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

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**Z. Naturforsch.** 57c, 81–84 (2002); received August 10/October 12, 2001

*Syphocampylus verticellatus*, Alkaloids Hydrochlorides, Antinociceptive Activity

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

**Introduction**

*Syphocampylus verticillatus* is a small shrub 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 Botânico 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, \(\beta\)-sitosterol and campsterol (77%, 20%, and 3%, respectively) and 20 mg of a mixture of \(\alpha\)- and \(\beta\)-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 \(\beta\)-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\(^\prime\)-methoxy-luteolin (17 mg)
that exhibits NMR spectra (in C6D6N) comparable to those of literature in dimso-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

1H NMR (300 MHz, C6D6N), 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′).

13C NMR (75 MHz, C6D6N), δ 182.5 (C-7), 165.7 (C-2), 164.3 (C-3′), 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-9′), 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).

1H NMR (300 Mz, CDCl3), 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′).

13C NMR (75 MHz, CDCl3), δ 102.3 (C-2′), 101.3 (C-7), 95.8 (C-6′), 94.7 (C-8′), 87.3 (C-5′), 71.0 (C-8), 69.4 (C-8), 64.4 (C-2), 64.2 (C-3′), 57.6 (C-3), 52.6 (C-5), 26.1 (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–C4 chain]+ (57), 170 [M–C6 chain]+ (100), 98 (96).

1H NMR (300 MHz, CDCl3), 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). 13C NMR (75 MHz, CDCl3), 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 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 accordance 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.), intracerebroventricularly (i.c.v.) or intrathecaelly (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 ID50 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 ID50 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 1H NMR, 13C 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 13C 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 ID₅₀ 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.

Acknowledgements.

The authors are grateful, for the financial support, to CNPq, PRONEX, FINEP from Brazil.

Table 1. Comparison of the mean ID₅₀ values for the antinociceptive action of alkaloid 1 and alkaloid 2 isolated from Syphocampylus verticillatus in the formalin pain model.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Route</th>
<th>Early phase [ID₅₀]b</th>
<th>Inhibition [%]</th>
<th>Late phase [ID₅₀]b</th>
<th>Inhibition [%]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alkaloid I*</td>
<td>i.p. (µmol/kg)</td>
<td>n.d.</td>
<td>36 ± 5</td>
<td>48.3 (39.6–59.1)</td>
<td>88 ± 6</td>
</tr>
<tr>
<td>Alkaloid II</td>
<td>i.p. (µmol/kg)</td>
<td>146.1 (114.9–193.6)</td>
<td>52 ± 3</td>
<td>112.6 (44.2–286.5)</td>
<td>60 ± 3</td>
</tr>
<tr>
<td></td>
<td>i.c.v. (nmol/site)</td>
<td>33.5 (13.1–85.6)</td>
<td>67 ± 6</td>
<td>56.2 (25.3–125.7)</td>
<td>74 ± 4</td>
</tr>
<tr>
<td></td>
<td>i.t. (nmol/site)</td>
<td>64.1 (50.5–81.2)</td>
<td>57 ± 4</td>
<td>51.2 (32.4–80.5)</td>
<td>59 ± 7</td>
</tr>
<tr>
<td></td>
<td>i.c.v. (nmol/site)</td>
<td>111.6 (87.0–143.6)</td>
<td>79 ± 3</td>
<td>69.9 (63.1–77.8)</td>
<td>79 ± 4</td>
</tr>
<tr>
<td></td>
<td>i.t. (nmol/site)</td>
<td>259.7 (222.5–302.3)</td>
<td>53 ± 5</td>
<td>89.7 (75.4–106.4)</td>
<td>56 ± 4</td>
</tr>
</tbody>
</table>

n.d., not determined. a Data from Santos et al., 1999b. The ID₅₀ values represent the dose of compound that inhibit the pain response by 50% in relation to the control value.


