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SESQUITERPENE LACTONES FROM *CALEA SEPTUPLINERVIA*

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Key Word Index—*Calea septuplinervia*; Asteraceae; Heliantheae; sesquiterpene lactones; eudesmanolides; flavonoids.

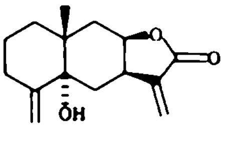
Abstract—Chemical analysis of *Calea septuplinervia* afforded two known flavonoids, acacetin and apigenin 7,4′-dimethyl ether, as well as the known eudesmanolide telekin. The new sesquiterpene lactone septuplinolide was shown to be a 7,8β-lactonized eudesmanolide.

INTRODUCTION

In continuation of our biochemical systematic studies of the genus *Calea*, we have chemically analysed *Calea septuplinervia* from Venezuela. Besides the flavones acacetin and apigenin 7,4′-dimethyl ether [1], the known eudesmanolide telekin (**1**) [2] was isolated. Telekin has previously been found in *C. szyszylowiczii* [3, 4]. The known flavonoids were identified by spectral comparison with authentic samples, and telekin by comparing spectral data with those reported in the literature [2–4]. The structure of the only new compound, which we named septuplinolide, was established by chemical and spectroscopic methods.

RESULTS AND DISCUSSION

Septuplinolide (**2**), $C_{15}H_{22}O_3$, is a crystalline compound (mp 171–172°) with an IR spectrum which indicated the presence of a hydroxyl group (3600 and 3480 cm^{-1}), a γ-lactone (1755 cm^{-1}) and a double bond (1665 cm^{-1}). The presence of an α-methylene-γ-lactone was corroborated by the ^{1}H NMR spectrum of **2** which exhibited two one-proton doublets at δ6.08 (H-13a) and 5.55 (H-13b), and a one-proton multiplet at δ2.96 (H-7). The assignments of the ^{1}H NMR signals of **2** were deduced from detailed spin decoupling experiments; the results are summarized in Table 1. Detailed ^{13}C NMR studies which involved proton-noise decoupling, single frequency off-resonance decoupling and heteronuclear multipulse DEPT experiments, corroborated the ^{1}H NMR assignments, and are summarized in Table 2. The ^{13}C NMR spectral data indicated the presence of four quaternary, three tertiary, six secondary and two primary carbon atoms, accounting for 15 carbon atoms in the septuplinolide molecule.



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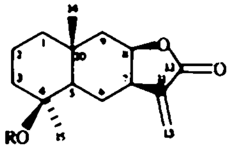

2 R = H
3 R = $CONHCOCCl_3$

Table 1. ^{1}H NMR spectral data* of septuplinolide (**2**)

	$CDCl_3$	Benzene-d_6
H-1	1.35 1.46†	1.08 1.30†
H-2	1.35 1.46†	1.08 1.30†
H-3a	1.78 *m*	1.50 *m*
H-3b	1.35 1.46†	1.08 1.30†
H-5	1.45 1.56†	1.08 1.25†
H-6a	1.94 2.10†	1.80 *ddd* (13.5; 7.2; 2.0)
H-6b	1.15 1.45†	1.01 *br dd* (13.5; 11.0)
H-7	2.96 *m*	2.25 *m*
H-8	4.44 *ddd* (5.0; 5.0; 2.0)	3.79 *ddd* (5.5; 5.0; 2.0)
H-9a	2.08 *dd* (15.5; 2.0)	1.74 *dd* (15.5; 2.0)
H-9b	1.43 *dd* (15.5; 5.0)	0.83 *dd* (15.5; 2.0)
H-13a	6.08 *d* (1.2)	6.04 *d* (1.2)
H-13b	5.55 *d* (1.2)	5.00 *d* (1.1)
H-14	0.94 *s*	0.76 *s*
H-15	1.08 *s*	0.78 *s*

*Spectra run at 200 MHz at ambient temperature. TMS was used as internal standard. Coupling constants (*J*) or line separations in Hz are given in parentheses.

†Obscured by other signals.

Due to the better resolved ^{1}H NMR signals in benzene-d_6 than in $CDCl_3$, the following discussion is based on the data obtained in C_6D_6. Double irradiation of the multiplet at δ2.25 (H-7) collapsed both one-proton doublets at δ6.04 (H-13a) and 5.00 (H-13b) to singlets, changed the three-fold doublet at δ3.79 (H-8) to a doublet of doublets, and collapsed both the three-fold doublet at δ1.80 (H-6a) to a doublet of doublets and the two-fold doublet at δ1.01 (H-6b) to a doublet. On the basis of chemical shift arguments, the three-fold doublet at δ3.79 was ascribed to the lactonic proton. Spin decoupling of the lactonic proton (δ3.79) changed H-7 (δ2.25) to a doublet of

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Table 2. ^{13}C NMR spectral data* of septuplinolide (2)

Carbon	δ, multiplicity
C-1	24.6 *t*†
C-2	19.3 *t*†
C-3	43.2 *t*‡
C-4	71.6 *s*
C-5	41.1 *d*
C-6	43.3 *t*‡
C-7	51.2 *d*
C-8	76.8 *d*
C-9	44.9 *t*‡
C-10	33.1 *s*
C-11	141.0 *s*
C-12	170.7 *s*
C-13	120.1 *t*
C-14	19.6 *q*
C-15	22.5 *q*

*The spectrum was obtained in $CDCl_3$ at ambient temperature at 50.32 MHz. Peak multiplicity was obtained by heteronuclear multipulse programs.

†,‡Assignments are interchangeable.

doublets, and collapsed both doublet of doublets at δ1.74 (H-9a) and 0.83 (H-9b) to doublets. In return, irradiation of either of the H-9 protons changed the other H-9 to a doublet and simplified the lactonic signal at δ3.79 (H-8). Double irradiation of the three-fold doublet at δ1.80 (H-6a) changed the H-7 multiplet at δ2.25, collapsed the doublet of doublets at δ1.01 (H-6b) to a doublet, and simplified the region δ1.08–1.30 (H-5). A similar change in the H-5 and H-7 signals and simplification of the H-6a signal (δ1.80) to a doublet of doublets were observed upon irradiation of the signal at δ1.01 (H-6b).

In the $CDCl_3$ ^{1}H NMR spectrum of **2**, the presence of two three-proton singlets at δ0.94 (H-14) and 1.08 (H-15), which correspond to an angular methyl group and to a methyl on a carbon bearing hydroxyl group, respectively, suggested a eudesmanolide-type skeleton with a hydroxyl group at C-4. Since the spin decoupling experiments (Table 1) clearly showed that the carbons bearing H-7 and the lactonic proton were both linked to a methylene group, the structure of **2** had to be formulated as a 7,8-lactonized eudesmanolide. The small coupling $J_{7,8}$ = 5.0 Hz indicated that the α-methylene-γ-lactone moiety was *cis*-fused. The CD spectrum of **2** showed a negative band at 252 nm indicative of a negative Cotton effect due to the $n \rightarrow \pi$ transition of the α-methylene-γ-lactone ring. Application of Geissman's rule [5], which predicts a negative Cotton effect for 12,8β-lactonized compounds, further supported the presence of a 12,8β-lactone in septuplinolide.

The configuration at C-4 in **2** was determined by *in situ* acylation of the hydroxyl group with trichloroacetylisocyanate [6]. The ^{1}H NMR spectrum of the trichloroacetylcarbamate **3** showed the presence of one NH signal at δ8.23 which further supported the presence of hydroxyl group in septuplinolide. The paramagnetic acylation shift of the C-4 methyl signal from δ1.08 in **2** to δ1.50 in **3** ($\Delta\delta$ = 0.42) was consistent with the presence of a hydroxyl group at C-4. The absence of a significant shift of the H-5 signal, which is assumed to be α as in other eudesmanolides isolated from *Calea* species, suggested a β-orientation of the C-4 hydroxyl group. Therefore, a stereostructure as shown in **2** can be formulated for septuplinolide.

EXPERIMENTAL

Calea septuplinervia Hieron. was collected on 11 December 1979 in La Mucuy, Venezuela, along the river on the left side of the road towards the gate of the National Park (L. Urbatsch, J. Pruski and N. Fischer, No. 3457, voucher deposited at L.S.U., U.S.A.). The air-dried plant material (830 g) was extracted and worked up as described before [7], providing 4.8 g of the crude terpenoid extract. CC of this crude syrup on silica gel, first with hexane–EtOAc mixtures and then with EtOAc–Me_2CO mixtures, provided 80 fractions of 200 ml each. Fractions 20–22 (117 mg) yielded 31 mg apigenin 7,4'-dimethyl ether as yellow needles upon evaporation of the solvent. Fractions 27–29 (120 mg) gave 80 mg acacetin (apigenin 4'-methyl ether).

Fraction 26 (55 mg) was purified by repetitive TLC on silica gel with $CHCl_3$–Me_2CO (9:1), affording 11 mg telekin (**1**), while fractions 36–38 (195 mg) gave 122 mg pure septuplinolide (**2**) as a colourless crystalline compound upon crystallization by vapour diffusion from petrol-$CHCl_3$ [8].

Septuplinolide (**2**). $C_{15}H_{22}O_4$, crystalline compound (mp 171–172°); UV λ_{max}^{MeOH} nm: end absorption; CD (MeOH; *c* 2.88 × 10^{-4}): $[\theta]_{211} + 3.7 \times 10^4$, $[\theta]_{252} - 5.8 \times 10^3$; IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3580 (OH), 3500 (*br*, OH), 1755 (γ-lactone), 1665 (double bond); EIMS (probe) *m/z* (rel. int.): 250 $[M]^+$ (0.8), 235 $[M - Me]^+$ (3.7), 233 $[M - OH]^+$ (5.3), 232 $[M - H_2O]^+$ (32.4), 217 $[M - H_2O - Me]^+$ (9.0), 204 $[M - H_2O - CO]^+$ (7.2), 189 $[M - H_2O - Me - CO]^+$ (7.1).

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