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CEPHALARIA SAPONIN A, A NEW BIDESMOSIDIC TRITERPENE SAPONIN FROM *CEPHALARIA TRANSSYLVANICA*

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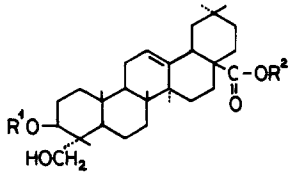
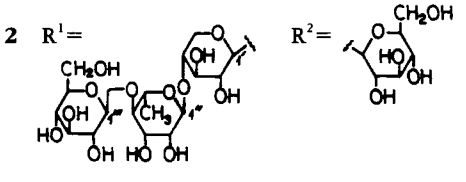
ABSTRACT.—A new bidesmosidic triterpene saponin, Cephalaria saponin A [**2**] was isolated from the MeOH extract of the flowers of *Cephalaria transsylvanica*. Its structure was determined as 3-*O*-{[β-D-glucopyranosyl(1→4)-α-L-rhamnopyranosyl(1→4)-β-D-xylopyranosyl]-[28-*O*-β-D-glucopyranosyl]}-3β,23-dihydroxy-12(13)-oleanen-28-oic acid.

Cephalaria transsylvanica L. (Dipsacaceae) is an annually flowering plant that has white- or lilac-colored flowers and grows in northwest Turkey, central Anatolia, south and central Europe, and some parts of Russia, Crimea, and Caucasia (1). The identification of several triterpene glycosides from *C. gigantea* has been reported (2,3). The present paper describes the isolation and structure elucidation of a bidesmosidic triterpene saponin [**2**] from the flowers of *C. transsylvanica*.

Cephalaria saponin A [**2**] was isolated by repetitive cc on Si gel from the dried flowers of *C. transsylvanica* and was purified by prep. tlc. The aglycone [**1**] was obtained by acidic hydrolysis with 2 N HCl-MeOH (1:1) and its structure was confirmed as hederagenin by ir, nmr, ms, and by comparison of tlc and physical properties with an authentic sample and literature values (4). The hydrolysate in the aqueous layer was examined by tlc [$CHCl_3$-MeOH-H_2O (65:35:10)+10% MeOH, lower phase] and paper chromatography [EtOAc-pyridine-H_2O (3.6:1:1.5); *n*-BuOH-EtOH-H_2O (2:1:1 and 4:1:5)]. These investigations showed the presence of glucose, rhamnose, and xylose. Gas chromatographic estimation of the silylated sugar mixture (5) gave a glucose-rhamnose-xylose (2:1:1) ratio.



1 $R^1=R^2=H$



2 $R^1=$ $R^2=$

After basic hydrolysis of **2**, an acidic saponin and glucose were obtained and the quantitative sugar analysis on this acidic glycoside gave the ratio glucose-rhamnose-xylose (1:1:1). This indicated that one glucose unit was at the C-28 position of the aglycone, that is, **2** is bidesmosidic. Further evidence for this conclusion was that one of the anomeric carbon signals (δ 95.7) (Table 1) resonated at relatively high field in the ^{13}C-nmr spectrum of compound **2**. The ^{13}C-nmr signal at δ 84.10 also indicated that the trisaccharide moiety was attached to C-3 of the aglycone (6,7).

In the negative-ion fabms spectrum of **2**, the expected molecular ion peak ($C_{53}H_{86}O_{22}$) was observed at *m/z* 1073 $[M-H]^-$. In addition, the peaks at *m/z* 911 (aglycone+pentose+deoxyhexose+hexose−$3H_2O$−H), 749 (aglycone+pentose+deoxyhexose−$2H_2O$−H) and 603 (aglycone+pentose−H_2O−H) indicated that the sugar chain, which consisted of xylose, rhamnose, and glucose, was linked to the C-3 hydroxyl group of the aglycone

TABLE 1. ^{13}C-Nmr Chemical Shifts of **2** in C_5D_5N.

Carbon	δ ^{13}C	Carbon	δ ^{13}C
C-1	38.8	3-*O* Sugars	
C-2	26.1	C-1′	105.1
C-3	84.1	C-2′	74.9
C-4	46.9	C-3′	76.1
C-5	47.6	C-4′	77.2
C-6	22.8	C-5′	64.1
C-7	32.9	C-1″	101.6
C-8	39.7	C-2″	72.2
C-9	48.3	C-3″	71.6
C-10	37.0	C-4″	77.9
C-11	23.5	C-5″	69.2
C-12	129.1	C-6″	18.1
C-13	143.5	C-1‴	104.9
C-14	42.0	C-2‴	75.3
C-15	27.9	C-3‴	78.2
C-16	23.8	C-4‴	71.0
C-17	46.6	C-5‴	77.8
C-18	41.7	C-6‴	62.7
C-19	46.7	28-*O* Sugar	
C-20	31.1	C-1	95.7
C-21	33.9	C-2	74.4
C-22	32.6	C-3	78.5
C-23	63.8	C-4	71.1
C-24	14.3	C-5	79.2
C-25	16.3	C-6	61.2
C-26	17.6		
C-27	26.3		
C-28	176.4		
C-29	33.5		
C-30	23.8		

and that the sequence is aglycone-xylose-rhamnose-glucose.

The ^{1}H-nmr spectrum of **2** displayed six tertiary methyl singlets at δ 0.86, 0.90, 0.97, 1.02, 1.06, and 1.13 and the methyl group of a rhamnose unit (3H, δ 1.43, d, J=6 Hz). Single-proton resonance signals at δ 4.36 (1H, d, J=7.8 Hz), 4.96 (1H, d, J=7.8 Hz), 5.10 (1H, d, J=1.8 Hz) and 6.24 (1H, d, J=8.1 Hz) revealed that one of the sugars (rhamnose) was linked in an α-glycosidic manner and the other three sugars were linked in a β-glycosidic fashion. This conclusion was also supported by the ^{13}C-nmr chemical shifts of the anomeric carbons (8).

Compound **2** was methylated (9) and then the methylated product was methanolyzed. Identification of the methylated methyl sugars were based on tlc, paper chromatography, and gc by comparison with reference methylated sugars and by gc-ms analyses (10,11). Methyl-2,3,4,6-tetra-*O*-methyl-β-D-glucopyranoside, methyl-2,3-di-*O*-methyl-α-L-rhamnopyranoside, and methyl-2,3-di-*O*-methyl-β-D-xylopyranoside were detected. These compound identifications led to the conclusion that the structure of Cephalaria saponin A [**2**] is 3-*O*-{[β-D-glucopyranosyl(1→4)-α-L-rhamnopyranosyl(1→4)-β-D-xylopyranosyl]-[28-*O*-β-D-glucopyranosyl]}-3β,23-dihydroxy-12(13)-oleanen-28-oic acid.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—The ^{1}H- and ^{13}C-nmr (APT) spectra were recorded on a Bruker 200 MHz spectrometer in pyridine-d_5 with TMS as internal reference. Ir spectra were taken as KBr pellets on a Bruker IFS-48 Ft-ir spectrometer. Optical rotations were measured on a Schmidt and Haensch Polartonic E polarimeter. Fabms spectra were recorded on a VG AutoSpec mass spectrometer. Gc and gc-ms analyses were carried out using a Hewlett-Packard GC (5890)-MSD (5917) combined system (column, 1 μm×0.25 mm×30 m, SPB-5; He, temperature 130–280; 3°/min), respectively.

PLANT MATERIAL.—The plant material was collected in Bornova, Izmir, Turkey, and identified by Prof. Dr. Özcan Secmen, Department of Botany, University of Ege. A voucher specimen (EGE 6584) is preserved in the Herbarium of Ege University.

EXTRACTION AND ISOLATION.—The air-dried and ground flowers of *C. transsylvanica* (1 kg) were extracted with 80% MeOH (4×5 liters). The combined extract was evaporated under reduced pressure at 40°. The resulting residue was extracted with hexane (2×1 liters) and $CHCl_3$ (2×1 liter) to remove non-glycosidic substances and a waxy residue was obtained (25 g). This was partitioned with EtOAc and H_2O. The H_2O-soluble part was extracted with *n*-BuOH and on evaporation afforded a gummy mixture (19 g). A part of this mixture (5 g) was subjected to cc over Si gel using a $CHCl_3$/MeOH/H_2O solvent system of gradually increasing polarity (65:25:10, 65:30:10, 65:35:10, lower phase) and four fractions (A, B, C, D) were collected. Fraction B (78 mg) was purified by prep. tlc to afford Cephalaria saponin A [**2**] (69 mg).

Cephalaria saponin A [**2**].—This saponin was obtained as a white amorphous powder; $[\alpha]D^{28}$ 9.1° (*c*=1.22, pyridine); ir (KBr) *ν* max 3460–3400, 3040, 1738, 1640, 885 cm^{-1}; negative-ion fabms *m/z* 1073 (M^+-H; hederagenin+2 glucose+rhamnose+xylose$-4H_2O-H$), 911 (hederagenin+glucose+rhamnose+xylose$-3H_2O-H$), 749 (hederagenin+xylose+rhamnose$-2H_2O-H$), 603 (hederagenin+xylose$-H_2O-H$; base peak); ^1H nmr (C_5D_5N) δ 6.24 (1H, d, *J*=8.0 Hz; anomeric proton of C-28 glc), 5.23 (1H, t, H-12), 5.10 (1H, d, *J*=1.8 Hz, H-1″), 4.96 (1H, d, *J*=7.8 Hz, H-1′), 4.36 (1H, d, *J*=7.8 Hz, H-1‴), 4.43–3.59 (overlapping signals of hydroxy groups), 1.43 (3H, d, *J*=6 Hz; methyl of rhamnose), 1.13, 1.01, 1.02, 0.97, 0.90, 0.86 (singlets, 6 methyl groups); ^{13}C nmr (APT) (C_5D_5N), see Table 1.

ACID HYDROLYSIS OF **2**.—Compound **2** (30 mg) in MeOH (5 ml) was hydrolyzed with 2 N HCl (5 ml) at 95° for 6 h. H_2O was added to the reaction mixture and the aglycone [hederagenin, **1**] was extracted with $CHCl_3$ and crystallized from MeOH (13.2 mg); mp 327–329° [lit. (4) mp 320–322°]; $[\alpha]D^{28}$ 78° (*c*=1.68, pyridine) [lit. (4) $[\alpha]D^{23}$ 81° (pyridine)]; ms *m/z* 472 $[M]^+$, 454 (M^+-H_2O), 426 (M^+-HCO_2H), 395 ($M^+-HCO_2H-CH_2OH$), 248 ($C_{16}H_{24}O_2$, base peak), 233 ($C_{15}H_{21}O_2$) and 189 ($C_{14}H_{21}$); ^1H nmr (C_5D_5N) δ 5.43 (1H, br t, H-12), 4.18 (1H, H-3), 3.73 (2H, br s, $-CH_2OH$), 1.28–0.86 (6 methyl singlets); ^{13}C nmr (APT) (C_5D_5N) consistent with the aglycone resonances of **2** (Table 1).

After acid hydrolysis, the aqueous phase was neutralized with Ag_2CO_3 and evaporated to dryness. The sugar mixture was analyzed by tlc, paper chromatography, and gc by comparison with authentic samples.

ALKALINE HYDROLYSIS OF **2**.—Compound **2** (15 mg) was heated in 1% KOH (5 ml) for 2 h at 110°. On cooling, the reaction mixture was neutralized with HCl (pH 6–7) and extracted with *n*-BuOH. The *n*-BuOH layer was evaporated to dryness to give an acidic saponin (9 mg), which was hydrolyzed with 2N HCl. The sugar mixture was silylated and then analyzed by gc. The H_2O layer was evaporated and paper chromatography of the residue revealed D-glucose.

METHYLATION OF **1** AND SUGAR IDENTIFICATION.—Compound **1** (20 mg) was dissolved in DMF. The solution was methylated at room temperature using Ag_2O (0.3 g) and CH_3I (1.5 ml). This procedure was repeated twice. The crude permethylated product (13 mg) was purified by prep. tlc using $CHCl_3$-EtOH (97:3) as solvent system.

The methylated saponin was methanolyzed for 5 h with 5% anhydrous methanolic HCl at 80°. The methylated methyl sugars were identified by comparison with authentic samples by tlc, gc, and gc-ms. Methyl-2,3,4,6-tetra-*O*-methyl-D-glucopyranoside (R_t 18 min, 31 sec): ms, *m/z* 187, 149, 101, 88 (base peak) 75, 73, 71; methyl-2,3-di-*O*-methyl-β-D-xylopyranoside (R_t 14 min, 27 sec): ms, *m/z* 161, 129, 101, 88 (base peak), 75, 74, 58 and methyl-2,3-di-*O*-methyl-α-L-rhamnopyranoside (R_t 12 min, 50 sec): ms, *m/z* 192, 161, 117, 88, 75 (base peak), 58, 43.

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