METHYLATION OF 2-ACETAMIDO-2-DEOXY-D-HEXOSES*

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ABSTRACT

Methyl iodide in the presence of the dimethylsulfinyl carbanion leads to complete *O*-methylation and *N*-methylation of 2-acetamido-2-deoxyglycosides. Thus, methylation of methyl 2-acetamido-2-deoxy-α-D-glucopyranoside and -galactopyranoside gave, respectively, methyl 2-deoxy-3,4,6-tri-*O*-methyl-2-(*N*-methylacetamido)-α-D-glucopyranoside and -galactopyranoside. Methylation, under these conditions, with methyl-^{14}C iodide showed that fully methylated 2-acetamido-2-deoxyglycosides, detectable in small amounts by radioautography, are obtained in quantitative yield. Methylation of methyl 2-acetamido-2-deoxy-6-*O*-trityl-α-D-hexopyranosides, followed by removal of the trityl group and acetylation gave methyl 6-*O*-acetyl-2-deoxy-3,4-di-*O*-methyl-2-(*N*-methylacetamido)-α-D-hexopyranosides. Separation and identification of these *N*-methylated 2-acetamido-2-deoxyhexosides was accomplished by g.l.c. and m.s. Acid hydrolysis of these derivatives gave the corresponding hydrochlorides.

INTRODUCTION

It has been repeatedly shown that rapid and complete per-*O*-methylation of simple and complex carbohydrates is achieved by using methyl iodide in the presence of dimethylsulfinyl carbanion as methylating agent, as first demonstrated by Hakomori[1]. Thus, this remarkable technique satisfies, in most cases, conditions previously difficult to fulfill, but necessary for the correct interpretation of the results and the unequivocal deduction of the structure of saccharides. The procedure can be performed on extremely small amounts of material, most easily when the permethylated product is isolated from the reaction mixture by retention on, and subsequent elution from, small charcoal columns[2]. The combination of this method of methylation with microtechniques of separation, characterization, and quantitative estimation of the methylated sugars, obtained after hydrolysis of the permethylated material, by t.l.c., g.l.c., and m.s., provides a very efficient tool for the determination of the structure of complex saccharides available only in very small amounts.

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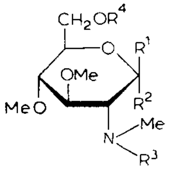
Since many complex saccharides of biological interest—glycosaminoglycans, glycolipids, glycoproteins—contain aminodeoxy sugars among other constituent units, it is of particular interest to examine the behavior of 2-amino-2-deoxyhexoses, and especially that of their *N*-acetyl derivatives, under these conditions of methylation, and to develop methods useful for the identification of the products which can be expected after permethylation and methanolysis, or hydrolysis, of the hexosamine-containing substances.

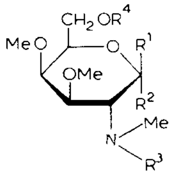
This paper describes (*a*) the methylation of methyl 2-acetamido-2-deoxy-α-D-glucopyranoside and -galactopyranoside which results in complete *N*- as well as *O*-methylation, (*b*) a micromethod of methylation using methyl-^{14}C iodide showing that per-*O*-methylated 2-(*N*-methylacetamido)-2-deoxyhexoses detectable by radioautography are obtained in quantitative yield from 2-acetamido-2-deoxyhexoses, (*c*) the synthesis of methyl 2-deoxy-3,4-di-*O*-methyl-2-(*N*-methylacetamido)-α-D-glucopyranoside and -galactopyranoside, (*d*) the separation of these derivatives by g.l.c. and the recording of their mass spectra, and (*e*) the hydrolysis of these derivatives which gives the corresponding 2-deoxy-2-methylaminohexose hydrochlorides*.

RESULTS AND DISCUSSION

In contrast to other classical or more recent methods of methylation which effect no or only partial *N*-methylation of *N*-acetyl derivatives of hexosamines[7-9], or require repeated or prolonged treatment with the reagent[3,4,10-12], the technique using methyl iodide in the presence of dimethylsulfinyl carbanion[1] leads not only to complete *O*-methylation, but also to quantitative *N*-methylation without loss of the *N*-acetyl group. The quantitative yield of the reaction, as shown by the use of methyl-^{14}C iodide and the counting of the radioactivity incorporated, and the relatively high volatility of the *N*-methylated derivatives which permits their easy separation



	R1	R2	R3	R4
1	H	OMe	Ac	Me
2	H	OMe	Ac	Ac
5	H,OH		H·HCl	Me
7	H	OMe	Ac	Tr
8	H	OMe	Ac	H
9	H,OH		H·HCl	H



	R1	R2	R3	R4
3	H	OMe	Ac	Ac
4	H	OMe	Ac	Me
6	H,OH		H·HCl	Me
10	H	OMe	Ac	Tr
11	H	OMe	Ac	H
12	H,OH		H·HCl	H

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*The synthesis and chromatographic properties of partially *O*-methylated 2-deoxy-2-methylamino-D-glucoses and -D-galactoses have been recently described by Gorin *et al*[3-5], who used Kuhn's procedure for methylation. H. Björndal *et al.*[6] have recently reported in a preliminary communication work showing *N*-methylation of hexosamines with Hakomori's reagent.

by g.l.c. and characterization by m.s., constitute interesting factors for the determination of the structure of hexosamine-containing substances.

The mass spectrum obtained for methyl 2-deoxy-3,4,6-tri-*O*-methyl-2-(*N*-methylacetamido)-α-D-glucopyranoside (**1**) is similar to the one published by Heyns and Muller[9] for the β-D- anomer. Consistent with the observations of Kochetkov and Chizhov[13], the corresponding galactosamine derivative produced a practically identical spectrum. The spectra of the isomeric methyl 6-acetyl-2-deoxy-3,4-di-*O*-methyl-2-(*N*-methylacetamido)-α-D-glucopyranoside (**2**) and -galactopyranoside (**3**) are also identical. Their fragmentation pattern is generally similar to that of the fully methylated compounds; the mass of the molecular ion (M^+ 319), however, is larger by 28 units, which corresponds to the additional carbonyl group at C-6. Of particular interest is the presence, in both derivatives, of peaks at *m/e* 287 and 256 which appear to result from the B splitting[9] of the molecule M−(MeOH+MeO). This yields fragments which retain the acetyl group at O-6, the *m/e* 256 fragment having a mass 28 units higher than the corresponding one at *m/e* 288, which appears in the fully methylated derivatives. In conclusion, these results indicate that this particular method of methylation promises to become as useful for studying the structure of hexosamine-containing substances as it is already for complex substances containing only neutral sugars.

Of particular interest is the use of methyl-^{14}C iodide for the methylation, followed by detection by radioautography of the chromatograms. This not only permits identification of the methylated carbohydrates on a very small scale, but also gives a quantitative estimation of their ratio in the original material by counting the radioactivity incorporated into each. The success of the procedure results from the quantitative yield in permethylated products given by this method of methylation. The application of this technique to the determination of the structure of hexosamine-containing oligosaccharides is being further investigated.

EXPERIMENTAL

General methods. — Melting points were determined between glass slides under a microscope and are corrected. Optical rotations were measured with a O. C. Rudolph and Sons 80Q3 photoelectric polarimeter. N.m.r. spectra were recorded with a Varian A-60 spectrometer. Chemical shift in deuterium oxide solution is reported as p.p.m. downfield from sodium 2,2-dimethyl-2-silapentane-5-sulfonate as internal standard. Silica gel for column chromatography, grade 950, 60–200 mesh, was a product from Davison Chemical, Baltimore, Maryland. Thin-layer chromatography was performed on plates coated with silica gel G (Uniplate^R, Analtech, Inc., Wilmington, Delaware). Elementary analyses were determined by Midwest Microlab, Inc., Indianapolis, Indiana. Gas–liquid chromatography was performed with a F and M 609 gas chromatograph. Combined gas–liquid chromatography–mass spectrometry was carried out on an LKB 9000 instrument with helium carrier gas, and OV-1 on Gaschrom Q (Applied Science Labs. State College, Pa.) at 175° as the stationary phase. Samples in the solid state, adsorbed on stainless-steel gauze[14] were injected into the flash heater at 218°. The molecular separator was maintained at 250° and the ion source at 270°. The mass-spectrometer ionizing current was 50 μA, and the ionizing energy 70 eV during the mass-spectral scans. Radioactivity was counted in a Packard Tri-Carb liquid scintillation spectrometer Model 3214.

*Methyl 2-deoxy-3,4,6-tri-*O*-methyl-2-(*N*-methylacetamido)-α-*D*-glucopyranoside* (**1**). — Under a stream of nitrogen and with stirring, a 50% suspension of sodium hydride in oil (1.6 g, Metal Hydrides, Beverly, Mass.) was dissolved in dimethyl sulfoxide (60 ml) maintained at 70°. To this solution, cooled to 20°, was added methyl 2-acetamido-2-deoxy-α-D-glucopyranoside[15] (1 g) dissolved in dimethyl sulfoxide (40 ml) and, after 0.5 h, methyl iodide (5 ml) was added dropwise. After 0.5–1 h, the mixture was diluted with water (300 ml) and a stream of nitrogen was bubbled through the mixture in order to evaporate the excess of methyl iodide. The solution was passed through a column (15 × 2.5 cm), prepared in water, of 2:1 Norit A–Celite 535 (prewashed in bulk with warm 6M hydrochloric acid, water, ethanol, and water). The solvent, salts, and byproducts of the reaction were eluted with water (500 ml) and 1:1 methanol–water (500 ml); then, pure methanol (300 ml) eluted the colorless oily product. It was purified by distillation under 0.05 Torr at a bath temperature of 120–140° (1.104 g, 89%), $[\alpha]_D^{26}$ +131° (*c* 0.81, methanol) lit.[10]: b.p. 128°/0.11 mm, $[\alpha]_D^{21}$ +93.5° (chloroform).

Anal. Calc. for $C_{13}H_{25}NO_6$: C, 53.59; H, 8.65; N, 4.81. Found: C, 53.12; H, 8.62; N, 4.76.

In order to evaluate the yield of the reaction when performed on a microscale, an equimolecular mixture of methyl β-D-galactopyranoside and of methyl 2-acetamido-2-deoxy-α-D-glucopyranoside was methylated with methyl-^{14}C iodide. The radioactivity incorporated into the methylated hexosamine derivative was compared to that incorporated into methyl tetra-*O*-methyl-β-D-galactopyranoside. Therefore, a solution containing methyl β-D-galactopyranoside (0.97 mg) and methyl 2-acetamido-2-deoxy-α-D-glucopyranoside (1.175 mg), *i.e.* equimolecular amounts, in dimethyl sulfoxide (0.5 ml) was added to a previously prepared solution of sodium hydride in oil (2 mg) in dimethyl sulfoxide (1 ml) kept at 30°. A stream of dry nitrogen carrying methyl-^{14}C iodide vapor was bubbled through the solution until about 50 mg had been carried over (the specific activity of methyl-^{14}C iodide was 1 mCi/3 ml). After keeping the mixture for 1 h at room temperature, water (4 ml) was added and nitrogen was bubbled through in order to evaporate the excess methyl iodide. Then nonradioactive methyl iodide (0.5 ml) was added and again evaporated under a stream of nitrogen in order to eliminate as much as possible of the methyl-^{14}C iodide. The aqueous solution was transferred onto a column containing a 2:1 mixture of charcoal–Celite (0.8 ml). The column was washed successively with water (35 ml) which eluted salts, with 1:1 methanol–water (1 ml), methanol (1 ml), and 2:1 chloroform–methanol (25 ml). The three last fractions containing the methylated carbohydrates were evaporated to dryness at low temperature in a rotatory evaporator. Portions of the residue, dissolved in methanol, were applied on plates coated with silica gel G and the thin-layer chromatogram was developed with 1:1 benzene–acetone. The radioactive material was shown by radioautography to be located in two spots (R_F 0.91 and 0.55). The position of the faster-moving spot coincided with that of a standard of methyl 2,3,4,6-tetra-*O*-methyl-β-D-galactopyranoside revealed with the anthrone–sulfuric acid reagent; the position of the slower-moving spot coincided with that of a standard of methyl 2-deoxy-3,4,6-tri-*O*-methyl-2-(*N*-methylacetamido)-α-D-glucopyranoside (**1**) revealed either with the copper acetate–phosphoric acid reagent[16], or by spraying with 6M hydrochloric acid, covering with a clean glass plate, heating for 1 h at 100°, spraying with aqueous ninhydrin buffered with pyridine–acetic acid, and heating again. This test produced a purple color. The areas corresponding to the two radioactive spots were separately scraped off the plate, and the silica was placed in vials with 10 ml of toluene scintillation fluid gelled with three drops of toluene diisocyanate[17]. The c.p.m. in the two spots, in a number of experiments, were equal within ±8%, thus indicating that, within the limits of experimental error, an equal amount of ^{14}C had been incorporated into the *N*-methylated hexosamine and into the galactoside. Since it has been shown, independently, that methylation of methyl β-D-galactopyranoside, under the same conditions, gives crystalline methyl 2,3,4,6-tetra-*O*-methyl-β-D-galactopyranoside in quantitative yield, it can be concluded that the hexosamine derivative is also obtained quantitatively.

*Methyl 2-deoxy-3,4,6-tri-*O*-methyl-2-(*N*-methylacetamido)-α-D-galactopyranoside* (**4**). — This derivative was prepared as described for the preparation of **1**, but starting from methyl 2-acetamido-2-deoxy-α-D-galactopyranoside[18]. The oily product, obtained in 72–85% yield, had $[\alpha]_D^{24}$ +144° (*c* 0.91, methanol).

Anal. Calc. for $C_{13}H_{25}NO_6$: C, 53.59; H, 8.65; N, 4.81. Found: C, 52.85; H, 8.78; N, 4.92.

*2-Deoxy-3,4,6-tri-*O*-methyl-2-methylamino-D-glucose hydrochloride* (**5**). — A solution of distilled compound **1** (384 mg), in 3M hydrochloric acid (15 ml) was heated for 3 h under reflux. The solution was evaporated to dryness *in vacuo*, and the remaining hydrochloric acid was removed by additions and evaporations of 1:1 toluene–ethanol. The crystalline residue was recrystallized from ethyl acetate and methanol–ether (232 mg), dec. *ca.* 200°, $[\alpha]_D^{25}$ $+123 \rightarrow +114°$ (*c* 0.78, water): lit.: $[\alpha]_D^{25}$ $+122 \rightarrow +113°$ (Ref. 3), $[\alpha]_D^{20}$ +105° (Ref. 10); $[\alpha]_D^{24}$ −108° for the L isomer (Ref. 11).

Anal. Calc. for $C_{10}H_{22}ClNO_5$: C, 44.20; H, 8.16; Cl, 13.05; N, 5.16. Found: C, 43.58; H, 7.95; Cl, 12.82; N, 5.07.

The n.m.r. spectrum showed the presence of one N–Me (2.76 p.p.m., singlet, 3H) as well as three O–Me groups (3.37, 3.54, 3.62 p.p.m.). The anomeric hydrogen appeared as a doublet (5.48 p.p.m., *J* 3.5 Hz) indicative of an α-orientation (dihedral angle 60° with H-2 of the ring). The spectrum is entirely consistent with structure **5**.

*2-Deoxy-3,4,6-tri-*O*-methyl-2-methylamino-D-galactose hydrochloride* (**6**). — This compound was prepared as described for **5** starting from **4**. The crystalline product was recrystallized from methanol–ether, dec. *ca.* 200°, $[\alpha]_D^{26}$ $+144 \rightarrow +131°$ (*c* 0.97, water); lit.[4]: dec. 165°, $[\alpha]_D^{25}$ $+142 \rightarrow +130°$.

Anal. Calc. for $C_{10}H_{22}ClNO_5$: C, 44.20; H, 8.16; Cl, 13.05; N, 5.16. Found: C, 44.19; H, 7.98; Cl, 13.04; N, 5.27.

*Methyl 2-deoxy-3,4-di-*O*-methyl-2-(*N*-methylacetamido)-6-*O*-trityl-α-*D*-glucopyranoside* (**7**). — The methylation of methyl 2-acetamido-2-deoxy-6-*O*-trityl-α-D-glucopyranoside[19] (1 g) was performed as described for the preparation of **1**. The reaction mixture was diluted with benzene (200 ml) and the solution washed thrice with saturated aqueous sodium hydrogen carbonate (300 ml) and with water. The organic layer was dried with sodium sulfate, the solvent evaporated *in vacuo*, and the syrupy residue was chromatographed on a column of silica gel (60 g) prepared in pentane. Elution was carried out with mixtures of increasing concentration of benzene in pentane, followed by mixtures of benzene and ether which eluted 850 mg (78%). After recrystallization from ether, m.p. 192–195°, $[\alpha]_D^{24}$ +95° (*c* 1.01, chloroform).

Anal. Calc. for $C_{31}H_{37}NO_6$: C, 71.66; H, 7.18; N, 2.69. Found: C, 71.84; H, 7.30; N, 2.72.

*Methyl 2-deoxy-3,4-di-*O*-methyl-2-(*N*-methylacetamido)-α-*D*-glucopyranoside* (**8**). — To a solution of **7** (506 mg) in glacial acetic acid (7 ml) at 100°, water (3.5 ml) was added dropwise with occasional stirring, and the mixture was kept for 90 min at 100°. The solution was evaporated to dryness, and the acid eliminated by repeated additions and evaporations of 1:1 ethanol–toluene. The residue was chromatographed on a column of silica gel (50 g) prepared in 1:1 benzene–ether. This solvent mixture eluted triphenylmethanol and then ethyl acetate–acetone eluted **8** as a syrup (206 mg, 75%), $[\alpha]_D^{25}$ +84° (*c* 0.79, chloroform).

Anal. Calc. for $C_{12}H_{23}NO_6$: C, 51.97; H, 8.36; N, 5.05. Found: C, 51.42; H, 8.76; N, 5.06.

Acetylation of **8** (40 mg) with acetic anhydride (0.3 ml) and pyridine (0.5 ml) for 24 h at room temperature gave the 6-*O*-acetyl derivative (**2**) which was purified by chromatography on silica gel (5 g). The oily product (38 mg), eluted with benzene–ether, had $[\alpha]_D^{26}$ +117° (*c* 0.67, methanol).

Anal. Calc. for $C_{14}H_{25}NO_7$: C, 52.65; H, 7.89; N, 4.38. Found: C, 52.24; H, 7.94; N, 4.10.

*2-Deoxy-3,4-di-*O*-methyl-2-methylamino-*D*-glucose hydrochloride* (**9**). — The hydrochloride **9** was obtained by hydrolysis of **7** or **8** with 3M hydrochloric acid for 3 h under reflux. The yield, in either case, varied between 45 and 55%. The crystalline product was recrystallized from ethanol–ether to give needles, dec. *ca.* 200°, $[\alpha]_D^{25}$ $+129 \rightarrow +104°$ (*c* 0.70, water); lit.[3]: dec. 199°, $[\alpha]_D^{25}$ $+120 \rightarrow +111°$.

Anal. Calc. for $C_9H_{20}ClNO_5$: C, 41.90; H, 7.82; Cl, 13.76; N, 5.43. Found: C, 41.91; H, 7.63; Cl, 13.90; N, 5.39.

*Methyl 2-deoxy-3,4-di-*O*-methyl-2-(*N*-methylacetamido)-6-*O*-trityl-α-*D*-galactopyranoside* (**10**). — This oily product was obtained in 77% yield by methylation of methyl 2-acetamido-2-deoxy-6-*O*-trityl-α-D-galactopyranoside[20] under conditions similar to those described for the preparation of **7**, $[\alpha]_D^{23}$ +53.5° (*c* 1.1, chloroform).

Anal. Calc. for $C_{31}H_{37}NO_6$: N, 2.69. Found: N, 2.45.

*Methyl 2-deoxy-3,4-di-*O*-methyl-2-(*N*-methylacetamido)-α-*D*-galactopyranoside* (**11**). — Hydrolysis of **10**, as described for the preparation of **8**, gave syrupy **11** in 76% yield, $[\alpha]_D^{24}$ +127° (*c* 0.75, chloroform).

Anal. Calc. for $C_{12}H_{23}NO_6$: C, 51.97; H, 8.36; N, 5.05. Found: C, 52.22; H, 8.56; N, 4.95.

Acetylation gave the syrupy 6-*O*-acetyl derivative **3**, $[\alpha]_D^{25}$ +137° (*c* 0.68, chloroform).

Anal. Calc. for $C_{14}H_{25}NO_7$: N, 4.38. Found, N, 4.16.

*2-Deoxy-3,4-di-*O*-methyl-2-methylamino-*D*-galactose hydrochloride* (**12**). — Compound **10** (395 mg) was hydrolyzed with a mixture of glacial acetic acid (7 ml) and water (3.5 ml) for 1.5 h at 100°. After addition of cold water, the cooled suspension was filtered to collect triphenylmethanol. The filtrate was evaporated to dryness, and the residue was heated for 3 h under reflux with 3M hydrochloric acid (13 ml). The solution was decolorized with charcoal, filtered through Celite, evaporated *in vacuo*, and the last traces of acid and water eliminated by addition and evaporation of toluene–ethanol. The crystalline residue (143 mg, 89%) was very hygroscopic. Part of it (67 mg) was acetylated with acetic anhydride and pyridine and the product was purified by chromatography on silica gel (5 g). The acetate was eluted as an impure oil with ethyl acetate.

The remainder of the hydrochloride (76 mg), in aqueous solution, was absorbed on a column of Dowex 50 (H^+). The column was washed with water and the product was eluted with M hydrochloric acid. The eluate, evaporated to dryness and dried by addition and evaporation of 1:1 ethanol–toluene, gave a hygroscopic, crystalline residue (76 mg), $[\alpha]_D^{27}$ $+131 \rightarrow +128°$ (*c* 0.82, water); lit.[4]: $[\alpha]_D^{25}$ +121°.

For further characterization, this product (59 mg) was benzoylated with benzoyl chloride (0.3 ml) in pyridine (5 ml) for 24 h at 4°. After addition of a small piece of ice, the solution was kept for 1 h and then poured onto crushed ice. The product was extracted with chloroform, and the solution washed three times with ice-cold M hydrochloric acid and three times with water, dried with sodium sulfate, and evaporated to dryness. The oily residue was purified by chromatography on silica gel (10 g) prepared in pentane. The syrupy benzoate was eluted (yield, 53%) with 9:1 and 4:1 benzene–ether, $[\alpha]_D^{23}$ +64° (*c* 0.60, chloroform).

Anal. Calc. for $C_{30}H_{31}NO_8$: N, 2.63. Found: N, 2.62.

Gas–liquid chromatography. — Gas–liquid chromatography of methyl 2-deoxy-3,4,6-tri-*O*-methyl-2-(*N*-methylacetamido)-α-D-glucopyranoside (**1**), methyl 2-deoxy-3,4,6-tri-*O*-methyl-2-(*N*-methylacetamido)-α-D-galactopyranoside (**4**), methyl 6-*O*-acetyl-2-deoxy-3,4-di-*O*-methyl-2-(*N*-methylacetamido)-α-D-glucopyranoside (**2**) and methyl 6-*O*-acetyl-2-deoxy-3,4-di-*O*-methyl-2-(*N*-methylacetamido)-α-D-galactopyranoside (**3**) was performed on two columns: (*a*) 3 m, 6 mm stainless-steel tubing with 3% ECNSS-M on 100/120 Gas Chrom Q (Applied Science, State College, Pa.) operated at 190° with a helium flow of 70 ml/min. (*b*) 1.8 m, 6 mm stainless-steel tubing with a 2.85% OV-1 on 100/120 Gas Chrom Q operated at 175°, with a helium flow of 70 ml/min. Table I shows the retention times in minutes of the four compounds. A minor peak was present in the chromatograms of both tri-*O*-methyl derivatives **1** and **4**. Their retention times and amounts are expressed as percentages of the total

TABLE I

GAS–LIQUID CHROMATOGRAPHY OF DI- AND TRI-*O*-METHYL ETHERS OF 2-ACETAMIDO-2-DEOXY-*O*-HEXOPYRANOSIDES

Compounds	*ECNSS column*			*O V-1 column*	
	Major peak Ret. time (min)	*Minor peak Ret. time (min)*	*Rel. area (%)*	*Major peak Ret. time (min)*	*Minor peak Ret. time (min)*
1	31	21	16	14	9
4	46	29	5	16	10
2	88			30	
3	140			32	

area under both major and minor peaks. In the case of the two di-*O*-methyl derivatives **2** and **3**, only one peak was observed.

Mass Spectrometry. — The mass spectra of the four compounds **1**–**4** are shown in Figs. 1–4. The mass spectra of the minor peaks present in the chromatograms of the

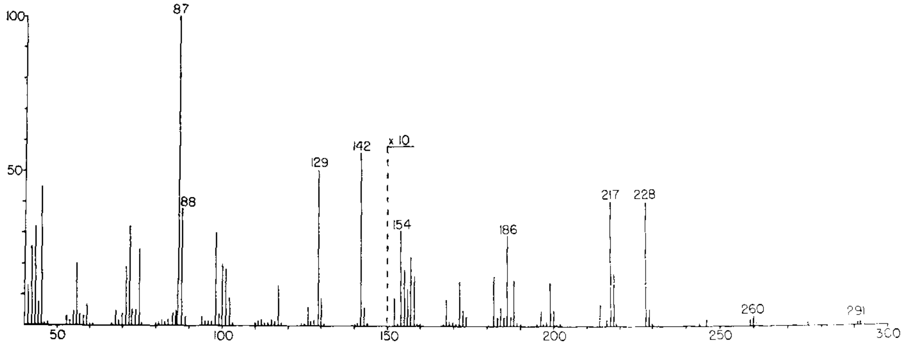

Fig. 1. Mass spectrum of methyl 2-deoxy-3,4,6-tri-*O*-methyl-2-(*N*-methylacetamido)-α-D-glucopyranosde (**1**).

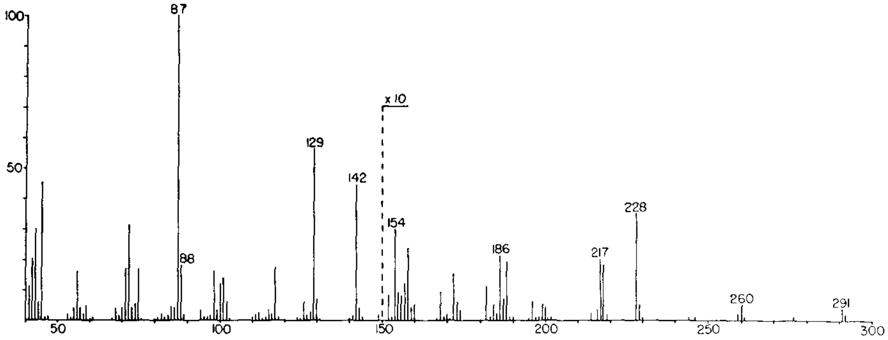

Fig. 2. Mass spectrum of methyl 2-deoxy-3,4,6-tri-*O*-methyl-2-(*N*-methylacetamido)-α-D-galactopyranoside (**4**).

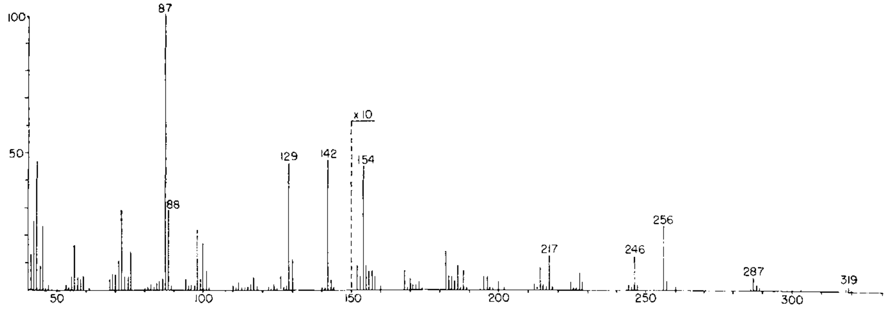

Fig. 3. Mass spectrum of methyl 6-*O*-acetyl-2-deoxy-3,4-di-*O*-methyl-2-(*N*-methylacetamido)-α-D-glucopyranoside (2).

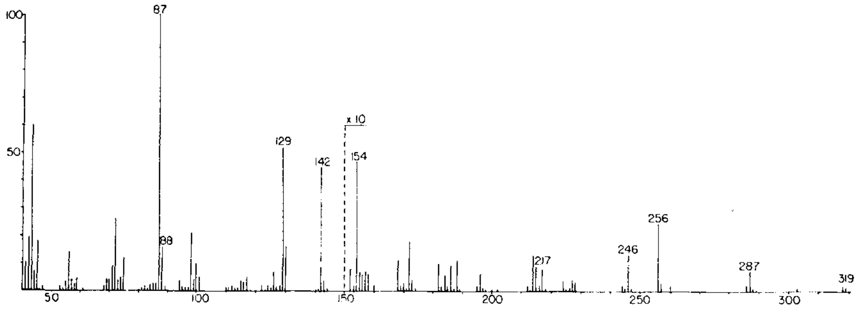

Fig. 4. Mass spectrum of methyl 6-*O*-acetyl-2-deoxy-3,4-di-*O*-methyl-2-(*N*-methylacetamido)-α-D-galactopyranoside (3).

tri-*O*-methyl derivatives were practically identical with those of the main ones. A possible explanation for these observations is that the minor peaks correspond to β-D anomers originating from a proportion of methyl 2-acetamido-2-deoxy-β-D-hexopyranosides difficult to separate from the α-D anomers in the starting materials. In the synthesis of the 3,4-di-*O*-methyl derivatives, however, the starting compounds were 6-*O*-trityl derivatives of the 2-acetamido-2-deoxy-α-D-hexopyranosides which are easy to obtain free of β-D anomers[7].

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REFERENCES

1 S. Hakomori, *J. Biochem.* (Tokyo), 55 (1964) 205.
2 A. Stoffyn, P. Stoffyn, and E. Märtensson, *Biochim, Biophys. Acta*, 152 (1968) 353.
3 P. A. J. Gorin and A. J. Finlayson, *Carbohyd. Res.*, 18 (1971) 269.
4 P. A. J. Gorin, *Carbohyd. Res.*, 18 (1971) 281.
5 P. A. J. Gorin and R. J. Magus, *Can. J. Chem.*, 49 (1971) 2583.
6 H. Björndal, C. G. Hellerqvist, B. Lindberg, and S. Svensson, *Angew. Chem.*, 9 (1970) 610.
7 R. W. Jeanloz, *Advan. Carbohyd. Chem.*, 13 (1958) 190.
8 R. Kuhn and H. Trischmann, *Chem. Ber.*, 96 (1963) 284.
9 K. Heyns and D. Muller, *Tetrahedron*, 21 (1965) 3151.
10 S. A. Barker, M. Stacey, and D. J. Tipper, *Nature*, 184 (1959) 1718.
11 J. Fried and H. E. Stavely, *J. Amer. Chem. Soc.*, 74 (1952) 5461.
12 R. U. Lemieux and R. J. Cushley, *Can. J. Chem.*, 41 (1963) 858.
13 N. K. Kochetkov and O. S. Chizhov, *Advan. Carbohyd. Chem.*, 21 (1966) 39.
14 E. Menini and J. K. Norymberski, *Biochem. J.*, 95 (1965) 1; E. Menini, J. C. Orr, R. Gibb, and L. L. Engel in R. Scholler (Ed.), *Gas Chromatography of Steroid Hormones*, Gordon and Breach, New York, 1968, p. 41.
15 R. C. G. Moggridge and A. Neuberger, *J. Chem. Soc.*, (1938) 745.
16 M. E. Fewster, B. J. Burns, and J. F. Mead, *J. Chromatogr.*, 43 (1969) 120.
17 J. N. Bollinger, W. A. Mallow, J. W. Register, Jr., and D. E. Johnson, *Anal. Chem.*, 39 (1967) 1508.
18 M. Stacey, *J. Chem. Soc.*, (1944) 272.
19 R. W. Jeanloz, *J. Amer. Chem. Soc.*, 74 (1952) 4597.
20 P. J. Stoffyn and R. W. Jeanloz, *J. Amer. Chem. Soc.*, 80 (1958) 5690.