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TRITERPENOID GLYCOSIDES FROM *CEPHALARIA TRANSSYLVANICA*

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Key Word Index—*Cephalaria transsylvanica*; Dipsacaceae; flowers; hederagenin; triterpene glycoside.

Abstract–On the basis of spectroscopic and chemical methods, the structures of two new triterpenoid glycosides, transsylvanoside E and F, isolated from *Cephalaria transsylvanica* have been established as 3-*O*-[β-D-xylopyranosyl (1 → 3)-α-L-rhamnopyranosyl (1 → 4)-β-D-glucopyranosyl (1 → 4)-β-D-glucopyranosyl (1 → 2)-β-D-xylopyranosyl]-3β,23-dihydroxy Δ^{12}-oleanen-28-carboxylic acid and 3-*O*-[β-D-glucopyranosyl (1 → 3)-α-L-rhamnopyranosyl (1 → 4)-β-D-xylopyranosyl]-28-*O*-[β-D-glucopyranosyl (1 → 4)-β-D-glucopyranosyl]-3β,23-dihydroxyΔ^{12}-oleanen-28-carboxylic acid, respectively. A new proglycoside was isolated from the cleavage of the ester–glycoside linkage and it's structure characterized as 3-*O*-[β-D-glucopyranosyl (1 → 3)-α-L-rhamnopyranosyl (1 → 4)-β-D-xylopyranosyl]-3β,23-dihydroxy Δ^{12}-oleanen-28-carboxylic acid.

INTRODUCTION

Some isolation studies have previously been carried out on *Cephalaria* species, which have been used as a folk medicine for their hypotermic, alleviative, relaxant and anti-infective activities [1, 2]. Two free triterpenoid acids [3] and some triterpene glycosides have been isolated from *C. transsylvanica* [4, 5]. In a preceding paper [6], we also reported on the antimicrobial and antifungal activities of this plant. As a continuation of studies on this plant, we present here the isolation of two new triterpenoid glycosides, named transsylvanoside E (**1**) and F (**2**).

RESULTS AND DISCUSSION

Repeated purification by column chromatography (silica gel 60) of the *n*-butanol-soluble fractions of the methanolic extract of the flowers of *C. transsylvanica* led to the isolation of two triterpene glycosides (**1**, **2**), identified by the Liebermann–Burchard test [7] and by the formation of a stable froth when shaken with water. Their IR spectra showed hydroxyl (3412–3395 cm^{-1}) and C=C double bond absorptions (1631 cm^{-1}). Compounds **1** and **3** showed carboxylic group absorptions (1693 and 1697 cm^{-1}) and the IR spectrum of **2** included an ester group absorption at 1728 cm^{-1}. Hydrolysis of **1** and **2** under acidic conditions afforded the same aglycone which was identified as hederagenin by comparison with an authentic sample by chemical and spectroscopic means [3, 8].

On acid hydrolysis **1** and **2** gave D-glucose, D-xylose and L-rhamnose by paper chromatography (solvent systems F and G) [9, 10]. Analysis of the silylated sugars by GC [11] gave the ratio glucose–rhamnose–xylose as 2:1:2 for **1** and 3:1:1 for **2**. The ^{13}C NMR spectra (Table 1) indicated the presence of anomeric carbon signals (δ101.4, 104.5, 104.7, 105.4 and 106.5) in **1** and (δ95.6, 101.4, 104.9, 105.2, 106.6) in **2** [12–15]. The signal at δ95.6 suggested that **2** had a 28-*O*-glycosidic linkage, which was further confirmed by the ester group absorption in the IR spectrum. The ^{13}C NMR signal of C-28 for **2** appeared at *ca* δ176.5, whereas the chemical shift of the corresponding carbon in hederagenin was *ca* δ180.1 [4]. The simultaneous presence of a 3-*O*-glycosidic linkage in **1** and **2** was easily seen by attendant downfield shifts at δ82.9 and 83.3 for C-3, whereas in hederagenin this carbon signal was observed at *ca* δ76.4 [8]. Thus **1** is 3-*O*-monodesmoside and **2** is a 3,28-bisdesmoside.

On alkaline hydrolysis, **2** afforded **3**. Compound **3** gave a peak for the deprotonated molecule, at *m/z* 911.1 $[M - H]^-$ and fragment ion peaks at *m/z* 749.5 $[M - H - Glc]^-$, 603.5 $[M - H - Glc - Rham]^-$ and 471 $[M - H - Glc - Rham - Xyl]^-$ in the negative ion FAB-mass spectrum. In the IR spectrum, besides the hydroxyl (3393 cm^{-1}) and C=C double bond absorptions (1632 cm^{-1}), the carbonyl group absorption was seen at 1697 cm^{-1}. ^{13}C NMR signals also confirmed the above inferences. In the ^1H NMR spectrum of **3**, the anomeric proton signals at δ5.00 (1H, *br s*), 5.03 (1H, *d*, J = 7.2 Hz) and 5.52 (1H, *d*, J = 7.6 Hz) led to the assignment of the anomeric configuration of two monosaccharide units as β and one as α. These were supported by their carbon signals (Table 1). Compound **3** was identified as

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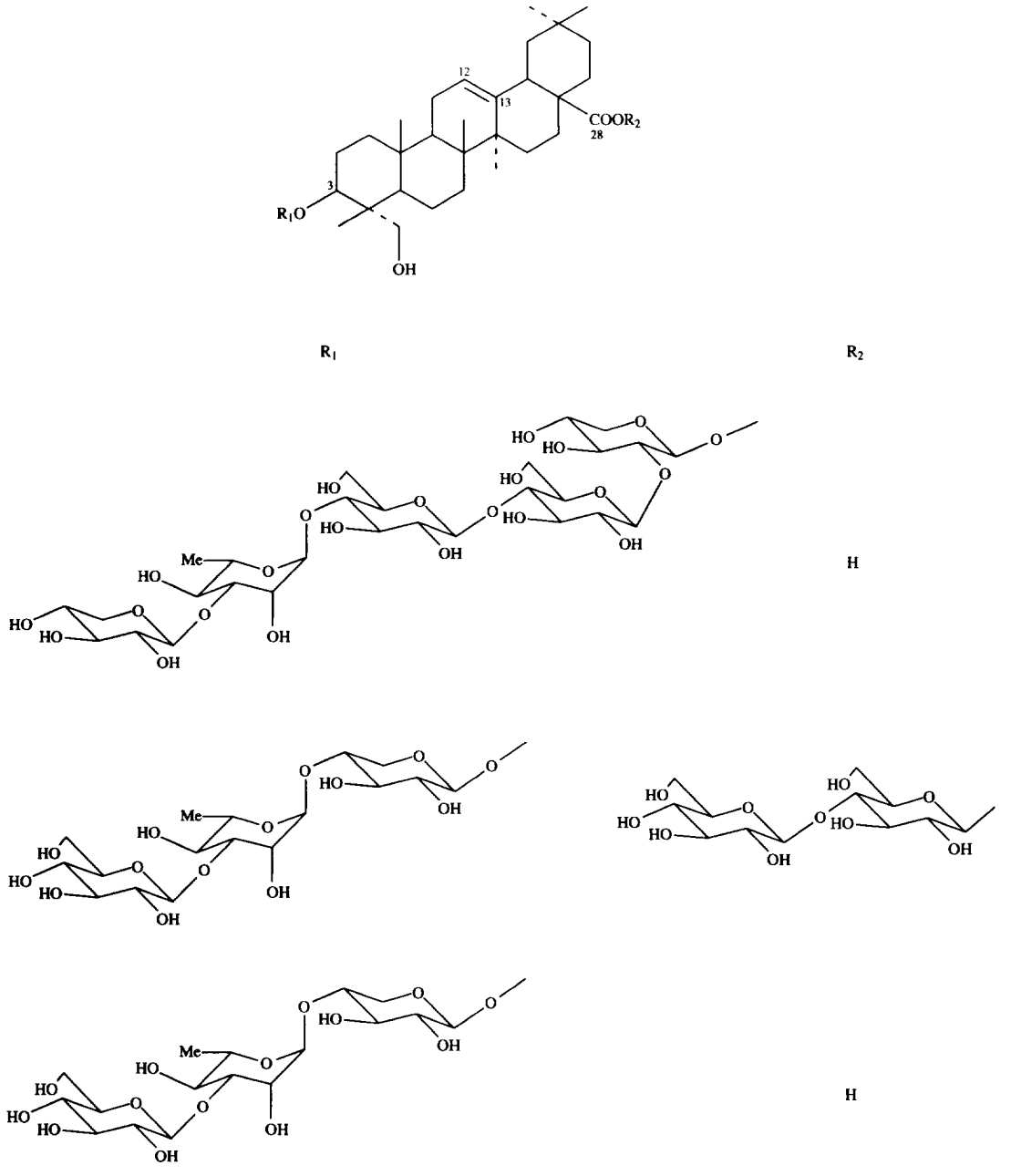

3-*O*-[*β*-D-glucopyranosyl (1 → 3)-α-L-rhamnopyranosyl (1 → 4)-*β*-D-xylopyranosyl]-3*β*, 23-dihydroxy Δ^{12}-oleanen-28-carboxylic acid.

The negative ion FAB-mass spectrum of **1** showed a quasimolecular ion peak at *m/z* 1205.2 $[M - H]^-$ in addition to peaks at *m/z* 1073.6 $[M - H - Xyl]^-$, 910.9 $[M - H - Xyl - Glc]^-$, 749.3 $[M - H - Xyl - 2Glc]^-$, 603 $(M - H - Xyl - 2Glc - Rham]^-$ and 471 $[M - H - 2Xyl - 2Glc - Rham]^-$ which indicated the sequence of the sugar chain. The anomeric configurations of four monosaccharide units as *β* and one as α were deduced from the anomeric proton signals at *δ*4.90 (1H, *br s*), 5.10 (1H, *d*, *J* = 7.2 Hz), 5.20 (1H, *d*, *J* = 7.7 Hz), 5.30 (1H, *d*, *J* = 7.8 Hz), and 5.47 (1H, *d*, *J* = 7.0 Hz), of its ^{1}H NMR spectrum. Hence **1** is 3-*O*-[*β*-D-xylopyranosyl (1 → 3)-α-L-rhamnopyranosyl (1 → 4)-*β*-D-glucopyranosyl (1 → 4)-*β*-D-glucopyranosyl (1 → 2)-*β*-D-xylopyranosyl]-3*β*, 28-dihydroxy Δ^{12}-oleanen-28-carboxylic acid.

The M_r was determined by the negative ion FAB-mass spectrum of glycoside **2** which gave a peak at *m/z* 1235.5 $[M - H]^-$. The fragment ion peaks were seen at *m/z* 1073.4 $[M - H - Glc]^-$, 911.1 $[M - H - 2Glc]^-$, 749.3 $[M - H - 3Glc]^-$, 603.5 $[M - H - 3Glc - Rham]^-$ and 471 $[M - H - 3Glc - Rham - Xyl]^-$ for **2**. They corresponded to the loss of sugars. The interglycosidic linkages of the 28-*O*-sugar chain were established by ^{13}C NMR spectroscopy (Table 1). The presence of downfield methylene signals at *δ*80.7 due to C-4 of the inner

Table 1. ^{13}C NMR spectral data of aglycone and sugar moieties of compounds **1**–**3** (pyridine-d_5, TMS as int. standard)

	C	1	2	3
Aglycone moiety				
	3	83.3	82.9	82.6
	12	122.5	120.0	122.2
	13	144.5	144.1	144.4
	23	67.3	69.3	65.9
	28	180.2	176.5	180.0
3-*O*-Sugar moieties				
Xyl	1	106.5	106.6	106.4
	2	78.2	75.1	74.6
	3	76.5	75.8	75.5
	4	69.6	78.4	78.2
	5	63.9	66.4	63.6
Glc	1	104.7	104.9	104.5
(→ ^{2}Xyl)	2	74.3	72.3	72.0
or	3	77.5	74.1	78.1
(→ ^{3}Rham)	4	80.2	70.8	71.0
	5	75.3	75.1	75.2
	6	61.2	62.5	62.2
Glc*	1	104.5		
(→ ^{4}Glc)	2	74.8		
	3	77.0		
	4	80.7		
	5	75.9		
	6	61.4		
Rham	1	101.4	101.4	101.1
(→ ^{4}Glc*)	2	71.7	71.4	71.3
or	3	81.3	81.1	80.9
(→ ^{4}Xyl)	4	72.9	72.9	72.6
	5	69.5	69.7	69.4
	6	18.4	18.4	18.1
Xyl*				
(→ ^{3}Rham)	1	105.4		
	2	74.0		
	3	76.6		
	4	70.7		
	5	66.5		
28-*O*-Sugar moieties				
Glc*	1		95.6	
	2		73.8	
	3		78.4	
	4		78.6	
	5		78.5	
	6		63.9	
Glc**				
(→ ^{4}Glc*)	1		105.2	
	2		75.3	
	3		77.9	
	4		71.6	
	5		76.6	
	6		61.9	

glucose in **2** revealed that this glucose was attached to another glucose at position C-4. The anomeric configurations of the sugars were fully defined by the NMR spectra. In the ^{1}H NMR spectrum, the anomeric proton signals at 6.25 (1H, *d*, J = 8.0 Hz), 5.89 (1H, *br s*), 5.54 (1H, *d*, J = 7.6 Hz), 5.07 (1H, *d*, J = 7.9), 5.02 (1H, *d*, J = 7.9 Hz) led to the assignments of the configurations of four monosaccharide units as β and one as α. On the basis of the above evidence, the structure of **2** was elucidated as 3-*O*-[β-D-glucopyranosyl(1 → 3)-α-L-rhamnopyranosyl (1 → 4)-β-D-xylopyranosyl]-28-*O*-[β-D-glucopyranosyl (1 → 4)-β-D-glucopyranosyl]-3β,23-dihydroxy Δ^{12}-oleanen-28-carboxylic acid.

EXPERIMENTAL

FAB-MS: negative ion mode, polyethylene glycol as matrix, VG 20-250 quadrupole mass spectrometer; EIMS: (70 eV ion beam energy, 200° ion source; ^{1}H (200 MHz) and ^{13}C (50 MHz) NMR: pyridine-d_5, TMS as int. standard; IR: KBr; GC: column: 0.52 μm × 0.32 mm × 25 m, HP-1; N_2, temp. 130–280°, 2° min^{-1}; CC: silica gel 60 (Merck 7743); prep. TLC silica gel 60 (Merck 7747); TLC: precoated silica gel 60 F_{254} plates (Merck 5554). Spots were visualized by spraying with 10 % H_2SO_4 followed by heating. PC: Schleicher and Schüll 2043 b chromatography paper was used in descending mode. For chromatographic studies the following solvent systems were used: A, $CHCl_3$–MeOH–H_2O (13:5:2); B, $CHCl_3$–MeOH–H_2O (13:7:2); C, $CHCl_3$–MeOH–H_2O (13:7:2 + 10 % MeOH); D, $CHCl_3$–MeOH (15:1); E, $CHCl_3$–EtOH (97:3); F, EtOAc–pyridine–H_2O (2:1:2); G, *n*-BuOH–EtOH–H_2O (2:1:1 and 4:1:5). For A–C solvent systems the lower phases were used.

Plant material. Cephalaria transsylvanica was collected in Bornova-İzmir (Turkey) in July and identified by the Herbarium Center of the Faculty of Science, University of Ege. A voucher specimen (No. 4517) is deposited in the same centre.

Isolation and purification. Dried and ground flowers of *C. transsylvanica* were extracted (× 3, 24 hr for all extractions) with 80% MeOH. After the removal of solvent under vacuum at ~ 40° a waxy residue remained. This was washed with hexane, Me_2CO and $CHCl_3$ successively to remove non-glycosidic substances. The BuOH-soluble fractions of this residue were saturated with H_2O. The BuOH layer was evapd under red. pres. at ~ 40°. A portion of this mixture (5 g) was repurified by repetitive CC over silica gel eluted with solvent systems A–C. Thus, **1** (94 mg) and **2** (45 mg) were obtained as amorphous powders.

3-O-[β-D-*Xylopyranosyl* (1 → 3)-α-L-*rhamnopyranosyl* (1 → 4)-β-D-*glucopyranosyl* (1 → 4)-β-D-*glucopyranosyl* (1 → 2)-β-D-*xylopyranosyl*]-3β,23-*dihydroxy* Δ^{12}-*oleanen*-28 *carboxylic acid* (**1**). $[\alpha]_D^{29}$ − 4.95° (MeOH; *c* 2.72). IR ν_{max} cm^{-1}: 3412 (OH), 1693 (CO_2H), 1631 (C=C); FAB-mass (negative mode) *m/z*: 1205.2 [M − H]$^-$, 1073.6 [M − H − Xyl]$^-$, 910.9 [M − H − Xyl − Glc]$^-$, 749.3 [M − H − Xyl − 2Glc]$^-$, 603 [M − H − Xyl − 2Glc −

Rham]$^-$, 471 [M − H − 2Xyl − 2Glc − Rham]$^-$; ^{1}H NMR (200 MHz, pyridine-d_5): δ5.47 (1H, *d*, J = 7.0 Hz, H-1 of terminal β-Xyl), 5.30 (1H, *d*, J = 7.8 Hz, H-1 of β-Glc), 5.20 (1H, *d*, J = 7.7 Hz, H-1 of β-Glc), 5.10 (1H, *d*, J = 7.2 Hz, H-1 of β-Xyl), 4.90 (1H, *br s*, H-1 of α-Rham), 5.45 (1H, *br s*, H-12), 3.27 (1H, *m*, H-3α), 1.50 (3H, *d*, J = 5.6 Hz, Me of Rham), 1.24 (3H, *s*, Me), 1.14 (3H, *s*, Me), 1.02 (3H, *s*, Me), 1.01 (3H, *s*, Me), 0.94 (6H, *s*, 2 × Me); ^{13}C NMR-APT (50 MHz, pyridine-d_5): Table 1.

3-O-[β-D-*Glucopyranosyl* (1 → 3)-α-L-*rhamnopyranosyl* (1 → 4)-β-D-*xylopyranosyl*]-28-O-[β-D-*glucopyranosyl* (1 → 4)-β-D-*glucopyranosyl*]-3β,23-*dihydroxy* Δ^{12}-*olean-en-28-carboxylic acid* (**2**). Amorphous powder, $[\alpha]_D^{29}$ − 3.69° (MeOH; *c* 1.21). IR ν_{max} cm^{-1}: 3395 (OH), 1728 (CO_2R), 1631 (C=C). FAB-mass (negative mode) *m/z*: 1235.5 [M − H]$^-$, 1073.4 [M − H − Glc]$^-$, 911.1 [M − H − 2Glc]$^-$, 749.3 [M − H − 3Glc]$^-$, 603.5 [M − H − 3Glc − Rham]$^-$, 471 [M − H − 3Glc − Rham − Xyl]$^-$. ^{1}H NMR (200 MHz, pyridine-d_5): δ6.25 (1H, *d*, J = 8.0 Hz, H-1 of ester β-Glc), 5.89 (1H, *br s*, H-1 of α-Rham), 5.54 (1H, *d*, J = 7.6 Hz, H-1 of β-Xyl), 5.07 (1H, *d*, J = 7.9 Hz, H-1 of terminal ester β-Glc), 5.02 (1H, *d*, J = 7.8 Hz, H-1 of β-Glc), 5.37 (1H, *br s*, H-12), 3.20 (1H, *m*, H-3α), 1.54 (3H, *d*, J = 5.8 Hz, Me of Rham), 1.19 (3H, *s*, Me), 1.15 (3H, *s*, Me), 1.12 (3H, *s*, Me), 0.98 (3H, *s*, Me), 0.86 (6H, *s*, 2 × Me); ^{13}C NMR-APT (50 MHz, pyridine-d_5): Table 1.

Alkaline hydrolysis of compounds **1** *and* **2**. Compounds **1** (30 mg) and **2** (20 mg) were dissolved separately in MeOH (5 ml). The solns were left overnight at room temp. after adding dry methanolic NaOMe up to pH 12–13. The reaction mixtures were neutralized with 2 M HCl and then concd to dryness *in vacuo*. The residues were extracted with *n*-BuOH to give 28 and 15 mg of hydrolysed compounds, respectively. Comparison of these new compounds with the original ones (solvent system A) gave a new glycoside (18 mg) (**3**), which was derived from **2**. Compound **1** remained unchanged after alkaline hydrolysis.

3-O-[β-D-*Glucopyranosyl* (1 → 3)-α-L-*rhamnapyranosyl* (1 → 4)-β-D-*xylopyranosyl*]-3β, 23-*dihydroxy* Δ^{12}-*olean-en-28-carboxylic acid* (**3**). $[\alpha]_D^{29}$ − 9.40° (MeOH; *c* 0.9). IR ν_{max} cm^{-1}: 3393 (OH), 1697 (CO_2H), 1632 (C=C). FAB-mass (negative mode) *m/z*: 911.1 [M − H]$^-$, 749.5 [M − H − Glc]$^-$, 603.5 [M − H − Glc − Rham]$^-$, 471 [M − H − Glc − Rham − Xyl]$^-$. ^{1}H NMR (200 MHz, pyridine-d_5): δ 5.52 (1H, *d*, J = 7.6 Hz, H-1 of β-Glc), 5.03 (1H, *d*, J = 7.2 Hz, H-1 of β-Xyl), 5.00 (1H, *br s*, H-1 of α-Rham), 5.40 (1H, *br s*, H-12), 3.27 (1H, *m*, H-3α), 1.53 (3H, *d*, J = 5.6 Hz, Me of Rham), 1.23 (3H, *s*, Me), 1.12 (3H, *s*, Me), 1.00 (3H, *s*, Me), 0.99 (3H, *s*, Me), 0.90 (6H, *s*, 2 × Me); ^{13}C NMR-APT (50 MHz, pyridine-d_5): Table 1.

Acid hydrolysis of glycosides. Solns of **1**–**3** (15 mg each) in 80% MeOH–C_6H_6 (1:1) (5 ml) were each refluxed for 6 hr at 95° after adding 2 M HCl (5 ml). The organic layer was evapd *in vacuo*. H_2O was added to the reaction mixture and the aglycone was extracted with $CHCl_3$. The $CHCl_3$ extract was evapd *in vacuo* and purified on a silica gel column (solvent system D) giving 6, 7 and 5 mg respectively. Mp 328° [3], $[\alpha]_D^{15}$ + 77° (MeOH; *c* 0.7) [3]. IR ν_{max} cm^{-1}: 3422 (OH), 1693 (CO_2H), 1630 (C=C). EIMS *m/z*: 472 [M]$^+$, 471 [M − H]$^+$ (100), 426 [M − HCO_2H]$^+$, 408 [M − HCO_2H − H_2O]$^+$, 395 [M − HCO_2H − CH_2OH]$^+$, 248, 233, 203, 189, 175.

The H_2O layers were combined and neutralized with a satd soln of Na_2CO_3 and concd to dryness for each glycoside. The residues were compared with standard sugars on TLC (solvent system C) and descending PC (solvent system F and G), which showed D-glucose, D-xylose and L-rhamnose in **1**–**3**. The H_2O layers were silylated with trimethyl chlorosilane and hexamethyldisilazane in pyridine under $CaCl_2$ tube for 1 hr at 60°. Analysis of the silylated sugars by GC gave the ratio glucose–rhamnose–xylose as 2:1:2 for **1**, 3:1:1 for **2** and 1:1:1 for **3**.

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