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DITERPENES FROM *FLEISCHMANNIA HYMENOPHYLLA* AND *BRICKELLIA LACINIATA*

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Key Word Index—*Fleischmannia hymenophylla*; *Brickellia laciniata*; Compositae, diterpenes; beyerane derivative; labdanes; sesquiterpene lactone, heliangolide.

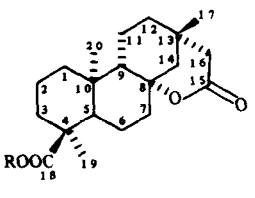
Abstract—The aerial parts of *Fleischmannia hymenolepis* gave in addition to phytol and sesamin a new derivative of beyerene named hymenophylloide. The aerial parts of *Brickellia laciniata* afforded several new labdane derivatives and a heliangolide. Structures were elucidated by spectroscopic methods and the absolute configuration of the labdanes were determined by CD measurements.

INTRODUCTION

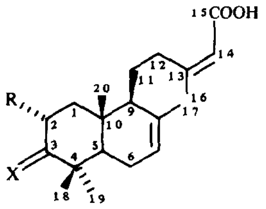
In continuation of our chemical investigations of representatives of the tribe Eupatorieae (Compositae) we have studied a further *Fleischmannia* species and *Brickellia laciniata*. The aerial parts of the latter have so far only been investigated for flavones [1] while the roots gave an acetylenic compound and a derivative of dehydronerolidol [2] which are widespread in the genus [3]. The results are presented in this paper.

RESULTS AND DISCUSSION

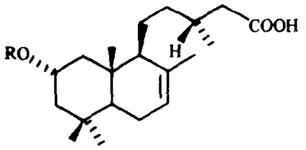
The extract of the aerial parts of *Fleischmannia hymenophylla*, a species growing in Costa Rica and Panama, gave phytol, sesamin and the diterpene **1**. The molecular formula of **1** indicated the presence of four oxygens ($C_{20}H_{30}O_4$), two of them being due to an acid. Accordingly, addition of diazomethane afforded a methyl ester (**1a**). The ^{13}C NMR spectrum (Table 1) showed that in addition to the acid a lactone group was present (δ171.9). Furthermore, the multiplicities required a tetracyclic compound. The ^{1}H NMR spectrum of **1** and **1a** (Table 1) displayed three methyl singlets which would be in agreement with a kaurane or beyerane derivative where the five membered ring was transformed to a δ-lactone as the IR spectrum excluded a γ-lactone. A pair of double doublets at δ2.33 and 2.22 in the spectrum of **1** were due to H-16 as *W*-couplings with H-14 and H-12, respectively, were responsible for the small couplings. Starting with these signals by using NOE difference spectroscopy and spin decoupling all signals could be assigned. The resulting sequences clearly showed that a diterpene lactone was present derived from beyeran-16-one by Bayer–Villiger-oxidation or from a pimarane derivative by transformation of the vinyl group to an acetic acid residue followed by lactone formation. The stereochemistry was deduced from the observed NOE's. Irradiation of H-17 gave clear effects with H-14 (4%), H-14′ (4%), H-16′ (6%) and H-16 (2%) indicating that the lactone oxygen was at C-8 and the methylene group (H-16) at C-13. Further NOE's were



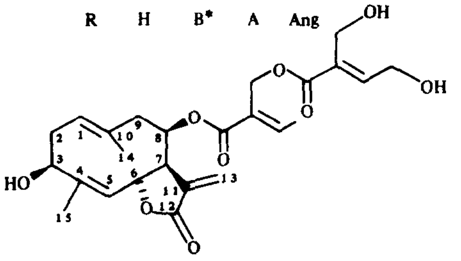
	1	1a
R	H	Me



	2	3
R	H	OA*
X	O	H_2

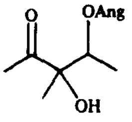


	4†	5	6	7
R	H	B*	A	Ang



8

*A =



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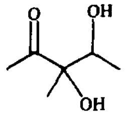

† **4a** is the 2-oxo methylester

Table 1 ^1H and ^{13}C NMR spectral data of **1** and **1a** ($CDCl_3$, 400 and 100.6 MHz respectively, δ-values)

H	**1**	**1a**	Multiplicity	C	**1**	**1a**	Multiplicity
5	1.76	1.74	*br dd*	1	38.4	38.5	*t*
6	1.87	1.85	*dddd*	2	17.5	17.5	*t*
6′	1.16	1.03	*m*	3	36.9	36.9	*t*
7	1.90	1.88	*dt*	4	47.3	47.5	*s*
7′	1.62	1.50	*m*	5	49.7	50.0	*d*
9	1.16	1.14	*br dd*	6	20.5	20.4	*t*
11	1.67	1.66	*m*	7	39.4	39.4	*t*
11′	1.26	1.25	*dddd*	8	82.6	82.6	*s*
12	1.67	1.60	*m*	9	56.1	56.1	*d*
12′	1.30	1.30	*ddd*	10	36.4	36.5	*s*
14	1.53	1.51	*dd*	11	18.1	18.1	*t*
14′	1.40	1.38	*dd*	12	38.9	38.9	*t*
16	2.33	2.32	*dd*	13	30.5	30.5	*s*
16′	2.22	2.20	*dd*	14	45.9	45.9	*t*
17	0.96	0.95	*s*	15	172.1	171.9	*s*
19	1.21	1.19	*s*	16	41.9	41.9	*t*
20	1.01	0.99	*s*	17	29.9	29.9	*q*
				18	*	179.3	*s*
				19	16.4	16.5	*q*
				20	15.1	15.1	*q*
OMe	--	3.65	*s*	OMe	–	51.9	*q*

*Not detected

J [Hz] 5,6 = 6,6′ = 9,11′ = 12; 5,6′ = 1.5, 6,7 = 6′,7 = 3, 7,7′ = 13, 9,11 = 11,12′ = 12,12′ = 11′,12′ ~ 14, 12,14 = 2, 14,14′ = 13, 14′,16 = 2.5, 16,16′ = 18

present between H-9 and H-5 (6%), H-20, H-6α (5%) and H-19 (5%) as well as between H-20, H-19 (5%) and H-6α (6%). As the signals of H-19 and H-20 could not been assigned unambiguously selective INEPT was used which connect H-17 with C-13, H-19 with C-4 and C-18 as well as H-20 with C-10. The ^{13}C NMR signals were assigned by 2D-techniques. The diterpene **1** we have named hymenophylloide.

The aerial parts of *Brickellia laciniata*, a species growing in the southwest of U.S.A. and Mexico, gave in addition to widespread compounds (Experimental) and 7-hydroxy-5,6E-dehydro-6,7-dihydro-α-farnesene, the labdane derivatives **2–6**, the angelate **7** [4] and the heliangolide **8**.

The structure of **2** could be deduced easily from its ^1H NMR spectrum (Table 2) which was similar to that of the corresponding 13*E*-isomer [5]. As expected the H-16 signal was shifted up field in the spectrum of **2**.

The ^1H NMR spectrum of **3** (Table 2) was in part similar to that of **2**. However, additional signals of an angelate residue and of two further methyl signals (δ 1.37 *s* and 1.36 *d*) as well as a new low field triplet of triplets at δ4.97 indicated that an ester of the corresponding 2α-hydroxy derivative of **2** was present. The highest peak in the mass spectrum (*m/z* 500) was due to $C_{30}H_{44}O_6$. However, a fragment *m/z* 419 corresponding to $C_{25}H_{39}O_5$ indicated that it was due to elimination of the angeloyloxy radical. Accordingly, *m/z* 500 was formed by loss of water. A fragment at *m/z* 302 most likely was due to loss of the acid which therefore was in agreement with the ^1H NMR signals indicating that the compound was the angelate of 2-methyl-2,3-dihydroxybutyric acid, probably formed by nucleophilic attack of angelate at the β-carbon of epoxyangelate.

The ^1H NMR data of **4** (Table 2) and its molecular formula ($C_{20}H_{34}O_3$) indicated that we were dealing with the 13,14-dihydro derivative of the alcohol corresponding to the ester **3**. Comparison with the ^1H NMR signals of the 3-oxo derivative isolated from *B veronicaefolia*, where the relative configuration at C-13 was established [6], indicated that the same configuration was very likely.

The ^1H NMR spectra of **5** and **6** (Table 2) showed that esters of **4** were present. While in the spectrum of **6** the signals of the ester part were identical with those of **3** in the spectrum of **5** those of the angelate group were missing. Accordingly, the mass spectrum showed a peak at *m/z* 438, corresponding to $C_{25}H_{42}O_6$. As expected an upfield shift of the methyl signals of this ester group was observed. Thus, the acids **5** and **6** both were closely related to **3**.

Esterification and oxidation of **4** gave the keto ester **4a**, which showed a positive Cotton-effect. Application of the octant rule supported the proposed absolute configuration, which agrees with that reported so far from all labdanes from *Brickellia* species.

In contrast to this the ketone **2** showed a weak negative Cotton-effect. Inspection of a Dreiding-model indicated that the axial 4-methyl group is probably responsible for this effect though the more distant large part of the molecule should lead to a positive effect.

The ^1H NMR spectrum of **8** at elevated temperature (Table 3) allowed the assignment of all signals by spin decoupling leading to a sequence which only agreed with the presence of a heliangolide with a 3β-hydroxy and 8β-acyloxy group. Inspection of the ester signals, their chemical shifts and couplings led to the proposed structure of the ester group. The observed data agreed well with those reported from other lactones having this ester

Table 2. ^{1}H NMR spectral data of **2–6** and **4a** ($CDCl_3$, 400 MHz, δ-values)

H	**2***	**3**	**4**	**4a†**	**5**	**6**
1α	1 51 *ddd*	‡	1 13 *dd*	2 37 *br d*	1 33 *dd*	1.22 *dd*
1β	2.20 *ddd*	‡	1 75 *br d*	2 10 *dd*	1.75 *br d*	1 81 *ddd*
2	2.26 *ddd* 2.70 *ddd*	4 97 *tt*	3 86 *dddd*	—	5 07 *dddd*	4.98 *dddd*
3α	—	‡	0 93 *dd*	2 15 *br d*	1.03 *dd*	1.09 *dd*
3β	—	‡	2 16 *br d*	2.48 *dd*	2.29 *br d*	2 09 *ddd*
7	5 43 *br s*	5 41 *br s*	5 38 *br s*	5 43 *br s*	5 83 *br s*	5.41 *br s*
14	5.67 *br s*	5 64 *br s*	2 23 *m*	2.28 *dd* 2 16 *dd*	2 22 *dd* 2 13 *dd*	2.26 *br d*
16	1 94 *br s*	1 90 *br s*	0 98 *d*	0 95 *d*	0 97 *d*	1 01 *d*
17	1 78 *br s*	1 74 *br s*	1 66 *br s*	1 69 *br s*	1 66 *br s*	1 67 *br s*
18	1 09 *s*	0.91 *s*	0 91 *s*	1.04 *s*	0.95 *s*	0.94 *s*
19	1 04 *s*	0 97 *s*	0 91 *s*	0 90 *s*	0 98 *s*	0 98 *s*
20	0 97 *s*	0 79 *s*	0.77 *s*	0 78 *s*	0 86 *s*	0 84 *s*
OR	—	6.04 *qq*	—	—	3 99 *q*	6.05 *qq*
		5.27 *q*			1 32 *s*	5 25 *q*
		1.91 *dq*			1.24 *d*	1.96 *dq*
		1 37 *s*				1.37 *s*
		1 84 *dq*				1.87 *dq*
		1 36 *d*				1.33 *d*

*H-12 2 78 *br ddd* (12, 12, 5) and 2 58 *br ddd* (12, 12, 5), ‡obscured; †OMe 3.66 *s*.

J[Hz]: Compound **2**· 1α,1β = 1α,2β = 2α,2β = 14, 1α,2α = 1β,2α = 1β,2β = 4; compounds **3–6** 1α,1β = 1α,2 = 2,3α = 3α,3β = 12, 1β,2 = 2,3β = 4, 13,14 = 6, 13,14′ = 8, 13,16 = 6 5; 14,14′ = 15; (compound **4a** 1α,1β = 3α,3β = 12 5; 1β,3β = 2 5

Table 3 ^{1}H NMR spectral data of **8** ($CDCl_3$, 60°, δ-values, J [Hz] in parenthesis)

H	
1	5.20 *m*
2	2.66 *ddd* (3, 9 5, 14)
2′	2.19 *ddd* (4, 7 5, 14)
3	4 48 *dd* (3, 4)
5	5.21 *dq* (11, 1 5)
6	6 36 *dd* (11, 2 5)
7	2 93 *br s*
8	5 30 *ddd* (3 5, 3, 1)
9	2 78 *dd* (3 5, 14 5)
9′	2 41 *dd* (3, 14 5)
13	6.33 *d* (2 7)
13′	5 73 *d* (2)
14	1 78 *br s*
15	1.73 *d* (1 5)
PR	7 06 *t* (6)
	6 93 *q* (7)
	4 93 *s* (2H)
	4.44 *d* (6, 2H)
	4 33 AB (2H)
	1 90 *d* (7, 3H)

residue. Furthermore, the corresponding 3-*O*-acetate has been reported from *Ageratina tristis* [7]; the spectral data are very similar.

The genera *Fleischmannia* and *Brickellia* are not very closely related. They are placed in different subtribes [8]. The isolation of sesamin from the *Fleischmannia* species support the previous findings, while the beyerene derivative **1** probably is less characteristic. These diterpenes are not very widespread in the Compositae. In the tribe Eupatorieae it has been reported from *Peteravenia* [9] and *Stevia* species [10]. Labdane derivatives and nerolidol derivatives are widespread in *Brickellia*. So far no sesquiterpene lactones have been isolated but the occurrence of a heliangolide like **8** probably has no chemotaxonomic relevance, as these lactones are widespread in the tribe showing relationship to the Heliantheae where similar lactones are present.

EXPERIMENTAL

Air-dried plant material was extd with Et_2O–MeOH–petrol (1·1 1) and the extracts obtained sepd as reported previously [11] The extract of 1 kg of aerial parts of *F. hymenophylla* (Klatt) K et R (collected near Ciudad Quesada, Costa Rica, voucher 125931, deposited in the National Herbarium of Costa Rica) gave by CC and TLC 15 mg phytol, 60 mg sesamin and a crude fr A 15% portion of the latter gave by HPLC (MeOH–H_2O, 3·1, always RP 8, flow rate, 3 ml/min) 20 mg **1** (R_t 8.1 min) The roots (225 g) gave 100 mg sesamin.

The extract of 580 g aerial parts of *B laciniata* A. Gray (voucher Boldt 34252, collected in October 1988, Hwy NM 137, Sitting Bull Falls, Carlsbad, New Mexico) gave by CC 50 mg caryophyllene, 25 mg germacrene D, 80 mg taraxasteryl acetate, 100 mg caryophyllenepoxide, 30 mg spathulenol, 90 mg 7-hydroxy-5,6*E*-dehydro-6,7-dihydro-α-farnene and two mixts. One-fifth of the first one gave by HPLC (MeOH–H_2O, 9 1) 10 mg 5,7-dihydroxyflavone, 40 mg **2** (R_t 6.5 min), 10 mg **3** (R_t 7 8 min), 50 mg **6** (R_t 10.5 min) and 60 mg **7** (R_t 14.2 min). One-tenth of the last fraction gave by HPLC (MeOH–H_2O, 17 3) 50 mg **8** (R_t 4 5 min), 100 mg **4** (R_t 7 7 min) and 150 mg **5** (R_t

10 5 min) Known compounds were identified by comparing the 400 MHz ^{1}H NMR spectra with those of authentic materials

Hymenophylloide (**1**) Colourless gum, IR $\nu_{max}^{CHCl_3}$ cm^{-1} 3560–3300, 1710 (CO_2H), 1730 (δ-lactone) MS *m/z* (rel int) 334 215 $[M]^+$ (8) (calc for $C_{20}H_{30}O_4$ 334 214), 289 $[M-CO_2H]^+$ (26), 274 $[289-Me]^+$ (24), 121 $[C_9H_{13}]^+$ (100) Addition of CH_2N_2 gave **1a**, colourless crystals, mp 143°; IR $\nu_{max}^{CHCl_3}$ cm^{-1} 1730, 1715 (CO_2R), MS *m/z* (rel int.): 348.230 $[M]^+$ (26) (calc for $C_{21}H_{32}O_4$ 348 230), 289 $[M-CO_2Me]^+$ (100), 229 (34), 121 (88), $[\alpha]_D^{24°}$ + 5 8 ($CHCl_3$, *c* 1 6)

3-*Oxo-labda*-7,13Z-*dien*-15-*oic acid* (**2**) Colourless gum, IR $\nu_{max}^{CHCl_3}$ cm^{-1}, 3500–2700, 1725, 1650 (C=C–COOH), 1700 (C=O), MS *m/z* (rel int). 318 200 $[M]^+$ (5) (calc for $C_{20}H_{30}O_3$ 318 220), 219 $[M-CH_2C(Me){=}CHCOOH]^+$ (35), 81 (100), 55 (85), ^{13}C NMR ($CDCl_3$, C-1–C-20) 35 8 *t*, 37 7 *t*, 217 1 *s*, 47 4 *s*, 51 2 *d*, 24 0 *t*, 121 8 *d*, 135 3 *s*, 54 2 *d*, 36 7 *s*, 25 9 *t*, 34 6 *t*, 160 6 *s*, 117 1 *d*, 171 7 *s*, 25 4 *q*, 21 9 *q*, 25 0 *q*, 22 1 *q*, 13 2 *q*, CD. $\Delta\varepsilon_{289}$ −0 1

2α-[3-*angeloyloxy-2-hydroxy-2-methyl butyryloxy*]-*Labda*-7,13Z-*dien*-15-*oic acid* (**3**) Colourless gum, IR $\nu_{max}^{CHCl_3}$ cm^{-1}. 3500–2700, 1720 (CO_2H), 1740 (CO_2R), MS *m/z* (rel int). 500 314 $[M-H_2O]^+$ (1) (calc. for $C_{30}H_{44}O_6$ 500 314), 419 $[M-OAng]^+$ (2), 302 $[M-RCO_2H]^+$ (7), 203 (41), 122 (70), 107 (100), 83 (88)

2α-*Hydroxylabd*-7-*en*-15-*oic acid* (**4**). Colourless gum, IR $\nu_{max}^{CHCl_3}$ cm^{-1}. 3600–2700, 1710 (CO_2H), MS *m/z* (rel int) 304.240 $[M-H_2O]^+$ (8) (calc for $C_{20}H_{32}O_2$ 304 240), 289 $[304-Me]^+$ (8), 122 (100), 107 (92), 95 (63), 81 (62) Addition of CH_2N_2 and oxidation with PCC in $CHCl_3$ gave the ketone **4a**, CD (MeCN) $\Delta\varepsilon_{291}$ +0 54

2α-[2,3-*dihydroxy-2-methylbutyryloxy*]-*Labd*-7-*en*-15-*oic acid* (**5**) Colourless gum, IR $\nu_{max}^{CHCl_3}$ cm^{-1}. 3500–2700, 1720 (CO_2H), MS *m/z* (rel. int) 438 $[M]^+$ (0 1), 304 240 $[M-RCO_2H]^+$ (18) (calc for $C_{20}H_{32}O_2$· 304 240), 289 $[304-Me]^+$ (15), 203 (36), 122 (100), 107 (82), 95 (46) Addition of CH_2N_2 gave the Me ester, colourless gum, IR $\nu_{max}^{CHCl_3}$ cm^{-1} 3480 (OH), 1720 (CO_2R); MS *m/z* (rel int) 452 $[M]^+$ (0 1), 421.294 $[M-OMe]^+$ (3) (calc. for $C_{25}H_{41}O_5$ 421.294), 319 $[M-OCOR]^+$ (35), 318 $[M-RCO_2H]^+$ (28), 303 $[318-Me]^+$ (22), 203 (88), 122 (100), 107 (96), 95 (48), ^{1}H NMR as **5**, except OMe 3 65 *s* and small shift differences

2α-[3-*Angeloyloxy-2-hydroxy-2-methyl butyryloxy*]-*labd*-7-*en*-15-*oic acid* (**6**) Addition of CH_2N_2 gave the Me ester, colourless gum, IR $\nu_{max}^{CHCl_3}$ cm^{-1}· 3620 (OH), 1735 (CO_2R), MS *m/z* (rel int.) 534 356 $[M]^+$ (1 5) (calc for $C_{31}H_{50}O_7$ 534 356), 503 $[M-OMe]^+$ (1), 318 $[M-RCO_2H]^+$ (54), 303 (20), 234 (37), 201 (66), 189 (42), 83 (100), ^{1}H NMR as **6**, except OMe 3 66 *s* and small shift differences

8β-[5-(4,5-*dihydroxytigloyloxy*)-*Tigloyloxy*]-*nobilin* (**8**) Colourless gum, IR $\nu_{max}^{CHCl_3}$ cm^{-1} 3600 (OH), 1760 (γ-lactone), 1725 (CO_2R), MS *m/z* (rel int) 246 126 $[M-RCO_2H]^+$ (3) (calc for $C_{15}H_{18}O_3$ 246 126), 228 $[246-H_2O]^+$ (5), 98 (86), 69 (100)

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