Antinociceptive Activity of the Natural Piperidine Alkaloid Hydrochlorides from *Syphocampylus verticellatus*

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In addition to 3′-methoxyluteolin and mixtures of sterols and triterpenes, the leaves of *Syphocampylus Verticellatus* yielded two piperidine alkaloid hydrochlorides, one of them has a novel structure. The alkaloids exhibit antinociceptive activity.

Introduction

Syphocampylus verticillatus is a small strub widely distributed at the border of the rivulets of the Paraná State (Brazil) whose leaves are used in the folk medicine (Correa 1931). In a previous paper (Miguel *et al.*, 1996) we described the X-ray structure determination of the main alkaloid occurring in the plant as hydrochloride. Successively, from the same plant, Biavatti (Biavatti *et al.*, 1998) reported the same alkaloid, isolated as free base, depending from the isolation process involving alkalinization of the extract.

Pharmacological studies have shown (Trentin *et al.*, 1997) that the hydroalcoholic extract of the leaves exhibits dose-related antinociceptive activity in several models of nociception in mice. Owing to the considerable pharmacological interest (Santos *et al.*, 1999) of that extract we have re-examined its composition. This paper describes the isolation of a second novel piperidine alkaloid hydrochloride and other components of leaves, as well as the antinociceptive activity of both alkaloid hydrochlorides.

Material and Methods

Plant material

The leaves of *Syphocampylus verticellatus* (Campanulaceae) were collected in January in São José dos Pinhais near to Curitiba, Paraná. The plant was identified by Gert Hatschbach Director of the Museu Botánico Municipal (Curitiba). Voucher specimens are deposited in the Herbario Municipal (Curitiba) under the cipher 68920.

Isolation and identification

Air dried leaves (10 kg) were powdered and macerated with 95% methanol at room temperature for approximately 14 days. After solvent removal under reduced pressure the extract was then suspended in water and successively partitioned with 500 ml. of each one of the following solvents: hexane, chloroform, ethyl acetate and butanol, respectively.

Part of the dry hexane fraction (5 g) was chromatographed on a silica gel column eluted with hexane-ethyl acetate gradient giving 320 mg of a mixture of stigmasterol, β-sitosterol and campesterol (77%, 20%, and 3%, respectively) and 20 mg of a mixture of α- and β-amyrin (60% and 40%, respectively) that were determined by GC-MS.

Part of the dry ethyl acetate fraction (10 g) was chromatographed on a silica gel column eluted with a gradient of methanol in ethyl acetate yielding β-sitosterol glucoside (24 mg) which was identified on the basis of NMR spectra data in comparison with those of an authentic sample available in our laboratory, and 3′-methoxy-luteolin (17 mg) that exhibits NMR spectra (in C_5D_5N) comparable to those of literature in dmso-d6 (Sakakibara *et al.*, 1976; Wagner *et al.*, 1976). The location of the OMe group was confirmed by a difference NOE experiment.

The dry *n*-butanol soluble portion (51.5 g) of the extract was adsorbed on silica gel washed with ethyl acetate, and then eluted with methanol. After evaporation of the methanol the fraction was chromatographed on silica gel column eluted with a gradient of methanol in ethyl acetate. The fractions eluted with MeOH:EtOAc 1:1 v/v afforded the alkaloid chloride **2** (100 mg) and **1** (3 g) successively.

3′-Methoxy luteolin

^{1}H NMR (300 MHz, C_5D_5N), δ 13.83 (s, OH-5), 7.66 (dd, *J* = 8.3 and 2.0 Hz; H-6), 7.62 (d, *J* = 2.0 Hz; H-2), 7.29 (d, *J* = 8.3 Hz; H-5′), 7.0 (s, H-2), 6.88 (d, *J* = 2.1 Hz; H-6), 6.78 (d, *J* = 2.0 Hz; H-8), 3.83 (s, OMe-3′). Difference NOE experiment: the selective irradiation at δ 3.87 (OMe-3′) enhanced the signal at δ 7.62 (H-2′). ^{13}C NMR (75 MHz, C_5D_5N), δ 182.5 (C-4), 165.7 (C-7), 164.3 (C-2), 162.9 (C-9), 158.3 (C-5), 152.4 (C-4′), 148.7 (C-3′), 122.1 (C-1′), 121.1 (C-6′), 116.7 (C-5′), 110.1 (C-2′), 104.7 (C-10), 104.0 (C-3), 99.8 (C-6), 94.7(C-8), 55.8 (OMe-3′).

N-Methyl-2,6-bis-[2-hydroxy-pentyl)-piperidine hydrochloride, **1**

White crystals, m.p. 75 °C (MeOH). EI-MS, *m/z* (rel. int.): 271 [M$^+$] (6), 184 (100), 98 (74), 96 (32). ^{1}H MNR (300 Mz, $CDCl_3$), δ 4.05–3.87 (m; H-2, H-6, H-8, H-8′), 2.95 (br s; OH), 2.68 (N–Me), 0.90 (t, *J* = 7 Hz; Me-11, Me-11′). ^{13}C NMR (75 MHz, $CDCl_3$), δ 69.4 (C-8, C-8′), 64.1 (C-2, C-6), 40.4 (C-7, C-7′), 37.6 (C-3, C-5), 26.1 (C-9, C-9′), 22.4 (C-4), 18.5 (C-10, C-10′), 14.0 (Me-11, Me-11′).

N-Methyl-2-(2-hydroxybutyl)-6-(2-hydroxypentyl-piperidine), **2**

White crystals, m.p. 55 °C (MeOH). EI-MS, *m/z* (rel. int.): 257 [M$^+$] (11), 184 [M–C_4 chain]$^+$ (57), 170 [M–C_5 chain]$^+$ (100), 98 (96). ^{1}H NMR (300 MHz, $CDCl_3$), δ 4.06–4.00 (m; H-8, H-8′), 3.96–3.86 (m; H-2, H-2′), 2.70 (s, N–Me), 0.93 (t, *J* = 7 Hz; Me-10′), 0.90 (t,*J* = 7 Hz, Me-10). ^{13}C NMR (75 MHz, $CDCl_3$), δ 71.0 (C-8′), 69.4 (C-8), 64.4 (C-2, C-6), 40.6 (C-7, C-7′), 37.9 and 37.4 (C-3, C-5), 31.2 (C-9′), 26.1 (N–Me), 24.5 (C-9), 22.7 (C-4), 18.5 (C-10), 14.0 (Me-11), 9.7 (Me-10′).

The antinociceptive action in the formalin-induced pain

Non-fasted male Swiss mice (25–35 g), housed at 22 ± 2 °C under a 12-h light/12-dark cycle and with access to food and water *ad libitum*, were used throughout the experiments. The experiments reported were carried out in accordance with the current guidelines for the care of laboratory animals and the ethical guidelines for investigations of experimental pain in conscious animals according to Zimmermann (1983).

Experiments were carried out in according with previous described method (Trentin *et al.*, 1997; Santos *et al.*, 1999). Briefly, animals were injected intraplantarly with 20 μl of 2.5% formalin solution (0.92% of formaldehyde), made up in phosphate-buffer solution (concentration: NaCl 137 mM, KCl 2.7 mM and phosphate buffer 10 mM), in the right hindpaw. Mice were treated with alkaloid by intraperitoneally (i.p., 10.2–102.3 μmol/kg), intracerebroventricularly (i.c.v.) or intrathecally (i.t.) (34.1–341.2 nmol/site) as described previously (Santos and Calixto, 1997; Santos *et al.*, 1999), 30, 25 and 15 min before formalin injection, respectively. Control animals received a similar volume of vehicle systemically (i.p., 10 ml/kg) or centrally (i.c.v. or i.t., 5 μl/site). When possible, the ID_{50} values were determined by linear regression from individual experiments using linear regression "GraphPad" software.

The alkaloid given by i.p., i.t. or by i.c.v. routes produced dose-related antinociception when assessed against the both phases of the formalin-induced analgesic response. The calculated mean ID_{50} values and the inhibition (%) for these effects are presented in Table 1.

Result and Discussion

The hexane fraction, obtained from the partition of the crude methanol (95%) extract of the dried leaves, showed the presence of β-sitosterol, stigmasterol, campesterol, α- and β-amirin that were identified by co-injection (HRGC) with authentic specimens.

The ethyl acetate fraction present β-sitosterol glycoside and 3′-methoxy luteolin identified specially by ^{1}H NMR, ^{13}C NMR and difference NOE experiment.

The butanol soluble fraction yielded two alkaloid hydrochlorides to which the structure **1** and **2** (Fig. 1) were attributed, respectively, on the basis of NMR data and for the main component (alkaloid 1) by X-ray diffraction method (Miguel *et al.*, 1996). The assignment of the ^{13}C NMR signals followed by the comparison with the data of literature for piperidine hydrochlorides (Eliel *et al.*, 1980) as well as for free piperidines (Krebs and Ramiarantsoa 1998; Eliel *et al.*, 1980).

In Table 1 are compared the mean ID_{50} values of both alkaloids for the antinociceptive action in the formalin model of pain. The behavior of both alkaloids was similar, however, while the alkaloid **1** gave a higher inhibition than alkaloid **2** on the second phase of the pain, alkaloid **2** seems more potent to inhibit the first phase corresponding to the neurogenic pain. This fact leads to believe that a study of structure and activity correlation should be important to obtain analogs with selective antinociceptive activity.

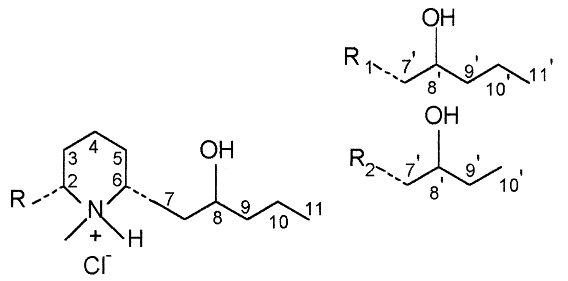

Fig. 1. Structures of alkaloid **1** (R_1) and **2** (R_2).

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Table 1. Comparison of the mean ID_{50} values for the antinociceptive action of alkaloid 1 and alkaloid 2 isolated from *Syphocampylus verticellatus* in the formalin pain model.

		Formalin test			
Compound	Route	Early phase $[ID_{50}]^b$	Inhibition [%]	Late phase $[ID_{50}]^b$	Inhibition [%]
Alkaloid I[a]	i.p. (µmol/kg)	n.d.	36 ± 5	48.3 (39.6– 59.1)	88 ± 6
	i.c.v. (nmol/site)	146.1 (114.9–193.6)	52 ± 3	112.6 (44.2–286.5)	60 ± 3
	i.t. (nmol/site)	33.5 (13.1– 85.6)	67 ± 6	56.2 (25.3–125.7)	74 ± 4
Alkaloid II	i.p. (µmol/kg)	64.1 (50.5– 81.2)	57 ± 4	51.2 (32.4– 80.5)	59 ± 7
	i.c.v. (nmol/site)	111.6 (87.0–143.6)	79 ± 3	69.9 (63.1– 77.8)	79 ± 4
	i.t. (nmol/site)	259.7 (222.5–302.3)	53 ± 5	89.7 (75.4–106.4)	56 ± 4

n.d., not determined. [a] Data from Santos *et al.*, 1999[b]. The ID_{50} values represent the dose of compound that inhibit the pain response by 50% in relation to the control value.

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