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ENT-LABDANES IN *EUPATORIUM BUNIIFOLIUM*

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Key Word Index—*Eupatorium buniifolium*; Asteraceae; *ent*-labdane diterpenoids.

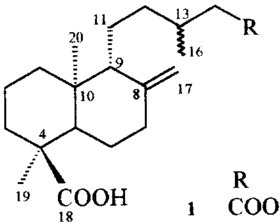
Abstract—*Eupatorium buniifolium* was investigated for diterpenoid content. Methyl-*ent*-labd-8(17)-en-18-oic acid-15-oate, 15-hydroxy-*ent*-labd-8(17)-en-18-oic acid, 15,16-epoxy-15-methoxy-*ent*-labd-8(17)-en-18-oic acid and 15-methoxy-*ent*-labd-8(17)-13-dien-18-oic acid methyl ester-16,15-olide were isolated from the aerial part of this plant and their structures were determined. The known compounds polyalthic acid and nivenolide were also identified. All these compounds were characterized on the basis of spectral analysis (including 2D NMR) and physical constants. © 1998 Elsevier Science Ltd. All rights reserved

INTRODUCTION

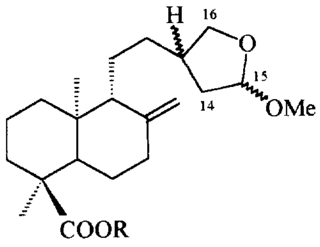
Eupatorium buniifolium Hooker et Arnott is a South American species spreading from the southern part of Bolivia, Brazil, Paraguay and Uruguay to the northern and central part of Argentina, growing as a weed in overgrazing fields. It is employed in folk medicine, used as a tincture and for its hepatoprotective and disinfectant properties [1]. On the other hand extracts of this plant have been shown to have antiherpetic activity [2]. Previous phytochemical studies on samples of this species from Entre Rios Province (Argentina) have reported the isolation of triterpenes, 6-methoxyflavonoids and 2′-oxygenated flavonoids [1], hydroxycinamic acids and their derivatives [1, 3] and flavonoid glycosides [3]. As part of our continuing search of plants belonging to the *Asteraceae* in the Cuyo region, we have collected *E. buniifolium* from 'El Durazno' in San Luis Province, where it is locally known as 'romerillo colorado' or 'chilca', and examined its constituents, resulting in the characterization of four new labdane-type diterpenoids (**1–4**) and the known polyalthic (**5**) [4] and nivenolide (**6**) [5] acids.

RESULTS AND DISCUSSION

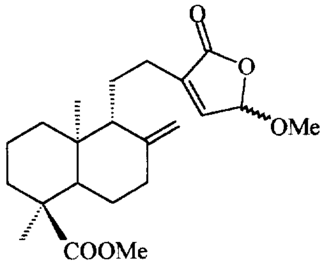
All the compounds isolated have a common framework, differing only at the side chain on C-9. The molecular connectivities in the decaline system were deduced from spectroscopic analysis comprising ^{1}H NMR, ^{13}C NMR, 2D experiments as well as the EI-mass spectrum. The optical rotation of all the isolated compounds, with negative values, suggested an *ent*-labdane nucleus. Compound **1**, $C_{21}H_{34}O_4$, $[M]^+$ at m/z 350, was isolated as an oil. The ^{13}C spectrum of **1** (Table 2) from the decaline system showed the resonances attributed to two methyls, five methylenes, two methines, two quaternary carbons, two olefinic carbons and one carboxylic carbon. The ^{1}H NMR spectrum (Table 1) was characteristic of labdane-type diterpenes with an exo-methylene group (δ 4.50 and 4.83, H-17 and H-17′) and two quaternary methyl groups (δ 0.70, H-20 and 1.14, H-19). These last values showed that the C-4 carboxyl group was equatorially oriented [5]. In addition, the chemical shift of the C-19 (δ 16.2) and C-20 (δ 14.5) carbons in the ^{13}C NMR spectra showed that both methyl groups were axial (high field due to γ-shielding) [6, 7, 8, 9]. Furthermore, the NOESY experiment showed that H-19 protons were spatially coupled with H-20. These results were in agreement with a labdane skeleton with a carboxyl group on C-18. The correlation between C-15 (δ 173.6) and the methyl protons of carboxymethyl group (δ 3.66), derived from the COLOC data (Table 3) was observed. Therefore, the position of the carboxymethyl group was assigned on C-14. The relative configuration of **1** has been assumed from the combined evidence of its ^{13}C NMR and ^{1}H NMR spectral data by comparison with those of closely related compounds and the negative optical rotation value [7, 9]. The co-occurrence of the established *ent*-labdanes **5** and **6** is an additional valuable argument for the assignment of such configuration to the novel compounds. Hence, compound **1** must be methyl-*ent*-labd-8(17)-en-18-oic acid-15-oate and it was present as a diastereomerically pure compound. Compound **2** was acetylated (Ac_2O-pyridine) to afford an epimeric mixture of monoacetate **2a**. The presence of this epimeric mixture could be explained by a non-stereospecific reduction of the double bond in the primary GGPP



R
1 COOMe
2 CH_2OH
2a CH_2OCOMe



3 R = H
3a R = Me



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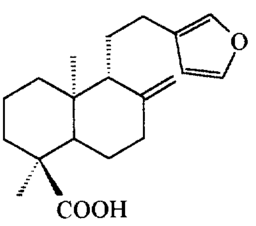

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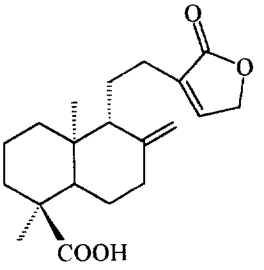

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cyclization product. It only differed from compound **1** in the replacement of the carboxymethyl group by the acetoxymethylene group as revealed by the ^1H NMR and ^{13}C NMR spectra (H-15, H-15′ δ 4.04; OCOCH_3 δ 2.01 and C-15 δ 63.0; OCOCH$_3$ δ 171.3, 21.0). Therefore, the structure of **2** was assigned as 15-hydroxy-*ent*-labd-8(17)-en-18-oic acid. The stereochemistry at C-13 remains unknown. Compound **3** was isolated from chromatographic fractions treated with diazomethane and studied as the methyl ester derivative (**3a**) and was present as an inseparable mixture of epimeric cyclic acetals. The exact mass of the $[M]^+$ ion was found to be *m/z* 364.2561, suggesting the molecular formula $C_{22}H_{36}O_4$. The three proton singlets in the ^1H NMR spectrum at δ 3.32, 3.34 and 3.66 indicated the presence of three OCH_3 groups, one of them a methyl ester. All the spectroscopical data observed showed a great resemblance to the data described for **1** and **2** with differences only in the side-chain structure. In fact the ^{13}C NMR spectrum indicated the presence of an acetal methine as double peaks occurring at δ 105.1 and 105.5, as well as another pair of peaks for a methylene carbon bearing oxygen, which formed part of a tetrahydrofuran ring at δ 71.5 and 72.4. On the other hand, the ^1H NMR spectrum showed the signals corresponding to a hemiacetal methyl (δ 3.32, 3.34) and the hemiacetal proton showed superimposed signals corresponding to 2 triplets (δ 4.95 and 4.98) and 2 *br d* (δ 4.94 and 4.95), that might indicate the presence of the 4 diastereomers. Two pair of triplets at δ 3.42–3.47 and 3.97–4.05 were ascribed to H-16 and H-16′ to the tetrahydrofuran ring. The relative configuration of **3a** has been assumed from the combined evidence of its ^{13}C NMR and ^1H NMR spectral data in comparison with those of closely related compounds, the presence of **5** and **6**, and the optical rotation negative value, together with biogenetic considerations. Therefore, from a combination of all of these data the novel structure of the mixture of epimeric acetals **3** was established as 15,16-epoxy-15-methoxy-*ent*-labd-8(17)-en-18-oic acid. The acidic nature of **3** was confirmed by its conversion to the methyl ester **3a** on treatment with ethereal diazomethane. However, since methanol was used during the procedure, it is possible that **3** was an artifact and that the true natural product was its hemiacetal analogue.

Compound **4** was isolated as a minor constituent and its structure was deduced from spectroscopical data. The ^1H NMR spectrum (Table 1) indicated that this compound differed from **6** by the replacement of an H-15 by a methoxy group as well as the methyl ester derivative of the carboxyl group at C-18. This was supported by the ^{13}C NMR spectrum that indicated the presence of an acetal methine as a single peak occurring at δ 102.4. The existence of a γ-lactone with α,β-unsaturation was confirmed by the appearance in the ^1H NMR spectrum of a doublet of doublets at δ 6.75, for the β-proton on a double bond conjugated with a carbonyl group. Its hemiacetal analogue would be the true natural product due to the employment of methanol in several steps of the purification procedure. All the spectroscopic and physical data observed for compound **6** were in agreement with those described for nivenolide [5], an *ent*-labdane with an ethyl cyclopentene lactone as side chain. Table 2 presents the full assignments for all ^{13}C NMR signals based on a combination of 1D and 2D-NMR techniques comprising DEPT and XH-CORR.

Table 1. ^{1}H NMR spectral data* of compounds **1, 2a, 3a, 4, 5** and **6**.

H	**1**	**2a**	**3a**	**4**	**5**	**6**
14				6.75 *dd* (2.7, 1.5)	6.25 *dd* (1.6, 0.9)	7.10 *q* (1.8)
15		4.04 *br t* (6.8)	4.94 *brd* (4.8), 4.95 *br d* (4.9) 4.95 *t* (2.8) and 4.98 *t* (2.9)	5.72 *dd* (2.7, 1.3)	7.34 *t* (1.6)	4.78 *q* (1.8)
16	0.95 *d* (6.6)	0.92 *d* (6.0)	3.42 *t* (7.9) and 3.47 *t* (7.9)		7.19 *br s*	
16′			3.97*t* (7.9) and 4.05 *t* (7.9)			
17	4.50 *br s*	4.47 *br s*	4.47 *br s*	4.57 *br s*	4.59 *br s*	4.59 *br s*
17′	4.83 *br s*	4.79 *br s*	4.81 *br s*	4.85 *br s*	4.87 *br s*	4.89 *br s*
19	1.14 *s*	1.13 *s*	1.13 *s*	1.14 *s*	1.15 *s*	1.13 *s*
20	0.70 *s*	0.70 *s*	0.68 *s*	0.71 *s*	0.72 *s*	0.71 *s*
15-OMe	3.66 *s*		3.32 *s* and 3.34 *s*	3.55 *s*		
18-OMe			3.66 *s*	3.64 *s*		
$OCOCH_3$		2.01 *s*				

* 200.13 MHz; $CDCl_3$; TMS as internal reference. Coupling constants (*J* in Hz).

Table 2. ^{13}C NMR spectral data* (DEPT, XH-CORR) of compounds **1, 2a, 3a, 4, 5** and **6**.

C	**1**	**2a**	**3a**	**4**	**5**	**6**
1	37.8 *t*	37.9 *t*	37.8 *t*	38.0 *t*	37.7 *t*	37.9 *t*
2	18.3 *t*	18.3 *t*	18.3 *t*	18.3 *t*	18.2 *t*	18.2 *t*
3	36.9 *t*	37.0 *t*	36.8 *t*	36.9 *t*	36.9 *t*	36.9 *t*
4	47.4 *s*	47.4 *s*	47.6 *s*	47.7 *s*	47.3 *s*	47.4 *s*
5	49.3 *d*	49.4 *d*	49.7 *d*	49.8 *d*	49.3 *d*	49.3 *d*
6	26.6 *t*	26.7 *t*	26.6 *t*	26.7 *t*	26.6 *t*	26.6 *t*
7	37.7 *t*	37.8 *t*	37.6 *t*	37.7 *t*	37.6 *t*	37.6 *t*
8	147.7 *s*	147.9 *s*	147.7 *s*	147.1 *s*	147.5 *s*	147.0 *s*
9	57.0 *d*	56.9 *d*	56.5 and 56.6 *d*	56.2 *d*	55.9 *d*	56.1 *d*
10	38.8 *s*	38.8 *s*	38.9 *s*	39.0 *s*	38.6 *s*	38.8 *s*
11	20.7 *t*	20.6 *t*	22.0 and 22.2 *t*	21.3 *t*	23.3 *t*	21.2 *t*
12	35.6 *t*	35.7 *t*	31.9 and 32.6 *t*	24.3 *t*	23.8 *t*	24.3 *t*
13	31.0 *d*	30.2 *d*	37.3 and 38.9 *d*	139.1 *s*	125.2 *s*	134.5 *s*
14	41.2 *t*	35.6 *t*	39.0 and 39.2 *t*	141.5 *d*	110.7 *d*	144.0 *d*
15	173.6 *s*	63.0 *t*	105.1 and 105.5 *d*	102.4 *d*	142.4 *d*	70.1 *t*
16	19.8 *q*	19.2 *q*	71.5 and 72.4 *t*	171.3 *s*	138.4 *d*	174.3 *s*
17	106.8 *t*	106.9 *t*	106.7 *t*	107.3 *t*	106.8 *t*	107.3 *t*
18	185.3 *s*	185.4 *s*	179.1 *s*	179.2 *s*	185.5 *s*	185.3 *s*
19	16.2 *q*	16.2 *q*	16.4 *q*	16.5 *q*	16.1 *q*	16.2 *q*
20	14.5 *q*	14.6 *q*	14.5 *q*	14.6 *q*	14.5 *q*	14.6 *q*
OCH3 (on C-15)	51.2 *q*		54.4 and 54.8 *q*	56.9 *q*		
OCH3 (on C-18)			51.7 *q*	51.9 *q*		
OCOCH3		21.0 *q*				
OCOCH3		171.3 *s*				

*50.3 MHz; $CDCl_3$.

Table 3. Selected COLOC correlations on compound **1**

C	correlated protons	C	correlated protons
1	H-20	12	H-16
3	H-19	13	H-16
4	H-19	14	H-16
5	H-19, H-20	15	15-OMe
7	H-17, H-17′	18	H-19
9	H-17, H-17′, H-20		
10	H-20		

EXPERIMENTAL

General

The ^{1}H NMR, ^{13}C NMR, COSY, HETCOR, NOESY phase sensitive and COLOC spectra were obtained on a Bruker AC-200 spectrometer with $CDCl_3$ as solvent and TMS as int. standard. Mps are uncorr. Optical rotations were recorded on a Perkin-Elmer 141 polarimeter. EIMS were collected at 70 eV on a GC-MS SHIMADZU QP-5000 and HR-EIMS were obtained with a VG-ZAB-BEQQ spectrometer at

LANAIS-EMAR-CONICET, University of Buenos Aires. CC was performed on silica gel G 70–230 mesh, Kieselgel 60 H, Sephadex LH-20 and MPLC (LiChroprep™ Si 60, 63–125 μm). TLC: silica gel 60 F_{254} using Hexane-EtOAc and $CHCl_3$-MeOH gradients, sprayed with H_2SO_4-HOAc-H_2O (4:20:1) followed by heating.

Plant material

Aerial parts of *Eupatorium buniifolium* H. et A. were collected on October 2, 1989 at 'El Durazno Alto', San Luis. The plant was identified by Ing. Luis A. Del Vitto, U.N.S.L., and a voucher is deposited at the U.N.S.L. Herbarium N° 134.

Extraction and isolation

The air-dried aerial parts (1.350 kg) were extracted with $CHCl_3$. The solvent was evaporated under red. pres. and the residue was then dissolved in MeOH and kept at 2°. After filtering to remove the ppt., the resulting soln. was diluted with H_2O (10, 20 and 30%) and was partitioned between petrol, $CHCl_3$ and EtOAc, respectively. The $CHCl_3$ soln. was evap. and the residue chromatographed on silica gel using *n*-hexane-EtOAc gradients. After several purification, compounds **1** (573 mg), **2** (36 mg), **3** (95 mg), **4** (5 mg), **5** (290 mg) and **6** (92 mg) were obtained.

Methyl-ent-labd-8(17)-*en*-18-*oic acid*-15-*oate* (**1**)

$[\alpha]_D^{25}$:-18.4 ($CHCl_3$; *c* 1.0). MS *m/z* (rel. int.): 350 $[M]^+$ (3), 319 (2), 318 (3), 305 (19), 304 (36), 291 (10), 289 (11), 221 (12), 177 (15), 161 (32), 121 (100), 95 (31), 81 (57), 67 (38), 55 (61), 43 (76).

15-*Acetoxy-ent-labd*-8(17)-*en*-18-*oic acid* (**2a**)

MS *m/z* (rel. int.): 364 $[M]^+$ (2), 318 (21), 303 (3), 304 (3), 305 (4), 221 (4), 181 (9), 177 (4), 161 (16), 121 (65), 95 (20), 81 (44), 67 (22), 55 (39), 43 (100).

15,16-*Epoxy*-15-*methoxy-ent-labd*-8(17)-*en*-18-*oic acid methyl ester* (**3a**)

MS *m/z* (rel. int.): 364.2561 $[M]^+$ (< 1%), 349 (2), 332 (5), 317 (2), 304 (2), 305 (2), 289 (2), 207 (5), 180 (12), 175 (4), 121 (100), 93 (12), 81 (12), 67 (11), 55 (28), 41 (30).

15-*Methoxy-ent-labd*-8(17)-13-*dien*-18-*oic acid methyl ester*-16,15-*olide* (**4**)

$[\alpha]_D^{25}$:-37.5° ($CHCl_3$; *c* 0.45). MS *m/z* (rel. int.): 376 $[M]^+$ (1), 358 (3), 345 (1), 344 (3), 248 (9), 189 (10), 128 (25), 121 (100), 105 (21), 91 (27), 95 (15), 96 (17), 81 (27), 79 (33), 67 (29), 59 (22), 55 (56), 41 (83).

15,16-*Epoxy-ent-labd*-8(17),13(16),14-*trien*-18-*oic acid* (**5**)

Crystals, mp 101° (lit 102°). $[\alpha]_D^{25}$:-40.9° (EtOH; c 1.4), lit:-46° (EtOH; *c* 5). MS *m/z* (rel. int.): 316 $[M]^+$ (70), 235 (4), 221 (6), 189 (28), 121 (80), 95 (40), 94 (38), 81 (100), 67 (34), 55 (30), 41 (34).

ent-Labd-8(17)-13-*dien*-18-*oic acid*-16,15-*olide* (**6**)

Crystals, mp 168° (lit 170-171°). $[\alpha]_D^{25}$: − 36.6° ($CHCl_3$; *c* 0.72), lit: − 38.4° ($CHCl_3$; *c* 3.25). MS *m/z* (rel. int.): 332 $[M]^+$ (< 1%), 314 (4), 286 (100), 271 (40), 235 (5), 175 (16), 189 (22), 121 (86), 95 (27), 81 (52), 67 (38), 55 (54), 41 (76).

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