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GERMACRANOLIDES, CALBERTOLIDES A, B AND C, FROM *CALEA BERTERIANA*

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Key Word Index—*Calea berteriana*; Asteraceae; Heliantheae; sesquiterpene lactones; germacranolides; heliangolide.

Abstract—The isolation and structure elucidation of two germacranolides, calbertolides A and B, and a heliangolide, calbertolide C, from *Calea berteriana* are reported. The structures of the new compounds were established by spectroscopic methods.

INTRODUCTION

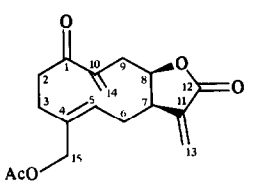
As a continuation of our chemical investigations of the genus *Calea* [1–3] of the tribe Heliantheae, subtribe Galinsoginae, we have studied *C. berteriana* from Venezuela. Besides heliangine (**4**) [4, 5] two new germacranolides (**1** and **2**) and a heliangolide (**3**) were found. The new compounds were characterized by spectroscopic methods.

RESULTS AND DISCUSSION

Calbertolide A (**1**), $C_{17}H_{20}O_5$, displayed in the 200 MHz ^{1}H NMR spectrum two one-proton doublets at δ6.32 (H-13a) and 5.63 (H-13b), and a multiplet at δ2.71 (H-7) that are characteristic of an α-methylene-γ-lactone. An IR band at 1760 cm^{-1} corroborated the presence of the γ-lactone moiety. A base peak at *m/z* 43 in the mass spectrum, together with a three-proton singlet at δ2.10 in the ^{1}H NMR spectrum of **1**, indicated the presence of an acetate side chain in calbertolide A. Detailed ^{1}H NMR double resonance experiments allowed the structural assignments which are summarized in Table 1.

Irradiation of the multiplet at δ2.71 (H-7) collapsed the two H-13 doublets at δ6.32 and 5.63 to singlets, simplified the doublet of doublet of doublets at δ4.31 (H-8) to a broadened doublet and also affected both multiplets at δ3.0–3.30 (H-6a) and 2.30–2.50 (H-6b). On the basis of chemical shift arguments the signal at δ4.31 was assigned to the proton on the lactonic carbon (H-8). Double irradiation at δ4.31 (H-8) sharpened the H-7 multiplet at δ2.71 and the two one-proton multiplets at δ3.34 (H-9a) and 2.20–2.50 (H-9b). In return, irradiation at δ2.40 (centre of the H-9b multiplet) collapsed the doublet of triplets at δ3.34 (H-9a) to a broadened singlet, simplified the H-8 signal at δ4.31 and sharpened the broad singlets at δ5.93 (H-14a) and 5.82 (H-14b). Irradiation of the centre of the H-6a multiplet (3.15) collapsed the broad doublet of doublets at δ5.35 (H-5) to a broadened doublet and simplified the region of δ2.30–2.50 (H-6b). Saturation of the centre of the H-6b signal at δ2.4 also collapsed the H-5 broad doublet of doublets at δ5.35 to a doublet and simplified the H-6a multiplet (δ3.0–3.3). Conversely, irradiation of the doublet of doublets at δ5.35 (H-5) narrowed both regions between δ3.0 and 3.3 (H-6a) and 2.3 and 2.5 (H-6b), and sharpened the broadened methylene singlet at δ4.56 (H-15).

The ^{1}H NMR spectral data presented above, together with the other spectroscopic evidence clearly indicated a germacranolide structure with an α,β-unsaturated γ-lactone, one endocyclic double bond, a conjugated exocyclic methylene and an acetate moiety. On the basis of chemical shifts arguments, the acetate group must be attached at C-15. The presence of an α,β-unsaturated ketone containing an exocyclic methylene (C-14) was deduced from its ^{1}H NMR spectrum which showed a broadened one-proton singlet at δ5.93 (H-14a) and a



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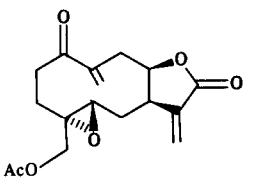

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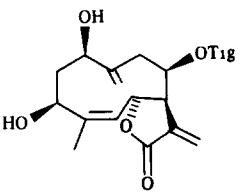

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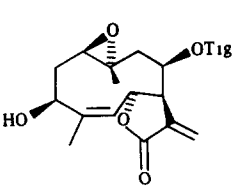

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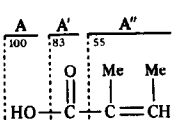

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Table 1. ^{1}H NMR spectral parameters* of compounds 1–3 (200 MHz, $CDCl_3$, TMS as int. standard)

H. No.	1	2	3
H-1	—	—	4.09 *dd* (11.5, 3.0)
H-2a	3.22 *dd* (13.0, 5.0)	3.04 *dd* (13.0, 5.5)	2.40 *ddd* (15.0, 11.5, 3.0)
H-2b	2 53†	2.46–1.60†	2 10†
H-3a	2.50–2.56†	1.49–2.63†	4 53 *dd* (5 2, 3.0)
H-3b	2.08–2.22†	1 40–1.55†	—
H-5	5.35 *br dd* (10.0, 6.3)	2 74 *dd* (10.5, 4.8)	5.35 *dd* (9.5, 1.0)
H-6a	3.0–3 3†	2 04–2.16†	6.38 *dd* (9.5, 2.0)
H-6b	2.30–2.50†	1.53–1.64†	
H-7	2.71 *m*	2.72–2.84 m	3.11 m
H-8	4 31 *ddd* (9.5, 4.5, 4.0)	4.59 *m*	5.18 *m*
H-9a	3.34 *dt* (12.5, 4.0, 1.5)	3.14 *dd* (12.5, 5.0)	2.91 *dd* (15.0, 6.2)
H-9b	2.20–2.50†	2.58† *m*	2.58 *dd* (15.0, 3 9)
H-13a	6.32 *d* (3.2)	6.34 *d* (3.0)	6.33 *d* (2.5)
H-13b	5.63 *d* (3.0)	5.60 *d* (2.8)	5 75 *d* (2.0)
H-14a	5.93 *br s*	6.26 *br s*	5.51 *br s*
H-14b	5.82 *d* (1 5)	6 09 *d* (1 0)	5.13 *br s*
H-15a	4.56 *br s*	4.71 *d* (12.0)	1.79 *br s*
H-15b		3.83 *dd* (12.0, 1.0)	
OR	2.10 *s*	2 16 *s*	6.82 *qq* (7.2, 2.0)
			1.78 *br s*
			1.76 *s*

*Values are in δ-values are figures in parentheses are coupling constants or line separation (J) in Hz.

†Obscured by other signals.

narrow doublet at δ5.82 (H-14b) characteristic of conjugated exocyclic methylene vinyl protons as exemplified by dehydroartemorin [6]. Based on the multiplicities of the vinyl proton H-5 (*dd*) and the lactonic proton H-8 (*ddd*), calbertolide A (**1**) can be represented as a 7,8-lactone since the spin decoupling experiments clearly indicated that the carbons bearing H-5 and H-8 were both linked to methylene groups. Although the conformational uncertainties in the germacranolide, **1**, do not permit unequivocal selection of steric dispositions, the small value of the coupling constant $J_{7,8} = 4$ Hz indicated an equatorial orientation of H-8 and, therefore, a *cis*-fusion of the 12,8-lactone. Stereomodel considerations suggested that the H-5 signal should be more strongly deshielded by the acetate carbonyl at C-15 when the 4,5-double bond is *cis* rather than *trans*. However, in calbertolide A (**1**), the chemical shift of H-5 corresponds well to those of structurally similar germacrolides reported [7, 8], suggesting a 4,5-*trans*-double bond.

Calbertolide B (**2**), $C_{17}H_{20}O_6$, was a gum which in the ^{1}H NMR spectrum showed signals typical of an α-methylene-γ-lactone (two one-proton doublets at δ6.34 and 5.60, and an H-7 multiplet at δ2.78). This was further confirmed by an IR absorption at 1770 cm^{-1}. Other IR bands indicated an ester (1730 cm^{-1}), and an α,β-unsaturated ketone (1680 cm^{-1}). The ester side chain had to be an acetate group on the basis of a characteristic three-proton singlet at δ2.16, together with a base peak at m/z 43 in the mass spectrum of compound **2**. The ^{1}H NMR spectrum of calbertolide B (**2**) (Table 1) was very similar to that of calbertolide A (**1**), except for the signals due to H-5 and H-15. The vinylic H-5 signal in **1** was replaced in **2** by a one-proton doublet of doublets at δ2.74, the chemical shift of which is in agreement with a proton on an epoxide ring. The two-proton broad singlet at δ4.56, corresponding to H-15 in **1**, appeared in **2** as a typical AB pattern suggesting a 4,5-epoxide moiety. On the basis of the above spectral data and the elemental composition, $C_{17}H_{20}O_6$, calbertolide B (**2**) can be formulated as the 4,5-epoxide derivative of calbertolide A.

Calbertolide C (**3**), $C_{20}H_{26}O_6$ was a gum with an IR spectrum showing the presence of absorption bands at 3420 (broad, hydroxyl groups), 1755 (γ-lactone), 1710 (unsaturated ester) and 1650 cm^{-1}. The α-methylene-γ-lactone grouping was further confirmed by the ^{1}H NMR spectrum of **3** which exhibited two one-proton doublets at δ6.33 (H-13a) and 5.75 (H-13b), and a multiplet at δ3.11 (H-7). The ester substituent was assigned to a tiglate group on the basis of the diagnostic ^{1}H NMR signals (a one-proton quartet of quartets at δ6.82, a three-proton broad singlet at δ1.78 and a three-proton singlet at δ1.76), together with characteristic mass spectral peaks at m/z 83 (**A'**) and 55 (**A"**). Assignments of the ^{1}H NMR spectrum signals were obtained by detailed spin decoupling experiments, the results being summarized in Table 1.

The ^{1}H NMR spectral data of calbertolide C (**3**) indicated the presence of two exocyclic methylene groups, two protons on carbons bearing a hydroxyl group, a methyl group on a double bond and a tiglate ester side chain A distinct feature of the ^{1}H NMR spectrum of calbertolide C (**3**) was the presence of H-7 as a narrow multiplet at δ3.11 ($J_{7,13a} = 2.5$ Hz and $J_{7,13b} = 2.0$ Hz).

These small couplings, together with the small coupling constant ($J_{6,7} = 2.0$ Hz), are typical absorptions for heliangolides which possess a 12,6α-lactone and a 4,5-*cis* double bond [9]. Comparison of the ^{1}H NMR spectrum of **3** with the co-occurring heliangine (**4**) showed that both were very similar with distinct differences in the H-1 signals which experienced a paramagnetic shift from δ2.65 in heliangine (**4**) to δ4.09 in compound **3**. Furthermore, a downfield shift of the Me-10 protons from δ1.50 in **4** to a pair of one-proton broadened singlets at 5.51 (H-14a) and 5.13 (H-14b) in calbertolide C (**3**) was observed. These differences clearly indicated the presence of an OH-1 group and an exocyclic methylene at C-10. Since the spectra of **3** and **4** were nearly superimposable for almost all of the signals, except those mentioned above, the stereochemistry must be the same on the chiral centres C-6–C-8 and C-10. The stereochemistry at C-1 was tentatively suggested on the basis of the coupling constants ($J_{1,2} = 11.5$ and 3.0 Hz) which indicated a β-orientation for the OH-1 group. This was further supported by the notion that **3** must be biogenetically derived from heliangine (**4**) by an elimination process which involves opening of the 1(10)-epoxide functions. A β-orientation of the OH-3 group in **3** was derived from the H-3 couplings as well as the deshielding effect of the O-3 upon H-6 (δ6.38), a chemical shift near the value observed for H-6 in heliangine (**4**) [4].

EXPERIMENTAL

Calea berteriana DC was collected during December 1981 in Venezuela in savanna *ca* 15 km W. of Upata (J. Pruski and J. Steyermark No. 1474) Voucher is deposited in the Louisiana State University, herbarium.

Dried leaves (154 g) were extracted and worked-up according to ref. [10] to yield 1 02 g crude syrup. The syrup was chromatographed over 75 g silica gel with petrol–Me_2CO mixtures of increasing polarity, followed by mixtures of Me_2CO–MeOH; 52 fractions of 50 ml each were collected.

Fractions 15 and 16 (18 mg) gave a mixture containing **1**, fractions 18 and 19 (30 mg) provided a mixture of **2** and heliangine (**4**), and fraction 20 contained **3**. Prep. TLC of the above mixtures using petrol–Me_2CO (63:35) yielded 1.5 mg pure **1**, 4 mg **2**, 5.5 mg **3** and 14 mg **4**.

Calbertolide A (**1**). Gum, $C_{17}H_{20}O_5$; UV λ_{max}^{MeOH} nm: 202 (ε1.49 × 10^4), 212 sh (ε1.28 × 10^4); CD (MeOH; *c* 2.0 × 10^{-4}): $[\theta]_{315}$ −1.22 × 10^3, $[\theta]_{245}$ −2.53 × 10^3, $[\theta]_{208}$ +1.63 × 10^5; IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1760 (γ-lactone), 1730 (acetate), 1675 (enone); EIMS (probe) *m/z* (rel. int): 304 $[M]^+$ (10.5), 262 $[M - CH_2CO]^+$ (23.9), 261 $[M - Ac]^+$ (2.9), 244 $[M - HOAc]^+$ (26.3), 226 $[M - HOAc - H_2O]$ (10.1), 216 $[M - HOAc - CO]^+$ (31.0), 201 $[M - HOAc - CO - Me]^+$ (18.8), 43 $[Ac]^+$ (77.1). (Calc. for $C_{17}H_{20}O_5$: 304.1310. Found: MS 304.1266.)

Calbertolide B (**2**). $C_{17}H_{20}O_6$; Gum UV λ_{max}^{MeOH} nm: 204 (ε1.1 × 10^4); CD (MeOH; *c* 2.5 × 10^{-4}): $[\theta]_{310}$ −1.79 × 10^2, $[\theta]_{249}$ −1.79 × 10^3, $[\theta]_{229}$ −3.26 × 10^3; IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1770 (γ-lactone), 1710 (acetate), 1680 (enone); EIMS (probe) *m/z* (rel. int.): 320 $[M]^+$, 278 $[M - CH_2CO]^+$ (1.5), 260 $[M - HOAc]^+$ (2.5), 242 $[M - HOAc - H_2O]^+$ (2.0), 232 $[M - HOAc - CO]^+$ (3.7), 217 $[M - HOAc - CO - Me]^+$ (4), 43 $[Ac]^+$ (100). (Calc. for $C_{17}H_{20}O_6$: 320.1260. Found: MS 320.1276.)

Calbertolide C (**3**). $C_{20}H_{26}O_6$: Gum, UV λ_{max}^{MeOH} nm: strong end absorption, last reading 207 (ε1.39 × 10^4); CD (MeOH; *c* 2.0 × 10^{-4}): $[\theta]_{246}$ +4.71 × 10^3, $[\theta]_{211}$ −4.14 × 10^4; IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3420 (br, OH), 1755 (γ-lactone), 1710 (unsatd ester), 1650 (double bond); EIMS (probe) *m/z* (rel. int.): 362 $[M]^+$, 262 $[M - A]^+$ (1.1), 244 $[M - A - H_2O]^+$ (2.0), 216 $[M - A - H_2O - CO]^+$ (2.2), 201 $[M - A - H_2O - COMe]^+$ (2.6), 83 $[A']^+$ (100.0), 55 $[A'']^+$ (23.6). (Calc. for $C_{15}H_{18}O_4$: 262.1205. Found: MS 262.1209.)

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