

SESQUITERPENE LACTONES OF *CALEA DIVARICATA* AND THE
MOLECULAR STRUCTURE OF LEPTOCARPIN ACETATE

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In continuation of our chemical studies of the genus *Calea* (1-3), we have investigated *Calea divaricata* Bentham from Venezuela for its secondary constituents. We report here the isolation and characterization of three flavones and seven sesquiterpene lactones. The structures were determined by nmr and mass spectral correlations with the data of known compounds. The molecular structure of Leptocarpin acetate (**9**) was established by single crystal X-ray diffraction and its absolute configuration derived from chiroptical data.

RESULTS AND DISCUSSION

Column chromatography of the crude extract of *C. divaricata* gave three known flavones that were identified by ^1H nmr, uv, and ms analyses and shown to be identical with *O*-methylacacetin (**1**), acacetin (**2**), and apigenin (**3**) by comparison with published data (4,5). Lactones **4** and **5** gave ^1H -nmr spectra and mass spectral fragmentations, which were identical with the respective data of atripliciolide angelate and 11,13-dihydroxy-11,13-atripliciolide angelate, previously isolated from *Calea pilosa* (6) and *Calea ternifolia* (7). Chemical and physical data of compound **6** indicated identity with budlein A, a lactone previously isolated from *Viguiera* species (8,9). Compound **7** was identical with 11,13-dihydro-11 α , 13-epoxyatripliciolide 8 β -*O*-angelate (7), and the nmr and ms data suggested that compound **8** represents leptocarpin, a heliangolide previously found in *Leptocarpha rivularia* (10). Decoupling experiments on compounds **8** and **9** demonstrated that lactone **9** is the acetate derivative of **8**. It had not been previously isolated as a natural product but was prepared from lep-

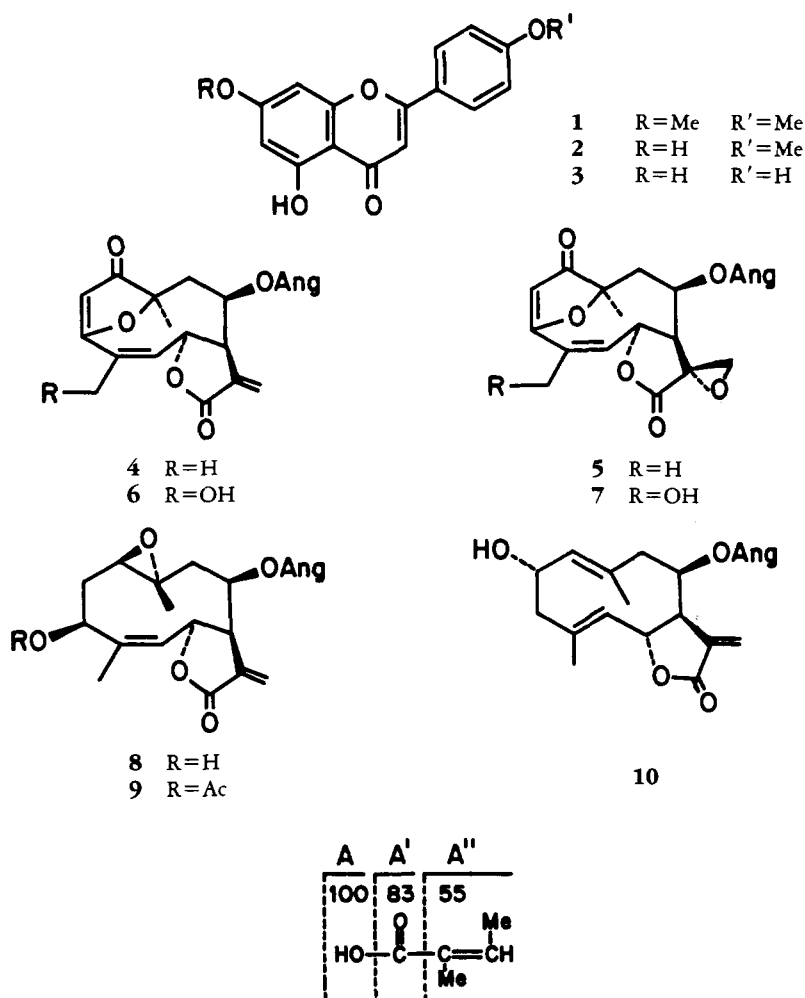
tocarpin (10). The ^{13}C -nmr, high field ^1H -nmr, ms, and cd spectral data of the heliangolide **9** are given in this paper since they were not previously described. The ^1H -nmr spectral data of **10** were identical with data reported for 2 α -hydroxyeupatolide-8-*O*-angelate, previously isolated from *Tithonia rotundifolia* (11).

The ^{13}C -nmr spectra for lactones **5**, **7**, **8**, **9**, and **10** were not previously reported and are therefore described here. The peak multiplicities were determined by off-resonance decoupling, and the assignments were confirmed by single frequency proton decoupling, as summarized in Table 1.

The chemistry of the sesquiterpene lactones present in *C. divaricata* agree well with that of other members of the genus *Calea* in that the furanoheliangolides with a 6 α ,12 *trans*-fused lactone and a 8 β -ester side-chain are the predominant lactones present. The occurrence of the germacrolide 2 α -hydroxyeupatolide-8-*O*-angelate (**10**) is significant because it is a likely biogenetic precursor for the eudesmanolides (3) and guaianolides (1) which are major constituents of several *Calea* species (1,3,12).

CRYSTAL STRUCTURE ANALYSIS OF LEPTOCARPIN ACETATE (**9**).—The molecular structure of leptocarpin acetate, determined by X-ray diffraction, is illustrated in Figure 1, and the atomic coordinates are listed in Table 2. Its conformation is typical for a heliangolide, being nearly identical to those of eupafornonin (13) and dihydroheliangine (14) (as the monochloroacetate).

Bond distances, which have individual standard deviations of 0.006-



0.011Å, are normal and agree well with those of eupafornin. The epoxide is symmetric, with C-O distances 1.442(6) and 1.449(7)Å, and C-C distance 1.466(8)Å. The angelate moiety is experimentally planar, with none of its seven heavy atoms lying more than 0.010(6)Å from their best plane.

The conformation of the α -methylene- γ -lactone ring is an intermediate form between the envelope with C7 at the flap and the half-chair with C12 on the pseudo-twofold axis. The cd spectrum of leptocarpin acetate exhibited a positive band at 240 nm indicative of a positive Cotton effect of the $n \rightarrow n^*$ transition of the α -methylene- γ -lactone group. The crystal structure showed the lactone ring to have a right-

handed chirality with positive torsion angles (O2=C12-C11=C13, +7.8°; C11-C7-C6-O1, +16.7°) for the lactone chromophore if the absolute configuration of **9** is that given in Figure 1. Application of Beecham's rule (15) and the extension by Cox and Sims (16) correlates the right-handed chirality of these torsion angles with a positive Cotton effect; thus, we suggest an absolute configuration for compound **9** as shown in Figure 1.

EXPERIMENTAL

C. divaricata was collected on December 19, 1979, in Estado Bolivar, Venezuela, about 165 km south of El Dorado (J. Pruski and J. Steyermark, voucher No. 1426 deposited at Louisiana State University, Baton Rouge, LA). Air-dried plant material (682 g) was extracted and worked

TABLE 1. ^{13}C -nmr Spectral Data^a of Compounds **7**, **8**, **9** and **10**

	7	8	9	10
C-1	205.19 s	60.68 d	60.10 d	133.89 d
C-2	104.74 d	32.53 t	30.32 t	69.21 d
C-3	182.41 s	72.22 d	72.75 d	48.16 t
C-4	138.43 s	141.65 s	138.12 s	142.64 s ^b
C-5	133.54 d	126.39 d	125.88 d	129.37 d
C-6	75.31 d	74.17 d	74.39 d	75.53 d
C-7	46.89 d	48.44 d	48.22 d	53.16 d
C-8	70.35 d	75.82 d	75.45 d	70.97 d
C-9	41.78 t	43.63 t	43.33 t	43.96 t
C-10	87.81 s	58.70 s	58.15 s	134.30 s ^b
C-11	135.87 s	126.93 s	126.21 s	125.58 s
C-12	172.27 s	169.58 s	169.20 s	169.47 s
C-13	49.92 t	124.74 t	124.88 t	121.21 t
C-14	20.73 q	22.94 q	22.83 q	20.48 q
C-15	62.14 t	20.33 q	20.16 q	18.60 q
C-1'	166.07 s	166.42 s	165.82 s	166.34 s
C-2'	125.77 s	137.42 s	136.83 s	136.43 s ^b
C-2'-Me	19.90 q	19.67 q	19.19 q	19.74 q
C-3'-Me	15.69 q	15.70 q	15.70 q	15.85 q
C-1''	—	—	169.09 s	—
C-2''	—	—	20.92 s	—

^aRun in CDCl_3 at 50.32 MHz at ambient temperature. Chemical shifts are given in ppm relative to TMS as determined by proton noise decoupling. Peak multiplicity was obtained by off-resonance decoupling (2.5 ppm above TMS). Assignments were confirmed by single frequency off-resonance proton decoupling.

^bAssignments interchangeable.

up as described previously (17), yielding 11.8 g of crude terpenoid syrup. The crude syrup was chromatographed on a silica gel column (500 g) with petroleum ether-EtOAc mixtures of increasing polarity first and then with EtOAc-Me₂CO mixtures (3%, 5%, 10%, 25%, and 50%); 75 200-ml fractions were collected.

Fractions 16-26 afforded 182 mg of *O*-methylacetin (**1**) as yellow needles on recrystallization from CHCl_3 -hexane. Fractions 31-32 (30 mg) gave 11 mg of **4** after purification by preparative tlc (silica gel, hexane-Me₂CO 5%). Fractions 33-35 (145 mg) provided 138 mg of impure **5**, which was recrystallized from hexane-EtOAc to give 120 mg of colorless crystals, mp 125° [Lit. (6): gum]. Fractions 36-37 yielded 68 mg of yellow

low crystals of **2**. Fractions 39-41 (870 mg) were purified by recrystallization from EtOAc-hexane yielding 630 mg of pure compound **9** as colorless prisms. Fractions 42-44 (230 mg) were rechromatographed on silica gel plates with hexane-Me₂CO (4:1) to give 61 mg of **8**. Fraction 45 afforded 7 mg of **3** as a yellow powder. Fractions 46-47 (120 mg) yielded 53 mg of compound **10** after preparative tlc (silica gel, hexane-Me₂CO, 4:1). Fractions 49-50 (400 mg) gave 260 mg of **6** after recrystallization from EtOAc-hexane. Fractions 51-53 (310 mg) afforded a mixture of compounds **6** and **7**, which after repeated preparative tlc yielded 73 mg of **7**.

Leptocarpin acetate (**9**), mp 210.5-211.0° [Lit. (10): 180-182°]. ^1H nmr (200 MHz, C_6D_6 ,

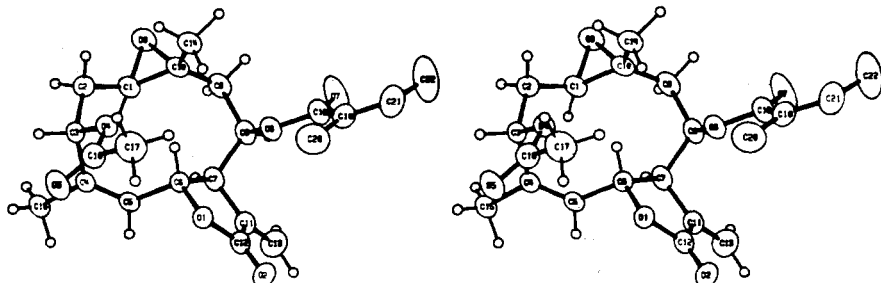


FIGURE 1. Stereoscopic representation of leptocarpin acetate. Hydrogen atoms of the angelate group have been omitted for clarity.

TABLE 2. Coordinates for Leptocarpin Acetate

Atom	x	y	z	Atom	x	y	z
O1	0.5979(2)	0.3008(2)	0.8633(2)	C9	0.9508(4)	0.2176(5)	0.7617(3)
O2	0.5046(3)	0.4335(3)	0.8002(2)	C10	0.9276(4)	0.1222(4)	0.8176(3)
O3	1.0336(3)	0.0853(3)	0.8596(2)	C11	0.7166(4)	0.4292(4)	0.8069(3)
O4	0.6855(3)	0.0570(2)	0.9562(2)	C12	0.5949(4)	0.3927(4)	0.8213(3)
O5	0.5480(3)	0.0656(3)	1.0623(2)	C13	0.7387(6)	0.5213(4)	0.7757(4)
O6	0.7542(3)	0.2516(3)	0.6992(2)	C14	0.8544(5)	0.0387(5)	0.7758(4)
O7	0.8420(3)	0.2886(5)	0.5717(2)	C15	0.7763(4)	0.2314(4)	1.1380(3)
C1	0.9440(4)	0.1320(4)	0.9136(3)	C16	0.5758(4)	0.0482(4)	0.9876(3)
C2	0.8930(4)	0.0612(4)	0.9830(3)	C17	0.4942(5)	0.0146(5)	0.9161(4)
C3	0.7725(4)	0.0915(4)	1.0197(3)	C18	0.7572(5)	0.2591(5)	0.6095(3)
C4	0.7579(4)	0.2065(4)	1.0412(3)	C19	0.6437(5)	0.2228(4)	0.5705(3)
C5	0.7344(4)	0.2796(3)	0.9832(3)	C20	0.5468(5)	0.1880(5)	0.6324(5)
C6	0.7184(4)	0.2696(4)	0.8846(3)	C21	0.6287(5)	0.2234(5)	0.4844(4)
C7	0.7986(4)	0.3437(4)	0.8311(3)	C22	0.7109(7)	0.2529(8)	0.4151(5)
C8	0.8529(4)	0.2958(4)	0.7477(3)				

TMS) 2.37 (dd, $J=10.0$; 4.5 Hz, H-1), 2.03 (dt, $J=15.5$; 4.5; 4.5 Hz, H-2a), 1.41 (ddd, $J=15.5$, 10.0; 2.5 Hz, H-2b), 4.94 (dd, $J=4.5$; 2.5 Hz, H-3), 4.71 (br dd, $J=11.0$; 1.2 Hz, H-5), 6.00 (dd, $J=11.0$; 2.0 Hz, H-6), 2.00 (m, H-7), 5.02 (m, $w_{1/2}=8.0$ Hz, H-8), 2.79 (dd, $J=15.0$; 4.5 Hz, H-9a), 1.00 (dd, $J=15.0$; 3.0 Hz, H-9b), 6.21 (d, $J=2.2$ Hz, H-13a), 5.12 (d, $J=2.0$ Hz, H-13b), 1.37 (s, H-14), 1.47 (d, $J=1.3$ Hz, H-15), 5.70 (qq, $J=7.2$; 1.5 Hz, H-3'), 1.97 (dq, $J=7.2$; 1.5 Hz, C-2'-Me), 1.81 (dq, $J=1.5$ Hz, C-3'-Me), 1.80 (s, OAc); cd (c 4.62×10^{-4} , MeOH): $[\theta]_{240} +6.16 \times 10^4$, $[\theta]_{212} -5.54 \times 10^5$; ms 70 eV, m/z (rel. int.) 362 (0.2; M-CH₂CO)⁺, 344 (0.2; M-HOAc)⁺, 304 (0.2; M-A⁺, 262 (3.1; M-CH₂CO-A⁺, 261 (13.0; M-A-CO₂)⁺, 244 (4.5; M-A-HOAc)⁺, 83 (100.0; A'⁺, 55 (27.1; A''⁺, 43 (14.5; CH₃CO)⁺.

X-RAY DATA.¹—A crystal of dimensions $0.44 \times 0.48 \times 0.64$ mm was used for data collection on Enraf-Nonius CAD4 diffractometer equipped with MoK α radiation and a graphite monochromator. *Crystal Data*: C₂₂H₂₈O₇ MW=404.5, orthorhombic space group P2₁2₁2₁, $a=11.441(2)$, $b=12.915(2)$, $c=15.091(2)$ Å, $Z=4$, $d_c=1.205$ g cm⁻³, $\lambda=0.71073$ Å, $\mu(\text{MoK}\alpha)=0.84$ cm⁻¹. Data were collected by ω -2 θ scans of variable speed, designed to yield $I \approx 50 \sigma(I)$ for all significant reflections. One octant of data having $1^\circ \leq \theta \leq 26.5^\circ$ was measured, yielding 2610 unique reflections of which 1538 had $I \geq 3\sigma(I)$ and were used in the refinement. Data reduction included corrections

for background, Lorentz, and polarization effects; no absorption correction was necessary.

The structure was solved by direct methods (MULTAN 78) (18) and refined by full matrix, weighted least squares methods. Nonhydrogen atoms were refined anisotropically, while hydrogen atoms were located in difference maps and included as fixed contributions with isotropic $B=5.0$ Å². A secondary extinction coefficient refined to a value of $1.06(2) \times 10^{-6}$. Convergence was achieved with $R=0.045$, $R_w=0.043$ based on observed reflections, and the maximum residual was $0.13 \text{ e } \text{Å}^{-3}$.

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¹Atomic coordinates for this structure have been deposited with the Cambridge Crystallographic Data Centre and can be obtained on request from Dr. Olga Kennard, University Chemical Laboratory, Lensfield Rd., Cambridge, CB2 1EW, UK.

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