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TRITERPENES FROM THE COMBINED LEAF AND STEM OF *LITHOSPERMUM CAROLINIENSE*

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Key Word Index—*Lithospermum caroliniense*; Boraginaceae; stem; triterpenes.

Abstract—An investigation of the combined leaf and stem of *Lithospermum caroliniense* afforded two new pentacyclic triterpenoids based on the olean-12-ene and taraxast-12-ene skeletal types. The structures of these compounds were elucidated on the basis of spectral analysis as 1α,3β,23-trihydroxyolean-12-ene-28-oic acid and 3α,19β,21α,23-tetrahydroxytaraxast-12-ene-28-oic acid. Copyright © 1996 Elsevier Science Ltd

INTRODUCTION

As part of our ongoing search for antineoplastic agents from plants, we have investigated *Lithospermum caroliniense* MacMill. (Boraginaceae). A literature search revealed that no previous phytochemical or biological study has been undertaken on this species. However, extensive investigations have been conducted on other *Lithospermum* species. For example, there are numerous reports of various species acting as antifertility agents [1–5]. Specific compounds frequently reported are quinoids, such as shikonen [6], flavonoids [7, 8], and pyrrolizidine alkaloids [9]. Triterpenoids have been encountered infrequently and include β-amyrin and lupeol [10]. In the course of our search for potential topoisomerase I inhibitory plant constituents [11], we have isolated from a methanol–chloroform (1:1) extract of the combined leaf and stem parts of *L. caroliniense* two new pentacyclic triterpenes (**1** and **2**) as inactive constituents. In this communication, we report the structures of these triterpenoids.

RESULTS AND DISCUSSION

The negative FAB-mass spectrum of **1** showed a quasi-molecular ion $[M-H]^-$ at *m/z* 487, indicating a molecular formula $C_{30}H_{48}O_5$. The broad-band decoupled ^{13}C and DEPT NMR spectra revealed 30 signals due to six methyl, 10 methylene, six methine and eight quaternary carbons. The olean-12-ene triterpene skeleton was interred from the chemical shifts of C-12 (δ 123.7) and C-13 (δ 144.9) [12, 13] and was also confirmed by the proton signal of H-18 at δ 3.26 (*dd*, J = 4.0 and 13.8 Hz) and the triplet-like signal of H-12 at δ 5.45. Proton and ^{13}C NMR spectral comparison of **1** with hederagenin, a 1-deoxy analogue of **1**, revealed close similarities except in ring A [14]. In the ^1H NMR spectrum of **1**, two doublets (1H each) at δ 3.71 and 4.21 (J = 10.0 Hz) were assigned to the hydroxymethyl group at C-23, while the signal at δ 4.24 (*dd*, J = 4.3 and 9.5 Hz) suggested a β-equatorial orientation of the hydroxyl group at C-3. In the ^1H–^1H COSY NMR spectrum of **1**, the C-1 and C-3 oxymethine protons were mutually coupled with the C-2 methylene protons. The substitution pattern in ring A was confirmed by observation of long-range HMBC correlations of H-2α (δ 2.28) with C-1 (δ 68.9) and C-3 (δ 78.2), H-3 (δ 4.24) with C-1, and H-23α (δ 3.71) with C-24 (δ 14.3) and C-3. The remaining ^{13}C NMR signals were assigned by further analysis of the HMBC and HMQC spectra of **1**. In a proton-decoupling experiment on **1**, irradiation of H-2α at δ 2.28 collapsed the double doublet signal of H-3 to a doublet (J = 9.5 Hz), while the multiplet centred at δ 4.22 was converted into a doublet (J = 4.4 Hz), thus proving that the C-1 hydroxyl group occupied an α-axial position. The ^{13}C NMR chemical shifts of C-23 (δ 66.5) and C-24 (δ 14.3) suggested an α-equatorial orientation of the hydroxymethyl group. It has also been noted previously that, in triterpenoids with C-4 attached hydroxymethyl groups, the 4β-methyl group as in **1** resonates about 10 ppm further upfield than the corresponding 4α-oriented methyl group (δ_C *ca* 24 ppm) [15, 16]. The structure of **1** was thus established as 1α,3β,23-trihydroxyolean-12-en-28-oic acid.

The molecular formula of **2** was deduced as $C_{30}H_{48}O_6$ from its high-resolution negative FAB-mass

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spectrum, which showed a quasi-molecular ion $[M-H]^-$ at m/z 503.3385 ($C_{30}H_{47}O_6$). A retro-Diels–Alder fragment at m/z 280 ($C_{16}H_{24}O_4$) suggested the presence of two hydroxyl groups and a carboxyl group in the D/E rings of **2** [17]. The ^{13}C NMR spectral data (Table 1) including DEPT revealed the presence of six methyl, nine methylene, seven methine and eight quaternary, carbons, ascribable to a triterpene skeleton. The ^1H NMR spectrum contained signals for six methyls (δ 0.87, 1.04, 1.30, 1.42 and 1.69), two methines geminal to hydroxyl groups (δ 4.16, H-3 and 4.28, H-21), oxymethylene protons (δ 3.76 and 3.93) and a vinyl proton (δ 5.60, H-12). In the ^1H NMR spectrum of **2** the broad singlet resonance of the C-3 methine proton at δ 4.16 indicated an α-axial orientation of the hydroxyl group at C-3. An AB system for the geminally coupled H_2-23 was evident at δ 3.76 and 3.43 (each *d*, $J = 10.8$ Hz). Analysis of HMQC and HMBC data for **2** coupled with comparison of the ^1H and ^{13}C NMR data for **2** with those for ilexolic acid A (**3**), isolated from *Ilex rotunda*, revealed similarities except in rings A and E [16]. The ^{13}C NMR chemical shifts of C-23 and C-24 at δ 71.0 and 17.6, respectively, were suggestive of the α-equatorial disposition of the C-23 hydroxymethyl group as reported for triterpenoids having a similar substitution pattern in ring A [18]. Closer inspection of the ^{13}C NMR spectral data for the ring-E carbons in **2** and **3** indicated further differences in the chemical shifts of C-19, C-20, C-21 and C-22, as well as in the C-29 and C-30 methyl group signal (Table 1). The ^{13}C NMR signal at δ 72.6 was assigned to the hydroxyl-bearing quaternary carbon C-19, while that at δ 66.1 was attributed to the C-21 methine carbon. Furthermore, the threefold doublet signal of H-21 at δ 4.28 ($J = 2.7$, 4.3 and 11.3 Hz) required an α-equatorial orientation of the hydroxyl group at C-21 in a *trans*-D/E ring as in taraxast-12-ene rather than in an urs-12-ene skeleton [19]. The structure of **2** was therefore determined as 3α,19β,21α,23-tetrahydroxytaraxas-12-en-28-oic acid.

Table 1. ^{13}C NMR chemical shifts of **1**, **2** and **3***

C	1	2	3†
1	68.9	38.3	39.0
2	47.7	26.2	27.6
3	78.2	78.8	73.6
4	42.2	40.8	42.9
5	47.9	43.3	48.8
6	18.5	18.3	18.9
7	33.2	33.0	33.4
8	40.0	40.1	40.5
9	48.2	47.6	47.9
10	43.6	43.1	37.3
11	23.6	24.0	24.2
12	123.7	127.9	128.6
13	144.9	140.0	140.2
14	38.5	42.3	42.3
15	28.3	29.0	29.6
16	23.9	26.7	27.4
17	46.6	48.3	49.2
18	42.2	54.4	54.6
19	46.4	72.6	74.9
20	30.9	42.2	50.6
21	34.2	66.1	67.6
22	33.2	42.5	48.6
23	66.5	71.0	68.0
24	14.3	17.6	13.2
25	14.3	16.6	16.1
26	17.3	17.1	17.4
27	26.1	24.5	24.7
28	180.2	180.1	180.4
29	33.2	16.8	27.8
30	23.7	16.6	11.5

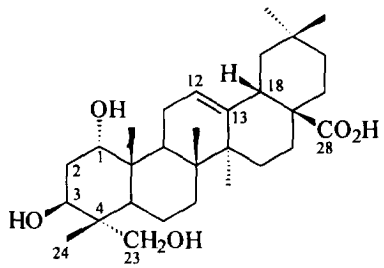
*Measured in pyridine-d_5 at 125 MHz.
†Taken from ref. [5].

EXPERIMENTAL

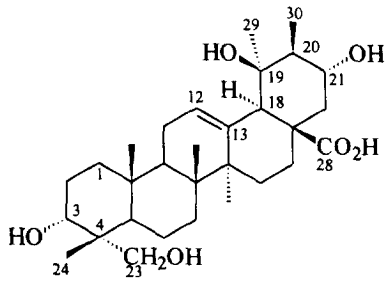
Mps: uncorr.; IR was recorded using a KBr disc. ^1H and ^{13}C NMR spectra were recorded with TMS as int. standard. For MS measurements, a VGZAB-E magnetic sector instrument with direct-probe insertion was used. For FABMS, Xe gas and nitrobenzyl alcohol as matrix were employed. The resolution used for accurate mass measurement was 10 000. Chromatographic sepns were carried out on silica gel 60. TLC spots were detected by spraying with phosphomolybdate reagent (5% phosphomolybdic acid in EtOH) and heating the plate to *ca* 100°. Preparative high-speed countercurrent chromatography was performed with a HSCC chromatograph (P.C. Inc., Potomac, MD) equipped with a prep. coil having a capacity of 420 ml.

Plant material. The combined leaf and stem of *L. caroliniense* were collected in Maple, Texas, U.S.A., in June 1992. A voucher specimen (A1886) representing this collection has been deposited in the John G. Searle Herbarium, Field Museum of Natural History, Chicago, Illinois.

Extraction and isolation. The dried and ground leaf and stem parts (0.847 kg) were extracted with MeOH–$CHCl_3$ (1:1) (2 × 2 l) under reflux. The extract was concd *in vacuo* at 40° and partitioned between $CHCl_3$–MeOH (4:1) and H_2O. The organic portion was concd, dissolved in MeOH–H_2O (9:1), and defatted with hexane. The 90% MeOH extract (3.94 g) was subjected to CC on silica gel (400 g; 70–230 mesh. E. Merck, Darmstadt, Germany), eluting the column initially with $CHCl_3$ followed by stepwise increments of polarity by gradual addition of MeOH. The fr. (0.715 g) containing **1** and **2** was eluted with 9–10% MeOH–$CHCl_3$. A 100 mg portion of this fr. was submitted to HSCCC sepn with the upper phase of hexane–EtOAc–MeOH–H_2O (1:2:1:1) being used as mobile phase. This sepn was conducted at 3–4 ml min^{-1} with the coil speed maintained at *ca* 700 rev min^{-1}. Frs (10–15 ml each) were collected. Pure **1** (20 mg) was eluted in frs 25–31, while the slightly less polar pure **2** (10 mg) was eluted in frs 15–17.



1



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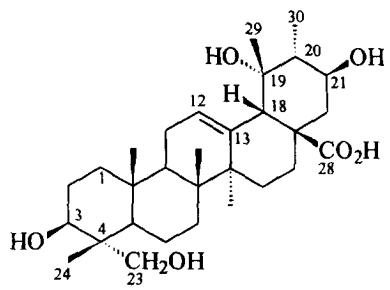

3

1α,3β,23-*Trihydroxyolean*-12-*en*-28-*oic acid* (**1**). Amorphous powder, mp 240–243°; $[\alpha]_D^{25}$ +39.3° (MeOH; *c* 0.3); IR ν_{max}^{KBr}: 3420, 2920, 1683, 1450, 1376, 1232, 1037, 930 cm^{-1}; HR-FABMS $[M-H]^-$ *m/z* 487.3420, calc. for $C_{30}H_{47}O_5$ $[M-H]^-$ 487.3424 ($\Delta=-0.7$ ppm); ^1H NMR (500 MHz, pyridine-d_5): δ 0.89, 0.97, 1.03, 1.05, 1.05, 1.12 (each 3H, *s*, 6× Me), 1.42 (1H, *m*, H-2β), 2.28 (1H, *m*, H-2α), 3.26 (1H, *dd*, $J=13.8$, 4.0 Hz, H-18), 3.71, 4.21 (2H, AB system, each *d*, $J=10.0$ Hz, H_2-23), 4.22 (1H, *m*, overlapped signal, H-1), 4.24 (1H, *dd*, $J=4.3$, 9.5 Hz, H-3), 5.45 (1H, *br s*, H-12); ^{13}C NMR data: Table 1.

3α,19β,21α,23-*Tetrahydroxytaraxast*-12-*en*-28-*oic acid* (**2**). Amorphous powder, mp 257–259°; $[\alpha]_D^{25}$ + 29.5° (MeOH; *c* 0.2); IR ν_{max}^{KBr}: 3410, 2920, 1683, 1450, 1376, 1263, 1232, 1198, 1149, 1037, 930 cm^{-1}; HR-FABMS $[M-H]^-$ *m/z* 503.3385, calc. for $C_{30}H_{47}O_5$ $[M-H]^-$ 503.3373 ($\Delta=-0.7$ ppm); ^1H NMR (500 MHz, pyridine-d_5): δ 0.87 (3H, *s*, Me-24), 1.04, 1.14 (6H, Me-25, Me-26), 1.30 (3H, *d*, $J=6.1$, Me-30), 1.42 (3H, *s*, Me-29), 1.69 (3H, *s*, Me-27), 3.05 (1H, *s*, H-18), 3.76, 3.93 (2H, AB system, each *d*, $J=10.8$ Hz, H_2-23), 4.16 (1H, *s*, H-3), 4.28 (1H, *ddd*, $J=11.4$, 4.3, 2.7 Hz, H-21), 5.60 (1H, *br s*, H-12); ^{13}C NMR data: Table 1.

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