ALKALOIDAL CONTENT OF FOUR BERBERIS SPECIES. STRUCTURE OF BERBERILaurine, A NEW BISBENZYL TETRAHYDROISOQUINOLINE

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ABSTRACT.—From Berberis hohiana, Berberis hummeliaefolia, Berberis laurina, and Berberis pacidentata, twenty-one isoquinoline alkaloids were isolated. B. laurina yielded a new compound, berberilaurine [1], a bisbenzyltetrahydroisoquinoline with two diarylether bridges in between C-7 to C-5' and C-11 to C-12'.

Local pharmacopoeiae still reveal sources of new potent drugs for treatment of parasitic diseases in developing countries. In this respect, several Bolivian Indian tribes have been recorded to treat cutaneous leishmaniasis with, among others, Berberidaceous plants. In vitro, crude extracts of Bolivian barberries show leishmanicidal and trypanocidal activities. This prompted us to examine the phytochemical content of these species. The present paper describes the alkaloid content of four species collected on the Bolivian altiplano: Berberis boliviunu Ledl., Berberis hohiana, and Berberis pascendentata Rusby.

RESULTS AND DISCUSSION

From these four species, 21 alkaloids were isolated of which one is new: berberilaurine [1], related to lauberine [2], a bisbenzyltetrahydroisoquinoline alkaloid of sub-group H with two diarylether bridges between C-7 to C-5' and C-11 to C-12'. [The bisbenzyltetrahydroisoquinoline alkaloids classification adopted here is the one introduced by Guinaudeau et al. (2).] Its structure was established from the following evidence.

Berberilaurine [1] is a levorotary labile compound, isolated in a small amount (8 mg). Its uv spectrum is that of a typical bisbenzyltetrahydroisoquinoline (1). A slight bathochromic shift in basic solution indicates the presence of one or more phenolic

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1. A. Fournet, unpublished results.
groups. The $^1$H-nmr spectrum of 1 (Figure 1) is essentially the same as that of 2 (2). It differs by signals near 3.90 ppm, accounting for only two methoxy groups (compared to three in 2), thus suggesting a structure in which a methoxyl of 2 is replaced by an hydroxyl. Assignments of the other signals were made by analogy with 2. The eims spectrum exhibits a molecular ion at $m/z$ 594 (C$_{36}$H$_{38}$O$_5$N$_2$) in agreement with this substitution pattern. The fragmentation of bisbenzyltetrahydroisoquinoline proceeds mainly by double benzylic cleavage of single and double charged molecular ions leading to single and double charged bisisoquinolic ions (3). The single charged bisisoquinolic ion appears at $m/z$ 368 and $m/z$ 367 by loss of H', while the double charged bisisoquinolic ion appears at $m/z$ 184 (base peak). This indicates that the upper half of the molecule bears one methoxyl and two hydroxy groups and, therefore, that the C-12 is substituted by a methoxyl. The position of the methoxyl in the bisisoquinolic moiety was determined by a 2D long-range $^1$H,$^1$H shift-correlated nmr experiment with a delay time of 125 msec. The two coupling cross peaks between methoxyls and aromatic protons in the ortho position are almost superimposed at coordinates 3.91–6.81 ppm and 3.95–6.79 ppm. Thus, the 12-OMe (coupled to H-13 appearing at 6.81 ppm) resonates at 3.91 ppm. The other methoxyl resonating at 3.95 ppm is coupled with the H-8’ (appearing at 6.79 ppm) and, therefore, is borne by the C-7’. Therefore, 1 has the structure of the 6-O-demethylauberine and was given the trivial name of berberrin.

\[ \text{Figure 1. } ^1\text{H-nmr chemical shifts of berberrin [1] at 300 MHz.} \]

The alkaloid composition of the four Berberis species is presented in Table 1. As expected, all species proved to be rich in berberine, above all in the roots. The roots of B. \textit{pandicentata} yielded more than 20 g/kg of berberine. The main tertiary alkaloids of B. \textit{baldiana}, B. \textit{baldianifolia}, and B. \textit{pandicentata} are bisbenzyltetrahydroisoquinolines of subgroups B and C. This composition also was seen in the majority of Berberis species previously analyzed (4). B. \textit{laurina} contains predominantly bisbenzyltetrahydroisoquinolines of subgroups D and H.

Another sample of B. \textit{laurina}, originating from Rio de la Plata, part of its known geographical distribution (5), was previously examined (6–8). There are differences between the alkaloidal compositions of these two specimens. The Rio de la Plata sample showed espinine and espinidine, which may be viewed as precursors of bisbenzyltetrahydroisoquinolines of subgroups D and H. The Bolivian sample contains the new alkaloid berberillaurine [1] but no single bridged bisbenzyltetrahydroisoquinoline. The chemical differences between the two specimens may be due to different edaphic conditions or different physiological stages at collection time, or may be genetically fixed.
Such variations in alkaloid content have already been observed in *Hern andia peltata* (9,10) and in *Alberisia pappana* (11,12). This fact leads one to be cautious about the taxonomic significance of the occurrence of particular compounds. The only relevant observation is that *B. laurina* is so far the only reported *Berberis* species with bisbenzyltetrahydroisoquinolines of subgroups D and H as major tertiary components.

The biological activities of some of the bisbenzyltetrahydroisoquinolines isolated in the course of this work will appear in separate papers (13,14).

**EXPERIMENTAL**

**GENERAL EXPERIMENTAL PROCEDURES.**—Uncorrected mp's were determined in capillary tubes on a Büchi 510 apparatus. Optical rotations were measured with a Schmidt-Haensch Polartronic I polarimeter. Uv spectra were recorded on a Beckman 530 spectrometer. Ir spectra were recorded on a Perkin-Elmer 580 apparatus. ¹H-nmr spectra were recorded on Varian EM 360 A (60 MHz) and Bruker AC 300 TF (300 MHz) spectrometers. Ms spectra were recorded on a Varian MAT 311 instrument.

**PLANT MATERIAL.**—*B. boliviana* roots (1.9 kg) and stems (1.9 kg) were collected in June 1985 along the Collana road, km 27.5, La Paz district, Bolivia, at 3900 m altitude, under the reference AF 718. *B. hemisfola* roots (0.9) were collected in October 1986 in the vicinity of Navao, Tarija district, Bolivia, at 900 m altitude, under the reference AF 718. *B. laurina* roots (0.4) were collected in August 1985 along the Collana road, km 11, La Paz district, Bolivia, at 3900 m altitude, under the reference AF 584. *B. paniculata* roots (0.58 kg) stems (0.8 kg) were collected in April 1986 along the Collana road, km 11, La Paz district, Bolivia, at 3900 m altitude, under the reference AF 624.

Voucher specimens are deposited at the Instituto Boliviana de Biologia de Altituda (I.B.B.A.) herbarium.

**EXTRACTION AND ISOLATION.**—After removing the lipids by petroleum ether, the powder was alkalized and extracted with CHCl₃ in a Soxhlet apparatus. The alkaloidal mixture was further purified by the usual acid-base treatment. Crude alkaloidal extracts yielded as follows: *B. boliviana* roots, 25 g/kg; *B. boliviana* stems, 7.9 g/kg; *B. hemisfola* roots, 4.1 g/kg; *B. laurina* roots, 5.25 g/kg; *B. paniculata* roots, 2.4 g/kg (and 20 g/kg berberine chloride, which precipitated in acidic water during workup); *P. tomentosa* stems, 2.4 g/kg. Alkaloids were then separated by cc. Columns were packed with Merck 60 column Si gel (art. 7734) and eluted with mixtures of C₃H₇/CHCl₃/MeOH of increasing polarity or packed with Merck 60 H tlc Si gel (art. 7736) and eluted with CHCl₃-MeOH-NH₄OH (99–92:1–8:0.1–0.5). Preparative tlc on Merck 60 HF₂₅₄ Si gel (art. 7735) was also performed in solvent systems of CHCl₃-

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**TABLE 1. Alkaloid Composition of the Four Analyzed Berberis Species.**

<table>
<thead>
<tr>
<th>Isolated Alkaloids</th>
<th><em>Berberis boliviana</em> (roots)</th>
<th><em>Berberis boliviana</em> (stems)</th>
<th><em>Berberis hemisfola</em> (roots)</th>
<th><em>Berberis laurina</em> (roots)</th>
<th><em>Berberis paniculata</em> (roots)</th>
<th><em>Berberis paniculata</em> (stems)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(+) N-Methylcoclaurine</td>
<td>M</td>
<td>1</td>
<td>m</td>
<td>M</td>
<td>M</td>
<td>M</td>
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<tr>
<td>Berberine</td>
<td>M</td>
<td>g</td>
<td>M</td>
<td>M</td>
<td>M</td>
<td>M</td>
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<tr>
<td>Palmatine</td>
<td>M</td>
<td>m</td>
<td>m</td>
<td>m</td>
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<tr>
<td>Protopine</td>
<td>m</td>
<td>m</td>
<td>m</td>
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<tr>
<td>(+) Dihydrodihydroxalosine</td>
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<td>m</td>
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<td>Thalifoline</td>
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<td>Notoxyhydrinine</td>
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<tr>
<td>(+) Berberine</td>
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<td>M</td>
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<td>(+) Oxyacanthine</td>
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<td>M</td>
<td>M</td>
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<td>(+) Homosarcaline</td>
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<td>(+) Allocomine</td>
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<tr>
<td>(+) Isotetrandrine</td>
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<td>M</td>
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<td>(+) Belarine</td>
<td>M</td>
<td>M</td>
<td>M</td>
<td>M</td>
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<tr>
<td>(+) Lauberline</td>
<td>M</td>
<td>M</td>
<td>M</td>
<td>M</td>
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<tr>
<td>(+) Lauberline (2)</td>
<td>M</td>
<td>M</td>
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<tr>
<td>(+) Palmitic acid</td>
<td>m</td>
<td>m</td>
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<tr>
<td>(+) Lauberline (1)</td>
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<tr>
<td>(+) Patagonine</td>
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</table>

* M = major alkaloid (more than 10% of the crude alkaloids); m = minor alkaloid (less than 10% of the crude alkaloids). Estimates are based on examination of the crude alkaloid mixtures by tlc.

IDENTIFICATION OF THE ISOLATED COMPOUNDS.—As the data of the known compounds correspond to those extensively published in the literature, they are not repeated. (+)-N-methyl coclaurine (80 mg): uv, eims, nmr data identical with those of Tomita et al. (15). Berberine chloride (20 g) and palmatine chloride (350 mg): uv, nmr, and nmr of the tetrahydro derivatives data identical with those of Kame-
tani (16). Protopine (120 mg): uv, ir, eims, and nmr data identical with those of Gözler and Shamma (18). Thaliloline (60 mg) and noroxyhydrastinine (45 mg): mp, ur, ir, nmr, eims, and nmr data identical with those of Guinaudeau and Shamma (17). (+)-Dihydrolinaresine (350 mg): mp, ur, ir, nmr, eims, and nmr data identical with those of Guha et al. (20) and Schiff (21, 22).

BERBERILLAURINE (1).—Berberilaaurine (8 mg): [α]D negative (c = 0.1, EtOH); uv A max (EtOH) 213, 229 sh, 290; (EtOH/NaOH) 217, 236 sh, 292; eims m/z [M]+ 594 (28) (C₃₉H₃₀O₁₀N₂), 593 (17), 368 (18), 367 (65), 353 (8), 192 (47), 190 (53), 184 (100), 176 (54), 168 (28), 162 (23); ¹H nmr see Figure 1.

LITERATURE CITED