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Sauroine—a novel *Lycopodium* alkaloid from *Huperzia saururus*

María Gabriela Ortega, Alicia Mariel Agnese and José Luis Cabrera*

Farmacognosia, Departamento de Farmacia, Facultad de Ciencias Químicas, Universidad Nacional de Córdoba, IMBIV (Instituto Multidisciplinario de Biología Vegetal, UNC-CONICET) Ciudad Universitaria, Córdoba 5000, Argentina

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Abstract—A novel *Lycopodium* alkaloid, sauroine (7,8-dihydroxylycopodine, **1**) was isolated from the aerial parts of the medicinal species *Huperzia saururus*. The structure and relative stereochemistry of **1** were elucidated on the basis of its spectral data.
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Species from the *Lycopodium* family have long been studied and many alkaloids have been reported so far.[1–4] As a part of our investigation in the search of bioactive substances from the autochthonous flora of Argentine, we studied *Huperzia saururus* (Lam.) Trevis. (= *Lycopodium saururus* Lam., = *Urostachis saururus* (Lam.) Herter) (Lycopodiaceae). In a previous paper, we demonstrated that the alkaloid extract obtained from the aerial parts of this species exhibited a marked in vitro anticholinesterase activity. In addition, in that opportunity we described the isolation and identification of seven *Lycopodium* alkaloids, 6-hydroxylycopodine, *N*-acetyllycodine, lycopodine, lycodine, *N*-methyllycodine, sauroxine, and clavolonine.[5] During the isolation procedure, investigation showed that there was a significant amount of a probable new alkaloid. In the present work, as a continuation of these studies the isolation of a hitherto unknown *Lycopodium* alkaloid (sauroine, **1**, Fig. 1) and its structure elucidation are reported.

Dried and crushed aerial parts[6] of *H. saururus* (3 kg) were extracted as previously described.[5] The total alkaloid extract (15.75 g) was submitted to gel filtration on Sephadex LH-20 (Farmacia, Sweden) and MeOH was used as mobile phase (1500 mL) to afford four major fractions. The second fraction (2.24 g) was separated by circular chromatography[7] on a mixture of silica gel and Al_2O_3 (70:30, 45 g) and eluted with toluene/$ClCH_3$/EtOH (26.5:41:32.5, 1000 mL). Five main fractions were obtained. The fourth fraction (0.36 g) was subjected to a silica gel column chromatography (14 g) and eluted with EtOH (300 mL). As a result eleven fractions were separated and fraction four was purified by preparative TLC on silica gel GF_{254}, eluted with cyclohexane/diethylamine (1:1, 70 mL) yielding 20 mg of **1** (R_f: 0.57).

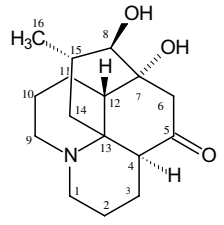

Figure 1. Sauroine-a novel metabolite from *Huperzia saururus*.

The structure of this new natural product was established by mass spectrometry and extensive spectroscopic analysis.

Compound **1**[8] presented a molecular ion at *m/z* 279 (1.2%) consistent with the formula $C_{16}H_{25}NO_3$ deduced from the HREIMS (found: 279.1842; calcd; 279.1835).

The base peak at *m/z* 206 corresponds to the loss of the bridge ring (C-8, C-14, C-15, and C-16) with a hydroxyl group, plus a hydrogen atom from C-12 indicated that **1** possess a lycopodine-type skeleton.[9]

The IR spectrum depicts the presence of a ketone (1700 cm^{-1}) and hydroxyl groups (3487–3365 cm^{-1}).

Keywords: *Lycopodium* alkaloid; 7,8-dihydroxylycopodine; *Huperzia saururus*.
* Corresponding author. Tel.: +54 0351 433 4163; fax: +54 0351 433 4127; e-mail: jcabrera@dqo.fcq.edu.ar

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Table 1. ^{1}H and ^{13}C NMR data for compound **1**

N	C (ppm)[a] DEPT HMQC	H (ppm)[b]	COSY	NOESY	HMBC
1	47.19 (CH_2)	Hα 3.34 (td, 14.1, 3.8)	1β, 2α, 2β	1β, 2α	C-2, C-9
		Hβ 2.61 (dd, 14.5, 5)	1α, 2α	1α, 2α, 2β	C-2, C-9, C-13
2	18.28 (CH_2)	Hα 1.45 (br d, 13.5)	2β, 1α, 1β, 3α, 3β	1α, 1β, 2β, 3α	C-1, C-3, C-4
		Hβ 1.72–1.96 (m)	2α, 1α, 1β, 3α, 3β	1β, 2α, 3α	C-3
3	19.05 (CH_2)	Hα 2–2.11 (m)	3β, 2α, 2β, 4	2α, 2β, 3β, 4	C-1, C-13
		Hβ 1.5–1.63 (m)	3α, 2α, 2β, 4	3α, 14 (*endo*)	C-2, C-4, C-1, C-13
4	41.98 (CH)	2.88 (dd, 11.7, 3)	3α, 3β	3α, 6α, 9α, 11α	C-3, C-5, C-13, C-14
5	209.4 (C)	—	—	—	H-4, H-6α,β
6	44.32 (CH_2)	Hα 2.49 (d, 15.7)	6β	4, 6β, 11α	C-5, C-7, C-4, C-8, C-12
		Hβ 2.81 (dd, 15.7, 1.4)	6α	6α	C-5, C-7, C-8, C-12
7	74.39 (C)	—	—	—	H-6α,β, H-8, H-12, H-11α
8	83.10 (CH)	3.19 (d, 9.5)	15, 14 (*endo*, *exo*)	14 (*exo*), 12, 16	C-7, C-15, C-6, C-12, C-16
9	46.94 (CH_2)	Hα 3.13 (td, 12.4, 3)	9β, 10α, 10β	4, 9β, 10α, 11α	C-10, C-11, C-13, C-1
		Hβ 2.72 (br d, 12.2)	9α, 10α, 10β	9α, 10α, 10β	C-10, C-11, C-13, C-14
10	25 (CH_2)	Hα, Hβ 1.72–1.96 (m)	10α, 10β, 9α, 9β, 11α, 11β	9α, 9β, 10α, 10β, 11α, 11β	C-9, C-11, C-12
11	19.31 (CH_2)	Hα 1.5–1.63 (m)	11β, 10α, 10β, 12	4, 6α, 11β	C-10, C-12, C-9, C-7, C-13
		Hβ 2–2.11 (m)	11α, 10α, 10β, 12	11α, 10α, 10β, 12	C-10, C-12, C-9, C-13
12	48.8 (CH)	1.72–1.96 (m)	11α, 11β	8, 11β, 14 (*exo*)	C-7, C-11, C-13, C-6, C-8
13	59.36 (C)	—	—	—	H-4, H-12, H-14 (*endo*, *exo*), H-1β, H-9α,β, H-11α,β, H-15
14	40.67 (CH_2)	H (*endo*) 2.57 (d, 8.5)	14 (*exo*), 15, 8, 16	3β, 14 (*exo*), 15, 16	C-15, C-13, C-4, C-8, C-12, C-16
		H (*exo*) 1.16–1.25 (m)	14 (*endo*), 15, 8, 16	8, 14 (*endo*), 12, 16	C-15, C-13, C-8, C-16
15	32.04 (CH)	1.16–1.25 (m)	8, 14 (*endo*, *exo*), 16	8, 14 (*endo*), 12, 16	C-8, C-14, C-16, C-13
16	19.22 (CH_3)	1.025 (d, 5.6)	15, 14 (*endo*, *exo*)	8, 14 (*endo*, *exo*), 15	C-15, C-8, C-14, C-13

[a] Solution in $CDCl_3$ referenced to $CHCl_3$ at 77.7.
[b] Solution in $CDCl_3$ referenced to $CHCl_3$ at 7.25.

The analyses of ^{1}H, ^{13}C, and DEPT NMR help us to elucidate its structure. ^{13}C NMR indicated 16 carbon signals and DEPT NMR (Table 1) showed the presence of 13 carbon signals: one methyl, four methines, and eight methylenes, the other three carbon signals correspond to three quaternary carbons. On comparison of the ^{13}C NMR spectrum with lycopodine,[10] the most important differences in our spectrum were the absence of the signals that correspond to one methylene group (8-CH_2) and one methine (7-CH) and the presence of two hydroxyl-bearing carbon signals at $\delta_C = 83.1$ (C-8) and 74.39 (C-7) that correspond at methine and quaternary signals, respectively. Furthermore, in contrast to those of lycopodine, the increases of $\delta_{C\text{-}6}$ and $\delta_{C\text{-}12}$ and decreases of $\delta_{C\text{-}5}$, $\delta_{C\text{-}11}$, and $\delta_{C\text{-}13}$ were consistent with the β-effect and γ-effect from 7-OH, respectively.[4,10] The carbon signals at $\delta_C = 209.4$ and 59.36 correspond to carbonyl (C-5) and quaternary function (C-13), respectively. HMQC experiment established that the carbon at $\delta_C = 74.39$ (C-7) and 59.36 (C-13) have not correlation with any proton.

The structure of **1** was validated by the 2D NMR (^{1}H–^{1}H COSY, HMQC, and HMBC) data (Table 1). The ^{1}H–^{1}H COSY (Fig. 2) revealed the connectivities of C-1 to C-3, C-9 to C-11, and C-8 to C-15, C-14, and C-16. There were no observed connectivities at C-6 with any vicinal proton. The important HMBC correlations (Fig. 2) led us to connect these three partial units. H-1 is connected to C-2, C-9, and C-13; H-4 to C-5, C-3, C-13, and C-14; H-6 to C-5, C-7, C-4, and C-8; H-14 *endo* to C-13, C-4, C-15, C-12, and C-16; H-10 to C-11, C-9, and C-12. The relative stereochemistry of **1** was built by the NOESY spectrum (Fig. 3; Table 1),

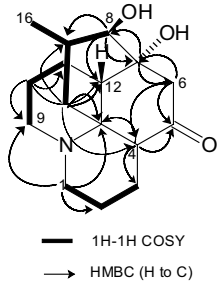

Figure 2. COSY and HMBC important correlations of **1**.

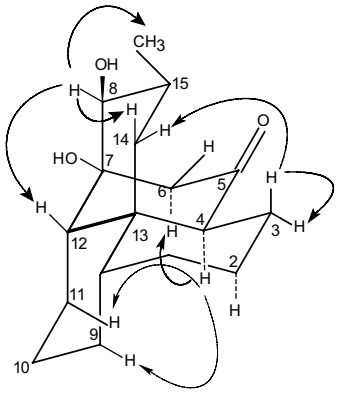

Figure 3. NOESY important correlations of **1**.

which showed the significant NOEs between H-4 with H-6α, H-9α, and H-11α, between H-8 with H-14 *exo*, H-12 and H-16, between H-3β with H-3α and H-14

endo, between H-6β and H-6α. Thus, the relative configuration of **1** was 7α,8-*endo*-dihydroxylycopodine.

In addition, biological activity of **1** was investigated in a similar mode that was done with the alkaloid extract[5] and by using erythrocyte membranes as source of acetylcholinesterase. Until a concentration of 10 μg/mL of **1** the alkaloid was not active in comparison with the 0.58 μg/mL of the extract.

Acknowledgements

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6. Plant material was collected in Pampa de Achala, Dpto. San Alberto, province of Córdoba, in November 1998, and identified by Dr. Gloria Barboza, Instituto Multidisciplinario de Biología Vegetal, Universidad Nacional de Córdoba. A voucher specimen is deposited at CORD as Altamirano No 684.
7. Circular chromatography was carried out on a Chromatotron Harrison Research, model 7924T, series no V46.
8. Compound **1**: $[\alpha]_D^{22}$ +26.2 (*c* 0.083, CH_3OH).
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