, YU A ZHDANOV, G N DOROFEENKO, AND L A UZLOVA, *Zh Obshch Khim*, 33 (1963) 3444, 35 (1965) 181, M N MIRZAYANOVA, L P DAVYDOVA, AND G I SAMOKHVALOV, *Dokl Akad Nauk SSSR*, 173 (1967) 367, B A DMITRIEV, N E BAIRAMOVA, L V BOKINOVSKIĬ, AND N K KOCHETKOV, *ibid*, 173 (1967) 350
4 D S POTGIETER AND D L MACDONALD, *J Org Chem*, 26 (1961) 3934

Carbohydrate Research
Elsevier Publishing Company, Amsterdam
Printed in Belgium

Preliminary communication

Reaction of glycosyl chlorides with silver tetrafluoroborate

K IGARASHI, T HONMA, AND J. IRISAWA
Shionogi Research Laboratory, Shionogi & Co. Ltd., Fukushima-ku, Osaka (Japan)

(Received June 30th, 1969)

Recently, there has been a marked increase in interest in the synthesis of fluorinated carbohydrates[1].

We have found a new method for the synthesis of glycosyl fluorides under very mild, homogeneous conditions When a glycosyl chloride (200 mg) was added to an ice-cold solution of silver tetrafluoroborate[2] (138 mg, *ca* 1 2 mol) in anhydrous ether or toluene (7 5 ml) under anhydrous conditions and with stirring, silver chloride was immediately precipitated. Cold, saturated, aqueous sodium hydrogen carbonate (3 ml) was then added, the organic layer was separated, and the aqueous layer was extracted with dichloromethane The combined organic solutions were washed with water, dried, and evaporated The product was crystallized from ether and light petroleum (b p. 30–50°) after preparative t l c on silica gel with benzene–ether (1 1) The results are summarized in Table I.

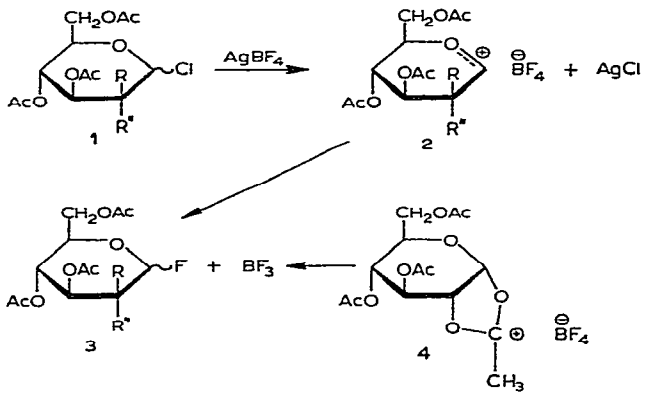
TABLE I

REACTION OF GLYCOSYL CHLORIDES WITH SILVER TETRAFLUOROBORATE[a,b]

Formula 1		*Anomeric configuration*	*Solvent*	*Reaction time (min)*	*Yield (%) of* 3 *isolated*	
R^1	R^2				α	β
H	Cl	α	Ether	15	60 5	17 7
			Toluene	60	76 3	~1[c,d]
H	Cl	β	Ether	15	62	15
			Toluene	60	77 2	~1[d]
Cl	H	α	Ether	15	70	18
			Toluene	60	91	~1[e,f]
Cl	H	β	Ether	15	72 8	19 8
			Toluene	60	91	~1[e,f]
H	OAc	α	Ether	15	49	24

[a]Molar ratio of glycosyl chloride and silver tetrafluoroborate was 1 1 2, unless otherwise stated [b]Unless otherwise stated, compounds obtained were proved to be identical with authentic samples (see ref 1) [c]Two molar equivalents of silver tetrafluoroborate were used because of the slower reaction rate [d]Quantitative analysis by g l c showed that α- and β-D-glucosyl fluorides were obtained in a ratio of 95 5 4 5 [e]Quantitative analysis by g l c showed that α- and β-D-mannosyl fluorides were obtained in a ratio of 98 9 1 1 [f]3,4,6-Tri-*O*-acetyl-2-chloro-2-deoxy-β-D-mannopyranosyl fluoride showed m p 88 5–89 5°, $[\alpha]_D^{21}$ −44 8 ±0 8° (*c* 1, chloroform)

When the reaction was carried out in ether, anomerization was not observed, and the α- and β-D-glycopyranosyl fluorides obtained correspond to the kinetically controlled products For reactions in toluene, the anomerization is so fast that an equilibrated mixture is obtained It is well known[3] that boron trifluoride not only forms a very stable complex with ether (but not with toluene) but also has a tendency to coordinate with reactive fluorine atoms of organic compounds to yield tetrafluoroborate ion. In ether, the reaction of glycopyranosyl fluorides **3** and boron trifluoride-etherate, giving the glycopyranosyl tetrafluoroborate ion-pair **2**, which is assumed to be the reaction intermediate, is prevented owing to the stability of the boron trifluoride-etherate but, in toluene, the reaction can easily take place.



An unexpected result was observed in the reaction of tetra-*O*-acetyl-α-D-glucopyranosyl chloride with silver tetrafluoroborate in ether The products were 2,3,4,6-tetra-*O*-acetyl-α- and -β-D-glucopyranosyl fluorides, but the cyclic acetoxonium tetrafluoroborate[4] **4** could not be obtained The glucosyl fluorides were also obtained in similar yields when compound **4** was dissolved in ether at 0°

Further investigations are in progress

REFERENCES

1 F MICHEEL AND A KLEMER, *Advan Carbohyd Chem*, 16 (1961) 85, N F TAYLOR, R F CHILDS, AND R V BRUNT, *Chem Ind* (London), (1964) 928, S COHEN, D LEVY, AND E D BERGMANN, *Chem Ind* (London), (1964) 1802, P W KENT AND J E G BARNETT, *J Chem Soc*, (1964) 2497, and references cited therein, C PEDERSEN, *Acta Chem Scand*, 20 (1966) 963, and references cited therein, L D HALL AND J F MANVILLE, *Can J Chem*, 47 (1969) 1, 19, 361, and 379, A D BARFORD, A B FOSTER, J H WESTWOOD, AND L D HALL, *Carbohyd Res*, 11 (1969) 287, and references cited therein

2 G. A OLAH AND H W QUINN, *J Inorg Nucl Chem*, 14 (1960) 295, K. HEYNS AND H PAULSEN, *Angew Chem*, 72 (1960) 349

3 H S BOOTH AND D R MARTIN, *Boron Trifluoride and Its Derivatives*, Wiley, New York, 1949, p 39

4 H. PAULSEN, W P TRAUTWEIN, F G ESPINOSA, AND K HEYNS, *Ber*, 100 (1967) 2822

Carbohydrate Research
Elsevier Publishing Company, Amsterdam
Printed in Belgium

Preliminary communication

Pyranoid compounds bearing parallel and cross-oriented dienol ether linkages

J KISS

Chemical Research Department, F Hoffmann-La Roche & Co , Ltd , Basel (Switzerland)

(Received September 20th, 1969, in revised form, November 6th, 1969)

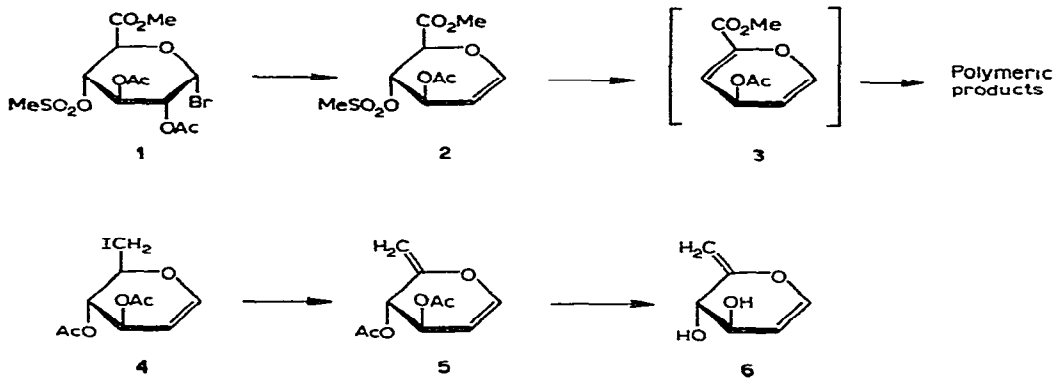
According to the stability principle of Brown and coworkers[1], 6-membered rings having an endocyclic double bond are more stable than those having an exocyclic double bond This situation contrasts with that for 5-membered rings having an olefinic linkage, for which an exocyclic double bond stabilizes, and an endocyclic one labilizes, the ring This stability principle has thus far been proved in the field of the carbocyclic olefins[2] We have studied Brown's stability principle for some isomeric sugar derivatives having an *endo-* or *exo*-cyclic enol ether linkage in the pyranoid or furanoid ring, and the results of these investigations will be reported elsewhere[3]

The question of ring stability in this field is important, because, during the past decade, some classes of natural products have been discovered that have a similar kind of (*a*) unsaturated, pyranoid structure containing an enol acetal linkage (*e g*, bacterial and enzymic degradation products of mucopolysaccharides[4], and enzymic degradation products of pectins[5]), or (*b*) unsaturated,furanoid structure containing an enol acetal linkage (*e g* [6], Angustmycin-A)

In the present communication are reported the preparation and characterization, and some preliminary indications concerning the stability, of some pyranoid compounds having a dienol ether linkage

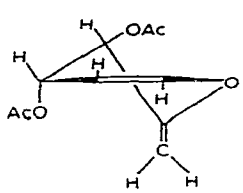
The dienol ether 3 (methyl 4-*O*-acetyl-2,6-anhydro-3,5-dideoxy-D-*glycero*-hex-2,5-dien-1-onate) having parallel double bonds was obtained by a mild β-elimination[7] of methanesulfonate at 20° caused by action of the theoretical amount of alkali (sodium methoxide or alkali acetate in methyl sulfoxide) on methyl 4-*O*-acetyl-2,6-anhydro-5-deoxy-3-*O*-(methylsulfonyl)-D-*lyxo*-hex-5-en-1-onate (2), m p 75–76°, $[\alpha]_D^{25}$ -34 3° (c 0 66, chloroform), obtained by reduction of the corresponding 1-deoxy-1-bromo-uronate[7] (1) with zinc–acetic acid by the method of E Fischer[8]

Compound 3 proved to be unstable, and it polymerized immediately, hence,we did not investigate (*a*) the extent to which spontaneous polymerization is the result of the two double bonds, which are parallel to each other, (*b*) the effect of the substituent on C-4, which is in an allylic position on both sides, or (*c*) the effect of the carboxyl group in this polymerization However, 4*H*-pyran[9], for example, is highly unstable at



room temperature in the presence of polymerization inhibitors in an atmosphere of inert gas at -70°, it can be stored for only a few days On the other hand, the 1,2-double bond of glycals prevents the pyranoid ring from being unstable Furthermore, the 4,5-unsaturated 4-deoxyhexopyranosiduronates[7] (similar to compound 2, which contains a bonded acrylic ester linkage in the pyranoid ring) are also stable

It was desired to test the stability of the pyranoid ring when the two enol ether double bond are cross-oriented to each other, *i e*, in a ring bearing *endo*- and *exo*-cyclic double bonds Such a system can be obtained from 3,4-di-*O*-acetyl-1,5-anhydro-2,6-dideoxy-6-iodo-D-*arabino*-hex-1-enitol (4), $[\alpha]_D^{25}$ -37° (*c* 0 42, chloroform), R_F 0 64 by t l c on Silica Gel with 3·7 (v/v) acetone–petroleum ether (b p 40–45°), by treating it with dry silver fluoride in dry pyridine by the method of Helferich and Himmen[10], to give the new compound 5, having *exo*- and *endo*-cyclic double bonds Compound 5 is stable it can be purified by column chromatography on Florisil or by vacuum distillation (b p 56–57°/0 02 torr) to give a colorless oil, n_D^{25} 1 4752, $[\alpha]_D^{25}$ -198° (*c* 0 58, chloroform) At 0°, it crystallizes, m p 40°, ν_{max}^{KBr} 3112, 898 (methylidene), 1754, 1230 (ester), 1674 and 1653 cm^{-1} (—C=C—), λ_{max}^{EtOH} 226 nm (ϵ_{mM} 8 6), o r d data (Cotton effect) $[\alpha]_{690}^{25}$ -126° (M_{690} -267°), $[\alpha]_{589}^{25}$ -178° (M_{589} -377°), $[\alpha]_{262}^{25}$ -1,378° (M_{262} -2,921°, min), $[\alpha]_{239}^{25}$ +2,880° (M_{239} +6,105°, sh), $[\alpha]_{220}^{25}$ +8 284° (M_{220} 17,562°, max), $[\alpha]_{217}^{25}$ +7,277° (M_{217} 15,427°) [*c* 0 210, 9mM, methanol (Dr K Noack)], c d data λ_{max}^{MeOH} 239 (Δ_ϵ +0 734), 215 (Δ_ϵ +6 23), λ_{min}^{MeOH} 235 (Δ_ϵ +0 576), λ_0^{MeOH}204 [25°, *c* 0 525 (Dr K Noack)]. The 100-MHz n m r. spectrum (obtained by Dr G. Englert) was measured in benzene-d_6 doublets at τ 3 8 (H-1), τ 5 18 and 5.44 (H-6 and H-6'), quartets at τ 4 39 (H-4), 4 78 (H-3) and 5 0 (H-2), $J_{1,2}$ 6 0 Hz, $J_{2,3}$ 5 0 Hz, $J_{3,4}$ 3 2 Hz, $J_{2,4}$ 1 5 Hz, $J_{6,6'}$ 1 3 Hz, $J_{1,6}$ 0 1–0 3 Hz, and $J_{1,3}$ 0 ± 0 3 Hz Hence, compound 5 exists in a half-chair (H_5^4) conformation



5

The mass-spectral behavior of 5 was studied (by Dr. W. Vetter) with an AEI-MS9 mass spectrometer. The main direction of fragmentation proved to be the loss of a molecule of acetic acid [the resulting fragment(s) having a mol.wt. of 152]; the product then decomposes by splitting off a molecule of ketene, giving stable system(s) having a mol.wt. of 110 (*e.g.*, a pyrillium ion having a π-electron sextet)[11].

3,4-Di-*O*-acetyl-1,5-anhydro-2,6-dideoxy-D-*threo*-hex-1,5-dienitol (5) is stable at room temperature (without the use of any polymerization inhibitor or an atmosphere of inert gas). The two acetyl groups can be split off by catalysis with sodium methoxide in methanol without decomposition or polymerization. The resulting 1,5-anhydro-2,6-dideoxy-D-*threo*-hex-1,5-dienitol (6) can be purified by column chromatography or by vacuum distillation [b.p. 90–95°(bath)/0.3 torr, Hickman still]; $[\alpha]_D^{25}$ -165° (*c* 0.2, chloroform); ν_{max}^{KBr} 3380, 1042 (OH), 1670, 1653 (–C=C–), and 868 cm^{-1} (methylidene); λ_{max}^{EtOH} 225 nm (ϵ_{mM} 10.3); o.r.d. data (Cotton effect): $[\alpha]_{690}^{25}$ -94° (M_{690} -120°), $[\alpha]_{589}^{25}$ -138° (M_{589} -176°), $[\alpha]_{240}^{25}$ -3,270° (M_{240} -4,185°, min), $[\alpha]_{227}^{25}$ 0°, $[\alpha]_{220}^{25}$ 13,878° (M_{220} 17,763°) [*c* 0.253, methanol (Dr. K. Noack)]; c.d. data: λ_{max}^{MeOH} 246 (Δ_ϵ +0.086), λ_{max}^{MeOH} 243 ($\Delta_\epsilon \pm 0°$), λ_{min}^{MeOH} 227–224 (Δ_ϵ -3.88), λ_{max}^{MeOH} 219–215 (Δ_ϵ -3.42), λ_{min}^{MeOH} 206 (Δ_ϵ -5.53) at 25° [(*c* 0.2528 (Dr. K. Noack)].

According to these preliminary observations, cross-orientation of two double bonds in a 6-membered ring system is a stabilizing factor; this phenomenon can be designated a type of "cross homoconjugative effect". From the point of view of the stability theory of Brown[1], it may be proposed that, in a 1,5-anhydro-2-deoxy-hex-1-enitol (glucal) system similar to a saturated, 5-membered ring system, the introduction of a second double bond in an endocyclic enol ether position labilizes, and, in an exocyclic position, stabilizes, the 6-membered ring (pyranoid) compounds.

We intend to test the validity of this suggested rule by study of further examples of substituted pyranosides, and also to investigate the same question in unsaturated, 5-membered ring, enol acetal furanoid systems by introducing a second double bond in the *endo*- and the *exo*-cyclic position[12].

REFERENCES

1 H. C. Brown, H. J. Brewster, and H. Shechter, *J. Amer. Chem. Soc.*, 76 (1954) 467.
2 R. B. Turner and R. H. Garner, *J. Amer. Chem. Soc.*, 80 (1958) 1424; M. Scholz and H.-J. Kohler, *Tetrahedron*, 25 (1969) 1863.
3 J. Kiss and F. Burkhardt, *Helv. Chim. Acta*, 52 (1969) in press, and further papers in preparation.
4 A. Linker, K. Meyer, and P. Hoffman, *J. Biol. Chem.*, 219 (1956) 13.
5 P. Albersheim, H. Neukom, and H. Deuel, *Helv. Chim. Acta*, 43 (1960) 1422.
6 H. Hoeksema, G. Slomp, and E. E. van Tamelen, *Tetrahedron Lett.*, (1964) 1878.
7 J. Kiss, *Carbohyd. Res.*, 10 (1969) 328.
8 E. Fischer, M. Bergmann, and H. Schotte, *Ber.*, 53 (1920) 510.
9 J. Straiting, H. J. Kaijer, E. Molenaar, and L. Brandsma, *Angew. Chem.*, 74 (1962) 465.
10 B. Helferich and E. Himmen, *Ber.*, 61 (1928) 1825.
11 P. A. Finan, R. J. Reed, W. Snedden, and J. M. Wilson, *J. Chem. Soc.*, (1963) 5945.
12 H. L. Rice, *J. Amer. Chem. Soc.*, 74 (1952) 3193.

Carbohydrate Research
Elsevier Publishing Company, Amsterdam
Printed in Belgium

Book review

Chemistry and Enzymology of Marine Algal Polysaccharides, by ELIZABETH PERCIVAL and RICHARD H MCDOWELL Academic Press Inc, London and New York, 1967, xii+219 pp, 60/-($12.00).

The long history of the study of marine algal polysaccharides has led to the gradual realization that these polysaccharides are somewhat different in composition from these polysaccharides of land plants Algae synthesize a wide variety of fascinating polysaccharides, some of which, such as the sulfated galactans, and fucoidan, are unique to the red and brown seaweeds, respectively. The general nature of the polysaccharides found in the common marine algae has been known for many years, and is described in numerous works of reference The more recent work, which is largely the result of rapidly developing investigations during the last decade, is widely scattered, and has been brought systematically into one volume for the first time in this book

Nine chapters are included; they are (1) Polysaccharides in living marine algae, (2) Elucidation of the structure of polysaccharides, (3) Food storage polysaccharides of the Phaeophyceae and Crysophyceae, (4) Other neutral polysaccharides, food reserve and structural, (5) Alginic acid, (6) and (7) Sulfated polysaccharides containing neutral sugars, (8) Polysaccharides containing uronic acid and ester sulfate, (9) Some comparisons of algal with other polysaccharides

The book has been written by Dr Elizabeth Percival (Royal Holloway College, London), a well-known international authority in the chemistry of marine algal polysaccharides, and Dr Richard H McDowell (Alginate Industries Ltd, London) The production is excellent; each chapter has an extensive bibliography in which the literature is covered well into 1967, and there is a good subject index

The emphasis in the book is on structure and the general relationships of polysaccharides as they occur in the algae, rather than on commercial products from seaweeds. However, some space is given to the properties and uses of alginates, agar, and carrageenan Attention is also given to the action of enzymes from various sources on some of the algal polysaccharides and products derived from them Although we are very far from understanding the complete metabolic processes involved in the life cycle of the algae, the book will provide a useful basis for further work

Although general knowledge of organic and physical chemistry is presumed, chapter (2) is devoted to a detailed explanation of all recent techniques employed in polysaccharide chemistry The book is intended for the chemist and biologist engaged in any aspect of phycological research, but it will also be of value to persons in those industries that make use of plant gums and mucilages.

Kyoto Technical University CHOJI ARAKI

ANNOUNCEMENTS

(1) The Vth International Symposium on Carbohydrate Chemistry will be held in Paris from 17th to 22nd August 1970 The programme will include 30 lectures and 12 round-table discussions The Symposium is open to all persons interested in the chemistry and biochemistry of carbohydrates.

Preliminary programmes and registration forms are available from the Secretary F Percheron, 4 Avenue de l'Observatoire, 75-Paris VI^{e}, France

(2) The Joint IUPAC-IUB Commission on Biochemical Nomenclature (CBN) decided in 1968 to include Enzyme Nomenclature within its field of work After carefully considering the document "Enzyme Nomenclature Recommendations (1964) of the International Union of Biochemistry on the Nomenclature and Classification of Enzymes, together with their Units and the Symbols of Enzyme Kinetics", CBN decided, at its 1969 meeting, that the time was appropriate for a revision and an extension of this report It has therefore set up a Committee which has been asked to work towards a revision of "Enzyme Nomenclature", including the addition of newly-described enzymes, by 1971 The convener of the Committee is Professor E C Webb, Department of Biochemistry, The University of Queensland, St Lucia, Brisbane, 4067 Australia It would be helpful to the Committee if all biochemists who have suggestions to make in this field, either of enzymes which are omitted from the existing report or who wish to draw attention to errors in the existing report or of improvements in the existing names, would send them directly to Prof E C Webb as soon as possible and preferably before March 31st, 1970

O HOFFMANN-OSTENHOF
Chairman, IUPAC-IUB Joint Commission
on Biochemical Nomenclature

CORRIGENDUM

Carbohyd Res , 10 (1969) page 314, equation (*1*) should read

$$e^{k_2 t}(1-\alpha) = \frac{2k_2 - (k_3^{(1)} + k_3^{(2)})}{2k_2 - 2(k_3^{(1)} + k_3^{(2)})} e^{-(k_3^{(1)} + k_3^{(2)} - k_2)t} - \frac{k_3^{(1)} + k_3^{(2)}}{2k_2 - 2(k_3^{(1)} + k_3^{(2)})}$$