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ALKALOIDAL CONTENT OF FOUR *BERBERIS* SPECIES. STRUCTURE OF BERBERILAURINE, A NEW BISBENZYLTETRAHYDROISOQUINOLINE

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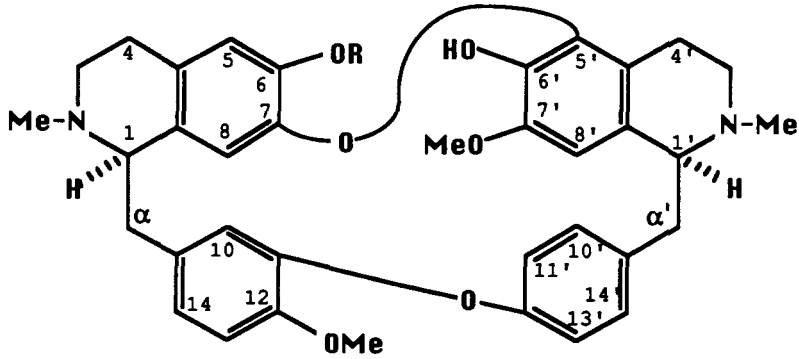
ABSTRACT.—From *Berberis boliviana*, *Berberis bumeliaefolia*, *Berberis laurina*, and *Berberis paucidentata*, twenty-one isoquinoline alkaloids were isolated. *B. laurina* yielded a new compound, berberilaurine [**1**], a bisbenzyltetrahydroisoquinoline with two diarylether bridges in between C-7 to 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.[1] 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 boliviana* Lechl., *Berberis bumeliaefolia* Schneid., *Berberis laurina* Billbg., and *Berberis paucidentata* 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



[1]A. Fournet, unpublished results.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 ($C_{36}H_{38}O_6N_2$) 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$^{\cdot}$, 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 hydroxyl 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*-demethyllauberine and was given the trivial name of berberilaurine.

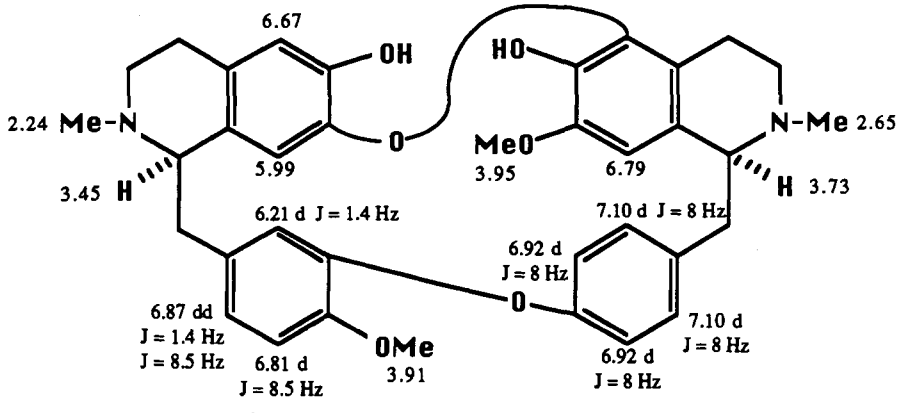


FIGURE 1. ^{1}H-nmr chemical shifts of berberilaurine [**1**] at 300 MHz.

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. paucidentata* yielded more than 20 g/kg of berberine. The main tertiary alkaloids of *B. boliviana, B. bumeliaefolia,* and *B. paucidentata* are bisbenzyltetrahydroisoquinolines of subgroups B and C. This composition also was seen in the majority of *Berberis* species previously analyzed (4). *B. laurina* contains predominantly bisbenzyltetrahydroisoquinolines of subgroups D and H.

Another sample of *B. 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 berberilaurine [**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.

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

Isolated Alkaloids	*Berberis boliviana* (roots)	*Berberis boliviana* (stems)	*Berberis bumeliaefolia* (roots)	*Berberis laurina* (roots)	*Berberis paucidentata* (roots)	*Berberis paucidentata* (stems)
(±)-*N*-Methylcoclaurine		m				
Berberine	M	M	M	M	M	M
Palmarine			M	m		
Protopine			m	m		
(±)-Dihydrolinaresine	m					
Thalifoline	m					
Noroxyhydrastinine		m				
(+)-Berbamunine		m				
(+)-Obaberine	M	M			M	M
(+)-Oxyacanthine	M	M	M		M	M
(+)-Homoaromoline	m			m		
(+)-Aromoline	m	m	m	m		
(+)-Isotetrandrine	M	M	m		M	M
(+)-Berbamine	M	M	M		M	M
(+)-Obamegine	m	m				
(+)-Thalrugosine	m			m		
(−)-Belarine				M		
(−)-*O*-7-Demethylisothalicberine				m		
(−)-Lauberine [**2**]				M		
(−)-Berberilaurine [**1**]				m		
(+)-Patagonine	m					

[a]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.

Such variations in alkaloid content have already been observed in *Hernandia peltata* (9,10) and in *Albertisia papuana* (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. ^{1}H-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 584. *B. bumeliaefolia* roots (0.9) were collected in October 1986 in the vicinity of Navaez, 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 meters altitude, under the reference AF 586. *B. paucidentata* 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 Biologica de Altituda (I.B.B.A.) herbarium.

EXTRACTION AND ISOLATION.—After removing the lipids by petroleum ether, the powder was alkalinized and extracted with $CHCl_3$ 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. bumeliaefolia* roots, 4.1 g/kg; *B. laurina* roots, 5.25 g/kg; *B. paucidentata* roots, 2.4 g/kg (and 20 g/kg berberine chloride, which precipitated in acidic water during workup); *B. paucidentata* 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 C_6H_6/$CHCl_3$/MeOH of increasing polarity or packed with Merck 60 H tlc Si gel (art. 7736) and eluted with $CHCl_3$-MeOH-NH_4OH (99–92:1–8:0.1–0.5). Preparative tlc on Merck 60 HF_{254} Si gel (art. 7735) was also performed in solvent systems of $CHCl_3$-

MeOH-NH_4OH (99–92:1–8:0.1–0.5), $CHCl_3$-MeOH-diethylamine (98–92:1–6:1–2), C_6H_6-EtOH-diethylamine (80:15:5, twice developed) or EtOAc-C_6H_6-MeOH-diethylamine (40:40:15:5, thrice developed).

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, eims, and nmr of the tetrahydro derivatives data identical with those of Kametani (16). Protopine (120 mg): uv, ir, 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 Gözler and Shamma (18). Thalifoline (60 mg) and noroxyhydrastinine (45 mg): uv, ir, eims, and nmr data identical with those of Krane and Shamma (19). (+)-Berbamunine (75 mg), (+)-obaberine (2.5 g), (+)-oxyacanthine (3.4 g), (+)-homoaromoline (57 mg), (+)-aromoline (63 mg), (+)-isotetrandrine (2.3 g), (+)-berbamine (2.8 g), (+)-obamegine (42 mg), (+)-thalrugosine (68 mg), (−)-belarine (75 mg), (−)-*O*-7-demethylisothalicberine (54 mg), and (−)-lauberine (125 mg): $[\alpha]^{25}D$, uv, eims, and nmr data identical with those of Guha *et al.* (20) and Schiff (21,22). (+)-Patagonine (10 mg): $[\alpha]^{25}D$ positive; uv, ir, eims, and nmr data identical with those of Guinaudeau *et al.* (23).

BERBERILAURINE [**1**].—Berberilaurine (8 mg): $[\alpha]^{25}D$ negative ($c = 0.1$, EtOH); uv λ max (EtOH) 213, 229 sh, 290; (EtOH/NaOH) 217, 236 sh, 292; eims *m/z* $[M]^+$ 594 (28) ($C_{36}H_{38}O_6N_2$), 593 (17), 368 (18), 367 (65), 353 (8), 192 (47), 190 (53), 184 (100), 176 (54), 168 (28), 162 (25); ^1H nmr see Figure 1.

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