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SESQUITERPENE LACTONES FROM *CALEA LEPTOCEPHALA*

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Key Word Index—*Calea leptocephala*; Asteraceae; Heliantheae; sesquiterpene lactones; germacranolides; furanoheliangolides; guaianolides.

Abstract—Chemical analysis of *Calea leptocephala* yielded seven sesquiterpene lactones three of which had not been previously reported as natural products. The new compounds were represented by a furanoheliangolide, 8β-angeloyloxy-9α-acetoxycalyculatolide, the germacranolide desacetylcalein A, and a guaianolide, 8β-angeloyloxyleptocephalide. The structures of the new compounds were established by spectroscopic methods.

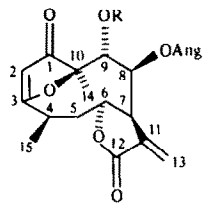
INTRODUCTION

In continuation of our biochemical systematic investigation of the genus *Calea* (Asteraceae, Heliantheae) [1–3], we have analysed *Calea leptocephala* from Michoacan, Mexico for its sesquiterpene constituents. Its chemical analysis yielded seven sesquiterpene lactones, three of which had not been previously isolated as natural products. The structures of the new compounds, which were shown to represent a furanoheliangolide, 8β-angeloyloxy-9α-acetoxycalyculatolide (**2**), the germacranolide desacetylcalein A (**3**) and the guaianolide 8β-angeloyloxyleptocephalide (**7**), were established by spectroscopic methods. The guaianolide **7** represents the first of this skeletal type so far isolated from Mexican *Calea* species [4].

RESULTS AND DISCUSSION

Compound **4** was identified as calein A, a sesquiterpene lactone widely distributed in the genus *Calea* [4], by comparison of its ^{1}H NMR and mass spectra with those of an authentic sample. Spectral data of lactone **5** showed its identity with 2,3-epoxycalein A which was first isolated from *C. ternifolia* var. *ternifolia* [5]. Compound **1** was identical with 8β-angeloyloxy-9α-hydroxy-calyculatolide, a furanoheliangolide with a known molecular structure previously isolated from *C. ternifolia* var. *calyculata* [6]. Lactone **6** was identical with erioflorin, a heliangolide earlier found in *Eriophyllum confertiflorum* [7] and *E. lanatum* [8].

8β-Angeloyloxy-9α-acetoxycalyculatolide (**2**), $C_{22}H_{26}O_8$, is a crystalline material (mp 181–182°) with an IR spectrum that indicated the presence of a γ-lactone (1755 cm^{-1}), a saturated ester (1740 cm^{-1}), an α,β-unsaturated ester (1715 cm^{-1}), double bonds (1645 cm^{-1}) and an enolic double bond (1595 cm^{-1}). The presence of an α-methylene-γ-lactone was corroborated by the ^{1}H NMR spectrum of compound **2** which exhibited two doublets at δ6.33 (H-13a) and 5.49 (H-13b), and a one-proton multiplet at 3.60 (H-7). Furthermore, diagnostic ^{1}H NMR signals indicated that the ester side chain was an angelate group and a three-proton singlet at δ2.26 suggested the presence of an acetate moiety (Table 1). These assignments were corroborated by strong mass spectral peaks at *m/z* 83 (A^2), 55 (A^3) and 43 (Ac). Further



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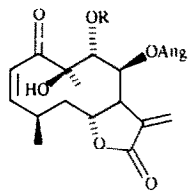
2 R = Ac



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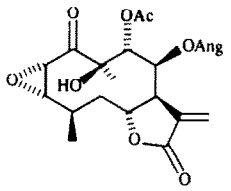

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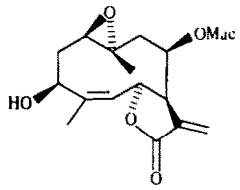

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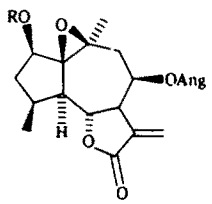

7 R = H
8 R = CONHCOCl$_3$

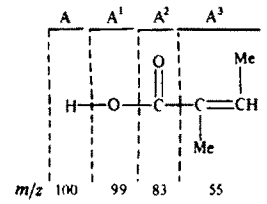

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Table 1. ^{1}H NMR spectral data of compounds 2, 3 and 7 (200 MHz, $CDCl_3$ [C_6D_6]*

H	2	3	7
2	5.58 *d* (2.0)	6.51 *d* (12.0)	3.71 [2.85] *dd* (11.0; 6.0)
3a	—	5.94 *t* (12.0)	2.76 [2.40] *dd* (15.5; 6.0)
3b	—		2.48–2.68† [2.00–2.30†]
4	3.06 *p* (7.0)	3.19 *ddq* (5.0)	2.60 [1.97] *m*
5a	2.61 *ddd* (14.0; 10.0; 7.0)	1.77† *m*	1.63 [0.65–0.80] *dd* (11.0; 4.5)
5b	2.06 *br d* (14.0)	1.46† *m*	
6	4.41 *br dd* (10.0)	4.47 *dd* (12.0; 5.0)	4.48 [3.94] *t* (11.0)
7	3.60 *m*	2.67 *m*	2.85 [1.86] *dddd* (11.0; 3.5; 3.2; 3.0)
8	5.04 *br d* (4.5)	5.44 *dd* (9.5; 2.5)	5.83 [5.45] *m*
9a	5.40 *d* (4.5)	3.93 *t* (9.5)	2.49 [2.10] *dd* (15.5; 3.0)
9b			1.55–1.75†
13a	6.33 *d* (3.2)	6.28 *d* (1.2)	6.21 [6.05] *d* (3.5)
13b	5.49 *d* (3.0)	5.79 *br s*	5.51 [5.12] *d* (3.2)
14	1.37 *s*	1.52 *s*	1.28 [0.84] *s*
15	1.39 *d* (6.5)	1.12 *d* (6.2)	1.32 [1.42] *d* (7.0)
OAng	6.16 *qq* (7.0; 1.5)	6.08 *qq* (7.0; 2.0)	6.14 [5.68] *qq* (7.0; 2.0)
	1.95 *dq* (7.0; 1.5)	1.95 *dq* (7.0; 2.0)	1.99 [1.91] *dq* (7.0; 2.0)
	1.82 *br*	1.85 *br*	1.87 [1.67] *q* (2.0)
OAc	2.26 *s*	—	—

*Figures in parentheses are coupling constants (J) or line separations in Hertz.
†Obscured by other signals.

assignments of the ^{1}H NMR signals of compound 2 were deduced from detailed double irradiation experiments (Table 1).

Comparison of the ^{1}H NMR spectrum of compound 2 with the product obtained from 8β-angeloyloxy-9α-acetoxyternifolin by chromate oxidation [9] showed that the two lactones were identical in spite of minor differences between the spectral parameters reported for the synthetic gum [9] and the newly isolated crystalline natural product 2. Because of the more detailed decoupling data of pure crystalline compound 2, its spectral data are included in Table 1. The ^{13}C NMR spectral data of compound 2 (Table 2) are also in complete agreement with the proposed structure.

Desacetylcalein A (3), $C_{20}H_{26}O_7$, is a crystalline compound (mp 198.5°) which exhibited in the ^{1}H NMR spectrum two broadened one-proton signals at δ6.28 (H-13a) and 5.79 (H-13b) coupled with a one-proton narrow multiplet at 2.67 (H-7) typical of a methylene-γ-lactone. The γ-lactone moiety was further corroborated by a strong IR absorption band at 1760 cm^{-1}. Other IR bands indicated the presence of hydroxyl groups (3530 and 3440 cm^{-1}), an α,β-unsaturated ester (1710 cm^{-1}), a ketone group (1690 cm^{-1}), and a double bond(s) (1630 cm^{-1}). The ester side chain was assigned an angelate group on the basis of typical ^{1}H NMR signals (Table 1), and strong mass spectral peaks at m/z 83 (A^2) and 55 (A^3). Detailed spin decoupling experiments allowed the assignments of all proton signals of compound 3 (Table 1).

Comparison of the ^{1}H NMR spectra of compound 3 with the co-occuring calein A (4) showed great similarities for most signals, clearly indicating that the two compounds have the same skeletal arrangement. The two lactones differed in that the acetate singlet in the ^{1}H NMR spectrum of calein A was missing in the spectrum of compound 3, and the H-9 signal, which appears as a doublet at δ5.59 in calein A, represents a triplet at 3.93 in compound 3. This suggested that lactone 3 bears a hydroxyl group at C-9. Addition of D_2O to 3, simplified the H-9 triplet at δ3.93 to a doublet, further corroborating the presence of a hydroxyl substituent at C-9. The configurations at C-6, C-7, C-8 and C-9 of compound 3 were evident from the ^{1}H NMR coupling constants which were correlated with dihedral angles obtained from stereomodels, and which were very similar to the ones of calein A (4). The coupling constant $J_{8,9}$ = 9.5 Hz indicated an α-orientation for the hydroxyl group attached to C-9. Therefore compound 3 represents the desacetyl derivative of calein A (4).

Table 2. ^{13}C NMR spectral data* of 2 (50.32 MHz, $CDCl_3$)

Carbon	δ, multiplicity	Carbon	δ, multiplicity
1	201.9 *s*	12	168.4 *s*
2	103.8 *d*	13	122.9 *t*
3	192.5 *s*	14	20.5 *q*‡
4	31.2 *d*	15	19.9 *q*‡
5	40.8 *t*	1′	164.8 *s*
6	74.7 *d*†	2′	125.6 *s*
7	47.0 *d*	3′	146.6 *d*
8	74.1 *d*†	4′	16.3 *q*§
9	73.7 *d*†	5′	15.8 *q*§
10	89.4 *s*	1″	168.6 *s*
11	139.7 *s*	2″	18.8 *q*

*Peak multiplicity was determined by heteronuclear multipulse programs (DEPT).
†‡§Assignments are interchangeable.

8β-Angeloyloxyleptocephalide (7), $C_{20}H_{26}O_6$, is a colourless crystalline compound (mp 172–172.5°) with an IR spectrum that indicated the presence of a hydroxyl group (3600 and 3465 cm^{-1}), a γ-lactone (1770 cm^{-1}), an unsaturated ester (1715 cm^{-1}), a double bond

(1650 cm^{-1}), and an epoxide ring (1245 and 870 cm^{-1}). Two one-proton doublets at δ6.21 (H-13a) and 5.51 (H-13b) and a one-proton multiplet at 2.85 (H-7) in the ^1H NMR spectrum of compound **7** further corroborated the presence of a α-methylene-γ-lactone. Diagnostic NMR signals and strong mass spectral peaks at m/z 83 (A^2) and 55 (A^3) indicated the presence of an angelate ester side chain. The assignment of the ^1H NMR signals were deduced from detailed double irradiation experiments in $CDCl_3$ and benzene-d_6 (Table 1) and suggested a guaianolide skeleton.

The chemical shift of H-2 (δ3.71) indicated a proton on a carbon bearing a hydroxyl group. This was verified by *in situ* acylation of compound **7** with trichloroacetyl isocyanate [10]. The ^1H NMR spectrum of the trichloroacetyl carbamate derivative (**8**) showed one broadened NH singlet at δ8.37 clearly demonstrating the presence of one hydroxyl group in compound **7**. Furthermore, the paramagnetic acylation shift of H-2 from δ3.71 in compound **7** to δ5.01 in **8** ($\Delta\delta$ = 1.30) was in complete agreement with a secondary hydroxyl group at C-2 [10]. The absence of further hydroxyls in compound **7** required an epoxide group. Since signals for protons on carbons bearing an epoxide oxygen were missing the epoxide ring had to be at tertiary carbons, suggesting a C-1(10)-epoxide. This was supported by a three-proton methyl singlet at δ1.28 which must correspond to the methyl group at C-10 [11].

The remaining signals to be assigned were a three-proton doublet at δ1.32 (J = 7.0 Hz) coupled to a one-proton multiplet at δ2.60, which was also coupled to the two H-3 and the H-5 signals. Therefore, the doublet at δ1.32 was ascribed to the methyl group at C-4 (H-15).

The relative configurations of the chiral centres in compound **7** were deduced from the ^1H NMR coupling constants which were correlated with the dihedral angles obtained from Dreiding models. The large coupling constants $J_{5,6}$ = 11.0 Hz and $J_{6,7}$ = 11.0 Hz, indicated antiperiplanar arrangements of H-5, H-6 and H-7. On the assumption that H-7 is α-oriented as in all sesquiterpene lactones isolated so far from higher plants [12], an α-orientation for H-5 and a β-orientation for H-6 must be concluded. Similarly, the small coupling constant $J_{7,8}$ = 3.0 Hz suggested a β-orientation for the angelate side chain at C-8. The configuration of the methyl group on C-4 was formulated as β on the basis of the coupling constant $J_{4,5}$ = 4.5 Hz which was in accord with a relative *cis*-disposition of H-4 and H-5. Based on the same arguments, the coupling constants $J_{2,3b}$ = 11.0 Hz and $J_{2,3a}$ = 6.0 Hz indicated a β-orientation for the hydroxyl group at C-2. The β-orientation of the 1(10)-epoxide ring was based on the chemical shifts comparisons of H-5 and H-6 with those of globicin [11], a guaianolide with an α-oriented 1(10)-epoxide group. The downfield shift of H-6 in compound **7** by a value of $\Delta\delta$ = 0.36 and the appearance of H-5 at the normal positions for this type of proton, can be best explained if the 1(10)-epoxide in compound **7** is β-oriented.

EXPERIMENTAL

Calea leptocephala Blake was collected on 3 August, 1978 in Michoacan, Mexico, 13.3 miles south of the junctions 120 and 37 along highway 37 (L. Urbatsch and J. Wussow, No. 3347; voucher deposited at L.S.U., U.S.A.). The air-dried plant material (515 g) was extracted and worked up by the procedure described earlier [13], and provided 9.0 g of the crude terpenoid extract. Column chromatography of the crude syrup on silica gel with $CHCl_3$–Me_2CO mixtures of increasing polarity provided 68 fractions of 200 ml each.

Prep. TLC (silica gel) of fraction 10 (105 mg) with $CHCl_3$–Me_2CO (9:1) yielded 60 mg of crude **2**. Crystallization by vapour diffusion [14] from petrol–$CHCl_3$ gave 43 mg of colourless crystalline 8β-angeloyloxy-9α-acetoxycaliculatolide (**2**). Fractions 11–12 (230 mg) afforded 82 mg of calein A (**4**) after purification by TLC (silica gel) with $CHCl_3$–Me_2CO (9:1). Fractions 13–15 (310 mg) yielded 51 mg of epoxycalein A (**5**) by the same process. Purification of fractions 17–18 (97 mg) by repetitive TLC on silica gel with $CHCl_3$–Me_2CO (9:1) yielded 45 mg of erioflorin (**6**) and 11 mg of desacetylcalein A (**3**). Repetitive purification of fractions 21–22 (65 mg) by TLC on silica gel with Me_2CO–$CHCl_3$ mixtures (1:9 and 1:4) afforded 35 mg of 8β-angeloyloxy-9α-hydroxycalyculatolide (**1**). Similarly, fractions 28–29 (52 mg) yielded 7 mg of 8β-angeloyloxyleptocephalide (**7**).

8β-Angeloyloxy-9α-acetoxycalyculatolide (**2**). $C_{22}H_{26}O_8$, mp 181–182°; UV λ_{max}^{MeOH} nm (ε, $\times 10^{-4}$): 212 (1.95), 258 (1.02); IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1755 (γ-lactone), 1740 (acetate), 1715 (unsatd ester), 1700 (ketone group), 1645 (double bond), 1595 (enolic double bond); EIMS (probe) m/z (rel. int.): 418 $[M]^+$ (2.0), 400 $[M-H_2O]^+$ (4.8), 376 $[M-CH_2CO]^+$ (8.6), 359 $[M-AcO]^+$ (1.4), 358 $[M-HOAc]^+$ (3.5), 277 $[M-CH_2CO-A^1]^+$ (15.9), 276 $[M-CH_2CO-A]^+$ (1.6), 259 $[M-AcO-A]^+$ (1.7), 83 $[A^2]^+$ (100.0), 55 $[A^3]^+$ (17.4), 43 $[Ac]^+$ (4.9).

Desacetylcalein A (**3**). $C_{20}H_{26}O_7$, mp 198.5°; UV λ^{MeOH} nm: end adsorption; IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3530 (OH), 3440 (OH), 1760 (γ-lactone), 1710 (unsatd ester), 1690 (ketone), 1630 (double bond); EIMS (probe) m/z (rel. int.): 378 $[M]^+$ (1.1), 360 $[M-H_2O]^+$ (1.6), 278 $[M-A]^+$ (0.2), 260 $[M-A-H_2O]^+$ (0.2), 245 $[M-A-H_2O-Me]^+$ (0.1), 83 $[A^2]^+$ (100.0), 55 $[A^3]^+$ (24.7).

8β-Angeloyloxyleptocephalide (**7**). $C_{20}H_{26}O_6$, mp 172.0–172.5°; UV λ^{MeOH} nm: end adsorption; IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3600 (OH), 1770 (γ-lactone), 1715 (unsatd ester), 1650 (double bond), 1245 (epoxide), 870 (epoxide); EIMS (probe) m/z (rel. int.): 362 $[M]^+$ (0.3), 344 $[M-H_2O]^+$ (0.1), 263 $[M-A^1]^+$ (5.0), 262 $[M-A]^+$ (0.7), 245 $[M-A^1-H_2O]^+$ (0.7), 244 $[M-A-H_2O]^+$ (0.4), 217 $[M-A^1-H_2O-CO]^+$ (0.8), 216 $[M-A-H_2O-CO]^+$ (0.6), 83 $[A^2]^+$ (100.0), 55 $[A^3]^+$ (27.1).

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