Carbohydrate Research, 176 (1988) 117–126
Elsevier Science Publishers B.V., Amsterdam – Printed in The Netherlands

NOVEL D-GLUCANS OBTAINED BY DIMETHYL SULFOXIDE EXTRACTION OF THE LICHENS *Letharia vulpina, Actinogyra muehlenbergii,* AND AN *Usnea* SP*

MARCELLO IACOMINI, PHILIP A. J. GORIN**, MADALENA BARON,
Departamento de Bioquímica, Universidade Federal do Paraná, Caixa Postal 19046, 81.504 Curitiba, Paraná, (Brazil)

ALEXANDER P. TULLOCH‡, AND MYTOSK MAZUREK
Plant Biotechnology Institute, National Research Council, Saskatoon, Saskatchewan S7N OW9 (Canada)

(Received October 10th, 1984; accepted in revised form, April 17th, 1987)

ABSTRACT

Extraction of certain lichens with cold dimethyl sulfoxide provided a β-D-glucan virtually free of contaminating α-D-glucan and galactomannan. Applied to *Letharia vulpina*, the method gave β-D-glucan, and extraction of the residue with hot water followed by cooling gave α-D-glucan. From *Usnea* sp. a β-D-glucan was isolated, but little α-D-glucan was present. Extraction of *Actinogyra muehlenbergii* provided a (1→6)-linked β-D-glucopyranan containing one acetyl group for every 8–9 glucosyl units, being present almost exclusively as monosubstituent at O-2, O-3, and O-4.

INTRODUCTION

Lichens generally contain one or two glucan components and a galactomannan. These can be fractionated, as in the case of *Cetraria islandica* (Iceland moss), by a hot-water extraction which solubilizes the two glucans; these may then be separated as the β-D-glucan is insoluble in cold water[1], whereas the α-D-glucan is soluble[2]. Hot aqueous alkaline extraction of the resulting lichen residue liberated the galactomannan component, which could be purified as the water-insoluble copper complex formed with Fehling solution[3]. During the course of analysis of the polysaccharide components of several lichens, new fractionation procedures were tested for the isolation of pure polysaccharides. These included the use of cold dimethyl sulfoxide in place of hot water in the initial step. Thus nine lichens were extracted, whose galactomannans were the subject of a previous investigation[4,5]. These were *Cladonia alpestris, Cladonia confusa, Cladonia amaurocraea, Parmelia*

*N.R.C. No. 24766.
**To whom correspondence should be addressed.
‡Deceased February 15th, 1987.

0008-6215/88/$ 03.50

sulcata, Letharia vulpina, Stereocaulon paschale, Peltigera aphthosa, Actinogyra muehlenbergii, and an *Usnea* sp. which grew on dead branches of trees. It was found that cold dimethyl sulfoxide extraction gave purer glucans than hot-water extraction when *L. vulpina, A. muehlenbergii*, and *Usnea* sp. were investigated. With the other lichens, neither procedure was effective in the extraction of pure glucans. We describe herein the isolation and characterization of the glucans from these three lichens.

RESULTS AND DISCUSSION

D-*Glucans of* L. vulpina. — *L. vulpina* was treated with hot water, and the extract concentrated to a small volume and then frozen. On thawing, an insoluble polysaccharide remained, and this was recovered in 2% yield. It contained glucose, galactose, and mannose in a 44:3:3 molar ratio, and the C-1 portion of its ^{13}C-n.m.r. spectrum (Fig. 1A) contained signals of equal area at the relatively high field of δ 102.0 and 101.0, indicating an α-D-glucopyranan structure[6]. Other signals at δ 79.8 and 82.9 could be attributed to substitutions at O-4 and O-3, respectively[7]. The polysaccharide (9% yield) from the mother liquor contained glucose, galactose, and mannose in an 89:4:6 molar ratio, and its low-field C-1 signals at δ 105.3, 104.6, and 104.2 corresponded to a β-D-glucopyranan structure[6] (Fig. 1B). A large signal at δ 80.4 suggested substitution at O-4, whereas a smaller one at δ 88.0 had a shift consistent[8] with a glucosyl residue at O-3.

Extraction of *L. vulpina* with cold dimethyl sulfoxide gave, in 12% yield, a

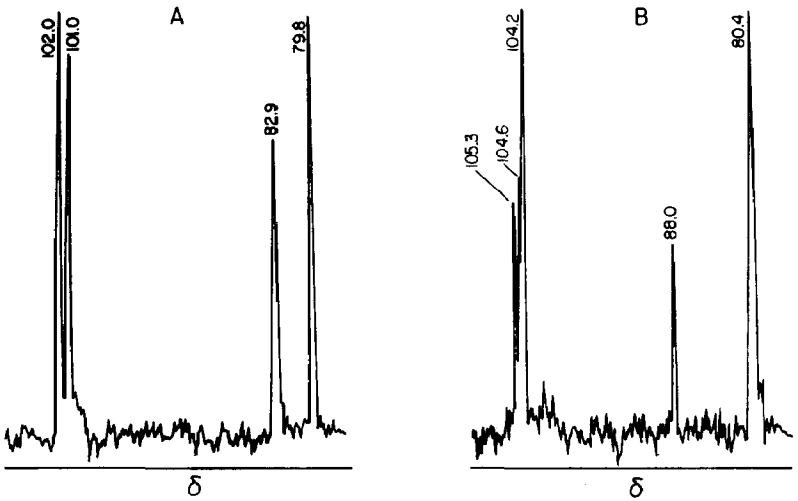

Fig. 1. ^{13}C-N.m.r. spectra (portion of C-1 and *O*-substituted carbon atoms) of: (A) Water-insoluble α-D-glucan of *L. vulpina*, obtained by hot-water extraction. (B) β-D-Glucan isolated from the supernatant. Numerical values (δ) are based on signal of external Me_4Si.

glucan which had only 2% each of mannose- and galactose-containing components. A β-D configuration was indicated by the low specific rotation of −8° and a ^{13}C-n.m.r. spectrum identical to that illustrated in Fig. 1B. Methylation analysis resulted in the detection, by g.l.c.–m.s., of 2,3,6- and 2,4,6-tri-*O*-methylglucitol acetates[9], showing the presence of (1→4)- and (1→3)-linkages in a 3:1 ratio. The linear β-D-glucopyranan structure, thus demonstrated, agreed with the detection, on Smith degradation incorporating mild hydrolytic conditions, of spots on a paper chromatogram corresponding to erythritol and 2-*O*-β-D-glucopyranosyl-D-erythritol. The absence of a glucobiosyl fragment indicated that these linkages are distributed throughout the chain, as in the main structure **1**. The presence of other trace structures having longer sequences of (1→4)-linkages is possible.

-β-D-Glc*p*-(1→3)-β-D-Glc*p*-(1→4)-β-D-Glc*p*-(1→4)-β-D-Glc*p*-(1→4)-

1

The chemical structure differs from that of lichenan obtained from Iceland moss which contains (1→4)- and (1→3)-linkages in a 7:3 ratio[10]. Also, the *L. vulpina* glucan differs since it is soluble in water.

The residue remaining after dimethyl sulfoxide extraction was treated with hot water, and following partial evaporation, freezing, and thawing, an insoluble fraction was formed. It was isolated in 4% yield and contained principally glucose (95%) with mannose (3%) and galactose (2%). Its ^{13}C-n.m.r. spectrum was identical to that of the water-insoluble α-glucan obtained from the hot-water extract (Fig. 1A). Methylation analysis showed that (1→3)- and (1→4)-linkages were present in a 6:5 ratio. A Smith degradation, carried out under mild hydrolysis conditions, gave spots on a paper chromatogram corresponding to 2-*O*-α-D-glucopyranosyl-D-erythritol and *O*-α-D-glucopyranosyl-(1→3)-*O*-α-D-glucopyranosyl-(1→2)-D-erythritol, but no erythritol, indicating that consecutive (1→4)-linkages were not present, and that the distribution of the linkages was regular throughout the chain. The glucan contains somewhat more of structure **2**, with alternate (1→3)- and (1→4)-linkages, than structure **3**, which has two consecutive (1→3)-linkages. Surprisingly, it resembles water-soluble isolichenan, which has been reported to contain linkages at O-3 and O-4 in ratios of 11:9 (ref. 11) and 3:2 (ref. 10).

-α-D-Glc*p*-(1→3)-α-D-Glc*p*-(1→4)-

2

-α-D-Glc*p*-(1→3)-α-D-Glc*p*-(1→3)-α-D-Glc*p*-(1→4)-

3

The aforementioned extractions did not remove all glucose-containing material since further treatment of the lichen residue with hot 2% aqueous

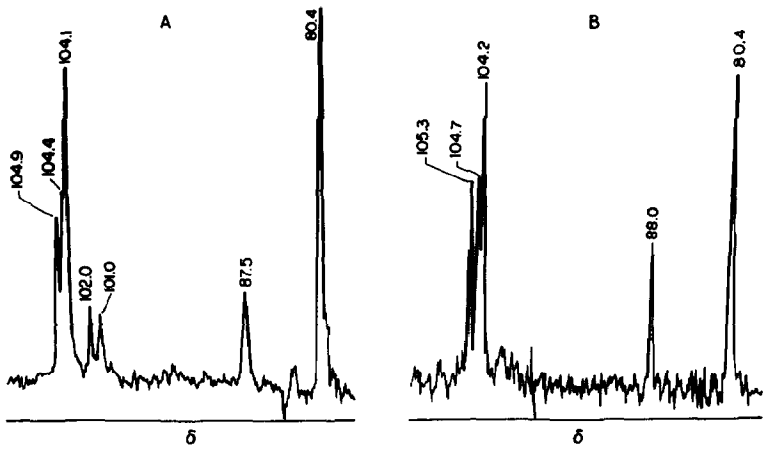

Fig. 2. ^{13}C-N.m.r. spectra (portion of C-1 and *O*-substituted carbon atoms) of: (A) Water-insoluble polysaccharide of *Usnea* sp., isolated by hot-water extraction. (B) β-D-Glucan extracted with cold dimethyl sulfoxide. Numerical values (δ) based on signal of external Me_4Si.

potassium hydroxide gave rise to a soluble fraction (21% yield) having mannose, galactose, and glucose in a 41:28:31 ratio.

D-*Glucans of* Usnea *sp.* — A water-insoluble D-glucan containing only 1% of mannose was obtained in 5.5% yield by the hot-water extraction method described above. From the C-1 portion of its ^{13}C-n.m.r. spectrum (Fig. 2A), it appeared to be a mixture with signals at δ 104.9, 104.4, 104.1, 87.5, and 80.4, corresponding to a β-D-glucan and two small ones at δ 101.0 and 102.0 arising from an α-D-glucan. The shifts and relative proportions of the signals suggest a β-D-glucan contaminated by a small amount of α-D-glucan, each component having the same chemical structure as the D-glucans of *L. vulpina*. The β-D-glucan could be obtained free of α-D-glucan (in 12% yield) by extraction with cold dimethyl sulfoxide. It proved to be identical with that of *L. vulpina* in terms of methylation data, Smith degradation, rotational measurements, and ^{13}C-n.m.r. spectrum (Fig. 2B). It contained 4% of mannose and 3% of galactose as impurities.

Structural variations occur in β-D-glucans of the genus *Usnea* in the ratios of (1→3)-to-(1→4) linkages. The linkage ratio of the D-glucan of *Usnea* sp. is 1:3, whereas that of *Usnea rubescens*[12] is 3:7 and resembles lichenan. It would be of interest to compare these glucans with the "lichenan" of *Usnea barbata*, which is advertized in the catalog of Sigma. A sample of the α-D-glucan of the *Usnea* sp. could not be obtained following hot-water extraction of the residue after dimethyl sulfoxide treatment, presumably because of its presence in a small proportion.

Partly acetylated (1→6)-linked β-D-glucopyranan from A. muehlenbergii. — Hot-water extraction of a rock tripe *(A. muehlenbergii)*, followed by cooling of the

extract, gave a copious precipitate. In order to isolate the polysaccharide, excess ethanol was added and the precipitated product (29% yield) was found to contain glucose, galactose, and mannose in a ratio of 93:3:4. The extraction process was not complete since, on hot aqueous potassium hydroxide extraction, a further 23% of polysaccharide was obtained having glucose (87%), galactose (6%), and mannose (8%) as components.

Since the hot-water extraction was not efficient, and as the glucan contained *O*-acetyl groups (^{13}C-n.m.r. spectroscopy; Fig. 3) which might migrate in hot water, cold dimethyl sulfoxide extraction seemed superior. The resulting glucan, having only 2% of mannose as impurity, was isolated in 35% yield after precipitation with excess ethanol. Methylation analysis indicated that all the glucopyranosyl residues were 6-*O*-substituted. They had the β-D configuration since the glucan had a low specific rotation of −42°, and since its ^{13}C-n.m.r. spectrum contained a low field C-1 signal at δ 104.6 (Fig. 3). A small proportion of acetyl groups were present as evidenced by signals at δ 22.1 (CH_3) and 175.3 (C=O). The presence of *O*-acetyl groups was confirmed by acid hydrolysis which liberated acetic acid, identified as its sodium salt by ^{13}C- and ^{1}H-n.m.r. spectroscopy (respective signals at δ 24.3 and δ 1.86).

Unacetylated (1→6)-linked β-D-glucopyranans were originally isolated from the related lichens *Umbilicaria pustulata* (L.) Hoffm. and *Umbilicaria hirsuta*[13–15]. Lightly *O*-acetylated (~2%) D-glucans have been isolated, by aqueous extraction followed by ethanol precipitation, from *Gyrophora esculenta* Miyoshi, *Lasallia papulosa* (Ach.) L.[16,17], *Umbilicaria angulata* Tuck., *Umbilicaria caroliniana* Tuck.,

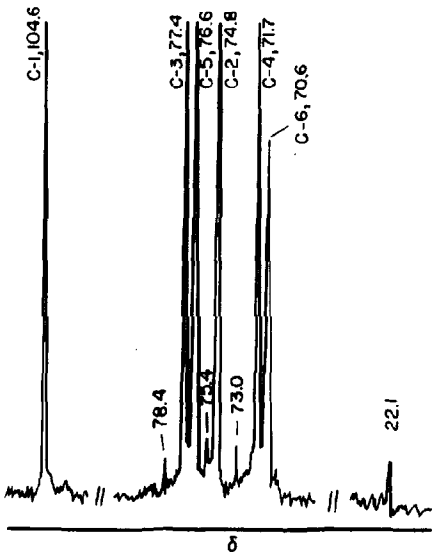

Fig. 3. ^{13}C-N.m.r. spectrum of partly acetylated (1→6)-β-D-glucopyranan, isolated by cold dimethyl sulfoxide extraction from *A. muehlenbergii*. Numerical values (δ) based on signal of external Me_4Si.

Umbilicaria polyphylla (L.) Baumg[18], and *Lasallia pennsylvanica*[12]. The product from *G. esculenta*[18] contained *O*-acetyl groups at O-3, as determined by the chemical method of Bouveng[19]. However, the polysaccharides were isolated *via* extraction with boiling water, a process that could cause *O*-acetyl migration.

The distribution of the *O*-acetyl groups in the D-glucan of *A. muehlenbergii* was also determined with the method of Bouveng[19], with some modifications. In the initial treatment with phenylisocyanate to form the phenylcarbamate derivative, dimethyl sulfoxide was used as the solvent because dissolution was not complete in the recommended *N,N*-dimethylformamide. In subsequent steps, Bouveng successively converted the phenylcarbamate into the *N*-methyl derivative, *O*-deacetylated with acid, removed the phenylcarbamate groups with lithium aluminum hydride in oxolane, and hydrolyzed the partly *O*-methylated polymer with acid[19]. In our procedure, the improvements of Corrêa *et al.*[20] were incorporated, in which the phenylcarbamate derivative was methylated under alkaline conditions, and the phenylcarbamate groups were then removed with methylsulfinyl carbanion. The resulting mixture of partly *O*-methylated glucoses was then reduced with sodium borohydride, followed by acetylation, and the mixture of *O*-methylglucitol acetates examined by g.l.c.–m.s.[21] using a capillary column of DB-210. Detected were acetates of glucitol (71%), and its 2-*O*- (20%), 3-*O*- (3%), 4-*O*- (5%), 2,3-di-*O*- (<1%), and 2,3,4-tri-*O*-methyl (<1%) derivatives, the positions of *O*-methylation corresponding to those of the *O*-acetyl groups in the original glucan. However, it is clear from the ^{13}C-n.m.r. spectrum of the glucan (Fig. 3), its *O*-acetyl content by the Hestrin method[22] and its saponification equivalent, which agrees with this value that corresponds to 1 acetyl group per 8–9 hexosyl units, that the aforementioned data are not quantitatively reliable.

A better evaluation of the quantitative distribution of the ester groups was obtained from the ^{13}C-n.m.r. spectrum of the glucan. This showed 6 signals corre-

TABLE I

DISPLACEMENT OF ^{13}C RESONANCES ON MONOACETYLATION OF METHYL β-D-GLUCOPYRANOSIDE

Derivative of methyl β-D-glucopyranoside	*Shift value and displacement[a] observed on O-acetylation (p.p.m.)*								
	C-1	*C-2*	*C-3*	*C-4*	*C-5*	*C-6*	*OCH_3*	*C=O*	*$COCH_3$*
Unsubstituted	103.6	73.45	76.2	70.0	76.2	61.1	57.5		
2-*O*-Acetyl	101.6[b]	74.2[b]	74.2[b]	70.0	76.4	61.0[b]	57.4[b]	173.7	20.8
	(*−2.0*)	(*+0.8*)	(*−2.0*)		(+0.2)	(−0.1)	(−0.1)		
3-*O*-Acetyl	103.4[b]	71.8[b]	77.8[b]	68.3	76.05	60.9[b]	57.65[b]	174.2	20.9
	(−0.2)	(*−1.7*)	(*+1.6*)	(*−1.7*)	(−0.1)	(−0.2)	(+0.1)		
4-*O*-Acetyl	103.8[b]	73.6[b]	74.3	71.6[b]	74.3	60.9[b]	57.8[b]	173.8	20.9
	(+0.2)	(+0.1)	(*−1.9*)	(*+1.6*)	(*−1.9*)	(−0.2)	(+0.3)		
6-*O*-Acetyl	103.7[b]	73.4[b]	75.9	69.8	73.7[b]	63.6[b]	57.6[b]	174.4	20.5
	(+0.1)	(−0.1)	(−0.3)	(−0.2)	(*−2.5*)	(*+2.5*)	(+0.1)		

[a]In parentheses. [b]Assignments confirmed by homonuclear, ^{1}H-decoupling series, followed by 2D ^{13}C–^{1}H plots.

sponding to those of a (1→6)-linked β-D-glucopyranan[23] with minor ones at δ 73.0, 75.4, and 78.4 (Fig. 3), attributable to *O*-acetylated carbons. These could be assigned with the aid of the above-mentioned Bouveng analysis[19] and consideration of downfield α- and upfield β-shifts occurring on acetylation[24] of appended hydroxyl groups of 2-*O*, 3-*O*, 4-*O*, and 6-*O*-acetyl derivatives of methyl β-D-glucopyranoside (see Table I). Thus, in the case of the 3-*O*-acetyl standard, the C-3 resonance underwent a downfield shift of 1.6 p.p.m., whereas those of adjacent C-2 and C-4 were each shifted upfield by 1.7 p.p.m. Applying these values to the glucan spectrum, the signal at δ 78.4 would arise from *O*-acetylated C-3 which suffered a downfield shift of 1.0 p.p.m. and was accompanied by a C-2 signal at δ 73.0 that was displaced upfield by 1.8 p.p.m. The corresponding C-4 signal was likely shifted upfield to be hidden by the large C-6 resonance at δ 70.6. The small signal at δ 75.4 was attributed to *O*-acetylated C-2 as it underwent a downfield α-shift of 0.6 p.p.m., comparable to +0.8 p.p.m. occurring on 2-*O*-acetylation of methyl β-D-glucopyranoside (Table I). Unfortunately, any *O*-acetylated C-4 signal in the spectrum would also contribute to that at δ 73.0, and accompanying C-3 and C-5 signals could be hidden by large signals at δ 76.6 and 74.8, respectively. However, any contribution of *O*-acetylated C-4 to the δ 73.0 signal is small, as its size is of the same magnitude as that of C-3 at δ 78.4. Thus, it appears that acetyl groups are mainly distributed between O-2 and O-3 in the glucan, with a smaller proportion at O-4.

This distribution of *O*-acetyl groups suggests two biosynthetic pathways. In one, the ester group is linked to each available position; in the other, one hydroxyl group only is originally substituted, and the migration takes place during later growth owing to the extremely high temperatures experienced by the lichen in summer.

EXPERIMENTAL

Methods. — Optical rotations were measured on 0.2% solution of polysaccharide in 2% aqueous NaOH.

^{13}C-N.m.r. spectra were recorded under the conditions previously described[3]. The determinations were carried out at 70° on nonacetylated polysaccharides dissolved in 1–3% NaOD in D_2O. Variations in the concentration of alkali gave rise to small differences in the chemical shifts of certain signals from sample to sample. The *O*-acetylated glucan was examined in D_2O.

The determination of the sugar composition, the methylation analysis, and the Smith degradation of polysaccharides were carried out as described previously[3].

Lichens. — *Usnea* sp. was collected from the lower dead branches of spruce trees growing on the west shore of Mountain Lake, near the township of Stanley Mission, Saskatchewan, Canada. *A. muehlenbergii* (a rock tripe, having the Cree name Asīnīwākonak) was found on prominent rock formations on the east shore of the lake. *L. vulpina* was collected from dead branches of softwood in the Lake Louise region, Alberta, Canada. It is also found in the boreal forests of North Saskatchewan.

Hot-water extraction and cold-water precipitation of glucans. — Samples of *L. vulpina* and *Usnea* sp. (1.0 g), previously extracted with cold 2:1 (v/v) benzene–ethanol, were treated with water (50 mL) for 6 h at 100°. After filtration, the filtrates were centrifuged to remove small particles and evaporated to 3 mL. The solutions were frozen and, after 1 h, they were kept in a refrigerator overnight to thaw. The precipitates were isolated by centrifugation and the pellets dispersed in cold water which was centrifuged. This process was repeated in order to remove soluble impurities. The supernatants were combined, evaporated to a small volume (2 mL), and the dissolved polysaccharide precipitated by addition to excess ethanol (10 mL).

Dimethyl sulfoxide extraction of L. vulpina, Usnea *sp., and* A. muehlenbergii. — Lichen samples (1.0 g), previously extracted with 2:1 (v/v) benzene–ethanol, were shaken in dimethyl sulfoxide (17 mL) for 45 h. The mixtures were filtered through glass wool, the residues washed with further dimethyl sulfoxide (5 mL), and the filtrates centrifuged to remove fine particles. Precipitation of the glucans was effected by addition to excess ethanol (200 mL).

Hot aqueous KOH extraction of L. vulpina. — Residual lichen remaining after successive cold dimethyl sulfoxide and hot-water extraction of *L. vulpina* (1.0 g) was treated with 2% aqueous KOH (17 mL) for 2 h at 100°. The solution was made neutral with acetic acid and filtered. The filtrate was added to excess ethanol (100 mL), and the resulting precipitate isolated.

Preparation of 2-O-, 3-O-, 4-O-, and 6-O-acetyl derivatives of methyl β-D-glucopyranoside. — The 2-*O*- and 3-*O*-acetyl derivatives were prepared, from their previously described 4,6-*O*-benzylidene derivatives[25], respectively by treatment with 80% acetic acid for 20 min at 100°, followed by evaporation of the solvent, partition of the residue between water and hexane, and evaporation of the aqueous layer. The 6-*O*-acetyl derivative was obtained as described in the literature[25].

Methyl 4-*O*-acetyl-β-D-glucopyranoside was synthesized starting from methyl 2,3-di-*O*-benzyl-β-D-glucopyranoside[26] as follows. A sample (700 mg) in *N,N*-dimethylformamide (0.5 mL) containing Ag_2O (0.5 g) was stirred with cooling (ice bath) and α-bromotoluene (0.25 mL) added dropwise. After 3 days, a little methanol was added, the mixture treated with excess dichloromethane, and the solution filtered. Evaporation of the filtrate gave a syrup that was fractionated on a column of silica gel (eluent: 5:1 v/v toluene–ethyl acetate). Fractions were obtained containing methyl 2,3,4,6-tetra-*O*-benzyl-β-D-glucopyranoside (130 mg), followed by methyl 2,3,6-tri-*O*-benzyl-β-D-glucopyranoside (140 mg), m.p. 66–67° (from ether–hexane), $[\alpha]_D^{25}$ −18° (*c* 2.4, chloroform); t.l.c. (3:1, v/v, toluene–ethyl acetate) R_F 0.73; lit.[27] m.p. 64–65°, $[\alpha]_D$ −17°; ^{13}C-n.m.r. ($CDCl_3$): δ 138.6, 138.0, 128.56, 128.45, 128.40, 128.13, 127.98, 127.84, 127.74 ($CH_2C_6H_5$), 104.9 (C-1), 84.1, 81.9, 75.3, 74.7, 74.2, 73.8, 71.7, 70.4, and 57.2 (OMe); these data correspond to cited values[27].

Anal. Calc. for $C_{28}H_{32}O_6$: C, 72.39; H, 6.94. Found: C, 72.14; H, 6.78.

The next fraction (0.35 g) contained methyl 2,3,4-tri-*O*-benzyl-β-D-gluco-

pyranoside (^{13}C-n.m.r. signal at δ 62.1 corresponding to C-OH-6); m.p. 85–86° (ether–hexane), $[\alpha]_D^{25}$ +9° (*c* 5, chloroform); t.l.c.: R_F 0.49; lit.[28,29] 91–92°, $[\alpha]_D$ +10°; ^{13}C-n.m.r. ($CDCl_3$): δ 138.6, 138.5, 138.0, 128.6, 128.53, 128.41, 128.10, 127.91, 127.69 ($CH_2C_6H_5$), 104.9 (C-1), 84.5, 82.5, 77.7, 75.7, 75.1, 74.9, 62.1 (unsubstituted C-6), and 57.4 (OMe).

Anal. Calc. for $C_{28}H_{32}O_6$: C, 72.39; H, 6.94. Found: C, 72.14; H, 6.95.

The 2,3,6-tri-*O*-benzyl derivative was acetylated in 1:1 (v/v) pyridine–acetic anhydride (2 mL) for 30 min at 100° to form the 4-acetate. The solution was evaporated to a syrup, which was *O*-debenzylated by shaking with 5% Pd–C (1.0 g) in acetic acid (20 mL) in the presence of H_2. After 24 h, fresh catalyst was added and the hydrogenolysis process repeated. The mixture was filtered, and the filtrate lyophilized to yield methyl 4-*O*-acetyl-β-D-glucopyranoside (0.13 g). As with the aforementioned 2-*O*- and 3-*O*-acetyl derivatives, the product was not subjected to a further crystallization step in order to minimize possible acetyl migration. Instead, they were examined immediately by ^{13}C-n.m.r. spectroscopy as D_2O solutions at ambient temperature (see Table I).

G.l.c.–m.s. analysis of acetates of glucitol and its partly O-methylated derivatives. — The partly *O*-acetylated β-D-glucan of *A. muehlenbergii* was subjected to a combination of the methods of Bouveng[19], but using dimethyl sulfoxide instead of *N,N*-dimethylformamide in the phenylcarbamylation step, and of Corrêa *et al.*[20], as described in the Results and Discussion section. The resulting mixture of glucose and its *O*-methyl derivatives was successively reduced in aqueous $NaBH_4$ and acetylated to give a mixture of *O*-methyl alditol acetates which was examined by g.l.c.–m.s. using a capillary column coated with DB-210 (0.25 mm i.d. × 30 m). The analyses were performed with a Model 4000 Finnigan unit, interfaced with an Incos 2300 Data System. The injections were made in the split mode at 50° and a rapid program (40°/min) to 220° (hold) was carried out. The carrier gas was He (linear velocity, 22 cm/s). E.i. mass spectra were obtained repetitively every 2 s by scanning from mass 40 to 420. The following acetates of glucitol and several *O*-methyl derivatives were detected having the following retention times and percentage areas: 2,3,4-tri-*O* (391 s, <1%), 2,3-di-*O* (477 s, <1%), 2-*O* (603 s, 20%), 3-*O* (633 s, 3%), and 4-*O*-methyl (703 s, 5%), and glucitol (778 s, 71%).

REFERENCES

1 J. J. BERZELIUS, *J. Chem. Phys.*, 7 (1815) 317–352.
2 P. KARRER AND B. JOOS, *Hoppe-Seyler's Z. Physiol. Chem.*, 141 (1924) 311–315.
3 P. A. J. GORIN AND M. IACOMINI, *Carbohydr. Res.*, 128 (1984) 119–132.
4 M. IACOMINI, C. L. SCHNEIDER, AND P. A. J. GORIN, *Carbohydr. Res.*, 142 (1985) 237–251.
5 P. A. J. GORIN AND M. IACOMINI, *Carbohydr. Res.*, 142 (1985) 253–267.
6 L. D. HALL AND L. F. JOHNSON, *J. Chem. Soc., Chem. Commun.*, (1969) 509–510.
7 I. YOKOTA, S. SHIBATA, AND H. SAITÔ, *Carbohydr. Res.*, 69 (1979) 252–258.
8 T. USUI, N. YAMAOKA, K. MATSUDA, K. TUZIMURA, H. SUGIYAMA, AND S. SETO, *Agric. Biol. Chem.*, 39 (1975) 1071–1076.
9 P. A. J. GORIN, E. M. GIBLIN, G. P. SLATER, AND L. HOGGE, *Carbohydr. Res.*, 106 (1982) 235–238.
10 N. B. CHANDA, E. L. HIRST, AND D. J. MANNERS, *J. Chem. Soc.*, (1950) 1951–1958.

11 K. H. MEYER AND P. GÜRTLER, *Helv. Chim. Acta*, 30 (1947) 761–765.
12 Y. NISHIKAWA, K. OHKI, K. TAKAHASHI, G. KURONO, F. FUKUOKA, AND M. EMORI, *Chem. Pharm. Bull.*, 22 (1974) 2692–2702.
13 B. DRAKE, *Biochem. Z.*, 313 (1943) 388–394.
14 B. LINDBERG AND J. MCPHERSON, *Acta Chem. Scand.*, 8 (1954) 985–988.
15 C. G. HELLERQVIST, B. LINDBERG, AND K. SAMUELSSON, *Acta Chem. Scand.*, 22 (1968) 2736–2737.
16 S. SHIBATA, Y. NISHIKAWA, T. TAKEDA, AND M. TANAKA, *Chem. Pharm. Bull.*, 16 (1968) 2362–2369.
17 Y. NISHIKAWA, M. TANAKA, S. SHIBATA, AND F. FUKUOKA, *Chem. Pharm. Bull.*, 18 (1970) 1431–1434.
18 Y. NISHIKAWA, T. TAKEDA, S. SHIBATA, AND F. FUKUOKA, *Chem. Pharm. Bull.*, 17 (1969) 1910–1916.
19 H. O. BOUVENG, *Acta Chem. Scand.*, 15 (1961) 87–95.
20 J. B. C. CORRÊA, F. REICHER, S. ODEBRECHT, AND M. L. DE OLIVEIRA, *An. Acad. Bras. Ciênc.*, 51 (1979) 429–433.
21 B. LINDBERG AND J. LÖNNGREN, *Methods Enzymol.*, 50 (1978) 3–33.
22 F. DOWNS AND W. PIGMAN, *Methods Carbohydr. Chem.*, 7 (1976) 241–243.
23 H. SAITÔ, T. OHKI, N. TAKASUKA, AND T. SASAKI, *Carbohydr. Res.*, 58 (1977) 293–305.
24 D. R. BUNDLE, H. J. JENNINGS, AND I. C. P. SMITH, *Can. J. Chem.*, 51 (1973) 3812–3819.
25 A. P. TULLOCH AND A. HILL, *Can. J. Chem.*, 46 (1968) 2485–2493.
26 J. C. DENNISON AND D. I. MCGILVRAY, *J. Chem. Soc.*, (1951) 1616.
27 P. J. GAREGG, H. HULTBERG, AND S. WALLIN, *Carbohydr. Res.*, 108 (1982) 97–101.
28 N. PRACDIĆ AND D. KEGLEVIĆ, *Tetrahedron*, 21 (1965) 1897–1901.
29 D. KEGLEVIĆ AND D. LJEVAKOVIĆ, *Carbohydr. Res.*, 64 (1978) 319–322.