589.
9. Rodriguez, M. C., Barluenga, J., Savona, G., Piozzi, F., Servettaz, O. and Rodriguez, B. (1984) *Phytochemistry* **23**, 1465.
10. Papanov, G. Y. and Malakov, P. Y. (1982) *Z. Naturforsch.* **37B**, 519.
11. Malakov, P. Y., Papanov, G. Y., Mollov, N. M. and Spassov, S. L. (1978) *Z. Naturforsch.* **33B**, 1142.
12. Rodriguez, M. C., Barluenga, J., Pascual, C., Rodriguez, B., Savona, G. and Piozzi, F. (1984) *Phytochemistry* **23**, 2960.
13. Eguren, L., Perales, A., Fayos, J., Rodríguez, B., Savona, G. and Piozzi, F. (1982) *J. Org. Chem.* **47**, 4157.
14. Pascual, C., Fernández, P., García-Alvarez, M. C., Marco, J. L., Fernández-Gadea, F., de la Torre, M. C., Hueso-Rodríguez, J. A., Rodríguez, B., Burno, M., Paternostro, M., Piozzi, F. and Savona, G. (1986) *Phytochemistry* **25**, 715.
15. Piozzi, F., Rodríguez, B. and Savona, G. (1987) *Heterocycles* **25**, 807.

Phytochemistry, Vol. 26, No. 10, pp. 2861–2863, 1987.
Printed in Great Britain.

0031–9422/87 $3.00 + 0.00

A NEW DITERPENOID FROM *ERICAMERIA LARICIFOLIA*

JOSEPH J. HOFFMANN, SHIVANAND D. JOLAD,* BARBARA N. TIMMERMANN, ROBERT B. BATES,† FERNANDO A. CAMOU† and TERUNA J. SIAHAAN†

University of Arizona, Office of Arid Lands Studies, Bioresources Research Facility, 250 E. Valencia Rd., Tucson AZ 85706, U.S.A.; *University of Arizona, College of Pharmacy, Tucson, AZ 85721, U.S.A.; †University of Arizona, Department of Chemistry, Tucson, AZ 85721, U.S.A.

(*Revised received* 29 *March* 1987)

Key Word Index—*Ericameria laricifolia*; Asteraceae; Astereae; Solidagininae; diterpenoid acids; labdanes; grindelanes.

Abstract—An acid fraction of the methylene chloride extract of *Ericameria laricifolia* gave, in addition to four known grindelanes, a new diterpenoid acid possessing an *ent*-labdane skeleton. Its structure, based on the spectral properties of its methyl ester derivative, has been determined as 15-succinyloxy-*ent*-labd-13*E*-en-8*β*-ol.

INTRODUCTION

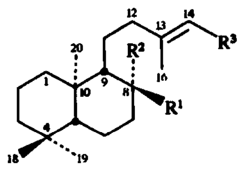
As part of our phytochemical investigations of the genus *Ericameria* (Asteraceae, Astereae) from the southwestern U.S.A. [1], we have now examined the resin acid constituents of *Ericameria laricifolia* (Gray) Shinners from New Mexico. *Ericameria*, a new world genus of perhaps 12 species, is often treated as a section of the genus *Haplopappus*. *E. laricifolia* is a resinous shrub that grows on desert hillsides from eastern California to western Texas. The chemistry of *Ericameria* is largely unknown. Four taxa were reported to produce flavonoid aglycones and glycosides [2, 3] as well as labdane diterpenoid acids [1]. In this paper we describe the isolation and characterization of a new and four previously reported labdanoids from *E. laricifolia.*

RESULTS AND DISCUSSION

The methylene chloride extract of the above-ground biomass gave an ether soluble fraction from which the sodium carbonate-soluble acid fraction was separated and methylated. Separation of the methylated product chromatographically yielded the new labdane **1b** and four known grindelane methyl esters: methyl 6-oxo-17-acetoxy-(**2b**) [4], 17-acetoxy-(**3b**) [4], 18-acetoxy-(**4b**) [1] and 17-isobutyroxy-(**5b**) [4] grindelate, identified by TLC and ^{1}H NMR spectral comparisons with authentic samples. Compounds **3b** and **4b** have very similar R_f vlues and were not separated from one another, but the amount of each present in the mixture of two was clear by ^{1}H NMR.

Structure of compound 1b

The IR ($CHCl_3$) spectrum of **1b** showed absorption for hydroxyl (3520 cm^{-1}), ester (1730 and 1155 cm^{-1}), =CH- (3010 and 838 cm^{-1}), -CH_2CO-(1405 cm^{-1}) and -$C(Me)_2$- (1380 and 1360 cm^{-1}) groups. The EI mass spectrum of **1b** allowed deduction of the structure except for stereochemistry. The molecular ion peak at *m/z* 422, which was barely discernible, was deduced from peaks at *m/z* (rel. int.) 291 $[M-O_2CCH_2CH_2COOMe]^+$ (1.7), 290 $[M-HOOCCH_2CH_2COOMe]^+$ (7.2), 275 $[290-Me]^+$ (5.1), 272 $[290-H_2O]^+$ (8.5) and 257 $[290-Me-H_2O]^+$ (8.8). These peaks, together with an intense peak at *m/z* 115 $[O^+\equiv CCH_2CH_2COOMe]$ (94.4%) suggested that **1b** was a methyl succinate derivative of a diterpene



	R¹	R²	R³	
1a	OH	$\overset{17}{\text{Me}}$	$\overset{15}{C}H_2-O-\overset{1'}{C}(=O)-\overset{2'}{C}H_2-\overset{3'}{C}H_2-\overset{4'}{C}OOH$	
1b	OH	Me	$CH_2-O-C(=O)-CH_2-CH_2-COOMe$	
6	OH	Me	CH_2OH	
7	OH	Me	CH_2OH	dihydro derivatives
8	OH	Me	COOMe	
9	Me	OH	CH_2OH	
10	Me	OH	COOMe	

diol. Peaks at m/z 115 and 290 $[M-HOOCCH_2CH_2COOMe]^+$ occur in the EI mass spectrum of all diterpenoid methyl succinates examined in our laboratories [unpublished results]. That the succinate and unsaturation functions were part of the C-9 side chain was deduced from another strong peak at m/z 192 (42.7 %) whose formation from m/z 290 was rationalized as shown in Fig. 1. The $M^{+\cdot}$ and m/z 291 ions could also produce m/z 192 in a similar manner. The further loss of a methyl radical from m/z 192 accounted for the most abundant peak at m/z 177 (82.6 %). The possibility of the hydroxyl group being at C-8 was also supported by the fragmentation sequence m/z 422 → m/z 291 $[M-C_5H_7O_4]^+$ → m/z 273 $[291-H_2O]^+$ (10.3 %) → m/z 149 [retro-Diels–Alder (RDA)] (23.8 %). The presence of a diagnostic peak at m/z 109 (52.2 %), formed by the elimination of a methyl radical from the left-half of the RDA fragment, suggested that the ring A in **1b** was unsubstituted. This peak is present in the EI mass spectrum of all 7-en labdanes/grindelanes having no substituent in ring A that we have studied [4]. Thus the position of the hydroxyl group at C-8 appeared to be the structural prerequisite for the formation of the 7-en ion (m/z 273, 10.3 %) and its further RDA breakdown. Another clue to the position of the hydroxyl group at C-8 in **1b** came from a pair of peaks at m/z 191 (39.4 %) and 81 (96.7 %) resulting from an allylically activated C-9/C-11 bond fission of the m/z 272 ion as shown in Fig. 1.

The ^{1}H NMR (250 MHz, $CDCl_3$–TMS) spectral parameters of **1b** were consistent with these findings and gave stereochemical information. The large side chain was defined by absorptions for H-12 (δ2.11, *t*, $J = 8.2$ Hz), H-14 (5.34 *t*, $J = 7.1$ Hz) H-15 (4.61, *dd*, $J = 12.5$, 7.0 Hz; 4.62, *dd*, $J = 12.5$, 7.0 Hz), H-16 (1.71, *s*, $W_{1/2} = 3.1$ Hz, indicating an *E* configuration for the 13,14 double bond [5]), H-2′ and H-3′ (2.64, *s*), and OMe (3.69, *s*). The chemical shifts of the four singlet methyl groups (Table 1) as compared with those of 8-hydroxylabdanes **6–10** show the constitution and relative configurations in **1b** to be as shown. In particular, the hydroxyl at C-8 must be equatorial to absorb at *ca* 0.80 as in **6–8** rather than at *ca* 0.95 as in **9** and **10**. The ^{13}C NMR shifts were also consistent with structure **1b**.

The absolute configuration depicted in **1b** is tentative on the basis that the most closely related natural products **6–10** are all *ent*-labdanes, and the most closely related ones **6** and **7**, like **1b**, have small positive optical rotations (Table 1).

EXPERIMENTAL

Plant material. The plant material used in this study was collected in New Mexico, Grant County, 17.5 miles southwest of Silver City in September, 1985. A voucher specimen (SPM 3099) has been deposited at ARIZ. All plant material was air-dried, ground to 3 mm particle size and stored at 5° prior to extraction.

Extraction. The ground whole plant of *E. laricifolia* (900 g) was extracted by percolation with CH_2Cl_2 at room temp. (24 hr) and the solvent removed. The dry extract (135 g) was stirred with Et_2O (2 l) for 2 hr, left in the refrigerator overnight and filtered. From the Et_2O- soluble filtrate, the Na_2CO_3 (5 % aq.) soluble portion was sepd, neutralized (25 % aq HCl), extracted with Et_2O, dried and solvent freed to give 76.6 g of the acid mixture.

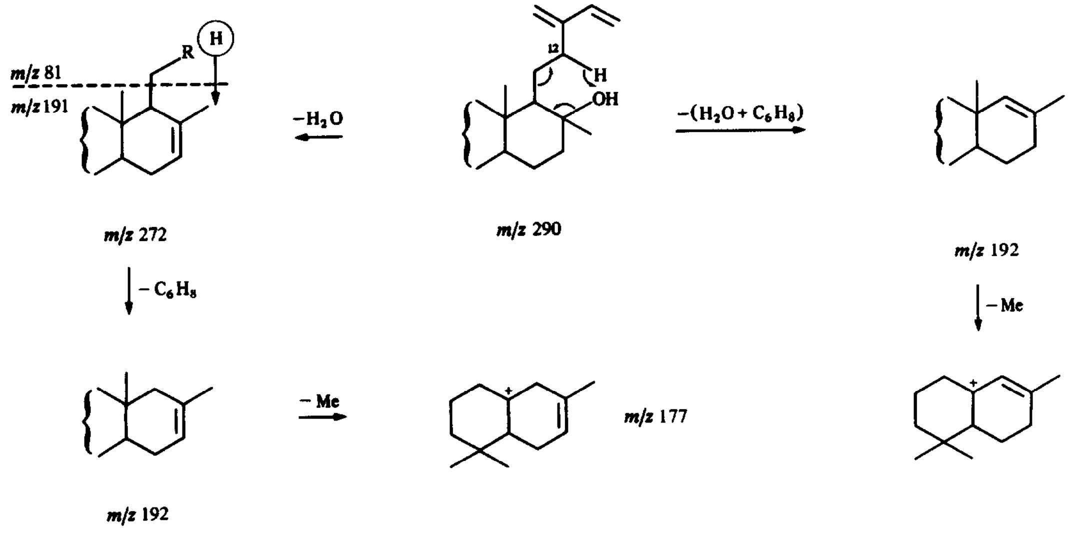


Table 1. Comparison of $[\alpha]_D$ ($CHCl_3$) and chemical shifts (δ) of the 17- to 20-methyl protons of **1b** and related labdanes (**6**–**10**)

Compound	17-Me	18-Me	19-Me	20-Me	$[\alpha]_D^0$	Ref.
1b	1.13	0.87	0.80*	0.79*	+3.6†	
6	1.13	0.87	0.80	0.80	+0.7	[6]
7	1.15	0.87	0.81	0.81	+3.0	[7]
8	1.15	0.87	0.81	0.81	−3.0	[7]
9	1.14	0.86	0.83	0.95	−32.0	[8]
10	1.13	0.86	0.82	0.94	−37.0	[8]

*May be interchanged.
†($CHCl_3$; *c* 4.0).

The dry acid mixture (74.6 g) was methylated according to the procedure described in ref. [9].

Isolation of compounds **1b**–**5b**. The isolation of these compounds was performed qualitatively. The methylated product (79 g) was dissolved in Et_2O, adsorbed over silica gel 60, submitted to silica gel 60 CC (1 kg packed in *n*-hexane) and eluted with *n*-hexane–Et_2O [1:1, fr. F1) and 1:3 (frs F2 and F3)], Et_2O (100%, fr. F4) and CH_2Cl_2–MeOH (1:1, fr. F5). Fraction F2 (27 g) was rechromatographed on silica gel 60 (300 g packed in CH_2Cl_2) eluting the column with CH_2Cl_2–EtOAc [50:1 (frs F6-F23), 1:1 (fr. F24)]. From fr. F8 (2.05 g) compounds **1b** and **2b** were isolated by repetitive silica gel PF 254 prep TLC using CH_2Cl_2–MeOH (50:1 and 45:1, multiple developments). A portion of the fr. F1 (1.2 g) was submitted to silica gel PF 254 prep. TLC [*n*-hexane–EtOAc (9:1)]. This gave six fractions, F25-F30. Compounds **3b** and **4b** (as a TLC homogeneous mixture) from fr. F29 and **5b** from fr. F28 were isolated by further prep. TLC.

15-*Succinyloxy*-ent-*labd*-13*E*-*en*-8*β*-*ol*. Colourless oil. Its $[\alpha]_D$, IR, ^1H NMR and MS data are described in the text. ^{13}C NMR (δ, $CDCl_3$–TMS): 39.6 (C-1), 18.3 (C-2), 42.7 (C-3), 33.1 (C-4), 56.0 (C-5), 20.4 (C-6), 44.4 (C-7), 74.2 (C-8), 61.1 (C-9), 39.1 (C-10), 23.3 (C-11), 41.8 (C-12), 143.5 (C-13), 117.8 (C-14), 61.7 (C-15), 16.5 (C-16), 23.6 (C-17), 33.3 (C-18), 21.4 (C-19), 15.4 (C-20), 172.3 (C-1'), 28.8 (C-2'), 29.1 (C-3'), 172.7 (C-4'), 51.7 (OMe).

Acknowledgements—We thank Dr Steven P. McLaughlin for plant collection and identification and Mr Peter Baker for mass spectral data. This investigation was supported by the National Science Foundation under Grant No. PCM-8304771 which we gratefully acknowledge.

REFERENCES

1. Dentali, S. J., Hoffmann, J. J., Jolad, S. D. and Timmermann, B. N. *Phytochemistry* **26**, (in press).
2. Urbatsch, L. E. and Wussow, J. R. (1979) *Brittonia* **31**, 265.
3. Clark, W. D., Wollenweber, E. (1984). *Z. Naturforsch.* **39c**, 1184.
4. Timmermann, B. N., Hoffmann, J. J., Jolad, S. D., Bates, R. B. and Siahaan, T. J. (1987). *Phytochemistry* **26**, 467.
5. Bory, S., Fetizon, M. and Laszlo, P. (1963) *Bull. Soc. Chim. Fr.* 2310.
6. Jefferies, P. R. and Payne, T. G. (1965) *Aust. J. Chem.* **18**, 1441.
7. Henrick, C. A., Jefferies, P. R. and Rosich, R. S. (1964) *Tetrahedron Letters* 3475.
8. Hugel, G., Oehlschlager, A. C. and Ourisson, G. (1966) *Tetrahedron* **8**, 203.
9. Timmermann, B. N., Hoffmann, J. J., Jolad, S. D., Schram, K. H., Klenck, R. E. and Bates, R. B. (1982) *J. Org. Chem.* **47**, 4114.