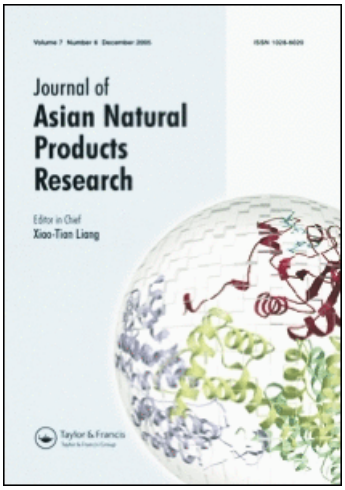

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Xylopyrine-C, a new cyclopeptide alkaloid from *Zizyphus xylopyra*

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Xylopyrine-C, a new 14-membered ring cyclopeptide alkaloid, has been isolated from the root bark of *Zizyphus xylopyra* together with a known alkaloid scutianine-C, and their structures were established by chemical and spectral evidences.

Keywords: *Zizyphus xylopyra*; Rhamnaceae; cyclopeptide alkaloids; scutianine-C; xylopyrine-C

1. Introduction

The plant *Zizyphus xylopyra* Willd. (Rhamnaceae) is distributed throughout India [1]. A number of peptide alkaloids [2] and other constituents [3–5] have earlier been reported from this plant. Here, we report the isolation of cyclopeptide alkaloids scutianine-C [6] and a new cyclopeptide alkaloid xylopyrine-C (**1**).

2. Results and discussion

Chromatographic separation of the crude base fraction of the root bark of *Z. xylopyra* followed by preparative TLC furnished the cyclopeptide alkaloids scutianine-C and xylopyrine-C (**1**).

Xylopyrine-C (**1**), $C_{36}H_{36}N_4O_4$ $[M]^+$ (588.2737), gave Dragendorff reaction for alkaloids. The IR spectrum of **1** was typical for cyclopeptide alkaloids and showed strong bands characteristic of secondary amide, styryl double bond, arylether, and N-CH_3 groups [7]. The UV spectrum exhibited a typical strong end absorption at 205 nm and a shoulder at 250 nm, characteristic of the styrylamine chromophore in the 14-membered ring containing cyclopeptide alkaloids [7].

The structure of the majority of the cyclopeptide alkaloids can largely be determined by their high-resolution mass spectra [8]. In view of this fact, the HR-MS analysis of **1** was applied to elucidate the structure.

The MS fragmentation pattern of alkaloid **1** closely resembled that of crenatine-A (**2**) [9]. The α-cleavage products of **1** gave ion peaks at *m*/*z* 455 (ion a) and the base peak at *m*/*z* 134 (ion b) due to cleavage of the amino acid residue outside the ring *N*-monomethylphenylalanine, whereas crenatine-A gave the base peak at *m*/*z* 114 (ion c) and the counterpart ion at *m*/*z* 455 (ion a). Further fragmentation of ion *m*/*z* 455 (ion a) of compounds **1** and **2** were identical. The characteristic fragments for the styrylamine unit at *m*/*z* 135 and phenylalanine at *m*/*z* 120 revealed the identity of the units forming the 14-membered heterocyclic ring of compound **1**. The fragment ions at *m*/*z* 497 and 455 represented the whole ring system and the ions at *m*/*z* 412, 371, 308, 278, 250, 224, 187 showed a linkage of the different units.

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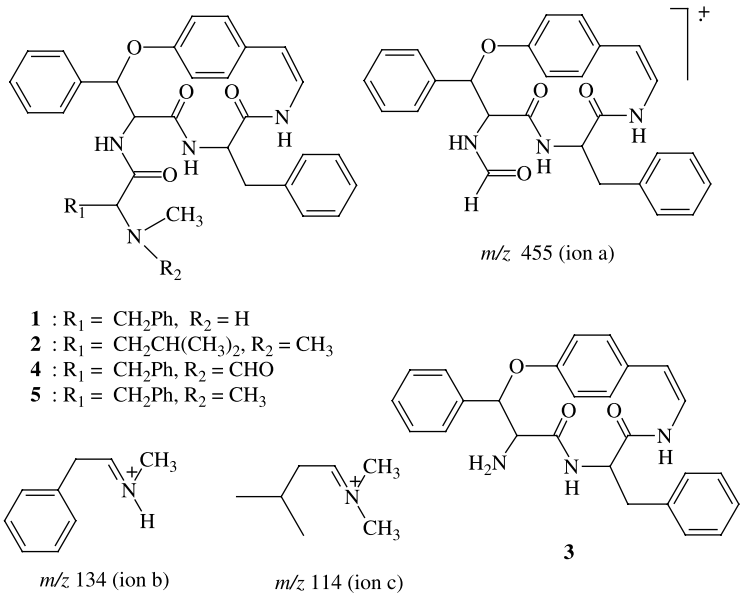

Figure 1. Structures of compounds **1**–**5** and main fragment ions a–c.

The elementary compositions of all the fragments were substantiated by HR-MS. Compound **1** thus differs from **2** only in its amino acid residues that are outside the ring. The identity of the ring bound and the amino acid residue outside the ring were proved, respectively, to be phenylalanine and *N*-monomethylphenylalanine in **1**, and phenylalanine and *N*,*N*-dimethylleucine in **2** by acid hydrolysis of **1** and **2** and co-PC comparison of the hydrolysate. Both compounds **1** and **2** furnished an identical compound **3** on partial hydrolysis (Figure 1).

Compound **3** after acid hydrolysis with 6 M HCl gave phenylalanine. The structure of xylopyrine-A was thus settled as **1**. The structure was further supported by formylation and methylation of **1** into compounds **4** and **5**.

3. Experimental

3.1 General experimental procedures

Melting points were determined on a Toshniwal apparatus and are uncorrected. IR spectra were recorded on a Perkin-Elmer spectrophotometer model 221 using KBr pellet. UV spectra were obtained using a Carry-14 spectrophotometer using spectral methanol. MS were recorded using a Kratos MS-50 mass spectrometer operating at 70 eV with the evaporation of sample in the ion source at 200°C, and $[\alpha]_D$ values in $CHCl_3$ at 20°C was carried out using a Perkin-Elmer polarimeter 141. Column chromatography was performed on silica gel columns (BDH, 60–120 mesh). TLC was performed on silica gel G (Merck, New Jersey, USA). Paper chromatography was conducted on a Whatman No. 1 paper; solvents for TLC: $CHCl_3$–MeOH (9:1) (solvent A), (4:1) (solvent B) and for PC: *n*-BuOH–HOAc–H_2O (4:1:5) (solvent C). Spots on paper chromatograms were detected using the ninhydrin reagent.

3.2 Plant material

The root bark of *Zizyphus xylopyra* was collected from Mirzapur District, UP, India and identified by Professor N.K. Dube, Department of Botany, Banaras Hindu University, Varanasi. A voucher specimen No. 13 of the sample has been kept in the Department.

3.3 Extraction and isolation

Dried root bark (5 kg) was 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. The acidic fraction was basified with ammonia and extracted with $CHCl_3$, which furnished a mixture of crude alkaloids (4.8 g). The crude alkaloidal fraction was chromatographed over a SiO_2 gel column eluting with a mixture of $CHCl_3$ and MeOH. The eluants from $CHCl_3$–MeOH (99:1) and (9:1) after purification with preparative TLC with solvents A and B furnished the compounds scutianine-C (12 mg) and xylopyrine-C (18 mg) (**1**).

3.3.1 Scutianine-C

Scutianine-C crystallized from MeOH as colorless granules, mp 264–266°C; R_f 0.47 (solvent A), 0.64 (solvent B); $[\alpha]_D^{20}$ − 230 (*c* 0.16, $CHCl_3$). UV (MeOH) λ_{max} (nm): 205 (strong end absorption); IR (KBr) ν_{max} (cm^{-1}): 3260 (—NH), 3020–2920 (—CH), 2890, 2790 (—NCH_3), 1652 (sec. amide), 1624 (—C═C—), 1610, 1500 (aromatic), 1230 (arylether); HR-MS *m/z*: 534.3220 $[M]^+$ (calcd for $C_{31}H_{42}N_4O_4$, 534.3206), 489, 308, 228, 218, 202, 190, 148, 135, 131, 120, 115, 114 (base peak), 97, 69. It was identified as scutianine-C by comparison with authentic sample [6] (mp 264–266°C, co-TLC and superimposable IR).

3.3.2 Xylopyrine-C (*1*)

Compound **1** crystallized from MeOH as colorless granules, mp 245–247°C; R_f 0.24 (solvent A), 0.44 (solvent B); $[\alpha]_D^{20}$ − 230 (*c* 0.18, $CHCl_3$). It showed UV (MeOH) λ_{max} (nm): 205 (strong end absorption), 250 sh; IR (KBr) ν_{max} (cm^{-1}): 3400 (—NH), 2950 (—CH), 2790 (—NCH_3), 1690, 1655 (sec. amide), 1620 (—C═C—), 1225, 1040 (arylether); HR-MS *m/z*: 588.2737 $[M]^+$ (calcd for $C_{36}H_{36}N_4O_4$, 588.2736), 497.2189 ($C_{29}H_{29}N_4O_4$), 455.1850 ($C_{27}H_{25}N_3O_4$), 412.1785 ($C_{26}H_{24}N_2O_3$), 371.1760 ($C_{24}H_{23}N_2O_2$), 308.1158 ($C_{18}H_{16}N_2O_3$), 278.1176 ($C_{18}H_{16}NO_2$), 250.1235 ($C_{17}H_{16}NO$), 215.0820 ($C_{12}H_{11}N_2O_2$), 224.1075 ($C_{15}H_{14}NO$), 187.0871 ($C_{11}H_{11}N_2O$), 135.0682 (C_8H_9NO), 131.0495 (C_9H_7O), 134.0970 ($C_9H_{12}N$, base peak), 120.0810 ($C_8H_{10}N$), 103.0544 (C_8H_7).

3.3.3 Hydrolysis of xylopyrine-C (*1*)

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

3.3.4 Partial hydrolysis of xylopyrine-C (*1*) and crenatine (*2*)

Compounds **1** (8 mg) and **2** (6 mg) were heated separately on a water bath with 4 ml of a mixture of conc. HCl–AcOH–H_2O (1:1:1) and after standard usual work up, an identical compound **3** was obtained as a colorless amorphous solid, MS *m/z*: 427.1895 $[M]^+$ ($C_{26}H_{25}N_3O_3$), 308, 278, 250, 224, 135. Compound **3** on hydrolysis with 6 M HCl in a sealed tube for 18 h at 120°C gave phenylalanine (co-PC with authentic sample).

3.3.5 Formylation of xylopyrine-C (*1*)

Compound **1** (7 mg) was reacted with HCOOH–Ac_2O and kept overnight at room temperature. After evaporation of the solvent and purification by preparative TLC (solvent B), *N*-formyl derivative **4** was obtained as a colorless amorphous solid, MS *m/z*: 616.2685 $[M]^+$ ($C_{37}H_{36}N_4O_5$).

3.3.6 Methylation of xylopyrine-C (*1*)

Compound **1** (9 mg) was treated with HCHO and $NaBH_4$ by slowly adding and checking the reaction mixture by TLC. After usual work up, *N*-methylated product **5** was obtained as a colorless amorphous solid, MS *m/z*: 602.2893 $[M]^+$ ($C_{37}H_{38}N_4O_4$).

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