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CYCLOPEPTIDE ALKALOIDS FROM *ZIZYPHUS NUMMULARIA*

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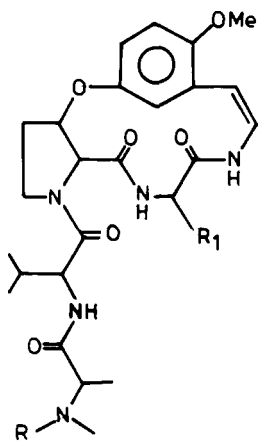
Zizyphus nummularia Wight and Arn (Rhamnaceae) is a thorny, small bush native to the Mirzapur District of India, where it grows in dry and arid regions (1). The fruit is used in bilious attacks and is considered to be cool and astringent. The leaves are used in scabies and other skin diseases. Smoke from the dried leaves is inhaled for the treatment of colds and coughs. A decoction of the bark is used as a hip bath for joint pains and as a gargle for sore throat and bleeding gums (2). As a part of our extended work on the alkaloids of the plants belonging to the Rhamnaceae we have examined the stem bark of *Z. nummularia*. About a dozen cyclopeptide alkaloids have so far been reported from the root bark of *Z. nummularia* (3-5) and the alkaloids nummularine-M, nummularine-N (6), and nummularine-O (7) from the stem bark of this plant. Here we report the isolation and characterization of nummularine-P [**1**], a new cyclopeptide alkaloid, along with a known peptide alkaloid, mauritine-D (8), from the stem bark of *Z. nummularia*. The structure of the new compound is related to sativanine-C [**3**] (9).

The ir spectrum of **1** displayed diagnostic peaks for the secondary amido group of the peptide linkage and absorptions attributed to styryl >C=C<,



		R	R_1
1	(nummularine-P)	H	$(CH_3)_2CHCH_2$-
2	(*N*-formyl nummularine-P)	H-C- (=O)	$(CH_3)_2CHCH_2$-
3	(sativanine-C)	H	$CH_3CH_2CH(CH_3)$-

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$-OCH_3$, -NMe, and -NH groups. The uv spectrum showed absorption maxima at 258 and 320 nm characteristic of a styrylamine chromophore in the 13-membered cyclopeptide alkaloids (10). The ^{1}H-nmr spectrum confirmed the presence of an N-CH_3 group, an OMe group, three D_2O exchangeable -NH signals, and a *cis* styrylamine moiety. The *cis* olefin proton adjacent to the aromatic ring of the 13-membered ring system appeared as a doublet at δ 5.90 (J=8.5 Hz). Four CH_3 groups centered at δ 0.65 as double doublets (J=5 Hz), and two methine protons as multiplets at δ 1.70 are assigned to valine and leucine moieties. A doublet for three protons at δ 1.34 (J=7 Hz) is assignable for a CH_3 of alanine. A signal at δ 5.60 as doublet of triplet is due to hydrogen attached to the carbon adjacent to the ether oxygen of the proline unit. The chemical shifts of three methylenes in the region of δ 2.60-3.65 appeared relatively downfield because they are linked to electronegative atoms. A seven proton complex pattern in the region δ 6.79-8.61 is assigned to Ar-*H*, >N-H, and one *cis* olefinic hydrogen of the styryl double bond, and five protons in the region δ 4.00-4.80 are due to one >N-H and four methine protons flanked by carbonyl and nitrogen functions. The presence of leucine, valine, and *N*-monomethyl alanine was confirmed by paper chromatography of the hydrolysate of **1** and comparison with authentic samples.

The molecular formula of **1** was determined by hrms as $C_{29}H_{43}N_5O_6$. The mass spectrum of **1** closely follows the fragmentation pattern of sativanine-C [**3**] (9) showing their gross structural similarity. The identity of each fragment ion was substantiated by hrms. The base peak at *m/z* 58 shows the amine fragment ion $C_3H_8N^+$ which could be due to *N,N*-dimethylglycine or *N*-monomethylalanine. Formation of the *N*-formyl derivative [**2**] (11) of **1** confirmed the terminal amino acid as *N*-monomethylalanine.

These findings established the structure **1** for nummularine-P which differs from that of sativanine-C [**3**] in having a leucine unit instead of isoleucine as an amino acid bound to the nitrogen of the styrylamine function. Nummularine-P is, therefore, an isomer of sativanine-C.

The second alkaloid, mauritine-D, was identified by spectral analysis, hydrolysis experiment, and direct comparison with authentic sample (co-tlc and superimposable ir).

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Melting points were measured with a Toshniwal melting point apparatus and are uncorrected. Ir and uv were recorded on Perkin-Elmer 221 and Cary 14 spectrophotometers respectively. ^{1}H nmr were obtained on a Bruker-HX 90 MHz spectrometer. Mass spectral analyses were performed on Kratos MS-30 and MS-50 mass spectrometers operating at 70 eV with evaporation of the sample in the ion source at about 200°. Tlc was carried out on silica gel G (BDH), and paper chromatography was performed on Whatman No. 1 paper.

PLANT MATERIAL.—The plant material was procured from Mirzapur district, UP, India, and its authenticity was verified by Prof. S.K. Roy, Department of Botany, B.H.U., Varanasi. A voucher specimen is kept in the Department of Medicinal Chemistry, I.M.S., B.H.U.

EXTRACTION AND ISOLATION.—Stem bark of *Z. nummularia* (5 kg) was repeatedly extracted with a mixture of C_6H_6-NH_4OH-MeOH (100:1:1). The bases were separated from the combined C_6H_6 extracts by shaking with 5% aqueous citric acid. The aqueous layer was basified with NH_4OH and extracted with $CHCl_3$, and the crude alkaloids (3.8 g) were obtained as a brown semi solid. It was chromatographed on a silica gel (135 g) column, eluting with solvents of increasing polarity, and all the collected fractions were monitored by tlc.

MAURITINE-D.—$CHCl_3$-MeOH (50:1) eluates on repeated crystallization from MeOH furnished an amorphous powder (10 mg); $[\alpha]^{20}D$ −256° (*c* 0.1, $CHCl_3$). It exhibited in its uv λ max (MeOH) strong end absorption with shoulders at 250 and 280 nm; ir ν max ($CHCl_3$) 3400 (-NH), 3010-2870 (CH), 2790 (-NCH_3), 1690 (>NHCO), 1630 (C=C), 1600 and 1500

(aromatic), 1214 and 1025 (phenol ether); ms *m/z* 597.80 [M^+], 540.3236, 484, 482, 441, 371, 370, 344, 343, 342, 274, 255, 235, 229, 227, 209, 203, 186, 181, 135, 114 (base peak), 96, 86, 68. Hydrolysis with 6N HCl indicated the presence of isoleucine, leucine, and *N,N*-dimethylisoleucine in the hydrolysate by comparison with authentic samples by paper chromatography.

NUMMULARINE-P [**1**].—$CHCl_3$-MeOH (10:1) eluates showed the presence of a mixture of two alkaloids. Repeated preparative tlc and crystallization furnished mauritine-D (3 mg) and colorless crystals of nummularine-P (18 mg), mp 143-144°; ir ν max ($CHCl_3$) 3382 (-NH), 2831 (-OCH_3), 2775 (-NCH_3), 1688 and 1640 (NHCO), 1610 (C=C), 1230 and 1035 (phenol ether); uv λ max (MeOH) 258 (log ϵ 3.99), 320 (log ϵ 3.90); 90 MHz ^{1}H nmr ($CDCl_3$, δ) 0.65 [*dd*, *J*=5.0 Hz, 2×H-C-(CH_3)$_2$], 1.34 (*d*, *J*=7 Hz, >C(H)CH_3), 1.70 [*m*, 2×$_H$>C(CH_3)$_2$], 2.47 (*s*, N-CH_3), 2.60-3.65 (complex pattern, 3×-CH_2), 3.77 (*s*, Ar-OCH_3), 4.00-4.80 (complex pattern, four methine *H* flanked between carbonyl and nitrogen function and one *NH*), 5.60 (*dt*, H_2C-C(O-Ar)-H(C-H)), 5.90 (*d*, *J*=8.5 Hz, *cis* olefinic *H*), 6.79-8.61 (complex pattern, 3×Ar-*H*, 3×N*H*, and one *cis* olefinic *H*); mol wt (ms) 557.3200, Calcd. for $C_{29}H_{43}N_5O_6$: 557.3213.

N-FORMYLNUMMULARINE-P [**2**].—*N*-formyl derivative **2** was prepared from **1** when treated with HCOOH/Ac_2O and kept overnight at room temperature. The solvent was evaporated, and the product was purified by preparative tlc in C_6H_6-Me_2CO (3:1), mp 168-169°; mol wt (ms) 585.3177, Calcd. for $C_{30}H_{43}N_5O_7$: 585.3163; ms *m/z* 585 (M^+, $C_{30}H_{43}N_5O_7$), 114, 86, 58.

HYDROLYSIS.—The compound **1** (4.5 mg) was hydrolyzed with 6N HCl in a sealed tube by heating at 120° for 10 h. Excess reagent was removed in vacuo and the residue taken up in H_2O for pc on Whatman No. 1. The amino acids were identified as leucine, valine, and *N*-monomethylalanine by comparison with authentic specimens using *n*-BuOH-HOAc-H_2O (4:1:5) as solvent system and ninhydrin as developing reagent.

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