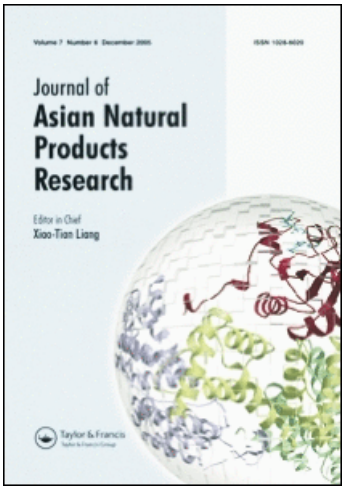

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Xylopyrine-F, a new cyclopeptide alkaloid from *Zizyphus xylopyra*

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A new 14-membered ring cyclopeptide alkaloid xylopyrine-F, together with known alkaloids nummularine-P and sativanine-H, has been isolated from the root bark of *Zizyphus xylopyra* and their structures were established by chemical and spectral evidences.

Keywords: *Zizyphus xylopyra*; Rhamnaceae; cyclopeptide alkaloids; nummualarine-P; sativanine-H; xylopyrine-F

1. Introduction

Zizyphus species (Rhamnaceae) are distributed throughout India and used in the Indian System of Medicine for the treatment of various diseases [1]. The isolation of several cyclopeptide alkaloids have also been reported from these species [2]. In this paper, we report the isolation and characterization of a new 14-membered cyclopeptide alkaloids, designated as xylopyrine-F (**1**) together with two known 13-membered ring cyclopeptide alkaloids nummularine-P [3] and sativanine-H [4] from the bark of *Zizyphus xylopyra*.

2. Results and discussion

Chromatographic separation of the crude base fraction of the bark of *Z. xylopyra* resulted in the isolation of xylopyrine-F (**1**). The IR spectrum was typical for cyclopeptide alkaloids and showed strong bands characteristic of secondary amide, styryl double bond, arylether, and NH groups. The UV spectrum exhibited typical strong end absorption band at 204 nm, characteristic of styrylamine chromophore in the 14-membered ring containing cyclopeptide alkaloids [5].

The structure of the majority of the cyclopeptide alkaloids can largely be determined mainly by their high-resolution mass spectra [6]. In view of this fact, the HR-MS analysis of compound **1** was applied to elucidate the structure (Figure 1).

The molecular formula of compound **1** was determined as $C_{29}H_{38}N_4O_4$ by HR-MS at *m*/*z* 506.2892 $[M]^+$. The MS fragmentation pattern of alkaloid **1** closely resembled that of integerrenine (**2**) [7] having only difference in their amino acid residue outside the ring. The α-cleavage products of **1** gave ion peaks at *m*/*z* 421 (ion a) and the base peak at *m*/*z* 86 (ion b) due to cleavage of the amino acid leucine outside the ring, whereas integerrenine gave the base peak at *m*/*z* 114 (ion c) for *N*,*N*-dimethylisoleucine and the counterpart ion *m*/*z* 421 (ion a). Further, fragmentation of the ion *m*/*z* 421 (ion a) of compounds **1** and **2** were identical. The ion peak at *m*/*z* 135 indicates the presence of a *p*-hydroxystyrylamine group and the ion peak at *m*/*z* 131 arises from etherified β-hydroxyphenylalanine.

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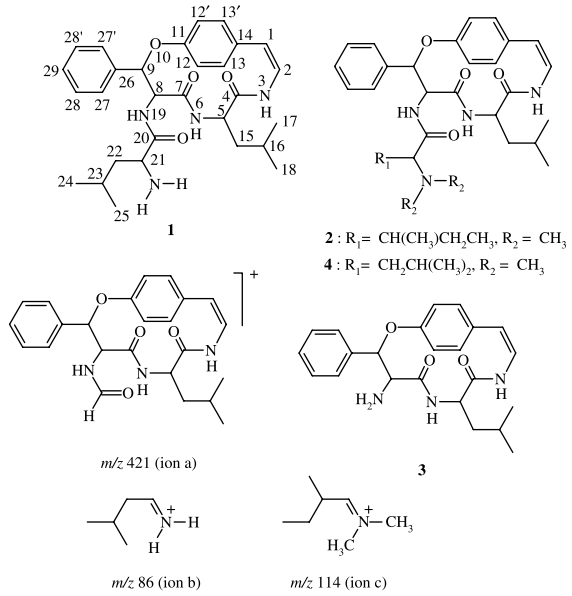

Figure 1. Structures of compounds **1**–**4** and ions a–c.

The ion *m/z* 86 (ion b) also represents the ring-incorporated amino acid residue, which is due to the fact that acid hydrolysis of **1** gave only leucine. The ion peaks at *m/z* 224, 274, and 337 indicated that the *p*-hydroxystyrylamine moiety is bonded with β-hydroxyphenylalanine and leucine; in addition, the ions at *m/z* 244 and 216 prove these two amino acid residues to be attached to each other. The ions at *m/z* 201 and 173 indicated the connection between leucine and β-hydroxyphenylalanine. All these fragment ions together with the ions at *m/z* 449, 419, 421, and 378 thus establish the 14-membered ring cyclopeptide alkaloid system in compound **1**, possessing leucine as a ring-incorporated amino acid residue and outside the ring. The elementary compositions of all the fragments were substantiated by HR-MS. Compound **1** thus differs from **2** only in its amino acid residues outside the ring, which were proved, respectively, to be leucine in **1** and *N*,*N*-dimethylisoleucine in **2** by acid hydrolysis and co-PC comparison of the hydrolysate. Partial hydrolysis of **1** and **2** yielded an identical compound **3**, $C_{23}H_{27}N_3O_3$ (*m/z* 393, $[M]^+$), which on hydrolysis gave ring-bound amino acid leucine. *N*-Methylation of **1** furnished compound **4**, $C_{31}H_{42}N_4O_4$ (*m/z* 534, $[M]^+$), identical to the reported compound discarine-C [8]. The structure of xylopyrine-F is thus settled **1**. ^{13}C NMR spectral data fully supported the structure of **1**.

3. Experimental

3.1 General experimental procedures

The melting points were determined on a Toshniwal apparatus and are uncorrected. IR spectra were recorded on a Perkin–Elmer spectrophotometer model 221 using KBr pellets. UV spectra were measured on a Carry-14 spectrophotometer using spectral methanol. MS data were recorded on a Kratos MS-50 mass spectrometer operating at 70 eV with evaporation of the sample in the ion source at 200°C and $[\alpha]_D^{20}$ in $CHCl_3$ at 20°C was carried out on a Perkin–Elmer polarimeter 141. ^{13}C NMR spectra were recorded using 100 MHz NMR spectrometers using $CDCl_3 + CD_3OD$ as the solvent. Column chromatography was carried out on silica gel columns (BDH, 60–120 mesh). TLC was performed on silica gel G (Merck). Paper chromatography was carried out on Whatman

No. 1 paper; solvents for TLC: $CHCl_3$–MeOH (9:1; solvent A), (2:1; solvent B) and for PC: *n*-BuOH–HOAc–H_2O (4:1:5; solvent C). Spots were detected on paper chromatograms using ninhydrin reagent.

3.2 Plant material

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

3.3 Extraction and isolation

Dried barks (6 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. The acidic fraction was basified with ammonia and extracted with $CHCl_3$, which gave a mixture of crude alkaloids (5.6 g). The crude alkaloidal fraction was chromatographed over SiO_2 gel column eluting with a mixture of $CHCl_3$ and MeOH. The eluants collected from $CHCl_3$–MeOH (9:1), (4:1), and (1:1) after purification with preparative TLC with solvents A and B furnished nummularine-P (22 mg), sativanine-H (12 mg) and xylopyrine-F (16 mg).

3.3.1 Nummularine-P

Nummularine-P crystallized with MeOH as colorless granules, mp 142–144°C; R_f 0.30 (solvent A) and 0.50 (solvent B). UV (MeOH) λ_{max} (nm): 258 and 320; IR (KBr) ν_{max} (cm^{-1}): 3350 (—NH), 2831 (—OCH_3), 2775 (—NCH_3), 1688 and 1640 (sec. amide), 1615 (—C═C—), 1230 (aryl ether); HRMS *m/z* 557.3200 $[M]^+$ (calcd for $C_{29}H_{43}N_5O_6$, 557.3213), 542, 500, 528, 514, 498, 457, 401, 400, 374, 373, 372, 304, 259, 233, 216, 209, 165, 96, 68, 58 (base peak). It was identified as nummularine-P by comparison with authentic sample [3] (mp 142–144°C, co-TLC and superimposable IR).

3.3.2 Sativanine-H

Sativanine-H crystallized from MeOH as colorless granules, mp 190–192°C; R_f 0.28 (solvent A) and 0.45 (solvent B). UV (MeOH) λ_{max} (nm): 263 and 318; IR (KBr) ν_{max} (cm^{-1}): 3320 (—NH), 2770 (—NCH_3), 1235 and 1050 (aryl ether), 2800 (—OMe), 1615 (—C═C—), 1650 and 1637 (sec. amide); HRMS *m/z* 557.3208 $[M]^+$ (calcd for $C_{29}H_{43}N_5O_6$, 557.3213), 500, 498, 457, 374, 373, 372, 233, 216, 209, 195, 185, 181, 165, 96, 58 (base peak). It was identified as sativanine-H by direct comparison with authentic sample [4] (mp 190–192°C, co-TLC and superimposable IR).

3.3.3 Xylopyrine-F (*1*)

Xylopyrine-F crystallized from MeOH as an amorphous solid; R_f 0.20 (solvent A), 0.40 (solvent B); $[\alpha]_D^{20}$ − 138 (*c* 0.20, $CHCl_3$). UV (MeOH) λ_{max} (nm): 204 (strong end absorption); IR (KBr) ν_{max} (cm^{-1}): 3400 (—NH), 2960 (—CH), 1645 (sec. amide), 1635 (—C═C—), 1550 (aromatic), 1220 and 1030 (aryl ether), HRMS *m/z* 506.2892 $[M]^+$ (calcd for $C_{29}H_{38}N_4O_4$, 506.2893), 491.2658 ($C_{28}H_{35}N_4O_4$), 505.2806 ($C_{29}H_{37}N_4O_4$), 449.2188 ($C_{25}H_{29}N_4O_4$), 421.1998 ($C_{24}H_{27}N_4O_4$), 419.1833 ($C_{24}H_{25}N_3O_4$), 378.1945 ($C_{23}H_{26}N_2O_3$), 337.1916 ($C_{21}H_{25}N_2O_2$), 274.1313 ($C_{15}H_{18}N_2O_3$), 244.1330 ($C_{15}H_{18}NO_2$), 201.0664 ($C_{11}H_9N_2O_2$), 224.1073 ($C_{15}H_{14}NO$), 216.1388 ($C_{14}H_{18}NO$), 173.0715 ($C_{10}H_9N_2O$), 135.0682 (C_8H_9NO), 131.0496 (C_9H_7O), 114.1288 ($C_7H_{16}N$), 103.0546 (C_8H_7), 86.0969 ($C_5H_{12}N$, base peak), 85.0891 ($C_5H_{11}N$); ^{13}C NMR ($CDCl_3 + CD_3OD$) δ 122.8 (C-1), 124.4 (C-2), 167.0 (C-4), 52.1 (C-5), 170.4 (C-7), 52.8 (C-8), 81.8 (C-9), 156.0 (C-11), 114.5 (C-12), 121.9 (C-12′), 132.9 (C-13), 130.4 (C-13′), 132.0 (C-14), 39.0 (C-15), 25.3 (C-16), 22.2 (C-17), 22.0 (C-18), 173.0 (C-20), 66.1 (C-21), 34.1 (C-22), 24.8

(C-23), 20.5 (C-24), 23.1 (C-25), 140.0 (C-26), 127.0 (C-27), 127.0 (C-27′), 128.4 (C-28), 128.4 (C-28′), 125.0 (C-29).

3.4 Hydrolysis of xylopyrine-F (1)

Compound **1** (7 mg) was heated in a sealed tube with 6 M HCl (1 ml) for 24 h at 120°C. The hydrolysate was examined by paper chromatography (solvent C), which showed one ninhydrin-positive spot identified as leucine on comparison with the authentic sample.

3.5 Partial hydrolysis of xylopyrine-F (1) and integerrenine (2)

Compounds **1** (7 mg) and **2** (6 mg) were heated separately on a water bath with 4 ml of a mixture of conc. $HCl-CH_3COOH-H_2O$ (1:1:1), and on usual work up they furnished an identical compound **3** as colorless amorphous solid, MS *m/z* 393.2052 $[M]^+$ ($C_{23}H_{27}N_3O_3$), 274, 244, 224, 216, 135. Compound **3** on hydrolysis with 6 M HCl in a sealed tube for 18 h at 120°C gave leucine (co-PC with authentic sample).

3.6 Methylation of xylopyrine-F (1)

Compound **1** (8 mg) was treated with HCHO and $NaBH_4$ by adding slowly and checking the reaction mixture by TLC. On usual work up, it furnished the *N*-methylated product **4** as a colorless amorphous solid, MS *m/z* 534.3118 $[M]^+$ ($C_{31}H_{42}N_4O_4$) identical to discarine-C [8] (co-TLC, HR-MS, superimposable IR).

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