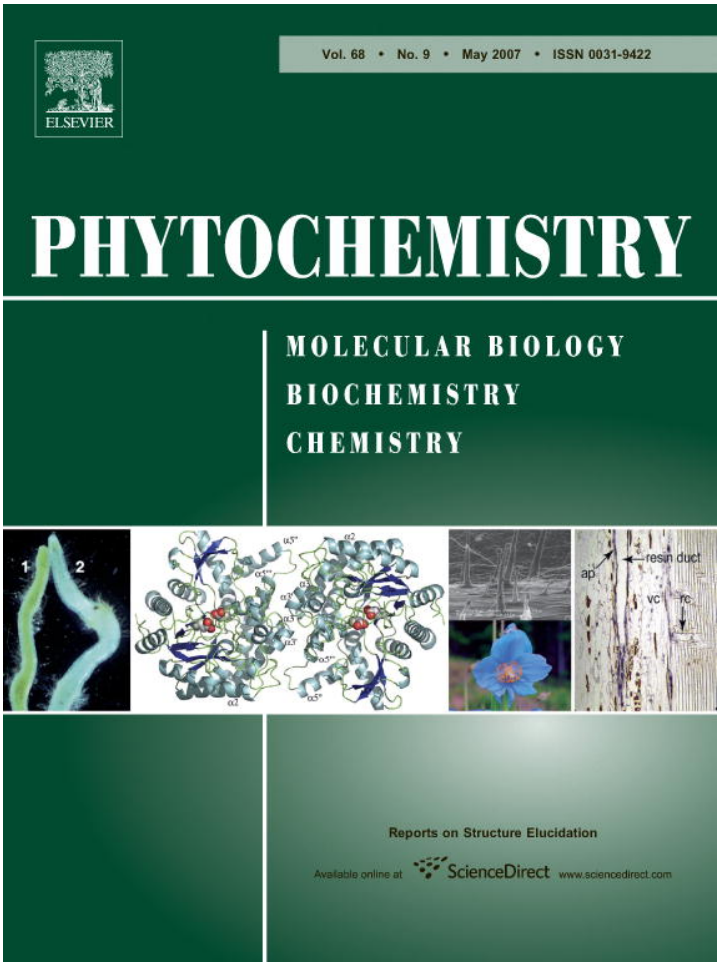

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Phytochemistry 68 (2007) 1295–1299
PHYTOCHEMISTRY
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Prenylflavonoids from *Flourensia fiebrigii*

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Received 24 August 2006; received in revised form 20 December 2006
Available online 26 February 2007

Abstract

Three compounds: (2*S*)-8-(3″-methylbut-2″-enyl)-7,3′,4′-trihydroxyflavanone, (2*S*)-8-(3″-methyl-4″-hydroxy-but-2″-enyl)-7,3′,4′-trihydroxyflavanone and (2*S*)-8-(3″-methyl-4″-hydroxy-but-2″-enyl)-5,3′,4′-trihydroxy-7-methoxyflavanone, along with five previously known compounds, were isolated from the aerial parts of *Flourensia fiebrigii*. Their structures were elucidated by application of various spectroscopic methods, including 1D and 2D NMR techniques.

Keywords: *Flourensia fiebrigii*; Asteraceae; 5-Deoxyflavonoids; Prenylated flavonoids

1. Introduction

The genus *Flourensia*, Asteraceae, comprises 25 species distributed throughout America. This genus seems to be characterized by the occurrence of prenylflavonoids and benzofuran derivatives (Bohlmann and Grenz, 1977; Bohlmann and Jakupovic, 1979; Bohlmann et al., 1984). In previous articles, we have reported the isolation of several prenylflavonoids and benzofuran derivatives from both *Flourensia campestris* Wedd. and *Flourensia riparia* Griseb. (Uriburu et al., 2004, 2005).

As part of our continuing program aimed towards the study of *Flourensia* species present in Argentina, we herein report the isolation and structural characterization of three new prenylflavanones from *Flourensia fiebrigii* Blake, as well as the presence of five previously known compounds. Their structures were elucidated by application of various spectroscopic methods, including the use of 1D and 2D NMR spectroscopy.

2. Results and discussion

The Et_2O extract of the aerial parts of *F. fiebrigii* yielded three new compounds, the prenylflavonoids **1**–**3**, together with other known ones: tremetone (Zalkow et al., 1979), 6-methoxytremetone (Castañeda et al., 1996), 8-prenyleriodictyol (Fukai and Nomura, 1990), 2-isopropenyl-3-oxyangeloyl-5-acetyl-*cis*-2,3-dihydrobenzofuran (Bohlmann and Dutta, 1979) and 5,3′-dihydroxyisobavachin-7-*O*-methyl ether (Bohlmann and Jakupovic, 1979).

Compound **1** was obtained as an amorphous solid, and the molecular formula was determined to be $C_{20}H_{20}O_5$ from analysis of its HREIMS and NMR spectroscopic data. The UV, ^1H and ^{13}C NMR spectra (Table 1) were consistent with a flavanone skeleton. In addition, both the lack of a downfield signal for a chelated phenolic group in the ^1H NMR spectrum and the absence of a batochromic shift when the UV spectrum was recorded with the addition of $AlCl_3$/HCl were indicative of a 5-deoxyflavanone. This was confirmed by the appearance of two *ortho*-coupled doublets ($J = 8.5$ Hz) at δ 7.75 and 6.53, each integrating for one proton, assigned to H-5 and H-6, respectively. It also displayed a set of characteristic signals for a γ,γ-dimethylallyl moiety which was located at C-8, based on the HMBC

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doi:10.1016/j.phytochem.2007.01.004

Table 1
^{1}H (500 MHz, *J* in Hz) and ^{13}C (125 MHz) spectroscopic data for compounds **1**–**3**

	1[a]		**2**[b]		**3**[b]	
	δH	δC	δH	δC	δH	δC
2	5.35 *dd* (2.9, 13.1)	79.2 *d*	5.43 *dd* (3.2, 12.0)	80.0 *d*	5.44 *dd* (3.2, 11.5)	79.4 *d*
3*ax*	2.97 *dd* (2.9, 17.0)	44.0 *t*	2.96 *dd* (12.0, 16.6)	43.9 *t*	3.11 *dd* (11.5, 17.0)	43.3 *t*
3*eq*	2.81 *dd* (13.1, 17.0)		2.74 *dd* (3.2, 16.6)		2.84 *dd* (3.2, 17.0)	
4		191.5 *s*		191.0 *s*		198.5 *s*
5	7.75 *d* (8.5)	126.5 *d*	7.58 *d* (8.7)	126.0 *d*		163.9 *s*
6	6.53 *d* (8.5)	110.6 *d*	6.61 *d* (8.7)	110.2 *d*	6.13 *s*	92.8 *d*
7		161.5 *s*		162.2 *s*		166.8 *s*
8		114.7 *s*		116.0 *s*		109.2 *s*
9		160.3 *s*		162.1 *s*		160.1 *s*
10		114.0 *s*		116.2 *s*		103.9 *s*
1′		131.9 *s*		132.2 *s*		132.1 *s*
2′	7.00 *brs*	113.4 *d*	7.09 *d* (1.7)	114.4 *d*	7.07 *brs*	114.5 *d*
3′		143.5 *s*		146.2 *s*		146.6 *s*
4′		143.5 *s*		146.2 *s*		146.6 *s*
5′	6.90 *brs*	115.4 *d*	6.84 *d* (8.1)	115.8 *d*	6.85 *brs*	115.9 *d*
6′	6.90 *brs*	118.9 *d*	6.86 *dd* (1.7, 8.1)	118.5 *d*	6.85 *brs*	119.0 *d*
1″	3.43 *brd* (7.0)	22.2 *t*	3.40 *brd* (7.0)	22.0 *t*	3.26 *brd* (7.3)	21.5 *t*
2″	5.23 *brt* (7.0)	120.9 *d*	5.50 *brt* (7.0)	123.2 *d*	5.40 *brt* (7.3)	123.6 *d*
3″		135.4 *s*		136.3 *s*		136.1 *s*
4″	1.77 *brs*	25.8 *q*	3.93 *d* (5.5)	68.2 *t*	3.90 *brs*	68.5 *t*
5″	1.78 *brs*	18.0 *q*	1.70 *brs*	13.5 *q*	1.67 *brs*	13.8 *q*
OCH_3					3.90 *s*	54.5 *s*
OH	5.97 *s*				8.15 *s*	
OH	5.59 *s*				7.91 *s*	
OH	5.40 *s*				12.21 *s* 5-OH	
4″-OH			3.87 *t* (5.5)			

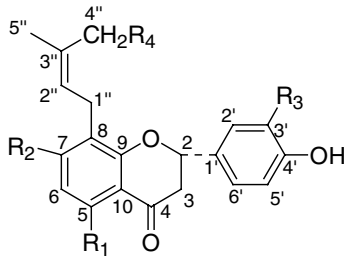
Assignments aided with HH-COSY, HSQC and HMBC.
[a] In $CDCl_3$.
[b] In CD_3COCD_3.

correlations from H-1″ through C-7, C-8, C-9. The aromatic signals of ring B also were evident as a two-proton singlet at δ 6.90 (H-5′ and H-6′) and an one-proton singlet at δ 7.00 (H-2′) (Harborne, 1994); additionally three D_2O exchangeable signals were observed (Table 1) corresponding to three hydroxy phenolic groups. The 3′,4′ dihydroxy substitution was also evident from the HMBC correlations from H-2 through C-1′, C-2′, C-6′, C-4, and from H-2′ through C-1′, C-6′, C-2, respectively. In the CD spectrum, a negative Cotton effect at 301 nm (Δε −1.16), and a positive Cotton effect at 332 (Δε +6.35) indicated the *S*-configuration at C-2 (Iinuma et al., 1994; Slade et al., 2005). Therefore, this new compound was characterized as (2*S*)-8-(3″-methylbut-2″-enyl)-7,3′,4′-trihydroxyflavanone (**1**).

Compound **2** was isolated as a gum and was assigned the molecular formula $C_{20}H_{20}O_6$ from analysis of its HRFABMS and NMR spectroscopic data. The UV spectrum in MeOH at 286 nm suggested a flavanone skeleton and the absorption maxima was also unaffected by addition of $AlCl_3$/HCl as for compound **1**. The ^{1}H NMR spectra was similar to that for **1** (Table 1), with the same substitution pattern, except for the presence of a 2H doublet at δ 3.93 (H-4″) attributable to a methylene carbinol allylic group. This allylic secondary hydroxyl group at δ 3.87, was unambiguously established to be at C-4″ by means of the COSY, HSQC and HMBC spectra. The attachment of the hydroxyprenyl moiety was determined to be at C-8 by the detection of HMBC correlations from H-1″ to C-7, C-8, C-9, C-3″. The absolute configuration at C-2 was considered to be *S* according to the results of the CD spectroscopic analysis (see Section 4). Consequently, the structure of **2** was concluded to be

(2*S*)-8-(3″-methyl-4″-hydroxy-but-2″-enyl)-7,3′,4′-trihydroxyflavanone.

Compound **3** was isolated as a gum, and gave a molecular ion at *m*/*z* 387.1446 in the HRFABMS consistent with the molecular formula $C_{21}H_{22}O_7$. The UV spectrum showed a λ_{max} at 288 nm typical of a flavanone skeleton. The ^{1}H NMR spectra was similar to that for **2** (Table 1), except for signals for a chelated phenolic group (δ 12.21) and a methoxyl group (δ 3.90) (Table 1). The phenolic group was confirmed to be at the C-5 position by the HMBC connectivities from the OH-5 through C-5, C-6, C-10. The methoxyl group was placed at C-7 on the basis of its HMBC correlation. The one proton singlet at δ 6.13 was assigned to H-6 due its HMBC connectivities with C-5, C-7, C-8, C-10. The ^{1}H and ^{13}C NMR spectroscopic data of the B ring were similar to those of compounds **1** and **2**, suggesting the same substitution pattern (Table 1). The absolute configuration at C-2 was again concluded to be *S* according to the analysis of CD spectroscopic data (see Section 4). Consequently, the structure of compound **3** was established as (2*S*)-8-(3″-methyl-4″-hydroxy-but-2″-enyl)-5,3′,4′-trihydroxy-7-methoxyflavanone.



	R_1	R_2	R_3	R_4
1	H	OH	OH	H
2	H	OH	OH	OH
3	OH	OMe	OH	OH

3. Concluding remarks

The chemical relationship of *Flourensia heterolepis* (Bohlmann and Jakupovic, 1979), *F. campestris*, *F. riparia* (Uriburu et al., 2004), and the results of this work in *F. fiebrigii*, suggest some chemotaxonomical relationships.

This is because several flavonoids found in these species exhibit an 8-prenylation at ring A. Therefore, this finding may support placing the genus *Flourensia* in the subtribe Ecliptinae (Bohlmann, 1990).

In the present study, identification of two 5-deoxyflavonoids is reported. This compound class represents a common feature in the family Leguminoseae, with many being C-8 prenyl derivatives (Rodrigues Garcez et al., 1988; Rao et al., 1994; Iinuma et al., 1995; Barron and Ibrahim, 1996; Magalhães et al., 1996). Until this study, the 5-deoxyflavanoids were only previously isolated from *F. heterolepis* (7-hydroxyflavanone and 7-methoxyflavanone) (Bohlmann and Jakupovic, 1979) and *F. campestris* (7,3′,4′-trimethoxyflavone) (Uriburu M.L. Ph.D. Thesis, Salta University, 2002) in the Asteraceae. However, homologues of them have also been reported as present in the *Achillea* (Ahmad et al., 1995) and *Calea* (do Nascimento et al., 2002; do Nascimento and de Oliveira, 2004) plant families.

As far as we know through this is also the first report of 5-deoxy-C-8-prenylflavanones in the Asteraceae.

4. Experimental

4.1. General experimental procedures

NMR spectra were recorded using Bruker Avance 500 or Bruker AC 200 spectrometer. Whereas FABMS and HRFABMS were determined using a VG-ZAB spectrometer. Desorption EIMS (DEIMS) and HREIMS (HRDEIMS) were obtained using a VG-7070 spectrometer, with UV spectra being measured with a GBC 918 spectrophotometer. IR spectra were recorded as KBr disks, using an IR-FT Bruker model IFS-88 spectrometer, and CD spectra were obtained with a Jasco 715.

4.2. Plant material

F. fiebrigii was collected in March 1999, Parque Nacional Los Cardones, Salta Province, Argentina. A voucher specimen (No. 11244) is on deposit at the Museum of the Facultad de Ciencias Naturales, Universidad Nacional de Salta, Salta, Argentina.

4.3. Extraction and isolation

The dried aerial parts of *F. fiebrigii* (1.6 kg) were extracted with EtOH–H_2O (98:2) (3.5 l) at 35 °C, for 96 h. The resulting extract was conc. under reduced pressure and the residue was suspended in MeOH–H_2O 9:1 (250 ml). After 24 h, the resulting suspension was filtered, the MeOH–H_2O solution was washed with hexane (3 × 200 ml), and the MeOH was evaporated in vacuo. The remaining aq. solution was extracted with Et_2O. The resulting Et_2O extract (3 g) was next fractionated by reversed-phase silica gel vacuum liquid chromatography eluting with MeOH–H_2O 7:3 and MeOH, respectively. The MeOH–H_2O 7:3 eluent was then submitted to silica gel flash chromatography eluting with a gradient of hexane–EtOAc, to afford seven subfractions (F1 through F7).

Tremetone (22.0 mg) and 6-metoxy-tremetone (12.0 mg) were obtained from F1 (hexane–AcOEt 9:1). Fraction F2 (hexane–EtOAc 4:1) was purified by reversed-phase semiprep. TLC (MeOH–H_2O 4:1) to afford 2-isopropenyl-3-oxyangeloyl-5-acetyl-*cis*-2,3-dihydrobenzofuran (11.0 mg).

Fraction F3 (hexane–EtOAc 7:3) was purified on Sephadex LH-20, eluting with MeOH, to afford 5,3′-dihydroxyisobavachin-7-*O*-methyl ether (15 mg). Fraction F4 (hexane–EtOAc 3:2) and F5 (hexane–EtOAc 1:1) were reunited and submitted to silica gel flash chromatography ($CHCl_3$–MeOH 10:0.5) to yield 8-prenyleriodictyol (5.0 mg) and (2*S*)-8-(3″-methylbut-2″-enyl)-7,3′,4′-trihydroxyflavanone (**1**, 13.0 mg). F6 (hexane-EtOAc 2:3) was subjected to silica gel flash chromatography using $CHCl_3$-MeOH followed by semiprep. TLC ($CHCl_3$–MeOH 5:1) (runs 3×), and finally purified by Sephadex LH-20 CC in MeOH to afford (2*S*)-8-(3″-methyl-4″-hydroxy-but-2″-enyl)-5,3′,4′-trihydroxy-7-methoxyflavanone (**3**, 3 mg). Fraction F7 (hexane–AcOEt 1:9) was submitted to flash chromatography (hexane–EtOAc 1:1) to give (2*S*)-8-(3″-methyl-4″-hydroxy-but-2″-enyl)-7,3′,4′-trihydroxyflavanone (**2**, 5 mg).

*4.4. (2S)-8-(3″-methylbut-2″-enyl)-7,3′,4′-trihydroxyflavanone (**1**)*

Amorphous powder, CD (MeOH; *c* 0.175): ($\Delta\varepsilon_{301}$ −11.16), ($\Delta\varepsilon_{332}$ +6.35); UV λ_{max}^{MeOH} nm ($\log\varepsilon$) : 286 (4.31), 308 sh; $\lambda_{max}^{MeOH+AlCl_3}$ nm : unchanged; $\lambda_{max}^{MeOH+NaOMe}$ nm : 339; $\lambda_{max}^{MeOH+NaOAc}$ nm : 336; IR ν_{max}^{KBr} cm^{-1} : 3400, 2966, 2925, 1651, 1599, 1522, 1441, 1286. For ^1H (500 MHz, $CDCl_3$) and ^{13}C (125 MHz) NMR spectroscopic data, see Table 1. DEIMS 70 eV *m/z* (rel. int.): 340 $[M]^+$ (86), 325 $[M–Me]^+$ (7), 297 $[M–C_3H_7]^+$ (68), 205 $[A_1+H]^+$ (30), 204 $[A_1]^+$ (14), 189 $[A_1–Me]^+$ (15), 176 (35), 161 $[A_1–CO–Me]^+$ (53), 149 $[A_1–C_4H_7]^+$ (97), 136 $[B_1]^+$ (100); HRDEIMS *m/z* 340.1300 $[M]^+$ (Calcd. for $C_{20}H_{20}O_5$ 340.1310).

*4.5. (2S)-8-(3″-methyl-4″-hydroxy-but-2″-enyl)-7,3′,4′-trihydroxyflavanone (**2**)*

Gum, CD (MeOH; *c* 0.045): ($\Delta\varepsilon_{301}$ −10.06), ($\Delta\varepsilon_{333}$ +6.00); UV λ_{max}^{MeOH} nm ($\log\varepsilon$) : 286 (4.31), 313 sh; $\lambda_{max}^{MeOH+AlCl_3}$ nm : no change; $\lambda_{max}^{MeOH+NaOMe}$ nm : 342, $\lambda_{max}^{MeOH+NaOAc}$ nm : 337; IR ν_{max}^{KBr} cm^{-1} : 3369, 2925, 1620, 1595, 1284. For ^1H (500 MHz, acetone-d_6) and ^{13}C (125 MHz) NMR spectroscopic data, see Table 1. FABMS *m/z* (rel. int.): 379 $[M+Na]^+$ (37), 357 $[M+H]^+$ (50), 339 $[(M+H)–H_2O]^+$ (24), 203 $[(A_1+H)–H_2O]^+$ (100), 149 $[(A_1)–C_4H_7O]^+$ (79); HRFABMS *m/z* 357.1334 from the $[M+H]^+$ (Calcd. for $C_{20}H_{21}O_6$ 357.1338).

*4.6. (2S)-8-(3″-methyl-4″-hydroxy-but-2″-enyl)-5,3′,4′-trihydroxy-7-methoxy-flavanone (**3**)*

Gum, CD (MeOH; *c* 0.065): ($\Delta\varepsilon_{293}$ −7.52), ($\Delta\varepsilon_{335}$ +1.76); UV λ_{max}^{MeOH} nm ($\log\varepsilon$) : 283(4.21), 333 sh; $\lambda_{max}^{MeOH+AlCl_3}$ nm : 312; $\lambda_{max}^{MeOH+AlCl_3+HCl}$ nm : no change; $\lambda_{max}^{MeOH+NaOMe}$ nm : no change; $\lambda_{max}^{MeOH+NaOAc}$ nm : no change; IR ν_{max}^{KBr} cm^{-1} : 3432, 2929, 1639, 1597, 1268. For ^1H (500 MHz, acetone-d_6) and ^{13}C (125 MHz) NMR spectroscopic data, see Table 1. FABMS *m/z* (rel. int.): 387 $[M+H]^+$ (100), 370 $[(M+H)–HO]^+$ (15), 233 $[(A_1+H)–H_2O]^+$ (40), 179 $[(A_1)–C_4H_7O]^+$ (20), 136 $[B_1]^+$ (7); HRFABMS *m/z* 387.1446 from the $[M+H]^+$ (Calcd. for $C_{21}H_{23}O_7$ 387.1443).

Acknowledgements

This work was supported with funds from the Consejo de Investigación, Universidad Nacional de Salta, Argentina. Work at the Universidad Nacional de Córdoba was supported by grants from CONICET and SECyT-UNC, Argentina. The NMR spectra were acquired at the Department of Chemistry facility Carnegie Mellon University (USA), and were purchased in part using funds from the NSF (CHE-0130903).

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