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Constituents of two *Flourensia* species

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Abstract

The MeOH extract of aerial parts of *Flourensia riparia* Grisebach (Asteraceae) afforded a sesquiterpene lactone, 4β-hydroxy-4,10α-dimethyl-7αH,8αH-eudesman-11-ene-8,12-olide, together with septuplinolide, its isomer at positions C-5 and C-10. In addition, known flavonoids, *p*-hydroxyacetophenone derivatives, carabrone and isoalantolactone were identified. Three known flavonoids and a benzofuran were isolated from *Flourensia campestris* Wedd.

Keywords: *Flourensia riparia*; *Flourensia campestris*; Asteraceae; Eudesmanolides; Benzofurans; Flavonoids; Prenylated flavonoids; *p*-Hydroxyacetophenone derivatives

1. Introduction

The genus *Flourensia* was placed by Stuessy (1977) in the subtribe Helianthinae but was later transferred together with *Encelia* to the subtribe Ecliptinae (Robinson, 1981). This genus comprises about 25 species distributed throughout America. Previous studies on this genus reported the isolation of flavonoids, eremophilanes and costic acid as well as a large variety of *p*-hydroxyacetophenone derivatives (Kingston et al., 1975; Guerreiro et al., 1979; Bohlmann and Jakupovic, 1979; Wollenweber and Yatskievych, 1985; Stuppner and Müller, 1994). Sesquiterpene lactones have only been described in *Flourensia macrophylla* (Bohlmann et al., 1984a,b).

As a result of a chemotaxonomic survey on the genus *Flourensia*, the present report deals with the isolation and structural elucidation of one 10α-methyl eudesmanolide together with several known compounds from *Flourensia riparia* Griseb., a species of North Western Argentina. *Flourensia campestris* Wedd. was also examined and found to contain some of the same compounds isolated from *F. riparia*. The known compounds were identified by comparison of their spectral properties with those reported in the literature.

2. Results and discussion

The MeOH extract of the aerial parts of a collection of *F. riparia* from Salta province (Argentina) yielded: 4β-hydroxy-4,10α-dimethyl-7αH,8αH-eudesman-11-ene-8,12-olide (**1**); septuplinolide (**2**) (Tada et al., 1993); isoalantolactone (Bohlmann et al., 1978); carabrone (Yoshioka et al., 1973); encecalin (Bjeldanes and Geissman, 1969); 6-methoxytremetone (Castañeda et al., 1996); euparone (Elsohly et al., 1974) 2,5-diacetyl-6-methoxybenzofuran (Bin et al., 1997) and *p*-hydroxyacetophenone. Most of the flavonoids isolated from this species carried an isoprenyl moiety at C-8. These are 8-prenylnaringenin (Stevens et al., 1997); exiguaflavanone K (Iinuma et al., 1994); 5,3′G-dihydroxyisobavachin-7-

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O-methyl ether (Bohlmann and Jakupovic, 1979); glabranin (Mitscher et al., 1983); 8-prenyldihydroisorhamnetin (Mc Cormick et al., 1986); glepidotin B (Mitscher et al., 1983) and scariosin (Ali Nia et al., 1992). Pinobanksin (Kuroyanagi et al., 1982) was also identified.

Compound **1** was assigned the molecular formula $C_{15}H_{22}O_3$ (HRDEIMS) and the IR spectrum (ν_{max} 3490, 1757 cm^{-1}) suggested the presence of a tertiary hydroxyl group and an α,β-unsaturated-γ-lactone.

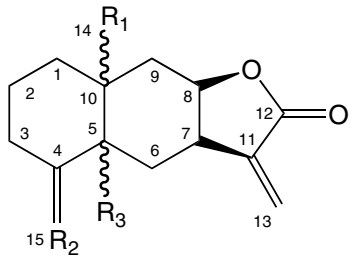
The presence of the α-methylene-γ-lactone moiety was also evident from the characteristic two one-proton doublet at δ 6.31 (*d*, $J = 3.6$ Hz, H-13a), and at δ 5.58 (*d*, $J = 3.2$ Hz, H-13b) in the ^1H NMR spectrum, and the signals corresponding to C-11 (δ 137.2), C-13 (120.3), and C-12 (δ 170.6) in the ^{13}C NMR spectrum.

The chemical shift at δ 4.75 (*ddd*, $J = 11.1$, 7.6, 6.3 Hz) was ascribed to the proton bound to C-8. This assignment was confirmed by the connectivities found in the COSY experiment. Thus, cross-correlation peaks observed in the COSY experiment as well as proton decoupling experiment led us to assign all the signals in the spectrum as well as their coupling constants (see Section 3). The ^{13}C NMR spectrum of **1** exhibited 15 signals and the DEPT experiment confirmed the presence of the hydroxylated quaternary carbon at δ 71.7, one oxymethine carbon at δ 75.7 (C-8), one carbonyl signal, two methyl carbons at δ 19.6 and 17.9, and five methylene groups.

The molecular formula of **1** was identical to that of septuplinolide (**2**) and both the IR profile and the ^{13}C NMR spectrum of compound **1** resembled those reported for septuplinolide (**2**) (Tada et al., 1993).

In the phase sensitive NOESY experiment, NOE cross-peaks between H-8α/H-7α, H-8α/H-14 (δ 0.92), H-7α/H-14, and H-15 (δ 1.15)/H-14 were observed, suggesting that these protons were on the same side of the molecule. Further interactions found between H-7 and H-6α at δ 1.79, and H-14/H-9α (δ 1.91) suggested that these protons are also in the same orientation.

The structure of septuplinolide (**2**) was previously confirmed by stereoselective synthesis as a *trans* decaline with C-8 *cis* annelation (Tada et al., 1993) and single X-ray diffraction analysis (Vargas et al., 1991). The NOEs between H-14β/H-15β and H-7α/H-8α confirmed the relative stereochemistry.

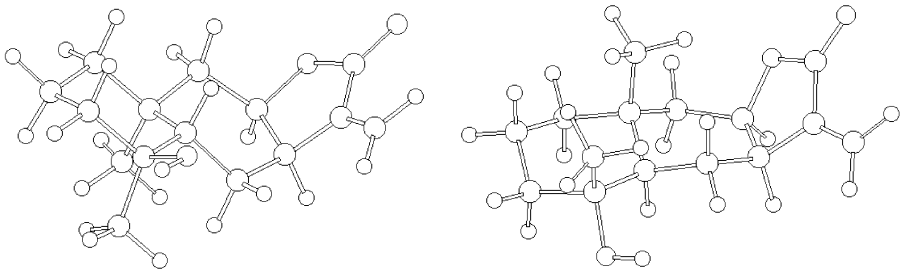


	R_1	R_2	R_3
1	α CH_3	α CH_3, β OH	β H
2	β CH_3	β CH_3 α OH	α H

Since the NOESY experiment suggested that both methyl groups in compound **1** are on the same plane as H-8 and H-7, the stereochemistry of the decalin ring system is most likely opposite that of septuplinolide (**2**).

The assignment of the relative stereochemistry of **1**, based on NOESY data, and the observed coupling constants, was further supported by the calculated possible low-energy conformation. The detection of the NOEs between H-6β (δ 2.34) and H-13b (δ 5.58), confirmed the close spatial proximity of both protons. The large coupling constants between H-5β at δ 1.28 with H-6α at δ 1.79 (13.2 Hz), and H-8α at δ 4.75 with H-9β at δ 0.98 (11.1 Hz), were in agreement with their axial position, and with the estimated coupling constants from dihedral angles, using generalized equations described by Hassnoot et al. (1980).

In order to explain the significant difference in the value of the allylic coupling constant $^4J_{7,13}$ between compound **1** (3.6 and 3.2 Hz) and septuplinolide (**2**) (1.1 and 1.1 Hz), molecular models for both compounds were generated using AM1 semiempiric calculations with MOPAC. The minimized structures are depicted in Fig. 1.



Compound **1** Septuplinolide (**2**)

Fig. 1. Three-dimensional structures of compound **1** and septuplinolide (**2**) generated by AM1 calculations using MOPAC.

The model for compound **1** showed that the exomethylene-γ-lactone group belongs to the pseudorotational *S*-type (Appendino et al., 1983). This conformation is a consequence of the inversion of the configuration at C-5 and C-10 with respect to septuplinolide (**2**). In this case, the bond C-7,C-11 adopts an axial orientation, leading to a value of −80.69° for the dihedral angle H-7,C-7,C-11,C-13. It is well known that the allylic coupling constant has its maximum value when the angle formed by the allylic proton and the plane of the double bond is near 90°. This is the reason why compound **1** shows a high value for the allylic $^4J_{7,13}$. On the other hand, septuplinolide (**2**) showed the bond C-7,C-11 in an *equatorial* orientation. The observed value of the dihedral angle H-7,C-7,C-11,C-13 for septuplinolide (**2**) was −49.34°, which agrees very well with the lower value observed for the coupling constant between H-7 and both H-13 protons.

All these data led us to assign the structure of **1**, as 4β-hydroxy-4,10α-dimethyl-7αH,8αH-eudesman-11-ene-8,12-olide.

One population of *F. campestris* from Cordoba Province (Argentina) was also studied, and resembled *F. riparia* in its chemical contents. The MeOH extract of the aerial parts of the plant contained no lactones, but gave 2,5-diacetyl-6-methoxybenzofuran; 5,3′-dihydroxyisobavachin-7-*O*-methyl ether; 8-prenyl dihydroisorhamnetin and the flavanone 8-prenyleriodictyol (Fukai and Nomura, 1990). The ^{13}C NMR spectrum data for 8-prenyl dihydroisorhamnetin that have not been previously reported are given in Section 3.

The chemistry of *F. campestris* is close to that of *F. heterolepis* (Bohlmann and Jakupovic, 1979) particularly in the co-occurrence of prenylated flavonoids and benzofuran derivatives. *F. riparia* seems to be related to *F. macrophylla* (Bohlmann et al., 1984a,b) based on the co-occurrence of prenylated flavonoids and sesquiterpene lactones of the eudesmanolide type. Similar lactones have been isolated from several genera from the Ecliptinae (Bohlmann et al., 1984b; Ciccio and Calzada, 1981; Herz and Kumar, 1979; Srivastava et al., 1990) and these findings may support the placing of *Flourensia* and *Encelia* in this subtribe.

3. Experimental

3.1. General

^{1}H and ^{13}C NMR spectra were recorded in a Bruker AC 200 spectrometer. HRDEIMS and EIMS (70 eV) were recorded on a Micromass double focusing magnetic sector mass spectrometer, model VG-7070 EHF. HPLC was performed with an SP thermGoseparation products Spectra Series P_{100} apparatus equipped with a Shodex R_1-71 refractive index and UV detector at 310 nm. Semipreparative HPLC was carried out using a YMC RP18 reversed phase column (250 × 20 mm) at 5 ml/min flow rate. UV spectra were obtained on a GBC 918 spectrophotometer. IR spectra were recorded on an IR-FT Bruker.

3.2. Plant material

Flourensia riparia Griseb. was collected in December 1995, in El Maray, Salta Province, Argentina and identified by Lazaro Novara. A voucher specimen (no. 10765) is deposited in the Museum of the Facultad de Ciencias Exactas, Universidad Nacional de Salta, Salta, Argentina.

Fluorensia campestris Wedd. was collected in December 1997, in Carlos Paz, Córdoba Province, Argentina and identified by Dr. Luis Ariza Espinar. A voucher specimen (CORD 3352) is on deposit at the Museo Botánico, Córdoba, Argentina.

3.3. Extraction and isolation

The air-dried aerial part of *F. riparia* (1.2 kg) was extracted with MeOH (3.5 l) at 50°. The resulting extract was concentrated under reduced pressure and partitioned with hexane–MeOH–H_2O (10:3:1). The polar phase was evaporated in vaccum and extracted with $CHCl_3$. The $CHCl_3$ extract was evaporated under reduced pressure and the residue (4 g) was purified by CC on silica gel, eluting successively with $CHCl_3$, $CHCl_3$–Me_2CO and Me_2CO, to give eight main fractions (F_1–F_8) which were submitted to semipreparative HPLC. Fractions F_1–F_5 were eluted with MeOH–H_2O (7:3), and fractions F_6–F_8 with MeOH–H_2O (7.5:2.5), to yield subfractions f_1–f_8. Subfraction f_1 afforded a 1:1 mixture (25 mg) of encecalin and 6-methoxytremetone. Subraction f_2 was purified by preparative TLC with toluene–MeOH (9.5:5) to gave carabrone (12 mg). Subraction f_3 yielded euparone (18.4 mg) and 2,5-diacetyl-6-methoxhybenzofuran (19.2 mg). Subfraction f_4 yielded glepidoptin B (13.7 mg). Subfraction f_5 gave exiguaflavanone K (21 mg), impure glabranin (5.4 mg) and pinobanksin (3.3 mg), these being purified by Sephadex LH-20 chromatography. Subfraction f_6 was subjected to flash chromatography eluted with a hexane–EtOAc gradient; the hexane–EtOAc (1:1) eluate with further purified by preparative TLC with C_6H_6–MeOH (9:1) gave septuplinolide (**2**) (1.9 mg) and a mixture of septuplinolide (**2**) and isoalantolactone (4.2 mg); the hexane–EtOAc (3:2) eluate was purified by Sephadex LH-20 chromatography to afford **1** (6 mg). Subfraction f_7 yielded 8-prenylnaringenin (4 mg), 5,3′-dihydroxyisobavachin-7-*O*-methyl ether (5 mg) and scariosin (1.7 mg). Subfraction f_8 was subjected to HPLC eluted with CH_3CN–H_2O (1:1) to yield 8-prenyl dihydroisorhamnetin (14 mg).

The EtOAc extract was fractionated by CC (silica gel), with $CHCl_3$ and $CHCl_3$–Me_2CO mixture of increasing polarity to yield *p*-hydroxyacetophenone (30 mg).

The air-dried aerial part of *F. campestris* (460 g) was extracted as described above. After partitioning, the $CHCl_3$ residue (3 g) was subjected to CC (silica gel) with C_6H_6 and C_6H_6–EtOAc mixtures of increasing polarity as eluent systems to give 2,5-diacetyl-6-methoxhybenzofuran (3.2 mg), 5,3′-dihydroxyisobavachin-7-*O*-methyl ether (19.2 mg), 8-prenyl dihydroisorhamnetin (5 mg) and 8-prenyleridioctyol (25 mg).

*3.3.1. 4β-Hydroxy-4, 10 α-dimethyl-7αH,8αH-eudesman-11-ene-8,12-olide (**1**)*

Gum. IR ν^{KBr} cm^{-1}: 3490, 2970, 2927, 1757, 1664. ^{1}H–NMR (200.13 MHz, $CDCl_3$): δ 0.92 (3H, *s*, H-14), 0.98 (1H, *m*, H-9β), 1.15 (3H, *s*, H-15), 1.28 (1H, *dd*, $J_{5\beta,6\alpha} = 13.2$ Hz, $J_{5\beta,6\beta} = 3.0$ Hz, H-5β), 1.79 (1H, *ddd*, $J_{6\alpha,6\beta} = 14.0$, $J_{6\alpha,5\beta} = 13.2$ Hz, $J_{6\alpha,7\alpha} = 6.2$ Hz, H-6α), 1.91 (1H, *dd*, $J_{9\alpha,9\beta} = 13.2$, $J_{9\alpha,8\alpha} = 6.3$ Hz, H-9α), 2.34 (1H, *ddd*, $J_{6\beta,6\alpha} = 14.0$ Hz, $J_{6\beta,5\beta} = 3.0$ Hz, $J_{6\beta,7\alpha} = 1.5$, H-6β), 3.28 (1H, *m*, H-7α), 4.75 (1H, *ddd*, $J_{8\alpha,9\beta} = 11.1$, $J_{8\alpha,7\alpha} = 7.6$, $J_{8\alpha,9\alpha} = 6.3$ Hz, H-8α), 5.58 (1H, *d*, $J = 3.2$ Hz, H-13b), 6.31 (1H, *d*, $J = 3.6$ Hz, H-13a). ^{13}C NMR (50.03 MHz, $CDCl_3$): δ 17.9 (*q*, C-14), 19.6 (*t*, C-2), 20.3 (*t*, C-1), 22.4 (*q*, C-15), 34.7 (*s*, C-10), 39.6 (*d*, C-5), 40.6 (*t*, C-3), 43.6 (*t*, C-6), 48.0 (*d*, C-7), 48.3 (*t*, C-9), 71.7 (*s*, C-4), 75.7 (*d*, C-8), 120.3 (*t*, C-13), 137.2 (*s*, C-11), 170.6 (*s*, C-12). HRDEIMS found 250.1566 $C_{15}H_{22}O_3$, requires 250.1569. DEIMS *m/z* (rel. int.): 250 $[M]^+$ (3), 232 $[M–H_2O]^+$ (32), 217 $[M–H_2O–CH_3]^+$ (6), 189 $[M-61]^+$ (4), 165 $[M-85]^+$ (21), 163 (12), 121 (17), 81 (32), 43 (100).

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