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TRITERPENOID SAPONINS OF *ELEUTHEROCOCCUS SENTICOSUS* ROOTS

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ABSTRACT.—From the roots of *Eleutherococcus senticosus*, two new glycosides of protoprimulagenin A have been isolated. Structures of these compounds have been elucidated on the basis of spectral data as 3β-{*O*-β-D-glucopyranosyl-(1→3)-*O*-β-D-galactopyranosyl-(1→4)-[*O*-α-L-rhamnopyranosyl-(1→2)]-*O*-β-D-glucuronopyranosyl} -16α-hydroxy-13β,28-epoxyoleanane [**1**] and 3β-{*O*-α-L-rhamnopyranosyl-(1→4)-*O*-α-L-rhamnopyranosyl-(1→4)-[*O*-α-L-rhamnopyranosyl-(1→2)]-*O*-β-D-glucopyranosyl-(1→x)-*O*-β-D-glucuronopyranosyl} -16α-hydroxy-13β,28-epoxyoleanane [**2**].

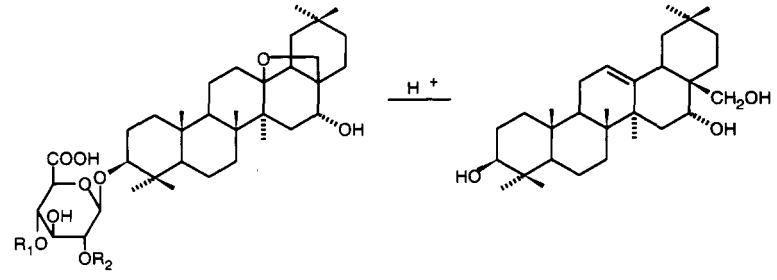
The family Araliaceae has been the subject of considerable phytochemical investigations due to the therapeutic activity of many of its plants. Triterpenoid saponins of dammarane and oleanane skeletons are considered to be responsible for the biological activity. However, only phenolic glycosides have been isolated from the roots of *Eleutherococcus senticosus* Rupr. et Maxim. (1–4). We have isolated from this material two new triterpenoid saponins (5) that have now been identified with the help of spectroscopic methods.

RESULTS AND DISCUSSION

The *n*-BuOH-soluble fraction of an MeOH extract from the roots of *E. senticosus* was subjected to cc over Si gel. The fraction eluted with $CHCl_3$-MeOH-H_2O (6:4:1) contained two saponins **1** and **2** and was purified on a Sephadex LH-20 column by elution with MeOH. Separation of both saponins was achieved by rotatory tlc on a Si gel disk.

Acid hydrolysis of **1** and **2** afforded the same genin **3**. The ms peaks observed at *m/z* 250 and 219 suggested the olean-12-ene skeleton with two hydroxyl groups on the D and E rings (6). Analysis of ^{1}H-nmr and ^{13}C-nmr spectral data on the basis of literature reports (7–11) allowed the identification of **3** as primulagenin A. Analysis of the aqueous phase obtained in the course of hydrolysis by tlc revealed the presence of rhamnose, glucose, galactose, and glucuronic acid (12).

Comparison of ^{13}C-nmr spectra of **1** and **3** showed that primulagenin A was not the aglycone of **1**. The spectrum of **1** did not exhibit signals due to C-12 and C-13 unsaturated carbons at 123.4 and 145.4 ppm, but an additional oxygenated carbon signal was



1 R_1=*O*-β-D-Glc-(1→3)-β-D-Gal-; R_2=α-L-Rha-

2 R_1=*O*-α-L-Rha-(1→4)-*O*-α-L-Rha-(1→4)-[*O*-α-L-Rha-(1→2)]-β-D-Glc-; R_2=H

3

observed at 88.4 ppm. This led to the conclusion that the actual aglycone of **1** has the structure of protoprimulagenin A (4). This structure was also supported by the ^{1}H-nmr spectrum, in which C-28 protons appeared as two doublets at 3.38 and 3.66 ppm with $J = 7.5$ Hz observed for 13β,28-epoxyoleanane compounds (13).

The positive ion fabms of **1** showed a peak corresponding to $[M + H]^+$ at *m/z* 1105. The negative ion fabms exhibited a molecular ion peak at *m/z* 1103 $[M - H]^-$ and fragment ions at *m/z* 941, 957, 779, and 795, which were attributed to the loss of terminal hexose, terminal deoxyhexose, hexose-hexose disaccharide, and to the loss of both terminal units (the hexose and deoxyhexose), respectively. The ion fragments at *m/z* 321 $[M - H - (2 \times 162) - \text{aglycone}]^-$ and 337 $[M - H - 162 - 146 - \text{aglycone}]^-$ indicated the linkage of deoxyhexose and hexose to glucuronic acid. Gc-ms analysis of the acetylated products of permethylation-methanolysis of **1** revealed the presence of methyl-2,3,4,6-tetra-*O*-methylglucopyranoside, methyl-2,3,4-tri-*O*-methylrhamnopyranoside, and methyl-3-*O*-acetyl-2,4,6-tri-*O*-methylgalactopyranoside. The ^{13}C-nmr spectrum of **1** showed four anomeric signals at 105.8, 102.8, 102.1, and 100.9 ppm, which were assigned to β-D-glucopyranose, β-D-galactopyranose, α-L-rhamnopyranose, and β-D-glucuronic acid, respectively (14–17). The carboxyl carbon of β-D-glucuronic acid was observed at 176.8 ppm. The signal at 88.4 ppm supported a 1↦3 linkage between glucose and galactose, while signals at 82.4 and 81.4 ppm indicated a 2,4 substitution of glucuronic acid (15–17). A detailed inspection of gc-ms results obtained for permethylation-methanolysis products showed, apart from permethylated monosaccharides, the presence of a small amount of permethylated disaccharides. The ms performed for a peak with Rt 23 min revealed a high mass ion at *m/z* 464 which could reflect the $[M - 60]^+$ ion of methyl-diacetyl-penta-*O*-methylgalactosyl-glucuronide. The ions at *m/z* 247 and 261 indicate acetyl-tri-*O*-methylhexose and acetyl-di-*O*-methylglucuronic acid methyl ester to be fragments of a disaccharide. The presence of ions at *m/z* 321, 382, and 189 supports a 1↦4 linkage between hexose and glucuronic acid (18).

Based upon the above data, the structure of saponin **1** was established as 3β-{*O*-β-D-glucopyranosyl-(1↦3)-*O*-β-D-galactopyranosyl-(1↦4)-[*O*-α-L-rhamnopyranosyl-(1↦2)]-*O*-β-D-glucuronopyranosyl}-16α-hydroxy-13β,28-epoxyoleanane.

Negative ion fabms of **2** showed a molecular ion at *m/z* 1233 $[M - H]^-$ and fragment ions at *m/z* 941, 795, 633, which reflected the elimination of two rhamnose, three rhamnose, and glucose plus three rhamnose units, respectively. The fragment *m/z* 633 was composed of genin and glucuronic acid. Permethylation-methanolysis followed by acetylation gave methyl-2,3,4-tri-*O*-methylrhamnopyranoside, methyl-4-*O*-acetyl-2,3-di-*O*-methylrhamnopyranoside, and methyl-2,4-diacetyl-3,6-di-*O*-methylglucopyranoside. The obtained results did not allow us to elucidate which carbon of glucuronic acid is substituted by the sugar chain. The structure of **2** can only be: 3β-{*O*-α-L-rhamnopyranosyl-(1↦4)-*O*-α-L-rhamnopyranosyl-(1↦4)-[*O*-α-L-rhamnopyranosyl-(1↦2)]-*O*-β-D-glucopyranosyl-(1↦x)- *O*-β-D-glucuronopyranosyl}-16α-hydroxy-13β,28-epoxyoleanane.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—^{1}H nmr (250 MHz) and ^{13}C nmr (75.469 MHz) were measured with a Bruker instrument with TMS as internal standard. Eims, hrms, positive mode fabms, and negative mode fabms were taken with MAT-711, CH5-DF Finnigan, and ZAB-E spectrometers. Mp's were uncorrected. Gc-ms was performed with a 5890 gas chromatograph with 5950B MSD, Hewlett-Packard.

PLANT MATERIAL.—The roots of *E. senticosus* were purchased by ROLIMPEX from the Soviet Union in 1982. The voucher specimen is maintained in the Institute of Medicinal Plants, Poznan, Poland.

EXTRACTION AND ISOLATION.—The powdered roots (2 kg) of *E. senticosus* were extracted exhaustively with hot MeOH. The extract was evaporated to a dark syrup that was suspended in H_2O and reextracted successively with *n*-hexane, Et_2O, EtOAc, and *n*-BuOH saturated with H_2O. The *n*-BuOH-soluble fraction was chromatographed over a Si gel (230–400 mesh, Merck) column by elution with a gradient of $CHCl_3$-MeOH-H_2O (70:25:3 to 60:40:10). The fraction eluted with $CHCl_3$-MeOH-H_2O (60:40:10) yielded, after evaporation, a mixture of crude saponins (2.5 g). The crude saponins were purified on a Sephadex LH-20 column by elution with MeOH. The mixture of saponins **1** and **2** (380 mg) was separated by rotatory tlc on a Si gel (60 PF_{254} with gypsum, Merck) disk by elution with $CHCl_3$-MeOH-H_2O (65:35:7).

SAPONIN **1**.—Colorless powder (180 mg): mp 255–258°; $[\alpha]^{22}D$ −45° (c = 0.4, MeOH); ir (KBr) ν max 3400, 2950, 1730, 1610, 1075 cm^{-1}; fabms *m/z* (rel. %) $[M+H]^+$ 1105 (10), $[M+Na]^+$ 1127 (12), $[M+K]^+$ 1143 (25), $[M+K-146]^+$ 997, $[M+K-162]^+$ 981 (7), $[M+K-146-162]^+$ 835 (5); negative fabms *m/z* (rel. %) $[M-H]^-$ 1103 (35), $[M-H-162]^-$ 941 (8), $[M-H-146]^-$ 957 (7), $[M-H-162-146]^-$ 795 (12), $[M-H-(2\times162)]^-$ 779 (5), $[M-H(2\times162)-\text{aglycone}]^-$ 321 (17), $[M-H-146-162-\text{aglycone}]^-$ 337 (10); ^{1}H-nmr (250 MHz, pyridine-d_5) δ ppm 3.24 (1H, m, H-3α), 3.38 (1H, d, J = 7.5 Hz, H-28a), 3.66 (1H, d, J = 7.5 Hz, H-28b), 4.06 (1H, m, H-16β); ^{13}C nmr see Table 1.

TABLE 1. ^{13}C-nmr Spectra of Primulagenin A [**3**] and Saponin **1**.

Carbon	Compound					
	3		**1**			
C-1	38.8[a]	40.0[b]	40.1[c]	Sugar moiety		
C-2	27.3	28.8	27.0	Glc	1	100.9
C-3	78.9	79.7	91.8		2	76.0
C-4	38.7	39.9	40.8		3	78.1
C-5	55.3	56.9	57.1		4	71.9
C-6	18.3	19.5	18.9		5	78.1
C-7	32.8	33.6	33.7		6	62.8
C-8	39.9	41.0	43.3	Gal	1	102.8
C-9	47.0	[d]	51.2		2	75.1
C-10	36.9	38.1	37.4		3	88.4
C-11	23.4	24.6	19.9		4	71.0
C-12	122.7	123.4	37.3		5	78.0
C-13	142.9	145.4	88.4		6	63.3
C-14	41.6	42.7	45.3	Rha	1	102.4
C-15	35.2	37.3	37.9		2	72.1
C-16	75.0	74.8	79.5		3	72.7
C-17	40.6	41.4	45.3		4	74.0
C-18	42.7	43.6	53.0		5	70.3
C-19	46.9	[d]	39.8		6	18.9
C-20	30.4	31.6	32.3	GlcA	1	105.8
C-21	34.7	35.0	35.0		2	82.4
C-22	25.5	27.6	30.5		3	76.4
C-23	28.1	30.1	28.2		4	81.4
C-24	15.7	16.4	16.8		5	76.9
C-25	15.6	16.2	16.8		6	176.8
C-26	17.2	17.6	18.9			
C-27	26.2	27.9	19.9			
C-28	70.8	71.2	78.7			
C-29	32.8	34.1	34.0			
C-30	23.4	25.1	24.9			

[a]In $CDCl_3$.
[b]In MeOH-d_4.
[c]In MeOH-d_4.
[d]Covered by solvent.

SAPONIN **2**.—Colorless powder (22 mg): mp 230° (dec); $[\alpha]^{22}D$ −15° (c = 0.5, MeOH); ir (KBr) ν max 3400, 2950, 1730, 1610, 1075 cm^{-1}; negative fabms *m/z* (rel. %) $[M-H]^-$ 1233 (30), $[M-H-(2\times146)]^-$ 941 (15), $[M-H-(3\times146)]^-$ 795 (15), $[M-H-(3\times146)-162]^-$ 633 (15); ^1H nmr (250 MHz, pyridine-d_5) δ ppm 3.22 (1H, m, H-3α), 3.40 (1H, d, J = 7.5 Hz, H-28a), 3.70 (1H, d, J = 7.5 Hz, H-28b), 4.08 (1H, m, H-16β).

ACID HYDROLYSIS OF **1** AND **2**.—A solution of **1** (60 mg) or **2** (18 mg) in 2 N HCl in 80% MeOH was refluxed for 2 h. Usual workup gave colorless needles of primulagenin A [**3**]: mp 225–229° (MeOH), [lit. (11) 229–232°]; hreims (80 eV) *m/z* 458.37713 ($C_{30}H_{50}O_3$); eims (80 eV) *m/z* (rel. %) 458 (27), $[M-H_2O]^+$ 440 (5), [rDA a]$^+$ 250 (94), [rDA a − CH_2OH]$^+$ 219 (100), [rDA b]$^+$ 207 (27); ^1H nmr (300 MHz, pyridine-d_5) δ ppm 0.92, 0.99, 1.02, 1.07, 1.09, 1.21, 1.81 (each s, 7 × Me), 3.50 (1H, t, J = 8 Hz, H-3α), 3.62 (1H, d, J = 11 Hz, H-28a), 3.83 (1H, d, J = 11 Hz, H-28b), 4.72 (1H, br s, H-16β), 5.43 (1H, m, H-12); ^{13}C nmr see Table 1.

An aqueous layer was neutralized with Ag_2CO_3, concentrated, and chromatographed on a Si gel plate (Merck) with a mixture of *n*-PrOH–EtOH–H_2O (7:2:1) as a mobile phase. Hydrolysis of saponin **1** gave glucose R_f 0.42, galactose R_f 0.50, rhamnose R_f 0.78, and glucuronic acid R_f 0.20. Hydrolysis of saponin **2** gave glucose R_f 0.42, rhamnose R_f 0.78, and glucuronic acid R_f 0.20.

PERMETHYLATION AND METHANOLYSIS OF **1** AND **2**.—To a solution of **1** or **2** (10 mg) in DMSO (1 ml), powdered and dried NaOH (25 mg) and MeI (0.5 ml) were added. The mixture was shaken at room temperature for 2 h, and $CHCl_3$ (1 ml) and H_2O (1 ml) were added to the reaction mixture. The organic layer was washed three times with H_2O (5 ml), dried over Na_2SO_4, and evaporated to dryness. The residue was dissolved in 2 N HCl in MeOH and refluxed for 2 h. Usual workup (19) gave a mixture of permethylated monosaccharides, which was acetylated with pyridine-Ac_2O (1:1) (0.4 ml) at room temperature overnight. Gc-ms analysis was performed with an HP-1 column (12 m × 0.2 mm) over the temperature range 100°–200° (6°/min). Saponin **1** under the above conditions gave methyl-2,3,4-tri-*O*-methylrhamnopyranoside Rt 7.98, 7.70; methyl-2,3,4,6-tetra-*O*-methylglucopyranoside Rt 9.61, 10.11; methyl-3-*O*-acetyl-2,4,6-tri-*O*-methylgalactopyranoside Rt 12.11, 12.60 min. The methyl ester of methyl-2-*O*-acetyl-3-*O*-methyl-4-*O*-(2,4,6-tri-*O*-methyl-3-*O*-acetylgalactopyranosyl)-glucuronopyranoside at Rt 23 min has also been detected: ms *m/z* (rel. %) 464 (1), 382 (13), 321 (14), 261 (31), 247 (2), 201 (27), 189 (3), 101 (14), 88 (79). Saponin **2** gave methyl-2,3,4-tri-*O*-methylrhamnopyranoside Rt 7.70, 7.98; methyl-4-*O*-acetyl-2,3-di-*O*-methylrhamnopyranoside Rt 11.1, 11.2; methyl-2,4-di-*O*-acetyl-3,6-di-*O*-methylglucopyranoside Rt 16.20, 16.23 min.

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ERRATA

For the paper by Uhrín and Proksa entitled "Conformational Analysis of 4-Methylraucubaininium and 4-Methylstrictaminium Chlorides," *J. Nat. Prod.*, **52**, 637 (1989), the authors request the following corrections:

The absolute configuration at C-2 and C-7 in structure **1** and C-2 in structure **2** was erroneously assigned as *S*; applying stereochemical rules correctly it should be *R*. The assignment of prochiral ligands at C-5, C-14, and C-21 should be changed: H_S should be H_R and vice versa throughout the paper.

The affiliation of John A. Beutler in the paper entitled "Dereplication of Phorbol Bioactives: *Lyngbya majuscula* and *Croton cuneatus*," *J. Nat. Prod.*, **53**, 867 (1990) was listed erroneously with the Laboratory of Drug Discovery Research & Development DTP, NCI due to author error. The correct affiliation should have been listed as PRI/DynCorp. Dr. Beutler is currently affiliated with the LDDRD.