A Novel Antiprotozoal Aminosteroid from Saracha punctata


Institut Français de Recherche Scientifique pour le Développement en Coopération (ORSTOM), Unité de Recherche No. 45, 209-213 rue La Fayette 75440 Paris Cedex 10, France, Laboratoire de Pharmacognosie, UPRESA 6013, Faculté de Pharmacie, 51 rue Cognacq-Jay, C1096 Reims Cedex, France, Instituto de Investigaciones Químicas, Universidad Mayor de San Andres, CP 303, La Paz, Bolivia, and Instituto Boliviano de Biología de Altura (IBBA), CP 717, La Paz, Bolivia

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A new aminosteroid, 3β-amino-22,26-epiminocholest-5-ene named sarachine (1), and two known flavonoids, eriodictyol (2) and 7-O-β-D-glucopyranosyl-eriodictyol (3), were isolated from the leaves of Saracha punctata. The alkaloid was found to inhibit the growth of Leishmania braziliensis promastigotes (100% at 25 μM) and of Trypanosoma cruzi epimastigotes in culture (50% at 25 μM) and showed a strong in vitro antiplasmodial activity with an IC50 of 25 nM.

In the course of a screening program for potential antiprotozoal drugs from Bolivian plants, an ethanolic extract from the leaves of Saracha punctata Ruiz et Pavón was screened for its activity against different strains of Leishmania (responsible for leishmaniasis), against Trypanosoma cruzi (the causative factor of Chagas' disease), and against the rodent malaria parasites Plasmodium vinckei and Plasmodium berghei. The genus Saracha belongs to the Solanaceae and consists of three species growing in Bolivia, limited to the humid highland forests named "Yungas", where it is rather common as a shrub.

Until very recently, no previous chemical work had been published on the genus, and we describe the isolation and structure elucidation of a new steroidal aminosteroid (1) and the identification of two known flavonoids (2 and 3). The leishmanicidal and antimalarial properties of the crude ethanolic extract of S. punctata and of the alkaloid 1 will be briefly discussed.

The ethanolic extract from the leaves of S. punctata was partitioned between n-butanol and water. The residue obtained after evaporation of the butanol was extracted with n-butanol to yield, after evaporation, one major compound (1), which we propose the trivial name sarachine. Compounds 2 and 3 were identified as the known compounds eriodictyol45 and 7-O-β-D-glucopyranosyl-eriodictyol by comparison of their spectral data with published values.

The molecular peak of sarachine (1) observed at m/z 398 in the EIMS suggested that the molecule contained two nitrogen atoms, and a molecular formula of C29H44N2O was proposed after examination of the 13C NMR spectrum. The gross features of the 1H and 13C NMR spectra suggested the steroid-like nature of the skeleton. With the mass spectral fragmentation of steroidal amines being well documented, it was possible to deduce that the prominent peak at m/z 98 was the result of a fragmentation between C-20 and C-22 of a 22,26-epiminocholestane and that the ion at m/z 56 belonged to an amino group at C-3. The 1H NMR spectrum of 1 showed two three-proton singlets at 0.70 and 1.00 ppm for the angular methyl groups CH3-18 and CH3-19; two three-proton doublets at 0.83 (J = 7.0 Hz) and 0.94 (J = 7.0 Hz), corresponding to the two secondary methyl groups CH3-27 and CH3-21; and one broad doublet at 3.32 (1H, J = 4.0 Hz) for the olefinic proton. These signals were those expected for a 6a-cholosterylamine. Four protons were observed between 2.2 and 3.1 ppm, attributable to four α-amino protons, which were attached to two methine amino carbons and one methylene amino carbon resonating between 52 and 69 ppm (HMQC). Most 13C NMR signals of 1 were assigned by analysis of HMQC and HMB correlations, except methylenes C-2, C-11, C-15, C-16, and C-23, which were attributed by comparison with literature data.

In the HMBC spectrum, the doublet of CH3-27 was correlated with the methylene C-26 carbon at δ 54.2; this carbon was linked to two coupled protons, one triplet at δ 2.26 (J = 11.5 Hz), and one broad doublet at δ 3.05 in the HMQC spectrum. The value of the 3J coupling constant suggested that H-25 was in the axial position. The chemical shift of C-27 at δ 19.4 confirmed that the terminal methyl was in an equatorial position as in isoteinemine and soflasifoline. The CH3-21 doublet exhibited a long-range H-C correlation with a proton at δ 2.49 (J = 11.0 Hz). The
last α-amino proton, a triplet of triplets (J = 11.0, 4.5 Hz) at δ 9.80 corresponded to the axially oriented hydrogen at C-3. The observation of a ROE effect between CH₃-18 and H-20 was in agreement with the amastigote survival usual macrophage survival.

Detailed analysis of ROE and inter-proton coupling constants in the pipedine ring of 1 allowed the determination of the relative configurations of C-22 and C-25 but not their absolute configurations. The determination of the C-22 and C-25 configurations in 22,26-epiminosteroids has an unpublished X-ray crystallographic study and a partial chain of desacetylmuldamine (or teinemine), nor does it have the configuration of isoteinemine. That of sarachine (1) on various strains of the promastigote alcoholic extract from the leaves of *Leishmania braziliensis* 2903 and at 25 pg/mL with the other strains. The crude extract did not display any activity for the intracellular amastigote form at 10 pg/mL and showed toxicity toward macrophage host cells (no survival). In conclusion, we have at hand an abundant source of a new steroid (1) with interesting preliminary biological activities. Work is in progress to understand its antimalarial mode of action and to secure a determination of the C-22 configuration.

Experimental Section

**General Experimental Procedures.** Optical rotations were determined with a Perkin–Elmer 241 polarimeter. 1H NMR and 13C NMR spectra were recorded with a Bruker AC 300 at 300 and 75 MHz, respectively, in CDCl₃. Two-dimensional NMR experiments were performed using standard Bruker microprograms. EIMS were obtained with an Autospec VG mass spectrometer. Si gel 60 (Geduran, 70-230 mesh, 300 at 300 and 200 at 200) was used for column chromatography. TLC was performed on precoated TLC plates with Si gel 60 (GF, Whatman).

**Parasites.** *Leishmania amazonensis* strain MHOM/BR/84/ CAY H-142 was originally isolated in the French Guiana Institut Pasteur. *Leishmania braziliensis* strain MHOM/BR/ 75/M 2903 was obtained from IBBA, a WHO reference laboratory; identifications were controlled by isoenzyme analysis. *Trypanosoma cruzi* strain Tulubuen was used. The strain was obtained from IBBA, and the identification was confirmed by isoenzyme analysis.

**Plant Material.** Samples from *S. punctata* were collected in the Bolivian "Yungas" at an elevation of 2700 m, in a place named "Sibera", 150 km from Cochabamba on the Santa Cruz road in August 1989. Herbarium specimens were identified and deposited in the U. M. S. A. National Herbarium of La Paz (voucher specimen Moretti 1458).

**Extraction and Isolation.** Dried ground leaves (0.7 kg) were successively extracted with petroleum ether and EtOH.

### Table 1. In Vitro Antileishmanial Activity on Promastigote and Amastigote Forms of *Leishmania* spp. and Trypanosidal Activity on Epimastigote Forms of *Trypanosoma cruzi* in the EtOH Extract from *Saracha punctata* and Sarachine (1)

<table>
<thead>
<tr>
<th></th>
<th>EtOH extract (µg/mL)</th>
<th>Sarachine (1) (µg/mL)</th>
<th>Pentamidine (µg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>100</td>
<td>50</td>
<td>25</td>
</tr>
<tr>
<td><em>T. cruzi</em> (%) inhib.</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td><em>L. braziliensis</em> 2903</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td><em>L. donovani chagasi</em> PFP7</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td><em>L. amazonensis</em> 143</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
</tbody>
</table>

amastigote survival: 0 0 0 0 100

macrophage survival: 0 0 0 0 ND 95

### Table 2. In Vivo Antimalarial Activity of EtOH Extract from *Saracha punctata* and Sarachine (1)

<table>
<thead>
<tr>
<th>Strain</th>
<th>Doses (mg/kg x days)</th>
<th>% Parasitemia on Day 4 (± S.E.M.)</th>
<th>% Suppression of Parasitemia</th>
<th>Mortality on Day 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>P. vinckeii petteri (279 BY)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td></td>
<td>73 ± 7</td>
<td></td>
<td></td>
</tr>
<tr>
<td>EtOH Extract</td>
<td>200 (x = 2 days)</td>
<td>1</td>
<td>96</td>
<td>5</td>
</tr>
<tr>
<td>100 (x = 2 days)</td>
<td>4 ± 2</td>
<td>83</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>50 (x = 4 days)</td>
<td>38 ± 12</td>
<td>48</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td>P. berghei (NK65)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>88 ± 4</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sarachine (1)</td>
<td>32</td>
<td>27 ± 9</td>
<td>69</td>
<td>3</td>
</tr>
<tr>
<td>16</td>
<td>64 ± 8</td>
<td>27</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>74 ± 5</td>
<td>16</td>
<td>0</td>
<td></td>
</tr>
</tbody>
</table>
in a Soxhlet apparatus. Evaporation of EtOH in vacuo gave a gum (220 g) that was dialyzed against pure H₂O and n-BuOH. Evaporation of the organic layer afforded a gum (220 g) that was dialyzed against pure H₂O. After freeze-drying, a part of the residue (54 g) was partitioned between H₂O and n-BuOH. Evaporation of the organic layer yielded 5.4 g of steroidal amine 1 as a greenish powder.

3/6-Amino-22,26-epiminocholest-5-ene (sarachine) (1): [α]D = -21.6° (c 0.5, MeOH); 1H NMR (CDCl₃, 300 MHz) δ 5.92 (1H, br d, J = 4.0 Hz, H-6), 5.05 (1H, m, H-26a), 2.90 (1H, tt, J = 11.0, 4.5 Hz, H-3), 2.49 (1H, dm, J = 11.0 Hz, H-22), 2.28 (1H, t, J = 11.5 Hz, H-26b), 2.15 (1H, dd, J = 13.0, 4.5, 2.0 Hz, H-4a), 2.05 (1H, td, J = 12.0, 2.0 Hz, H-4b), 1.99 (1H, m, H-12a), 1.98 (2H, m, H-7), 1.58 (3H, m, H-18, H-16a, H-34a), 1.61 (3H, h-8), 1.15-1.46 (4H, m, H-15, 16b, H-25), 1.25-1.10 (2H, m, H-23), 1.20 (1H, br d, J = 2.0 Hz, H-16a), 0.94 (1H, m, H-9), 0.83 (3H, d, J = 6.0 Hz, H-24a), 1.68 (1H, m, H-8), 1.65-1.50 (4H, m, H-15, 16b, H-25), 1.25-1.10 (2H, m, H-23), 1.20 (1H, br d, J = 2.0 Hz, H-16a), 0.94 (1H, m, H-9), 0.83 (3H, d, J = 6.0 Hz, H-24a), 1.68 (1H, m, H-8), 1.65-1.50 (4H, m, H-15, 16b, H-25), 1.25-1.10 (2H, m, H-23), 1.20 (1H, br d, J = 2.0 Hz, H-16a), 0.94 (1H, m, H-9), 0.83 (3H, d, J = 6.0 Hz, H-24a), 1.68 (1H, m, H-8), 1.65-1.50 (4H, m, H-15, 16b, H-25), 1.25-1.10 (2H, m, H-23), 1.20 (1H, br d, J = 2.0 Hz, H-16a), 0.94 (1H, m, H-9), 0.83 (3H, d, J = 6.0 Hz, H-24a), 1.68 (1H, m, H-8), 1.65-1.50 (4H, m, H-15, 16b, H-25), 1.25-1.10 (2H, m, H-23), 1.20 (1H, br d, J = 2.0 Hz, H-16a), 0.94 (1H, m, H-9), 0.83 (3H, d, J = 6.0 Hz, H-24a), 1.68 (1H, m, H-8), 1.65-1.50 (4H, m, H-15, 16b, H-25), 1.25-1.10 (2H, m, H-23), 1.20 (1H, br d, J = 2.0 Hz, H-16a), 0.94 (1H, m, H-9), 0.83 (3H, d, J = 6.0 Hz, H-24a), 1.68 (1H, m, H-8), 1.65-1.50 (4H, m, H-15, 16b, H-25), 1.25-1.10 (2H, m, H-23), 1.20 (1H, br d, J = 2.0 Hz, H-16a), 0.94 (1H, m, H-9), 0.83 (3H, d, J = 6.0 Hz, H-24a), 1.68 (1H, m, H-8), 1.65-1.50 (4H, m, H-15, 16b, H-25), 1.25-1.10 (2H, m, H-23), 1.20 (1H, br d, J = 2.0 Hz, H-16a), 0.94 (1H, m, H-9), 0.83 (3H, d, J = 6.0 Hz, H-24a), 1.68 (1H, m, H-8), 1.65-1.50 (4H, m, H-15, 16b, H-25), 1.25-1.10 (2H, m, H-23), 1.20 (1H, br d, J = 2.0 Hz, H-16a), 0.94 (1H, m, H-9), 0.83 (3H, d, J = 6.0 Hz, H-24a), 1.68 (1H, m, H-8), 1.65-1.50 (4H, m, H-15, 16b, H-25), 1.25-1.10 (2H, m, H-23), 1.20 (1H, br d, J = 2.0 Hz, H-16a), 0.94 (1H, m, H-9), 0.83 (3H, d, J = 6.0 Hz, H-24a), 1.68 (1H, m, H-8), 1.65-1.50 (4H, m, H-15, 16b, H-25), 1.25-1.10 (2H, m, H-23), 1.20 (1H, br d, J = 2.0 Hz, H-16a), 0.94 (1H, m, H-9), 0.83 (3H, d, J = 6.0 Hz, H-24a), 1.68 (1H, m, H-8),

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### References and Notes


