New Triterpenoid Pentasaccharides from *Androsace saxifragifolia*

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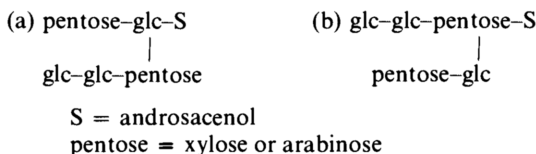
Two new triterpenoid pentasaccharides, saxifragifolins C and D, isolated from the aerial part of *Androsace saxifragifolia* were characterized as androsacenol 3-*O*-{β-D-xylopyranosyl-(1 ⟶ 2)-β-D-glucopyranosyl-(1 ⟶ 4)-[β-D-glucopyranosyl-(1 ⟶ 4)-β-D-glucopyranosyl-(1 ⟶ 2)]-α-L-arabinopyranoside} (**6**) and cyclamiretin A 3-*O*-{β-D-xylopyranosyl-(1 ⟶ 2)-β-D-glucopyranosyl-(1 ⟶ 4)-[β-D-glucopyranosyl-(1 ⟶ 4)-β-D-glucopyranosyl-(1 ⟶ 2)]-α-L-arabinoside} (**7**). The structures were elucidated by a combination of field desorption mass spectrometry, chemical degradation, and ^{1}H and ^{13}C n.m.r. spectroscopy.

In previous communications [1,2] the isolation and characterization of the sapogenols androsacenol (**1**), cyclamiretin A (**2**), and cyclamiretin D (**3**) and the saponins saxifragifolin A (**4**) and saxifragifolin B (**5**) from *A. saxifragifolia* have been reported. Further work on the saponin mixture of the plant led to the isolation of two new triterpenoid pentasaccharides, saxifragifolin C and saxifragifolin D. This paper reports the isolation and structure elucidation of these two glycosides.

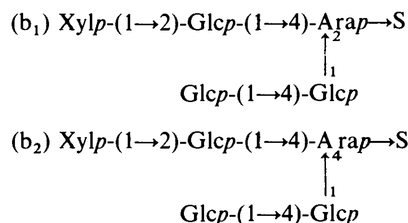
Results and Discussion

The ethanolic extract of the aerial part of *A. saxifragifolia* on repeated chromatographic purification on silica gel column afforded, besides fractions A and B whose characterization has already been reported,[2] a third fraction, C, which gave positive colour tests for triterpenoid glycosides. This fraction, although apparently homogeneous by t.l.c., was found to be a mixture of two compounds by h.p.l.c., and could successfully be separated by preparative h.p.l.c. to afford saxifragifolin C (**6**) and saxifragifolin D (**7**) using a reversed-phase Spherisorb S-10-ODS column as stationary phase and MeOH–water (70:30) as mobile phase. Acid hydrolysis of compound (**6**) led to the formation of an aglycone characterized as androsacenol (**1**) and to monosaccharide constituents identified as D-glucose, D-xylose, and L-arabinose by comparison with authentic samples; the absolute configuration is assumed for glucose and xylose constituents and that of arabinose was determined by its isolation and by a study of its properties (see Experimental section).

Field desorption mass spectrometry [3–7] (FDMS) was employed for the determination of the molecular weight and the sequence of the monosaccharide units in the glycone portion of saxifragifolin C (**6**). The FD spectrum displayed ion peaks at *m/z* 1 319 and 1 303 formed by cationization of the molecule with K^+ and Na^+ respectively. As is usually the case the $[M + K]^+$ ion peak was smaller than that due to $[M + Na]^+$. Although the FDMS spectrum did not show the molecular ion peak, the appearance of the $[M + K]^+$ and $[M + Na]^+$ ion peaks helped in the determination of the molecular weight of saxifragifolin C (**6**). The fragment ions at *m/z* 1 157 and 1 141 may be ascribed respectively to the loss of a terminal glucose unit from $[M + K]^+$ and $[M + Na]^+$, and the ions at *m/z* 1 187 and 1 171 are assigned to the loss of a terminal pentose (xylose or arabinose) from $[M + K]^+$ and $[M + Na]^+$ respectively. The discernible fragment ions at *m/z* 1 025 and 1 009 are attributed respectively to the loss of a terminal glucose–pentose disaccharide and/or both a terminal pentose and terminal glucose unit from $[M + K]^+$ and $[M + Na]^+$, while the fragment ions at *m/z* 995 and 979 are ascribed to the loss of a terminal glucose–glucose disaccharide and/or both terminal glucose units from the potassium and sodium cation complexes respectively. The FD mass spectrum does not show any fragment ion corresponding to the loss of a trisaccharide unit from either $[M + K]^+$ or $[M + Na]^+$, which suggests that a branch point in the glycone occurs on the monosaccharide directly attached to the aglycone. Thus from the FDMS studies one of the following two monosaccharide sequences may be proposed for the carbohydrate moiety of saxifragifolin C.



Treatment of saxifragifolin C with sodium metaperiodiate followed by hydrolysis liberated L-arabinose as the only sugar constituent, suggesting that the arabinose moiety of saxifragifolin C contains no vicinal diol and is linked to the aglycone either through positions 1, 2, and 4 or through positions 1 and 3. Moreover, permethylation of saxifragifolin C with NaH–MeI in hexamethylphosphoric triamide (HMPA) yielded the permethylate assigned structure (**8**), which on acid hydrolysis liberated 2,3,4,6-tetra-*O*-methyl-D-glucose, 3,4,6-tri-*O*-methyl-D-glucose, 2,3,6-tri-*O*-methyl-D-glucose, 2,3,4-tri-*O*-methyl-D-xylose, and 3-*O*-methyl-L-arabinose. Consequently the monosaccharide sequence of the carbohydrate moiety of saxifragifolin C was shown to be (b), which itself represents a mixture of two isomers (b_1) and (b_2). Moreover, this methylation analysis and the ^{13}C n.m.r. data of saxifragifolin C (Table) suggested the pyranose-ring forms for the monosaccharides.



Saxifragifolin C on partial hydrolysis with 2M-HCl in Bu^nOH on a steam-bath for 2 h afforded a mixture of prosapogenins which was permethylated and subjected to p.l.c. to yield permethylates assigned structures (**9**), (**10**), and (**11**) containing four, three, and three monosaccharides respectively as revealed by ^{1}H n.m.r. spectroscopy. The permethylate (**9**) on acid hydrolysis yielded 2,3,4,6-tetra-*O*-methyl-D-glucose, 2,3,6-tri-*O*-

(1) R = H, R′ = OAc
(2) R = H, R′ = H
(11) R = Me, R′ = OAc
(4) R = H, R′ = OAc
(5) R = H, R′ = H
(10) R = Me, R′ = OAc
(9) R = Me, R′ = OAc
(6) R = H, R′ = OAc
(7) R = H, R′ = H
(8) R = Me, R′ = OAc
(12) R = Me, R′ = H

(3)

methyl-D-glucose, and 3-*O*-methyl-L-arabinose. The permethylate (**10**) on hydrolysis liberated 2,3,4,6-tetra-*O*-methyl-D-glucose, 2,3,6-tri-*O*-methyl-D-glucose, and 3,4-di-*O*-methyl-L-arabinose, whereas the permethylate (**11**) on similar acid hydrolysis furnished 2,3,4,6-tetra-*O*-methyl-D-glucose and 3-*O*-methyl-L-arabinose. Thus the formation of the permethylate (**10**) was helpful in distinguishing between the possible isomers (b_1) and (b_2), and the monosaccharide sequence of the glycone portion of saxifragifolin C was established as (b_1).

β-Configuration ($^4C_1$ conformation) for the three glucopyranosyl units and the xylopyranose, and α-configuration ($^1C_4$ conformation) for the arabinopyranoside, were inferred from the *J*-values of the respective anomeric protons in the ^{1}H n.m.r. spectra of the permethylates (**8**), (**9**), (**10**), and (**11**) (see Experimental section). The attachment of the carbohydrate moiety at the C-3 position of the aglycone (**1**) was revealed from the ^{13}C n.m.r. chemical shifts of saxifragifolin C (Table). Assignment of the ^{13}C δ-values of compound (**6**) were made by comparison with those of the sapogenin and the appropriate methyl glycosides [8] by the application of the known chemical-shift rules [9,10] and glycosylation shifts.[8,11–15]

On the basis of the foregoing evidence, the structure of saxifragifolin C is proposed to be androsacenol 3-*O*-β-D-xylopyranosyl(1 ⟶ 2)-β-D-glucopyranosyl-(1 ⟶ 4)-[β-D-glucopyranosyl-(1 ⟶ 4)-β-D-glucopyranosyl-(1 ⟶ 2)]-α-L-arabinopyranoside (**6**)

Saxifragifolin D on acid hydrolysis afforded two sapogenins and three monosaccharides. The two sapogenins were identified as cyclamiretin A (**2**) and cyclamiretin D (**3**) by comparison of physical and spectral properties with authentic samples, and the monosaccharides were identified as D-glucose, D-xylose, and L-arabinose. The ^{13}C n.m.r. spectrum of saxifragifolin D (Table)

Table. Chemical shifts δ_C/p.p.m. ($\pm$0.1) of androsacenol[1] (**1**), cyclamiretin A[2] (**2**), saxifragifolin C (**6**), and saxifragifolin D (**7**) (C_5D_5N)

Carbon	(1)	(2)	(6)	(7)	Carbon	(6)	(7)
1	38.0	39.1	39.2	38.8	A-1	104.3[a]	104.2[a]
2	28.3	28.3	26.7	26.7	A-2	79.1	79.1
3	78.2	78.0	89.1	89.2	A-3	71.5[b]	71.6[b]
4	39.6	39.5	39.7	39.8	A-4	75.8[c]	75.8[c]
5	55.8	55.7	55.7	55.6	A-5	64.7	64.6
6	18.3[a]	18.6[a]	18.5[c]	18.5[c]	G-1	103.9[a]	104.0[a]
7	32.7[b]	32.9[b]	33.2[a]	33.2[a]	G-2	85.5	85.5
8	42.9	42.5	42.5	42.7	G-3	71.9[b]	71.8[b]
9	47.6	47.8	47.5	47.6	G-4	71.0[b]	71.0[b]
10	37.3	37.3	36.8	36.8	G-5	78.7	78.7
11	19.2[a]	19.3[a]	19.2[c]	19.2[c]	G-6	62.9	63.0
12	31.8[b]	31.8[b]	31.7[a]	31.6[a]	X-1	107.3	107.2
13	86.1	86.4	86.4	86.0	X-2	75.5[c]	75.5[c]
14	44.6	44.7	44.5	44.6	X-3	77.9	77.8[d]
15	34.4	34.6	34.4	34.4	X-4	70.5	70.5
16	73.2	77.2	73.4	73.5	X-5	66.9	66.9
17	46.5[c]	44.7	46.5	44.1	G′-1	104.7[a]	104.6[a]
18	50.6	50.6	50.4	50.4	G′-2	75.5[c]	75.5[c]
19	36.8	37.0	36.8	36.9	G′-3	76.2	76.3
20	47.6[c]	48.0	48.3	48.2	G′-4	76.2	76.2
21	30.1[b]	37.0	30.8[a]	36.8	G′-5	77.9	77.9[d]
22	74.1	33.8	73.9	33.3	G′-6	62.4	62.5
23	28.8	28.7	28.0	28.0	G″-1	105.2[a]	105.1[a]
24	16.6[d]	16.6	16.4[b]	16.3[b]	G″-2	74.6	74.6
25	16.4[d]	16.6	16.6[b]	16.6[b]	G″-3	78.7	78.6
26	18.7	18.2	18.8	18.7	G″-4	71.8[b]	71.8[b]
27	20.1	19.6	19.8	19.8	G″-5	77.9	77.9[d]
28	70.3	78.0	70.0	77.6	G″-6	62.4	62.4
29	24.2	24.8	24.1	24.0			
30	205.3	207.2	205.3	206.6			
22-OAc	169.8		169.9				
	20.4		20.9				

G, G′, G″ = glucose, X = xylose, A = arabinose.
[a,b,c,d] Assignments within a column may be interchanged.

revealed that cyclamiretin A (**2**) is the genin present, indicating that cyclamiretin D (**3**) is an artifact formed by acid-catalysed rearrangement of compound (**2**) during hydrolysis as reported previously.[16]

The FD mass spectrum of saxifragifolin D displayed peaks at *m/z* 1 261 and 1 245, assignable to $[M + K]^+$ and $[M + Na]^+$ respectively, and other fragment ions analogous to those in compound (**6**) at *m/z* 1 129, 1 113, 1 099, 1 083, 967, 951, 937, and 921. The glycone moiety of compound (**11**) may also be shown to be identical with that of compound (**6**) by similarity of the ^{13}C values of the carbohydrate moieties of both the glycosides and by permethylation studies. Moreover attachment of the pentasaccharide moiety at C-3 of cyclamiretin A (**2**) was deduced by comparison of the ^{13}C data of compound (**2**) with those of saxifragifolin D keeping in mind the glycosylation-shift values. Thus saxifragifolin D was characterized as cyclamiretin A 3-*O*-{β-D-xylopyranosyl-(1 ⟶ 2)-β-D-glucopyranosyl-(1 ⟶ 4)-[β-D-glucopyranosyl-(1 ⟶ 4)-β-D-glucopyranosyl-(1 ⟶ 2)]-α-L-arabinopyranoside} (**7**).

Experimental

All m.p.s were measured on a Kofler hot-stage apparatus and are uncorrected. Optical rotations were measured on a Perkin-Elmer automatic polarimeter. ^1H N.m.r. spectra were recorded on a JEOL FX-100 (99.6 MHz) instrument in $CDCl_3$ or C_5D_5N with $SiMe_4$ as internal standard. ^{13}C N.m.r. spectra were recorded on a JEOL FX-100 Fourier-transform spectrometer operating at 25.05 MHz for solutions in C_5D_5N ($SiMe_4$ as internal standard). I.r. spectra were recorded in Nujol mulls on a Perkin-Elmer model 177 instrument. Electron-impact mass spectra were recorded on a Hitachi model RMU-6L mass spectrometer. Field-desorption mass spectra were recorded on a JEOL D-300 instrument with data system JMA-2000, with scan time 20 s. Field-desorption emitters were prepared by high-temperature activation of 10 μm diameter tungsten wire. FD Emitters with an average length of 30 μm for the carbon microneedles were used as standards. The ionization efficiency and the adjustment of the FD emitter were determined by means of the acetone peak at *m/z* 58 in the field-ionization mode. All FD spectra were produced at an ion-source pressure of 3×10^{-7} Torr, and an ion-source temperature of 60—70 °C; the ion-source potentials were +2 kV for the field anode and −5 kV for the slotted cathode plate. The samples were desorbed by direct heating (emitter heating current 23 mA) without emission control. Methanol was used as solvent for the compounds. In general, samples of 1×10^{-5} g were applied to the standard emitter *via* a syringe. G.l.c. was performed on a Hewlett-Packard model 5730A instrument on a column of (i) ECNSS-M 3% on Gas Chrom Q at 190 °C for alditol acetates, or (ii) OV-225 on Gas Chrom Q at 195 °C for partially methylated alditol acetates. High-performance liquid chromatography (h.p.l.c.) was performed on a Spectra-Physics model SP 8000B instrument with a column of Spherisorb S-10-ODS and a Micromeritics 771 refractive-index detector in MeOH–water (7:3) as solvent. T.l.c. was carried out on silica gel G (B.D.H.) with the following solvent systems: (A) benzene–$CHCl_3$–EtOAc–MeOH (1:7:1:1), (B) $CHCl_3$–MeOH–water (64:36:2). Paper chromatography for sugars was performed on Whatman paper No. 1 with the solvent systems (C) Bu^nOH–

C_5H_5N–water (6:4:3), (D) Bu^nOH–EtOH–water (4:1:5 upper layer).

Isolation of Saxifragifolin C (**6**) *and Saxifragifolin D* (**7**).—The air-dried and powdered aerial part of the plant *A. saxifragifolia* (1 kg) was successively extracted with light petroleum (boiling range 60—80 °C), $CHCl_3$, and 90% ethanol. A portion (50 g) of the total ethanol extract (80 g) was chromatographed on silica gel as described previously [2] and the fraction eluted with $CHCl_3$–MeOH (85:15) yielded saxifragifolin C and saxifragifolin D. Elution of the column with $CHCl_3$–MeOH (80:20) yielded a fraction (1.8 g) which, on further purification by rechromatography, afforded a t.l.c.-homogeneous glycoside (0.6 g). This glycoside fraction was found to be a mixture of two compounds by h.p.l.c. Preparative h.p.l.c. of this glycoside mixture with reversed-phase Spherisorb S-10-ODS column with the solvent system MeOH–water (7:3) and flow rate 3 ml min^{-1} afforded *saxifragifolin C* (**6**) (0.25 g), m.p. 280—283 °C; $[\alpha]_D$ −5.5° (*c* 0.5 in MeOH) (Found: C, 56.2; H, 7.6. $C_{60}H_{96}O_{29}$ requires C, 56.24; H, 7.55%) and *saxifragifolin D* (**7**) (0.17 g), m.p. 272—275 °C; $[\alpha]_D$ −6.9° (*c* 0.6 in MeOH) (Found: C, 56.9; H, 7.7. $C_{58}H_{94}O_{27}$ requires C, 56.94; H, 7.75%).

Hydrolysis of Saxifragifolin C (**6**).—Compound (**6**) (75 mg) was hydrolysed with 2M-HCl in aqueous MeOH (80%; 25 ml) at boiling water-bath temperature for 3 h. The hydrolysate was worked up in the usual manner[1] and the residue, on chromatographic purification over silica gel followed by crystallization from EtOAc, yielded a sapogenol (12 mg), m.p. 260—262 °C, which was characterized as androsacenol (**1**) from its physical and spectral characteristics.[1]

The filtrate from the hydrolysate was neutralized with Ag_2CO_3 and filtered. A portion of the filtrate was concentrated under reduced pressure and tested for carbohydrates by paper chromatography with solvent system C. D-Glucose, D-xylose, and L-arabinose were identified using authentic specimens. That the arabinose was the L-enantiomer was confirmed by its actual isolation by preparative paper chromatography and comparison of its specific rotation with that of authentic L-arabinose. The identification of the monosaccharides were also confirmed by g.l.c. of the carbohydrate mixture on column (i) after preparation of their alditol acetates by reduction with $NaBH_4$ followed by acetylation in the usual way.

Periodate Oxidation of Saxifragifolin C (**6**) *and Hydrolysis of the Product.*—To a solution of compound (**6**) (25 mg) in 90% EtOH (3 ml) was added dropwise a solution of sodium metaperiodate (25 mg) in water (2 ml), and the mixture was stirred at 15 °C for 3 h, kept at room temperature overnight, and worked up in the usual manner. The residue was hydrolysed with 2M-HCl. The aqueous phase was tested for carbohydrates by paper chromatography. Only L-arabinose was identified.

Permethylation of Saxifragifolin C (**6**) *and Hydrolysis of the Product.*—A solution of compound (**6**) (40 mg) in HMPA (6 ml) was treated with NaH (250 mg) and MeI (6 ml) at room temperature for 3 h. The reaction mixture was extracted with diethyl ether and the extract was worked up in the usual manner to yield a gummy residue, which was chromatographed over silica gel with EtOAc–light petroleum (b.p. 60—80 °C) (4:6) as eluant to give permethylate (**8**) (20 mg) as powder, m.p. 108—110 °C; $\delta(CDCl_3)$ 0.80 (3 H, s), 0.88 (3 H, s), 0.97 (3 H, s), 1.15 (3 H, s), 1.28 (3 H, s), 2.01 (3 H, s, 22-OAc), 4.35 (1 H, d, *J* 5 Hz, 1-H of arabinose unit), 4.38 (1 H, d, *J* 6 Hz, 1-H of glucose unit), 4.42 (1 H, d, *J* 7 Hz, 1-H of glucose unit), 4.44 (1 H, d, *J* 7 Hz, 1-H of glucose unit), 4.74 (1 H, d, *J* 7 Hz, 1-H of xylose unit), 5.04 (1 H, m, 22-H), and 9.44 (1 H, s, 30-H).

The permethylated product (**8**) (10 mg) was hydrolysed on being heated under reflux with 2M-HCl in aqueous MeOH (5 ml) for 3 h. The reaction mixture was then cooled, evaporated to dryness under reduced pressure, diluted with water, and filtered. The filtrate was neutralized with Ag_2CO_3 and filtered. The filtrate was concentrated, and the product was reduced with $NaBH_4$ and worked up in the usual manner. The residue was acetylated with Ac_2O–pyridine (1:1) at water-bath temperature for 1 h, dried *in vacuo* over fused $CaCl_2$, and subjected to g.l.c. analysis on column (ii). Five peaks were detected and identified according to their retention times relative to that of the alditol acetate of authentic 2,3,4,6-tetra-*O*-methyl-D-glucose (R_t-values). The five peaks were identified as the alditol acetates of 2,3,4,6-tetra-*O*-methyl-D-glucose (R_t 1.0), 2,3,4-tri-*O*-methyl-D-xylose (R_t 0.53), 3,4,6-tri-*O*-methyl-D-glucose (R_t 1.83), 2,3,6-tri-*O*-methyl-D-glucose (R_t 2.30, and 3-*O*-methyl-L-arabinose (R_t 1.50) by comparison with R_t-values of authentic samples.[17,18]

Partial Hydrolysis of Saxifragifolin C (**6**) *and Methylation of the Prosapogenin Mixture followed by their Separation.*—Compound (**6**) (35 mg) was heated in 2M-HCl (3 ml) and Bu^nOH (3 ml) on a steam-bath for 2 h. To the reaction mixture were added Bu^nOH (40 ml) and water (15 ml) and the mixture was shaken. The Bu^nOH layer was separated, then washed with water, and evaporated under reduced pressure to give a prosapogenin mixture (24 mg). This was methylated in the same way as for (**6**) in the preceding experiment to give a resinous substance (27.2 mg), which was subjected to preparative t.l.c. (p.l.c.) [silica gel; developer benzene–acetone (3:1)] to give three fractions. Each fraction was further purified by p.l.c. to give the pure permethylates (**9**) (6.5 mg), (**10**), (4.2 mg), and (**11**) (3.4 mg).

Permethylate (**9**). $\delta_H(CDCl_3)$ 4.37 (1 H, d, *J* 5 Hz, 1-H of arabinose unit), 4.40 (1 H, d, *J* 7 Hz, 1-H of glucose unit), 4.42 (1 H, d, *J* 7 Hz, 1-H of glucose unit), and 4.45 (1 H, d, *J* 7 Hz, 1-H of glucose unit). On acid hydrolysis it yielded 2,3,4,6-tetra-*O*-methyl-D-glucose, 2,3,6-tri-*O*-methyl-D-glucose, and 3-*O*-methyl-L-arabinose, identified by g.l.c. analysis of the alditol acetates.

Permethylate (**10**). $\delta_H(CDCl_3)$ 4.36 (1 H, d, *J* 5 Hz, 1-H of arabinose unit), 4.41 (1 H, d, *J* 6.5 Hz, 1-H of glucose unit), and 4.44 (1 H, d, *J* 7 Hz, 1-H of glucose unit). This, on hydrolysis, liberated 2,3,4,6-tetra-*O*-methyl-D-glucose, 2,3,6-tri-*O*-methyl-D-glucose, and 3,4-di-*O*-methyl-L-arabinose [R_t 1.37 in column (i) at 190 °C].

Permethylated (**11**). $\delta_H(CDCl_3)$ 4.37 (1 H, d, *J* 5 Hz, 1-H of arabinose unit) and 4.41 (1 H, d, *J* 7 Hz, 1-H of glucose unit). The compound (**11**), on acid hydrolysis, furnished 2,3,4,6-tetra-*O*-methyl-D-glucose and 3-*O*-methyl-L-arabinose.

Hydrolysis of Saxifragifolin D (**7**).—Compound (**7**) (100 mg) was refluxed with 2M-HCl in aqueous MeOH (20 ml) for 4 h and the mixture was then worked up in the usual manner. Chromatographic separation of the sapogenols, and crystallization of the products from EtOAc, yielded two compounds, cyclamiretin A (**2**) (11 mg) and cyclamiretin D (**3**) (19 mg).

Permethylation of Compound (**7**) *and Hydrolysis of the Product.*—A solution of compound (**7**) (50 mg) in HMPA (7 ml) was treated with NaH (350 mg) and MeI (7 ml) at room temperature for 3 h. The reaction mixture was worked up and the residue was purified by chromatography on a silica gel column with EtOAc–light petroleum (b.p. 60—80 °C) (4:6) as eluant to yield the permethylate (**12**) as a powder (25 mg), $\delta(CDCl_3)$ 0.80 (3 H, s), 0.88 (3 H, s), 0.96 (3 H, s), 1.08 (3 H, s), 1.15 (3 H, s), 4.34 (1 H, d, *J* 5 Hz, 1-H of arabinose unit), 4.38 (1 H, d, *J* 6 Hz, 1-H of glucose unit), 4.40 (1 H, d, *J* 7 Hz, 1-H of

glucose unit), 4.44 (1 H, d, J 7 Hz, 1-H of glucose unit), 4.76 (1 H, d, J 7 Hz, 1-H of xylose unit), and 9.55 (1 H, s, 30-H).

The permethylate (**12**) (10 mg) was hydrolysed with 2M-HCl in aqueous MeOH (8 ml) on water-bath for 3 h. The reaction mixture was worked up in the usual manner and filtered. The filtrate was concentrated, neutralized with Ag_2CO_3, and converted into alditol acetates as above. G.l.c. analysis on column (ii) showed five peaks again corresponding to alditol acetates of 2,3,4,6-tetra-*O*-methyl-D-glucose, 2,3,4-tri-*O*-methyl-D-xylose, 3,4,6-tri-*O*-methyl-D-glucose, 2,3,6-tri-*O*-methyl-D-glucose, and 3-*O*-methyl-L-arabinose.

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