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DITERPENES OF *ERICAMERIA LINEARIFOLIA*

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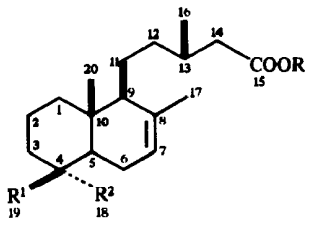
Key Word Index—*Ericameria linearifolia*; Asteraceae; labdane acids; labdane stereochemistry; grindelane stereochemistry.

Abstract—The isolation and characterization of two new bicyclic labdane diterpenes, 18α-succinyloxy-labd-7-en-15-oic acid and 8,17H-7,8-dehydropinifolic acid, and four known grindelane diterpenes from the aerial parts of *Ericameria linearifolia* are described. The configuration of C-4 substituents in related labdanes is discussed briefly.

INTRODUCTION

Ericameria linearifolia (D.C.) Urb. & Wuss. (Asteraceae, Astereae, Solidagininae) is a conspicuously resinous shrub that occurs in the arid regions of the south western United States and northern Baja, California. Although *Ericameria* has been treated as a section in the genus *Haplopappus*, it is now recognized at a generic level based on morphological, chemical and phenological studies [1]. So far, only flavonoids have been identified from five *Ericameria* taxa, including eight aglycones and glycosides from *E. linearifolia* [1–3].

As part of our phytochemical investigations of desert plants for biologically active compounds, we have isolated and spectroscopically characterized six diterpene acids (**1a–6a**) as methyl esters (**1b–6b**) from the methanol extract of the aerial parts of *E. linearifolia*. These include two new cativic compounds (**1b** and **2b**) and four grindelic compounds (**3b–6b**). During the course of our stereochemical study of these new diterpenes by way of ^{1}H NMR comparisons with related diterpenes, we observed discordance in the assignment of $-CH_2OR$ substituent at C-4 in many previously reported labdanes. These findings are also discussed in this paper.



	R	R¹	R²
1a	H	Me	$CH_2OC(=O)-CH_2-CH_2-COOH$ (1′, 2′, 3′, 4′)
1b	Me	Me	$CH_2OC(=O)-CH_2-CH_2-COOMe$
2a	H	Me	COOH
2b	Me	Me	COOMe
2c	H	COOH	Me

RESULTS AND DISCUSSION

The methanol extract of the aerial parts of *E. linearifolia* gave an ethyl ether-soluble fraction from which the sodium carbonate-soluble acid fraction was separated and methylated. Silica gel column chromatography of the methylated product gave a series of fractions from which two new (**1b** and **2b**) and four previously reported (**3b–6b**) diterpenes were separated by preperative TLC. The identity of the known compounds, methyl (**3b**), methyl 18-acetoxy-(**4b**), methyl 6-oxo-(**5b**), and methyl 6,8(17)-dehydro-(**6b**) grindelate, was established by TLC, IR, mass spectrometric and NMR data, as well as GC R_t comparisons with authentic samples.

Compound **1b** showed IR (neat) absorptions at 1738, 1164 (ester C=O) and 846 (C=C) cm^{-1}. The EI mass spectrum of **1b** (*m/z* 450, $[M]^+$) $C_{26}H_{42}O_6$ by high resolution was so informative that it allowed us to deduce the structure of **1b** except for its stereochemistry. The presence of the succinate group, which accounts for four of the six oxygen atoms, was clearly marked by the appearance of two characteristic intense peaks at *m/z* 115 $[\overset{+}{O}\equiv CCH_2CH_2COOMe]$ and *m/z* 318 $[M-HOOCCH_2CH_2COOMe]^+$. These fragments (*m/z* 115 and $[M-132]$) were constantly encountered in the mass spectra of succinyl derivatives of all diterpenes isolated in our laboratories (unpublished results). The succinate group was assumed to be at C-4, replacing either the C-18 or C-19 methyl of the *gem*-dimethyl group. This assumption was based on the absence of the *gem*-dimethyl characteristic doublet in the IR. The remaining two oxygen atoms were involved in a COOMe function, which was located at the end of the C-9 side chain. This was deduced as follows: the appearance of two strong peaks at *m/z* 122 (C_9H_{14}) and *m/z* 123 (C_9H_{15}) corresponded to the loss of 74 ($C_3H_6O_2$) and 73 mass units ($C_3H_5O_2$) from the characteristic right-hand half of the retro-Diels–Alder (RDA) fragmentation peak at *m/z* 196 ($C_{12}H_{20}O_2$) via β-cleavage with and without transfer of a γ-hydrogen atom,

Table 1. ^{1}H NMR (90 MHz) chemical shifts of compounds **1b** and **2b** (δ, $CDCl_3$-TMS)

	1b		**2b**	
H-7	5.34	1H, *br*, $W_{1/2}$ = 9 Hz	5.30	1H, *br*, $W_{1/2}$ = 8 Hz
H-16	0.96	3H, *d*, *J* = 6.6 Hz	0.96	3H, *d*, *J* = 6.2 Hz
H-17	1.66	3H, *br s*	1.64	3H, *br s*
H-18	3.75	2H, *d*, *J* = 2.2 Hz	—	
H-19	0.90	3H, *s*	1.21	3H, *s*
H-20	0.79	3H, *s*	0.78	3H, *s*
COOMe	3.66	3H, *s*	3.63	3H, *s*
	3.68	3H, *s*	3.66	3H, *s*
H-2′ } H-3′ }	2.63	4H, *s*	—	

respectively. These peaks suggested that **1b** belonged to the labd-7-en series with the COOMe group in the right-hand half and the succinate group in the left-hand half of the RDA moieties. This indicated the diacid nature of **1a**. The size and location of the side chain ($C_7H_{13}O_2$) at C-9 was deduced from the base peak at *m/z* 189 ($C_{14}H_{21}$) whose production from *m/z* 318 ($C_{21}H_{34}O_2$) corresponded to the allylically activated C-9–C-11 bond cleavage. The above data clearly suggested that **1b** possessed a cativic acid skeleton in which one of the 4,4-geminal methyls was replaced by a succinate group.

The ^{1}H NMR signals (Table 1) of **1b** fully supported the above findings. The various Me signals, two quaternary, one tertiary and an allylic, together with a signal for an olefinic proton were strongly reminiscent of Δ^7-labdanes, especially those of cativic acid (Me ester). The additional signals in **1b** conformed very well with the additional succinate group. The assignment of an equatorial C-4α succinate group in **1b** followed from ^{1}H NMR spectral comparisons with related labdanes [4, 5]. Stereostructure **1a** is suggested for the new diacid, 18α-succinyloxy-labd-7-en-15-oic acid. The ^{13}C NMR of its methyl ester (Table 2) was in full agreement with structure **1b**.

The structure of **2b** was deduced by correlation with **1b** which differed from **2b** only at C-4. By comparing their IR and NMR (Tables 1 and 2) spectra, which showed striking similarities, and from the characteristic series of peaks in the EI mass spectrum of **2b**, which lacked the peaks corresponding to the succinate moiety, the structure of **2b** was determined easily. The difference of 86 mass units between the M_r of **1b** (*m/z* 450, $[M]^+$) and **2b** (*m/z* 364, $[M]^+$), which corresponded to $C_4H_6O_2$ by high resolution analysis of **1b** ($C_{26}H_{42}O_6$) and **2b** ($C_{22}H_{36}O_4$), immediately suggested that the succinate group in **1b** was replaced by COOMe in **2b**. This was confirmed from ^{1}H and ^{13}C NMR (Tables 1 and 2) analyses of **2b** which were very similar to those of **1b** but lacked C-18 succinate absorptions. That **2b** contains the B-ring component with the same features as **1b** was clear from two ubiquitous peaks at *m/z* 122 (base) and *m/z* 123 (44.2%), derived from the right half of the RDA fragment as discussed above under **1b**. This close correspondence also suggested that the stereochemistries of **1b** and **2b** were the same. Additional support for placing the C-4 COOMe in the equatorial (α) configuration came from the IR of **2b** in which the 1247 cm^{-1} band was more intense than that at 1150 cm^{-1} [6]. ^{1}H NMR spectral comparisons with related labdanes [7–10] were in agreement with this assignment. Therefore, compound **2a**, which is the C-4 epimer of **2c** [11], is 8,17H-7,8-dehydropinifolic acid.

Table 2. ^{13}C NMR (22.5 MHz) chemical shifts for compounds **1b** and **2b** (δ, $CDCl_3$-TMS)

Carbon	**1b***	**2b**
1	38.7	38.5
2	18.0	18.3
3	39.4	39.5
4	36.6	46.9
5	44.6	45.5
6	24.4	24.4
7	121.7	121.8
8	135.3	135.5
9	55.2	55.6
10	36.7	36.7
11	23.9	25.7
12	36.2	37.4
13	31.3	31.4
14	41.4	41.4
15	173.6	173.4
16	20.0	20.0
17	22.0	21.9
18	73.2	178.9
19	17.6	17.0
20	14.1	14.1
MeO	51.2, 51.7	51.1, 51.7

*Succinate carbons appear at 172.2, 172.6, 29.1 and 29.4.

While determining the C-4 stereochemistry of **1b**, we noticed that the ^{1}H NMR chemical shifts (δ) reported for $-CH_2OR$ (R=H, Me or Ac) substituents at C-4 in some labdane diterpenes disagreed with the stereochemistry shown and/or discussed. A literature review of ^{1}H NMR data of several labdanes (Table 3) clearly showed the difference in chemical shift of *ca* 0.4 ppm between the axial and equatorial orientations of $-CH_2OR$ substituents at C-4 [12]. Thus, the δ values reported for $-CH_2OR$ substituents at C-4 in discoidic acid methyl ester (R = H, 3.66) [13], cordobic acid methyl ester (R = H, 3.58) and its derivatives, 7-*epi* (R = H, 3.56), monoacetate (R = Ac, 4.03) and diacetate (R = Ac, 4.01) [14] represent axial (C-19 substituted) and not equatorial orientations as depicted. Similarly the δ values reported for $-CH_2OR$ at C-4

Table 3. Reported ^{1}H NMR chemical shifts (δ, $CDCl_3$-TMS) for C-4 substituted ($-CH_2OR$) labdanes

R = H or Me		R = Ac	
equatorial	axial	equatorial	axial
3.11 [17]	3.55 [7]	3.83 [17]	4.17 [29]
3.25 [17]	3.73 [10]	3.74 [15]	4.12 [29]
3.22 [7]	3.70 [29]	3.74 [8]	4.12 [29]
3.23 [8]	3.63 [29]	3.75 [4]	4.12 [5]
3.23 [10]	3.64 [29]	3.75 [5]	4.10 [30]
3.24 [4]	3.65 [29]	3.66 [26]	4.02 [7]
3.26 [5]	3.67 [29]	3.79 [28]	
3.18 [26]	3.68 [29]	3.75 [28]	
3.22 [27]	3.69 [29]		
3.27 [27]	3.60 [27]		
3.27 [28]			
3.28 [28]			

in 19-hydroxygrindelic acid (R = H, 3.26) [15], 19-hydroxyisolambertianol (R = H, 3.26) [16], 19-hydroxy-17-acetoxy-*ent*-cativic acid methyl ester (R = H, 3.26) [17] and a 19-hydroxy substituted friedolabdane (R = H, 3.37) [17] correspond to equatorial (C-18 substituted) and not axial configurations as stated.

The configurations of **1b** and **2b** were assumed to be normal labdanes based on the data presented and their positive OR values. Although previous work indicated that grindelic acid, which has a large negative OR value, was a normal labdane [18–20], X-ray analysis has established a normal labdane configuration for a grindelane and a labdane with positive OR values [21, 22], and an *ent*-labdane configuration for two labdanes with negative OR values [23, 24]. Recent work by Jakupovic *et al.* [17] stated that **3a** belonged to the *ent*-labdane series. With this evidence it now appears that all previously reported grindelanes (with negative OR values) might belong to the *ent*-labdane series.

EXPERIMENTAL

Plant material. E. linearifolia was collected from two localities in Mojave County, Arizona, during May 1985; 36.5 km SE and 3.2 Km NW of Kingman. Herbarium specimens have been deposited in the Herbarium at the University of Arizona, Tucson. All plant material was air-dried, ground to 3 mm particle size and stored at 5° prior to extn.

Extraction and fractionation. Milled aerial parts (0.5 kg) were extd exhaustively with MeOH in a Soxhlet extractor (24 hr). The solvent-free ext. (115 g) was stirred (1 hr) with Et_2O (1 l), left in the freezer (1 hr), filtered and the residue extd with more Et_2O (800 ml). From the combined Et_2O-sol material, an aq. Na_2CO_3 (5%) sol phase was sepd, acidified (25% aq. HCl), and extd with Et_2O. The dry acidic fraction (55.6 g) was methylated with MeI in Me_2CO–K_2CO_3 [25] and the resulting Me ester mixt (45 g) sepd by CC [EM silica gel 60 (1,125 g), *n*-hexane–EtOAc (49:1), and gradually increasing the concn of EtOAc] into a total of 71 fractions (200 ml each). Based on their TLC profiles, similar fractions were combined to yield 20 fractions. TLC and GC analyses of these showed that fractions 4, 6, and 7 contained **6b**, **3b**, and **2b**, respectively, and fractions 12 and 13 contained **1b**, **4b**, and **5b**. These compounds were isolated and purified by prep. TLC (silica gel 60 PF-254) with *n*-hexane–Me_2CO and *n*-hexane–EtOAc solvent systems.

18α-*Succinyloxy-labd-7-en-15-oic acid methyl ester* (**1b**). Colourless oil. IR ν_{max}^{neat} cm^{-1}: 1738, 1437, 1364, 1201, 1164, 1003, 846; EIMS (probe), 70 eV, *m/z* (rel. int.): 450 $[M]^+$ (4.2), 319 (17.6), 318 (48.0), 303 (35.6), 271 (20.8), 203 (14.3), 196 (4.9), 190 (21.2), 189 (100), 175 (20.3), 161 (16.6), 148 (20.4), 147 (20.4), 133 (26.3), 123 (42.0), 122 (46.4), 119 (53.3), 115 (40.0), 109 (47.3), 107 (28.4), 95 (32.6), 81 (31.4), 69 (23.6), 55 (47.6), high resolution MS: *m/z* 450.3010 (calc for $C_{26}H_{42}O_6$, 450.2981).

$$[\alpha]_{24°}^{\lambda} = \frac{589 \quad 578 \quad 546 \quad 463 \quad 365}{+15.45 \quad +16.00 \quad +18.31 \quad +31.93 \quad +48.61}$$

($CHCl_3$; *c* 2.95)

For NMR consult Tables 1 and 2.

8,17*H*-7,8-*Dehydropinifolic acid methyl ester* (**2b**). Colourless oil. IR ν_{max}^{neat} cm^{-1}: 1739, 1726, 1436, 1247, 1184, 1150, 1110, 879; EIMS (probe), 70 eV, *m/z* (rel. int.): 364 $[M]^+$ (4.2), 349 (6.3), 305 (22.3), 304 (33.4), 289 (19.6), 273 (6.4), 257 (15.1), 235 (38.6), 203 (15.2), 189 (16.2), 175 (67.4), 173 (19.3), 161 (19.1), 149 (18.8), 135 (27.0), 123 (44.2), 122 (100), 121 (72.5), 109 (86.9), 107 (53.9), 95 (63.1), 81 (53.5), 69 (43.5), 55 (59.2), high resolution MS: *m/z* 364.2585 (calc for $C_{22}H_{36}O_4$, 364.2614).

$$[\alpha]_{24°}^{\lambda} = \frac{589 \quad 578 \quad 546 \quad 463 \quad 365}{+16.44 \quad +17.00 \quad +19.40 \quad +31.75 \quad +45.93}$$

($CHCl_3$; *c* 4.34)

For NMR consult Tables 1 and 2.

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