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ENT-LABDANES FROM *AUSTROBRICKELLIA PATENS*

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Key Word Index—*Austrobrickellia patens*; Compositae; diterpenes; *ent*-labdane derivatives; sesquiterpenes; caryophyllenic acid.

Abstract—The aerial parts of *Austrobrickellia patens* afforded five new labdane derivatives. In addition a caryophyllenic acid and several known compounds were isolated.

INTRODUCTION

The recently discovered small South American genus *Austrobrickellia* [1] is placed in the subtribe *Alomiinae* (tribe Eupatorieae) [2]. The species is characterized by very slender corallas with short, rather erect lobes, large clavate style branches and an enlarged hairy mode at the base of the style. The five ribbed achenes have been of primary significance in their placement. This feature along with the smaller cylindrical carpopodium, the scarcely fringed pappus setae and the rigidly divergent branching continue to distinguish the group from *Brickellia* and related genera. So far only a small collection from Bolivia has been studied chemically [3]. As no typical compounds were isolated we decided to investigate a sample from Argentina. In addition to known compounds an acid derived from caryophylleneepoxide and five labdane derivatives were isolated.

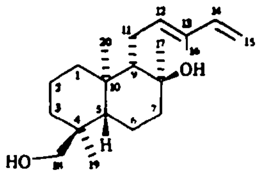
RESULTS AND DISCUSSION

The aerial parts of *Austrobrickellia patens* (D. Don. ex H. et A.) K. et R. afforded caryophyllene, bicyclogermacrene, spathulenol, 4β-hydroxygermacra-1(10)*E*,5*E*-diene, 3-angeloyloxy-6-methoxytremetone, lupeyl acetate, taraxasterylacetate, caryophyllene-epoxide (**6**), the corresponding acid **7** as well as the five labdane derivatives **1**–**5**.

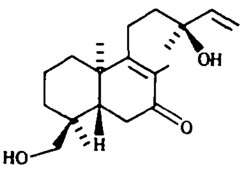
The structure of **7** was deduced from the ^{1}H NMR spectral data of the methyl ester (see Experimental). Spin decoupling allowed the assignment of all signals which were in part close to those of caryophyllene-epoxide. One methyl group was replaced by the carbomethoxy group which had to be placed at C-11. The configuration at this centre followed from a clear NOE between H-12, H-1 and H-10. The orientation of the carbomethoxy group followed from the downfield shifts of H-9α (Δ = 0.69 ppm) and H-10α (Δ = 0.68 ppm) when compared with the shifts of caryophyllene-epoxide.

The structure of **1** followed from the ^{1}H NMR spectrum (see Experimental) which was close to that of the corresponding 6,18-dihydroxyabienol derivative from a *Koanophyllon* species [4]. The stereochemistry at C-4 and C-8 was deduced from the chemical shifts of H-18 and H-17 respectively. The *E*-configuration of the Δ^{12}-double bond followed from the shift of H-14.

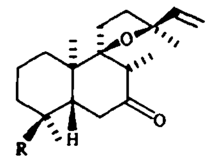
Compounds **2**–**5** were closely related as followed from the ^{1}H NMR spectra. In all cases the characteristic signals of a vinyl group and of three methyls at saturated carbons were present. In the spectrum of the main compound (**3**) a methyl doublet and a pair of doublets for a hydroxymethylene group were detected. By spin decoupling all signals were assigned though some were overlapped multiplets. The methyl doublet collapsed to a singlet on irradiation of a doublet quartet at δ2.55. Furthermore a signal at δ2.21 (H-6β) was sharpened. As the latter showed



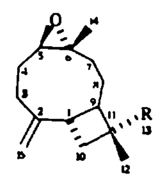
1



2



3 R = CH_2OH
4 R = CHO
5* R = CO_2H



6 R = Me
7* R = CO_2H

*5a and 7a are the corresponding methyl esters couplings with signals at δ2.43 (*dd*, H-6α) and 2.36 (*dd*, H-5) the presence of a keto group at C-7, which was indicated by an IR band at 1710 cm^{-1}, was established. The ether bridge between C-9 and C-13 followed from a group of multiplets between 1.75 and 2.08 (4H) which showed no couplings with other signals similar to the ^{1}H NMR data of grindelic acid derivatives [5]. The ^{13}C NMR data (see Experimental) agreed well with structure **3**. The fragmentation pattern in the MS of **3** supported the proposed structure. In addition to the elimination of isoprene (*m/z* 252) and water (*m/z* 302), the base peak (*m/z* 180) was most likely formed as the result of RDA (fission of the bands between C-9 and C-10 as well as between C-5 and C-6). Inspection of a model allowed the assignment of the stereochemistry by NOE difference spectroscopy. Clear effects were observed between H-20, H-17, H-2α, H-6α, H-19 and H-11, between H-19, H-20, H-18, H-2α and H-6α, between H-17, H-11′ and H-20 as well as between H-16, H-8, H-14 and H-15t. These effects which required C-8-methyl and C-4-hydroxymethylene axial, established the configurations at C-4, C-5, C-8, C-9, C-10 and C-13 and assigned the methyl groups. A W-coupling between H-6β and H-8 also indicated the configuration at C-8. The absolute configuration was supported by a negative Cotton effect at $\Delta\varepsilon$ 275 nm which, by using the octant rule, indicated an *ent*-labdane derivative.

The structures of **4** and **5**, which was transformed to their methyl ester **5a**, were readily deduced from the ^{1}H NMR data (see Experimental). The presence of a carbonyl group at C-18 caused a downfield shift of the H-5 and the H-19 signals while the remaining ones were unaffected. The mass spectra showed identical behaviour. In both cases the base peak was *m/z* 180 as the result of a RDA.

The spectral data of **2** differed markedly from those of **3**–**5**, though again several ^{1}H NMR signals were close to those of **3**–**5**. The absence of an H-8 signal and the replacement of the methyl doublet by an olefinic methyl signal indicated that **2** was an isomer of **3** where the ether ring was opened and a double bond was formed. Accordingly, the mass spectroscopic behaviour was changed completely. Now the base peak (*m/z* 203) was most likely formed by loss of water and of the side chain. A UV maximum at 244 nm agreed with the presence of a substituted cyclohexenone. The changed conformation of the diterpene **2** led to a change of the Cotton effect which, however, also agreed with the presence of an *ent*-labdane if the octant rule was applied. Most likely **2** is the precursor of **3**. The roots only gave known compounds (see Experimental).

The isolation of *ent*-labdane derivatives from *Austrobrickellia* supported the relationship with *Brickellia* where this type of diterpene is most widespread [6–10]. However, so far in no species of the latter genus have special compounds like **1**–**5** been reported. Furthermore dehydronerolidol derivatives, which seem to be typical for *Brickellia* [7], have not been detected in *A. patens*. Two other genera, which are placed in the same subtribe, contain labdane derivatives more closely related to those of *Brickellia* [11, 12]. Further investigations may give a clearer picture of the chemotaxonomy of this subtribe.

EXPERIMENTAL

The air dried plant material (400 g, collected in Feb. 1985 in Argentina, Voucher RMK 9451, deposited in the U.S. National Herbarium, Washington) was extracted with MeOH–Et_2O–petrol (1 : 1 : 1) and worked-up as reported previously [13]. By CC (silica gel) six fractions were taken: (1) petrol, (2) Et_2O–petrol (1:9), (3) Et_2O–petrol (3:7), (4) Et_2O–petrol (1:1), (5) Et_2O and (6) Et_2O–MeOH (9:1). Prep. TLC (SiO_2, PF 254) of fraction 1 gave 35 mg caryophyllene and 35 mg bicyclogermacrene. Prep. TLC of fraction 2 (Et_2O–petrol, 1:9) gave 100 mg taraxasteryl acetate, 30 mg lupeyl acetate and 100 mg **6**. Prep. TLC of fraction 3 (C_6H_6–CH_2Cl_2, 1:1) gave 15 mg spathulenol, 20 mg 4β-hydroxygermacra-1(10)*E*,5*E*-diene and 20 mg **6**. Prep. TLC of fraction 4 (C_6H_6–CH_2Cl_2–Et_2O, 2:2:1) gave 5 mg *cis*-3-angeloyloxy-6-methoxytremetone and a crude fraction which gave by HPLC (MeOH–H_2O, 17:3, always RP 8 *ca* 100 bar, flow rate *ca* 3 ml/min) 1 mg **4** (R_t 4.4 min). Fraction 5 gave, after TLC and HPLC (MeOH–H_2O, 17:3), 19 mg **3** (R_t 4.7 min). The ^{1}H NMR spectrum of fraction 6 indicated the presence of acids. After esterification by addition of CH_2N_2, prep. TLC (Et_2O) gave three fractions (6/1–6/3). HPLC of 6/1 (MeOH–H_2O, 7:3) gave 2.6 mg **7a** (R_t 5.3 min) and 15 mg **5a** (R_t 6.0 min). HPLC of 6/2 (MeOH–H_2O, 7:3) gave 1.2 mg **1** (R_t 21.3 min) and HPLC of 6/3 (MeOH–H_2O, 7:3) afforded 7.7 mg **2** (R_t 7.8 min). Prep. TLC of the extract of the roots (60 g) gave 10 mg 11-hydroxy-6-methoxytremetone, 25 mg dammadienyl acetate, 12 mg stigmasterol and 10 mg 11-hydroxy-11,12-dihydroobliquin [14]. Known compounds were identified by comparing the 400 MHz ^{1}H NMR spectra with those of authentic material.

18-*Hydroxyablenol* (**1**). Colourless oil; IR $\nu_{\max}^{CCl_4}$ cm^{-1}: 3620 (OH); MS *m/z* (rel. int.): 306 $[M]^+$ (1), 288.245 $[M-H_2O]^+$ (12) (calc. for $C_{20}H_{32}O$: 288.245), 257 $[288-CH_2OH]^+$ (12), 207 $[288-C_6H_9]^+$ (40), 134 (89), 81 $[C_6H_9]^+$ (100); ^{1}H NMR (400 MHZ, $CDCl_3$): δ2.32 and 2.20 (*ddd* (*br*), H-11), 5.59 (*t* (*br*), H-12), 6.34 (*dd*, H-14), 4.90 (*d*, H-15c), 5.06 (*d*, H-15t), 1.78 (*s*, H-16), 1.19 (*s*, H-17), 3.42 and 3.13 (*d* (*br*), H-18), 0.89 (*s*, H-19), 0.75 (*s*, H-20); [*J* (Hz): 9,11 = 9,11′ = 11,12 = 11′,12 = 6; 11,11′ = 15; 14,15c = 11; 14,15t = 17; 15c,15t = 1; 18,18′ = 11].

13,18-*Dihydroxy-7-oxo*-ent-*labda*-8,14-*diene* (**2**). Colourless oil; IR $\nu_{\max}^{CCl_4}$ cm^{-1}: 3640, 3610 (OH), 1660, 1620 (C=CC=O), 3090, 935 ($CH=CH_2$); UV: $\nu_{\max}$ 244 nm (Et_2O); MS *m/z* (rel. int.): 320.235 $[M]^+$ (8) (calc. for $C_{20}H_{32}O_3$: 320.235), 305 $[M-Me]^+$ (6), 302 $[M-H_2O]^+$ (8), 271 $[302-CH_2OH]^+$ (31), 203 (100); CD (MeCN): $\Delta\varepsilon_{373} = +0.26$, $\Delta\varepsilon_{354} = +0.72$, $\Delta\varepsilon_{341} = +0.75$, $\Delta\varepsilon_{326} = +0.35$; $[\alpha]_D^{24} = +25$ ($CHCl_3$; *c* 0.7); ^{1}H NMR ($CDCl_3$): δ1.93 (*ddd* (*br*), H-1α) 1.53 (*ddd* (*br*), H-1β), 1.36 (*ddd* (*br*), H-3β), 2.05 (*dd*, H-5), 2.34 (*dd*, H-6α), 2.41 (*dd*, H-6β), 2.25 (*m*, H-11), 5.93 (*dd*, H-14), 5.13 (*dd*, H-15c), 5.26 (*dd*, H-15t), 1.33 (*s*, H-16), 1.72 (*s*, H-17), 3.40 and 3.10 (*dd* (*br*), H-18), 0.84 (*s*, H-19), 1.10 (*s*, H-20); [*J* (Hz): 1α,1β = 1β,2α = 2α,2β = 2α,3β = 3α,3β = 13; 1α,2α = 1β,2β = 2α,3α = 2β,3β = 4; 5,6α = 13; 5,6β = 5; 6α,6β = 17; 14,15c = 11; 14,15t = 17; 15c,15t = 1; 18,18′ = 11; 18,OH = 18′,OH = 4].

18-*Hydroxy-7-oxo-9β,13β-epoxy*-ent-*labd*-14-*ene* (**3**). Colourless oil; IR $\nu_{\max}^{CCl_4}$ cm^{-1}: 3630 (OH), 1710 (C=O), 3090, 925 ($CH=CH_2$); MS *m/z* (rel. int.): 320.235 $[M]^+$ (26) (calc. for $C_{20}H_{32}O_3$: 320.235), 305 (3), 302 (6), 252 $[M-\text{isoprene}]^+$ (19), 180 $[C_{11}H_{16}O_2, \text{RDA}]^+$ (100), 165 (27), 109 (45); $[\alpha]_D^{24} = -9.5$ ($CDCl_3$; *c* 1.0); CD (MeCN): $\Delta\varepsilon_{275} = -0.1$; ^{1}H NMR ($CDCl_3$): δ1.27 (*d* (*br*), H-1α), 1.50 (*ddd* (*br*), H-1β), 1.62 (*ddddd*, H-2α), 1.53 (*m*, H-2β), 1.30 (*d* (*br*), H-3α), 1.41 (*ddd* (*br*), H-3β), 2.36 (*dd*, H – 5), 2.43 (*dd*, H-6α), 2.21 (*ddd* (*br*), H-6β), 2.55 (*dq*, H-8), 2.08 and 1.85 (*m*, H-11), 1.90–1.75 (*m*, H-12), 5.92 (*dd*, H-14), 4.93 (*dd*, H-15c), 5.11 (*dd*, H-15t), 1.25 (*s*, H-16), 1.20 (*d*, H-17), 3.33 and 3.09 (*d*, H-18), 0.80 (*s*, H-19), 1.09 (*s*, H-20); [*J* (Hz): H-1α–H-3β see compound **2**; 5,6α = 6α,6β = 14; 5,6β = 2.5; 6β,8 = 1.5; 8,17 = 8; 14,15c = 11; 14,15t = 17; 15c,15t = 1; 18, 18′ = 11]; ^{13}C NMR ($CDCl_3$, C-1–C-20): 32.4 *t*, 17.2 *t*, 35.3 *t*, 37.1 *s*, 39.7 *d*, 38.0 *t*, 215.4

s, 57.5 *d*, 93.5 *s*, 41.7 *s*, 28.9 *t*, 34.8 *t*, 83.5 *s*, 143.9 *d*, 111.0 *t*, 27.6 *q*, 16.4 *q*, 71.5 *t*, 16.7 *q*, 18.1 *q*.

7,18-*Dioxo-9β,13β-epoxy-*ent*-labd-14-ene* (**4**). Colourless oil; IR $\nu_{\max}^{\mathrm{CCl_4}}$ cm^{-1}: 1730 (CHO), 1720 (C=O); MS *m/z* (rel. int.): 318.220 $[\mathrm{M}]^+$ (17) (calc. for $C_{20}H_{30}O_3$: 318.220), 303 (6), 300 (6), 289 (5), 285 (8), 271 (10), 180 (100), 109 (98); ^{1}H NMR ($CDCl_3$): δ2.66 (*dd*, H-5), 2.51 (*dd*, H-6α), 1.78 (*ddd*, H-6β), 2.62 (*dq*, H-8), 2.11 (*m*, H-11, 12), 5.97 (*dd*, H-14), 4.99 (*dd*, H-15c), 5.13 (*dd*, H-15t), 1.30 (*s*, H-16), 1.21 (*d*, H-17), 9.23 (*s*, H-18), 1.13 (*s*, H-19), 1.08 (*s*, H-20); [*J* (Hz): see 3].

*Methyl-7-oxo-9β,13β-*epoxy-ent-*labd-14-en-18-oate* (**5a**). Colourless gum; IR $\nu_{\max}^{\mathrm{CCl_4}}$ cm^{-1}: 1730 (CO_2R), 1720 (C=O), 3090, 1640, 930 ($CH{=}CH_2$); MS *m/z* (rel. int.): 348. 230 $[\mathrm{M}]^+$ (27) (calc. for $C_{21}H_{32}O_4$: 348.230), 316 $[\mathrm{M} - \mathrm{MeOH}]^+$ (6), 180 (100), 109 (61), 107 (59); $[\alpha]_D^{24} = +13$ ($CHCl_3$; *c* 0.9); ^{1}H NMR ($CDCl_3$): δ2.91 (*dd*, H-5), 2.50 (*dd*, H-6α), 1.88 (*ddd*, H-6β), 2.57 (*dq*, H-8), 2.11 (*m*, H-11, H-12), 5.96 (*dd*, H-14), 4.96 (*dd*, H-15c), 5.16 (*dd*, H-15t), 1.28 (*s*, H-16), 1.19 (*d*, H-17), 1.18 (*s*, H-19), 1.09 (*s*, H-20); [*J* (Hz): see 3].

Methyl-5β, 6α-epoxycaryophyllen-13-oate (**7a**). Colourless oil; IR $\nu_{\max}^{\mathrm{CCl_4}}$ cm^{-1}: 1730 (CO_2R); MS *m/z* (rel. int.): 264.172 $[\mathrm{M}]^+$ (5) (calc. for $C_{16}H_{24}O_3$: 264.172), 246 (5), 235 (8), 232 (6), 109 (72), 107 (74), 93 (88), 81 (86), 79 (85), 55 (100); ^{1}H NMR ($CDCl_3$): δ2.68 (*ddd*, H-1), 2.36 (*ddd*, H-3α), 2.14 (*m*, H-3β), 2.26 (*m*, H-4α) 1.31 (*m*, H-4β), 2.88 (*dd*, H-5), 1.03 (*ddd*, H-7α), 2.09 (*ddd*, H-7β), 1.82 (*dddd*, H-8α) 1.55 (*dddd*, H-8β), 2.46 (*dd* (*br*) H-9), 2.30 (*dd*, H-10α), 1.78 (*dd*, H-10β), 1.31 (*s*, H-12), 1.20 (*s*, H-14), 5.05 and 4.93 (*d*, (*br*), H-15); [*J* (Hz): 1,9 = 10; 1,10α = 11; 1,10β = 8; 3α,3β = 13; 3α,4α = 4; 3α,4β = 7.5; 4α,5 = 4; 4β,5 = 10.5; 7α,7β = 13; 7α,8α = 5; 7α,8β = 13; 7β,8α = 3; 7β,8β = 4; 8α,8β = 15; 8β,9 = 10; 10α,10β = 11].

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