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Triterpenoid Glycosides from the Roots of *Tetrapanax papyriferum* K. KOCH. III.[1] Structures of Four New Saponins

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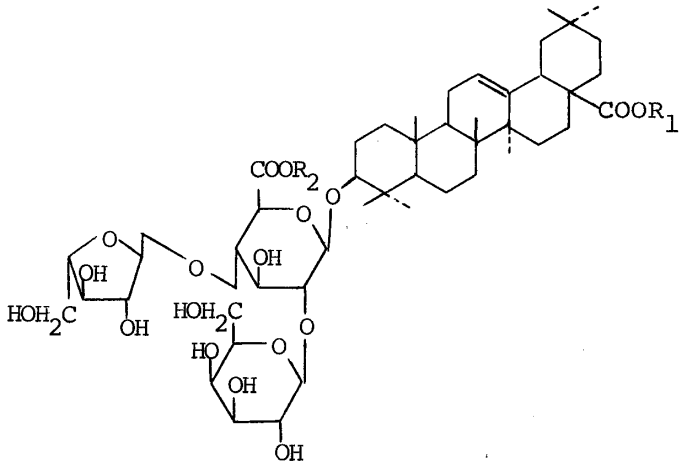
Six compounds were isolated from the roots of *Tetrapanax papyriferum* K. KOCH. and characterized as β-D-glucopyranosyl oleanate-(3)-[α-L-arabinofuranosyl-(1→4)]-[β-D-galactopyranosyl-(1→2)]-methyl-(β-D-glucopyranosid)uronate (**1**), α-L-rhamnopyranosyl-(1→4)-β-D-glucopyranosyl-(1→6)-β-D-glucopyranosyl oleanate-(3)-α-L-arabinofuranosyl-(1→4)-methyl-(β-D-glucopyranosid)uronate (**2**), β-D-glucopyranosyl oleanate-(3)-β-D-galactopyranosyl-(1→2)-methyl-(β-D-glucopyranosid)uronate (**3**), methyl oleanate-(3)-[α-L-arabinofuranosyl-(1→4)]-[β-D-galactopyranosyl-(1→2)]-methyl-(β-D-glucopyranosid)uronate (**4**), β-sitosterol β-D-glucopyranoside (**5**) and oleanolic acid-(3)-β-D-galactopyranosyl-(1→2)-β-D-fucopyranoside (**6**) on the bases of chemical and spectroscopic evidence.

Keywords——*Tetrapanax papyriferum*; Araliaceae; oleanolic acid; oleanolic acid glucuronide; bisdesmoside

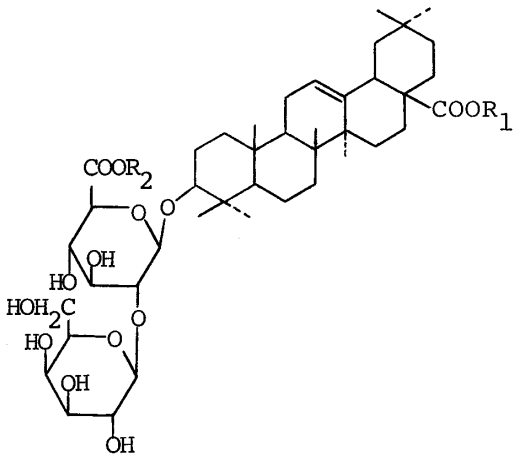
In the previous paper, we described the isolation and structural elucidation of several prosapogenins obtained from the roots of *Tetrapanax papyriferum* K. KOCH.[1] Further investigation of the chemical constituents of the title plant resulted in the isolation of four new triterpenoid saponins, along with two known compounds. In this paper, we wish to report the evidence which led to the establishment of the structures of these new saponins.

The crude saponin fraction, obtained from the methanolic extract of the roots of this plant, was further separated by droplet counter-current chromatography (DCCC) to give fractions R-1, R-2, R-3 and R-4. The study on the isolated glycosides from fractions R-2 and R-3 was reported previously.[2] Fraction R-1 was shown to be a mixture of highly polar saponins, and it was methylated with a solution of diazomethane. The methylated mixture thus obtained was repeatedly subjected to silica gel column chromatography to give compounds R-1a, R-1b, R-1c and R-1d. Fraction R-4 was chromatographed on a silica gel column to afford compounds R-4a and R-4b.

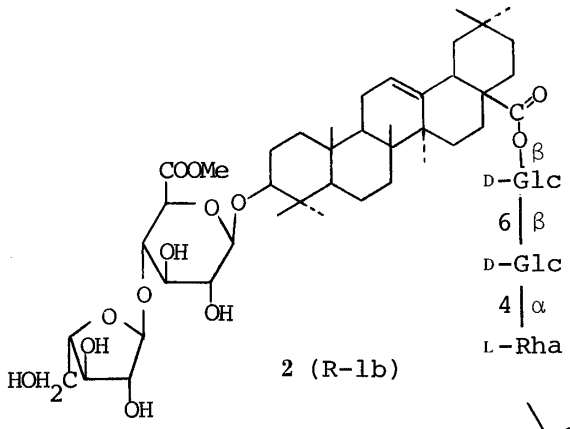
R-1a (**1**) was obtained as colorless needles. The infrared (IR) spectrum (1735 cm^{-1}) shows the presence of an ester group. In the proton nuclear magnetic resonance (^{1}H-NMR) spectrum of **1**, the signals at δ 3.82 (3H, s) and 5.41 (1H, d, J=8 Hz) can be assigned to a carbomethoxyl group and an ester-linked anomeric proton.[3] On acidic hydrolysis, R-1a afforded oleanolic acid as the sapogenin, and glucuronic acid, galactose, arabinose and glucose as the sugar components (molar ratio, 1 : 1 : 1 : 1). Furthermore, alkaline hydrolysis of R-1a gave 1,6-anhydroglucopyranose and a prosapogenin which was identical with Rb-2 (**7**)[1] (Chart 1), suggesting that R-1a is a glucosyl ester of Rb-2. This suggestion was supported by the carbon-13 nuclear magnetic resonance (^{13}C-NMR) specturm of **1** (Table I). The signals of the glucose moiety were in agreement with those for methyl β-D-glucopyranoside. The signals of the part other than the glucose were consistent with those of Rb-2. The anomeric carbon signal of the glucose was observed at 95.6 ppm, showing that the glucose was linked to the 28-



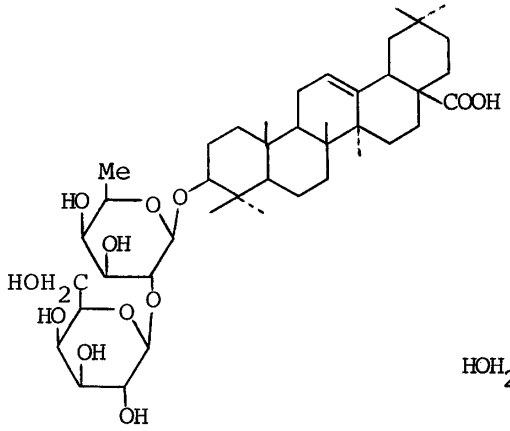
1 : R_1= Glc, R_2= Me (R-1a)
7 : R_1= H, R_2= H (Rb-2)
8 : R_1= Me, R_2= Me



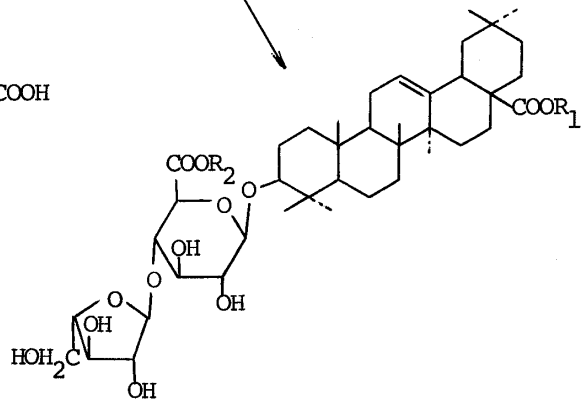
3 : R_1= Glc, R_2= Me (R-1c)
12 : R_1= H, R_2= H (Rb-4)
13 : R_1= Me, R_2= Me



2 (R-1b)



6 (R-4b)



9 : R_1= R_2= H (R-3)
10 : R_1= R_2= Me

Chart 1

TABLE I. ^{13}C-NMR Chemical Shifts (Pyridine-d_5)

	1	7	2	9	11	3	12	4	6
Oleanolic acid									
C- 3	89.5	89.2	89.3	88.9	78.9	89.2	89.1	89.4	89.2
C-13	144.0	144.5	144.0	144.4	144.1	144.1	144.5	178.0	179.8
C-28	176.5	179.6	176.5	179.6	176.5	176.5	179.8	178.0	179.8
								51.6	
Glucuronic acid									
C-1	106.3	106.3	106.7	106.5		107.1	106.5	106.6	
C-2	82.5	82.9	74.9	75.0		83.7	83.7	82.7	
C-3	74.1	75.7[a]	75.7	75.9		76.9[a]	77.1	74.7[a]	
C-4	79.0	78.5	78.3	78.5		72.8	72.9	78.4	
C-5	74.6[a]	74.6	74.9	75.7		74.9	74.8	74.9[a]	
C-6	170.0	171.8	170.2	172.0		170.0	172.0	170.1	
–COOMe	52.4		52.4			52.0		52.4	
Galactose									
C-1	104.8	104.6				105.2	105.1	105.0	106.6
C-2	74.2	74.2				74.6	74.5	74.4	74.6
C-3	75.4	75.4[a]				76.7[a]	76.8	75.6	76.5
C-4	69.4	69.4				69.4	69.4	69.5	69.3
C-5	74.7[a]	76.2[b]				77.4	77.6	76.6	77.2
C-6	61.3	61.2				61.3	61.3	61.4	61.3
Arabinose									
C-1	108.6	108.4	108.6	108.3				108.7	
C-2	82.3	82.3	82.5	82.1				82.7	
C-3	76.7	76.5[b]	77.1[a]	76.4				76.9	
C-4	86.9	87.2	86.9	87.4				87.2	
C-5	62.4[b]	62.5	62.5	62.5				62.5	
Glucose									
C-1	95.6		95.4		95.7	95.7			
C-2	73.9		73.6		74.1	74.1			
C-3	78.3		78.3		78.2	78.8			
C-4	71.0		70.6		70.3	71.1			
C-5	78.6		76.7		76.8	79.2			
C-6	62.3[b]		69.0		69.5	62.2			
Glucose									
C-1			104.4		105.0				
C-2			74.9		75.2				
C-3			76.3[a]		76.8				
C-4			78.3		78.2				
C-5			77.7		77.0				
C-6			61.1		62.2				
Rhamnose									Fucose
C-1			102.4		102.7				105.2
C-2			72.4		72.5				83.2
C-3			72.3		72.5				76.6
C-4			73.5		73.8				74.2
C-5			70.2		70.3				72.4
C-6			18.4		18.6				17.3

Assignments indicated by *a*) or *b*) may be reversed in each column.

carboxyl group in ester form.[3] The β-glucosyl linkage of the glucose was indicated by the ^{1}H-NMR spectrum of **1**, based on the observation of the anomeric proton signal at 5.41 ppm (doublet with $J=8$ Hz). Based on this evidence, the structure of R-1a was established as β-D-glucopyranosyl oleanate-(3)-[α-L-arabinofuranosyl-(1→4)]-[β-D-galactopyranosyl-(1→2)]-methyl-(β-D-glucopyranosid)uronate (**1**) (Chart 1).

R-1b (**2**) was obtained as a white powder. Acidic hydrolysis of **2** afforded oleanolic acid as the sapogenin, and glucuronic acid, arabinose, rhamnose and glucose as the sugar components (molar ratio, 1 : 1 : 1 : 2). Furthermore, **2** was hydrolyzed with KOH and the reaction mixture was extracted with butanol. Treatment of the butanol extract with diazomethane afforded a dimethyl ester which was identical with the dimethyl ester of R-3 (**10**).[2] The aqueous layer was hydrolyzed with acid and analyzed by gas-liquid chromatography (GLC) and thin-layer chromatography (TLC), showing the presence of rhamnose and glucose. These results suggested that R-1b is a ester composed of R-3 (**9**) as the acid part, and rhamnose and glucose as the alcohol part. This was supported by analysis of the ^{1}H- and ^{13}C-NMR spectra of R-1b as follows. The signal at 5.33 ppm (1H, d, J=8 Hz) in the ^{1}H-NMR spectrum of **2** can be attributed to the anomeric proton of glucose linked to the 28-carboxyl group of R-3 in ester form. The signal of the anomeric carbon of the glucose was observed at 95.4 ppm, supporting the view that the glucose is linked to C-28 in ester form. Furthermore, a comparison of the ^{13}C-NMR spectrum of R-1b with those of known compounds shows that the signals due to sugar moieties of R-1b are in good agreement with those of authentic R-3[3] and synthetic α-L-rhamnopyranosyl-(1→4)-β-D-glucopyranosyl-(1→6)-β-D-glucopyranosyl oleanate (**11**).[4]

Based on the above observations, the structure of R-1b was established as α-L-rhamnopyranosyl-(1→4)-β-D-glucopyranosyl-(1→6)-β-D-glucopyranosyl oleanate-(3)-α-L-arabinofuranosyl-(1→4)-methyl-(β-D-glucopyranosid)uronate (**2**). It is very interesting that the structure of the sugar moiety at C-28 is the same as in the papyriosides L-2a, b, c, and d which were isolated from the leaves of this plant.[5-7]

R-1c (**3**) was obtained as a white powder. On acid hydrolysis, **3** afforded oleanolic acid as the sapogenin, and glucuronic acid, galactose and glucose as the sugar components. Alkaline hydrolysis of **3** gave a 1,6-anhydroglucopyranose[8] and a prosapogenin which was identical with Rb-4 (**12**).[1] The prosapogenin was further reacted with diazomethane to produce a dimethyl ester (**13**) which was identical with the dimethyl ester of Rb-4. The ^{13}C-NMR spectrum of R-1c, except the signals due to the glucose moiety, was in agreement with those of Rb-4 (**12**). The anomeric carbon signal of the glucose was observed at 95.7 ppm, indicating that the glucose is linked to the 28-carboxyl group in ester form, and the other signals of the glucose were coincident with those of methyl β-D-glucopyranoside. The β-configuration of the glucose was also indicated by the ^{1}H-NMR spectrum, based on the observation of the anomeric proton signal at 5.38 ppm (doublet with J=7.5 Hz). Based on these observations, the structure of R-1c was established as β-D-glucopyranosyl oleanate-(3)-β-D-galactopyranosyl-(1→2)-methyl-(β-D-glucopyranosid)uronate (**3**).

R-1d (**4**) was identified as the dimethyl ester of Rb-2 (**7**)[1] by comparison of the mp, $[\alpha]_D$ and ^{13}C-NMR spectrum with those of an authentic sample. Further, R-4a (**5**) was identified as β-sitosterol β-D-glucopyranoside.

R-4b (**6**) was obtained as a white powder. On acid hydrolysis, **6** afforded oleanolic acid, galactose and fucose. In the ^{13}C-NMR spectrum of **6**, the signals of the sugar moiety are in good agreement with those for methyl β-D-galactopyranoside and methyl β-D-fucopyranoside,[9] but the C-2 signal of fucose was shifted downfield to 83.2 ppm, suggesting that the terminal galactose is linked to C-2 of the fucose, according to the glycosidation shift rule.[10] This suggestion was supported by the methylation analysis as follows: the methylation of **6** by Hakomori's method[11] followed by methanolysis and analysis by GLC showed the presence of methyl 2,3,4,6-tetra-*O*-methylgalactopyranoside and methyl 3,4-di-*O*-methylfucopyranoside.[12] The β-linkages of the two sugars were indicated by the ^{1}H-NMR spectrum of **6** based on the observation of the anomeric proton signals at 4.21 (1H, d, J=7 Hz) and 4.37 ppm (1H, d, J=7 Hz). Based on the above observations, the structure of R-4b was established as oleanolic acid-(3)-β-D-galactopyranosyl-(1→2)-β-D-fucopyranoside (**6**).

Compounds **1**, **2**, **3** and **6** are new triterpenoid saponins.

Experimental

The melting points were measured on a Yanagimoto microscopic hot plate and are uncorrected. IR spectra were taken with a JASCO IRA 2 spectrometer. Optical rotations were determined with a Union PM-201 polarimeter. GLC was performed on a Shimadzu GC-6A gas chromatograph. ^{1}H-NMR spectra were measured on a JEOL JNM-MH-100 spectrometer and ^{13}C-NMR spectra were measured on a JEOL JNM-FX-100 spectrometer, using tetramethylsilane (TMS) as an internal standard; chemical shifts are given in (ppm). Mass spectra (MS) were recorded on a Shimadzu LKB-9000 computer GCMSPAC-300M mass spectrometer. TLC was performed on precoated Kieselgel 60F 254 plates (Merck), and Kieselgel 60 (Merck) was used for column chromatography. Solvent A, the lower layer of $CHCl_3$–MeOH–H_2O (65 : 35 : 10); solvent B, benzene–acetone (4 : 1); solvent C, $CHCl_3$–MeOH (4 : 1).

Isolation of Saponins from the Roots of *Tetrapanax papyriferum* K. Koch.——The roots (1.3 kg) were extracted with methanol, and the methanolic extract thus obtained was distributed between butanol and water. The butanol extract was dissolved in a minimal amount of methanol and then dropped into ether. The precipitate was collected and dried to give the crude saponin (44.1 g).

The crude saponin (5 g) was further fractionated by DCCC [solvent system, $CHCl_3$–MeOH–H_2O (35 : 65 : 40), the upper layer as the mobile phase and the lower layer as the stationary phase]. The fractions that flowed from the DCCC apparatus were monitored by TLC and combined according to the results of TLC to give four fractions: R-1 (*Rf* value 0.1) (400 mg), R-2 (*Rf* 0.16) (1.8 g),[2] R-3 (*Rf* 0.21) (1.8 g),[2] and R-4 (*Rf* 0.7) (800 mg).

Fraction R-1 was reacted with diazomethane and then was chromatogrphed on a silica gel column with solvent A to give saponins R-1a (**1**) (92 mg), R-1b (**2**) (84 mg), R-1c (**3**) (90 mg) and R-1d (**4**) (95 mg).

Fraction R-4 was subjected to silica gel column chromatography [solvent, $CHCl_3$–MeOH (50 : 1), (30 : 1), (15 : 1) and (5 : 1)] to give compounds R-4a (**5**) (300 mg) and R-4b (**6**) (210 mg).

R-1a (1)——Colorless needles (MeOH), mp 226—228 °C, $[\alpha]_D^{22}$ −21.8 ° (*c*=0.5, MeOH). IR ν_{max}^{KBr} cm^{-1}: 3600—3200, 1735. ^{1}H-NMR (CD_3OD) δ: 3.82 (3H, s), 5.28 (1H, m) 5.41 (1H, d, *J*=8 Hz). *Anal.* Calcd for $C_{54}H_{86}O_{23}\cdot 3H_2O$: C, 56.04; H, 8.01. Found: C, 56.39; H, 8.06.

Acid Hydrolysis of 1——R-1a (**1**) (2 mg) was hydrolyzed by heating it in 2 N H_2SO_4–dioxane–H_2O for 5 h. The reaction mixture was diluted with water and extracted with ether. The water layer was divided into two parts. One part was evaporated to dryness *in vacuo*. Trimethylsilylation followed by GLC (2% OV-1 on Chromosorb WAW; column temperature, 160 °C, N_2 flow rate, 60 ml/min) showed the presence of galactose, arabinose and glucose in a ratio of 1 : 1 : 1. The other part was analyzed according to Hulyalkar and Perry,[13] and was found to contain glucuronic acid, retention time 10.41 min (2,3,5,6-tetra-*O*-trimethylsilyl-L-gulono-1,4-lactone). The ether extract was chromatographed on a column of silica gel to give an aglycone, which was identical with authentic oleanolic acid.

Alkaline Hydrolysis of 1——R-1a (**1**) (80 mg) was hydrolyzed by heating it in 1 N KOH for 5 h. The reaction mixture was distributed between butanol and water. 1,6-Anhydroglucopyranose was detected from the water layer. The butanol extract was reacted with diazomethane and the resulting product (yield 90%) was identified as the dimethyl ester of Rb-2 (**8**) by TLC and ^{1}H-NMR.

R-1b (2)——White prisms (MeOH), mp 203—205 °C, $[\alpha]_D^{22}$ −27.0 ° (*c*=0.6, MeOH). IR ν_{max}^{KBr} cm^{-1}: 3600—3160, 1735. ^{1}H-NMR (CD_3OD) δ: 3.79 (3H, s), 4.39 (1H, d, *J*=7.5 Hz), 4.85 (1H, br s), 4.92 (1H, br s), 5.24 (1H, m), 5.33 (1H, d, *J*=8 Hz). *Anal.* Calcd for $C_{60}H_{96}O_{27}\cdot 4H_2O$: C, 54.55; H, 7.93. Found: C, 54.28; H, 7.86.

Acid Hydrolysis of 2——**2** (2 mg) was hydrolyzed by heating in 2 N H_2SO_4–dioxane–H_2O for 5 h. From the resulting products, oleanolic acid as the sapogenin, and glucuronic acid, rhamnose, arabinose and glucose (molar ratio, 1 : 1 : 1 : 2) as the sugar components were identified by the same procedure as described for R-1a.

Alkaline Hydrolysis of 2——**2** (10 mg) was hydrolyzed by heating in 1 N KOH for 6 h and then the reaction mixture was distributed between butanol and water. Rhamnose and glucose (molar ratio, 1 : 2) as the sugar components were identified from the aqueous layer. The butanol extract was reacted with a solution of diazomethane in ether and the resulting product (yield 90%) was identified as the dimethyl ester of R-3 (**10**) by TLC and ^{1}H-NMR.

R-1c (3)——A white powder, mp 190—193 °C, $[\alpha]_D^{22}$ −8.7 ° (*c*=0.5, MeOH). IR ν_{max}^{KBr} cm^{-1}: 3600—3150, 1730. ^{1}H-NMR (CD_3OD) δ: 3.77 (3H, s), 5.24 (1H, m), 5.38 (1H, d, *J*=7.5 Hz). *Anal.* Calcd for $C_{49}H_{78}O_{19}\cdot 3H_2O$: C, 57.41; H, 8.26. Found: C, 57.73; H, 8.28.

Acid Hydrolysis of 3——**3** (2 mg) was hydrolyzed by heating in 2 N H_2SO_4–dioxane–H_2O for 5 h. The resulting products were identified as oleanolic acid, glucuronic acid, galactose and glucose.

Alkaline Hydrolysis of 3——**3** (10 mg) was hydrolyzed by heating in 1 N KOH for 5 h and then the reaction mixture was distributed between butanol and water. 1,6-Anhydroglucopyranose was identified from the water layer, and confirmed by its conversion into glucose on acid treatment. The butanol extract was reacted with diazomethane and the resulting product was identified as the dimethyl ester of Rb-4 (**13**) (yield 92%) by TLC and ^{1}H-NMR.

Identification of R-1d (4)——Compound R-1d (**4**) was identified as the dimethyl ester of Rb-2 by comparison of the mp, $[\alpha]_D$ and ^{13}C-NMR spectrum of **4** with those of an authentic sample.

Identification of R-4a (5)——Compound R-4a was identified as β-sitosterol-β-D-glucopyranoside by comparison of the mp. $[\alpha]_D$ and ^{13}C-NMR spectrum of **5** with those of an authentic sample. Acid hydrolysis of R-4a afforded an aglycone which was identical with β-sitosterol.

R-4b (6)——A white powder, mp 149—152 °C, $[\alpha]_D^{22}$ +33.1 ° (c=0.2, MeOH). IR ν_{max}^{KBr} cm^{-1}: 3600—3200, 1730. ^{1}H-NMR (CD_3OD) δ: 4.21 (1H, d, J=7.0 Hz), 4.37 (1H, d, J=7.0 Hz), 5.32 (1H, m). *Anal.* Calcd for $C_{42}H_{68}O_{12}\cdot 3H_2O$: C, 61.59; H, 9.11. Found: C, 61.37; H, 9.01.

Acid Hydrolysis of 6——**6** (1 mg) was hydrolyzed by heating in 2 N H_2SO_4–dioxane–H_2O for 5 h. The resulting products were identified as oleanolic acid, galactose and fucose as described before.

Methylation Analysis of 6——**6** (2 mg) was permethylated by Hakomori's method to give a permethylate of **6**. MS m/z: 219, 393. ^{1}H-NMR ($CDCl_3$) δ: 4.19 (1H, d, J=8 Hz, anomeric H of fucose), 4.65 (1H, d, J=7 Hz, anomeric H of galactose), 3.46 (6H), 3.50, 3.53, 3.56, 3.58 (each 3H) (all OMe).

The permethylate thus obtained was methanolyzed by refluxing it in 5% HCl–MeOH for 5 h. The resulting products were analyzed by GLC (10% diethylene glycol succinate, Chromosorb W, 160 °C), showing the presence of methyl 2,3,4,6-tetra-*O*-methylgalactopyranoside and methyl 3,4-di-*O*-methylfucopyranoside.

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