Pergamon

Phytochemistry Vol. 49, No. 7, pp. 2167–2169, 1998

Printed in Great Britain
0031-9422/98/$ - see front matter

PII: S0031-9422(98)00416-6

2″-*O*-CAFFEOYLORIENTIN FROM *VITEX POLYGAMA*

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(*Received* 3 *April* 1998)

Key Word Index—*Vitex polygama*; Verbenaceae; *C*-glucosylflavones; 2″-*O*-caffeoylorientin.

Abstract—A new flavone *C*-glucoside: 2″-*O*-caffeoylorientin was isolated from the leaves of *Vitex polygama* and identified by spectroscopic methods, together with orientin, *iso*-orientin, vitexin, isovitexin, luteolin, quercetin, quercetin 3-*O*-methylether and *p*-hydroxybenzoic acid. © 1998 Published by Elsevier Science Ltd. All rights reserved

INTRODUCTION

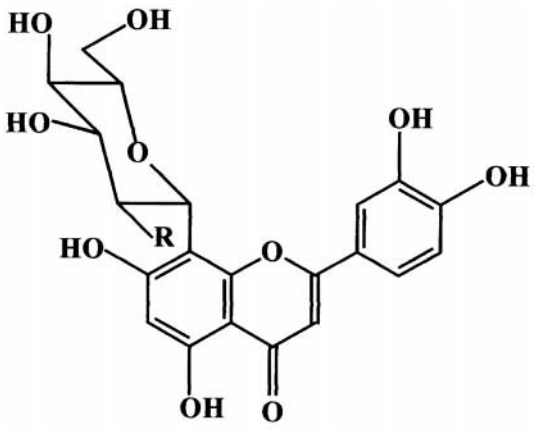
Vitex species (Verbenaceae) are small trees or shrubs which occur in tropical and subtropical regions. Plants of this genus are known to contain *C*-glucosyl flavones, ecdysteroids, diterpenes and iridoids [1]. In Europe, fruits of *V. agnus-castus* are used to treat menstrual disorders [2] while in China, the oil from the fruits of *V. negundo* var. *cannabifolia* is used to treat bronchitis [3]. The genus is well represented in Brazil, occurring from the Amazon region (*V. triflora* Vahl, *V. odorata* Hub.) to the state of Rio Grande do Sul (*V. montevidensis* Cham.). The species, *V. polygama* Cham. is found mainly in the states of Bahia, Minas Gerais and Rio de Janeiro. Bark and fruits of this plant are used in traditional medicine as emenagogue and diuretic and from the leaves, hentriacontane, nonacosane, tetracosanoyl acetate, ethyl caffeate and (+)-spatulenol have been reported [4].

In this paper we describe the isolation and identification of a new flavone *C*-glucoside: 2″-*O*-caffeoylorientin (**1**), together with orientin (**2**), *iso*-orientin, vitexin, isovitexin, luteolin, quercetin, quercetin 3-methyl ether, and *p*-hydroxybenzoic acid, which have been isolated for the first time from this species.

RESULTS AND DISCUSSION

Compound **1** and the other constituents were obtained from the ethyl acetate fraction of a methanolic leaf extract of *V. polygama* by repeated column chromatography. The known flavonoids as well as *p*-hydroxybenzoic acid were identified by comparison of their physicochemical data with those reported in literature and/or comparison with authentic samples.

A positive FAB-MS spectrum **1** showed molecular ions at *m/z* 611 $[(M + H)^+]$ and at *m/z* 633 $[(M + Na)^+]$, consistent with the molecular for-



1 R = Caffeoyl

2 R = H

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mula $C_{30}H_{26}O_{14}$. The ^{1}H-NMR spectrum of **1** was very similar to that of orientin (**2**), except for a set of signals indicative of a caffeoyl moiety: δ 7.79 (d, J = 16 Hz, H‴-8), 6.45 (*d*, J = 16 Hz, H‴-7), 6.89 (*dd*, J = 8 and 2 Hz, H‴-6), 7.20 (*d*, J = 8 Hz, H‴-5) and 7.35 ppm (*d*, J = 2 Hz, H‴-2).

Comparison of the ^{1}H-NMR data of **1** and **2** showed that for **1** the signal of H-2″ (H-2″ Glc) was shifted to δ 6.67 ppm (*t*, J = 10 Hz) when compared to the same one for **2** (δ 4.22–5.2 ppm) indicating that the caffeoyl moiety is attached to C-2″ of glucose. This finding is confirmed by the homonuclear ^{1}H–^{1}H correlation (COSY) NMR spectrum of **1**. Starting from the anomeric proton of glucose (δ 6.06 ppm, *d*, J = 10 Hz) a correlation peak was found only with the signal at δ 6.67 ppm, confirming the acylation position at C-2″. The anomeric proton observed at δ 6.06 ppm as a doublet with J = 10 Hz is indicative of a β-configuration for the glucose. In the aglycone moiety signals at δ 6.90 (s) and 6.67 (s) were assigned to protons H-6 and H-3 of the flavone ring A and signals at δ 7.93 (*dd*, J = 8 and 2 Hz), 7.12 (*d*, J = 8 Hz) and 8.35 (*d*, J = 2 Hz) were attributed to H-6′, H-5′and H-2′, respectively, on the flavone ring B, according to their coupling constants.

Comparison of the ^{13}C-NMR data of **1** and **2** shows that major differences between the two compounds are due to acylation of the glucose moiety of **1**. Carbon resonance values of **1** (Table 1) were assigned by comparison with the analogous data of **2** on the basis of chemical shift considerations, analyzing the β effects due to acylation [5]. Differences in chemical shifts between **1** and **2**: C-1″(−1.6 ppm), C-2″(+0.7 ppm) and C-3″(−2.7 ppm), are indicative of the acylation on C-2″ of the glucose moiety. Also, as shown in Table 1, comparison of the aglycone resonance values of C-6 and C-8 of the three compounds confirms that **1** is a caffeoyl derivative of orientin and not of *iso*-orientin.

Table 1. ^{13}C-NMR spectral data (75 MHz, Pyridine-d_5, TMS) of *C*-glucosylflavones isolated from the leaves of *Vitex polygama*

C	**1**	**2**	*iso*-orientin
Aglycone moiety			
2	165.24	165.34	164.99
3	103.22	103.33	103.78
4	182.94	183.14	182.95
5	163.93*	165.09	164.75
6	98.61	99.37	110.05
7	162.42	162.80	162.13
8	105.19	105.33	94.64
9	157.78	157.37	157.49
10	103.91	105.92	104.74
1′	121.65	123.13	122.72
2′	119.99*	120.46	119.55
3′	147.31	147.54	147.54
4′	151.30	151.68	151.68
5′	116.71*	117.06	116.90
6′	115.32*	115.44	114.53
Sugar moiety			
1″	74.01	75.59	75.33
2″	73.14	72.42	72.49
3″	78.16	80.84	80.71
4″	72.00	72.35	72.04
5″	83.56	83.41	83.01
6″	62.61	63.14	62.96
Caffeoyl moiety			
1‴	121.65		
2‴	116.32*		
3‴	145.24		
4″	147.31		
5‴	115.32*		
6‴	114.83		
7‴	147.20		
8‴	162.52		
9‴	166.52		

*Interchangeable values.

EXPERIMENTAL

General

^{1}H and ^{13}C-NMR were recorded at 300 and 75 MHz with TMS as int. standard on a Varian Gemini-300. FAB-MS were recorded in a positive mode (*meta*-nitrobenzylalchool + NaCl matrix) in a VG Autospect spectrometer. Si gel 60 (70-270 mesh, Macherey-Nagel) was used for CC and Si gel 60F254 (Merck) plates for TLC. Substances were detected by UV light or in the presence of NH_4OH vapor or by spraying with H_2SO_4–H_2O–Formol (2:1:1) followed by heating.

Plant material

Vitex polygama Cham. was collected in fruit at Maricá, Rio de Janeiro, Brazil. It was identified by Dr. Fátima Regina Salimena-Pires from Universidade Federal de Juiz de Fora, Juiz de Fora, Minas Gerais, where a voucher specimen is deposited.

Extraction and isolation

Leaves of *V. polygama* (3.0 kg) were extracted with EtOH. After filtration and concentration under red. press. the aq. residue was sequentially extracted with C_6H_6, CH_2Cl_2, EtOAc and *n*-BuOH. Part of the EtOAc extract (4.9 g) was chromatographed on Si gel eluted with EtOAc–Me_2CO–H_2O (25:8:2)–(5:2:1) (organic layer) affording 15 fractions of 150 ml each. Luteolin, quercetin, quercetin 3-*O*-methyl ether and *p*-hydroxybenzoic acid were isolated by Si gel CC ($CHCl_3$ with increasing amounts of EtOAc) from fraction 2. Fraction 4 afforded vitexin and orientin, after Si gel CC (EtOAc–Me_2CO–H_2O, 25:8:2) and, fractions 6–8 afforded isovitexin and orientin by Si gel CC ($CHCl_3$–MeOH–H_2O, 65:28:7,5). 2″-*O*-caffeoylorientin (**1**) was obtained (260 mg) after repeatedly Si gel CC (EtOAc–Me_2CO–H_2O, 25:8:2) of fraction 3 (640 mg).

*2″-O-Caffeoylorientin (**1**)*

^{1}H-NMR (pyridine-d_5, TMS): δ 6.06 (*d*, J = 10 Hz, H-1″); 6.45 (*d*, J = 16 Hz, H-7‴); 6.67 (*t*, J = 10 Hz, H-2″); 6.67 (*s*, H-3); 6.89 (*dd*, J = 8 and 2 Hz, H-6‴); 6.90 (*s*, H-6); 7.12 (*d* J = 8 Hz, H-5′); 7.20 (*d*, J = 8 Hz, H-5‴); 7.35 (*d*, J = 2 Hz, H-2‴); 7.79 (*d*, J = 16 Hz, H-8‴); 7.93 (*dd*, J = 8 and 2 Hz, H-8‴); 8.35 (*d*, J = 2 Hz, H-2″) and 14.05 (*sl*, OH-5). ^{13}C-NMR: Table 1. Fab MS m/z positive peaks (%):633 (4) [(M + Na)+]; 611 (3) [(M + H)+]; 533 (2); 511 (2); 435 (4); 329 (17); 308 (12); 289 (10); 258(5); 192 (7); 176 (68), 165 (15). UVλ_{max}^{MeOH}:249, 272 (*sh*), 295 (*sh*), 336; +$AlCl_3$ 273, 302 (*sh*), 358; +$AlCl_3$+HCl 279, 299, 339; +$AlCl_3$+H_3BO_3 249, 272 (*sh*), 295 (*sh*), 339.

Acknowledgements—We are indebted to Dr Helmut Duddeck from Institut fuer Organische Chemie, Universitaet Hannover, for performing FAB-MS spectra. One of us (S. G. L.) is indebted to Fundação Universitária José Bonifácio (FUJB-UFRJ) for a grant (Proc. No. 6090-9).

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