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TWO SESQUITERPENE LACTONES OF *CALEA TERNIFOLIA* VAR. *CALYCULATA*

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Key Word Index—*Calea ternifolia* var. *calyculata* (syn. *Calea hypoleuca*); Compositae; Heliantheae; Galinsoginae; sesquiterpene lactones; modified heliangolides.

Abstract—Chemical analysis of *Calea ternifolia* var. *calyculata* yielded the known sesquiterpene lactone calein A, as well as two new modified heliangolides which we named 8β-angeloyloxy-9α-acetoxyternifolin and 8β-angeloyloxy-9α-[2-methylbutanoyloxy]-ternifolin. The structures of the new compounds were established by spectroscopic methods. Support for the involvement of the ternifolin-type germacranolides in the biogenesis of the furan-type medium ring lactones was provided by chromate oxidation.

INTRODUCTION

The genus *Calea* of the tribe Heliantheae, subtribe Galinsoginae is by far the largest genus of the subtribe [1]. In recent years it has received increasing attention related to taxonomic [2–6] and chemical studies [7–11].

In continuation of our biochemical systematic investigations of taxa belonging to the tribe Heliantheae we have analysed *Calea ternifolia* HBK var. *calyculata* (B. L. Rob.) Wussow and Urbatsch of section Calea from Chiapas, Mexico for their sesquiterpene lactone constituents. Besides the known calein A (**1**) [8] two new medium ring lactones were found. These types of compound had previously been suggested [11] to play a key role in the biogenesis of the caleins [8] and calaxin-type (**6**) medium rings. This biogenetic assumption was supported by an acid-mediated chromate oxidation of 8β-angeloyloxy-9α-acetoxyternifolin‡ (**2**) to give a furan-type germacranolide.

RESULTS AND DISCUSSION

The two new compounds displayed ^{1}H NMR and mass spectral signals which were nearly identical except for absorptions that indicated differences in the ester groups attached to the two molecules. Compound **3**, $C_{25}H_{36}O_9$, displayed, in the 200 MHz ^{1}H NMR spectrum, two one-proton doublets at δ 6.29 ($J_{7,13a}$ = 2.0 Hz, H-13a) and 5.79 ($J_{7,13b}$ = 2.0 Hz, H-13b) and a broad multiplet at 2.60 (H-7) that are characteristic of an α-methylene-γ-lactone. An IR absorption at 1765 cm^{-1} corroborated the presence of a γ-lactone moiety. Detailed ^{1}H NMR double resonance experiments together with mass spectral patterns allowed the major structural assignments of **3**. Strong mass spectral peaks at *m/z* 85 (**A′**), 57 (**A″**), 83 (**B′**) and 55 (**B″**) together with diagnostic ^{1}H NMR absorptions (Table 1) indicated the presence of an α-methylbutanoate and angelate moiety in **3**. This was also supported by IR bands at 1745 (**B**) and 1725 cm^{-1} (**A**). Further IR bands at 3450 and 1710 cm^{-1} suggested the presence of hydroxyl and ketone function(s). This accounted for eight of the nine oxygens in **3**.

Irradiation of the multiplet at δ 2.60 (H-7) changed the doublet of doublets at 5.94 (H-8, $J_{7,8}$ = 1.5 Hz) to a doublet, simplified the triple doublet at 4.92 (H-6, $J_{7,6}$ = 3.5 Hz) to a doublet of doublets and collapsed the two H-13 doublets at 5.79 and 6.29 to singlets. On the basis of chemical shift arguments [7] the absorption at δ 4.92 was assigned a proton at a lactonic carbon whereas the signals centered at 5.94 were ascribed to a proton at a carbon carrying an ester group. Double irradiation at δ 5.94 collapsed the doublet at 5.85 (H-9, $J_{8,9}$ = 10.5 Hz) and the chemical shift suggested the attachment of the second ester moiety to C-9.

Irradiation at δ 4.92 (H-6) sharpened the H-7 multiplet at 2.60 to a broad singlet and affected one-proton multiplets centered at 1.62 (H-5b) and 2.12 (H-5a) which were coupled to a multiplet at 1.75 (H-4). In return, irradiation of the centre of H-4 affected the two H-5 absorptions, collapsed the three-proton doublet at 1.14 (C-4-Me) to a singlet and simplified the threefold doublet at 4.31 (H-3, $J_{3,4}$ = 2.5 Hz). Saturation of the H-3 signal at 4.31 collapsed the two doublets at 2.95 (H-2b, $J_{2b,3}$ = 6.5 Hz) and 3.25 (H-2a, $J_{2a,3}$ = 10 Hz) to an A,B-pattern ($J_{2a,2b}$ = 18 Hz). The chemical shift of H-3 suggested the attachment of a hydroxyl group to C-3 and the absorptions near δ 3 of the two geminally coupled protons at C-2 indicated their positioning next to a carbonyl function.

This accounted for all atoms in compound **3** except

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‡The name ternifolin is reserved for the medium ring lactone without C-8 and C-9 substituents.

Table 1. ^{1}H NMR spectral data of compounds **2**, **3** and **6** at 200 MHz (TMS as int. standard, $CDCl_3$)

	2	**3**	**6**
H-2a	3.25 *dd* (18; 10.5)*	3.25 *dd* (18; 10)	5.58 *d* (1.2)
H-2b	2.96 *dd* (18; 6.5)	2.95 *dd* (18; 6.5)	—
H-3	4.34 *ddd* (10.5; 6.5; 2.5)	4.31 *ddd* (10; 6.5; 2.5)	—
H-4	†	1.75†	3.05 *p* (7)
H-5a	†	2.12 *ddd* (4; 6.5; 16)	2.57 *m*
H-5b	†	~ 1.6†	2.10 *m*
H-6	4.90 *ddd* (6.5; 6.5; 3.5)	4.92 *ddd* (6.5; 6.5; 4)	4.45 *dd* (10; 4.5)
H-7	2.59 *brs*	2.60 *m*	3.59 *m*
H-8	5.88 *dd* (10; 1.5)	5.94 *dd* (10.5; 1.5)	5.06 *dd* (5; 1)
H-9	5.54 *d* (10)	5.85 *d* (10.5)	5.40 *d* (5)
H-13a	6.31 *d* (2.0)	6.29 *d* (2.0)	6.33 *d* (3.5)
H-13b	5.80 *d* (2.0)	5.79 *d* (2.0)	5.50 *d* (3)
Me-4	1.14 *d* (6.5)	1.14 *d* (6.5)	1.40 *d* (7)
Me-10	1.28 *s*	1.27 *s*	1.37 *s*
OAc	2.06 *s*	—‡	2.26 *s*
OAng	6.12 *qq* (7.5; 1.5)	6.13 *qq* (7.5; 1.5)	6.16 *qq* (7.5; 1.5)
	1.94 *dq* (7.5; 1.5)	1.95 *dq* (7.5; 1.5)	1.96 *dq* (7.5; 1.5)
	1.77 *q* (1.5)	1.77 *q* (1.5)	1.82 *q* (1.5)

*Numbers in parentheses are coupling constants or line separations in hertz.
†Obscured by other signals.
‡Chemical shift data of the 2-methylbutanoate group: 2.38 *sext* (H-2'); 1.42 *dq* (2H-3'); 1.16 *d* (6.5 Hz, Me-2'); 0.88 *t* (3H, Me-3').

one of each of carbon, oxygen, hydrogen and a methyl group which was indicated by a three-proton singlet at δ 1.28 in the ^{1}H NMR spectrum.

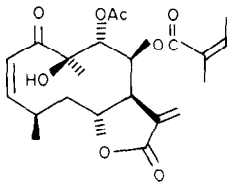
Since most *Calea*-derived sesquiterpene lactones possess a C-1 carbonyl and a hydroxyl at C-10 [10] the structure of the new compound could be tentatively formulated as a 10-membered ring (**3**) exclusive of stereochemistry and sites of attachment of the two ester groups. Due to the great similarity of the medium ring ^{1}H NMR proton absorptions of **2** and **3**, compound **2** must possess the same ring skeleton and differ from **3** by the absence of the 2-methylbutanoate group and the presence of an acetate moiety.

The stereochemistry of C-6, C-7, C-8 and C-10 of compounds **2** and **3** was assigned as H-6β, H-7α, H-8α and Me-10β by chemical transformation of **2** to a zexbrevin-type compound. Acid-mediated chromate-oxidation of **2** provided compound **6** via compounds **4** and **5**. Compound **6** exhibited medium ring proton signals nearly identical with zexbrevin (**7**) [12,13]. In both compounds **2** and **3**, the large coupling constant ($J_{8,9}$ = 10 Hz) indicated antiperiplanar orientation of H-8 and H-9 suggesting H-9β in compounds **2** and **3**.

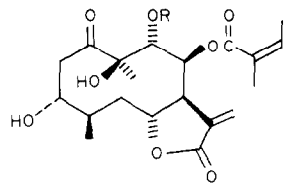
The deshielding of the acetate methyl (singlet at δ 2.26) in compound **6** and therefore in **2** is similar to analogs which had been prepared by acetylation of 9α-hydroxylfuranogermacranolides [14]. Therefore, in compound **2** the attachment of the acetoxy moiety is tentatively assigned to C-9 and the angelate to C-8. In compounds **2** and **3** the ^{1}H NMR chemical shifts of the angelate proton are the same within experimental error suggesting a similar chemical environment or the attachment of the angelate group to C-8 and the α-methylbutanoate moiety to C-9 in **3**.

The stereochemistry at C-3 and C-4 was tentatively assigned by correlation of the dihedral angles of the medium ring protons with the experimentally observed *J*-values by application of the Karplus correlation [15]. Using stereomodels, two major conformations were considered in this treatment. One with a downward-orientation of the C-1 carbonyl and the other with the C-1 carbonyl being oriented upward. In both conformations the skeleton of the medium ring was fixed around C-6 and C-9 so that the proton dihedral angles were: H-9/H-8 ~ 180°; H-8/H-7 ~ 80°; and H-7/H-6 ~ 140°; these angles correlated very well with the experimental *J*-values of the ^{1}H NMR absorptions. The dihedral angles of H-2α, H-2β, H-3 and H-4 in a conformation with an orientation of the C-1 carbonyl function below the plane of the medium ring correlated poorly with the observed proton couplings in all four configurational isomers at C-3 and C-4. The four possible configurational relationships at the chiral centers C-3 and C-4 in a conformation with an upward oriented C-1 carbonyl function were considered next. The dihedral angles H-4α/H-3β $\approx$ 110°; H-3β/H-2α $\approx$ 170°; H-3β/H-2β $\approx$ 50° were derived from model considerations and correlated best with the experimental *J*-values suggesting a 3α-OH and 4β-Me in **2** and **3**. A C-4 β-methyl would be in accord with the stereochemistry found in the calein-type compounds isolated from several *Calea* species [7–11]. Their stereochemistry is based on the neurolenin skeleton whose structure was established by X-ray analysis [16].

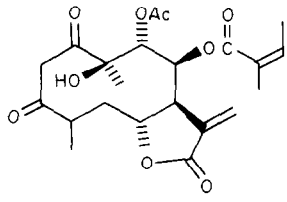
The finding of a β-oriented C-4 group is contrary to the results obtained in the acid-mediated oxidation of **2** which gave a zexbrevin-type compound (**6**) which based on literature data [12, 13] should possess a C-4α-methyl group. It is possible that the initial oxi-



1 Calein A



2 R = Ac
3 R = 2-Methylbutanoate



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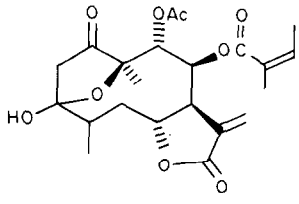

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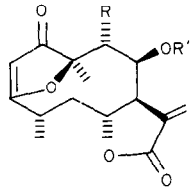
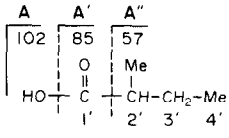
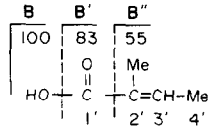


6 R = OAc, R' = Ang
7 R = H, R' = Me Acr





dation intermediate, the diketone (**4**), could undergo an acid-catalysed isomerization at C-4 with the methyl group adopting an α-configuration.

EXPERIMENTAL

^{1}H NMR spectra were determined at 200 MHz in $CDCl_3$, with TMS as int. standard. MS were taken at 70 eV by direct inlet.

Calea ternifolia was collected on July 29, 1978 in Chiapas, Mexico 0.9 miles south-east of Teopsco town square along Highway 190 (L. Urbatsch, No. 3333, voucher deposited at LSU, U.S.A.). Dried leaves (1 kg) were extracted and worked-up as previously described [17], providing 6.2 g of crude syrup which was chromatographed over Si gel using petrol and mixtures of petrol–EtOAc (10, 20, 25, 50, 75%) as eluant; 100 ml fractions were taken and fractions were monitored by TLC.

Fractions 17–18 provided 30 mg of calein A (**1**) which was identical with authentic material by ^{1}H NMR and MS analysis. Fraction 26 gave 50 mg of **3** and fractions 27–28 yielded 120 mg **2**.

8β-Angeloyloxy-9α-acetoxyternifolin (**2**). $C_{22}H_{30}O_9$, glass; UV λ_{max}^{EtOH} nm: 213 (ϵ 1.86×10^4); CD (MeOH; *c* 1.52×10^{-4}): $[\theta]_{212}-8.5\times10^4$, $[\theta]_{250}+5.33\times10^3$, $[\theta]_{290}+3.03\times10^3$; IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3425 (OH), 1745 (γ-lactone), 1730 (acetate), 1710 (ketone), 1695 (α, β-unsaturated ester); MS *m/z* (rel. int.): 438 $[M]^+$, 420 $[M-18]^+$ (0.4), 402 $[M-36]^+$ (5.1), 278 $[M^+-A-B]^+$, 83 $[B']^+$ (30), 55 $[B'']^+$ (100), 43 $[Ac]^+$ (18.9). (Calc. for $C_{22}H_{30}O_9$: 438.1889. Found: MS 438.1888.)

8β-Angeloyloxy-9α-(2-methylbutanoyl)-ternifolin (**3**). $C_{25}H_{36}O_9$, mp 55–60° (Et_2O); UV λ_{max}^{EtOH} nm: 213 (ϵ 2.55×10^4); CD (MeOH; *c* 2.08×10^{-4}): $[\theta]_{225}-4.88\times10^3$, $[\theta]_{253}+6.68\times10^2$, $[\theta]_{290}-5.76\times10^2$; IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3450 (OH), 1765 (γ-lactone), 1745 (ester), 1725 (α, β-unsaturated ester), 1710 (ketone), 1650 (double bond); MS *m/z* (rel. int.): 480 $[M]^+$, 462 $[M-18]^+$ (0.2), 444 $[M-36]^+$ (1), 378 $[M-A]^+$ (6.2), 85 $[A']^+$ (25.5), 83 $[B']^+$ (100), 57 $[A'']^+$ (23.9), 55 $[B'']^+$ (29.7). (Calc. for $C_{25}H_{36}O_9$: 480.2359. Found: MS 480.2370.)

Oxidation of **2**. A soln of 50 mg of **2** in Me_2CO containing a few drops of Jones' reagent was stirred at 0° until the orange colour persisted. The residue was diluted with H_2O and extracted with Et_2O. The solvent was evaporated *in vacuo* and the crude product purified by prep. TLC (petrol–EtOAc, 7 : 3). The main fraction gave 3 mg of compound **6**, $C_{22}H_{26}O_8$, gum; UV λ_{max}^{EtOH} nm: 213 (ϵ 1.91×10^4), 259 (ϵ 9.73×10^3); CD (MeOH; *c* 4.78×10^{-4}): $[\theta]_{221}-7.17\times10^3$, $[\theta]_{260}+2.38\times10^3$, $[\theta]_{298}+6.12\times10^2$; IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1760 (γ-lactone), 1740 (ester), 1703 (α, β-unsaturated ester), 1690 (α, β-unsaturated ketone), 1590 (enolic double bond); MS *m/z* (rel. int.): 418 $[M]^+$, 277 (21.5), 125 (19.9), 83 (100), 55 (30.7), 43 $[Ac]^+$ (15). (Calc. for $C_{22}H_{26}O_8$: 418.1625. Found: MS 418.1657.)

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