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A C-glucosylflavone from leaves of *Piper lhotzkyanum*

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Abstract

The dichloromethane fraction obtained from the methanolic extract of leaves of *P. lhotzkyanum* afforded a C-glucosylflavone (kaplanin) together with sakuranetin, methyl 4-methoxydihydroferulate and a mixture of methyl ferulate and dihydroferulate derivatives. Spectrometric methods were used to elucidate the structures of these compounds. © 2000 Elsevier Science Ltd. All rights reserved.

Keywords: Piper lhotzkyanum; Piperaceae; Leaves; Kaplanin; Flavonoid

1. Introduction

The family Piperaceae principally comprises five genera (*Piper*, *Peperomia*, *Lepianthes*, *Macropiper* and *Trianaeopiper*) and about 1400 species with worldwide distribution (Mabberley, 1993). Species of the genus *Piper* and *Peperomia* are found in abundance in Brazil (700 and 600, respectively) (Joly, 1977), some of which are used in folk medicine (Duke, 1985; Corrêa, 1984). Phytochemical investigation of Piperaceae species has afforded a diverse range of flavonoids, especially those with an unsubstituted B-ring. Many dihydrochalcones have also been isolated, but C-glycosylflavonoids are rare (Parmar et al., 1997). *Piper lhotzkyanum* Kunth (syn. *P. inversum* C.DC., *P. aromaticum* C.DC., *P. damazii* C.DC., *P. bennetianum* C.DC., *P. santa-barbaranum* C.DC.) is a shrub 1–3 m high, commonly found in humid forests with low incidence of light, in Southeast Brazil (Guimarães et al., 1992). Previous phytochemical investigation of *P. lhotzkyanum* yielded (*E*) and (*Z*) derivatives of 4-hydroxy-3-(3′,7′-dimethyl-1′-oxo)-2′,6′-octadienylbenzoic acid, a mixture of sesquiterpenes and *E*-phytol, and a new chromene (lhotzchromene) from hexane extract of the leaves (Moreira et al., 1998). Column chromatography of the dichloromethane fraction obtained from the methanolic extract of the leaves afforded 5-hydroxy-7-methoxy-8-C-β-glucosylflavone (kaplanin, **1**) as well as the known compounds methyl 4-methoxydihydroferulate (**2**), reported here for the first time in the family Piperaceae; sakuranetin (**3**), previously isolated from *P. aduncum* (Parmar et al., 1997); and a mixture of (**2**) with the C6–C3 derivatives 4-methoxydihydroferulic acid (**4a**), ethyl 4-methoxydihydroferulate (**4b**) and methyl ferulate (**4c**). The isolated substances were identified using spectroscopic analysis (1D and 2D ^{1}H- and ^{13}C-NMR, UV). The mixture was analysed by GC/MS and the substances were identified by comparison of their mass spectra with literature data, and by analysis of their mass fragmentation patterns (McLafferty and Stanffer, 1989).

2. Results and discussion

Compound **1** showed $[M + H]^+$ at m/z 431.4210 in its HR-FABMS, corresponding to the molecular formula $C_{22}H_{22}O_9$. The UV spectrum of compound **1** showed signals at $\lambda_{max}(\log \varepsilon)$ 213.3 (2.6), 287.2 (1.8) and

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330.0 (0.3). A bathochromic shift (60 nm) of band I in the UV spectrum was observed upon addition of $AlCl_3$, indicating a 5-hydroxy-substituted flavonoid. NaOH (0.1 N) or fused NaOAc treatment had no effect on the UV spectrum, indicating the absence of hydroxyl group at position 7 (A ring) or 4′ (B ring) (Mabry et al., 1970). The ^{1}H-NMR spectrum displayed signals for methoxyl protons at δ 3.95 (3H, *s*), an olefinic proton at δ 6.80 (1H, *s*), and a singlet and a multiplet due to aromatic protons at δ 6.48 (1H) and δ 7.6 – 8.2 (5H), respectively, which together suggested an unsubstituted B ring and a trisubstituted A ring. The presence of only one anomeric signal in the ^{1}H-NMR (4.98) and ^{13}C-NMR (72.02) spectra (72.02) suggested **1** to be a monosaccharide derivative. The large H-1′ and H-2′ coupling constant (10 Hz) for the anomeric proton indicated a β-configuration. The ^{1}H–^{1}H COSY spectrum supported this configuration. The ^{1}H-NMR spectrum also showed signals for carbinolic protons at δ 3.0–4.98 due to the sugar moiety (10H) and for a phenolic proton at δ 13.2 (5-O$\underline{\text{H}}$). The ^{13}C-NMR and HETCOR spectra of **1** showed signals at δ 95.85 and 105.97 due to carbons 6 and 8, indicating a C-substitution at carbon 8 of ring A. Also observed were signals at δ 62.9 and 82.5 arising from the glycosyl moiety. The ^{13}C-NMR assignments of **1** (Table 1) were compared to those published for 5-hydroxy-7-methoxyflavone (**5**), vitexin-7-glucoside (**6**), isovitexin (**7**), orientin (**8**), isoorientin (**9**), swertisin (**10**) and isoswertisin (**11**), and confirmed this compound to be 5-hydroxy-7-methoxy-8-C-β-glucosylflavone (Davoust et al., 1980; Chopin et al., 1984). To the best of our knowledge, this is the first occurrence in the family Piperaceae of a C-glycosylflavonoid with an unsubstituted B-ring.

Table 1
^{13}C-NMR spectral data for compound **1**[a], 5-hydroxy-7-methoxyflavone (**5**)[b], vitexin-7-glucoside (**6**), isovitexin (**7**), orientin (**8**), isoorientin (**9**), swertisin (**10**) and isoswertisin (**11**)[c]

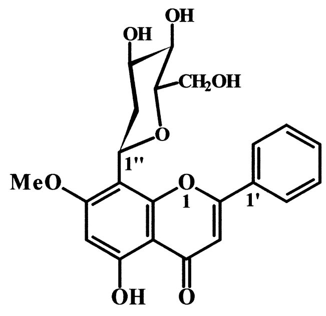
Position	1	5	6	7	8	9	10	11
2	165.0	163.5	164.2	163.9	164.0	163.6	164.0	164.5
3	105.5	105.4	102.4	102.9	102.5	102.8	103.1	102.5
4	183.6	182.1	182.3	182.1	181.9	181.8	182.3	182.4
4a	106.5	105.0	–[d]	103.5	104.0	103.3	104.1	105.9
5	163.2	161.3	160.7	160.8	160.0	160.2	160.9	161.0
6	95.9	98.2	98.5	109.0	97.9	108.5	109.8	95.1
7	165.2	165.4	162.6	163.3	162.2	163.0	164.2	163.5
8	105.9	92.8	107.3	93.7	104.4	93.6	90.7	104.8
8a	156.8	157.4	155.0	156.3	156.0	156.2	156.9	155.3
1′	132.3	130.6	121.2	121.1	122.0	121.4	121.1	121.6
2′	127.9	126.5	128.8	128.6	113.8	113.1	128.5	129.2
3′	129.9	129.2	115.8	116.0	145.5	145.4	116.0	116.0
4′	132.8	132.1	161.4	161.4	149.3	149.4	161.4	161.4
5′	129.9	129.2	115.8	116.0	115.6	116.0	116.0	116.0
6′	127.9	126.2	128.8	128.6	119.4	119.0	128.6	129.2
1″	72.0		73.9		73.3	72.9	72.9	
2″	74.5		71.4		70.6	70.3	70.3	
3″	80.3		78.5		78.4	78.6	79.3	
4″	72.5		69.9		70.5	70.0	71.4	
5″	82.5		81.4		81.7	81.3	81.5	
6″	62.9		61.5		61.4	61.2	62.0	
O$\underline{\text{C}}$$H_3$	56.9	56.7	–	–	–	–	–	

[a] **1** = acetone-d_6.

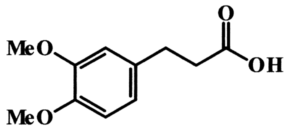
[b] **5** = $CDCl_3$.

[c] **6**–**11** = DMSO-d_6.

[d] Signal not reported.



(1)



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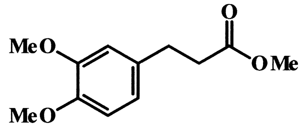
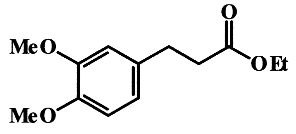
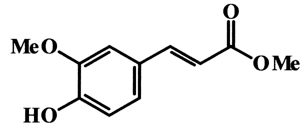


(2) 60.86%



(4b) 5.89%



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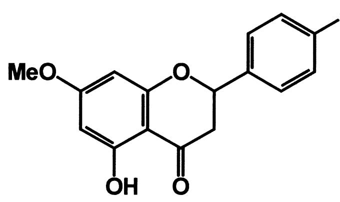

(3)

3. Experimental

3.1. General

UV spectra were recorded on a Shimadzu UV-1601 following the procedures for the identification of flavonoids (Mabry et al., 1970). Mass spectra were obtained on a VG Autospect operated at 70 eV. High resolution positive FAB-mass spectra were recorded using a glycerol matrix on a VG 7070E mass spectrometer. ^{1}H-NMR (200 MHz),^{13}C-NMR (50.2 MHz), APT, ^{1}H–^{1}H-COSY, and HETCOR spectra were recorded in acetone-d_6 or $CDCl_3$ on a Varian Gemini 200 spectrometer, using TMS as int. standard.

3.2. Separation

Silica gel (Merck); GC was performed using a Varian Star 3400 gas chromatograph, fused silica capillary column (DB-5, 30 m × 0.20 mm), H_2 as carrier gas and temp. programming from 60 to 270°C (3°C/min); GC-MS was performed using a HP5890 SII gas chromatograph coupled to a VG Autospect mass spectrometer at 70 eV, fused silica capillary column (DB-1, 30 m × 0.20 mm), He as carrier gas and temp. programming from 60 to 270°C (3°C/min).

3.3. Plant material

Leaves of *P. lhotzkyanum* were collected near Teresopolis, Rio de Janeiro, Brazil, in September 1996. Voucher samples (RB 323487) are deposited in the Herbarium of Rio de Janeiro Botanical Garden, Rio de Janeiro, Brazil.

3.4. Extraction and isolation

Dried and powdered leaves of *P. lhotzkyanum* (280 g) were extracted successively with hexane and methanol at room temperature. The MeOH extracts were evaporated to dryness under red. pres., then redissolved in H_2O/MeOH (7:3) and extracted sucessively with hexane, CH_2Cl_2, EtOAc and *n*-BuOH. The CH_2Cl_2 fraction (9.33 g) was fractionated on silica gel column eluted with a CH_2Cl_2–MeOH gradient. A fraction eluted with pure dichloromethane afforded a mixture of ferulates (3 mg) which were analysed and identified by GC/MS (Table 2). Compound **2** (10 mg) was eluted from the silica gel column with a mixture of CH_2Cl_2–MeOH 1%, and purified over XAD-2 column eluted with MeOH. Compound **3** (15 mg) and compound **1** (28 mg) were eluted from a silica gel column with a mixture of CH_2Cl_2–MeOH (2.5 and 20%, respectively) and were purified by recrystallization from hexane and EtOAc.

3.4.1. Kaplanin *1*

Yellow amorphous solid (CH_2Cl_2–MeOH 20%). HR-FABMS (positive mode) *m*/*z*: 431.4210 $[M + H]^+$; $C_{22}H_{22}O_9$ + H requires, 431.4206; UV λ_{max}^{MeOH} nm (log ε) MeOH: 213.3 (2.6), 287.2 (1.8), 330.0 (0.3); + $AlCl_3$: 204.8 (1.1), 220.4 (1.1), 281.0 (0.8), 326.6 (0.4), 391.6 (0.2); + NaOMe: 211.4 (3.1), 278.6 (1.8), 364.6 (0.4); ^{1}H-NMR spectral data (200 MHz, $CDCl_3$): δ 3.95 (3H, *s*, 7-OC$\underline{H}_3$), 4.98 (1H, *d*, J = 10 Hz, H″-1; glucosyl), 3.0–4.2 (10H, *m*, H-glucosyl), 6.48 (1H, *s*, H-6), 6.80 (1H, *s*, H-3), 7.6–8.2 (5H, *m*, H-2′-H-6′), 13.2 (1H, *brs*, 5-O$\underline{H}$); ^{13}C-NMR spectral data (50.2 MHz, $CDCl_3$): see Table 1.

3.4.2. Compound *2*

Amorphous solid (CH_2Cl_2–MeOH 1%). UV λ_{max}^{MeOH} nm (log ε) MeOH: 228.4 (0.3), 279.0 (0.1); EIMS *m*/*z* (rel. int.): 224 $[M]^+$ (35), 164 (18), 151 (100), 91 (20), 77 (30) 65 (20); ^{1}H-NMR spectral data (200 MHz, $CDCl_3$): δ 2.60 (2H, *t*, J = 7 Hz, H-1′); 2.90 (2H, *t*, J = 7 Hz, H-2′); 3.68 (3H, *s*, O=C–O–$\underline{C}H_3$); 3.84 (3H, *s*, O–CH_3); 3.86 (3H, *s*, O–CH_3); 6.72 (1H, *d*, J = 2 Hz, H-2); 6.74 (1H, *dd*, J = 2.8 Hz, H-6); 6.8 (1H, *d*, J = 8 Hz, H-5); ^{13}C-NMR spectral data (50.2 MHz, $CDCl_3$): δ 30.43 (C-1′), 35.82 (C-2′), 51.42 (O=C–O–$\underline{C}H_3$), 55.65, 55.75 (2 × OCH_3), 111.19 (C-5), 111.53 (C-2), 119.93 (C-6), 132.99 (C-1), 147.36 (C-4), 148.74 (C-3), 173.21 ($\underline{C}$=O).

3.4.3. Compound *3*

White solid (CH_2Cl_2–MeOH 2.5%). MS, UV and ^{1}H- and ^{13}C-NMR data were identical with published data (Liu et al., 1992).

Table 2
Identified substances in the mixture of C6–C3 derivatives

Substance	RT1[a]	RT2[b]	(%)	MS
4a[c]	35.05	28.40	21.99	210 [M]+ (35), 150 (25), 137 (100), 77 (25), 65 (20)
2[d]	36.77	29.40	60.86	224 [M]+ (35), 164 (20), 151 (100), 91 (20), 77 (25), 65 (18)
4b[e]	38.95	31.78	5.89	238 [M]+ (35), 164 (32), 151 (100), 91 (20), 77 (27), 65 (17)
4c[f]	40.57	33.75	5.09	208 [M]+ (100), 177 (75), 145 (60), 133 (30), 117(31), 105 (28), 89 (55), 77 (56), 51 (77)

[a] RT1 = RT in minutes from GC/MS.
[b] RT2 = RT in minutes from GC.
[c] **4a** = 4-Methoxydihydroferulic acid.
[d] **2** = Methyl 4-methoxyferulate.
[e] **4b** = Ethyl 4-methoxyferulate.
[f] **4c** = Methyl ferulate.

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