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STRUCTURE OF CUCUMARIOSIDE G_2, A NOVEL NONHOLOSTANE GLYCOSIDE FROM THE SEA CUCUMBER *EUPENTACTA FRAUDATRIX*

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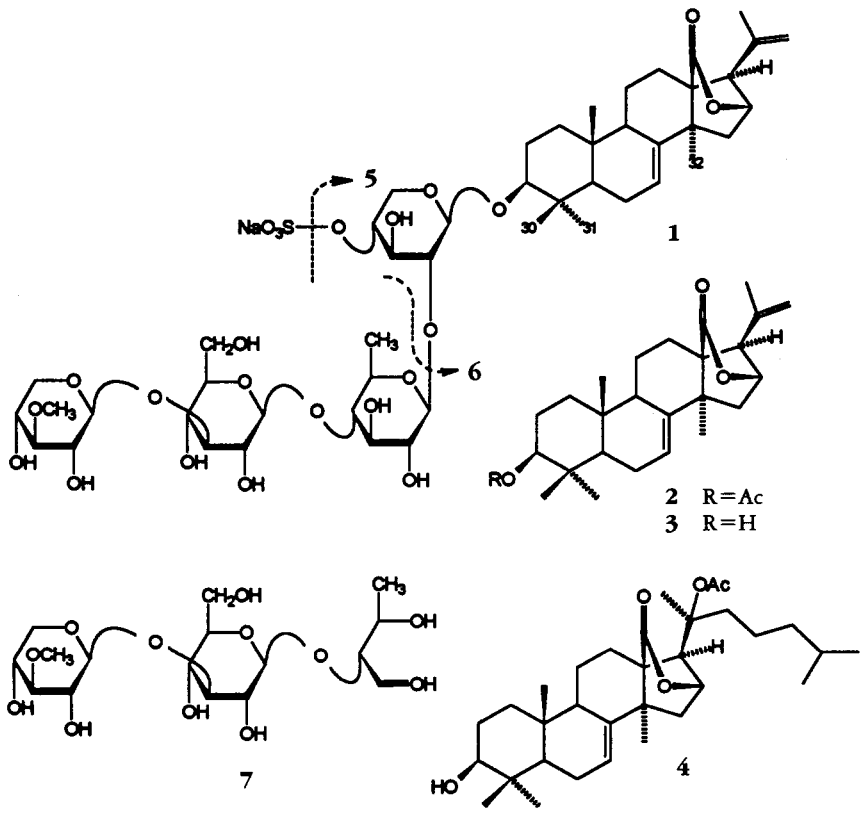
ABSTRACT.—The new triterpene glycoside cucumarioside G_2 [**1**] has been isolated from the sea cucumber *Eupentacta fraudatrix*. Glycoside **1** is the first triterpene glycoside with the 23,24,25,26,27-pentanorlanostane type of aglycone. Its structure has been established by chemical transformations as well as ^{13}C- and ^{1}H-nmr, eims, and liquid sims studies.

In continuation of our studies on triterpene glycosides from the sea cucumber *Eupentacta fraudatrix* (Djakonov et Baranova) (Sclerodactylidae, Dendrochirotida), collected from the Gulf of Posiet in the Sea of Japan (1–3), we isolated cucumarioside G_2 [**1**] as a minor component of a glycoside fraction (4). Herein we report full details of the isolation and structure elucidation of **1**.

Glycoside **1** was isolated from an EtOH/$CHCl_3$ extract of *E. fraudatrix* by



cc on Polychrom-1 (powdered Teflon) and Si gel, followed by hplc on a Zorbax-ODS column.

Selective hydrolysis of **1** with a 2 N H_2SO_4/toluene mixture followed by acetylation gave the acetylated derivative **2**. We named the genin **3**, on which compounds **1** and **2** are based, as posietogenin in accordance with the location where *E. fraudatrix* was collected.

The chemical shifts of H-7, H-9β, H-15, H-16, H-17, CH_3-10, CH_3-4β, CH_3-4α, and CH_3-14 in the ^{1}H-nmr spectrum of **2** (Table 1) are similar to those of onekotanogenin **4**, the 25,26-dihydro derivative of the aglycone of psolusoside B, described earlier from sea cucumbers belonging to the genus *Psolus* (5,6). NOe irradiation at 1.38 ppm (CH_3-14) enhanced signals at 2.89 ppm (H-17) and 5.55 ppm (H-7), demonstrating the presence of a 7(8)- double bond. The zero value of $J_{16,17}$ correlates with that of onekotanogenin [**4**]. The chemical shift of H-16 (Table 1) suggested the presence of an 18(16)- lactone. Signals for CH_3-20 (δ 1.76, d), H-22 (δ 4.90, m), and H-22′ (δ 4.95, m) were indicative of an isopropenyl fragment in **2**.

The resonances of C-1 through C-19, and of C-28 through C-30 in the ^{13}C-nmr spectrum of **1** (Table 2) correspond to analogous signals in the ^{13}C-nmr spectrum of the polycyclic system of psolusoside B (6). However, three signals were observed at 139.9, 113.9, and 21.9 ppm which do not belong to an 18(16)-lactone lanostane cyclic system, and were consistent with the presence of an isopropenyl unit. The number of signals in the ^{13}C-nmr spectrum of the aglycone moiety of **1** demonstrated the presence of 25 carbon atoms. Comparison of the spectral data with those of **4** suggested that the isopropenyl group must be attached to C-17. The proposed structure **3** for posietogenin was also confirmed by the molecular ion peak at *m/z* 426 in the eims of the acetate **2**.

Glycoside **1** gave D-xylose, D-glucose, D-quinovose, and D-3-*O*-methylxylose (1:1:1:1) as well as sulfuric acid after acid hydrolysis. The solvolysis of **1** led to the desulfated derivative **5**. The position of a sulfate group at C-4 of the xylose residue was determined by comparison of the ^{13}C-nmr spectra of glycoside **1** and derivative **5**. The peak at *m/z* 1095 $[M_{Na}+Na]^+$ in the positive-liquid sims of **1**, analysis of its nmr data, and sugar analysis all confirmed the presence of four monosaccharide units, a sulfate group and posietogenin [**3**] as the genuine aglycone in the glycoside. All signals of the carbohydrate chain in the ^{13}C-nmr spectrum of **1** are identical to those of the known cucumarioside G_1, previously isolated from *E. fraudatrix* (3), and correspond with the saccharide arrangement shown in structure **1**.

To check the sugar sequence we carried out Smith degradation of **1** (7). As a result we obtained the progenin **6**, containing xylose, and the bioside derivative **7**, containing 3-*O*-methylxylose and glucose (1:1). These data, in combination with ^{13}C-nmr information, confirm unambiguously the structure of the carbohydrate chain in **1**. In addition, the formation of progenin **6**, containing a xylose residue, showed that the first sugar is xylose. The second sugar, destroyed un-

TABLE 1. ^{1}H-Nmr Spectra of Derivative **2** in $CDCl_3$.

Proton	δ (*J*)	Proton	δ (*J*)	Proton	δ (*J*)
H-3α	4.47 dd (8.5 Hz)	H-16.....	4.69 d (2.5 Hz)	CH_3-4α ...	0.93 s
H-7	5.55 m	H-17.....	2.89 s	CH_3-14....	1.38 s
H-9β	2.67 m	CH_3-10....	0.93 s	H-22.....	4.90 m
H-15α	1.95 B dd (13.4 and 2.0 Hz)	CH_3-20....	1.76 d (2.5 Hz)	H-22′	4.95 m
H-15β	2.09 A d (13.4 Hz)	CH_3-4β ...	0.89 s	OAc	2.06 s

TABLE 2. ^{13}C-Nmr Data of Cucumarioside G_2 [**1**] and the Desulfated Derivative **5**.[a]

Carbon	**1**[b]	**1**[c]	**5**[b]	Carbon	**1**[b]	**1**[c]	**5**[b]
C-1	35.9	35.3	36.0	C^1_1	104.7	103.5	105.5
C-2	26.9	26.3	27.3	C^1_2	82.5	81.2	84.3
C-3	89.0	88.2	89.1	C^1_3	75.3	74.2	78.1
C-4	39.4	[d]	39.6	C^1_4	75.0	74.2	70.7
C-5	47.7	46.8	47.7	C^1_5	63.9	62.9	66.7
C-6	23.4	22.8	23.4	C^2_1	104.7	103.8	105.6
C-7	122.5	121.6	122.7	C^2_2	76.1	74.8	76.2
C-8	147.4	146.8	147.4	C^2_3	75.8	74.5	75.9
C-9	46.6	46.8	46.5	C^2_4	86.5	85.8	87.4
C-10	35.6	34.9	35.7	C^2_5	71.5	70.2	71.6
C-11	23.1	22.6	23.0	C^2_6	18.0	17.4	18.2
C-12	20.2	19.5	20.2	C^3_1	104.2	102.9	104.9
C-13	57.0	56.0	56.8	C^3_2	73.7	72.5	73.8
C-14	46.2	45.6	46.0	C^3_3	87.0	85.8	87.7
C-15	44.0	43.3	43.9	C^3_4	69.8	68.7	70.0
C-16	80.9	79.8	80.5	C^3_5	77.5	76.3	77.9
C-17	59.2	58.3	59.2	C^3_6	61.9	60.8	62.3
C-18	181.2	179.9	180.5	C^4_1	105.4	104.3	106.1
C-19	23.9	23.6	24.0	C^4_2	74.3	73.2	74.6
C-20	139.9	139.5	139.8	C^4_3	86.9	85.8	87.4
C-21	21.9	21.6	21.9	C^4_4	69.6	68.2	69.6
C-22	113.9	113.1	114.0	C^4_5	66.6	65.6	67.1
C-30	17.2	16.6	17.3	OCH_3	60.4	59.6	60.6
C-31	28.8	28.2	28.7				
C-32	34.1	33.9	34.1				

[a]The notation C^1, C^2, etc. refers to the first carbohydrate unit, second carbohydrate unit, etc.
[b]Spectrum run in C_5D_5N.
[c]Spectrum run in $(CD_3)_2SO$.
[d]Signal overlapped with solvent signal.

der Smith degradation, is quinovose, because the terminal bioside fragment **7** contains glucose and 3-*O*-methylxylose. The terminal position of 3-*O*-methylxylose follows from ^{13}C-nmr data. Moreover, the sequence of the sugar residues in **1** correlated with the positive-liquid sims and negative-liquid sims fragmentations (Figures 1 and 2, respectively). Indeed, in the positive-liquid sims (glycerol matrix), significant fragment peaks at *m/z* 1111 $(M_K+Na)^+$, 1095 $(M_{Na}+Na)^+$, 1089 $(M_K+H)^+$, and 1073 $(M_{Na}+H)^+$ for **1** as well as the $(M+Na)^+$ peak of a desulfated derivative **5** at *m/z* 993 were observed. After addition of NaCl to a cucumarioside G_2 [**1**] suspension in glycerol, only peaks at *m/z* 1095 and 993 were detected in this part of the spectra. The sugar sequence of **1** is based on the observation of fragment peaks at *m/z* 949 $[M_{Na}+Na-3\text{-}O\text{-Me-xyl}]^+$; 847 $[m/z\ 993-3\text{-}O\text{-Me-xyl}]^+$; 331 [3-*O*-Me-xyl-glc+Na]$^+$; 477 [3-*O*-Me-xyl-glc-qui+Na]$^+$; 609 [3-*O*-Me-xyl-glc-qui-xyl+Na]$^+$ in the positive-liquid sims. The positive-liquid sims of the desulfated derivative **5** was again indicative of the sugar sequence in **1**. Thus, the loss of sugar units from the ion at *m/z* 971 $[M^+-H]$ led to ions at *m/z* 825, 663, 517, and 385 $[AglOH_2]^+$, corresponding with a step-by-step elimination of 3-*O*-methylxylose, glucose, quinovose, and xylose, respectively. The loss of H_2O from the ion at *m/z* 385 gave the ion at *m/z* 367, and the loss of H_2O and CO_2 gave the ion at *m/z* 323 (Figure 1).

A molecular ion was observed at *m/z* 1049 $[M_{Na}-Na]^-$ in the negative-liquid sims of **1**. Fragmentation of this ion with cleavage of glycoside bonds led to ions at *m/z* 903 and 885 [*m/z* 903$-H_2O$], 741 and 723 [*m/z* 741$-H_2O$], and 595 and

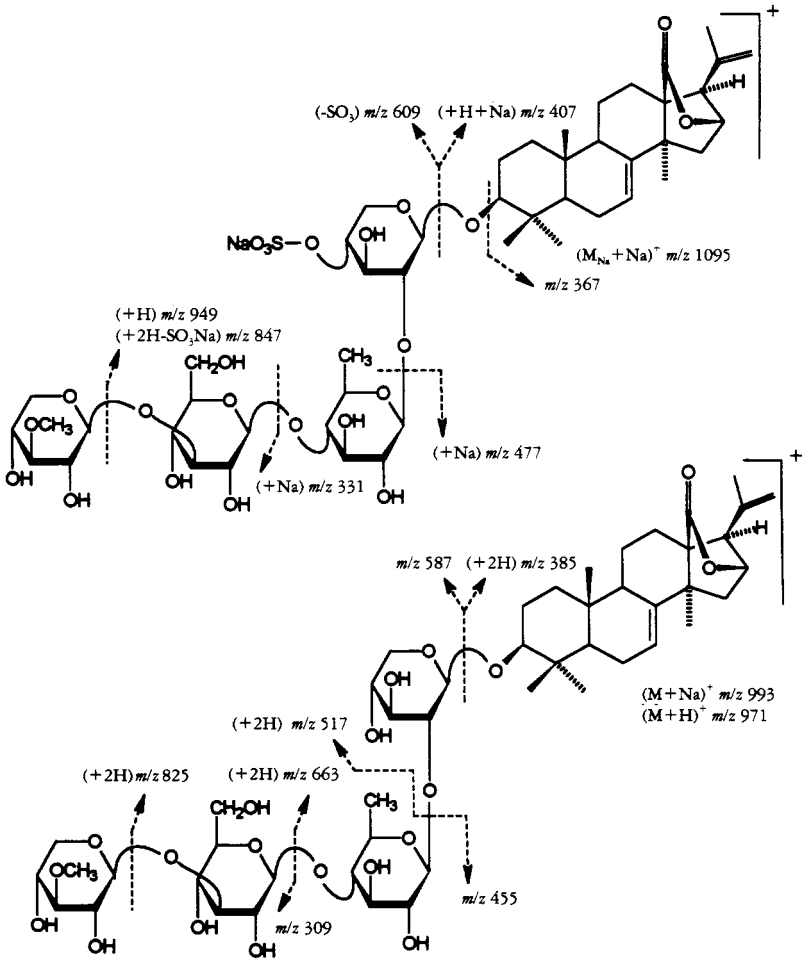


FIGURE 1. Positive-liquid sims fragmentation of cucumarioside G_2 [**1**] and its desulfated derivative **5**.

577 [*m/z* 595−H_2O] (Figure 2). Cleavage of the C_5-O and C_1-C_2 bonds in unsulfated sugar residues is also characteristic in the negative fabms of frondoside A (8). The anion of the carbohydrate chain of **1** was observed as a small peak at *m/z* 681, while the sulfated xylose residue gave rise to a series of anions at *m/z* 195, 211, and 227.

On the basis of all the above-mentioned data, the structure of cucumarioside G_2 [**1**] was established as an 18(16)-lactone of 23,24,25,26,27-pentanor-3-*O*-[3-*O*-methyl-β-D-xylopyranosyl-(1→3)-β-D-glucopyranosyl-(1→4)-β-D-quinovopyranosyl-(1→2)-4′-*O*-sodium sulfate-β-D-xylopyranosyl]-lanosta-7(8),20(22)-dien-3β-ol. No glycoside with an 18(16)-lactone and a shortened side-chain in the aglycone moiety has previously been isolated from sea cucumbers, and thus **1** is representative of a new structural type of holothurian glycosides.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—All mps were determined with a Kofler-Thermogenerate apparatus. Specific rotations were measured on a Perkin-Elmer 141 polarimeter. ^{1}H- and ^{13}C-nmr spectra were obtained on a Bruker WM-250 spectrometer at 250 and 62.7 MHz in C_5D_5N, $(CD_3)_2SO$ and $CDCl_3$, with TMS as an internal reference (δ=0). Eims were taken on a LKB-2091 spectrometer (direct inlet probe, ionizing energy 70 eV). Na^+, as a major ion at the sulfate group, was determined with an AA-780 atomic absorption spectrometer. Gc analysis was

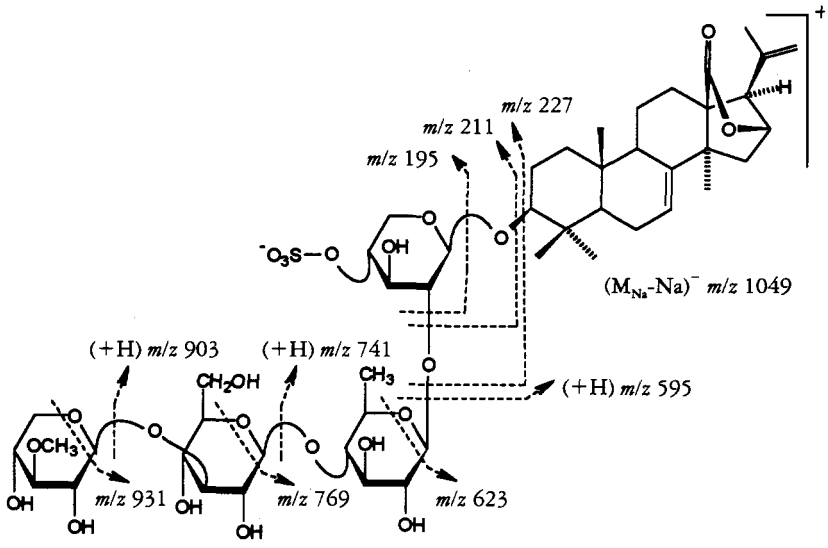


FIGURE. 2. Negative-liquid sims fragmentation of cucumarioside G_2 [**1**].

carried out on a Tsvet-110 apparatus, using a glass column 0.3×150 cm with 1.5% QF-1 as stationary phase and the following experimental conditions: Ar as the carrier gas, 60 ml/min, column temperature 110→225° (5°/min). For gc-ms we used a LKB 9000S apparatus and a column (0.3×300 cm with 1.5% QF-1) using He as carrier gas (50 ml/min). The conditions selected for analyses were: injection port 275°, molecular separator 265°, ion source 255°, column 110→210°, 4°/min, ionizing voltage 70 eV. Hplc was performed with a Dupont 8800 chromatograph equipped with a RIDK-102 differential refractometer (Laboratorni Pristroje, Prague). Positive- and negative-liquid sims were obtained on a MX 1310 spectrometer, equipped with an Institute of Automatic Instrumentation of the Russian Academy of Sciences (St. Petersburg) liquid sims source. The target was bombarded with Cs^+ ions of 7 kV energy, with the liquid matrix being glycerol or glycerol with NaCl as additive.

ANIMAL MATERIAL.—The sea cucumber *Eupentacta fraudatrix* was collected in Troitsa Bay (Gulf of Posiet, Sea of Japan) near the Marine Experimental Station of the Pacific Institute of Bioorganic Chemistry, Far East Division of the Russian Academy of Sciences, from a depth of 0.5–1.5 m in May 1990. The sea cucumber was identified by Dr. V.S. Levin (Institute of Marine Biology of the Far East Division of Russian Academy of Sciences). A voucher specimen (N 100590h) is on deposit in the marine specimen collection of the Pacific Institute of Bioorganic Chemistry, Russia.

EXTRACTION AND ISOLATION.—The fresh sea cucumbers (1000 specimens, 237 g dry wt) were cut and immediately extracted under reflux with a mixture of $CHCl_3$-EtOH (1:1) for 1 h, then with EtOH for 1 h. Combined extracts were evaporated and chromatographed on a column with Polychrom-1 (powdered Teflon) (Biolar, Latvia), previously filled with EtOH and washed with H_2O. Inorganic salts and polar impurities were eluted with H_2O. The glycoside fraction (9.8 g) was eluted from the column with 50% EtOH, and purified by cc on Si gel in a $CHCl_3$-EtOH-H_2O (100:100:17) system. A fraction containing a mixture of compounds in the cucumarioside G group (3.5 g) was thereby obtained. Glycoside **1** (450 mg) was purified from this fraction by hplc on a Zorbax-ODS column (4.8×250 mm, 1 ml/min) by eluting with 70:30 and then 73:27 H_2O-Me_2CO. Cucumarioside G_2 [**1**], mp 234–235°, $[\alpha]^{20}D$ −46° (c=0.1, pyridine); positive-liquid sims (glycerol matrix) *m/z* 1111 (10), 1095 (21), 1089 (66), 1073 (5), 993 (34), 943 (7), 847 (7), 723 (18), 609 (45), 477 (100), 407 (23), 389 (32), 367 (47), 349 (25), 331 (40); glycerol+NaCl matrix *m/z* 1095 (78), 993 (22), 949 (6), 847 (5), 669 (19), 606 (44), 477 (100), 389 (36), negative-liquid sims glycerol matrix *m/z* 1049 (2), 931 (0.2), 903 (0.21), 885 (0.3), 769 (0.6), 741 (0.7), 723 (0.8), 681 (0.5), 623 (1.5), 595 (1.4), 577 (0.6), 227 (49); ^{13}C nmr, see Table 2.

ACID HYDROLYSIS OF **1** FOLLOWED BY ACETYLATION.—Glycoside **1** (100 mg) was treated with 20 ml of a 2 N H_2SO_4-toluene (1:1) mixture at 100° for 2 h. The toluene layer was removed and the H_2O layer was extracted with $CHCl_3$ (3×5 ml). The $CHCl_3$ and toluene extracts were combined and concentrated under reduced pressure. The residue was acetylated with 4 ml of pyridine-Ac_2O (1:1) and after workup in the usual manner

was subjected to hplc on a Altex-Ultrasphere-Si column (10 mm×25 cm), with elution at 2 ml/min by the system hexane-EtOAc (8:1), to afford 4 mg of the derivative **2**, mp 265–267°, $[\alpha]^{20}D$ −67° (c=0.1, $CHCl_3$); ^1H nmr, see Table 1.

DESULFATION OF CUCUMARIOSIDE G_2 [**1**].—Cucumarioside G_2 (25 mg) in a pyridine-dioxane (1:1) mixture was heated at reflux for 1 h. After concentration under reduced pressure, the residue was chromatographed on a Si gel column with $CHCl_3$-MeOH (3:1). The yield of the desulfated derivative **5** was about 20 mg. Derivative **5** exhibited: mp 190–192°, $[\alpha]^{20}D$ −74° (c=0.1, pyridine), positive-liquid sims m/z 993 (0.3), 971 (0.6), 825 (2), 733 (2), 663 (1), 587 (4), 517 (5), 455 (6), 385 (34), 367 (100), 309 (31); ^{13}C nmr, see Table 2.

SUGAR ANALYSIS OF CUCUMARIOSIDE G_2 [**1**].—Compound **1** (5 mg) was dissolved in 5 ml of 2 N HCl, and the mixture was heated at reflux for 2 h. Then, 5 ml of H_2O was added to the mixture. The aglycone so produced was removed by extraction with $CHCl_3$. The aqueous layer was neutralized with Dowex (HCO_3^-), the resin was filtered off, and the H_2O layer was concentrated. To the dry residue, 1 ml of pyridine and 2 mg of $NH_2OH.HCl$ were added. The mixture was heated at 100° for 1 h. Then, 1 ml of Ac_2O was added and the mixture was heated at 100° for 1 h. The solution was concentrated and the resulting aldononitrile peracetates were analyzed by gc-ms, with xylose, quinovose, glucose, and 3-*O*-methylxylose (1:1:1:1) identified.

DEGRADATION OF GLYCOSIDE **1** BY THE SMITH METHOD.—Glycoside **1** (10 mg) was treated with 30 mg of $NaIO_4$ in 10 ml of H_2O for 72 h. The solution was loaded onto a Polychrom-1 column, washed with H_2O, and the product was recovered by elution with 50% EtOH. $NaBH_4$ (25 mg) was added to the aqueous EtOH eluate. The mixture was stirred at 20° for 2 h, adjusted to pH 5.0 by adding HOAc, and concentrated under vacuum. The residue obtained was dissolved in 10 ml of 0.5 N HCl, and allowed to stand at room temperature for 2 h. The reaction mixture containing progenin **6** and the disaccharide derivative **7** was subjected to chromatography on a column with Polychrom-1. Aqueous (containing derivative **7**) and alcoholic (containing progenin **6**) eluates were concentrated, hydrolyzed, and analyzed for monosaccharide compositions as described above. Xylose was identified in derivative **6** and 3-*O*-methylxylose and glucose (1:1) were identified in **7**.

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