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DAMMARANE-TYPE TRITERPENES FROM *CORDIA SPINESCENS*

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Key Word Index—*Cordia spinescens*; Boraginaceae; dammarane-type triterpene.

Abstract—Two new triterpenes, 3α,6β,25-trihydroxy-20(*S*),24(*S*)-epoxydammarane and 3α-acetoxy-6β,25-dihydroxy-20(*S*),24(*S*)-epoxydammarane, were isolated from the methanol extract of the leaves of *Cordia spinescens*, together with cabraleadiol. © 1997 Elsevier Science Ltd

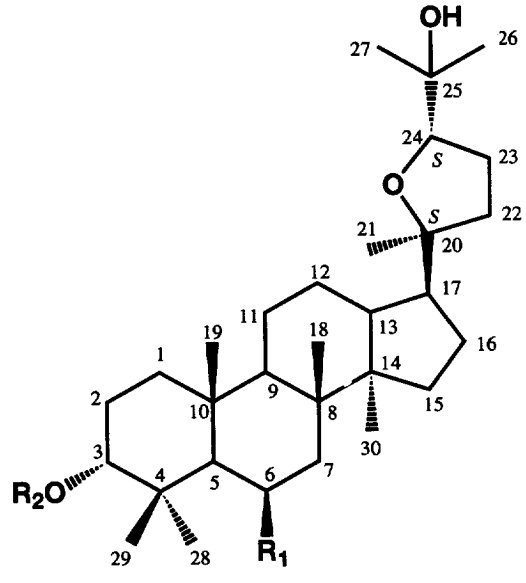
INTRODUCTION

Cordia spinescens L. is a shrub native to humid thickets and forests from western and southern Mexico to Venezuela and Peru. Infusions of the roots and leaves of this plant are used by Indians of northwestern Venezuela to relieve fever and headache [1], also the powdered stem bark is used externally for wound healing [2]. Several flavonoids, terpenoid benzoquinones and polyphenols have been isolated from the genus *Cordia* [3]. In this paper, we report on the isolation and structure elucidation of two new dammarane-type triterpenes, 3α,6β,25-trihydroxy-20(*S*),24(*S*)-epoxydammarane (**1**) and 3α-acetoxyl-6β,25-dihydroxy-20(*S*),24(*S*)-epoxydammarane (**2**) from *C. spinescens*.

RESULTS AND DISCUSSION

The MeOH extract of the leaves of *C. spinescens* was partitioned into hexane-, CH_2Cl_2-, EtOAc-, BuOH- and H_2O-soluble fractions. Repeated CC of the CH_2Cl_2-soluble fraction afforded three dammarane-type triterpenes (**1–3**). The ^1H and ^{13}C NMR spectral data of **3** (Tables 1 and 2) were in good agreement with those reported for cabraleadiol [4]. The structures of **1** and **2** were determined as follows.

Compound **1** was assigned the molecular formula $C_{30}H_{52}O_4$ (EI-MS *m/z* 476 $[M]^+$). The IR spectrum showed the presence of a hydroxyl group (3450 cm^{-1}). The ^1H NMR spectrum (Table 1) showed signals for eight tertiary methyls and three oxymethines at δ 3.37 (*br s*, H-3), 3.65 (*dd*, *J* = 10.2, 5.2 Hz, H-24) and 4.42 (*br s*, H-6). The ^{13}C NMR spectrum (Table 2) analysed by the aid of DEPT experiment, indicated the presence of eight methyls, four sp^3 quaternary carbons, and five oxygen-bearing carbons: C-6 (δ 69.4, *d*), C-25 (δ 70.2 *s*), C-3 (δ 77.6, *d*), C-24 (δ 86.3, *d*) and C-20 (δ 86.6 *s*). From the ^1H NMR spectrum, a hydroxyl group was assigned the α-configuration (*br s*) and located at C-3. The EI-mass spectrum of **1** showed a



1 R_1=OH, R_2=H
2 R_1=OH, R_2=Ac
3 R_1=R_2=H

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Table 1. ^{1}H NMR spectral data for compounds **1–3** ($CDCl_3$, 500 MHz)

H	1	2	3
H_3-18	1.32 (*s*)	1.33 (*s*)	0.97 (*s*)
H_3-19	1.23 (*s*)	1.24 (*s*)	0.86 (*s*)
H_3-21	1.16 (*s*)	1.17 (*s*)	1.15 (*s*)
H_3-26	1.12 (*s*)*	1.12 (*s*)*	1.11 (*s*)*
H_3-27	1.20 (*s*)*	1.20 (*s*)*	1.19 (*s*)*
H_3-28	1.03 (*s*)	0.94 (*s*)	0.94 (*s*)
H_3-29	1.23 (*s*)	1.29 (*s*)	0.84 (*s*)
H_3-30	0.86 (*s*)	0.90 (*s*)	0.89 (*s*)
CH_3CO	—	2.08 (*s*)	—
H-3	3.37 (*br s*)	4.59 (*br s*)	3.40 (*br s*)
H-6	4.42 (*br s*)	4.43 (*br s*)	—
H-24	3.65 (*dd*, J = 10.2, 5.2 Hz)	3.66 (*dd*, J = 10.2, 5.2 Hz)	3.64 (*dd*, J = 10.2, 5.2 Hz)

* Interchangeable values in each vertical column.

Table 2. ^{13}C NMR spectral data for compounds **1–3** ($CDCl_3$, 125 MHz)

C	1	2	3
1	35.8 *t*	36.4 *t*	33.6 *t*
2	25.3 *t*	22.8 *t*	25.3 *t*
3	77.6 *d*	79.7 *d*	76.3 *d*
4	38.4 *s*	37.6 *s*	37.6 *s*
5	49.7 *d*	50.8 *d*	49.5 *d*
6	69.4 *d*	68.9 *d*	18.2 *t*
7	42.9 *t*	42.9 *t*	35.1 *t*
8	39.6 *s*	39.6 *s*	40.6 *s*
9	51.1 *d*	51.1 *d*	50.6 *d*
10	36.9 *s*	36.8 *s*	37.2 *s*
11	21.7 *t*	21.7 *t*	21.6 *t*
12	27.1 *t*	27.0 *t*	27.0 *t*
13	41.7 *d*	41.7 *d*	42.7 *d*
14	50.1 *s*	50.1 *s*	50.1 *s*
15	31.5 *t*	31.5 *t*	31.4 *t*
16	25.8 *t*	25.8 *t*	25.8 *t*
17	49.5 *d*	49.7 *d*	49.8 *d*
18	16.3 *q*	16.3 *q*	15.5 *q*
19	17.5 *q*	17.4 *q*	16.0 *q*
20	86.6 *s*	86.6 *s*	86.6 *s*
21	27.3 *q*	27.3 *q*	27.2 *q*
22	34.6 *t*	34.6 *t*	34.7 *t*
23	26.3 *t*	26.3 *t*	26.3 *t*
24	86.3 *d*	86.4 *d*	86.2 *d*
25	70.2 *s*	70.3 *s*	70.2 *s*
26	24.0 *q**	24.0 *q**	24.0 *q**
27	27.8 *q**	27.8 *q**	27.9 *q**
28	28.1 *q*	27.7 *q*	28.3 *q*
29	24.3 *q*	24.0 *q*	22.1 *q*
30	16.7 *q*	16.8 *q*	16.5 *q*
$COOCH_3$		170.8 *s*	
$COOCH_3$		21.4 *q*	

Multiplicity of carbon signals were verified by DEPT experiments.

* Interchangeable values in each vertical column.

characteristic fragment ion at m/z 143 (100%) suggesting the presence of a hydroxyisopropyl-methyltetrahydroxyfuran side chain $[C_8H_{15}O_2]^+$ in the molecule [5, 6]. The above mentioned data were in accordance with those reported for epoxydammarane-type triterpenes [5–9]. From these observations, **1** was considered to be a hydroxy cabraleadiol [4]. The spin system, H-5 (δ 1.09)/H-6 (δ 4.42)/H-7a (δ 1.83) and H-7b (δ 1.48) seen in the ^{1}H ^{1}H COSY spectrum of **1**, and the relative downfield shifts of H_3-18, 19 and 29 (when compared with those of **3**) [4] suggested a β-hydroxyl substitution at C-6 (Table 1). This was further substantiated by NOESY experiment where spatial correlations were seen between H-6 (δ 4.42), H-5 (δ 1.09) and H_3-28 (δ 1.03).

As to the configuration at C-20 and C-24 of the side chain, both were assigned as *S* by comparing the chemical shifts of H-24 (δ 3.65, *dd*), H_3-26 (δ 1.12, *s*) and H_3-27 (δ 1.20, *s*) of **1** with those reported for 20*S* and 20*R* compounds [H_3-26 and H_3-27 of the latter compounds tend to have identical or quite similar (within 0.03 ppm) chemical shift values] [4, 8–11]. The chemical shift and coupling pattern of H-24/C-24 (δ 3.65, *dd*/δ_C 86.3) in **1** were comparable to those seen in **3** and in their C-24(*R*) epimers (δ 3.71 *t*/δ_C 83.2) such as ocotillone-II, ocotillol-II and eichlerianic acid [4] and suggested a 24*S* configuration in **1**. From the foregoing findings, compound **1** was concluded to be 3α,6β,25-trihydroxy-20(*S*),24(*S*)-epoxydammarane.

Most of the ^{1}H and ^{13}C NMR spectra of **2** (Tables 1 and 2) were similar to those of **1** except for the presence of signals for an additional acetyl group (δ_H 2.08; δ_C 170.8 and 21.4). This was evident from the IR (1721 cm^{-1}) and the EI-MS (m/z 518 $[M]^+$ and 459 $[M—CH_2OO]^+$) spectra. The downfield shifts of H-3/C-3 in **2** (δ_H 4.59/δ_C 79.7) compared to those of **1**, suggested possible acylation of C_3—OH (Tables 1 and 2). This was unambiguously confirmed by HMQC, HMBC and NOESY experiments. Accordingly, the structure of **2** was determined to be 3α-acetoxy-6β,25-dihydroxy-20(*S*),24(*S*)-epoxydammarane.

EXPERIMENTAL

General. Mps: uncorr.; Optical rotations: 25°; IR: $CHCl_3$; NMR: given in δ values; EI-MS and HR-

MS: 70 eV; CC: silica gel (Kieselgel 60, 70–230 mesh, Merck), ODS (Cosmosil 140 C_{18}-OPN, Nacalai Tesque), and Florisil (60–100 and 100–200 mesh, Floridin): LiChroprep Si 60 (Size A and Size B, Merck); TLC: silica gel 60 F_{254} (0.25 mm, Merck), and spots were visualized after spraying with anisaldehyde-H_2SO_4 followed by heating.

Plant material. The leaves of *Cordia spinescens* L. were collected from the Panama Canal area, Panama during July 1994 and identified by Mrs Carmen Galdames, Alex Espinoza and Eduardo Valdes. A voucher specimen is on deposit at the herbarium of the University of Panama.

Extraction and isolation of compounds. Dried leaves (1.5 kg) were extracted with MeOH (8 l × 6) at room temp., and the solvent was removed under red. pres. to give a residue (115 g). A part of the MeOH extract (89 g) was suspended in H_2O (500 ml) and shaken with solvents of increasing polarity to give hexane-soluble (24 g), CH_2Cl_2-soluble (10 g), EtOAc-soluble (6 g), BuOH-soluble (7 g) and H_2O-soluble (33 g) frs. The CH_2Cl_2-soluble fr. was subjected to CC on Florisil with increasing percentages of MeOH in $CHCl_3$ (0–100%) to give four frs (A–D). CC/silica gel of fr. A (C_6H_6–Me_2CO, 50:1 to 1:1) followed by MPLC/silica gel (C_6H_6–Me_2CO, 25:1) afforded **2** (15 mg) and **3** (6 mg). Repeated CC of fr. B on silica gel (C_6H_6–Me_2CO, 7:3) and ODS (70% aq. MeOH to MeOH) followed by MPLC/silica gel (C_6H_6–Me_2CO, 22:3) afforded **1** (14 mg).

3α,6β,25-Trihydroxy-20(S),24(S)-epoxydammarane (**1**). Yellowish oil, $[\alpha]_D$ −5.9° (*c* 0.67, $CHCl_3$). IR ν_{max} cm^{-1}: 3450; ^1H and ^{13}C NMR: Tables 1 and 2; EI-MS *m/z* (rel. int.): 476 $[M]^+$ (0.03), 440 (15), 399 (30), 381 (25), 143 $[C_8H_{15}O_2]^+$ (100), 125 (72); HR-MS *m/z* 476.3911 $[M]^+$, Calcd for $C_{30}H_{52}O_4$: 476.3865.

3α-Acetoxy-6β,25-dihydroxy-20(S),24(S)-epoxydammarane (**2**). Yellowish oil, $[\alpha]_D$ −15.0° (*c* 0.79, $CHCl_3$). IR ν_{max} cm^{-1}: 3450, 1721; ^1H and ^{13}C NMR: Tables 1 and 2; EI-MS *m/z* (rel. int.): 518 $[M]^+$ (0.02), 503 $[M—Me]^+$ (16), 500 $[M—H_2O]^+$ (4), 459 $[M—CH_3COO]^+$ (0.8), 399 (78), 381 (31), 143 $[C_8H_{15}O_2]^+$ (100), 125 (58); HR-MS *m/z* 503.3777 $[M—Me]^+$, calcd for $C_{31}H_{51}O_5$: 503.3736; 500.3880 $[M—H_2O]^+$, calcd for $C_{32}H_{52}O_4$: 500.3866.

Cabraleadiol (**3**). Colourless needles, mp 168–174° (lit. [4] 171–173°). $[\alpha]_D$ +13.5° (*c* 0.24, $CHCl_3$, (lit. [12] +13.6 (*c* 1, $CHCl_3$)). ^1H and ^{13}C NMR: Tables 1 and 2; EI-MS *m/z* (rel. int.): 460 $[M]^+$ (2.2), 445 (11), 427 (15), 401 (40), 383 (85), 191 (68), 143 $[C_8H_{15}O_2]^+$ (100), 125 (85).

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