Pergamon 0031-9422(94)00900-7 Phytochemistry, Vol. 38, No. 6, pp. 1481 1485, 1995

0031 9422/95 $9.50 + 0.00

ISOLATION OF TRITERPENE SAPONINS FROM *GYPSOPHILA CAPILLARIS*

M. HANI A. ELGAMAL, HESHAM S. M. SOLIMAN, MOHAMED S. KARAWYA,* BOZHANA MIKHOVA† and HELMUT DUDDECK‡§

National Research Centre, Laboratory of Natural Products, Dokki-Cairo, Egypt; *Department of Pharmacognosy, Faculty of Pharmacy, Cairo University, Kasr Elaini, Cairo, Egypt; †Institute of Organic Chemistry, Bulgarian Academy of Sciences, BG-1113 Sofia, Bulgaria; ‡Institut für Organische Chemie, Universität Hannover, Schneiderberg 1B, D-30167 Hannover, Germany

(*Received in revised form* 17 *October* 1994)

Key Word Index—*Gypsophila capillaris*; Caryophyllaceae; gypsogenin; gypsogenic acid; glucose; galactose; 1D and 2D correlated NMR spectroscopy; NOE; homo- and heteronuclear selective irradiation experiments; FAB-MS.

Abstract—Four novel triterpenoid saponins have been isolated from *Gypsophila capillaris*. Three were monodesmosidic: 3β-hydroxyolean-12-en-23,28-dioic acid 28-*O*-[β-D-glucopyranosyl-(1 → 3)-β-D-glucopyranosyl-(1 → 2)][β-D-galactopyranosyl(1 → 6)]β-D-glucopyranoside, 3β-hydroxyolean-12-en-23-oxo-28-oic acid 28-*O*-[β-D-glucopyranosyl-(1 → 2)-β-D-galactopyranosyl-(1 → 3)][β-D-glucopyranosyl(1 → 6)] β-D-galactopyranoside, 3β-hydroxyolean-12-en-23,28-dioic acid 28-*O*-[β-D-glucopyranosyl-(1 → 2)-β-D-galactopyranosyl-(1 → 3)][β-D-glucopyranosyl(1 → 6)]β-D-galactopyranoside. The fourth was bisdesmosidic: 3β-hydroxyolean-12-en-23,28-dioic acid 23-*O*-β-D-glucopyranosyl-28-*O*-[β-D-glucopyranosyl-(1 → 2)-β-D-galactopyranosyl-(1 → 3)][β-D-glucopyranosyl-(1 → 6)] β-D-galactopyranoside. The structures of the compounds were elucidated by 1D and 2D NMR techniques and from other spectroscopic evidence.

INTRODUCTION

Gypsophila capillaris (Forssk.) C. Chr. (= G. rockejeka Del.), Caryophyllaceae, is a perennial herbaceous plant. It has been collected from the eastern Egyptian desert between Cairo and Suez. This plant is expected to contain terpenoidal saponins with potential molluscicidal [1] and/or antiviral [2] activity. We have reported the first two saponins isolated from this plant as gypsogenic acid (3β-hydroxyolean-12-en-23,28-dioic acid) 28-*O*-β-D-glucopyranosyl(1 → 6)β-D-glucopyranoside and quillaic acid (3β,16α-dihydroxyolean-12-en-23-oxo-28-oic acid) 28-*O*-[β-D-glucopyranosyl(1 → 2)][β-D-galactopyranosyl(1 → 6)]β-D-glucopyranoside [3]. In the present paper we report four further saponins with related structures.

RESULTS AND DISCUSSION

After hydrolysis of **1**–**4** the aglycones were easily identified as gypsogenin (**I**) and gypsogenic acid (**II**), respectively by comparison of their ^{13}C NMR spectra with literature data [4, 5] and by chromatographic comparison with authentic samples. The ^{13}C NMR chemical shifts we found are very close to the reported values so we refrain from listing them in Table 1 except for the values of H-3 and C-3 which appear in the region of the sugar resonances. In addition, the hydrolysis afforded glucose and galactose in each case as determined by GC (persilylated) and TLC comparison with the authentic monosaccharides.

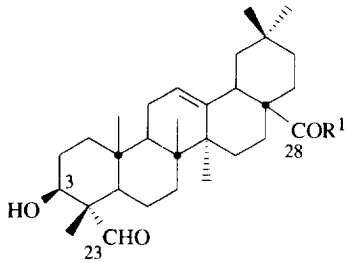
Structural determinations of the four saponins were mainly based on NMR spectral assignments which were confirmed by homo- and heteronuclear COSY, by ^{1}H, ^{1}H, ^{1}H RELAY, by homonuclear NOE-difference experiments and by measurements using homo- (^{1}H, ^{1}H) or heteronuclear (^{1}H, ^{13}C) selective irradiation [6, 7]. The value and the limits of the NOE-difference spectroscopy in differentiating glucose from galactose and for establishing the position of the interglycosidic linkages has been discussed by us before [3].

The general procedure of the structure determinations by evaluating the NMR spectra is summarized as follows:

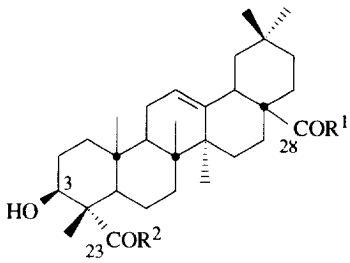
(a) The anomeric proton (H-1) signals were all doublets (J = 7 to 8 Hz) so that all sugars are in β-pyranose configuration in the saponins. Correlation with the anomeric carbon signals (^{1}H, ^{13}C COSY) afforded the anomeric ^{13}C signal positions.

(b) The H-3/C-3 signals of the aglycone could be found by inspecting the ^{1}H, ^{1}H and ^{1}H, ^{13}C COSY spectra of the saponins. Those of H-3 are in the typical region of sugar protons but can be separated from them by looking at their ^{1}H, ^{1}H COSY cross-peaks; they are the only ones which do not have coupling partners in that region but

§Author to whom correspondence should be addressed.



I (gypsogenin, if R^1 = OH)



II (gypsogenic acid, if $R^1 = R^2$ = OH)

	aglycone	R^1	R^2
1	**II**	O-glc[**A**]-(6←1)-gal[**D**] | (2←1)-glc[**B**]-(3←1)-glc[**C**]	OH
2	**I**	O-gal[**A**]-(6←1)-glc[**D**] | (3←1)-gal [**B**]-(2←1)-glc[**C**]	
3	**II**	O-gal[**A**]-(6←1)-glc[**D**] | (3←1)-gal [**B**]-(2←1)-glc[**C**]	OH
4	**II**	O-gal[**A**]-(6←1)-glc[**D**] | (3←1)-gal [**B**]-(2←1)-glc[**C**]	O-glc[**E**]

only with signals at smaller δ values (H-2). The chemical shifts of the C-3 carbons, compared with those of the respective ones in the free aglycone, showed that they are not glycosidated. The carboxyl functions are also indicative (see Table 1).

(c) Identification of the other sugar protons: the H-2 proton signals in the sugar units were found by looking at the cross-peaks of the H-1 signals in the ^{1}H, ^{1}H COSY spectrum. Likewise, the ^{1}H, ^{1}H, ^{1}H RELAY spectrum displays cross-peaks not only for the H-2 but also for the H-3 signals. H-3 signals were also seen along with H-5 signals as NOE responses when the H-1 atoms are irradiated. Both H-3 and H-5 are in *syn-diaxial* position with respect to H-1 providing significant NOE intensities in most cases. It is fortunate that in our saponin spectra most H-5 signals were well separated from the other sugar signals appearing at lowest frequencies (smallest δ values). ^{1}H, ^{1}H COSY cross-peaks of the H-5 with the two H-6 and with H-4 allow us to find these remaining signals. The pairs of H-6 can also be seen in the ^{1}H, ^{13}C COSY spectra because they belong to methylene groups. In some cases the NOE responses were not satisfactory. Here, we applied 1D ^{1}H, ^{1}H COSY and ^{1}H, ^{1}H, ^{1}H RELAY experiments using selective irradiation of the H-1 and H-5 signals by Gaussian-shaped pulses [8]. Whereas irradiation on H-1 or H-5 in such 1D COSY measurements afforded the position of H-2 or H-4 and H-6/H-6′, respectively, the 1D RELAY spectra showed the H-2 and H-3 signals.

(d) Identification of the sugars: generally, it is not possible to discriminate glucose and galactose by their ^{1}H and ^{13}C chemical shifts. However, the H-3 signals are indicative if they can be seen as NOE responses (H-1 irradiated). In glucose they are relatively broad triplets due to their couplings with the *antiperiplanar* H-4 and H-5. In contrast, the H-3 signals are rather narrow doublets in the galactose because the H-3/H-4 coupling is only very small since the respective torsional angle (H-3/C-3/C-4/H-4) is close to 90° [9]. In cases where the NOE responses were too weak this information could be obtained from the multiplicities of the H-3 signals in the 1D RELAY spectra.

(e) Glycosidic linkages between the β-glucoses and β-galactoses: all C-1 chemical shifts have $\delta > 100$ except if the sugar is attached to the C-23 or C-28 carboxylic group. Then, the chemical shift is δ93 to 95 if C-1 is attached to the 28-COO. In one case, glucose **E** in **4** with C-1 attached to 23-COO, this value is slightly higher (δ96.5). The H-1 signals in sugars attached to –COO– have always chemical shifts $\delta > 6$, in the others between δ5.73 and 4.89. If a C-6 signal is at δ68 to 69 it carries another sugar residue; otherwise the signal is at δ62 to 64.

Table 1. ^1H and ^{13}C chemical shifts of the sugars and some diagnostic atoms of the aglycone in the saponins **1**–**4** (in pyridine-d_5); δ7.19 for the lowest-frequency signal.

		1		**2**		**3**		**4**	
		^1H	^{13}C	^1H	^{13}C	^1H	^{13}C	^1H	^{13}C
A	A1	6.08	93.4	6.18	94.8	6.18	94.6	6.18	94.8
	A2	4.66	77.5	4.28	73.1	4.29	73.0	4.29	73.1
	A3	4.23	78.5	4.28	88.0	4.29	87.5	4.29	87.9
	A4	4.60	69.9	4.28	69.1	4.33	68.9	4.37	69.2
	A5	3.93	77.1	4.08	76.8	4.09	76.7	4.08	76.8
	A6	4.49/4.26	68.8	4.48/4.32	68.8	4.48/4.29	68.7	4.49/4.29	68.9
B	B1	5.73	104.1	5.30	105.7	5.31	105.3	5.34	105.8
	B2	4.02	76.1	4.03	83.6	4.05	83.1	4.06	83.7
	B3	4.18	84.5	4.09	78.1	4.10	78.1*	4.12	78.1*
	B4	4.18	70.9	4.08	71.2	4.09	71.2	4.14	71.3
	B5	4.02	76.1	3.86	78.5	3.89	78.3	3.92	78.5‡
	B6	4.64/4.29	63.7	4.42/4.22	62.3	4.42/4.22	62.2	4.45/4.26	62.3†
C	C1	5.18	106.2	5.26	105.7	5.27	105.4	5.29	105.8
	C2	3.98	72.6	4.01	76.3	4.01	76.0	4.04	76.4
	C3	4.09	78.2	4.10	77.9	4.12	77.8	4.12	78.0*
	C4	4.18	70.9	4.10	71.1	4.12	70.4	4.16	71.1
	C5	3.78	78.5	3.88	78.3*	3.90	77.9*	3.90	78.4‡
	C6	4.58/4.43	62.0	4.53/4.30	62.4	4.53/4.31	62.2	4.55/4.35	62.5
D	D1	4.89	102.7	4.95	102.5	4.97	102.4	4.95	102.6
	D2	4.00	75.8	4.03	75.6	4.05	75.4	4.06	75.6
	D3	4.18	78.2	4.25	77.9	4.24	77.8	4.24	78.0*
	D4	4.19	70.6	4.13	70.8	4.14	70.6	4.19	70.8
	D5	3.73	78.2	3.75	78.2*	3.76	78.0*	3.77	78.3
	D6	4.36/4.29	62.0	4.37/4.29	62.1	4.38/4.29	62.0	4.39/4.31	62.1†
E	E1							6.46	96.5
	E2							4.19	74.4
	E3							4.26	78.7
	E4							4.35	70.9
	E5							3.98	79.4
	E6							4.36/4.35	62.0
H/C-3 (aglyc.)		4.64	75.5	4.04	71.6	4.05	75.4	4.62	75.0
C-23 (aglyc.)			180.9		207.3		180.7		177.7
C-28 (aglyc.)			176.5		176.3		176.3		176.3

^1H, ^1H coupling constants are as expected [9] for glucose and galactose and are not included in this table.
Signals marked by *, †, ‡ may be interchanged.

If a methine carbon signal is well above δ80 it belongs to a C-3 carbon bearing another sugar. Interglycosidic linkages are revealed by the NOE-difference spectra; irradiating anomeric protons produces NOE responses of the protons across the glycosidic bond. In addition, selective INEPT experiments [10, 11] were successful. Selectively irradiating the anomeric proton by a soft pulse provided the ^{13}C signal of the carbon atom to which this sugar is linked. Since the identity of the responding carbon is known, the interglycosidic linkages could be determined unequivocally.

Saponin **4** is the only bisdesmosidic glycoside. It turned out that its carboxyl group C-28 carries the same tetrasaccharide as **3**. This is clearly proven by essentially the same ^1H and ^{13}C chemical shifts and signal assignments resulting from the experiments described above. However, an extra glucose is attached to C-23 as can be seen from the difference in the chemical shifts of C-23 of the aglycones in **3** and **4**.

The positive-ion FAB mass spectra gave the expected peaks for the expected aglycone, mono-, di-, tri-, tetra- and pentaglycoside ions (see Experimental).

To our knowledge, **1**–**4** have not been reported before in the literature. We have already pointed out that some related compounds have been isolated which carry sugar residues at C-3 or C-28 or both [3, 12, 13].

EXPERIMENTAL

General. Mps: uncorr. IR spectra in KBr. ^1H and ^{13}C NMR spectra were recorded in pyridine-d_5 using Bruker AM-400 (^1H: 400.1 MHz; ^{13}C: 100.6 MHz) and AM-300 (^1H: 300.1 MHz; ^{13}C: 75.4 MHz) spectrometers. Standard Bruker software has been applied for the multipulse NMR experiments. FAB-mass spectra were obtained with a VG Autospec (in lactic acid). CC was performed using silica gel 60 (140–270 mesh); TLC with silica gel 60 F_{254} plates (Merck); GC with a column SE-54

(Macherey & Nagel) and FID detector, carrier gas was N_2. Elemental analyses have not yet been performed in order to save the material for biological studies.

Isolation. Gypsophila capillaris (Forssk.) growing in the eastern Egyptian desert between Cairo and Suez has been collected and identified by Prof. L. Bolous (National Research Centre (NRC), Cairo). A voucher specimen is kept in the herbarium of the NRC. The powder form of the whole plant (aerial parts and roots, 2.5 kg) was extracted with MeOH, and the solvent was removed under red. pres. The methanolic extract was then dissolved in water and extracted with Et_2O. The defatted aq. layer was exhaustively extracted with *n*-BuOH, and the solvent was removed under red. pres. The *n*-BuOH extract (35 g) was chromatographed on a silica gel column and eluted by a mixt. of $CHCl_3$–MeOH–H_2O (100:10:1). For further elution the $CHCl_3$ content was reduced stepwise until a mixt. composition of 10:10:1 was reached. Finally, pure MeOH was used.

The fr. collected with a solvent mixt. (40:10:1) gave 1.4 g of a crude product from which we isolated two novel saponins described in our previous communication [3].

When the composition of the solvent mixt. reached (30:10:1), 0.9 g of a crude product was eluted. TLC of this fr. using $CHCl_3$ MeOH H_2O (18:11:2.7) showed one major spot ($R_f = 0.40$) with little impurity. Purification was achieved by medium-pressure reversed-phase chromatography (RP-8, 56% MeOH) followed by filtration over Sephadex LH-20 (Pharmacia, Sweden) with MeOH to isolate **2** (90 mg) in pure form. The fr. collected with a solvent mixt. (20:10:1) gave 1.6 g of a crude product. TLC of this fr. using $CHCl_3$ MeOH H_2O (18:11:2.7) showed the existence of two spots ($R_f = 0.31$ and 0.29, respectively). Medium-pressure reversed-phase chromatography (RP-8, 48% MeOH) and, finally, purification using Sephadex LH-20 allowed separation of **3** (180 mg) and **1** (165 mg) in pure form.

The crude fr. 1.1 g eluted by the solvent mixt. (10:10:1) showed a major spot ($R_f = 0.26$) on TLC using $CHCl_3$–MeOH–H_2O (18:11:2.7). This fr. was treated like the previous crude frs but with RP-8 and 51% MeOH followed by filtration on Sephadex LH-20 allowing isolation of **4** (150 g) in pure form.

Alkaline hydrolysis of **1** *to* **4**. Compounds **1**–**4** (20 mg) were refluxed with 5% KOH for 3 hr. The soln was neutralized with diluted HCl, extracted with *n*-BuOH satd with H_2O. The *n*-BuOH extract contained gypsogenin in the case of **2** and gypsogenic acid in the case of **1**, **3** and **4** ($R_f = 0.32$ and 0.52, respectively, using $CHCl_3$–MeOH, 9:1).

Acidic hydrolysis. The aq. methanolic soln of each compound from **1**, **2**, **3**, or **4** (15 mg each) was separately treated with 9% HCl under reflux for 6 hr. Working up as usual gave gypsogenic acid and gypsogenin, respectively. The aq. layer was neutralized with barium carbonate for the sugar test.

(3β-*Hydroxyolean*-12-*en*-23,28-*dioic acid* 28-O-[β-D-*glucopyranosyl*-(1 → 3)-β-D-*glucopyranosyl*-(1 → 2)][β-D-*galactopyranosyl*(1 → 6)]β-D-*glucopyranoside* (**1**). Mp 227–229°. $[\alpha]_D^{20} = +2.2$ (MeOH; *c* 13.8 mg ml^{-1}). IR ν_{max}^{KBr} cm^{-1}: 3408 (OH), 1715 (C=O), 1078 (C–O). FAB-MS (molecular weight: $C_{54}H_{86}O_{25}$) *m/z*: 487 [aglycone + H]$^+$; 649 [aglycone + 1hexose + H]$^+$; 811 [aglycone + 2hexoses + H]$^+$; 973 [aglycone + 3hexoses + H]$^+$; 1135 [aglycone + 4hexoses + H]$^+$.

(3β-*Hydroxyolean*-12-*en*-23-*oxo*-28-*oic acid* 28-O-[β-D-*glucopyranosyl*-(1 → 2)-β-D-*galactopyranosyl*-(1 → 3)][β-D-*glucopyranosyl*(1 → 6)]β-D-*galactopyranoside* (**2**). Mp 212–214°. $[\alpha]_D^{20} = +12.5$ (MeOH; *c* 14.2 mg ml^{-1}). IR ν_{max}^{KBr} cm^{-1}: 3402 (OH), 1724 (C=O), 1075 (C–O). FAB-MS (molecular weight: $C_{54}H_{86}O_{24}$) *m/z*: 471 [aglycone + H]$^+$; 633 [aglycone + 1 hexose + H]$^+$; 795 [aglycone + 2 hexoses + H]$^+$; 957 [aglycone + 3 hexoses + H]$^+$; 1119 [aglycone + 4 hexoses + H]$^+$.

(3β-*Hydroxyolean*-12-*en*-23,28-*dioic acid* 28-O-[β-D-*glucopyranosyl*-(1 → 2)-β-D-*galactopyranosyl*-(1 → 3)][β-D-*glucopyranosyl*(1 → 6)]β-D-*galactopyranoside* (**3**). Mp 230–232°. $[\alpha]_D^{20} = +10.5$ (MeOH; *c* 12 mg ml^{-1}). IR ν_{max}^{KBr} cm^{-1}: 3408 (OH), 1715 (C=O), 1076 (C–O). FAB-MS (molecular weight: $C_{54}H_{86}O_{25}$) *m/z*: 487 [aglycone + H]$^+$; 649 [aglycone + 1hexose + H]$^+$; 811 [aglycone + 2 hexoses + H]$^+$; 973 [aglycone + 3 hexoses + H]$^+$; 1135 [aglycone + 4hexoses + H]$^+$.

(3β-*Hydroxyolean*-12-*en*-23,28-*dioic acid* 23-O-[β-D-*glucopyranosyl*-28-O-[β-D-*glucopyranosyl*-(1 → 2)-β-D-*galactopyranosyl*(1 → 3)][β-D-*glucopyranosyl*(1 → 6)]β-D-*galactopyranoside* (**4**). Mp 225–227°. $[\alpha]_D^{20} = +4.5$ (MeOH; *c* 14.4 mg ml^{-1}). IR ν_{max}^{KBr} cm^{-1}: 3402 (OH), 1731 (C=O), 1074 (C–O). FAB-MS (molecular weight: $C_{60}H_{96}O_{30}$) *m/z*: 487 [aglycone + H]$^+$; 649 [aglycone + 1hexose + H]$^+$; 811 [aglycone + 2hexoses + H]$^+$; 973 [aglycone + 3hexoses + H]$^+$; 1135 [aglycone + 4hexoses + H]$^+$; 1297 [aglycone + 5hexoses + H]$^+$.

Acknowledgements—H. S. M. Soliman thanks the German Academic Exchange Service (DAAD) for a scholarship in the frame of the channel program between the governments of Germany and Egypt. B. Mikhova thanks the Deutsche Forschungsgemeinschaft for a fellowship at Hannover university. Financial support was provided by Deutsche Forschungsgemeinschaft and Fonds der Chemischen Industrie.

REFERENCES

1. Borel, C., Gupta, M. P. and Hostettmann, K. (1987) *Phytochemistry* **26**, 2685; Borel, C., Gupta, M. P. and Hostettmann, K. (1987) *Helv. Chim. Acta* **70**, 570.
2. Frechet, D., Christ, B., Monegier du Sorbier, B., Fischer, H. and Vuilhorgne, M. (A. Nattermann and Cie GmbH), *Ger. Offen.* DE 4,017,766 (cl. c07h 15/24), 05 Dec. 1991, Appl. 01 Jun. 1990; 4 pp.
3. Elgamal, M. H. A., Soliman, H. S. M., Karawya, M. S. and Duddeck, H. (1994) *Nat. Prod. Lett.* **4**, 217.
4. Frechet, D., Christ, B., Monegier du Sorbier, B., Fischer, H. and Vuilhorgne, M. (1991) *Phytochemistry* **30**, 927.
5. Agrawal, P. K. and Jain, D. C. (1992) *Progr. NMR Spectrosc.* **24**, 1.

6. Croasmun, W. R. and Carlson, R. M. K. (eds) (1994) *Two-Dimensional NMR Spectroscopy, Applications for Chemists and Biochemists*, 2nd edn. VCH Publishers, New York.
7. Martin, G. E. and Zektzer, A. S. (1988) *Two-Dimensional Methods for Establishing Molecular Connectivity*. VCH Publishers, New York.
8. Kessler, H., Oschkinat, H., Griesinger, C. and Bermel, W. (1986) *J. Magn. Reson.* **70**, 106.
9. Altona, C. and Haasnoot, C. A. G. (1980) *Org. Magn. Reson.* **13**, 417.
10. Bax, A. and Freeman, R. (1982) *J. Am. Chem. Soc.* **104**, 1099.
11. Bax, A. (1984) *J. Magn. Reson.* **57**, 314.
12. Dev, S. (ed.) (1989) *Handbook of Terpenoids*, pp. 433–435, 452. CRC Press, Boca Raton, FA.
13. Mahato, S. B. and Nandy, A. (1991) *Phytochemistry* **30**, 1357.