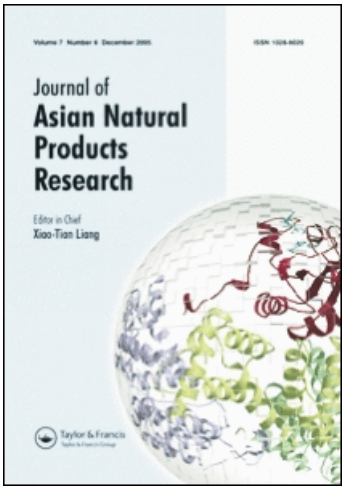

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Peptide alkaloids from *Zizyphus sativa* bark

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Two new cyclopeptide alkaloids, sativanine-N and sativanine-O were isolated from the stem bark of *Zizyphus sativa* and their structures were established by spectral analysis.

Keywords: *Zizyphus sativa*; Rhamnaceae; Cyclopeptide alkaloids; Sativanine-N; Sativanine-O

1. Introduction

In continuation of our work on *Zizyphus sativa* [1], we report here the isolation of two new cyclopeptide alkaloids designated sativanine-N and sativanine-O, from the MeOH fraction of the stem bark of the plant.

2. Results and discussion

Chromatographic methods of the crude base fraction of the methanolic extract of the stem bark of *Zizyphus sativa* followed by preparative TLC furnished the sativanine-N (**1**) and sativanine-O (**2**) compounds.

Sativanine-N (**1**), $C_{26}H_{38}N_4O_5$ (M^+, 486.2842) and sativanine-O (**2**), $C_{32}H_{34}N_4O_5$ (M^+, 554.2530) gave Dragendorff reaction for alkaloids [1]. The IR spectra of **1** and **2** were typical for peptide alkaloids and showed strong bands characteristic of secondary amide, styryl double bond, arylether and aromatic methoxyl groups [2]. Their UV spectrum exhibited absorption bands for 2,5-dialkoxystyrylamine chromophore in the 13-membered ring containing cyclopeptide alkaloids [3].

The structure of the majority of the peptide alkaloids can largely be determined by their high-resolution mass spectra [4]. In view of this fact, the HRMS analysis of **1** and **2** were applied to elucidate their structures.

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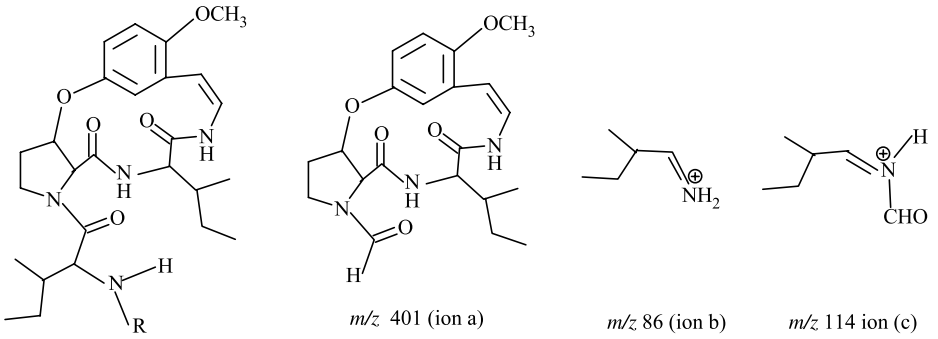
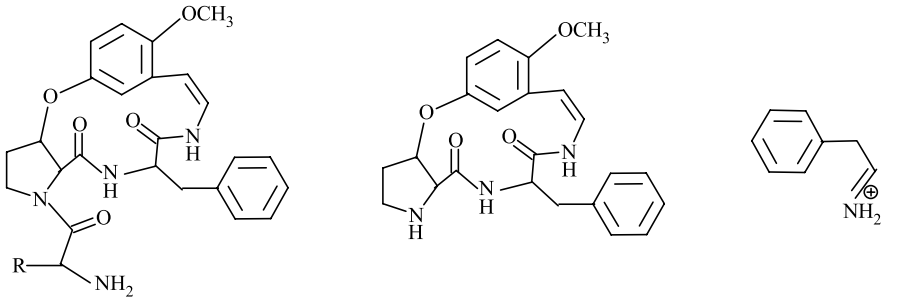
The mass spectroscopic fragmentation pattern of alkaloid **1** closely resembled that of sativanine-K (**3**) [5], having difference only in the end amino acids. The α-cleavage products of **1** gave ion peaks at *m/z* 401 (ion a) and the base peak at *m/z* 86 (ion b), due to cleavage of the terminal amino acid isoleucine, whereas sativanine-K gave an evident fragment peak at *m/z* 114 (ion c) and the counterpart ion *m/z* 401 (ion a). Further fragmentation of ion *m/z* 401 (ion a) of compounds **1** and **3** were identical. The characteristic fragments for the methoxy styrylamine unit at *m/z* 165, isoleucine at *m/z* 114 and *m/z* 86 and hydroxyproline at *m/z* 96 and *m/z* 68 revealed the identity of the units forming the 13-membered heterocyclic ring of compound **1**. The fragment ions of *m/z* 401, 400, 374, 373, 372 represented the whole ring system and the ions *m/z* 259, 233, 216, 209, 181 showed the linkage of the different units. The elementary composition of all the fragments were substantiated by HRMS. The identity of ring bound and terminal amino acids were proved to be isoleucine by acid hydrolysis of **1** and PC comparison of the hydrolysate. The structure of sativanine-N was thus settled as **1**. The structure was further supported by deformylation of **1** into sativanine-K (**3**) (figure 1).



m/z 401 (ion a) *m/z* 86 (ion b) *m/z* 114 ion (c)

1: R= H

3: R= CHO



2: R=— CH_2 — Ph **5** *m/z* 120 (ion d)

4: R=— CH_2 — CH—$(CH_3)_2$

Figure 1. The structures of compound **1**–**5** and fragment ions (**a**–**d**).

The mass fragmentation pattern of sativanine-O (**2**) closely resembled that of nummularine-S (**4**) [6]. The α-cleavage product of the terminal amino acid phenylalanine formed the base at *m/z* 120 (ion d). Like nummularine S, the characteristic fragments for the methoxystyrylamine unit at *m/z* 165, phenylalanine at *m/z* 120 and hydroxyproline at *m/z* 96 revealed the identity of the units forming the 13-membered heterocyclic ring of compound **2**. The fragment ions at *m/z* 435, 434, 408, 407 and 406 represented the whole ring system and ions at *m/z* 338, 259, 233, 216 and 215 showed the linkage of the different units. Compound **2** thus differs from **4** only in their end amino acids. Compound **2** contains phenylalamine as end amino acid whereas **4** contains leucine as end amino acid, as evidenced by high-resolution mass spectrometric analysis and hydrolysis experiments. Both compounds **2** and **4** furnished identical compound **5** on partial hydrolysis. Based on these findings, sativanine-O was proved to have the structure **2** (figure 1).

3. Experimental

3.1 General experimental procedures

Melting points were determined on a Toshniwal apparatus and are uncorrected. IR were recorded on a Perkin-Elmer spectrophotometer model 221 in KBr pellet. UV spectra were measured on a Carry-14 spectrophotometer using spectral methanol. MS was performed on a Kratos MS-50 mass spectrometer operating at 70 eV with evaporation of sample in the ion source at 200°C and $[\alpha]_D$ in MeOH at 20°C was carried out on a Perkin-Elmer polarimeter 141. Column chromatography was carried out on silica gel columns (BDH, 60–120 mesh), TLC was performed on silica gel G (Merck). Paper chromatography was conducted on Whatman No. 1 paper; solvents for TLC: $CHCl_3$—MeOH (1:2) (solvent A), (1:4) (solvent B), (8:1) (solvent C), C_6H_6—EtOAc—MeOH (3:1:1) (solvent D) and for PC: n-BuOH—HOAc—H_2O (4:1:5) (solvent E), were detected on paper chromatograms with ninhydrin reagent.

3.2 Plant material

Stem barks of the plant *Zizyphus sativa* were collected from Mirzapur U.P. District, India and identified by Dr N.K. Dube of the Department of Botany, Banaras Hindu University. A voucher specimen of the sample is kept in the Department.

3.3 Extraction and isolation

Dried stem barks (4 kg) were powdered and repeatedly extracted with a mixture of C_6H_6—NH_4OH—MeOH (100:1:1). The total extract was concentrated under reduced pressure and extracted with 7% aqueous citric acid. A mixture of crude alkaloids (2.5 g) was obtained from acidic fraction by extracting with $CHCl_3$. The crude alkaloidal fraction was chromatographed over SiO_2 gel column eluting with a mixture of $CHCl_3$ and MeOH. The eluants from $CHCl_3$—MeOH (1:8) furnished two major spots on TLC which were separated by preparative TLC with solvents A and B into the compounds sativanine-N (9 mg) (**1**) and sativanine-O (11 mg) (**2**).

3.4 Sativanine-N (1)

Compound **1** crystallised from MeOH as colourless granules, mp 176–78°C, Rf 0.65 (solvent C), $[\alpha]_D^{20}$ −158 (c, 0.18, $CHCl_3$). It showed UV (MeOH) λ_{max} nm: 270 (log ∈ 2.78), 322 (log ∈ 2.58), IR (KBr) $\nu_{max}\,cm^{-1}$: 3400 (—NH), 2960–2860 (—CH), 2790 (—OMe), 1675, 1650 (sec. amide), 1630 (—C═C—), 1240, 1050 (arylether), HRMS: *m/z* 486.2842 (M^+, calcd for $C_{26}H_{38}N_4O_5$, 486.2841), 401.1956 ($C_{21}H_{27}N_3O_5$), 400 ($C_{21}H_{26}N_3O_5$), 374.2054 ($C_{20}H_{28}N_3O_4$), 373.2008 ($C_{20}H_{27}N_3O_5$), 372.1896 ($C_{20}H_{26}N_3O_5$), 259.1084 ($C_{14}H_{15}N_2O_3$), 233.1278 ($C_{13}H_{17}N_2O_2$), 216.1022 ($C_{13}H_{14}NO_2$), 209.1284 ($C_{11}H_{17}N_2O_2$), 181.1342 ($C_{10}H_{17}N_2O$), 165.0794 ($C_9H_{11}NO_2$), 114.0920 ($C_6H_{12}NO$), 96.0454 ((C_5H_6NO), 86.0971 ($C_5H_{12}N$), 68.0508 (C_4H_6N).

3.5 Hydrolysis of sativanine-N (1)

Alkaloid **1** (4 mg) was heated in a sealed tube with 6 N HCl (1 ml) for 24 h at 120°C. The hydrolysate was examined by PC (solvent E) which showed two ninhydrin positive spots identified as isoleucine and hydroxyproline by comparison with authentic samples.

3.6 Deformylation of sativanine-K (3)

Sativanine-K(**3**) (5 mg) was treated with 0.5 N HCl in MeOH at room temperature for a period of 45 h. The solvent was evaporated, basified with NH_3 and extracted with $CHCl_3$. The $CHCl_3$ extract was purified by preparative TLC (solvent B) which on crystallisation furnished deformylated compound, mp 175–77°C identical with sativanine-N (**1**) (mmp, co-TLC and superimposable IR).

3.7 Sativanine-O (2)

Compound **2** crystallised from MeOH as colourless granules, mp 224–26°C, Rf 0.42 (solvent D), $[\alpha]_D^{20}$ −195 (c, 0.15, $CHCl_3$). It showed UV (MeOH) λ_{max} nm: 270 (log ∈ 2.80), 320 (log ∈ 2.40), IR (KBr) $\nu_{max}\,cm^{-1}$: 3400 (—NH), 2960–2860 (—CH), 2790 (—OMe), 1670,1640 (sec. amide), 1625(—C═C—), 1235, 1050 (arylether), HRMS: *m/z* 554.2530 (M^+, calcd for $C_{32}H_{34}N_4O_5$, 554.2529), 463.1983 ($C_{25}H_{27}N_4O_5$), 435.1784 ($C_{24}H_{25}N_3O_5$), 434.1718 ($C_{24}H_{24}N_3O_5$), 408.1910 ($C_{23}H_{26}N_3O_4$), 407.1825 ($C_{23}H_{25}N_3O_4$), 406.1748 ($C_{23}H_{24}N_3O_4$), 338.1270 ($C_{19}H_{18}N_2O_4$), 259.1084 ($C_{14}H_{15}N_2O_3$), 233.1278 ($C_{13}H_{17}N_2O_2$), 216.1020 ($C_{13}H_{14}NO_2$), 215.1180 ($C_{13}H_{15}N_2O$), 165.0795 ($C_9H_{11}NO_2$), 120.0825 ($C_8H_{10}N$), 96.0455 (C_5H_6NO), 68.0510 (C_4H_6N).

3.8 Hydrolysis of sativanine-O (2)

Alkaloid **2** (5 mg) was heated in a sealed tube with 6 N HCl (1 ml) for 24 h at 120°C. The hydrolysate was examined by PC (solvent E) which showed two ninhydrin-positive spots identified as phenylalanine and hydroxyproline by comparison with authentic samples.

3.9 Partial hydrolysis of sativanine-O (2) and nummularine-S (4)

Compounds **2** (8 mg) and **4** (6 mg) were heated separately on water both with 4 ml of a mixture of conc. HCl—CH_3COOH—H_2O (1:1:1) and on usual work up they furnished identical compound **5**, as colourless amorphous solid, MS: *m/z* 407 (M^+, $C_{23}H_{25}N_3O_4$).

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