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LABDANE DITERPENES FROM *BRICKELLIA GLOMERATA**

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Key Word Index—*Brickellia glomerata*; Compositae; Eupatorieae; labdane type diterpenes; *enantio*-oliveric acid; 12-oxolambertianic acid; demethyl pinusolide; lambertianic acid.

Abstract—The leaves of *B. glomerata* afforded three new labdane diterpenes. Their structures and stereochemistry were established by spectroscopic methods and chemical transformations.

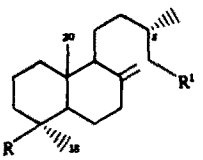
INTRODUCTION

In continuation of our chemical studies of members of the genus *Brickellia* (Eupatorieae) [1–4], we have undertaken the study of *B. glomerata* and have isolated in addition to the known lambertianic acid, three new labdane type diterpenes whose structures were established as enantio-oliveric acid (**1a**), 12-oxolambertianic acid (**2b**) and demethyl pinusolide (**3a**).

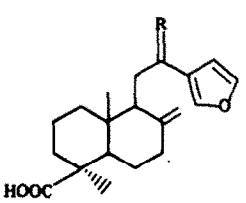
RESULTS AND DISCUSSION

Compound **1a** was the major constituent of the leaves of *B. glomerata*, $C_{20}H_{32}O_4$, $[M]^+$ at m/z 336, was isolated as a gum. The presence in **1a** of carboxylic acid group(s) were shown by IR absorptions at 3500–2380 and 1695 cm^{-1}, and by the formation of the dimethyl ester (**1b**) upon treatment with excess of diazomethane. It also contained an exocyclic methylene group (IR absorptions at 1640 and 890 cm^{-1}). The ^1H NMR spectrum of **1a** showed signals due to two tertiary methyl groups (δ0.55 and 1.27) and one secondary methyl group (δ0.95, *d*, J = 6.5 Hz). The presence of the exocyclic methylene group was confirmed by absorptions at δ4.47 and 4.80 (*br s*, 1H each).

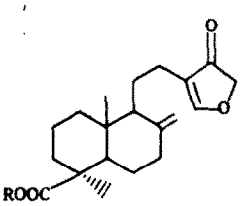
The hindered nature of one of the carboxylic groups in **1a** followed from the formation of the mono ester **1c**, obtained by Fischer esterification, while alkaline hydrolysis of **1b** gave the isomeric mono-ester **1d**. Additional proof for the axial configuration of the carboxyl group at C-4 was provided by reduction of **1a** with lithium aluminium hydride to the diol **1e**. The ^1H NMR spectrum of **1e**, showed two doublets at δ3.36 and 3.75 (AB system) characteristic of an axial hydroxy methylene group at C-4 [5–6].



1a R = R^1 = COOH
1b R = R^1 = COOMe
1c R = COOH, R^1 = COOMe
1d R = COOMe, R^1 = COOH
1e R = R^1 = CH_2OH



2a R = H_2
2b R = O



3a R = H
3b R = Me

*Contribution No. 844 from Instituto de Química, UNAM.

The stereochemistry at C-13 and the absolute configuration of **1a** were established by comparison of the ^{1}H NMR data of **1b** with those reported for dimethyl oliverate [7], which were identical, but the optical rotation had opposite sign. Hence **1a** must be (13*S*)-labd-8(17)-en-15,19-dioic acid (*enantio*-oliveric acid).

12-Oxo-lambertianic acid (**2b**) was a gum, $C_{20}H_{26}O_4$ ($[M]^+$ at *m/z* 330), which exhibited bands in the IR spectrum at 3700–2400 and 1690 cm^{-1} corresponding to a carboxylic acid group. The structure of **2b** clearly followed from the ^{1}H NMR spectrum which was very similar to that of lambertianic acid (**2a**) [8], differing only with respect to the furan ring signals which were downfield shifted, suggesting a β-ketofuran grouping [9]. This assumption was confirmed by the presence in the electron impact mass spectrum of the base peak at *m/z* 95 [9]. The axial orientation of the carboxyl group at C-4 was deduced from the typical position of the C-10 methyl signal [8, 10].

A third isolated compound (**3a**) was a gum with $[M]^+$ at *m/z* 332 indicating the formula $C_{20}H_{28}O_4$. The IR spectrum showed the presence of a carboxylic acid (3450–2400 and 1693 cm^{-1}) and a γ-lactone (1753 cm^{-1}). The structure of **3a** followed from the ^{1}H NMR spectrum which showed a great resemblance to the data described for nivenolide [11].

The stereochemistry of the carboxyl group at C-4 and the absolute configulation of **3a**, was established by conversion to the methyl ester **3b**, whose ^{1}H NMR and optical rotation data were identical to those previously published for pinusolide [12].

EXPERIMENTAL

Brickellia glomerata Fernald, was collected near Amacuzac, on the toll road Amacuzac–Iguala (59.5 km), state of Morelos, in December 1983. A voucher specimen has been deposited in the Herbarium of the University of Texas at Austin, Texas. Dried leaves (940 g) were extracted with CH_2Cl_2 and the extract was evaporated *in vacuo* to give a gummy residue (388 g). A 10.5 g sample was chromatographed over 110 g silica gel packed in CH_2Cl_2, 125 ml fractions being collected as follows: 1–9 (CH_2Cl_2), 10–18 (CH_2Cl_2–Me_2CO, 19:1), 19–22 (CH_2Cl_2–Me_2CO, 9:1).

Fractions 4 and 5 on standing afforded 300 mg of **2a** as a colourless solid, mp 124° (lit. 126–127° [8]), $[\alpha]_D^{25} = +45.5°$ ($CHCl_3$; *c* 0.47) (lit. $[\alpha]_D^{22} = +55°$ (EtOH) [8]). Fractions 10 and 11 (1.72 g) were rechromatographed in 100 g of silica gel using CH_2Cl_2 and mixtures of CH_2Cl_2–Me_2CO as eluants. Initial fractions were further purified by prep. TLC (CH_2Cl_2–Me_2CO 19:1, × 2) gave **2a** [8], **2b** and **3a**.

12-Oxo lambertianic acid (**2b**). A gum, IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3700–2400, 1690, 890. ^{1}H NMR (80 MHz, $CDCl_3$): δ0.70 (3H, *s*, H-20), 1.27 (3H, *s*, H-18), 4.36 (1H, *br s*, H-17), 4.72 (1H, *br s*, H-17'), 6.76 (1H, *br s*, H-14), 7.41 (1H, *t*, *J* = 1.5 Hz, H-15), 8.05 (1H, *br s*, H-16). EIMS (probe) 70 eV *m/z* (rel. int.): 330 $[M]^+$ (9), 220 $(M - Me - 95]^+$ (9), 121 $(C_9H_{13}]^+$ (18), 95 $(+O{\equiv}C{-}C_4H_3O]$ (100).

Demethyl pinusolide (**3a**). Colourless gum; IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3450–2400, 1754, 1694, 895: ^{1}H NMR (80 MHz, $CDCl_3$), δ0.60 (3H, *s*, H-20), 1.25 (3H, *s*, H-18), 4.55 (1H, *br s*, H-17), 4.85 (1H, *br s*, H-17'), 4.75 (2H, *br s*-H15), 7.05 (1H, *br s*, H-14): EIMS (probe) 70 eV *m/z* (rel. int.): 332 $[M]^+$ (5), 314 $(M - H_2O]^+$ (7), 286 $(M - CH_2O_2]^+$ (19), 271 $(M - CH_2O_2 - Me]^+$ (17), 121 $(C_9H_{13}]^+$ (51), 91 (62), 41 (100). Compound **3a** on esterification with CH_2N_2 afforded **3b**. The ^{1}H NMR spectrum was identical to that previously published for pinusolide (**3b**) [12].

Fractions 13–16 exhibited one major spot and were combined to give 1.3 g of crude **1a**. A 100 mg sample was purified by prep. TLC using CH_2Cl_2–Me_2CO (19:1) as eluant and afforded 60 mg of *enantio*-oliveric acid (**1a**) as a colourless gum. $[\alpha]_D^{25} + 30.5°$ (EtOH; *c* 0.59); IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3500–2380, 1695, 1640, 890; ^{1}H NMR (80 MHz, $CDCl_3$): δ0.55 (3H, *s*, H-20), 0.95 (3H, *d*, *J* = 6.5 Hz, H-16), 1.27 (3H, *s*, H-18), 4.47 (1H, *br s*, H-17), 4.80 (1H, *br s*, H-17'). EIMS, 70 eV, *m/z* (rel. int.): 336 $[M]^+$ (3), 318 $(M - H_2O]^+$ (8), 300 $[M - 2H_2O]^+$ (5), 290 $[M - HCOOH]^+$ (22), 121 $[C_9H_{13}]^+$ (100), 55 $[C_4H_7]^+$ (55).

enantio-*Dimethyl oliverate* (**1b**). Esterification of crude **1a** (616 mg) with excess of CH_2N_2 provided 300 mg of the ester **1b** after TLC purification (CH_2Cl_2–Me_2CO, 19:1). Colourless gum. $[\alpha]_D^{25} + 49.5°$ ($CHCl_3$; *c* 0.58); IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1720, 890; ^{1}H NMR (80 MHz, $CDCl_3$): δ0.50 (3H, *s*, H-20), 0.94 (3H, *d*, *J* = 6.0 Hz, H-16), 1.18 (3H, *s*, H-18), 3.60 (3H, *s*, O – Me), 3.65 (3H, *s*, O – Me), 4.46 (1H, *br s*, H-17), 4.81 (1H, *br s*, H-17').

enantio-*Oliveric acid*, 15-*monomethyl ester* (**1c**). Compound **1a** (291 mg) in MeOH and a drop of conc H_2SO_4 was refluxed for 1.3 hr and worked-up as usual to give, after TLC purification, 273 mg of **1c**. Colourless oil. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3500–2500, 1730, 1698, 1650, 892. ^{1}H NMR (80 MHz, $CDCl_3$): δ0.60 (3H, *s*, H-20), 1.24 (3H, *s*, H-18), 0.93 (3H, *d*, *J* = 6.0 Hz, H-16), 3.65 (3H, *s*, O – Me), 4.45 (1H, *br s*, H-17), 4.80 (1H, *br s*, H-17').

enantio-*Oliveric acid*, 19-*monomethyl ester* (**1d**). To a soln of 247 mg of **1b** in 20 ml MeOH, 127 mg NaOH was added. The reaction mixture was refluxed for 2 hr and worked-up as usual to give, after TLC purification (CH_2Cl_2–Me_2CO, 19:1) 196 mg **1d** as a colourless oil. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3500–2500, 1712, 890. EIMS 70 eV, *m/z* (rel. int.): 350 $[M]^+$ (6), 290 $[M - 60]^+$ (31), 121 $[C_9H_{13}]^+$ (100). ^{1}H NMR (80 MHz, $CDCl_3$): δ0.50 (3H, *s*, H-20), 0.97 3H, *d*, *J* = 6.0 Hz, H-16), 1.18 (3H, *s*, H-18), 3.60 (3H, *s*, O – Me), 4.46 (1H, *br s*. H-17), 4.81 (1H, *br s*, H-17').

(13*S*)-*Labd*-8(17)-*en*-15,19 *diol* (**1e**). To a soln of **1d** (189 mg) in 30 ml dry Et_2O was added $LiAlH_4$ (391 mg) in small amounts, the reaction being monitored by TLC. After 1 hr the reaction was worked-up and the residue purified by TLC (CH_2Cl_2–Me_2CO, 19:1), yielding 65 mg of diol **1e** as colourless crystals, mp 98–100°, $[\alpha]_D^{25} + 35°$ ($CHCl_3$; *c* 0.439), IR $\nu_{max}^{CHCl_3}$ cm^{-1} 3615, 1645, 890: EIMS 70 eV *m/z* (rel. int.): 308 $[M]^+$ (5), 290 $(M - H_2O]^+$, 277 $[M - CH_2OH]^+$ (80), 121 $[C_9H_{13}]^+$ (60), 95 (100), 55 (92). ^{1}H NMR (80 MHz, $CDCl_3$): δ0.64 (3H, *s*, H-20), 0.90 (3H, *d*, *J* = 6.0 Hz, H-16), 0.97 (3H, *s*, H-18), 3.65 (2H, *t*, *J* = 6.5 Hz, H-15), 3.36 (1H, *d*, *J* = 11.5, H-19), 3.75 (1H, *d*, *J* = 11.5 Hz, H-19'), 4.47 (1H, *br s*, H-17), 4.77 (1H, *br s*, H-17').

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A NOVEL WITHANOLIDE FROM *DATURA METEL*

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Key Word Index—*Datura metel*; Solanaceae; withanolide; Daturilin.

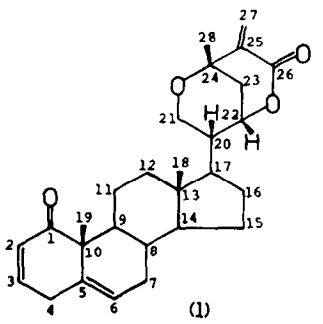
Abstract—A novel withanolide, daturilin, has been isolated from the alcohol soluble extract of the fresh leaves of *D. metel* and its structure established as 1-oxo-21,24*S*-epoxy-(20*S*,22*S*)-witha-2,5,25-trienolide through spectral studies including 2D NMR.

INTRODUCTION

In an earlier communication the isolation and structure elucidation of a new tropane alkaloid datumetine [1] has been reported from the alcoholic extract of the leaves of *D. metel*. L. Extended studies in the neutral constituents of the same plant material have led to the isolation and structure elucidation of a novel withanolide, daturilin (**1**). A few withanolides have been reported earlier from other species of *Datura* eg. *D. quercifolia*, [2–6], *D. ferox* [7], *D. stramonium* [8] and its variety *violaceae* [9], but this is the first instance of the isolation of a withanolide from *D. metel*. Further, although the C-21, C-24 epoxide ring has been reported in the triterpenes of tirucallane skeleton [10, 11], there is no precedence for an epoxide ring in a withanolide skeleton.

RESULTS AND DISCUSSION

From the acid-insoluble fraction of the alcoholic extract of *D. metel* leaves, a colourless crystalline constituent daturilin (**1**) mp 206° has been obtained through solvent fractionation and preparative thin layer chromatography. The molecular ion $[M]^+$ at *m/z* 436 and high resolution MS corresponded to the molecular formula $C_{28}H_{36}O_4$ and the prominent peaks at *m/z* 269 and 268 were indicative of M-side chain fragment with and without transfer of hydrogen. The intensity and position of the UV band at 218 nm (ε 16318) indicated two α,β-unsaturated carbonyl chromophores without extended conjugation. The IR spectrum exhibited bands at 1680 and 1720 cm^{-1} for α,β-unsaturated ketone and α,β-unsaturated δ-lactone groupings respectively [12], both characteristic of withanolides. IR and MS spectra did not show the presence of any hydroxyl group in **1**, which was also confirmed through negative results of attempted acetylation. In the low field region of the ^1H NMR spectrum of **1**, signals related to five vinylic proton were observed. Two one-proton doublets of double doublets at δ5.78 (J = 10.11, 3.24, 1.23 Hz) and δ6.69 (J = 10.11, 5.02, 2.49 Hz) have been assigned to H-2 and H-3 respectively. H-6 appeared as a double triplet at δ5.60 (J = 6.06, $W_{1/2}$ = 1.71 Hz) and the remaining two vinyl protons resonating at δ5.99 and δ6.56 as narrow doublets (J = 0.78 Hz) were indicative of H-27a and H-27b of the exo double bond in the side



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