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Kaempferol glycosides from *Siparuna apiosyce*

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Abstract

The kaempferol derivative 3, 7-di-*O*-methyl-4′-*O*-β-[α rhamnosyl (1→6)]-glucopyranoside (siparunoside) was isolated from the leaves of *Sparuna apiosyce*. Its structure was established by extensive NMR studies. The alkaloids reticuline and liriodenine were also isolated from the leaves along with the kaempferol derivative tiliroside. Benzylisoquinoline alkaloids were isolated from the wood (liriodenine) and wood bark (liriodenine, laurotetanine, *N*-methyl-laurotetanine, reticuline), together with a mixture of *cis* and *trans*-*N*-feruloyltyramines. 3,7,4′-tri-*O*-methylkaempferol was isolated from all organs.

Keywords: *Siparuna apiosyce* De Candolle; Monimiaceae; Kaempferol-3,7-dimethylether-4′-*O*-β; -[α-rhamnosyl(1→6)]-glucopyranoside; Siparunoside; Kaempferol 3-*O*-β-(6″-*p*-coumaroyl) glucopyranoside; Tiliroside; 3,7,4′-tri-*O*-methylkaempferol

1. Introduction

The genus *Siparuna* belongs to the family Monimiaceae, order Laurales sensu Dahlgren (1980). This family comprises about 34 genera, five of which occur in Brazil, and 440 species widely spread in the Southern Hemisphere, mainly in tropical and subtropical regions of the Americas (Hutchinson, 1967). Recently, *Siparuna* was considered to belong to the family Siparunaceae (Renner et al. 1997). Plants of the family Monimiaceae are used by local people in the treatment of gastrointestinal disorders (*Peumus boldus* and *Siparuna apiosyce*), skin diseases, and for colds, fever, headache and rheumatism (*S. apiosyce*) (Corrêa, 1926, 1978) as well as in the healing of snakebites (Lopez et al., 1990, 1993). They are considered to have tonic, stimulant, digestive and carminative properties (Peckolt and Peckolt, 1920; Corrêa, 1926, 1978; Occhioni and Lyra, 1948). Indians of the Amazonian tribe "Jammadi" use leaves of *Siparuna guianensis,* brewed into tea, for rheumatic pains (Prance, 1972). In Brazil, *S. apiosyce* is a popular medicine, commonly called "Limão Bravo" (wild lemon), due to its strong odour of lemon and to the shape of its fruits (Corrêa, 1926, 1978). The leaves of this species are described in the first Brazilian Pharmacopoeia (Dias da Silva, 1926) and are also included in the Pharmacopoeia of the State of São Paulo (1917) (Stellfeld, 1955) where other species are described. Limão Bravo is sold by many pharmaceutical companies in the form of cough drops and syrup.

There are some references in the literature on the chemistry of some Central American species of *Siparuna*: *S. dresslerana* (Gerard et al. 1986), *S. gilgiana* (Chiu et al., 1982), *S. griseo-flavescens* (Lopez et al., 1993), *S. nicaraguensis, S. pateliformis* (Gerard et al., 1986), *S. pauciflora* (Lopez et al. 1988) and *S. tonduziana* (Lopez et al., 1990), concerning benzylisoquinoline alkaloids. Alkaloids were also found in *S. guianensis* (Bráz-Filho et al., 1976). Terpenoids of the cadinane type are the major constituents from the Ecuadorian species *S. macrotepala* (El-Seedi et al. 1994).

We have reported earlier (Leitão et al., 1999) the isolation of 3,7,4′-tri-*O*-methylkaempferol, **1** (in all organs of *S. apiosyce*) and tiliroside, **2**. In this work we describe the isolation and structural determination of siparunoside, **3**, a new methylated kaempferol glycoside.

2. Results and discussion

The MeOH extract of leaves of *S. apiosyce* was partitioned against hexane, and the hexane-soluble fraction,

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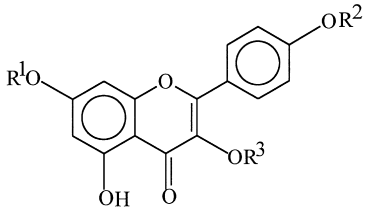

1 $R^1 = R^2 = R^3 = CH_3$

2 $R^1 = R^2 = H$, $R^3 =$ (6¨ - *O* - *p*- coumaroyl) - glucose

3 $R^1 = R^3 = CH_3$, $R^2 =$ – β – [α–rhamnosyl (1→6)] - glucopyranosyl

after purification by column chromatography, afforded sitosterol glucoside (Sakakibara et al., 1983) and 3,7,4′-tri-*O*-methylkaempferol, **1** (Calvert et al., 1979), as a major compound. The $CHCl_3$ fraction gave, after purification, the same flavonol as well as the oxoaporphine liriodenine. The EtOAc fraction was also purified by CC, affording tiliroside, **2** (Nikaido et al., 1987), and a new derivative of kaempferol, siparunoside, **3**, as an amorphous powder.

The ^1H NMR spectrum of **3** exhibited signals for a kaempferol moiety, two methoxyl groups (δ 3.98 and 3.80 ppm) and signals for the anomeric protons of two sugars (δ 5.69, *d*, $J = 7.2$ Hz and δ 5.48, *br s*). The UV spectrum of **3** showed maxima at 314.4, 268.6 and 208.2 nm, a bathochromic shift with $AlCl_3$ (free 5-OH) and no shift with NaOAc (substituted 7-OH). The locations of the methoxyl groups at positions 3 and 7 were determined by DIFFNOE experiments. Irradiation of the signal at δ 3.98 (3-OCH_3) enhanced the signal for H-2′, 6′ (δ 8.24 ppm) and irradiation at δ 3.80 ppm showed NOEs to H-6 (δ 6.62 ppm) and H-8 (δ 6.75 ppm). This methoxyl group was then assigned to position C-7. The ^{13}C chemical shifts for these two methoxyl groups (Table 1) are in accordance with those in the literature for fully aromatic rings A or B of flavonoids (δ 55.9 ppm) and C-3 methoxyl groups (δ 60.0 ppm) (Calvert et al., 1979). Complete and unequivocal ^1H and ^{13}C assignments are reported in Tables 1 and 2, as a result of HETCOR, long range HETCOR and INEPT experiments. These data agree with structure **3**. In particular, the location of glucose at C-4′ was confirmed by selective irradiation at δ 5.69 (H-1″), which showed its long range connectivity with the carbon signal at 160.3 (C-4′) in the INEPT experiments. The coupling constant of 7.2 Hz indicates a β configuration for glucose. Moreover, the C-6″ carbon signal of glucose in the ^{13}C NMR spectrum was shifted downfield (δ 68.0 ppm); thus, it was concluded that the rhamnosyl moiety was attached to C-6 of glucose. Acid catalyzed hydrolysis of 3 [by microanalytical procedure, directly on TLC plates with standards (Kuster, 1994)] showed that glucose and rhamnose were liberated. Therefore, compound **3** was characterized as kaempferol-3, 7-di-O-methyl-4′-*O*-β- [α-rhamnosyl (1→6)]-glucopyranoside, briefly named siparunoside.

Table 1
NMR spectral data for siparunoside from HETCOR; δ ppm (*J*, Hz)

Carbon number	C	H	L.R.
2	155.8	–	8.24
3	139.1	–	3.98
4	179.0	–	–
5	162.2	–	6.62
6	98.3	6.62, *d* (2.1)	6.75
7	165.8	–	3.80
8	92.5	6.75, *d* (2.1)	–
9	157.0	–	6.75
10	106.3	–	6.62
1′	124.2	–	7.54
2′,6′	130.5	8.24, *d* (9)	–
3′,5′	116.9	7.54, *d* (9)	–
4′	160.3	–	–
1″	101.7	5.69, *d* (7.2)	–
2″	78.4	4.41, *m*	–
3″	73.8	4.30, *m*	–
4″	69.8	4.41, *m*	–
5″	72.7	4.76, *d* (10)	-
6″	68.0	4.15, *m*	–
1‴	102.4	5.48, *br s*	–
2‴	72.1		–
3‴	77.3	4.30, *m*	–
4‴	71.5	4.02, *t*	–
5‴	74.6	4.41, *m*	–
6‴	18.4	1.61, *d* (6)	–
3-OCH_3	60.0	3.98, *s*	–
7-OCH_3	55.9	3.80, *s*	–

Table 2
INEPT data for siparunoside

Irradiated resonance	Proton	Connected carbon	
		^3J	^2J
8.24	H-2′, H-6′	C-2, C-4′	–
7.53	H-3′, H-5′	C-1′	C-4′
6.75	H-8	–	C-7, C-9
6.62	H-6	C-10	C-5
5.69	H-1″	C-4′	–

It is interesting to note that all flavonoids isolated from this species thus far show the same substitution pattern namely a 3,5,7,4′- tetraoxygenated framework, therefore showing them to be derivatives of kaempferol. It is also noteworthy that 3,7,4′-tri-*O*-methylkaempferol is present in all organs of *S. apiosyce*.

The BuOH extract from the leaves of *S. apiosyce* afforded, upon purification on Sephadex LH-20, the alkaloid reticuline. Other benzylisoquinoline alkaloids were isolated from the MeOH extract from the wood (liriodenine) and wood bark (laurotetanine, *N*-methyllaurotetanine, reticuline and liriodenione) of *S. apiosyce*. The MeOH extract of the wood of this plant also afforded *trans*-*N*-feruloyltyramine, while a mixture of

both *cis*- and *trans*- forms was isolated from the wood bark. The compounds were identified by their spectral data (Guinaudeau et al. 1975) and by comparison of their retention times (HPLC) and UV spectra with those of authentic samples of *cis*- and *trans*-*N*-feruloyltyramines, isolated from other Monimiaceae sources (*Mollinedia*) (Brito, 1998).

3. Experimental

3.1. Plant material

S. apiosyce De Candolle was collected at Vista Chinesa, Rio de Janeiro, Brazil, in October 1993 and identified by Maria Veronica Leite Pereira (UFRRJ). A voucher specimen is deposited at the Herbarium of FEEMA-Vista Chinesa (RJ).

3.2. Extraction and isolation of the constituents from leaves

Leaves of *S. apiosyce* (390 g) were exhaustively extracted with hexane and methanol consecutively. Evaporation in vacuo gave 15.0 g of the hexane extract and 35.5 g of the MeOH extract. The latter was partitioned between H_2O and hexane, $CHCl_3$, EtOAc and *n*-BuOH, in this order. The hexane-soluble fraction from the MeOH extract (3.9 g) was fractionated by CC over silica gel, eluted with $CHCl_3$ and increasing amounts of MeOH (up to $CHCl_3$/MeOH 10%). A total of 61 frs. (10 ml each) were collected. Frs. 10–19 (198 mg, eluted in $CHCl_3$) were purified by silica gel CC with benzene affording 3,7,4′-tri-*O*-methylkaempferol (41 mg). Frs. 58–60 (84 mg, eluted with $CHCl_3$/MeOH 10%) afforded sitosterol glucoside (60 mg). Part of the $CHCl_3$ fraction (6.0 g) of the MeOH extract was fractionated by silica gel CC eluted with CH_2Cl_2 with increasing amounts of EtOAc (CH_2Cl_2 up to CH_2Cl_2/EtOAc 80%). Frs. 2–19 afforded 3,7,4′-tri-*O*-methylkaempferol (22.8 mg). Frs. 19–22 (CH_2Cl_2/EtOAc 5%) were purified on Amberlite XAD-2 (MeOH, EtOAc) and the MeOH frs. afforded liriodenine (16 mg).

The EtOAc fraction (1.34 g) from the MeOH extract was fractionated by CC over silica gel, eluted with $CHCl_3$ with increasing amounts of MeOH. Frs. 70–74 (73 mg, eluted with $CHCl_3$/MeOH 20%) afforded tiliroside (64 mg). Frs. 75–83 (77 mg, $CHCl_3$/MeOH 20%) contained tiliroside. Frs. 84–90 (80 mg, eluted with MeOH) was purified on Sephadex LH-20 with MeOH as eluent. Frs. 5–6 (23 mg) afforded siparunoside, **3** (23 mg).

The BuOH fraction from the MeOH extract was purified on Amberlite XAD-2 with MeOH as eluent. Frs. 3–4 were purified in Sephadex LH-20 with MeOH, affording the alkaloid reticuline (5.8 mg) in frs. 4–6.

3.3. Extraction and isolation of the compounds from wood and wood bark of S. apiosyce

Wood (2.820 kg) and wood bark (950 g) of *S. apiosyce* were extracted, separately with hexane and MeOH.

Part of the MeOH extract of the wood (4.0 g) was fractionated by silica gel CC eluted with CH_2Cl_2 with increasing amounts of EtOAc and EtOAc with increasing amounts of MeOH, affording 46 frs. Frs. 10–23 (CH_2Cl_2/EtOAc 60%–EtOAc 100%) were purified by silica gel CC eluted with CH_2Cl_2, EtOAc and MeOH. Fraction 1 was purified by flash chromatography over silica gel (CH_2Cl_2/EtOAc/MeOH 9:0,5:0,5) yielding *trans*-*N*-feruloyltyramine in frs. 5–7 (19 mg). Frs. 7–20 (CH_2Cl_2/EtOAc 10%) afforded liriodenine (18 mg).

The MeOH extract (45.3 g) of the wood bark was suspended in MeOH/H_2O 9:1 and extracted with EtOAc and *n*-BuOH. Part of the EtOAc fraction (9.6 g) was purified over silica gel CC, eluted with a solvent gradient from hexane to EtOAc and EtOAc to MeOH with frs. of 150 ml each. Frs. 1–14 (eluted with hexane/EtOAc 50%) showed the presence of 3,7,4′-tri-*O*-methylkaempferol by comparison with samples isolated from the leaves. Frs. 6–14 (880 mg, eluted with hexane/EtOAc 80%) and frs. 15–20 (220 mg, eluted with EtOAc), after several purifications over silica gel CC resulted in the isolation of a mixture of *cis*- and *trans*-*N*-feruloyltyramines (20 mg). Frs. 21–24 (eluted with EtOAc/MeOH 20%) were purified by CC on silica gel, using $CHCl_3$/MeOH 10% up to MeOH 100% as eluents. Final purification of frs. 2–6 on preparative TLC plates (silica gel, $CHCl_3$/MeOH 10%) afforded liriodenine (19 mg). Fraction 25 (eluted with EtOAc/MeOH 30%) was purified on silica gel CC using a gradient of EtOAc and MeOH (from 10% to 50%). This procedure afforded *N*-methylaurotetanine (48.5 mg). Frs. 36–39 (100 mg, eluted with EtOAc/MeOH 50%) were purified on Sephadex LH-20 with MeOH, affording reticuline (4.5 mg). Frs. 43–48 (143 mg, eluted with MeOH) were purified by counter-current chromatography in a HSCCC apparatus with H_2O/EtOAc/BuOH 10:9:1 as solvent system (organic phase as mobile phase), 2 ml/min. Frs. 19–22 afforded laurotetanine (14 mg).

3.4. HPLC analysis of the EtOAc fraction

The EtOAc fraction of the MeOH extract of the wood bark of *S. apiosyce* was analysed by HPLC equipped with a photodiode array detector. The sample (13.6 mg) was diluted in 1 ml MeOH and 20 μl were injected. The elution system was composed of two solvents:A sodium perchlorate 0.2 M: perchloric acid 60%, 1000:0.2) and B acetonitrile. The elution gradient was linear (A:B 9:1) for 45 min and then A:B (1:9), A:B (6:4) and A:B (9:1). Identification of the alkaloids mentioned above and of

the two amides was accomplished by direct comparison of their UV spectra with those of authentic samples.

3.5. Siparunoside, **3** (Kaempferol 3,7-dimethylether-4′-O-β-[α-rhamnosyl (1→6)]-glucoside)

Yellow amorphous powder. $[\alpha]_D^{20}$ −26.0° (MeOH, *c* 0.1); HR-FABMS (positive mode) *m/z*: 623.5851 $[M+H]^+$; $C_{29}H_{34}O_{15}+H$ requires, 623.5900); UV $\lambda_{max.}$ (MeOH): 314.4, 268.6, 208.2; + $AlCl_3$ 391.0, 342.8, 301.20, 277.2, 209.2; + NaOAc 314.0, 268.8, 211.4; ^1H NMR (pyridine-d_5, 300 MHz) and ^{13}C NMR (pyridine-d_5, 75 Mhz) spectral data (see Table 1).

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