Triterpenoid Saponins from Bellis sylvestris, I. Structures of the Major Deacylsaponins

Thomas Schöpke, Karl Hiller, Victor Wray, K.-D. Köppel, Kazuo Yamasaki, and Ryoji Kasai

J. Nat. Prod., **1994**, 57 (9), 1279-1282• DOI: 10.1021/np50111a016 • Publication Date (Web): 01 July 2004

Journal of Natural Products
Vol. 57, No. 9, pp. 1279-1282, September 1994

TRITERPENOID SAPONINS FROM *BELLIS SYLVESTRIS*, I. STRUCTURES OF THE MAJOR DEACYLSAPONINS

THOMAS SCHÖPKE,* KARL HILLER,

Humboldt-Universität zu Berlin, Fachbereich Pharmazie, Goethestr. 54, D-13086 Berlin, Germany

VICTOR WRAY, K.-D. KÖPPEL,

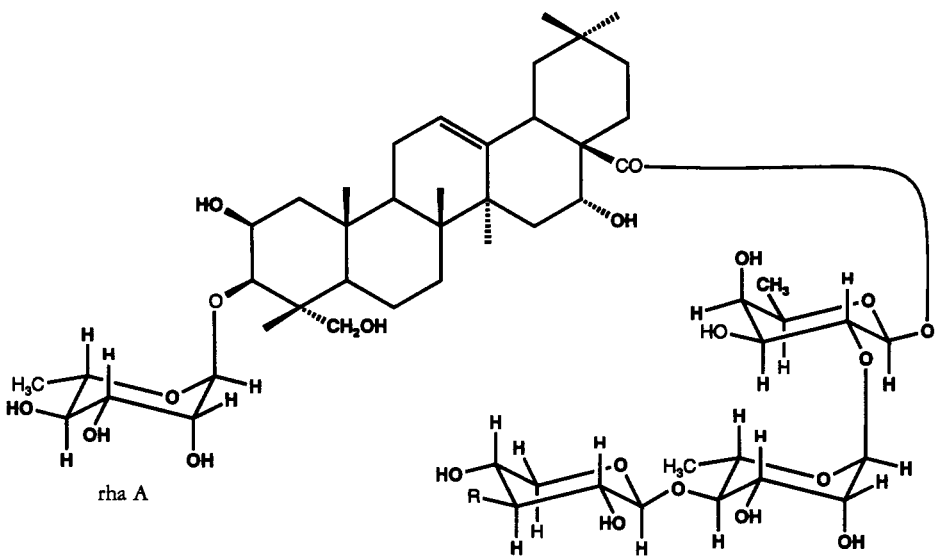
Gesellschaft für Biotechnologische Forschung mbH, Mascheroder Weg 1, D-38124 Braunschweig, Germany

KAZUO YAMASAKI, and RYOJI KASAI

Hiroshima University, School of Medicine, Institute of Pharmaceutical Sciences, Hiroshima 734, Japan

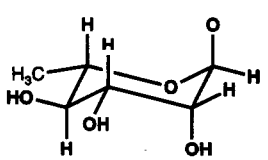
ABSTRACT.—Two major saponins from *Bellis sylvestris* have been isolated and their structures determined, mainly by high-field nmr spectroscopy. One of these [**2**] was identical with bellissaponin BS1 from *Bellis perennis*, while the second is a new triterpenoid saponin [**1**], named besysaponin C_{12}, and identified as 3-*O*-α-L-rhamnopyranosyl-2β,3β,16α,23-tetrahydroxyolean-12-en-28-oic acid 28-*O*-β-D-xylopyranosyl(1→4)-α-L-rhamnopyranosyl(1→2)-β-D-fucopyranoside.

The genus *Bellis* includes about ten species, all of which are small annual or perennial herbs. Most *Bellis* species are endemic to restricted regions and only *B. perennis* (the common daisy), *B. annua*, and *B. sylvestris* are widespread in their distribution. *Bellis sylvestris* Cyrill (Asteraceae), the autumn daisy, is native to the Mediterranean region. Flowering begins in



Besysaponin C_{12} [**1**] R= OH

Besysaponin C_{14} [**2**] R=



autumn and reaches a maximum in winter.

In previous investigations we have elucidated the structure of seven new triterpenoid saponins from *Bellis perennis* (1–4). The present paper describes the isolation and structure elucidation of the two major deacylsaponins obtained from the herb of *Bellis sylvestris*, among them a new deacylated saponin [**1**].

Two compounds (besysaponin C_{12} [**1**] and besysaponin C_{14} [**2**]) were isolated from the mild alkaline hydrolysate of the saponin mixture obtained from *B. sylvestris* as described in the Experimental.

Comparison of **1** and **2** with the deacylsaponins obtained from *Bellis perennis* L. by tlc indicated that **2** was identical with bellissaponin BS1. A molecular ion at *m/z* 1243.6 $[M+Na]^+$ in the fabms and agreement of the ^{13}C-nmr chemical shifts (see Table 2) confirmed this identification.

In contrast, **1** has not been found in *B. perennis*. Acid hydrolysis and tlc identification according to Kartnig and Wegschaider (5) gave polygalacic acid (2β,3β,16α,23-tetrahydroxyolean-12-en-28-oic acid) as the aglycone, and rhamnose, fucose, and xylose as sugar constituents. The fabms afforded a molecular ion at *m/z* 1097.4 $[M+Na]^+$ and a positive-ion hrfabms ion occurred at *m/z* 1097.5508 $[C_{53}H_{86}O_{22}+Na]^+$.

The ^{13}C- and ^1H-nmr spectra of **1** showed signals of four anomeric carbons and protons. By means of the ^1H-nmr, ^{13}C-nmr, DEPT, COSY, HMQC, and HMBC spectra, all of the proton and carbon resonances of **1** could be assigned

TABLE 1. ^1H-Nmr Chemical Shifts of Compound **1** (ppm).

Proton	Aglycone		Sugar	
H-1A	2.06	Rhamnose A	H-1	4.91, br s
H-1B	1.23		H-2	3.95, dd, J=1.6/3.3 Hz
H-2	4.26, d, J=3.3 Hz		H-3	3.82, d, J=3.3 Hz
H-3	3.98, d, J=3.7 Hz		H-4	3.42, t, J=9.5 Hz
H-5	1.38		H-5	3.87
H-6A	1.55		CH_3-6 . . .	1.29, d, J=6.2 Hz
H-6B	1.49	Xylose	H-1	4.54, d, J=7.7 Hz
H-7A	1.68		H-2	3.30, dd, J=7.7/6.4 Hz
H-7B	1.44		H-3	3.37
H-9	1.68		H-4	3.52, ddd
H-11A	2.03		H-5A . . .	3.91, t, J=11 Hz
H-11B	1.97		H-5B	3.91, t, J=5.7 Hz
H-12	5.36, t, J=5.4 Hz	Rhamnose B	H-1	5.46, d, J=1.7 Hz
H-15A	1.74, dd, J=3.5/14.9 Hz		H-2	3.97, dd, J=1.7/3.9 Hz
H-15B	1.51, dd, J=2.6/14.9 Hz		H-3	3.89, d, J=3.9 Hz
H-16	4.53		H-4	3.58, d, J=9.4 Hz
H-18	2.98, dd, J=4.2/14.3 Hz		H-5	3.84
H-19A	2.33, t, J=13.7 Hz		CH_3-6 . . .	1.36, d, J=6.1 Hz
H-19B	1.07	Fucose	H-1	5.34, d, J=8.2 Hz
H-21A	1.99		H-2	3.85
H-21B	1.21		H-3	3.73
H-22A	1.95		H-4	3.61, d, J=3.4 Hz
H-22B	1.82, dd, J=4.7/13.4 Hz		H-5	3.73, dd, J=3.4/6.4 Hz
H-23A	3.37		CH_3-6 . . .	1.27, d, J=6.4 Hz
H-23B	3.28			
CH_3-24 . . .	0.94			
CH_3-25 . . .	1.36			
CH_3-26 . . .	0.87			
CH_3-27 . . .	1.43			
CH_3-29 . . .	0.92			
CH_3-30 . . .	0.99			

TABLE 2. ^{13}C-Nmr Data of Compounds **1** and **2** and of Bellissaponin BS1 in CD_3OD.

Carbon	1	2	BS1		Carbon	1	2	BS1
C-1 ...	45.14	45.06	45.0	Rhamnose A	C-1 ...	104.17	104.08	103.9
C-2 ...	71.88	71.91	70.1		C-2 ...	72.33	72.58	72.6
C-3 ...	82.4	82.40	84.5		C-3 ...	72.24	72.26	72.3
C-4 ...	43.39	43.41	42.9		C-4 ...	74.00	73.95	74.6
C-5 ...	48.04	48.37	48.4		C-5 ...	70.32	70.31	70.4
C-6 ...	19.0[a]	19.02	19.0		C-6 ...	18.00	17.87	18.0
C-7 ...	33.74	33.60	33.7	Xylose	C-1 ...	107.07	107.07	107.1
C-8 ...	40.84	40.84	41.0		C-2 ...	76.06	76.39	76.2
C-9 ...	48.41	48.37	48.4		C-3 ...	78.13	84.27	84.6
C-10 ..	37.71	37.72	37.7		C-4 ...	71.09	68.76	70.1
C-11 ..	24.63	24.60	24.6		C-5 ...	67.24	67.14	66.0
C-12 ..	123.54	123.56	123.6	Rhamnose B	C-1 ...	101.09	101.31	101.2
C-13 ..	144.73	144.60	144.4		C-2 ...	71.88	72.21[b]	72.0
C-14 ..	42.91	42.92	43.4		C-3 ...	72.33	72.26[b]	72.4
C-15 ..	36.50	36.41	36.5		C-4 ...	84.27	84.49	82.7
C-16 ..	74.64	74.62	75.0		C-5 ...	68.72	69.83	68.8
C-17 ..	50.01	50.09	50.2		C-6 ...	18.29	17.93	18.3
C-18 ..	42.31	42.31	42.2	Fucose	C-1 ...	95.11	95.00	95.1
C-19 ..	47.97	47.96	48.0		C-2 ...	74.13	74.49	74.0[c]
C-20 ..	31.29	31.28	31.2		C-3 ...	76.64	76.64	76.4[c]
C-21 ..	34.44	36.51	36.4		C-4 ...	73.58	73.49	73.5
C-22 ..	31.97	31.92	31.7		C-5 ...	72.66	71.90	72.0
C-23 ..	65.76	65.85	67.1		C-6 ...	16.50	16.52	16.4
C-24 ..	14.73	14.94	14.9	Rhamnose C	C-1 ...		102.44	102.4
C-25 ..	18.00	18.00	18.0		C-2 ...		72.19[b]	71.6
C-26 ..	17.84	17.87	17.9		C-3 ...		72.26[b]	72.3
C-27 ..	27.28	27.23	27.3		C-4 ...		73.95	74.0
C-28 ..	177.23	177.24	177.3		C-5 ...		69.97	69.9
C-29 ..	33.38	33.37	33.3		C-6 ...		18.38	18.3
C-30 ..	24.87	24.86	25.0					

[a]Taken from couplings with H-6A and H-6B in the HMQC spectrum.
[b]Assignments may be interchanged.
[c]The assignments of these shifts have been interchanged compared to the previously published data (1).

as shown in Tables 1 and 2, confirming that **1** is a bisdesmosidic saponin of polygalacic acid having two rhamnoses and fucose and xylose as sugar components. Cross-peaks in the HMBC nmr spectrum were observed between H-1 (4.91 ppm) of one rhamnose (rha A) and C-3 of the aglycone (82.4 ppm) and C-1 (104.17 ppm) of rha A and H-3 (3.98 ppm) of polygalacic acid, between H-1 (5.34 ppm) of fucose and C-28 (177.23 ppm) of polygalacic acid, between H-2/C-2 (3.85/74.13 ppm) of fucose and C-1/H-1 (101.09/5.46 ppm) of the second rhamnose (rha B), and between H-4/C-4 (3.58/84.27 ppm) of rha B and C-1/H-1 (107.07/4.54 ppm) of xylose. Also, $J_{H1\text{-}H2}$ coupling constants of 7.7 and 8.2 Hz demonstrated that xylose and fucose occur as the β-anomers having the $^4C_1$ configuration. The $J_{C1\text{-}H1}$ values of 170 (rha A) and 173 Hz (rha B) and $J_{H4\text{-}H5}$ values of 9.5 (rha A) and 9.4 Hz (rha B) clearly indicated the presence of the α-anomers in $^1C_4$ configurations.

The gc of the L-cysteine methyl ester derivatives prepared according to Ref. (6) indicated that rhamnose is present as the L-enantiomer and that xylose and fucose are present as D-enantiomers. Hence, besysaponin C_{12} [**1**] has the structure 3-*O*-α-L-rhamnopyranosyl-2β,3β,16α,23-tetrahydroxyolean-12-en-28-oic acid 28-*O*-β-D-xylopyranosyl(1→4)-α-L-rhamnopyranosyl(1→2)-β-D-fucopyranoside.

The only difference between structures **1** and **2** is the absence of the terminal rhamnose in compound **1**. Nevertheless compound **1** could not be detected in *B. perennis*, suggesting that a taxonomic classification of the genus *Bellis* from a chemical point of view is possible.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—1D (^{1}H, ^{13}C, DEPT) and 2D [^{1}H-^{1}H-COSY, HMBC (^{1}H detected multiple-bond ^{13}C multiple-quantum coherence-spectroscopy), HMQC (^{1}H detected heteronuclear multiple-quantum coherence-spectroscopy)] nmr spectra were recorded in CD_3OD at 300 K on a Bruker AM 600 nmr spectrometer (^{1}H-nmr 600.14 MHz, ^{13}C-nmr 150.91 MHz) as described previously (2), and mass spectra were measured on a Kratos MS 50 HRS mass spectrometer. Tlc was carried out on Si gel 60 plates or foils (Merck) and cc on Sephadex® LH-20 (Pharmacia) and Si gel 60, 0.063–0.2 μm (Merck).

PLANT MATERIAL.—Whole plants of *B. sylvestris* were collected during December 1992 at Cerasomma, near to Lucca, Italy at an altitude of about 100 m. The material was dried at 50–60°. A voucher specimen is deposited at the herbarium of the Department of Pharmacy, Humboldt University, Berlin (number Scho-2).

EXTRACTION AND ISOLATION.—A 400-g quantity of the dried plant material was refluxed twice for 1 h with 80% MeOH (3000 ml). The MeOH was removed under reduced pressure and the residue was diluted with H_2O to 750 ml. The extract was defatted twice with $CHCl_3$ and extracted four times with *n*-BuOH. The dried *n*-BuOH extract was dissolved in MeOH and dropped into an excess of Et_2O giving 17.3 g of a brown, powdery crude glycoside mixture.

A 16-g aliquot of the crude glycoside mixture was subjected to Sephadex® LH-20 cc (MeOH) giving seven saponin containing fractions (yield 11.7 g). Saponin fraction 3 (the major saponin fraction, 2 g) was hydrolyzed with 2000 ml of 1% KOH for 2 h at room temperature. After neutralization with HCl the deacylated saponins were extracted four times with *n*-BuOH (500 ml each) giving 1.8 g of a residue. The deacylated saponins were separated by cc on Si gel ($CHCl_3$-MeOH-H_2O, 10:3:1, lower layer) giving 113 mg of compound **1** and 600 mg of compound **2**.

DETERMINATION OF THE ABSOLUTE CONFIGURATION OF THE SUGARS.—The determination was performed according to Ref. (6) using about 4 mg of compound **1**. Gc conditions: column, J&W Scientific DB-17 (30 m×0.25 mm i.d., film thickness 0.25 μm), 250° oven temperature, 280° injection port and detector temperature, carrier gas, He (22.3 liters/h). Retention times: D-xylose 9.11 min (L-xylose 9.72 min), L-rhamnose 9.97 min, D-fucose 10.57 min (L-fucose 11.31 min).

Besysaponin C_{12} [**1**].—Brownish, amorphous powder; hrfabms (in glycerol) *m/z* $[M+Na]^+$ 1097.5508 (calcd 1097.5514); tlc R_f 0.54 ($CHCl_3$-MeOH-H_2O, 7:4:1); ^{1}H nmr, see Table 1; ^{13}C nmr, see Table 2.

Besysaponin C_{14} [**2**].—Brownish, amorphous powder; tlc R_f 0.47 ($CHCl_3$-MeOH-H_2O, 7:4:1); ^{1}H nmr aglycone, δ 0.79, 0.88, 0.92, 0.95, 1.33, 1.39 (6×CH_3), 2.95 (dd, J=ca. 4 and 14 Hz, H-18), 5.34 (t, J=ca. 4 Hz, H-12), sugar methyl protons δ 1.22 (d, J=ca. 6.5 Hz), 1.25 (d, J=ca. 6.5 Hz), 1.26 (d, J=ca. 6.5 Hz), 1.32 (d, J=ca. 6.5 Hz); sugar anomeric protons, δ 4.49 (d, J=7.8 Hz), 4.87 (br s), 5.15 (d, J=1.8 Hz), 5.32 (d, J=8.0 Hz), 5.37 (d, J=1.8 Hz); ^{13}C nmr, see Table 2.

ACKNOWLEDGMENTS

The authors wish to thank Dr. Gianni Bedini, Botanical Garden, University of Pisa, Italy, for the identification of the plant material, R. Christ, GBF Braunschweig, for the hrfabms measurement, and Margot Janka, Humboldt University, Berlin, Department of Pharmacy, for technical assistance.

LITERATURE CITED

1. K. Hiller, T. Schöpke, V. Wray, and H.-R. Schulten, *Pharmazie*, **43**, 850 (1988).
2. T. Schöpke, V. Wray, B. Rzazewska, and K. Hiller, *Phytochemistry*, **30**, 627 (1991).
3. T. Schöpke, V. Wray, A. Kunath, and K. Hiller, *Phytochemistry*, **31**, 2555 (1992).
4. T. Schöpke, V. Wray, A. Kunath, and K. Hiller, *Pharmazie*, **45**, 870 (1990).
5. T. Kartnig and O. Wegschaider, *J. Chromatogr.*, **61**, 375 (1971).
6. S. Hara, H. Okabe, and K. Mihashi, *Chem. Pharm. Bull.*, **35**, 501 (1987).

Received 11 January 1994