Sulfated Pregnane Glycosides from *Periploca graeca*[⊥]

Tiziana Siciliano,[†] Ammar Bader,[‡] Nunziatina De Tommasi,[§] Ivano Morelli,[†] and Alessandra Braca*,[†]

Dipartimento di Chimica Bioorganica e Biofarmacia, Università di Pisa, Via Bonanno 33, 56126 Pisa, Italy, Faculty of Pharmacy, Al-Zaytoonah Private University of Jordan, P.O. Box 130, 11733 Amman, Jordan, and Dipartimento di Scienze Farmaceutiche, Università di Salerno, Via Ponte Don Melillo, 84084 Fisciano (SA), Italy

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Six new pregnane glycosides, four of them sulfated derivatives, were isolated from small branches of *Periploca graeca*. The compounds were identified as 16α-[(6-*O*-sulfo-β-D-glucopyranosyl)oxy]pregn-5-en-20-ol-3β-yl *O*-(2-*O*-acetyl-β-D-digitalopyranosyl)-(1→4)-β-D-cymaropyranoside (**1**), 16α-[(6-*O*-sulfo-β-D-glucopyranosyl)oxy]pregn-5-en-20-ol-3β-yl *O*-β-D-oleandropyranosyl-(1→4)-β-D-oleandropyranoside (**2**), 16α-[(6-*O*-sulfo-β-D-glucopyranosyl)oxy]pregn-5-en-20-ol-3β-yl *O*-β-D-oleandropyranoside (**3**), 16α-[(6-*O*-sulfo-β-D-glucopyranosyl)oxy]pregn-5-ene-3β,20-diol (**4**), 20-*O*-[(β-D-glucopyranosyl-(1→6)-β-D-glucopyranosyl-(1→2)-β-D-digitalopyranosyl)oxy]pregn-5-en-16β-ol-3β-yl *O*-β-D-digitalopyranosyl-(1→4)-β-D-cymaropyranoside (**5**), and calogenin 3-*O*-β-D-digitalopyranoside-20-*O*-β-D-canaropyranoside (**6**). Three pregnane glycosides, previously reported from the genus *Periploca*, were also isolated. Structures were established on the basis of spectroscopic analyses, including 1D and 2D NMR experiments, HRESIMS, elemental analysis, and chemical degradation.

Pregnanes in Asclepiadaceae plants have been studied actively since the 1960s, and many pregnanes and pregnane glycosides that are biological precursors of cardiac glycosides were discovered in several genera.[1] As a part of our studies on the biologically active metabolites from Asclepiadaceae plants, we selected plants belonging to the genus *Periploca*. *P. graeca* L. is a perennial liana occurring wild in southeast European and west Asian countries.[2] The decoction of the plant's leaves and bark is used in Italian folk medicine to prepare cardiotonic, hypertensive, diuretic, and purgative remedies.[3] Previous study on the aerial parts of the plant led to the isolation of flavonoids,[4] while cardenolides and pregnane glycosides, some of which showed antitumoral activity, have been reported from the genus *Periploca*.[5]

In the present investigation on *P. graeca* small branches, we report the isolation and structural characterization of six new pregnane glycosides, four of them being sulfated compounds. In addition, three known pregnane glycosides, previously reported from *Periploca* genus, were also isolated.

Results and Discussion

The methanol, chloroform−methanol, and chloroform extracts of small branches of *P. graeca* were separately chromatographed to afford nine pregnane glycosides.

Compound **1** showed a quasi-molecular ion peak at *m/z* 921 $[M]^-$ and significant fragments at *m/z* 861 $[M - 60]^-$, 719 $[M - 202]^-$, and 557 $[M - 202 - 144 - 18]^-$ in the negative ESIMS. From the high-resolution negative ESIMS, elemental analysis, and NMR spectral data its molecular formula was deduced to be $C_{43}H_{69}O_{19}SNa$. Data from the ^{13}C NMR spectrum (see Table 1) suggested a glycoside structure. The ^1H NMR of the aglycon portion showed signals for two singlet methyl groups (δ 0.84 and 1.08), one doublet methyl group (δ 1.32, $J = 6.0$ Hz), one olefinic proton at δ 5.40 (1H, br d, $J = 4.5$ Hz), and three signals at δ 3.56 (1H, m), 3.86 (1H, br q, $J = 6.0$ Hz), and 4.13 (1H, ddd, $J = 8.0, 6.5, 2.2$ Hz) corresponding to a secondary oxygenated carbon. The ^{13}C NMR spectrum showed for the aglycon moiety 21 signals that could be unambiguously correlated to the corresponding proton chemical shifts by HSQC experiment. The complete elucidation of the aglycon structure of **1** as pregn-5-ene-3β,16α,-20-triol was then achieved by HMBC and 2D-ROESY experiments and compared with NMR data reported in the literature.[6] In particular an ROE effect was observed between H-16 (δ 4.13) and Me-18 (δ 0.83), indicating that the relative stereochemistry of the hydroxyl group at C-16 was α. The sugar portion of **1** showed ^1H NMR signals corresponding to three anomeric protons at δ 4.26, 4.52, and 4.89, as well as signals attributable to two doublet methyls at δ 1.20 and 1.33, two methoxy groups at δ 3.41 and 3.45, and one acetyl group at δ 2.09. All these data indicated that **1** had three sugars, one of them being a 2,6-dideoxysugar and another being a 6-deoxysugar. The proton coupling network of each sugar residue was derived from a combination of 1D and 2D NMR experiments (1D-TOCSY, DQF-COSY, HSQC, and HMBC), which indicated that a β-D-cymaropyranose, a 2-*O*-acetyl-β-D-digitalopyranose, and a β-D-glucopyranose were present (Table 2). The presence of 2-*O*-acetyl-β-D-digitalopyranose was also confirmed by a HMBC correlation between the signal at δ 171.7 ($-OCOCH_3$) and δ 5.10 ($H\text{-}2_{dig}$). The configurations of the sugar units were assigned after hydrolysis of **1** with 1 N HCl. The hydrolysate was trimethylsilated, and GC retention times of each sugar were compared with those of authentic sugar samples prepared in the same manner. The absence of any glycosidation shift for the 2-*O*-acetyl-β-D-digitalopyranose suggested that this sugar was the terminal unit. Direct evidence for the sugar sequence and their linkage sites was derived from the results of the HMBC experiment, which showed unequivocal correlation peaks between δ 4.89 and 79.2 ($H\text{-}1_{cym}$−C-3), δ 4.52 and 84.4 ($H\text{-}1_{dig}$−$C\text{-}4_{cym}$), and δ 4.26 and 80.6 ($H\text{-}1_{glc}$−C-16). The low-field resonance of the $C\text{-}6_{glc}$ carbon (68.4 ppm) along with the low-field resonances of the $H\text{-}6a_{glc}$ and $H\text{-}6b_{glc}$ signals (δ 4.34 and 4.11, respectively) suggested the location of the sulfate moiety. To confirm the position of this

[⊥] Dedicated to the memory of Prof. Ivano Morelli.

* To whom correspondence should be addressed. Tel: +39-050-2219688. Fax: +39-050-2219660. E-mail: braca@farm.unipi.it.
[†] Università di Pisa.
[‡] University of Jordan.
[§] Università di Salerno.

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Table 1. ^{1}H and ^{13}C NMR Data for Aglycon Moieties of Compounds **1**, **5**, and **6** (CD_3OD, 600 MHz)[a]

	1		**5**		**6**	
position	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C
1a	1.90 m	38.4	1.89 m	38.2	1.91 m	38.5
1b	1.10 m		1.11 m		1.10 m	
2a	1.91 m	30.4	1.90 m	30.0	1.93 m	30.4
2b	1.56 m		1.54 m		1.62 m	
3	3.56 m	79.2	3.52 m	78.9	3.55 m	79.7
4a	2.35 br t (9.0)	39.9	2.34 br t (9.0)	39.9	2.43 br t (9.0)	39.6
4b	2.18 dd (10.5, 2.5)		2.21 dd (10.5, 3.0)		2.28 dd (10.0, 2.5)	
5		141.8		141.6		140.5
6	5.40 br d (4.5)	122.6	5.42 br d (4.0)	122.8	5.40 br d (4.5)	122.6
7a	2.01 m	32.6	2.08 m	34.7	2.08 m	28.0
7b	1.64 m		1.60 m			
8	1.53 m	32.2	1.53 m	32.6	2.18 m	39.0
9	1.02 m	51.8	1.01 m	51.2	0.90 m	51.6
10		37.5		37.4		37.5
11	1.53 m	21.5	1.54 m	21.5	1.32 m	23.5
12a	2.19 m	40.9	1.94 m	40.0	2.03 m	37.7
12b	1.32 m		1.22 m		1.72 m	
13		43.7		42.2		51.0
14	1.43 m	54.4	1.00 m	55.0		85.6
15a	1.81 m	33.6	2.21 m	35.5	1.61 m	33.0
15b	1.52 m		1.17 m		1.32 m	
16a	4.13 ddd (8.0, 6.5, 2.2)	80.6	4.67 ddd (8.0, 5.5, 2.0)	72.2	1.76 m	32.1
16b					1.57 m	
17	1.47 dd	65.6	1.42 dd	62.4	1.81 m	52.2
18	0.84 s	13.9	0.88 s	14.2	0.80 s	14.6
19	1.08 s	19.4	1.03 s	19.2	1.06 s	19.5
20	3.86 br q (6.0)	69.7	3.99 br q (6.0)	80.3	3.80 br q (6.5)	83.4
21	1.32 d (6.0)	23.8	1.41 d (6.0)	22.0	1.29 d (6.5)	17.4

[a] *J* values are in parentheses and reported in Hz; chemical shifts are given in ppm; assignments were confirmed by DQF-COSY, 1D-TOCSY, HSQC, and HMBC experiments.

sulfate residue, the solvolysis of **1** was carried out, using dioxane−pyridine, to obtain the desulfated derivative **1a**.[7] ESIMS and ^{1}H NMR spectra of this derivative were recorded and compared with those of the related sulfate. As expected, the positive ESIMS of **1a** gave a quasi-molecular ion at *m/z* 865 $[M + Na]^+$ (80 amu less than compound **1**). Upfield shifts of H-6a$_{glc}$ from δ 4.34 to 3.90 and of H-6b$_{glc}$ from δ 4.11 to 3.72 confirmed the location of the sulfate group at C-6$_{glc}$. Thus, **1** was determined to be 16α-[(6-*O*-sulfo-*β*-D-glucopyranosyl)oxy]pregn-5-en-20-ol-3*β*-yl *O*-(2-*O*-acetyl-*β*-D-digitalopyranosyl)-(1→4)-*β*-D-cymaropyranoside.

The negative HRESIMS of compound **2** gave a $[M]^-$ peak at *m/z* 863.4093, corresponding to the molecular formula $C_{41}H_{67}O_{17}S$ (calcd 863.4099). The presence of Na was determined by EDS analysis (see Experimental Section). Inspection of the NMR spectral data showed that the aglycon moiety of **2** was identical to that of **1**, while the sugar portion was the point of difference. In addition to the aglycon signals, the ^{13}C NMR (see Table 2) exhibited 20 signals ascribable to the saccharide portion made up of two 3-*O*-methyl-2,6-dideoxyhexopyranosyl and one hexopyranosyl units. Also the ^{1}H NMR spectrum (Table 2) supported the above results by the presence of three anomeric protons at δ 4.26, 4.68, and 4.72, two methyl doublets at δ 1.29 and 1.31, and two *O*-methoxy groups at δ 3.46. Also in this case, complete assignments of proton and carbon chemical shifts of the sugar portion were accomplished by HSQC, HMBC, DQF-COSY, and 2D-HOHAHA experiments and allowed the identification of the sugars as a terminal *β*-D-oleandropyranosyl as well as an inner *β*-D-oleandropyranosyl, and a 6-sulfate-*β*-D-glucopyranosyl unit. The configuration of the sugar units was determined as reported for compound **1**. The location of the sulfate residue was confirmed in the same way as **1** (ESIMS of the desulfated derivative at *m/z* 807 $[M + Na]^+$). Therefore, the structure of **2** was established as 16α-[(6-*O*-sulfo-*β*-D-glucopyranosyl)oxy]pregn-5-en-20-ol-3*β*-yl *O*-*β*-D-oleandropyranosyl-(1→4)-*β*-D-oleandropyranoside.

The ESIMS of compound **3** (HRESIMS: *m/z* 719.3307, corresponding to $C_{34}H_{55}O_{14}SNa$ by elemental analysis) showed a $[M]^-$ at *m/z* 719 and a prominent fragment at *m/z* 557 $[M - 144 - 18]^-$ (cleavage of one dideoxyhexose unit and one water molecule). Analysis of NMR data of compound **3** and comparison with those of **2** showed **3** to differ from **2** only in the absence of the terminal oleandropyranose unit. Therefore, the structure 16α-[(6-*O*-sulfo-*β*-D-glucopyranosyl)oxy]pregn-5-en-20-ol-3*β*-yl *O*-*β*-D-oleandropyranoside was assigned to compound **3**.

Compound **4** was assigned the molecular formula $C_{27}H_{43}O_{11}SNa$, as shown by its HRESIMS data (*m/z* 575.2521) in combination with the ^{13}C NMR spectrum and elemental analysis. The ^{13}C and ^{13}C DEPT spectra showed 27 signals, of which six were assigned to the sugar portion. Analysis of NMR data for this sugar moiety of compound **4** and comparison with those of **2** showed **4** to differ from **2** in the absence of the saccharidic moiety at C-3. From the foregoing evidence, **4** was deduced to be 16α-[(6-*O*-sulfo-*β*-D-glucopyranosyl)oxy]pregn-5-ene-3*β*,20-diol.

The ESIMS of compound **5** ($C_{54}H_{90}O_{24}$) showed $[M + Na]^+$ at *m/z* 1146 and fragment ions at *m/z* 984 $[(M + Na) - 162]^+$, 822 $[(M + Na) - (162 + 162)]^+$, and 643 $[(M + Na) - (162 + 162 + 160 + 18)]^+$. The ^{13}C and ^{13}C DEPT NMR spectra showed 54 signals, of which 33 were assigned to the sugar portion and 21 to the pregnane skeleton. Comparing the NMR data of the aglycon portion of **5** showed that this compound was very similar to **1**, with the differences between **5** and **1** being the chemical shifts of the signals of ring D (see Table 1). The aglycon moiety was identified as pregn-5-ene-3*β*,16*β*,20-triol by extensive 1D and 2D NMR experiments and by comparison with data already reported in the literature.[6] The sugar portion of **5** contained, in the ^{1}H NMR (Table 3), five anomeric proton

Table 2. ^{1}H and ^{13}C NMR Data for Sugar Moieties of Compounds **1**, **2**, and **3** (CD_3OD, 600 MHz)[a]

	1		**2**		**3**	
position	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C
D-Cym 1	4.89 dd (9.5, 2.0)	96.9				
2a	1.56 br dd (16.0, 12.0)	36.5				
2b	2.09 br dd (16.0, 3.5)					
3	3.86 q (3.5)	77.8				
4	3.27 dd (9.5, 3.5)	84.4				
5	3.86 dq (9.5, 6.0)	69.6				
6	1.20 d (6.0)	18.3				
-OMe	3.45 s	58.0				
acetyl-D-Dig 1	4.52 d (8.0)	103.7				
2	5.10 dd (9.5, 8.0)	72.3				
3	3.36 dd (9.5, 3.5)	82.6				
4	3.89 dd (3.5, 1.5)	68.5				
5	3.69 dq (6.5, 1.5)	71.7				
6	1.33 d (6.5)	16.4				
-OCOCH$_3$	2.09 s	20.7				
		171.7				
-OMe	3.41 s	56.8				
D-Ole I 1			4.68 dd (8.5, 1.5)	98.7	4.70 dd (8.5, 1.5)	99.7
2a			2.26 m	37.9	2.30 m	38.4
2b			1.39 m		1.36 m	
3			3.40 m	80.0	3.25 m	82.3
4			3.18 t (9.5)	83.9	3.00 t (9.5)	77.0
5			3.36 dq (9.5, 6.0)	72.0	3.28 dq (9.5, 6.5)	73.7
6			1.31 d (6.0)	18.2	1.29 d (6.5)	18.8
-OMe			3.46 s	57.4	3.38 s	57.7
D-Ole II 1			4.72 dd (8.5, 1.5)	101.2		
2a			2.35 m	37.4		
2b			1.33 m			
3			3.22 m	81.5		
4			2.99 t (9.0)	76.8		
5			3.27 dq (9.0, 6.0)	73.1		
6			1.29 d (6.0)	18.3		
-OMe			3.46 s	57.4		
D-Glc 1	4.26 d (8.0)	102.0	4.26 d (8.0)	102.0	4.26 d (8.0)	102.7
2	3.17 dd (9.5, 8.0)	74.9	3.18 dd (9.5, 8.0)	74.8	3.17 dd (9.0, 8.0)	75.6
3	3.37 t (9.5)	77.7	3.36 t (9.5)	77.7	3.38 t (9.0)	78.6
4	3.32 t (9.5)	71.5	3.32 t (9.5)	71.4	3.33 t (9.0)	72.0
5	3.43 m	75.8	3.42 m	75.9	3.43 m	76.6
6a	4.34 dd (12.0, 3.5)	68.4	4.35 dd (12.0, 3.0)	68.3	4.33 dd (12.0, 3.5)	69.0
6b	4.11 dd (12.0, 5.0)		4.12 dd (12.0, 5.0)		4.11 dd (12.0, 5.5)	

[a] *J* values are in parentheses and reported in Hz; chemical shifts are given in ppm; assignments were confirmed by DQF-COSY, 1D-TOCSY, HSQC, and HMBC experiments.

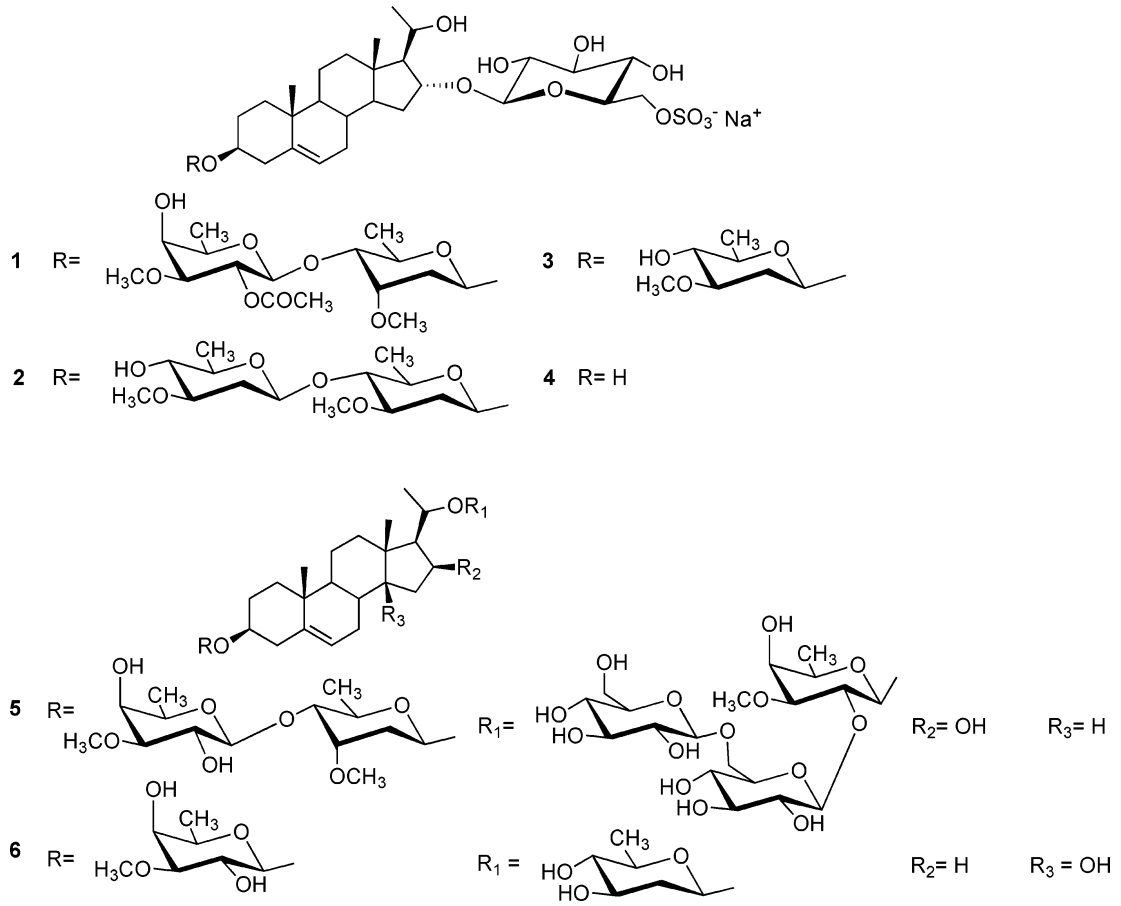
signals. The structures of the oligosaccharide moieties were deduced using 1D TOCSY and DQF-COSY experiments. In the HSQC experiments glycosidation shifts were observed for C-4$_{cym}$ (83.5 ppm), C-2$_{digII}$ (76.3 ppm), and C-6$_{glcI}$ (69.0 ppm). The absence of any ^{13}C glycosidation shift for digitalopyranosyl and glucopyranosyl moieties suggested that these sugars were terminal units. The exact sugar sequence was obtained by HMBC cross-peaks that showed correlation between H-1$_{cym}$−C-3, H-1$_{digI}$−C-$_{4cym}$, H-1$_{digII}$−C-20, H-1$_{glcI}$−C-2$_{digII}$, and H-1$_{glcII}$−C-6$_{glcI}$. The configuration of the sugar units was determined as reported for compound **1**. Thus, **5** was identified as 20-*O*-[(*β*-D-glucopyranosyl-(1→6)-*β*-D-glucopyranosyl-(1→2)-*β*-D-digitalopyranosyl)-oxy]pregn-5-en-16*β*,20-diol-3*β*-yl *O*-*β*-D-digitalopyranosyl-(1→4)-*β*-D-cymaropyranoside. The isomer at C-16 of **5** was previously isolated from *P. sepium*.[6]

Compound **6** showed the molecular formula $C_{34}H_{56}O_{10}$ unequivocally established by ESIMS (pseudomolecular ion peak at *m/z* 1271 $[2M + Na]^+$), NMR data, and elemental analysis. It showed two anomeric protons and carbons (Table 3). The structure of the sugar unit was determined by NMR data as one terminal *β*-D-digitalopyranose and one terminal *β*-D-canaropyranose, and their configurations were elucidated in the same manner as compound **1**. The aglycon was identified as calogenin from a careful analysis of its NMR spectra and by comparison with literature data.[8,9] Attachment of the digitalose residue at C-3 was indicated by the significant downfield shift observed for this carbon in **6** relative to the corresponding signal in calogenin and was subsequently confirmed from a long-range correlation between C-3 (δ 79.7) and H-1$_{dig}$ (δ 4.34) in the HMBC spectrum. In the same way, the *β*-D-canaropyranose moiety was located at C-20 from the long-range correlation between C-20 (δ 83.4) and H-1$_{can}$ (δ 4.67). Thus, the structure of **6** was established as calogenin 3-*O*-*β*-D-digitalopyranoside-20-*O*-*β*-D-canaropyranoside.

Three known pregnane glycosides, 20-*O*-[(*β*-D-glucopyranosyl-(1→6)-*β*-D-glucopyranosyl-(1→2)-*β*-D-digitalopyranosyl)oxy]pregn-5-en-16*β*-ol-3*β*-yl *O*-[(2-*O*-acetyl)-*β*-D-digitalopyranosyl-(1→4)-*β*-D-cymaropyranoside (**7**), 20-*O*-[(*β*-D-glucopyranosyl-(1→6)-*β*-D-glucopyranosyl-(1→2)-*β*-D-digitalopyranosyl)oxy]pregn-5-en-16α-ol-3*β*-yl *O*-[(2-*O*-acetyl)-*β*-D-digitalopyranosyl-(1→4)-*β*-D-cymaropyranoside (**8**), and 20-*O*-[(*β*-D-glucopyranosyl-(1→6)-*β*-D-glucopyranosyl-(1→2)-*β*-D-digitalopyranosyl)oxy]pregn-5-en-3*β*-yl *O*-[(2-*O*-acetyl)-*β*-D-digitalopyranosyl-(1→4)-*β*-D-cymaropyranoside (**9**), were isolated and identified on the basis of their spectroscopic data and by comparison with those reported in the literature.[10]

Glycosides containing sulfated residues have been identified previously in the plant kingdom,[11] but the occurrence of sulfated glycosides as pregnane derivatives is a very unusual finding.

Chart 1



Experimental Section

General Experimental Procedures. Optical rotations were measured on a Perkin-Elmer 241 polarimeter equipped with a sodium lamp (589 nm) and a 1 dm microcell. Elemental analyses were obtained using a Carlo Erba 1106 elemental analyzer and from a JEOL JSM-5600LV electron microscope, using the attached energy-dispersive X-ray spectrometer (EDS). IR spectra were recorded on a Mattson 3000 FTIR spectrometer with NaCl pellets. A Bruker DRX-600 NMR spectrometer using the UXNMR software package was used for NMR experiments.[12] HRESIMS spectra were obtained from a Bruker ESI microTOF spectrometer, while ESIMS spectra were obtained from a LCQ Advantage ThermoFinnigan spectrometer, equipped with Xcalibur software. MPLC separations were carried out on a Pye Unicam PU4010 pumping system; HPLC separations were conducted on a Shimadzu LC-8A series pumping system equipped with a Waters R401 refractive index detector and a Shimadzu injector.

Plant Material. The small branches of *P. graeca* were collected at Marina di Vecchiano (Pisa Province), Italy, on June 2001 and identified by Dr. A. Bader, Al-Zaytoonah Private University of Jordan, Amman. A voucher specimen (no. 04/6733) is deposited at Herbarium Horti Botanici Pisani (Nuove Acquisizioni), Pisa, Italy.

Extraction and Isolation. The dried, powdered small branches of *P. graeca* (480 g) were defatted with *n*-hexane and successively extracted for 48 h with $CHCl_3$, $CHCl_3$–MeOH (9:1), and MeOH, by exhaustive maceration (3 × 2 L), to give 6.7, 8.6, and 16.0 g of the respective residues. The MeOH extract was partitioned between *n*-BuOH and H_2O, to afford a *n*-BuOH-soluble portion (4.0 g). The *n*-BuOH residue was submitted to a Sephadex LH-20 column using MeOH as eluent to obtain six major fractions (A–F) by TLC results on silica 60 F_{254} gel-coated glass sheets with *n*-BuOH–AcOH–H_2O (60:15:25) and $CHCl_3$–MeOH–H_2O (40:9:1). Fraction A (100 mg) was purified by reversed-phase HPLC on a C_{18} μ-Bondapak column (30 cm × 7.8 mm, flow rate 2.0 mL min^{-1}) with MeOH–H_2O (2.9:2.1) to afford pure compounds **2** (11.0 mg) and **1** (18.0 mg). The $CHCl_3$–MeOH residue (8.0 g) was chromatographed on Sephadex LH-20 using MeOH as eluent; fractions of 8 mL were collected and grouped into eight major fractions (1–8), in the same way as the *n*-BuOH extract. Fraction 1 (170 mg) was purified by reversed-phase HPLC on a C_{18} μ-Bondapak column (30 cm × 7.8 mm, flow rate 2.0 mL min^{-1}) with MeOH–H_2O (7:3) to yield pure compounds **5** (4.0 mg), **7** (8.0 mg), and **8** (7.5 mg). Fraction 3 (450 mg) was purified by reversed-phase MPLC over C_8 Si gel (Lichroprep RP-8, 40–63 μm, 2.6 × 46 cm), using as eluent MeOH–H_2O mixtures (flow rate 1.5 mL min^{-1}). The chromatography was carried out at increasing polarity starting from MeOH–H_2O (1:4), followed by MeOH–H_2O (3:7), MeOH–H_2O (2:3), MeOH–H_2O (1:1), MeOH–H_2O (3:2), MeOH–H_2O (7:3), and finally MeOH–H_2O (4:1), yielding compound **3** (14 mg) together with four major groups (1–4). Group 2 (120 mg) was successively chromatographed over reversed-phase HPLC on a C_{18} μ-Bondapak column (30 cm × 7.8 mm, flow rate 2.0 mL min^{-1}) with MeOH–H_2O (4.5:5.5) to give pure compound **4** (7.0 mg). Group 3 (80 mg) was purified by reversed-phase HPLC on a C_{18} μ-Bondapak column (30 cm × 7.8 mm, flow rate 2.0 mL min^{-1}) with MeOH–H_2O (7.5:2.5) to give pure compound **9** (10.0 mg). Finally, the $CHCl_3$ extract was purified by a flash silica gel column (6.0 × 16.0 cm), eluting with $CHCl_3$ followed by increasing concentrations of MeOH (between 1% and 100%) in $CHCl_3$. Fractions of 30 mL were collected, analyzed by TLC (silica gel plates, in $CHCl_3$ or $CHCl_3$–MeOH (99:1, 98:2, 97:3, 9:1, 8:2)), and grouped into 10 fractions. Fraction 5 (90 mg) was subjected to reversed-phase HPLC on a C_{18} μ-Bondapak column (30 cm × 7.8 mm, flow rate 2.0 mL min^{-1}) with MeOH–H_2O (6.5:3.5) to yield pure compound **6** (12 mg).

Compound 1: white solid; $[\alpha]_D^{25}$ −37° (*c* 0.1, MeOH); IR (NaCl) ν_{max} 3450, 2903, 1732, 1643, 1253, 1220, 1076 cm^{-1};

Table 3. ^{1}H and ^{13}C NMR Data for Sugar Moieties of Compounds **5** and **6** (CD_3OD, 600 MHz)[a]

	5		**6**	
position	δ_H	δ_C	δ_H	δ_C
D-Cym 1	4.90 dd (9.5, 2.0)	96.7		
2a	1.56 br dd (16.0, 12.0)	36.2		
2b	2.08 br dd (16.0, 3.5)			
3	3.87 q (3.5)	78.4		
4	3.30 dd (9.5, 3.5)	83.5		
5	3.85 dq (9.5, 6.0)	68.5		
6	1.30 d (6.0)	16.5		
-OMe	3.46 s	56.8		
D-Dig I 1	4.32 d (8.0)	106.1	4.34 d (8.0)	102.7
2	3.54 dd (9.5, 8.0)	71.8	3.51 dd (9.0, 8.0)	72.0
3	3.15 dd (9.5, 4.0)	84.3	3.15 dd (9.0, 3.5)	84.5
4	3.85 dd (4.0, 1.5)	68.5	3.85 dd (3.5, 1.5)	68.4
5	3.64 dq (6.5, 1.5)	71.6	3.63 dq (6.0, 1.5)	71.5
6	1.82 d (6.5)	16.5	1.30 d (6.0)	16.5
-OMe	3.47 s	58.0	3.49 s	57.0
D-Dig II 1	4.42 d (8.5)	104.2		
2	3.94 dd (9.5, 8.5)	76.3		
3	3.43 dd (9.5, 3.5)	85.3		
4	3.90 dd (3.5, 1.5)	70.1		
5	3.61 dq (6.0, 1.5)	71.4		
6	1.34 d (6.5)	16.6		
-OMe	3.47 s	58.0		
D-Glc I 1	4.78.d (8.0)	103.8		
2	3.23 dd (9.5, 8.0)	75.0		
3	3.36 t (9.5)	78.0		
4	3.32 t (9.5)	71.5		
5	3.43 m	77.0		
6a	3.78 dd (12.0, 5.0)	69.0		
6b	4.21 dd (12.0, 3.5)			
D-Glc II 1	4.50 d (8.5)	104.2		
2	3.24 dd (9.5, 8.5)	75.1		
3	3.36 t (9.5)	78.1		
4	3.31 t (9.5)	71.6		
5	3.50 m	77.6		
6a	3.89 dd (12.0, 3.5)	62.7		
6b	3.67 dd (12.0, 5.0)			
D-Can 1			4.67 dd (9.0, 1.5)	102.5
2a			1.53 m	40.6
2b			2.19 m	
3			3.51 m	71.0
4			2.91 t (9.5)	78.4
5			3.27 dq (9.5, 6.0)	73.0
6			1.29 d (6.0)	17.4

[a] *J* values are in parentheses and reported in Hz; chemical shifts are given in ppm; assignments were confirmed by DQF-COSY, 1D-TOCSY, HSQC, and HMBC experiments.

^{1}H and ^{13}C NMR, see Tables 1 and 2; ESIMS *m/z* 921 $[M]^-$, 861 $[M - 60]^-$, 719 $[M - 60 - 142]^-$, 557 $[M - 60 - 142 - 144 - 18]^-$; HRESIMS *m/z* 921.4148 (calcd for $C_{43}H_{69}O_{19}S$ 921.4154).

Compound 2: white solid; $[\alpha]_D^{25}$ +32° (*c* 0.1, MeOH); IR (NaCl) ν_{max} 3453, 2890, 1736, 1640, 1251, 1218, 1077 cm^{-1}; NMR data for the aglycon moiety are identical to those of compound **1**; ^{1}H and ^{13}C NMR data of the sugar moiety, see Table 2; ESIMS *m/z* 863 $[M]^-$, 719 $[M - 144]^-$, 557 $[M - 144 - 144 - 18]^-$, 241 $[glc - 6 - SO_3]^-$; HRESIMS *m/z* 863.4093 (calcd for $C_{41}H_{67}O_{17}S$ 863.4099).

Compound 3: white solid; $[\alpha]_D^{25}$ −17° (*c* 0.1, MeOH); IR (NaCl) ν_{max} 3460, 2900, 1732, 1643, 1456, 1377, 1253, 1221, 1163, 1078, 1014 cm^{-1}; NMR data for the aglycon moiety are superimposable on those of compound **1**; ^{1}H and ^{13}C NMR data of the sugar moiety, see Table 2; ESIMS *m/z* 719 $[M]^-$, 557 $[M - 144 - 18]^-$; HRESIMS *m/z* 719.3307 (calcd for $C_{34}H_{55}O_{14}S$ 719.3313).

Compound 4: white powder; $[\alpha]_D^{25}$ −20° (*c* 0.1, MeOH); IR (NaCl) ν_{max} 3455, 2900, 1734, 1643, 1252, 1221, 1077 cm^{-1}; NMR data for the aglycon moiety are identical to those of compound **1**; ^{1}H NMR of the sugar moiety (CD_3OD, 600 MHz) δ 3.17 (1H, dd, J = 9.5, 8.0 Hz, H-2), 3.30 (1H, t, J = 9.5 Hz, H-4), 3.37 (1H, t, J = 9.5 Hz, H-3), 3.42 (1H, m, H-5), 4.12 (1H, dd, J = 12.0, 5.0 Hz, H-6a), 4.26 (1H, d, J = 8.0 Hz, H-1), 4.35 (1H, dd, J = 12.0, 3.5 Hz, H-6b); ^{13}C NMR of the sugar moiety (CD_3OD, 600 MHz) δ 68.5 (C-6), 71.7 (C-4), 74.9 (C-2), 75.9 (C-5), 77.8 (C-3), 101.9 (C-1); ESIMS *m/z* 575 $[M]^-$, 241 $[glc - 6 - SO_3]^-$; HRESIMS *m/z* 575.2521 (calcd for $C_{27}H_{43}O_{11}S$ 575.2526).

Compound 5: white solid; $[\alpha]_D^{25}$ −10° (*c* 0.05, MeOH); *anal*. C 57.70%, H 8.10%, calcd for $C_{54}H_{90}O_{24}$, C 57.74%, H 8.08%; ^{1}H and ^{13}C NMR, see Tables 1 and 3; ESIMS *m/z* 1146 $[M + Na]^+$, 984 $[M + Na - 162]^+$, 822 $[M + Na - 162 - 162]^+$, 643 $[M + Na - 162 - 162 - 160 - 18]^+$.

Compound 6: white powder; $[\alpha]_D^{25}$ −24° (*c* 0.1, MeOH); *anal*. C 65.40%, H 9.01%, calcd for $C_{34}H_{56}O_{10}$, C 65.36%, H 9.03%; ^{1}H and ^{13}C NMR, see Tables 1 and 3; ESIMS *m/z* 1279 $[2M + Na]^+$.

Solvolysis of Sulfated Compounds 1−4. A solution of each compound (5 mg) in dioxane (1 mL) and pyridine (1 mL) was heated at 130 °C for 5 h in a stoppered reaction vial. The residue, which was obtained by evaporation of the solvents to dryness under reduced pressure, was partitioned between H_2O and $CHCl_3$ (1:1).[7] The aqueous solution was dried under reduced pressure, affording the desulfated new, related compounds.

Acid Hydrolysis of Compounds 1−6. A solution of compounds **1**−**6** (2.0 mg each) in 1 N HCl (1 mL) was stirred at 80 °C in a stoppered reaction vial for 4 h. After cooling, the solution was evaporated under a stream of N_2. Each residue was dissolved in 1-(trimethylsilyl)imidazole and pyridine (0.2 mL), and the solution was stirred at 60 °C for 5 min. After drying the solution, the residue was partitioned between water and $CHCl_3$. The $CHCl_3$ layer was analyzed by GC using a 1-Chirasil-Val column (0.32 mm × 25 m). Temperatures of the injector and detector were 200 °C for both. A temperature gradient system was used for the oven, starting at 100 °C for 1 min and increasing up to 180 °C at a rate of 5 °C/min. Peaks of the hydrolysate were detected by comparison with retention times of authentic samples after treatment with 1-(trimethylsilyl)imidazole in pyridine.

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