Journal of Natural Products
Vol. 58, No. 7, pp. 1100-1102, July 1995

A BENZYLISOQUINOLINE ALKALOID FROM *BERBERIS VIRGETORUM*

CHUAN LIU, CHRISTOPHER W.W. BEECHER,*

Program for Collaborative Research in the Pharmaceutical Sciences, Department of Medicinal Chemistry and Pharmacognosy, College of Pharmacy, University of Illinois, Chicago, Illinois 60612

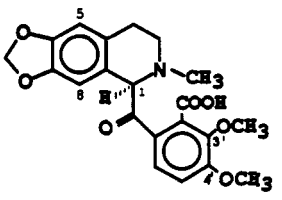
and SHOUXUN ZHAO

Phytochemistry Division, China Pharmaceutical University, Nanjing, JS 210009, People's Republic of China

ABSTRACT.—Column chromatography of an ethanol extract from the whole plant of *Berberis virgetorum* resulted in the isolation of a novel benzylisoquinoline alkaloid, (−)-berbervirine [**1**], together with three known compounds, namely, berberine, jatrorrhizine, and noroxyhydrastinine. The structure of the new compound was determined as (−)-6,7-dimethylenedioxy-3′,4′-dimethoxyl-α-carbonyl-2′-carboxylbenzylisoquinoline by consideration of spectral and cd evidence.

The whole plant of *Berberis virgetorum* Schneid. (Berberidaceae), mainly grown in Jiangsu, Jiangxi, Guangdong, and Guangxi in the People's Republic of China, has been used in the treatment of dysentery, diarrhea, stomatitis, throat infections, and hepatitis in folk medicine (1). Only berberine has been reported as an alkaloid constituent of this plant (1). Further investigation of the whole plant has led to the identification of jatrorrhizine, noroxyhydrastinine, and a novel benzylisoquinoline alkaloid. This communication describes the isolation and characterization of this new benzylisoquinoline alkaloid, (−)-berbervirine [**1**], from this plant.

(−)-Berbervirine [**1**], $C_{21}H_{21}O_7N$, was isolated as light-pink needles by cc of an EtOH extract of the whole plant of *B. virgetorum*, showing a hrms molecular ion at *m/z* 399.4045 (calcd 399.4130), and uv absorptions at 250 (log ε 4.36) and 333 nm (log ε 1.03), which were suggestive of a benzylisoquinoline alkaloid skeleton. This conclusion was further supported by a fragment ion at *m/z* 190 (2), which corresponded to the *N*-methylisoquinolinium cation with one methylene substituent.



1

The ir absorptions at 3200–2400, 1750, and 1700 cm^{-1}, along with ms fragment ions at *m/z* 354 and 218 and ^{13}C-nmr chemical shifts at δ 166.63 and 187.24, suggested that both a carbonyl and a carboxylic acid group were present in the structure. A bathochromic shift in the uv spectrum upon addition of base further supported the probability that a carboxylic acid function was present.

In the ^{1}H- and ^{13}C-nmr spectra, a three-proton resonance at δ 2.50 and a one-carbon resonance at δ 45.36 showed the presence of a $N\text{-}CH_3$ substituent. One two-proton singlet at δ 6.00 and a one-carbon signal at δ 101.60 suggested the presence of a methylenedioxy unit. When compared with the nmr data of some known benzylisoquinoline alkaloids, this methylenedioxy was assigned at the C-6–C-7 position of ring A, which agrees with the probable biogenesis (3,4).

Two three-proton singlets at δ 3.90 and 3.95 and two carbon signals at δ 56.43 and 61.63 provided evidence that there are two methoxyl groups in ring C. Based on a comparison with known compounds, the proton signal at δ 3.90 was assigned as OMe-4′ and at δ 3.95 as

OMe-3′ (5). The aromatic protons displayed chemical shifts at δ 6.70 (1H, s), 7.25 (1H, s), 7.30 (1H, d, J=8.0 Hz), and 7.50 (1H, d, J=8.0 Hz). The chemical shift at δ 10.32 (1H, s, disappeared in D_2O) further indicated that there was a carboxylic acid group in this structure.

A nOe nmr experiment was performed to define the substitution on ring C and to assign the chemical shifts of the methoxyl group. The OMe-4′ singlet at δ 3.90 and the lowfield H-5′ singlet at δ 7.50 displayed reversible nOes (8% and 5%). Irradiation of OMe-3′ at δ 3.95 did not enhance any signals. Due to the deshielding by the carboxylic acid group at C-2′ of ring C, the C-3′ methoxy group had higher chemical shifts (δ_H 3.95; δ_C 61.63) than the C-4′ methoxy group (δ_H 3.90; δ_C 56.43). Morever, the cd spectrum of **1** showed prominent maxima at 221 and 290 nm and was generally similar to analogous data of pseudolaudanine, which is of known absolute configuration (6). Thus, it follows that alkaloid **1** has the C-1 *R* configuration and that the structure is (−)-6,7-dimethylenedioxy-3′,4′-dimethoxyl-α-carbonyl-2′-carboxylbenzylisoquinoline. This compound has been given the trivial name berbervirine.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Mps were obtained on a PHMK 79/2212 micro-plate melting point meter and are uncorrected. Nmr spectra (FT300Q instrument) were recorded at 300 MHz for proton and 90 MHz for carbon resonances. Ir spectra were obtained on a Nicolet IR-400 instrument (KBr disks). Eims were obtained on a Nicolet FTMS 2000 instrument at 70 eV.

PLANT MATERIAL.—The plant *Berberis virgetorum* was identified by Associate Professor Sun-Liang Gong of the Department of Pharmacognosy of China Pharmaceutical University. A voucher specimen was deposited in the Phytochemistry Division, China Pharmaceutical University, Nanjing, People's Republic of China.

EXTRACTION AND ISOLATION.—Air-dried and ground whole plant material of *B. virgetorum* (3 kg) was extracted with EtOH. Concentration of the extract *in vacuo* afforded a residue (40 g). The concentrated extract was then mixed with silicaceous earth containing 2% Na_2CO_3, and chromatographed on the column with 2% Na_2CO_3. The column was washed with Et_2O, $CHCl_3$, and MeOH, respectively. Noroxyhydrastinine was identified in the fraction obtained from $CHCl_3$-MeOH (50:1). Compound **1** was eluted from the column with $CHCl_3$-MeOH (100:3). Berberine and jatrorrhizine were crystallized from MeOH. Berberine, jatrorrihizine, and noroxyhydrastinine were all identified by their spectral data (ir, uv, ms), tlc and mixed-melting points as well as by comparison with authentic samples (7).

(−)-Berbervirine [**1**].—A crystalline isolate [**1**] (6 mg) obtained from $CHCl_3/CH_3OH$, light-pink, easily soluble in warm MeOH or $CHCl_3$; mp 202–204°; $[\alpha]_D$ −200° (c=1.625, $CHCl_3$); uv (MeOH) λ max (log ε) 230 (4.21), 250 (4.36), 295 (1.40), 333 (1.03) nm; (MeOH+OH^-) λ max (log ε) 245 (4.50), 276 (sh, 3.32), 297 (1.50), 334 (1.10) nm; ir (KBr) ν max 3200–2400 (OH), 1750 (C=O, carboxyl), 1700 (C=O, carbonyl) cm^{-1}; ^{1}H nmr ($CDCl_3$, 300 MHz) δ 10.32 (1H, br s, disappeared in D_2O, -COOH), 7.50 (1H, d, J=8.0 Hz, H-5′), 7.30 (1H, d, J=8.0 Hz, H-6′), 7.25 (1H, s, H-8), 6.70 (1H, s, H-5), 6.00 (2H, s, -OCH_2O-), 3.95 (3H, s, OMe-3′), 3.90 (3H, s, OMe-4′), 3.78 (1H, s, H-8), 2.50 (3H, s, N-Me); ^{13}C nmr (DMSO-d_6, 300 MHz) δ 187.24 (s, C-α), 166.63 (s, COO-2′), 157.02 (s, C-4′), 149.77 (s, C-3′), 146.00 (s, C-6), 145.54 (s, C-7), 136.22 (s, C-8a), 123.69 (s, C-4a), 123.31 (s, C-1′), 121.64 (s, C-2′), 119.18 (d, C-6′), 116.61 (d, C-5′), 110.75 (d, C-5), 109.84 (d, C-8), 101.60 (t, OCO-6, 7), 61.63 (q, OC-3′), 61.53 (d, C-1), 56.43 (q, OC-4′), 50.46 (t, C-3), 45.36 (q, N-C), 29.02 (t, C-4); eims (70 eV) m/z $[M]^+$ 399 (8), 354 (12), 218 (45), 190 (100); hreims m/z 399.4045, calcd for $C_{21}H_{21}O_7N$, 399.4130. Relevant nmr nOes are OMe-4′ to H-5′ (8%) and H-5′ to OMe-4′ (5%); cd (MeOH) Δε (nm) (c=0.0017 g/ml) +0.1 (327), +1.2 (290), −3.01 (282), −2.24 (268.5), +1.43 (221), −27.6 (207); tlc ($CHCl_3$-Me_2CO, 6:1) R_f 0.61.

ACKNOWLEDGMENTS

The Instrumentation Center of China Pharmaceutical University provided facilities for the measurement of uv, ir, ^{1}H-nmr, and ms spectra. Dr. Lining Cai and Dr. Kyaw Zaw, Department of Medicinal Chemistry and Pharmacognosy, College of Pharmacy, University of Illinois at Chicago, provided assistance in the measurement of ^{1}H- and ^{13}C-nmr data, and Prof. Cunyi Sheng, Shanghai Institute of Drug Materials, determined the cd spectrum.

LITERATURE CITED

1. Nanjing College of Pharmacy, "Chinese Traditional Medicine Part II," People's Health Press, Beijing, 1976, p. 272.

2. M. Shamma, "The Isoquinoline Alkaloids." Academic Press, New York, 1972, p. 80.
3. E. McDonald, in: "Heterocyclic Compounds—Isoquinoline, Vol. 38. Part One." Ed. by G. Grethe, John Wiley and Sons, New York, 1981, pp. 285 and 297.
4. R.H.F. Manske, in: "The Alkaloids, Vol. IV." Ed. by R.H.F. Manske and H.L. Holmes, Academic Press, New York, 1954, p. 1.
5. G. Blasko, D.J. Gula, and M. Shamma, *J. Nat. Prod.*, **45**, 105 (1982).
6. B. Gözler, B. Kivcak, T. Gözler, and M. Shamma, *J. Nat. Prod.*, **53**, 666 (1990).
7. C. Liu and S.-X. Zhao, *J. China Pharm. Univ.*, **20**, 261 (1989).

Received 3 January 1995