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NUMMULARINE-O, A CYCLOPEPTIDE ALKALOID FROM *ZIZYPHUS NUMMULARIA*

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Key Word Index—*Zizyphus nummularia*; Rhamnaceae; peptide alkaloids; jubanine-B and nummularine-O.

Abstract—In addition to the known peptide alkaloid jubanine-B, a new peptide alkaloid, nummularine-O, has been isolated from the stem bark of *Zizyphus nummularia* and their structures have been elucidated by chemical and spectroscopic methods.

INTRODUCTION

A number of cyclopeptide alkaloids have been reported from the root bark [1–3] and stem bark [4] of *Zizyphus nummularia* (family Rhamnaceae). We report here the isolation and characterization of a new cyclopeptide alkaloid, nummularine-O, together with a known peptide alkaloid, jubanine-B [5].

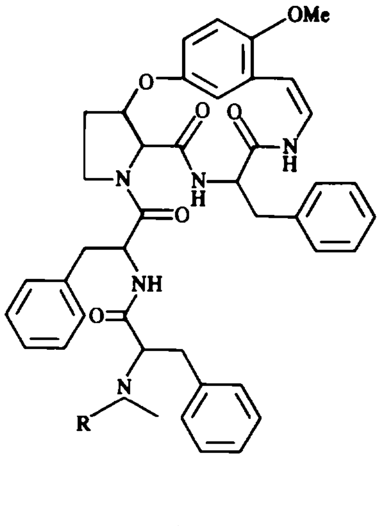
RESULTS AND DISCUSSION

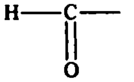
Column chromatography of the alkaloid fraction on silica gel followed by repeated preparative TLC gave small amounts of nummularine-O and jubanine-B. The molecular formula of nummularine-O was determined by high-resolution mass spectrometry as $C_{42}H_{45}N_5O_6$. The IR spectrum exhibited bands for -NH, C-H valence stretching, -OMe, -NMe, amide, $>C{=}C<$ and phenol ether. The UV spectrum showed absorption maxima at 265 and 320 nm, characteristic of 13-membered cyclopeptide alkaloids [6]. On acid hydrolysis it gave phenylalanine and *N*-monomethyl phenylalanine. Because of the small amount of compound isolated a good and interpretable ^{1}H NMR spectrum could not be measured. Nevertheless, singlets for N–CH_3 and O–CH_3 at δ2.4 and 3.67, respectively, could be clearly recognized.

The mass spectrum of **1** closely resembled that of jubanine-B (**3**). It is a homologue of jubanine-B, the main difference in the fragment ions being that the $[M]^+$ and all fragments carrying the end amino acid were 14 mu lower while the rest of the fragments were the same as those in the mass spectrum of jubanine-B. The base peak at *m/z* 134 indicates the amine fragment ion $[C_9H_{12}N]^+$ which could be due to *N*-monomethyl phenylalanine. The formation of *N*-formylnummularine-O (**2**) from **1** confirmed the presence of *N*-monomethyl phenylalanine as the end amino acid. The elementary composition of all fragment ions was substantiated by high-resolution mass measurements. These data suggested that nummularine-O possesses structure **1**, which differs from jubanine-B only in having *N*-monomethyl phenylalanine instead of *N,N*-dimethyl phenylalanine as the end amino acid.

The second alkaloid, jubanine-B, mp 97–100°, was identified by spectral analysis, hydrolysis and direct comparison with an authentic specimen (mmp, co-TLC and superimposable IR).

This is the first report of the presence of the new peptide alkaloid nummularine-O and the known peptide alkaloid jubanine-B in the stem bark of *Z. nummularia*.



	R
Nummularine - O (**1**)	H
N- Formyl nummularine - O (**2**)	 $\mathrm{H{-}C({=}O){-}}$
Jubanine - B (**3**)	Me

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EXPERIMENTAL

Stem bark of *Z. nummularia* (5 kg) collected from the Mirzapur district, U.P., India was repeatedly extracted with a mixture of C_6H_6–NH_4OH–MeOH (100:1:1) and the crude alkaloids (3.8 g) were obtained in the usual manner [7]. The crude alkaloids were chromatographed over silica gel (135 g), eluting with increasingly polar $CHCl_3$–MeOH mixtures. The homogeneity of the collected fractions was analysed by TLC. The eluants from $CHCl_3$–MeOH (5:1) indicated it to be a mixture of three main components. The alkaloids jubanine-B and nummularine-O were separated in a pure state by repeated prep. TLC using $CHCl_3$–Me_2CO–MeOH (1:1:1.5) and C_6H_6–EtOAc–MeOH (3:1:0.4).

Nummularine-O. Crystallized from MeOH as a colourless powder (9 mg), mp 159–161°; $[\alpha]_D^{20}$ $-239°$ (*c* 0.2; MeOH) and showed UV λ_{max}^{MeOH} nm (log ε): 270 (3.54), 318 (3.36); IR ν_{max}^{KBr} cm^{-1}: 3360 (–NH), 3000–2900 (C–H valence streching), 2835 (–OMe), 2735 (–NMe), 1670 and 1635 (sec. amide), 1618 ($>C{=}C<$), 1590 and 1505 (aromatic), 1220 and 1025 (phenol ether); ^{1}H NMR ($CDCl_3$): δ2.40 (*s*, –NMe), 3.67 (*s*, –OMe); MS *m/z*: 715.00 $[M]^+$, 624, 582, 580, 539, 435, 434, 408, 407, 406, 378, 338, 309, 281, 269, 259, 243, 233, 216, 165, 134 (base peak), 120, 96, 68. The alkaloid (4 mg) was hydrolysed with 6 N HCl (10 hr) in a sealed tube and the hydrolysate examined by PC (*n*-BuOH–HOAc–H_2O, 4:1:5). Phenylalanine and *N*-monomethyl phenylalanine were identified by comparison with authentic samples.

N-*Formylnummularine-O*. *N*-Formyl derivative was prepared from **1** by treatment with HCO_2H–Ac_2O overnight [8]. The solvent was evaporated and the product purified by prep. TLC, which gave colourless crystals of *N*-formylnummularine-O (**2**), mp 149°; MS *m/z*: 743.3127 $[M]^+$, 162, 134, 106.

Jubanine-B. Crystallized from MeOH as an amorphous powder (7.5 mg), mp 97–100°, $[\alpha]_D^{20}$ $-215°$ (*c* 0.28; MeOH) and showed UV λ_{max}^{MeOH} nm (log ε): 270 (3.54), 318 (3.36); IR ν_{max}^{KBr} cm^{-1}: 3355 (–NH), 2860 (–OMe), 2780 (–NMe), 1680 and 1635 (sec. amide), 1610 ($>C{=}C<$), 1200 and 1020 (phenol ether); MS *m/z*: 729.3520 $[M]^+$, 638, 582, 580, 539, 435, 434, 408, 407, 406, 392, 323, 295, 269, 259, 243, 216, 165, 148 (base peak), 120, 96, 68. On acid hydrolysis with 6 N HCl, it gave *N,N*-dimethyl phenylalanine and phenylalanine, which were identified by PC comparison with authentic samples.

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