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SYNTHESIS OF THE 1,2′:1′,2-DIANHYDRIDE OF 3,4-DI-*O*-ACETYL-β-L-RHAMNOPYRANOSE AND METHYL 3,4-DI-*O*-ACETYL-α-D-GALACTOPYRANURONATE FROM 2-*O*-(α-D-GALACTOPYRANOSYLURONIC ACID)-L-RHAMNOPYRANOSE

TSUYOSHI FUJIWARA AND KIYOSHI ARAI

Laboratory of Chemistry, Institute for Natural Science, Nara University, Horai-cho 1230, Nara (Japan)

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ABSTRACT

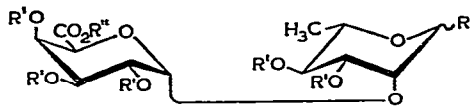
The title compound was synthesized from 2-*O*-(α-D-galactopyranosyluronic acid)-L-rhamnopyranose (**1**), showing that the dianhydride is a cyclization product of **1**. Formation of the dianhydride reached a maximum within 4 h, in both 2.5 and 5% methanolic hydrogen chloride at 90°.

INTRODUCTION

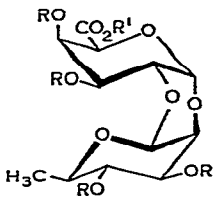
Dianhydrides of sugars, of which there are several, are unusual compounds. Di-D-fructopyranose 2,1′:2′,1-dianhydride[1–3], D-fructofuranose D-fructopyranose 2,1′:2′,1-dianhydride[4,5], di-D-fructofuranose 2,1′:2′,1-dianhydride[6], di-D-fructofuranose 2,1′:2′,4-dianhydride[7,8], and di-L-sorbopyranose 2,1′:2′,1-dianhydride[9] are examples of those that have been reported. All these dianhydrides are diketose dianhydrides, and only one dianhydride containing aldose residues had been reported[10], until the title compound (**3**) was described[11]. It was reported[11] that the dialdose dianhydride derivative (**3**) of **2** had been obtained by treatment, with methanolic hydrogen chloride, of the disaccharide fraction from the acid hydrolyzate of the water-soluble polysaccharide of *Phellodendron amurense* Rupr., followed by acetylation. The possibility that this dialdose dianhydride (**2**) was formed from **1** by the action of hydrogen chloride was presented, and in order to confirm this assumption, formation of the dianhydride **2** from **1** has now been achieved. A survey of the conditions favorable to the formation of **2** was also conducted.

RESULTS AND DISCUSSION

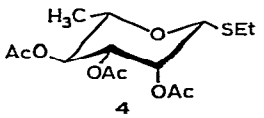
Analysis of the disaccharides that constitute the disaccharide fraction (Fraction E_5) in the hydrolyzate of the water-soluble polysaccharide was achieved by both paper chromatography and g.l.c. Fraction E_5 was separated into four compounds by paper chromatography with solvent system *1*. Fraction E_5 consisted of two main disaccharides (A, R_{Rha} 0.71; and C, R_{Rha} 0.52) and small proportions of two other disaccharides (B, R_{Rha} 0.62; and D, R_{Rha} 0.44). Authentic 2-*O*-(α-D-galactopyranosyl-



1 R = OH, R' = H, R'' = H
6 R = OMe(β), R' = H, R'' = Me
7 R = OMe(β), R' = Ac, R'' = Me



2 R = H, R' = H
3 R = Ac, R' = Me



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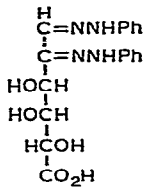

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uronic acid)-L-rhamnopyranose (**1**), prepared from the polysaccharide from flaxseed, had R_{Rha} 0.73, and the specific rotation of compound A was consistent with that of authentic **1**. Therefore, it was concluded that compound A was 2-*O*-(α-D-galactopyranosyluronic acid)-L-rhamnopyranose (**1**).

In order to confirm this conclusion, a color-development test on compound A by the triphenyltetrazolium chloride (TTC) reagent[12], paper chromatography with solvent system *2*, and g.l.c. analysis were performed. Compound A did not react with the TTC reagent. As the TTC reagent reacts with reducing sugars, except 2-*O*-substituted sugars, to give a red color[12], compound A has a (1→2)-bond. In paper chromatography with solvent system *2*, compound A did not move, but remained at the origin, showing that A contained a uronic acid. Compound A was collected by preparative paper-chromatography. It was completely hydrolyzed with trifluoroacetic acid (TFA), and the products were subjected to g.l.c.; the results showed that material A consisted of a compound of galacturonic acid and rhamnose in the molecular ratio of 1 : 1.

The hydrolyzate from compound A was separated into two fractions on a column of Dowex-1 X-8. The fraction eluted with water was evaporated, and converted into its thioglycoside triacetate (**4**), the melting point, specific rotation, and i.r. spectrum of which agreed with those of authentic ethyl 2,3,4-tri-*O*-acetyl-1-thio-β-L-rhamnopyranoside. The acidic fraction, eluted with 0.2M acetic acid, was evaporated, and the residue converted into its phenylosazone (**5**), the melting point, specific rotation, and i.r. spectrum of which agreed completely with those of authentic D-*lyxo*-hexosulosuronic acid 1,2-bis(phenyhydrazone). These results showed that compound A was composed of D-galacturonic acid and L-rhamnose residues.

Compound A was methylated with methylsulfinyl carbanion[13], and the product

TABLE I

RELATIVE RETENTION-TIMES IN THE G.L.C. OF PARTIALLY METHYLATED SUGARS (IN THE FORM OF THE ALDITOL ACETATES DERIVED THEREFROM)

Position of OMe	*Relative retention-times*				
	Authentic D-*glucose*	*Reference value*[14]			1
		D-*Glucose*	D-*Galactose*	L-*Rhamnose*	
2,3	5.65	5.39		0.98	
3,4				0.92	0.93
2,3,4	2.49	2.48	3.41	0.46	3.23
2,3,5			3.28		
2,3,6	2.52	2.50	2.42		
2,4,6			2.28		
2,5,6			3.25		
3,4,6			2.50		
2,3,4,6	1.00	1.00	1.25		

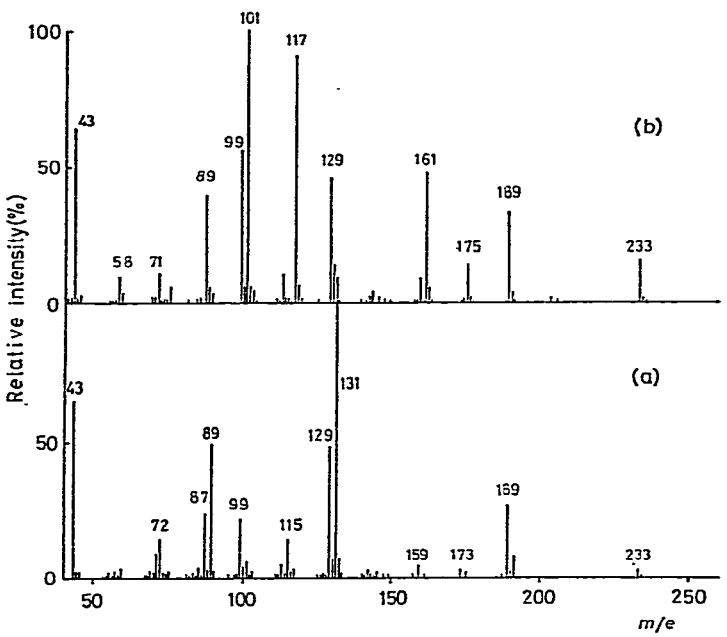

Fig. 1. Mass spectra of (a) Peak I, and (b) Peak II (see text).

treated with sodium borohydride to reduce the methyl ester of the galacturonic acid residue. The completely methylated disaccharide was hydrolyzed, the products were converted into the alditol acetates, and these were analyzed by g.l.c. and by g.l.c.–m.s. There was good agreement between the relative retention-times of the products and the reference values[14] for the authentic sugars (see Table I). There were two peaks (Peaks I and II) in the chromatogram of the methylated derivative of compound A.

Peak I had a relative retention-time of 0.9, and Peak II, 3.23. The reference value[14] showed that Peak I was the alditol acetate derivative from either 2,3- or 3,4-di-*O*-methyrhamnose, and Peak II was that from either 2,3,4- or 2,3,5-tri-*O*-methyl-galactose. In order to clarify this point, the g.l.c.-mass spectra were measured (see Fig. 1). The absence of peaks of *m/e* 117 and 203, and the presence of those of *m/e*

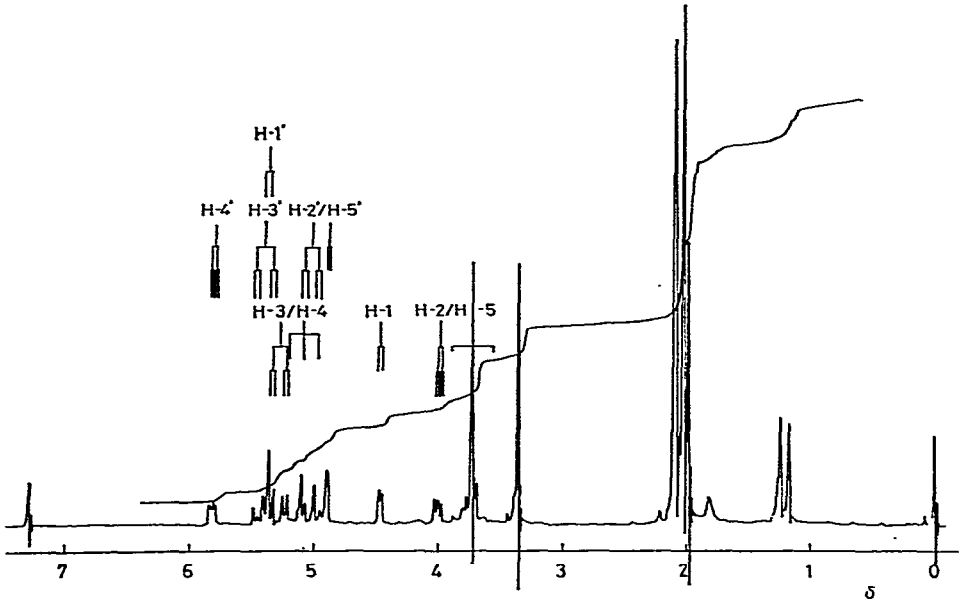

Fig. 2. N.m.r. spectrum of methyl 3,4-di-*O*-acetyl-2-*O*-(methyl 2,3,4-tri-*O*-acetyl-α-D-galacto-pyranosyluronate)-β-L-rhamnopyranoside (7).

TABLE II

THE N.M.R. PARAMETERS OF METHYL 3,4-DI-*O*-ACETYL-2-*O*-(METHYL 2,3,4-TRI-*O*-ACETYL-α-D-GALACTO-PYRANOSYLURONATE)-β-L-RHAMNOPYRANOSIDE (7)

Chemical shifts (δ)		*Coupling constants* (Hz)	
H-1	4.45	$J_{1,2}$	2.0
H-2	4.00	$J_{2,3}$	3.0
H-3	5.28	$J_{3,4}$	10.0
H-4	5.10	$J_{4,5}$	10.0
H-5	3.75	$J_{5,6}$	6.0
H-6	1.23		
[a]H-1′	5.35	[a]$J_{1',2'}$	3.5
H-2′	5.03	$J_{2',3'}$	10.0
H-3′	5.28	$J_{3',4'}$	3.2
H-4′	5.80	$J_{4',5'}$	1.5
H-5′	4.89		

[a]Primed numbers refer to the galacturonic acid residue.

131 and 189 in the spectrum of Peak I showed that it was the alditol acetate from 3,4-di-*O*-methylrhamnose. Similarly, the presence of peaks of *m/e* 117, 161, 189, and 233 in the mass spectrum of Peak II showed that it was not the alditol acetate from 2,3,5- but that from 2,3,4-tri-*O*-methylgalactose. The ratio of the areas of Peaks I and II on the chromatogram was 1.05:1.0, showing that the disaccharide formed by reduction of compound A was a 2-*O*-galactopyranosyl-rhamnose.

Compound A was esterified and glycosidated with methanolic hydrogen chloride, and the product (**6**) acetylated. The n.m.r. spectrum of the acetate (**7**) of **6** is given in Fig. 2 and Table II. The $J_{1',2'}$ value (3.5 Hz) showed that the bond between the rhamnose residue and the galacturonic acid residue was α. The $J_{2,3}$, $J_{3,4}$, and $J_{4,5}$ values showed that the L-rhamnose residue was in the $^1C_4$(L) conformation. Similarly, the $J_{2',3'}$, $J_{3',4'}$, and $J_{4',5'}$ values showed that the D-galacturonic acid residue was in the $^4C_1$(D) conformation. The configuration of C-1 of the L-rhamnoside residue could not be determined from the n.m.r. data, but the specific rotation of **7** showed that it had the β configuration. Therefore, **7** was methyl 3,4-di-*O*-acetyl-2-*O*-(methyl 2,3,4-tri-*O*-acetyl-α-D-galactopyranosyluronate)-β-L-rhamnopyranoside, and compound A was 2-*O*-(α-D-galactopyranosyluronic acid)-L-rhamnopyranose (**1**).

A solution of compound **1** in methanolic hydrogen chloride was boiled under reflux, and the product was acetylated. The acetate was chromatographed on a column of magnesol–Celite with benzene–*tert*-butanol. The fractions containing dianhydride **3** were pooled, and **3** was crystallized from 1:1 ethanol–ether. The melting point, specific rotation, and n.m.r. and i.r. spectra (see Fig. 3) agreed completely with those of the 1,2′:1′,2-dianhydride of 3,4-di-*O*-acetyl-β-L-rhamnopyranose and methyl 3,4-di-*O*-acetyl-α-D-galactopyranuronate that had been synthesized from Fraction E_5, showing that **2** was a cyclization product of **1**, and that **2**

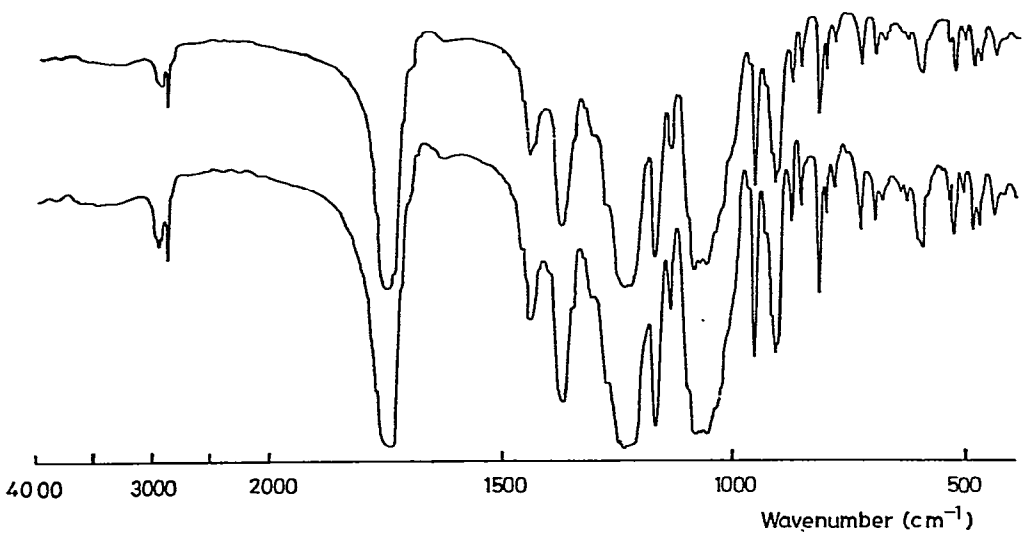

Fig. 3. I.r. spectra (KBr) of **3** obtained from Fraction E_5 (lower), and from **1** (upper).

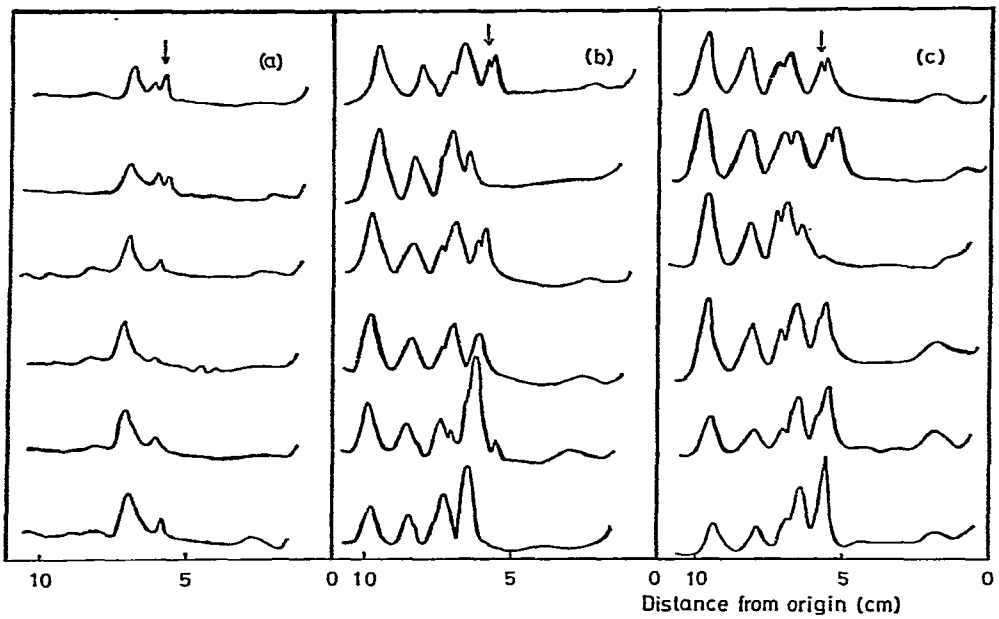

Fig. 4. T.l.c. of the acetylated, methanolyzation products from **1**. (Compound **1** was treated with (a) 1, (b) 2.5, or (c) 5% methanolic hydrogen chloride at 90°. The periods of treatment were 1, 2, 4, 6, 12, and 24 h, respectively, from bottom to top. Arrows indicate the positions of **3**.)

had been formed by intermolecular loss of one molecule of water from between OH-1 and OH-2′ of **1**. Therefore, it was concluded that **2** was formed from the compound **1** that was contained in Fraction E_5.

In order to study the conditions under which **2** is formed, various conditions, including reaction times, and concentrations of hydrogen chloride and of **1**, were tested. The results are given in Fig. 4. The concentrations of hydrogen chloride and the reaction times affected the formation of the dianhydride **2**. With 5% hydrogen chloride and a sugar concentration of 50 mg/ml, formation of the dianhydride increased with the reaction-time during the first several hours, and then ceased after 4–5 h. A decrease in the amount of dianhydride formed was not observed, even after 24 h. With 2.5% hydrogen chloride, the kinetics of formation of the dianhydride were almost the same as with 5% hydrogen chloride. The formation of dianhydride was very low with 1% hydrogen chloride; and the formation of other products was also very low, showing that the reaction proceeded very slowly in methanol containing 1% hydrogen chloride. Formation of the dianhydride was tested under the foregoing conditions, but at a concentration of sugar of 0.5 mg/ml. Formation of the dianhydride again occurred, and the concentration of the sugar had no effect on the yield of the dianhydride. These results showed that the most favorable conditions for the formation of the dianhydride **2** were with 2.5–5% hydrogen chloride during 4 h at 90°.

As has been already shown, the dianhydride **2** is a cyclization product of **1**,

but the question remained as to why the second bond was formed between OH-1 and OH-2′. When a solution of a sugar in methanolic hydrogen chloride is boiled under reflux, one molecule of water is released from OH-1 and the methanol, under the influence of the acid, to give methyl glycosides. In the case of dianhydride **2**, one molecule of water is released from OH-1 and OH-2′ in the same molecule, but the methanol is not involved. When free rotation occurs around C-2–O–C-1′, the hydroxyl group that comes into closest proximity to OH-1, where a new bond could form, is OH-2′. Therefore, the dianhydride **2** is also formed from **1**, in addition to methyl glycosides.

EXPERIMENTAL

Paper chromatography. — Paper chromatography was conducted on Whatman No. 1 paper with the following solvent systems; solvent *1*, 6:3:1 1-butanol–acetic acid–water; *2*, 6:4:3 1-butanol–pyridine–water. R_{Rha} values refer to rates of movement relative to that of L-rhamnose as unity.

Fraction E_5. — Fraction E_5 was obtained from the partial, acid hydrolyzate of the water-soluble polysaccharide of *Phellodendron amurense* Rupr. by use of a column of carbon–Celite as previously described[11].

Preparation of 2-O-(α-D-galactopyranosyluronic acid)-L-rhamnopyranose (**1**) *from flaxseed.* — Compound **1** was prepared from flaxseed by the method of Tipson *et al.*[15]. Ground flaxseed (2 kg) was extracted with water (10 liters), and the water-soluble polysaccharide (yield 110 g) was precipitated by the addition of ethanol. The precipitate (90 g) was hydrolyzed with 0.5M sulfuric acid (900 ml) for 9 h at 100°. The acids were neutralized with barium carbonate, the suspension filtered, and the polysaccharide precipitated from the filtrate with ethanol. Repeated precipitations gave the barium salt of **1** as a white, amorphous powder, $[\alpha]_D^{20}$ +75.3° (*c* 0.24, water). Treatment of the barium salt of **1** with Amberlite IR-120 (H^+) ion-exchange resin gave free acid **1**, $[\alpha]_D^{20}$ +67.5° (*c* 0.6, water).

Complete hydrolysis of disaccharide **1**. — Complete hydrolysis of disaccharide **1** (100 mg) was achieved by heating it for 3 h at 90° in 2M trifluoroacetic acid (TFA; 2 ml).

Ethyl 2,3,4-tri-O-acetyl-1-thio-β-L-rhamnopyranoside (**4**) *and* D-lyxo-*hexos-2-ulosuronic acid 1,2-bis(phenylhydrazone)* (**5**). — Material A (60 mg), isolated from Fraction E_5, was hydrolyzed completely with 2M TFA (1.5 ml), and the solution evaporated with addition of ethanol. The residue was dissolved in water (2 ml), and separated into two fractions by means of a column of Dowex-1 X-8 (AcO^-) ion-exchange resin. Fractions of unretained material eluted with water were pooled, and evaporated to a syrup which was dissolved in conc. hydrochloric acid (0.2 ml), and ethanethiol (0.2 ml) was added at room temperature. After 1 h, the mixture was made neutral with conc. ammonium hydroxide, and evaporated, and the residue acetylated. Crystallization from ethanol gave pure, crystalline **4** (5.2 mg), m.p. 108.2–108.9°; $[\alpha]_D^{17}$ +57.9° (*c* 0.19, chloroform).

Anal. Calc. for $C_{14}H_{22}O_7S$: S, 9.86; mol. wt. 334. Found: S 9.83; mol. wt. (mass spectrometry), 334 (M^+).

Acidic fractions, eluted with 0.2M acetic acid, were pooled and evaporated, and the residue was dissolved in water (1 ml); then, sodium acetate (40 mg) and phenylhydrazine hydrochloride (20 mg) were added, and the mixture was heated on a boiling-water bath for 1 h, and cooled. The crystals produced were collected, and recrystallized from methanol by gradual addition of water, to give pure **5** (4.1 mg); m.p. 141.7–142.1°; $[\alpha]_D^{17}$ +31.5→+10.1° (24 h; *c* 0.19, methanol).

Anal. Calc. for $C_{19}H_{22}N_4O_5$: N, 15.05. Found: N, 14.63.

Methylation analysis and g.l.c.–m.s. analysis. — Material A (10 mg) was methylated with methylsulfinyl carbanion and methyl iodide as already reported[11]. The methylation product was treated with sodium borohydride, and the ether successively hydrolyzed with 90% formic acid and 0.25M sulfuric acid. The hydrolyzate was reduced with sodium borohydride, and the alditols acetylated. G.l.c. of the resulting alditol acetates was performed at 180° with a column (1.5 m) of 5% of ECNSS-M on Gaschrom Q. G.l.c.–mass spectra were measured with a column (1 m) of 5% of OV-1 on Shimalite W, and the other conditions were the same as those already described[11].

Preparation of the 1,2′:1′,2-dianhydride (**3**) *of 3,4-di-*O*-acetyl-β-*L*-rhamnopyranose and methyl 3,4-di-*O*-acetyl-α-*D*-galactopyranuronate from* **1**. — Compound **1** (950 mg) in 2.5% methanolic hydrogen chloride (20 ml) was boiled for 5 h under reflux. The mixture was made neutral with silver carbonate, the suspension was filtered, and the filtrate was evaporated to a pale-yellow syrup. This syrup was acetylated with 1:1 acetic anhydride–pyridine for 3 days at room temperature, and the mixture was poured onto ice, and extracted with chloroform. The extract was successively washed with water, 10% hydrochloric acid, water, saturated sodium hydrogencarbonate, and water, and evaporated to a syrup which was transferred to the top of a column of 5:1 magnesol–Celite. Elution was performed with benzene–*tert*-butanol. The dianhydride fractions were pooled, and evaporated to a syrup which crystallized on addition of 1:1 ethanol–ether. Recrystallization from 1:1 ethanol–ether gave pure, crystalline **3** (28.2 mg); m.p. 250.3° (dec.); $[\alpha]_D^{17}$ +155.7° (*c* 3.1, chloroform).

Anal. Calc. for $C_{21}H_{28}O_{14}$: C, 50.00; H, 5.60; mol. wt., 504. Found: C, 49.51; H, 5.58; mol. wt. (m.s.), 445 (M^+ −59).

Estimation of the yield of **3** *from* **1** *by successive treatment with methanolic hydrogen choride and acetic anhydride–pyridine.* — A solution of **1** in 1, 2.5, or 5% methanolic hydrogen chloride was heated for 1–24 h at 90°. Two concentrations of **1** (50 and 0.5 mg/ml) were tested. The solution was then evaporated to dryness, followed by repeated addition and evaporation of methanol. The residue was acetylated with 1:1 acetic anhydride–pyridine during 24 h at room temperature. A small volume of methanol was then added, and the solution was evaporated to a syrup which was dissolved in chloroform. An aliquot equivalent to 1 mg of **1** was spotted on a t.l.c. plate of silica gel, and developed with 4:1 benzene–acetone. The

plate was treated with 10% sulfuric acid, and the absorbance at 500 nm was scanned with a Shimazu Chromatoscanner CS-900.

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