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POLYHYDROXYLATED TRITERPENES FROM *SENECIO PSEUDOTITES*

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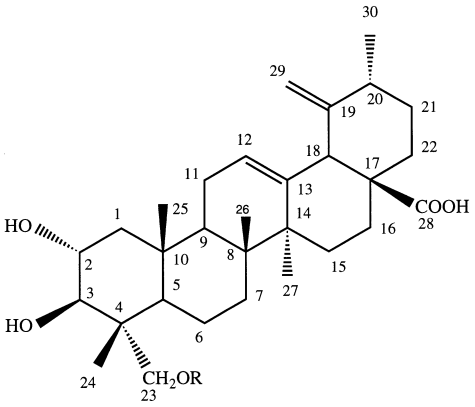
Key Word Index—*Senecio pseudotites*; Asteraceae; triterpenes; 2α,3β,23-trihydroxy-urs-12,19(29)-dien-28-oic-acid; 2α,3β,23-trihydroxy-urs-12,19(29)-dien-28-oic-acid-23-acetyl ester; 23-(*trans-p*-coumaroyloxy)-2α,3β,-dihydroxy-urs-12,19(29)-dien-28-oic-acid.

Abstract—Three new polyhydroxylated triterpenes were isolated from the chloroform-methanol extract of the leaves of *Senecio pseudotites*. Their structures were elucidated as 2α,3β,23-trihydroxy-urs-12,19(29)-dien-28-oic-acid; 2α,3β,23-trihydroxy-urs-12,19(29)-dien-28-oic-acid-23-acetyl ester; 23-(*trans-p*-coumaroyloxy)-2α,3β-dihydroxy-urs-12,19(29)-dien-28-oic-acid by NMR spectroscopy. © 1998 Elsevier Science Ltd. All rights reserved

INTRODUCTION

The leaves of *Senecio pseudotites* Griseb. have many traditional uses in South American popular medicine as a diuretic, antiashmatic and vermifugal drug [1]. In the course of our research on South American Asteraceae, (in a previous work) we described the isolation of chalcones from the methanol extract of the plant [2]. Here we report on the isolation and structural determination of three polyhydroxylated triterpenes (compounds **1**–**3**) of the ursane series with a novel 2α,3β,23-trihydroxy-12,19(29)-dien-28-oic acid skeleton from the chloroform–methanol (9:1) extract.



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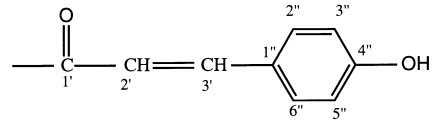
2 R = -COCH$_3$

3 R = 

RESULTS AND DISCUSSION

Compounds **1**–**3** were purified by Sephadex LH-20 column and RP HPLC from the chloroform–methanol (9:1) extract of the leaves of *Senecio pseudotites*. The molecular formulas ($C_{30}H_{46}O_5$ for **1**, $C_{32}H_{48}O_6$ for **2** and $C_{39}H_{52}O_7$ for **3**) were determined by negative ion FAB mass spectra as well as ^{13}C, and ^{13}C DEPT NMR analysis, which also indicated their triterpenic nature. The following NMR data suggested the structural features of urs-12-en-28-oic acid for compounds **1**–**3**: the olefinic hydrogen at δ 5.25 (1H, *m* H-12), a doublet signal for one of the methyls (δ 1.03, *d*, $J = 6.4$ Hz, Me-30), C-12 and C-13 resonances at δ 127.04 and 140.50, the carbonyl carbon resonance at δ 181.70 (C-28). The ^1H NMR spectrum of **1** showed also signals at δ 3.70 (*ddd*, $J = 11.0$, 12.0, 3.0 Hz) and 2.90

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Table 1. ^{13}C NMR data (600 MHz, CD_3OD) of compounds **1–3**

Carbon	DEPT	**1**	**2**	**3**
1	CH_2	49.00	49.00	49.00
2	CH	67.50	68.00	67.90
3	CH	78.80	77.80	77.75
4	C	42.00	41.03	41.03
5	CH	49.04	48.60	48.60
6	CH_2	19.30	19.30	19.00
7	CH_2	32.80	33.00	33.05
8	C	40.30	40.20	40.25
9	CH	47.30	47.10	47.50
10	C	38.00	37.52	37.68
11	CH_2	25.02	24.80	24.80
12	CH	127.04	128.80	128.94
13	C	140.50	140.00	140.10
14	C	41.00	41.00	41.20
15	CH_2	28.51	28.00	28.02
16	CH_2	25.30	26.03	26.05
17	C	48.00	48.02	48.04
18	CH	51.01	51.01	51.05
19	C	154.09	154.00	154.02
20	CH	38.10	38.10	38.10
21	CH_2	32.52	32.46	32.48
22	CH_2	38.02	38.00	38.00
23	CH_2	64.10	67.05	67.08
24	CH_3	14.50	14.00	14.02
25	CH_3	16.90	16.90	16.91
26	CH_3	17.00	17.10	17.12
27	CH_3	24.10	23.80	23.81
28	C	181.70	182.00	182.04
29	CH_2	107.90	107.72	107.80
30	CH_3	21.51	21.45	21.46
$\underline{C}OCH_3$			172.20	
$CO\underline{CH_3}$			21.40	
1′	C			168.70
2′	CH			115.12
3′	CH			145.80
1″	C			125.30
2″	CH			130.50
3″	CH			116.00
4″	C			160.35
5″	CH			116.00
6″	CH			130.50

(d, $J = 11.0$ Hz) ascribable respectively to the 2β- and 3α-protons on the carbons bearing hydroxyl functions. An AB doublet signal at δ 3.50 and 3.62 ($J = 11.5$ Hz) indicated the presence of a hydroxymethyl group. The ^{13}C NMR spectrum confirmed the existence of two –CHOH groups (δ 67.50 and 78.80) attributable to C-2 and C-3 positions and of one –CH_2OH group (δ 64.10) instead of typical Me-23 or Me-24 signals of an ursane skeleton [3]. The chemical shifts of C-4 (δ 42.00) and Me-24 (δ 14.50) led to the assignment of the –CH_2OH at C-23α position. Furthermore the other carbon signals assignable to rings A, B, C, and D were in agreement with those reported for asiatic acid (2α,3β,23-trihydroxyurs-12-en-28-oic acid) [4, 5] (Table 1). However the NMR spectra of **1**, compared with those of asiatic acid, lacked a doublet Me Signal (Me-29) and contained a complex proton signal at δ 4.65 and 4.74 (2H, *br s*) in the ^1H- as well as an sp^2 quaternary carbon (δ 154.09) and a CH_2 = (δ 107.90) in the ^{13}C NMR spectrum, ascribable to an *exo*-methylene group. The location of the *exo*-cyclic double bond at C-19(29) was suggested by the absence of the Me-29 signal and confirmed by the resonances of the vicinal carbons C-18, C-20 and C-21. In fact the signals of C-18 (δ 51.01) and C-20 (δ 38.10) were shifted to upfield by *ca.* δ -4.7 and -0.5 whereas that of C-21 (δ 32.52) was shifted to lowfield by *ca.* δ $+1.3$ with respect to compounds with the ursolic acid skeleton [3–6]. As reported in previous papers replacement of a Me by an *exo*-methylene group induces similar shifts in ursolic acid derivatives [6, 7]. Thus the structure 2α,3β,23-trihydroxy-urs-12,19(29)-dien-28-oic-acid was assigned to compound **1**.

The ^{13}C NMR spectra of compounds **2** and **3** showed the signals ascribable to rings A, B, C, D, and E almost superimposable to those of **1** and the presence of an acetyl in **2** and a *p*-coumaroyl group in **3** (Table 1). The ^1H NMR spectrum of **2** showed a signal for COO<u>Me</u> (δ 2.01); signals ascribable to a *p*-disubstituted aromatic ring (AA′BB′-system with doublet like signals centered at δ 7.55 and 6.88, $J_{ortho} = 8$ Hz) and to the vinylic protons of a *trans*-double bond (δ 7.73 and 6.44, $J_{trans} = 16.0$ Hz) were present in the spectrum of **3**. These data together with ^{13}C NMR resonances in the aromatic zone led to the identification of a *trans-p*-coumaroyl moiety [8]. Since the proton and carbon signals of the -CH_2OH at C-23 group were typically shifted downfield (δ_H 4.00 and 4.65; δ_C 67.0) with respect to **1**, the C-23 position must be esterified by the acetyl or the coumaroyl residues. The position of the acetyl and *p*-coumaroyl residues was confirmed by the results of the HMBC spectra which showed clear long-range correlation peaks between the carbonyl carbon (δ 172.2) of the acetyl and CH_2OH at C-23 signals (δ 4.00 and 4.65) of the aglycone in **2**, and the carbonyl carbon (δ 168.70) and both CH_2OH at C-23 and H-2′ (δ 7.55) signals of the coumaric acid residue in **3**. Thus compound **2** was 2α,3β,23-trihydroxy-urs-12,19(29)-dien-28-oic-acid-23-acetyl ester and compound **3** was 23-(*trans-p*-coumaroyloxy)-2α,3β,-dihydroxy-urs-12,19(29)-dien-28-oic-acid.

EXPERIMENTAL

NMR: CD_3OD, Bruker DRX-600 spectrometer; DEPT, and HMBC [9] experiments were performed using the UXNMR software package; chemical shifts are expressed in δ (ppm) referring to solvent peaks: δ_H 3.34 and δ_C 49.0 for CD_3OD. Optical rotations were measured on a Perkin-Elmer 192 polarimeter equipped with a sodium lamp (589 nm) and a 10 cm microcell. FAB-MS were recorded in the negative ion mode.

Plant material

The leaves of *S. pseudotites* were collected in Perù in October 1995 and identified by Prof. E. Cerrate. A voucher sample is deposited at the Herbario of the Museo de Historia Natural "J. Prado" de la Universidad Nacional Mayor de San Marcos, Lima, Perù.

Extraction and isolation

The powdered, dried leaves of *S. pseudotites* (500 g) were extracted successively with petrol ether (8.5 g), $CHCl_3$ (21.3 g), $CHCl_3$–MeOH (9:1) (10.6 g) and MeOH (31.3 g). Part of the $CHCl_3$ –MeOH (9:1) extract (6 g) was chromatographed on a Sephadex LH-20 column using MeOH as eluent. Frs (9 ml) were collected and checked by TLC [Silica gel plates in, $CHCl_3$–MeOH–H_2O (80:18:2) and $CHCl_3$–MeOH (8:2)]. Frs 18–24 (250 mg), containing the crude triterpenic mixture were submitted to reversed-phase HPLC on a C18 μ-Bondapak column (30 cm × 7.8 mm, flow rate 2.5 ml min^{-1}) using MeOH–H_2O (9:1) as the eluent to yield pure compounds **1** (12.0 mg, R_t = 13.7 min), **2** (9.7 mg, R_t = 16.9 min) and **3** (11.8 mg, R_t = 20.5 min).

Compound **1**. $[\alpha]_D^{25} = +15$ (MeOH, *c* 1); negative FAB-MS ($C_{30}H_{46}O_5$): *m/z* 485 $[M–H]^-$; ^{1}H NMR (CD_3OD): δ 0.73 (3H, *s*, Me-24), 0.91 (3H, *s*, Me-26), 1.07 (3H, *s*, Me-25), 1.03 (3H, *s*, Me-30), 1.21 (3H, *s*, Me-27), 2.90 (1H, *d*, *J* = 11 Hz, H-3α), 3.50 (1H, *d*, *J* = 11.5 Hz, Ha-23), 3.62 (1H, *d*, *J* = 11.5 Hz, Hb-23), 3.70 (1H, *ddd*, *J* = 11, 12, 3 Hz, H-2β), 4.65–4.74 (2H, *br s*, $=CH_2$), 5.27 (1H, *m*, H-12). ^{13}C NMR see Table 1.

Compound **2**. $[\alpha]_D^{25} = +18$ (MeOH, *c* 1); negative FAB-MS ($C_{32}H_{48}O_6$): *m/z* 527 $[M–H]^-$; ^{1}H NMR (CD_3OD): δ 0.75 (3H, *s*, Me-24), 0.92 (3H, *s*, Me-26), 1.03 (3H, *s*, Me-30), 1.05 (3H, *s*, Me-25), 1.21 (3H, *s*, Me-27), 2.01 (3H, *s*, COOMe), 2.91 (1H, *d*, *J* = 11 Hz, H-3α), 3.72 (1H, *ddd*, *J* = 11, 12, 3 Hz, H-2β), 4.00 (1H, *d*, *J* = 11 Hz, Ha-23), 4.65 (1H, *d*, *J* = 11 Hz, Hb-23), 4.63–4.68 (2H, *br s*, $=CH_2$), 5.27 (1H, *m*, H-12). ^{13}C NMR see Table 1.

Compound **3**. $[\alpha]_D^{25} = +16$ (MeOH, *c* 1); negative FAB-MS ($C_{39}H_{52}O_7$): *m/z* 631 $[M–H]^-$; ^{1}H NMR (CD_3OD): δ 0.75 (3H, *s*, Me-24), 0.91 (3H, *s*, Me-26), 1.03 (3H, *s*, Me-30), 1.06 (3H, *s*, Me-25), 1.20 (3H, *s*, Me-27), 2.90 (1H, *d*, *J* = 11 Hz, H-3α), 3.70 (1H, *ddd*, *J* = 11, 12, 3 Hz, H-2β), 4.00 (1H, *d*, *J* = 11 Hz, Ha-23), 4.65 (1H, *d*, *J* = 11 Hz, Hb-23), 4.68–4.74 (2H, *br s*, $=CH_2$), 5.25 (1H, *m*, H-12), 6.44 (1H, *d*, *J* = 16.0 Hz, H-2′), 6.88 (2H, *d*, *J* = 8 Hz, H-3″ and H-5″), 7.55 (2H, *d*, *J* = 8 Hz, H-2″ and H-6″), 7.73 (1H, *d*, *J* = 16.0 Hz, H-3′). ^{13}C NMR see Table 1.

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