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TRITERPENE SAPONINS FROM *SCABIOSA ROTATA*

TURHAN BAYKAL, TAYFUN PANAYIR, DENIZ TASDEMIR,† OTTO STICHER,† IHSAN ÇALIS‡*

Department of Pharmacognosy, Faculty of Pharmacy, Gazi University, TR-06330 Ankara, Turkey; † Swiss Federal Institute of Technology (ETH) Zurich, Department of Pharmacy, CH-8057 Zürich, Switzerland; ‡ Department of Pharmacognosy, Faculty of Pharmacy, Hacettepe University, TR-06100 Ankara, Turkey

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Key Word Index—*Scabiosa rotata*; Dipsacaceae; Triterpenoid Saponosides; Scabrioside A; Scabrioside B; Scabrioside C; Scabrioside D; Pomolic acid.

Abstract—Four new allose-containing triterpenoid saponosides, scabriosides A, B, C and D were isolated from the roots of *Scabiosa rotata*. Their structures were established as 3-*O*-*β*-D-xylopyranosyl-28-*O*-[*β*-D-allopyranosyl(1 → 6)-*β*-D-glucopyranosyl]-pomolic acid, 3-*O*-[*α*-L-rhamnopyranosyl(1 → 2)-*β*-D-xylopyranosyl]-28-*O*-[*β*-D-allopyranosyl(1 → 6)-*β*-D-glucopyranosyl]-pomolic acid, 3-*O*-[*α*-L-rhamnopyranosyl (1 → 2)-*α*-L-arabinopyranosyl]-28-*O*-[*β*-D-allopyranosyl(1 → 6)-*β*-D-glucopyranosyl]-pomolic acid, and 3-*O*-[*β*-D-glucopyranosyl(1 → 3)-*α*-L-rhamnopyranosyl(1 → 2)-*β*-D-xylopyranosyl]-28-*O*-[*β*-D-allopyranosyl(1 → 6)-*β*-D-glucopyranosyl]-pomolic acid, respectively, by the help of spectral evidence (IR, 1D- and 2D-NMR, FAB-MS). © 1998 Elsevier Science Ltd. All rights reserved

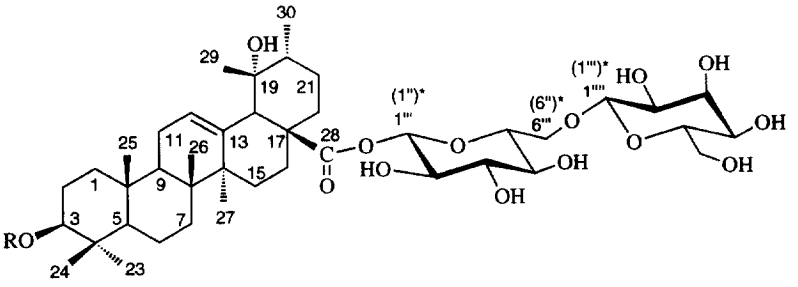
INTRODUCTION

Scabiosa rotata Bieb. (Dipsacaceae) is a wide spread plant in the flora of Turkey. Earlier investigations performed on this plant have resulted in the description of anatomical and morphological characters, the identification of chemical constituents and antifungal activities of the crude saponin fractions [1]. We now report the isolation and structure elucidation of four urs-12-ene type triterpenoid bidesmosidic saponosides.

RESULTS AND DISCUSSION

The methanolic extract of the roots of *Scabiosa rotata* was separated into several fractions, which were subjected to repeated column chromatography on normal and reversed phase silica gel, affording triterpene bidesmosidic saponosides, scabriosides A–D. Saponin **1** was obtained as an amorphous colourless powder. The FAB mass spectrum of **1** showed a quasimolecular $[M+Na]^+$ peak at *m/z* 951 corresponding to a molecular formula of $C_{47}H_{76}O_{18}$. IR absorptions at 3420, 1740 and 1635 cm^{-1} indicated the presence of hydroxyl (OH), ester carbonyl (C=O), and double bond (C=C) functionalities. The ^1H and ^{13}C NMR spectral data supported that **1** had a triglycosidic structure (Tables 1 and 2). The ^{13}C NMR spectrum of **1** revealed 47 carbon signals of which seventeen were assigned to a pentose and two hexose units and the remaining 30 signals to a triterpenoid skeleton. The $\Delta^{12(13)}$ functionality of the triterpenoid aglycone was deduced from the resonance of the sp^2 carbons C-12 (tertiary carbon) at δ 129.7 and C-13 (quaternary carbon) at δ 139.6. Their chemical shifts were also useful to distinguish between urs-12-ene and olean-12-ene analogs [2, 3]. The complete interpretation of the ^1H NMR spectrum was based on a 2D ^1H–^1H-homonuclear COSY experiment. The ^1H NMR spectrum of **1** exhibited resonances for the anomeric protons of the sugar moiety at δ 4.27 (*d*, J = 7.4 Hz), 5.28 (*d*, J = 7.9 Hz), 4.67 (*d*, J = 7.9 Hz) which were assigned to the anomeric protons of D-xylose, D-glucose and D-allose, respectively. Acid hydrolysis of **1** yielded glucose, allose and xylose, confirming this result. The anomeric proton signals of **1** indicated the anomeric configuration of the sugar units to be *β*. *β*-D-Allose is a rare sugar and has only been reported for a few saponins [4, 5]. The proton and the corresponding carbon resonances assigned to *β*-D-allose were in good accordance to those reported [6, 7]. The signal of C-28 at δ 178.6 further confirmed the IR absorption at 1740 cm^{-1} indicating the presence of an

‡* Author to whom correspondence should be addressed. Tel: 00 90 312 310 35 45/1089. Fax: 00 90 312 311 47 77. E-mail: acalis@dominet.in.com.tr



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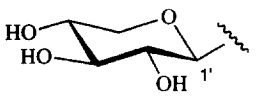
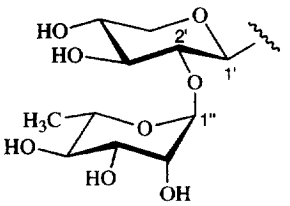


Scabrioside A (**1**)*



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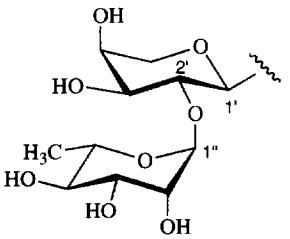

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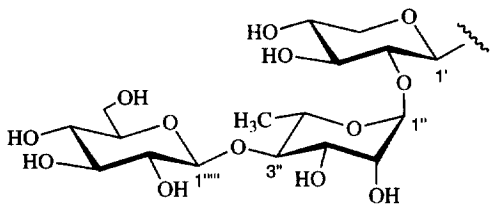

Scabrioside D (**4**)

ester group rather than a free acid group [8]. In addition, the shifts observed on the carbons of the β-D-glucose unit, particularly the values at δ 95.9 of the anomeric carbon (C-1″) were in agreement with a site of glycosylation at the 28-carboxyl group. Assignments for all proton and carbon resonances (see Tables 1 and 2) were achieved by COSY, HMQC and HMBC experiments. The ^{13}C NMR spectrum of **1** exhibited significant glycosidation shifts for C-3 (δ 90.8 *d*) of aglycone and C-6′ (δ 79.5 *d*) of β-D-glucose. These results supported the presence of a bidesmosidic structure. On the other hand, the ester linkage between the carboxy group located on C-28 of the aglycone moiety and the β-D-glucose unit was also evident from the chemical shift value of the anomeric proton observed for the β-D-glucose moiety (δ 5.28 *d*, $J = 7.9$ Hz). An HMBC experiment performed on **1** established the interglycosidic connectivities, showing correlations between C-3 (δ 90.8 *d*) of the aglycone and the anomeric proton (H-1″; δ 4.27 *d*) of β-D-xylose, C-28 (δ 178.6 *s*) of the aglycone and the anomeric proton (H-1′; δ 5.28) of β-D-glucose, C-6″ of β-D-glucose and the anomeric proton (H-1‴; δ 4.67 *d*) of β-D-allose. After the assignment of the ^{13}C NMR signals of the sugar moiety the resonances remaining for the aglycone of **1** were for seven methyl (six tertiary and one secondary), nine methylene, six methine and eight quaternary carbons. The carbon and proton resonances for the aglycone moiety of **1** indicated an elemental formula of $C_{30}H_{48}O_4$, implying seven

Table 1. ^{1}H NMR spectral data for scabiosides A (**1**), B (**2**), C (**3**) and D (**4**)

H	**1*** δ ppm, *J* (Hz)	**2** δ ppm, *J* (Hz)	**3** δ ppm, *J* (Hz)	**4** δ ppm, *J* (Hz)
1	1.64†, 1.00†	1.62†, 0.98†	1.64†, 1.00†	1.64†, 1.00†
2	1.80†, 1.70†	1.80†, 1.70†	1.80†, 1.70†	1.80†, 1.70†
3	3.13†	3.10 *dd* (11.4, 4.1)	3.12 *dd* (11.4, 4.1)	3.10 *dd* (11.4, 4.0)
5	0.79†	0.78†	0.78†	0.78†
6	1.53†	1.53†	1.53†	1.53†
7	1.34†	1.33†	1.32†	1.33†
9	1.70†	1.68†	1.70†	1.68†
11	1.98†, 1.34†	1.97†, 1.32†	1.96†, 1.30†	1.96†, 1.32†
12	5.29 *br s*	5.27 *br s*	5.29 *br s*	5.26 *br s*
15	1.85†, 1.03†	1.85†, 1.00†	1.85†, 1.02†	1.85†, 1.04†
16	2.60 *m*, 1.65†	2.57 *m*, 1.62†	2.61 *m*, 1.62†	2.60 *m*, 1.62†
18	2.53 *s*	2.51 *s*	2.52 *s*	2.51 *s*
20	1.38†	1.35†	1.40†	1.40†
21	1.70†	1.70†	1.70†	1.70†
22	1.74†	1.72†	1.75†	1.75†
23	1.05 *s*	1.02 *s*	1.02 *s*	1.03 *s*
24	0.84 *s*	0.84 *s*	0.84 *s*	0.84 *s*
25	0.95 *s*	0.93 *s*	0.96 *s*	0.94 *s*
26	0.78 *s*	0.75 *s*	0.78 *s*	0.78 *s*
27	1.32 *s*	1.30 *s*	1.32 *s*	1.31 *s*
29	1.20 *s*	1.19 *s*	1.20 *s*	1.18 *s*
30	0.93 *d* (6.6)	0.91 *d* (6.6)	0.93 *d* (6.6)	0.91 *d* (6.6)
	Xylose	Xylose	Arabinose	Xylose
1′	4.27 *d* (7.4)	4.36 *d* (7.0)	4.56 *d* (6.7)	4.35 *d* (7.0)
2′	3.18 *dd* (7.4, 8.8)	3.42†	3.77†	3.33†
3′	3.54†	3.45†	3.75†	3.40†
4′	3.45†	3.43†	3.80†	3.48†
5′	3.83†,	3.85†,	3.82†,	3.84†,
	3.20†	3.16 *dd* (10.5, 9.0)	3.48 *dd* (10.5, 9.0)	3.16 *dd* (11.0, 9.7)
		Rhamnose	Rhamnose	Rhamnose
1″		5.30†	5.10 *d* (1.7)	5.26†
2″		3.93 *dd* (1.7, 3.4)	3.88 *dd* (1.7, 3.4)	4.24 *dd* (1.7, 3.4)
3″		3.72†	3.70†	3.91†
4″		3.40†	3.40†	3.57†
5″		3.92†	3.80†	3.94†
6″		1.18 *d* (6.2)	1.20 *d* (6.2)	1.22 *d* (6.2)
	Glucose	Glucose	Glucose	Glucose
*(1″) 1‴	5.28 *d* (7.9)	5.26 *d* (7.8)	5.27 *d* (7.8)	5.26 *d* (7.8)
(2″) 2‴	3.33†	3.34†	3.35†	3.32†
(3″) 3‴	3.42†	3.42†	3.40†	3.40†
(4″) 4‴	3.45†	3.45†	3.45†	3.45†
(5″) 5‴	3.50†	3.48†	3.49†	3.48†
(6″) 6‴	4.10 *br d* (11.2)	4.08 *br d* (11.2)	4.10 *br d* (11.2)	4.10 *br d* (11.2)
	3.74 *dd* (11.2, 4.5)	3.72 *dd* (11.2, 4.4)	3.74†	3.74†
	Allose	Allose	Allose	Allose
(1‴) 1⁗	4.67 *d* (7.9)	4.65 *d* (7.9)	4.67 *d* (7.9)	4.65 *d* (7.9)
(2‴) 2⁗	3.30†	3.32†	3.33†	3.32†
(3‴) 3⁗	4.04 *t* (2.9)	4.02 *t* (2.9)	4.04 *t* (2.9)	4.02 *t* (2.9)
(4‴) 4⁗	3.49†	3.48†	3.49†	3.49†
(5‴) 5⁗	3.68†	3.66†	3.66†	3.65†
(6‴) 6⁗	3.83†, 3.66†	3.83†, 3.65†	3.83†, 3.67†	3.83†, 3.66†
				Glucose (Terminal)
1⁗′				4.47 *d* (7.2)
2⁗′				3.31†
3⁗′				3.40†
4⁗′				3.34†
5⁗′				3.35†
6⁗′				3.86†, 3.70†

† Signal patterns are unclear due to overlapping.
‡ Assignments were based on COSY, TOCSY, HMQC, HSQC-TOCSY and HMBC.

Table 2. ^{13}C NMR spectral data for scabriosides A (**1**), B (**2**), C (**3**) and D (**4**) (75.5 MHz, CD_3OD, δ-values)

C	1*	2	3	4
1	39.6 *t*	40.1 *t*	40.0 *t*	40.1 *t*
2	28.5 *t*	27.2 *t*	27.2 *t*	27.2 *t*
3	90.8 *d*	90.2 *d*	90.7 *d*	90.4 *d*
4	40.2 *s*	40.3 *s*	40.3 *s*	40.3 *s*
5	57.0 *d*	57.3 *d*	57.0 *d*	57.2 *d*
6	19.5 *t*	19.5 *t*	19.5 *t*	19.5 *t*
7	34.1 *t*	34.1 *t*	34.1 *t*	34.1 *t*
8	41.3 *s*	41.3 *s*	41.3 *s*	41.3 *s*
9	47.8 *d*	47.8 *d*	47.8 *d*	47.8 *d*
10	37.9 *s*	37.9 *s*	37.9 *s*	37.9 *s*
11	24.7 *t*	24.7 *t*	24.7 *t*	24.7 *t*
12	129.7 *d*	129.7 *d*	129.7 *d*	129.7 *d*
13	139.6 *s*	139.6 *s*	139.6 *s*	139.6 *s*
14	42.6 *s*	42.6 *s*	42.6 *s*	42.6 *s*
15	29.7 *t*	29.7 *t*	29.7 *t*	29.7 *t*
16	26.5 *t*	26.5 *t*	26.5 *t*	26.5 *t*
17	48.7 *s*	48.8 *s*	48.8 *s*	48.8 *s*
18	54.8 *d*	54.9 *d*	54.9 *d*	54.9 *d*
19	73.7 *s*	73.7 *s*	73.7 *s*	73.8 *s*
20	42.8 *d*	42.8 *d*	42.8 *d*	42.8 *d*
21	28.5 *t*	27.2 *t*	27.2 *t*	27.1 *t*
22	38.2 *t*	38.2 *t*	38.2 *t*	38.2 *t*
23	28.6 *q*	28.5 *q*	28.7 *q*	28.6 *q*
24	17.7 *q*	17.7 *q*	17.7 *q*	17.7 *q*
25	16.1 *q*	16.2 *q*	16.2 *q*	16.1 *q*
26	17.0 *q*	17.2 *q*	17.1 *q*	17.1 *q*
27	27.1 *q*	27.1 *q*	27.7 *q*	27.1 *q*
28	178.6 *s*	178.6 *s*	178.6 *s*	178.6 *s*
29	24.7 *q*	24.7 *q*	24.7 *q*	24.7 *q*
30	16.6 *q*	16.6 *q*	16.6 *q*	16.6 *q*
1′	107.2 *d*	106.2 *d*	104.7 *d*	106.5 *d*
2′	75.5 *d*	78.8 *d*	76.8 *d*	78.9 *d*
3′	78.0 *d*	78.6 *d*	73.0 *d*	78.4 *d*
4′	71.2 *d*	71.6 *d*	70.2 *d*	71.7 *d*
5′	67.0 *t*	66.4 *t*	63.6 *t*	66.5 *t*
1″		102.0 *d*	102.0 *d*	101.7 *d*
2″		72.0 *d*	72.2 *d*	70.9 d
3″		72.1 *d*	72.2 *d*	82.9 *d*
4″		74.0 *d*	73.9 *d*	72.6 *d*
5″		70.1 *d*	68.3 *d*	70.1 *d*
6″		18.0 *q*	18.0 *q*	18.2 *q*
*(1″) 1‴	95.9 *d*	95.9 *d*	95.9 *d*	95.9 *d*
(2″) 2‴	73.8 *d*	73.7 *d*	73.8 *d*	73.7 *d*
(3″) 3‴	78.1 *d*	78.2 *d*	78.2 *d*	78.2 *d*
(4″) 4‴	71.0 *d*	71.0 *d*	71.0 *d*	71.0 *d*
(5″) 5‴	77.9 *d*	77.9 *d*	77.9 *d*	77.8 *d*
(6″) 6‴	69.6 *t*	69.6 *t*	69.6 *t*	69.6 *t*
(1‴) 1⁗	102.5 *d*	102.5 *d*	102.5 *d*	102.5 *d*
(2‴) 2⁗	72.4 *d*	72.5 *d*	72.4 *d*	72.5 *d*
(3‴) 3⁗	72.7 *d*	72.8 *d*	72.8 *d*	72.8 *d*
(4‴) 4⁗	68.9 *d*	68.9 *d*	68.9 *d*	68.9 *d*
(5‴) 5⁗	75.5 *d*	75.5 *d*	75.5 *d*	75.5 *d*
(6‴) 6⁗	63.1 *t*	63.1 *t*	63.1 *t*	63.1 *t*
1⁗′				105.8 *d*
2⁗′				75.4 *d*
3⁗′				77.9 *d*
4⁗′				71.2 *d*
5⁗′				77.8 *d*
6⁗′				62.4 *t*

degrees of unsaturation of which two were assigned to a double bond, and a carbonyl functionality, and the remaining indicated that **1** is pentacyclic. Additionally, six tertiary methyl and one secondary methyl resonances were observed. A pair of signals at δ 129.7 *d* (C-12) and 139.6 *s* (C-13) was characteristic for the double bond of an urs-12-ene type structure. Furthermore, carbon signals at δ 90.8 *d* (C-3), and 73.7 *s* (C-19) were consistent with assignments to hydroxylated carbons. Finally, a singlet proton signal at δ 2.53 and the corresponding carbon resonance at δ 54.8 were assigned to H-18 and C-18, respectively. These results clearly supported the presence of pomolic acid (3β,19α-dihydroxyurs-12-ene-28-oic acid) as the aglycone for **1**. HMBC of **1** exhibited correlations between C-17/H-18, C-18/H_3-29, C-19/H-18, C-19/H_3-29, C-19/H_3-30, C-20/H-18, C-20/H_3-29, C-20/H_3-30, C-21/H_3-30, C-28/H-18 and C-29/H_3-30 for ring E of the molecule, confirming the proposed structure for the aglycone moiety. Further correlations are shown in Fig. 1. The ^1H and ^{13}C NMR spectral data assigned to the sapogenol moiety were also in good agreement to those reported for pomolic acid [9, 10]. ROESY of **1** showed the expected ROE correlations between the anomeric proton (H-1′) of β-D-xylose and H-3 of aglycone moiety, anomeric proton (H-1‴) of β-D-allose and H-6 of β-D-glucose, H-3 and H_3-23, H-18 and H_3-29, H-18 and H-12, H-12 and H_3-26, H-12 and H_3-29, H_3-25 and H_3-26 confirming the proposed structure. Thus, the structure of saponin **1** was established as 3-*O*-β-D-xylopyranosyl-28-*O*-[β-D-allopyranosyl(1 → 6)-β-D-glucopyranosyl]-pomolic acid, for which the trivial name scabrioside A is proposed.

Compound **2** was obtained as an amorphous colourless powder, and exhibited a quasimolecular $[M+Na]^+$ peak at *m/z* 1097 corresponding to a molecular formula of $C_{53}H_{86}O_{22}$. The IR spectrum of **2** showed absorptions at 3420, 1740 and 1635 cm^{-1} which indicated the presence of hydroxyl, ester carbonyl, and double bond functionalities, respectively. The ^1H NMR spectrum of **2** (Table 1) was very similar to that of **1** except for the extra signals at δ 5.27 (*d*, *J* = 1.7 Hz, H-1″), 3.92 (*dd*, *J* = 1.7, 3.4 Hz, H-2″), 3.72 (*dd*, *J* = 3.4, 10 Hz, H-3″) and 1.20 (3H, *d*, *J* = 6.2 Hz, H-6″) which were attributed to the presence of an α-L-rhamnose unit in addition to the anomeric protons of β-D-xylose (δ 4.36, *d*, *J* = 6.2 Hz), β-D-glucose (δ 5.26, *d*, *J* = 7.8 Hz) and β-D-allose (δ 4.65, *d*, *J* = 7.9 Hz). Compound **2** gave glucose, allose, xylose and rhamnose on acid hydrolysis. The ^{13}C NMR spectrum of **2** (Table 2) revealed fifty-three carbon resonances. This spectral evidence suggested that **2** could be a pomolic acid tetraglycoside. On comparison of the ^{13}C NMR spectrum with that of **1**, compound **2** showed only a set of additional signals of a terminal α-L-rhamnopyranosyl unit which was deduced to be attached at C-2 of the D-xylose of **1**. All proton and carbon assignments were based on 2D-NMR experiments (COSY, HMQC and HMBC), and an obvious chemical shift at δ 78.8 assigned to C-2 of

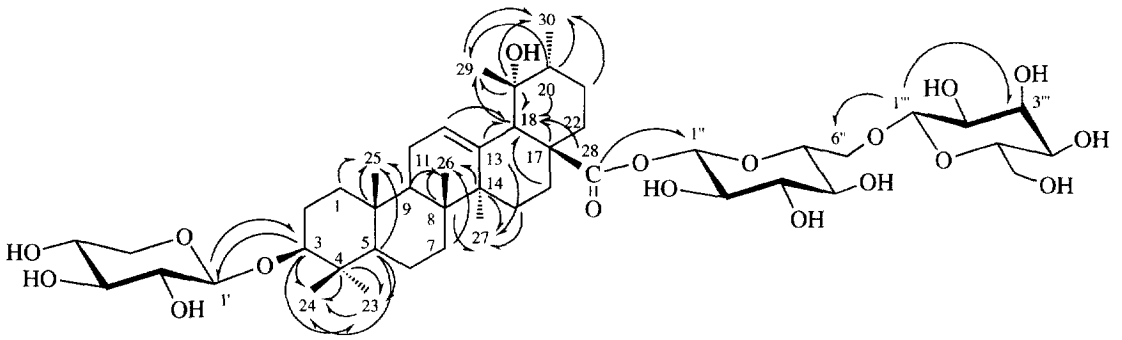

Fig. 1. Heteronuclear multiple bond correlations (HMBC) for **1**. Arrows point from carbon to proton.

the D-xylose unit supported this suggestion. A HSQC-TOCSY experiment performed with **2** confirmed the site of glycosidation of the terminal α-L-rhamnopyranosyl unit at C-2 of the D-xylose, since the carbon signal at δ 78.8 showed correlations to H-2′ and H-1′ of the D-xylose unit. Furthermore, all sites of glycosidations were also established by a HMBC experiment showing long-range correlations between C-1′ (δ 106.2 *d*) of the D-xylose and H-3 (δ 3.10, *dd*, J = 11.4 and 4.1 Hz) of the aglycone, C-2′ (δ 78.8 *d*) of the D-xylose and H-1″ (δ 5.29, *br s*) of α-L-rhamnose, C-28 (δ 178.6 *s*) of the aglycone and H-1‴ of the D-glucose (δ 5.26 *d*, J = 7.8 Hz), and C-6‴ (δ 69.6 *t*) of the D-glucose and H-1⁗ (δ 4.65, *d*, J = 7.9 Hz) of terminal D-allose unit. Consequently, the structure of compound **2** was established as 3-*O*-[α-L-rhamnopyranosyl(1 → 2)-β-D-xylopyranosyl]-28-*O*-[β-D-allopyranosyl(1 → 6)-β-D-glucopyranosyl]-pomolic acid, for which the trivial name scabrioside B is proposed.

Compound **3** was obtained as an amorphous colourless powder. The molecular formula ($C_{53}H_{86}O_{22}$) deduced from the peak at *m/z* 1097 $[M+Na]^+$ in the FAB mass spectrum and the absorptions in the IR of **3** was the same as that of **2**. The ^1H and ^{13}C NMR spectra of **3** displayed many similarities with those of **2**, especially for the resonances assigned to pomolic acid, β-D-glucose, α-L-rhamnose and β-D-allose (Tables 1 and 2). But, the set of additional protons apart from the anomeric proton at δ 4.56 (*d*, J = 4.0 Hz, H-1′) and the corresponding carbon signals were in agreement with the presence of a pentose unit which was identified as α-L-arabinose by COSY and HMQC experiments. The sugar components were confirmed by TLC and GC-MS detection of glucose, allose, arabinose and rhamnose. Although the carbon resonances indicated the same glycosidation pattern as found for **2**, HMBC performed with **3** made clear the connectivities showing the long-range correlations between C-28 (δ 178.6 *s*) of the aglycone and H-1‴ of the β-D-glucose (δ 5.27 *d*, J = 7.8 Hz), and C-6‴ (δ 69.6 *t*) of the β-D-glucose and H-1⁗ (δ 4.67, *d*, J = 8.0 Hz) of terminal β-D-allose unit and C-2′ (δ 76.8 *d*) of α-L-arabinose and H-1″ (δ 5.1 *d*, J = 1.5 Hz) of α-L-rhamnose. The glycosidation of α-L-arabinose unit at C-3 of the aglycone was confirmed by a ROESY experiment showing an intense ROE cross-peak between H-3 (δ 3.12, *dd*, J = 11.3 and 4.1 Hz) of the aglycone and H-1′ of α-L-arabinose. Further correlations were also observed between H-3 and H-5 (δ 0.79), H-3 and H_3-23 (δ 1.02), H-1′ of α-L-arabinose and H-1″ of α-L-rhamnose, H-1⁗ of β-D-allose and H-6‴ of β-D-glucose, confirming the proposed structure. Thus, compound **3** was established as 3-*O*-[α-L-rhamnopyranosyl(1 → 2)-α-L-arabinopyranosyl]-28-*O*-[β-D-allopyranosyl(1 → 6)-β-D-glucopyranosyl]-pomolic acid, for which the trivial name scabrioside C is proposed.

Compound **4** was the most polar among the saponosides isolated. In the ^1H NMR spectrum of **4** an additional anomeric proton resonance was observed at δ 4.47 (*d*, J = 7.2 Hz, H-1⁗) compared to compound **2** (Table 1). Apart from this resonance, COSY and HMQC experiments made clear that the set of carbon signals (δ 105.8 *d*, 75.4 *d*, 77.9 *d*, 71.2 *d*, 77.8 *d*, 62.4 *t*; C-1⁗′–C-6⁗′, respectively) corresponding to an additional sugar moiety belonging to a terminal β-D-glucopyranosyl unit (Table 2). This was also supported by the FAB mass spectrum, in which a quasimolecular ion peak was observed at *m/z* 1259 $[M+Na]^+$ (calc. for $C_{59}H_{96}O_{27}$). In the ^{13}C NMR spectrum of **4**, the presence of a typical carbon signal at δ 82.9 suggested that the additional sugar unit was attached to C-3″ of the rhamnose moiety, since no chemical shifts were observed for the carbon signals belonging to the xylose, glucose and the allose units in comparison to those of **2**. An HMBC experiment made clear all interglycosidic connectivities showing correlations between C-3 (δ 90.4) of the aglycone and H-1′ (δ 4.35) of xylose, C-2′ (δ 78.9) of xylose and H-1″ (δ 5.25) of rhamnose, C-3″ (δ 82.9) of rhamnose and H-1⁗′ (δ 4.47) of the terminal glucose unit, C-28 (δ 178.6) of the aglycone and H-1‴ (δ 5.26) of the inner glucose, and C-6‴ (δ 69.6) of the inner glucose and H-1⁗′ (δ 4.65) of allose. Based on the above data, compound **4** was established as 3-*O*-[β-D-glucopyranosyl(1 → 3)-α-L-rhamnopyranosyl(1 → 2)-β-D-xylopyranosyl]-28-*O*-[β-D-allopyranosyl(1 → 6)-β-

D-glucopyranosyl]-pomolic acid, for which the trivial name scabrioside D is proposed.

EXPERIMENTAL

General

IR Spectra (cm^{-1}); Perkin-Elmer 2000 FT-IR as pressed KBr disks. Optical rotations were recorded with a Perkin-Elmer 241 polarimeter using MeOH as solvent. 1D and 2D NMR spectra were recorded using a Bruker AMX-300 instrument. FAB-MS were recorded using a ZAB2-SEQ mass spectrometer. MPLC: LiChroprep C18, 40 μm (Merck); Labomatic column (1.8 × 35.2 cm); LEWA-M5 pump; Rheodyne injector; LKB 1700 Minirac collector; CC: Silica gel 60 (230–400 mesh, SDS). TLC: Silica gel F_{254} (Merck; for glycosides) and cellulose F_{254} (Merck; for sugars) plates; detection of saponins by spraying with 30% H_2SO_4/H_2O and of sugars by aniline phthalate reagent followed by heating at 100° for 5–10 min.

Plant material

Scabiosa rotata Bieb. was collected from Çankiri on the highway to Ilgaz. A voucher specimen has been deposited in the Herbarium of the Department of Pharmacognosy, Faculty of Pharmacy, Gazi University, Ankara.

Extraction

The air dried and powdered roots (1.5 kg) of *Scabiosa rotata* were first extracted with petrol (6 ×). The defatted material was then extracted with MeOH several times at room temp. The combined MeOH extract was evaporated *in vacuo* to leave a crude extract (141 g, yield 9.4%). The crude extract was dissolved in MeOH and the saponins were precipitated by dropwise addition of cold acetone while stirring. This procedure was repeated 3 × to yield the crude saponin (96 g, yield 6.4%).

Isolation of scabriosides A (**1**)*, B* (**2**)*, C* (**3**)*, and D* (**4**)

An aliquot of crude saponin (20.2 g) was applied to vacuum liquid chromatography on normal-phase silica gel material (Chromagel, 40–60 μm, 220 g) using $CHCl_3$–MeOH (80:20; 200 ml) and $CHCl_3$–MeOH–H_2O mixtures (80:20:1, 80:20:2, 75:25:2.5, 70:30:3, 65:35:3.5*, 60:40:4, and 50:50:5; each 200 ml, *400 ml, 100 ml/fr.). According to the TLC control of the frs, they were collected into 6 main frs (frs A–F: fr. A 1–5, 1120 mg; fr. B 6–8, 337 mg; fr. C 9–11, 734 mg; fr. D 12–13, 1180 mg; fr. E 14–16, 3.42 g; fr. F 17–18, 2.2 g). Fr. D was subjected to medium pressure liquid chromatography (MPLC) on LiChroprep C-18 using H_2O–MeOH mixtures of increasing amounts of MeOH (50–80% MeOH) to give compound **1** (49 mg). Fr. F was subjected to VLC on reversed phase material using H_2O and H_2O–MeOH mixtures of increasing amounts of MeOH (5–20%) and MeOH as eluents. This fr., rich in saponins eluted with MeOH (880 mg), was further subjected to open column chromatography on a normal-phase silica gel and eluted with $CHCl_3$–MeOH–H_2O mixtures (80:20:2, 75:25:2.5, 70:30:3, and 60:40:4) of increasing polarity to yield compounds **3** (86 mg), **2** (53 mg) and **4** (86 mg), respectively. Fr. E was also rich in compounds **2** and **3**.

Scabrioside A (**1**). Amorphous colourless compound. $[\alpha]_D^{20}$ −22.9° (MeOH, *c* 0.38). IR ν_{max}^{KBr}: 3420 (OH), 1740 (ester CO), 1635 (C=C) and 1070 (C—O—C) cm^{-1}. ^1H and ^{13}C NMR (^1H: 300 MHz; ^{13}C: 75.5 MHz; CD_3OD): see Tables 1 and 2. FAB-MS: *m/z* 951 $[M+Na]^+$ (calc. for $C_{47}H_{76}O_{18}$).

Scabrioside B (**2**). Amorphous colourless compound. $[\alpha]_D^{20}$ −35.2° (MeOH, *c* 0.5). IR ν_{max}^{KBr}: 3420 (OH), 2935 (C—H), 1735 (ester CO), 1635 (C=C), 1455, 1390 and 1070 (C—O—C) cm^{-1}. ^1H and ^{13}C NMR (^1H: 300 MHz; ^{13}C: 75.5 MHz; CD_3OD): see Tables 1 and 2. FAB-MS: *m/z* 1097 $[M+Na]^+$ (calc. for $C_{53}H_{86}O_{22}$).

Scabrioside C (**3**). Amorphous colourless compound. $[\alpha]_D^{20}$ −33.9° (MeOH, *c* 0.54). IR ν_{max}^{KBr}: 3420 (OH), 2935 (C—H), 1735 (ester CO), 1635 (C=C), 1455, 1390 and 1070 (C—O—C) cm^{-1}. ^1H and ^{13}C NMR (^1H: 300 MHz; ^{13}C: 75.5 MHz; CD_3OD): see Tables 1 and 2. FAB-MS: *m/z* 1097 $[M+Na]^+$ (calc. for $C_{53}H_{86}O_{22}$).

Scabrioside D (**4**). Amorphous colourless compound. $[\alpha]_D^{20}$ −23° (MeOH, *c* 0.5). IR ν_{max}^{KBr}: 3420 (OH), 2935 (C—H), 1735 (ester CO), 1635 (C=C), 1455, 1390 and 1070 (C—O—C) cm^{-1}. ^1H and ^{13}C NMR (^1H: 300 MHz; ^{13}C: 75.5 MHz; CD_3OD): see Tables 1 and 2. FAB-MS: *m/z* 1259 $[M+Na]^+$ (calc. for $C_{59}H_{96}O_{27}$).

Acid hydrolysis of compounds **1**, **2** *and* **3**. Compound **1**, **2** or **3** (each 5–6 mg) was refluxed in 2N HCl solution (2 ml) at 100° for 3 h cooled and filtered. The filtrate was neutralized passing it through Dowex Cl^- form) and evaporated. The residue was examined for sugars by TLC (EtOAc–pyridine–AcOH–H_2O, 36:36:7:21) and GC-MS according to the method given in Ref. [6].

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