Folotsoside A, a New Pregnane Glycoside from Folotsia sarcostemmoides

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J. Nat. Prod., **1991**, 54 (6), 1672-1676• DOI: 10.1021/np50078a031 • Publication Date (Web): 01 July 2004

Journal of Natural Products
Vol. 54, No. 6, pp. 1672-1676, Nov-Dec 1991

FOLOTSOSIDE A, A NEW PREGNANE GLYCOSIDE FROM *FOLOTSIA SARCOSTEMMOIDES*

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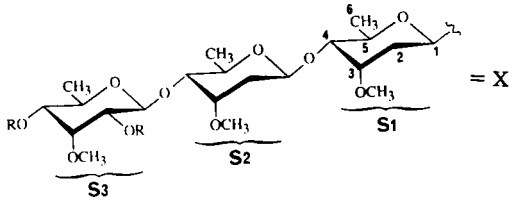
ABSTRACT.—From the aerial parts of *Folotsia sarcostemmoides*, a new pregnane ester triglycoside designated as folotsoside A [**1**] was isolated, accompanied by paeonol (2-hydroxy-4-methylacetophenone). The structure of folotsoside A was established as 12-*O*-benzoyl-lineolon 6-desoxy-3-*O*-methyl-β-D-allopyranosyl-(1→4)-β-D-cymaropyranosyl-(1→4)-β-D-cymaroside on the basis of chemical and spectroscopic evidence.

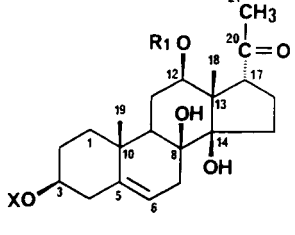
Folotsia sarcostemmoides Constantin et Bois (Asclepiadaceae) is an endemic climbing plant growing in the southern region of Madagascar, in the Reserve Naturelle of Beza-Mahafaly (1). This plant was chosen for study because of its use as a Malagasy traditional herbal remedy for the treatment of respiratory infections, constipation, fever and rickets, among other ailments (1,2). The present paper describes the structure elucidation of a new pregnane ester triglycoside, folotsoside A [**1**], that was obtained from the aerial parts of *F. sarcostemmoides*. Prior to our work, no previous laboratory investigations on this species have been reported.

RESULTS AND DISCUSSION

The molecular formula of **1** was determined as $C_{49}H_{72}O_{16}$ from its fabms and ^{13}C-nmr spectral data. The presence of a triglycosyl moiety was suggested by the observation of anomeric chemical shifts at δ 4.52, 4.69, and 4.79 in the ^1H-nmr spectrum and at δ 96.2, 99.7, and 102.3 in the ^{13}C-nmr spectrum of compound **1**.

Acid hydrolysis of **1** resulted in the production of the aglycone **2** and two different sugars. The presence of a benzoyl unit in **2** was evident from its uv (λ max 230, 273 nm), eims (*m/z* 105), and ^{13}C-nmr (δ 128.3, 129.5, 130.1, 132.9, and 165.2) spectral data. From the interpretation of its ^{13}C-nmr spectrum it was considered that the basic skeleton of **1** was composed of twenty-one carbons, indicating that the molecule was a pregnane benzoyl ester triglycoside. Furthermore, ^{13}C-nmr data showed that **2** contained four hydroxyl groups, a double bond, and a keto group (acetyl group) in its structure.





1 R=H, R_1=Bz
3 R=Ac, R_1=Bz

2 X=H, R_1=Bz
4 X=H, R_1=H

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A number of ring C/D-*cis* polyoxypregnane derivatives have been isolated from the plants of the Asclepiadaceae (3–

9), Ranunculaceae (10), and Scrophulariaceae (11) plant families, and their ^{13}C-nmr spectral parameters have been studied (5–7, 12). By reference to published ^{13}C-nmr data, it was noticed that the position of the double bond in **2** was at Δ^5 (δ 117.4 and 141.1) and that an acetyl group at C-17 was in the α configuration (δ 32.0 and 209.7) (5–7, 12). The position of a hydroxyl group, to which a benzoyl unit was attached, was deduced as occurring at C-12 in the β configuration, because the chemical shift at δ 4.93 was observed as a doublet of doublets (J = 11.1, 4.8 Hz) in the ^{1}H-nmr spectrum of **2**. Also, characteristic acylation shifts (13,14) were observed when the ^{13}C-nmr spectra of **2** and lineolon [**4**] (deacylcynanchogenin) were compared (i.e., C-12 was shifted by +4.5 ppm, C-11 by −3.6 ppm, and C-13 by −1.0 ppm) (12,13). Thus, aglycone **2** was identified as 12-*O*-benzoyl-lineolon (4,10).

From the ^{13}C-nmr spectrum of **1**, the number of carbons belonging to the sugar units was determined as twenty-one, including three methyl groups (δ 18.0, 18.3, 18.6), three methoxyl groups (δ 58.0, 58.1, 62.2), and two methylene carbons (δ 35.2, 35.7). From this information, it was apparent that two of the sugars were of the 2,6-didesoxy-3-*O*-methyl type and the other was a 6-desoxy-3-*O*-methyl sugar. In the ^{1}H-nmr spectrum, the coupling constants of the three anomeric protons [δ 4.52 (d, J = 7.8 Hz), 4.69 (dd, J = 8.1, 1.6 Hz), 4.79 (dd, J = 8.4, 1.3 Hz)] suggested that these three sugar units were all in the β-D form. The positive fabms of **1** exhibited a base peak at *m/z* 759, indicating that its terminal saccharide unit (S3) was the 6-desoxy-3-*O*-methyl sugar. A homo-decoupling experiment (360 MHz) was applied to determine the structure of this terminal sugar. Thus, irradiation of the anomeric proton (δ 4.59, d, J = 8.0 Hz) transformed H-2 to a broad singlet. The H-3 proton (δ 3.78, dd, J = 3.2, 3.0 Hz) was changed to a doublet (J = 3.0 Hz) by irradiation of H-2, and H-4 at δ 3.27 (dd, J = 9.6, 2.8 Hz) became a doublet (J = 8.5 Hz) when H-3 was irradiated. When the methyl proton at δ 1.28 (d, J = 6.1 Hz) was irradiated, the H-5 signal (δ 3.91, dq, J = 9.5, 6.2 Hz) was simplified as a doublet (J = 9.3 Hz). Finally, irradiation of H-5 converted H-4 to a doublet (J = 1.6 Hz). Consequently, the terminal sugar (S3) was deduced as 6-desoxy-3-*O*-methyl-β-D-allopyranose. This assignment was supported by consideration of the ^{13}C-nmr chemical shifts of its acetate **3**, that is, typical acylation shifts (2–3 ppm upfield shifts of neighboring carbons) (14) were observed at C-1, C-3, and C-5 of S3 as a result of the presence of two acetyl groups (2-OAc and 4-OAc).

The inner sugars (S1 and S2) were both determined as β-D-cymaropyranose by the interpretation of their proton coupling constants, homo-decoupling data and selective INEPT experiments (15). In the latter context, irradiation ($^3J_{CH}$ = 6 Hz) of three anomeric protons at δ 4.52, 4.69, and 4.79 resulted in selective enhancements, in turn, of carbons at δ 82.7 (S2-4 or S1-4), 82.6 (S1-4 or S2-4), and 78.0 (C-3). Analogous irradiation ($^3J_{CH}$ = 4 Hz) of the three methoxy protons at δ 3.36, 3.39, and 3.59 selectively enhanced the signals at δ 77.1 (S1-3 or S2-3), 77.0 (S2-3 or S1-3), and 80.8 (S3-3), respectively. In a similar manner, irradiation of the three methyl protons at δ 1.16, 1.20, and 1.22 enhanced, respectively, carbon peaks at δ 82.6 (S1-4 or S2-4) and 68.5 (S1-5 or S2-5), 82.7 (S2-4 or S1-4) and 68.6 (S2-5 or S1-5), and 72.9 (S3-4) and 70.8 (S3-5). It has been known that certain Asclepiadaceae and Apocynaceae plants contain cymarose as a sugar constituent of steroidal or pregnane glycosides (5–7, 9, 11, 16, 17), and ^{13}C-nmr data of their cymarosides have been reported (5–7, 16, 17). The chemical shifts of two inner sugars (S1 and S2) were consistent with such ^{13}C data. Therefore, the structure of

compound **1** was established as 12-*O*-benzoyl-lineolon 6-desoxy-3-*O*-methyl-β-D-allopyranosyl-(1→4)-β-D-cymaropyranosyl-(1→4)-β-D-cymaroside.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Melting points (uncorrected) were determined on a Kofler hot-stage apparatus. Optical rotations were taken with Perkin-Elmer model 141 and 241 polarimeters. Uv spectra were obtained on a Beckman DU-7 spectrometer, and ir spectra on a Nicolet MX-1 interferometer. ^{1}H-nmr and ^{13}C-nmr spectra were measured in $CDCl_3$ with TMS as internal standard, employing a Varian XL-300 instrument operating at 300 MHz and 75.6 MHz, respectively. ^{1}H-^{1}H COSY and ^{1}H-^{13}C HETCOR nmr experiments were also performed on the Varian XL-300, using standard Varian pulse sequences. Selective INEPT nmr experiments were conducted on a Nicolet NT-360 spectrometer (90.8 MHz). Mass spectra were measured on a Varian MAT 112S double-focusing mass spectrometer at either 70 or 20 eV. Fabms were obtained using a Finnegan MAT 90 instrument.

PLANT MATERIAL.—Aerial parts of *F. sarcostemmoides* were collected by one of us (PR) in the Southern region of Madagascar in June 1989. Herbarium specimens representing this collection are deposited in the Institut Malgache de Recherches Appliquées, Antananarivo, Madagascar.

EXTRACTION AND ISOLATION.—The air-dried and powdered plant material (500 g) was extracted by maceration with MeOH to afford a crude extract (38.8 g). A portion (35 g) was chromatographed on a column of Si gel (1 kg) using $CHCl_3$ and $CHCl_3$ containing increased amounts of MeOH as eluents. Elution with 1% MeOH in $CHCl_3$ afforded paeonol (2-hydroxy-4-methoxyacetophenone) (4.1 g, 0.82% w/w). Elution with 3% MeOH in $CHCl_3$ gave 18 fractions (300 ml each) showing similar tlc profiles. These combined fractions (8.5 g) were subjected to additional cc over Si gel (300 g), eluted with EtOAc/MeOH mixtures of increasing polarity. Elution with 3% MeOH in EtOAc afforded pure folotsoside A [**1**] (0.76 g, 0.152% w/w) which crystallized from MeOH/Et_2O.

ACID HYDROLYSIS OF FOLOTSOSIDE A [**1**].—Compound **1** (300 mg) was dissolved in EtOH (2 ml) and hydrolyzed by reflux with 2 N H_2SO_4 (40 ml) for 1 h. The solution was diluted with H_2O (100 ml) and extracted with $CHCl_3$ (100 ml × 3) to afford a residue which was chromatographed over Si gel (50 g) with petroleum ether-Me_2CO (1:1) as eluent to give compound **2** (100 mg). The aqueous phase was neutralized by $BaCO_3$, and two distinct sugar spots were observed by tlc.

CHARACTERIZATION OF COMPOUNDS **1**–**3**.—*Folotsoside A* [**1**].—Mp 209° (colorless prisms, MeOH/Et_2O); $[\alpha]^{20}D$ +19.0° (*c* = 1.0, $CHCl_3$); uv λ max (EtOH) (log ε) 230 (4.09), 273 (2.85) nm; ir ν max (KBr) 3500, 1720 (ester) cm^{-1}; ^{1}H nmr (300 MHz, $CDCl_3$) δ 1.06 (3H, s, Me-19), 1.16 (3H, d, *J* = 6.3 Hz, Me), 1.20 (3H, d, *J* = 6.0 Hz, Me), 1.22 (3H, d, *J* = 6.3 Hz, S3-Me), 1.59 (3H, s, Me-18), 1.97 (3H, s, Me-20), 3.36 (3H, s, OMe), 3.39 (3H, s, OMe), 3.59 (3H, s, S3-OMe), 3.69 (1H, dd, *J* = 3.0, 3.0 Hz, S3 H-3), 4.52 (1H, d, *J* = 7.8 Hz, S3 H-1), 4.69 (1H, dd, *J* = 8.1, 1.6 Hz, S2 H-1), 4.79 (1H, dd, *J* = 8.4, 1.3 Hz, S1 H-1), 4.88 (1H, dd, *J* = 11.1, 4.8 Hz, H-12), 5.30 (1H, br s, H-6), 7.36 (2H, dd, *J* = 7.8, 7.2 Hz, H-4′), 7.48 (1H, dd, *J* = 7.5, 7.2 Hz, H-5′), 7.90 (2H, d, *J* = 8.4 Hz, H-3′), (360 MHz, $CDCl_3$) sugar moiety δ 2.10 (1H, m, S1 H-2), 2.17 (1H, m, S2 H-2), 3.18–3.24 (2H, m, S1 H-4, S2 H-4), 3.27 (1H, dd, *J* = 9.6, 2.8 Hz, S3 H-4), 3.47–3.58 (3H, m, H-3, S1 H-5 or S2 H-5, S3 H-2), 3.78 (1H, dd, *J* = 3.2, 3.0 Hz, S3 H-3), 3.80 (1H, br d, *J* = 3.3 Hz, S1 H-3), 3.82 (1H, br d, *J* = 4.0 Hz, S2 H-3), 3.85 (1H, dq, *J* = 9.6, 6.2 Hz, S2 H-5 or S1 H-5), 3.91 (1H, dq, *J* = 9.5, 6.2 Hz, S3 H-5), 4.59 (1H, d, *J* = 8.0 Hz, S3 H-1), 4.76 (1H, dd, *J* = 9.7, 1.6 Hz, S2 H-1), 4.85 (1H, dd, *J* = 8.1, 1.3 Hz, S1 H-1); ^{13}C nmr see Table 1; eims *m/z* (rel. int.) 346 (genin) (8), 328 (14), 161 (21), 145 (49), 113 (48), 97 (82), 43 (100); negative fabms *m/z* (rel. int.) $[M-H]^-$ 915 (100); positive fabms *m/z* (rel. int.) $[M-sugar]^+$ 759 (100).

12-O-Benzoyl-lineolon [**2**].—White powder; $[\alpha]^{20}D$ −48.0° (*c* = 0.1, $CHCl_3$); uv λ max (EtOH) (log ε) 230 (4.13), 273 (2.87) nm [lit. (4) $[\alpha]D$ −62.6° (MeOH); uv λ max (EtOH) (log ε) 230 (4.13), 273 (3.06) nm]; ir ν max (KBr) 3460, 1720 (ester) cm^{-1}; ^{1}H nmr (300 MHz, $CDCl_3$) δ 1.11 (3H, s, Me-19), 1.64 (3H, s, Me-18), 2.00 (3H, s, Me-20), 3.56 (1H, m, H-3), 4.93 (1H, dd, *J* = 11.1, 4.8 Hz, H-12), 5.35 (1H, br s, H-6), 7.41 (2H, dd, *J* = 7.8, 7.2 Hz, H-4′), 7.53 (1H, dd, *J* = 7.5, 7.2 Hz, H-5′), 7.95 (2H, d, *J* = 7.8 Hz, H-3′); ^{13}C nmr see Table 1; eims *m/z* (rel. int.) 346 (genin) (23), 105 (99), 43 (100).

Folotsoside A acetate [**3**].—Compound **1** (300 mg) was acetylated overnight with 5 ml of Ac_2O-C_5H_5N (1:1) at room temperature to afford compound **3** (310 mg): mp 198° (colorless prisms, MeOH/Et_2O); $[\alpha]^{20}D$ −20.0° (*c* = 0.4, $CHCl_3$); uv λ max (EtOH) (log ε) 230 (4.00), 273 (2.82) nm; ir ν max (KBr) 3460, 1750 (acetate), 1720 (ester) cm^{-1}; ^{1}H nmr (300 MHz, $CDCl_3$) δ 1.07 (3H, s, Me-19), 1.13 (3H, d, *J* = 6.3 Hz, Me),

TABLE 1. ^{13}C-nmr Spectra of Compounds **1–4**.[a]

Carbon	Compound			
	1[b]	**2**[b]	**3**[b]	**4**[c]
C-1 (S1-1)	38.9 t (96.2 d)	38.7 t	38.8 t (96.1 d)	38.2
C-2 (S1-2)	29.0 t (35.7 t)[d]	30.7 t	28.9 t (35.6 t)[d]	30.8
C-3 (S1-3)	78.0 d (77.1 d)[d]	71.8 d	77.9 d (77.0 d)[d]	70.3
C-4 (S1-4)	38.9 t (82.6 d)[d]	41.9 t	38.8 t (82.5 d)[d]	42.1
C-5 (S1-5)	141.2 s (68.5 d)[d]	141.1 s	141.2 s (68.5 d)[d]	139.1
C-6 (S1-6)	117.5 d (18.6 q)[d]	117.4 d	117.4 d (18.2 q)[d]	117.9
C-7 (S1-3-OMe)	34.6 t (58.1 q)[d]	34.4 t	34.5 t (58.2 q)[d]	34.2
C-8 (S2-1)	74.8 s (99.7 d)	74.8 s	74.8 s (99.6 d)	73.3
C-9 (S2-2)	44.1 d (35.2 t)[d]	43.9 d	44.0 d (35.5 t)[d]	43.7
C-10 (S2-3)	37.4 s (77.0 d)[d]	37.1 s	37.3 s (77.4 d)[d]	36.3
C-11 (S2-4)	24.4 t (82.7 d)[d]	24.3 t	24.3 t (83.6 d)[d]	27.9
C-12 (S2-5)	72.5 d (68.6 d)[d]	72.3 d	72.4 d (68.1 d)[d]	67.8
C-13 (S2-6)	55.5 s (18.3 q)[d]	55.4 s	55.4 s (18.0 q)[d]	56.4
C-14 (S2-3-OMe)	86.8 s (58.0 q)[d]	86.6 s	86.6 s (58.0 q)[d]	86.3
C-15 (S3-1)	33.5 t (102.3 d)	33.3 t	33.4 t (99.9 d)	33.4
C-16 (S3-2)	21.7 t (73.1 d)	21.5 t	21.6 t (73.8 d)	20.8
C-17 (S3-3)	59.8 d (80.8 d)	59.7 d	59.7 d (76.6 d)	60.0
C-18 (S3-4)	15.1 q (72.9 d)	15.0 q	15.0 q (72.4 d)	13.8
C-19 (S3-5)	18.9 q (70.8 d)	18.9 q	18.8 q (67.5 d)	17.8
C-20 (S3-6)	209.8 s (18.0 q)[d]	209.7 s	209.6 s (17.5 q)	209.3
C-21 (S3-3-OMe)	32.1 q (62.2 q)	32.0 q	31.9 q (61.5 q)	31.4
C-1′ (S3-2-OCOMe)	165.3 s	165.2 s	165.1 s (169.4 s)[d]	
C-2′ (S3-2-OCO*Me*)	130.3 s	130.1 s	130.2 s (20.8 q)	
C-3′ (S3-4-OCOMe)	129.6 d	129.5 d	129.5 d (169.7 s)[d]	
C-4′ (S3-4-OCO*Me*)	128.4 d	128.3 d	128.3 d (20.8 q)	
C-5′	133.0 d	132.9 d	132.9 d	

[a]Measured at 75.6 MHz, δ TMS = 0.
[b]Obtained in $CDCl_3$.
[c]Values for this compound are from Yamagishi *et al.* (12). Obtained in DMSO-d_6.
[d]May be reversed with a close signal in the same column.

1.16 (6H, d, J = 6.0 Hz, Me, S3-Me), 1.61 (3H, s, Me-18), 1.98 (3H, s, Me-20), 2.03 (3H, s, OAc), 2.05 (3H, s, OAc), 3.38 (3H, s, OMe), 3.40 (3H, s, OMe), 3.42 (3H, s, OMe), 3.51 (1H, m, H-3), 4.53 (1H, dd, J = 9.6, 2.8 Hz, S3 H-4), 4.68 (1H, dd, J = 9.5, 2.7 Hz, S3 H-2), 4.72 (1H, br d, J = 8.1 Hz, S2 H-1), 4.75 (1H, d, J = 8.0 Hz, S3 H-1), 4.80 (1H, br d, J = 8.1 Hz, S1 H-1), 4.90 (1H, dd, J = 11.0, 4.6 Hz, H-12), 5.32 (1H, br s, H-6), 7.38 (2H, dd, J = 7.8, 7.2 Hz, H-4′), 7.50 (1H, dd, J = 7.6, 7.2 Hz, H-5′), 7.93 (2H, d, J = 7.8 Hz, H-3′); ^{13}C nmr see Table 1.

Paeonol (2-hydroxy-4-methoxyacetophenone) was obtained as a colorless oil and identified by comparison of its spectroscopic (uv, ir, ^{1}H-nmr, ^{13}C-nmr, ms) data with published values (18–20).

ACKNOWLEDGMENTS

PR was awarded a fellowship under the Fulbright Program. We thank the Nuclear Magnetic Resonance and Mass Spectrometry Laboratories of the Research Resources Center, University of Illinois at Chicago, for expert assistance and for the provision of spectroscopic equipment used in this study. We thank Mr. R.B. Dvorak, of the Department of Medicinal Chemistry and Pharmacognosy, University of Illinois at Chicago, for recording the fabms spectral data. We are also grateful to Mr. L.B.S. Kardono for running the selective INEPT nmr experiments.

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Received 16 May 1991