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A BIDESMOSIDIC TRITERPENE SAPONIN FROM *CEPHALARIA TRANSSYLVANICA*

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Key Word Index—*Cephalaria transsylvanica*; Dipsacaceae; flowers; cephalaria saponin B; hederagenin.

Abstract—The structure of cephalaria saponin B, isolated from *Cephalaria transsylvanica* was elucidated as 3-*O*-{[β-D-xylopyranosyl(1 → 4)-α-L-rhamnopyranosyl(1 → 2)-β-D-xylopyranosyl]-[28-*O*-β-D-glucopyranosyl]}-3β,23-dihydroxy-12(13)-oleanene-28-oic acid.

INTRODUCTION

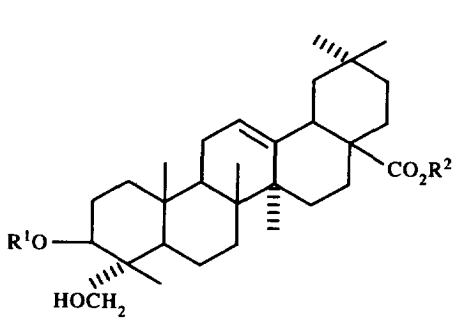
The genus *Cephalaria* is represented by 28 species in the flora of Turkey [1]. In earlier studies different compounds (e.g. alkaloids, triterpene saponins, flavonoids, iridoids) were identified from some *Cephalaria* species and their pharmacological activities were reported [2]. In a previous report [3] we described the isolation and identification of a new bidesmosidic triterpene saponin from flowers of *Cephalaria transsylvanica* L. The present communication describes the isolation and structure elucidation of a new bidesmosidic triterpene saponin; cephalaria saponin B (**2**) from the flowers of *C. transsylvanica*.

RESULTS AND DISCUSSION

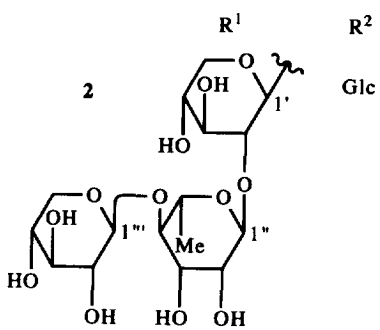
The new saponin **2** gave hederagenin (**1**) as the aglycone and D-glucose, L-rhamnose and D-xylose as the sugar moieties on acid hydrolysis. The sugars were determined by PC and GC comparison with reference sugars. The aglycone was identified by IR, NMR and mass spectroscopic analyses. The identity was confirmed by comparison of TLC and physical properties with an authentic sample and with literature values [4].

The anomeric carbon signals (δ105.6, 103.4, 101.7 and 96.8) observed in the ^{13}C NMR spectrum of **2** showed that glucose and two xylose were engaged in a β-glycosidic linkage, whereas rhamnose was α-glycosidic. This result was also supported by ^{1}H NMR chemical shifts of anomeric protons (see Experimental). GC analyses of the silylated sugar mixture [5] gave a glucose–rhamnose–xylose (1:1:2) ratio.

The appearance of an ester adsorption band in the IR spectrum of **2** (ν_{max} = 1732 cm^{-1}) and a carboxyl carbon signal (δ179.7) showed the presence of an ester linkage. Moreover, one of the anomeric carbon signals (δ96.8) resonating at rather high field in the ^{13}C NMR spectrum indicated that one of the sugars was at the C-28 position of the aglycone. After basic hydrolysis of **2** an acidic saponin and glucose were obtained. Quantitative sugar analysis on this acidic saponin gave the ratio rhamnose–xylose (1:2) and indicated saponin **2** was bidesmosidic. In addition, the ^{13}C NMR signal at δ81.0



	R1	R2
1	H	H



showed the trisaccharide sugar moiety to be attached at C-3 of the aglycone [6].

In the negative ion FAB-mass spectrum of **2** the expected molecular ion peak ($C_{52}H_{84}O_{21}$) was observed at *m/z* 1043 $[M - H]^-$. In addition, the peaks at *m/z* 881 [aglycone+2 pentose+deoxyhexose $- 3H_2O - H$], 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 and was linked to the C-3 hydroxyl group of the aglycone, was in the sequence aglycone-xylose-rhamnose-xylose.

Compound **2** was methylated [7] and then the methylated product was methanolysed. Identification of the methylated methyl sugars was based on TLC, PC and GC by comparison with reference methylated sugars and by GC-mass spectral analyses [8, 9]. Methyl-2,3,4,6-tetra-*O*-methyl-β-D-glucopyranoside, methyl-2,3,4-tri-*O*-methyl-β-D-xylopyranoside, methyl-3,4-di-*O*-methyl-β-D-xylopyranoside and methyl-2,3-di-*O*-methyl-α-L-rhamnopyranoside were detected.

On the basis of the above evidence, the structure of *Cephalaria saponin* B (**2**) was elucidated to be; 3-*O*-{[β-D-xylopyranosyl (1 → 4)-α-L-rhamnopyranosyl (1 → 2)-β-D-xylopyranosyl]-[28-*O*-β-D-glucopyranosyl]}-3β,23-dihydroxy-12(13)-oleanene-28-oic acid.

EXPERIMENTAL

General. The ^1H and ^{13}C NMR(APT) spectra were recorded on a Bruker 200 MHz spectrometer in pyridine-d_5 with TMS as int. standard. IR spectra were taken as KBr pellets on a Bruker IFS-48 FTIR spectrometer. Optical rotations were measured on a Schmitdt + Haensch Polartonic E polarimeter. EIMS were recorded on a Kratos MS-80 and negative ion FAB-MS were recorded on a Jeol VG 20–250 MS spectrometer. GC and GC-MS analyses were carried out using a Hewlett-Packard-439 gas chromatography (column: 3 mm × 1 m, N_2, 10% SE-30; temp. 110–270°, 2° min^{-1}) and a Hewlett-Packard GC (5890)-MSD(5917) system (column: 1 μm × 0.25 mm × 30 m, SPB-5, He, temp. 130–280°, 3° min^{-1}), respectively. Plant material was collected in Bornova-Izmir, Turkey.

Isolation of saponin. The air-dried and ground flowers of *C. transsylvanica* (1 kg) were extracted with 80% MeOH (4 × 5 l). After evapn of the MeOH, the liquor was extracted with hexane (2 × 1 l) and $CHCl_3$ (2 × 1 l) 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 evapn afforded a gummy mixt. (19 g). A part of this mixt. (5 g) was subjected to repeated CC on silica gel with $CHCl_3$–MeOH–H_2O (13:4:2, lower phase) solvent system to yield a saponin (75 mg) which was purified by PLC using the same solvent system to give cephalaria saponin B (**2**) (62 mg).

Cephalaria saponin B (**2**). The saponin was obtained as an amorphous powder; $[\alpha]_D^{28}$ − 65.47 (pyridine; *c* 0.37). IR ν_{max}^{KBr} cm^{-1}: 3434, 2959, 2929, 1732, 1603, 1395, 887. FAB-MS (− ve ion) *m/z*: 1043 [M − H; hederagenin + 2xylose + rhamnose + glucose $- 4H_2O - H$], 881 [hederagenin + 2xylose + rhamnose $- 3H_2O - H$], 749 [hederagenin + xylose + rhamnose $- 2H_2O - H$, base peak], 603 [hederagenin + xylose $- H_2O - H$]. ^1H NMR (pyridine-d_5): δ6.27 (1H, *d*, 8.0 Hz anomeric proton of C-28 Glc), 5.20 (1H, *t*, H-12), 5.08 (1H, *d*, 1.8 Hz, H-1″), 4.88 (1H, *d*, 7.8 Hz, H-1′), 4.72 (1H, *d*, 7.6 Hz, H-1‴), 4.39–3.66 (overlapped signals of CH-O and CH_2-O groups), 1.42 (3H, *d*, 6.7 Hz, methyl of rhamnose), 1.15, 1.08, 1.03, 0.96, 0.90 and 0.88 (singlets, 6 methyl groups). ^{13}C NMR(APT) (pyridine-d_5): δ179.7 (C=O), 145.1 and 132.2 (2 olefinic C), 105.6 103.4, 101.7 and 96.8 (4 anomeric C), 81.0–69.2 (15 CH-O-), 67.7–61.4 (4 CH_2-O), 47.9, 47.0 and 42.2 (3 methine C), 46.2–25.1 (6 quaternary C and 10 methylene C), 32.8–13.3 (7 methyl groups).

Acidic hydrolysis of compound **2** *and isolation and identification of the aglycone as hederagenin* (**1**). The saponin (35 mg) in methanol (5 ml) was hydrolysed with 2 M HCl (5 ml) at 95° for 6 hr. H_2O was added to the reaction mixt. and the aglycone was extracted with $CHCl_3$ and crystallized from MeOH (16 mg); mp 327–329° (lit. [4] mp 320–322°); $[\alpha]_D^{28}$ 78 (pyridine; *c* 1.68) (lit. [4] $[\alpha]_D^8$ 81 (pyridine). MS *m/z*: 472 $[M]^+$, 454 $[M - H_2O]^+$, 426 $[M - HCO_2H]^+$, 395 $[M - HCO_2H - CH_2OH]^+$, 248 ($C_{16}H_{24}O_2$, retro-Diels–Alder fragment, base peak), 233 ($C_{15}H_{21}O_2$) and 189 ($C_{14}H_{21}$). ^1H NMR (pyridine-d_5): δ5.43 (1H, *t*, H-12), 4.18 (1H, *t*, 7.1 Hz, H-3), 3.73 (2H, *br s*, $-CH_2-OH$), 1.28–0.86 (6 methyl singlets). ^{13}C NMR-APT (pyridine-d_5): δ179.7 (C=O), 144.3 and 122.3 (2 olefinic C), 73.2 (C-3), 67.2 (C-23), 48.5, 47.8 and 41.7 (3 methine C), 46.3–18.8 (6 quaternary and 10 methylene C), 46.1–18.8 (6 methyl C).

After acidic hydrolysis, the aq. phase was neutralized with Ag_2CO_3 and evapd till dryness. The sugar mixt. was analysed by TLC, PC and GC by comparison with authentic samples.

Alkaline hydrolysis. Compound **2** (10 mg) was heated in 1% KOH (5 ml) for 2 hr at 110°. After cooling, the reaction mixt. was neutralized (pH 6–7) with HCl and extracted with *n*-BuOH. The *n*-BuOH layer was evapd to dryness to give an acidic saponin (6 mg) which was hydrolysed with 2 M HCl. The sugar mixt. was silylated and then analysed by GC. The H_2O layer was evapd. Paper chromatography of the residue revealed D-glucose.

Methylation and identification of methylated sugars. The saponin **2** (15 mg), was dissolved in DMF. The soln was methylated at room temp. using Ag_2O (0.2 g) and MeI (1 ml). This procedure was repeated twice. The crude permethylated product was purified by PLC using $CHCl_3$–EtOH (97:3) to yield 10 mg of material.

The methylated saponin was methanolysed for 5 hr 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-β-D-glucopyranoside, MS *m/z*: 187, 149, 101, 88 (base peak), 75, 73, 71; methyl-2,3,4-tri-*O*-methyl-β-D-xylopyranoside, MS *m/z*: 176, 101, 88 (base peak), 75, 45; methyl-3.4-di-*O*-methyl-β-D-xylopyranoside, MS *m/z*: 161, 129, 101, 88 (base peak), 75, 74, 58

and methyl-2,3-di-*O*-methyl-α-D-rhamnopyranoside, MS *m/z*: 192, 161, 117, 88 (base peak), 75, 58, 43.

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