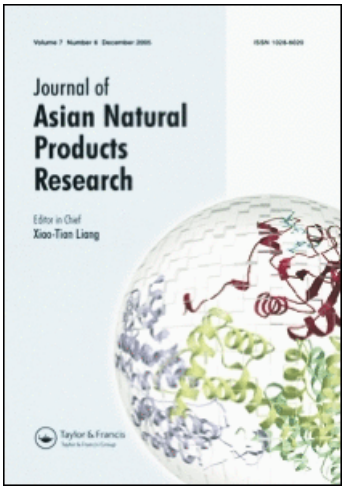

Journal of Asian Natural Products Research
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713454007

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To cite this Article Pandey, Manoj B. , Singh, Ashok K. , Singh, Jagdish P. , Singh, Virendra P. and Pandey, Vidya B.(2008) 'Three new cyclopeptide alkaloids from *Zizyphus* species', Journal of Asian Natural Products Research, 10: 8, 709 — 713
To link to this Article: DOI: 10.1080/10286020802016024
URL: http://dx.doi.org/10.1080/10286020802016024

Journal of Asian Natural Products Research
Vol. 10, No. 8, August 2008, 709–713

Three new cyclopeptide alkaloids from *Zizyphus* species

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(*Received 26 February 2007; final version received 28 July 2007*)

Two new cyclopeptide alkaloids, xylopyrine-D and xylopyrine-E, from *Zizyphus xylopyra* and a new alkaloid, jubanine-E, from *Zizyphus jujuba* have been isolated and their structures were established by chemical and spectral evidences.

Keywords: *Zizyphus xylopyra*; *Zizyphus jujuba*; Rhamnaceae; xylopyrine-D and xylopyrine-E; jubanine-E

1. Introduction

The aerial and root barks, leaves, and fruits of *Zizyphus* species (Rhamnaceae) are distributed throughout India and used in Indian System of Medicine for the treatment of various diseases [1–2]. Chemical investigation of different *Zizyphus* species has led to the isolation of several cyclopeptide alkaloids [3]. In a continuation of our work on cyclopeptide alkaloids from Rhamnaceae [3], we report herein the isolation and characterization of previously undescribed cyclopeptide alkaloids, xylopyrine-D (**1**) and xylopyrine-E (**2**) from the bark of *Zizyphus xylopyra* and jubanine-E (**3**) from the bark of *Zizyphus jujuba.*

2. Results and discussion

Chromatographic purification of the crude base fractions of the bark of *Z. xylopyra* and the bark of *Z. jujuba* followed by preparative TLC resulted in the isolation of xylopyrine-D (**1**) and xylopyrine-E (**2**) from *Z. xylopyra* and jubanine-E (**3**) from *Z. jujuba*. The IR spectra of **1**–**3** were typical for peptide alkaloids and showed strong bands that were characteristic of secondary amide, styryl double bond, arylether, and aromatic methoxyl groups. Their UV spectra exhibited absorption maxima for 2,5-dialkoxystyrylamine chromophore in the 13-membered ring containing cyclopeptide alkaloids [4].

The structure of the majority of the peptide alkaloids can be determined mainly by their high-resolution mass spectra [5]. In view of this fact, the HRMS analyses of compounds **1**–**3** were applied to elucidate their structures.

The molecular formula of compounds **1**–**3** was determined, respectively, as $C_{30}H_{38}N_4O_5$ (*m*/*z* 534.2841 $[M]^+$), $C_{28}H_{34}N_4O_5$ (*m*/*z* 506.2529 $[M]^+$), and $C_{33}H_{36}N_4O_5$ (*m*/*z* 568.2684 $[M]^+$) by HRMS. The MS fragmentation pattern of alkaloids **1**–**3** closely resembled that of nummularine-S (**4**) [6], differing only in their terminal amino acids. The α-cleavage fragment ions of **1**–**3** gave an identical ion peak at *m*/*z* 435 (ion a) and base peaks due to terminal amino acids, respectively, at *m*/*z* 100 (ion b) for *N*,*N*-dimethylvaline, *m*/*z* 72 (ion c) for valine, and *m*/*z* 134 (ion

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ISSN 1028-6020 print/ISSN 1477-2213 online

DOI: 10.1080/10286020802016024
http://www.informaworld.com

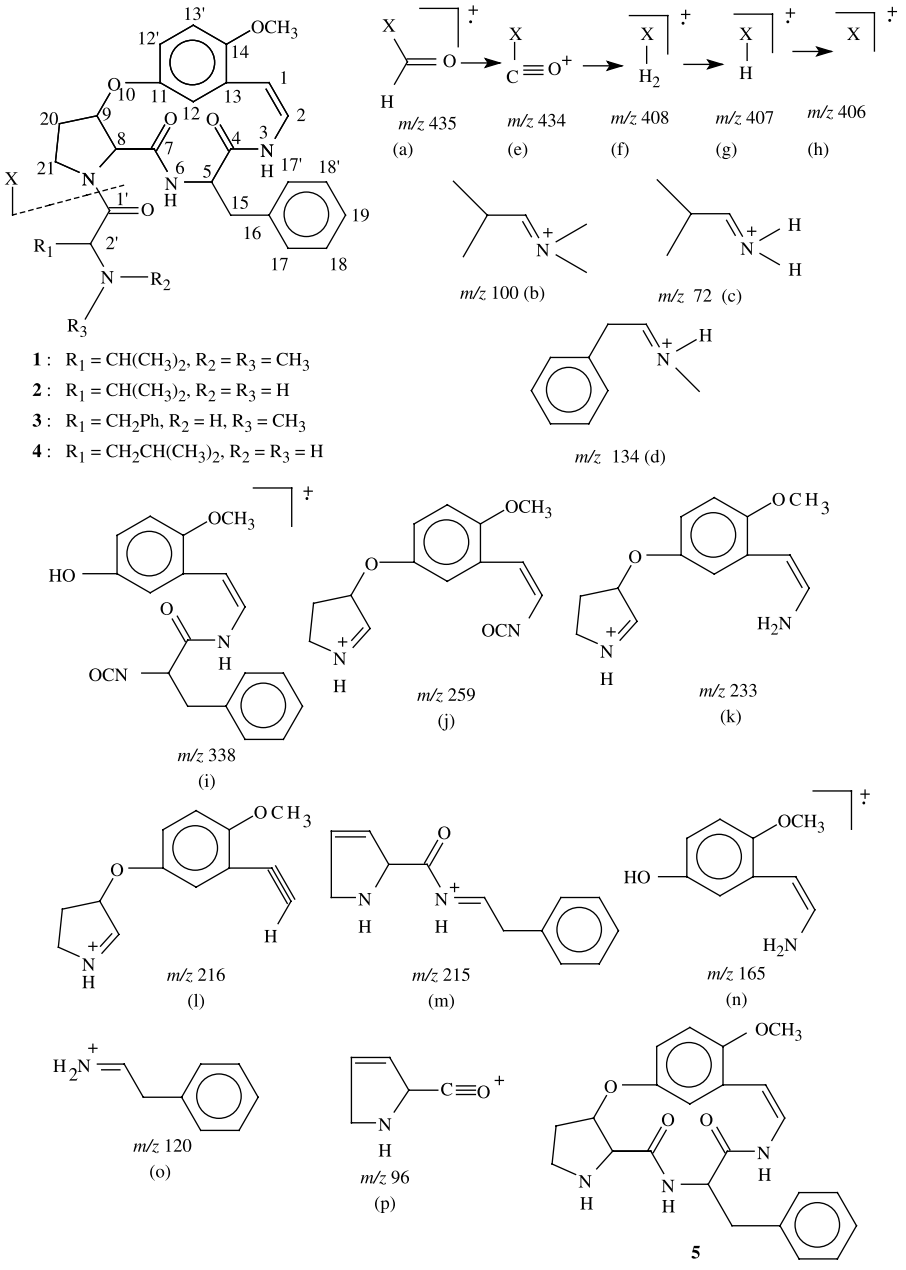


Figure 1. The structures of **1**–**5** and ions a–p.

d) for *N*-monomethylphenylalanine (Figure 1). A further fragmentation of ions at *m/z* 435 (ion a) of compounds **1**–**3** were identical (Table 1) to that of nummularine-S (**4**). The fragment ions at *m/z* 491, 463, and 477 obtained, respectively, in compounds **1**–**3** arises through radical scission of the residue R_1 [4]. The ring scission and the following scission at the amide groups in **1**–**3** exhibited characteristic fragments for a methoxystyrylamine unit at *m/z* 165 (ion n), phenylalanine at *m/z* 120 (ion o), and hydroxyproline at *m/z* 96 (ion p), revealing the

Table 1. HRMS data of compounds **1**–**3** and their fragment ions.

1	**2**	**3**
Mass, *m/z* (*relative intensity %*, *molecular formula*)		
534.2841 $[M]^+$(8, $C_{30}H_{38}N_4O_5$)	506.2529 $[M]^+$(10, $C_{28}H_{34}N_4O_5$)	568.2684 $[M]^+$(9, $C_{33}H_{36}N_4O_5$)
100.1126 (100, $C_6H_{14}N$, base peak)	72.0813 (100, $C_4H_{10}N$, base peak)	134.0969 (100, $C_9H_{12}N$, base peak)
491.2294 (14, $C_{27}H_{31}N_4O_5$)	463.1983 (12, $C_{25}H_{27}N_4O_5$)	477.2137 (13, $C_{26}H_{29}N_4O_5$)
435.1780 (8, $C_{24}H_{25}N_3O_5$)	435.1782 (7, $C_{24}H_{25}N_3O_5$)	435.1778 (9, $C_{24}H_{25}N_3O_5$)
434.1701 (5, $C_{24}H_{24}N_3O_5$)	434.1700 (4, $C_{24}H_{24}N_3O_5$)	434.1701 (5, $C_{24}H_{24}N_3O_5$)
408.1910 (2, $C_{23}H_{26}N_3O_4$)	408.1909 (3, $C_{23}H_{26}N_3O_4$)	408.1910 (3, $C_{23}H_{26}N_3O_4$)
407.1825 (10, $C_{23}H_{25}N_3O_4$)	407.1822 (9, $C_{23}H_{25}N_3O_4$)	407.1826 (9, $C_{23}H_{25}N_3O_4$)
406.1746 (3, $C_{23}H_{24}N_3O_4$)	406.1740 (4, $C_{23}H_{24}N_3O_4$)	406.1748 (3, $C_{23}H_{24}N_3O_4$)
215.1182 (8, $C_{13}H_{15}N_2O$)	215.1181 (8, $C_{13}H_{15}N_2O$)	215.1181 (6, $C_{13}H_{15}N_2O$)
259.1090 (4, $C_{14}H_{15}N_2O_3$)	259.1080 (5, $C_{14}H_{15}N_2O_3$)	259.1088 (5, $C_{14}H_{15}N_2O_3$)
233.1283 (4, $C_{13}H_{17}N_2O_2$)	233.1280 (5, $C_{13}H_{17}N_2O_2$)	233.1281 (4, $C_{13}H_{17}N_2O_2$)
216.1020 (10, $C_{13}H_{14}NO_2$)	216.1015 (11, $C_{13}H_{14}NO_2$)	216.1018 (12, $C_{13}H_{14}NO_2$)
338.1270 (4, $C_{19}H_{18}N_2O_4$)	338.1271 (4, $C_{19}H_{18}N_2O_4$)	338.1268 (5, $C_{19}H_{18}N_2O_4$)
165.0782 (55, $C_9H_{11}NO_2$)	165.0780 (50, $C_9H_{11}NO_2$)	165.0780 (53, $C_9H_{11}NO_2$)
120.0818 (20, $C_8H_{10}N$)	120.0811 (18, $C_8H_{10}N$)	120.0820 (20, $C_8H_{10}N$)
96.0465 (10, C_5H_6NO)	96.0459 (9, C_5H_6NO)	96.0464 (9, C_5H_6NO)
68.0503 (32, C_4H_6N)	68.0500 (30, C_4H_6N)	68.0503 (30, C_4H_6N)

identity of the unit forming a 13-membered heterocyclic ring of the molecule and the ions at *m/z* 338 (ion i), 259 (ion j), 233 (ion k), 216 (ion l), and 215 (ion m) showing the linkage of the different units (Figure 1). The fragment ions at *m/z* 435 (ion a), 434 (ion e), 408 (ion f), 407 (ion g), and 406 (ion h; Figure 1) represented the whole ring system in **1**–**3**. The elementary composition of all the fragments were substantiated by HRMS. The terminal amino acids were confirmed as *N*,*N*-dimethylvaline in **1**, valine in **2**, and *N*-monomethylphenylalanine in **3** by hydrolysis and partial hydrolysis and paper chromatography comparison of the hydrolysate with authentic compounds. Partial hydrolysis of **1**–**4** gave identical compound **5**. Based on these findings, the structures of xylopyrine-D, xylopyrine-E, and jubanine-E were settled, respectively, as **1**–**3** in Figure 1, which differed in their terminal amino acids from nummularine-S (**4**) in having *N*,*N*-dimethylvaline in **1**, valine in **2**, and *N*-monomethylphenylalanine in **3** instead of leucine in **4**. The structures of **1**–**3** were further supported by ^{13}C NMR spectral data (Table 2). The isolation of xylopyrine-D, xylopyrine-E, and jubanine-E provide a new addition to the growing list of 13-membered ring cyclopeptide alkaloids.

3. Experimental

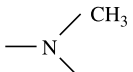
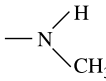
3.1 General experimental procedures

Melting points were determined on a Toshniwal apparatus and are uncorrected. The UV spectra were taken on a Carry-14 spectrophotometer using spectral methanol. The IR spectra were recorded on a Perkin–Elmer spectrophotometer model 221 with KBr pellets. ^{13}C NMR spectra were carried out on 100 MHz NMR in $CDCl_3 + CD_3OD$. MS was performed on a Kratos MS-50 mass spectrometer operating at 70 eV with the evaporation of the sample in the ion source at 200°C and $[\alpha]_D$ in $CHCl_3$ at 25°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, NJ, USA); paper chromatography was conducted on Whatman No. 1 filter paper; solvents for TLC were $CHCl_3$–MeOH (8:1; solvent A), (4:1; solvent B) and solvent for PC was *n*-BuOH–HOAc–H_2O (4:1:5; solvent C); paper chromatogram was developed with ninhydrin reagent.

3.2 Plant material

Barks of *Z. xylopyra* and *Z. jujuba* were collected from Mirzapur District (Uttar

Table 2. ^{13}C NMR spectral data of compounds **1**–**3** (100 MHz).

Carbon	**1**	**2**	**3**
1	106.0	106.2	106.0
2	121.0	121.0	121.3
4	167.0	166.9	166.8
5	60.0	60.5	60.4
7	170.0	170.0	169.8
8	64.0	64.2	64.4
9	76.0	76.3	76.2
11	151.0	151.0	151.6
12	111.0	111.4	111.6
12′	118.0	118.6	117.8
13	124.0	123.5	124.0
13′	114.0	114.0	114.0
14	152.0	152.0	150.8
15	34.0	33.8	35.0
16	140.0	140.2	141.0
17	129.0	129.2	130.0
17′	129.0	129.2	130.0
18	127.0	126.8	127.0
18′	127.0	126.8	127.0
19	125.0	125.0	125.0
20	33.0	33.0	32.5
21	47.0	47.0	46.4
—OCH_3	55.0	55.5	55.4
Terminal region	*N*,*N*-dimethylvaline	valine	*N*-monomethylphenylalanine
C-1′	171.0	170.8	172.0
C-2′	68.0	68.5	71.0
C-3′	30.0	30.0	33.6
C-4′	19.0	19.2	140.0
C-5′	18.0	18.0	128.8
C-6′	–	–	127.8
C-7′	–	–	125.0
C-8′	–	–	127.8
C-9′	–	–	128.8
	42.0	–	–
	42.0	–	–
	–	–	41.8

Pradesh, India) and identified by Professor N.K. Dube (Department of Botany, Banaras Hindu University, Varanasi, India). The voucher specimens (No. 13 and 14) of the samples are deposited in this department.

3.3 Extraction and isolation

The dried and powdered barks (4.5 kg) of *Z. xylopyra* were 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 (2.8 g) was obtained from the acidic fraction by basifying and extracting with $CHCl_3$. The crude alkaloidal fraction was chromatographed over SiO_2 gel column by eluting with a mixture of $CHCl_3$ and MeOH. The eluants from $CHCl_3$ and $CHCl_3$–MeOH (4:1) on

preparative TLC with solvents A and B furnished, respectively, alkaloids xylopyrine-D (17 mg; **1**) and xylopyrine-E (15 mg; **2**). The dried powdered barks of *Z. jujuba* (5 kg) were extracted in the usual manner and the crude base (3.0 g) was chromatographed over SiO_2 gel column. The $CHCl_3$ eluants, on preparative TLC with solvent A, gave jubanine-E (12 mg; **3**).

3.3.1 Xylopyrine-D (*1*)

Compound **1** was crystallized from MeOH as colorless granules, mp 231–233°C; R_f 0.42 (solvent A), 0.62 (solvent B); $[\alpha]_D^{25}$ − 168 (*c* 0.16, $CHCl_3$); UV λ_{max} (MeOH, nm): 270 (log ε 2.81), 320 (log ε 2.24); IR ν_{max} (KBr, cm^{-1}): 3265 (—NH), 2960–2860 (—CH), 2830 (—OMe), 2785 (—NMe), 1680 and 1635 (sec. amide), 1620 (—C═C—), 1240 and 1050 (arylether); HRMS: *m/z* 534.2841 $[M]^+$ (calcd for $C_{30}H_{38}N_4O_5$, 534.2842) and other data: see Table 1; ^{13}C NMR spectral data: see Table 2.

3.3.2 Xylopyrine-E (*2*)

Compound **2** was crystallized from MeOH as colorless granules, mp 253–255°C; R_f 0.35 (solvent A), 0.58 (solvent B); $[\alpha]_D^{25}$ − 210 (*c* 0.18, $CHCl_3$); UV λ_{max} (MeOH, nm): 268 (log ε 2.83), 320 (log ε 2.26); IR ν_{max} (KBr, cm^{-1}): 3260 (—NH), 2880–2960 (—CH), 2790 (—OMe), 1680 and 1630 (sec. amide), 1615 (—C═C—), 1240 and 1050 (arylether); HRMS: *m/z* 506.2529 $[M]^+$ (calcd for $C_{28}H_{34}N_4O_5$, 506.2529) and other data: see Table 1; ^{13}C NMR spectral data: see Table 2.

3.3.3 Jubanine-E (*3*)

Compound **3** was crystallized from MeOH as colorless granules, mp 216–218°C; R_f 0.25 (solvent A), 0.38 (solvent B); $[\alpha]_D^{25}$ − 135 (*c* 0.18, $CHCl_3$); UV λ_{max} (MeOH, nm): 270 (log ε 2.80), 320 (log ε 2.40); IR ν_{max} (KBr cm^{-1}): 3400 (—NH), 2960–2860 (—CH), 2790 (—OMe), 1670 and 1640 (sec. amide), 1625 (—C═C—), 1235 and 1050 (arylether); HRMS: *m/z* 568.2684 $[M]^+$ (calcd for $C_{33}H_{36}N_4O_5$, 568.2685) and other data: see Table 1; ^{13}C NMR spectral data: see Table 2.

3.4 Hydrolysis of compounds 1–3

Compounds **1**–**3** were separately hydrolyzed with 6 M HCl in a sealed tube for 24 h at 120°C. The hydrolysates were examined by paper chromatography. Compound **1** furnished *N*,*N*-dimethylvaline and phenylalanine, compound **2** gave valine and phenylalanine, and compound **3** furnished *N*-monomethylphenylalanine and phenylalanine by spraying with ninhydrin and co-PC with authentic samples.

3.5 Partial hydrolysis of compounds 1–4

Compounds **1**–**4** were heated on a water bath separately for 6 h with 6 ml of a mixture of $HCl–AcOH–H_2O$ (1:1:1). All the compounds furnished identical compound **5** as an amorphous solid (co-TLC, co-MS, and superimposable IR); HRMS: *m/z* 407.1825 $[M]^+$ (calcd for $C_{23}H_{25}N_3O_4$, 407.1845), 338, 259, 233, 216, 215, 165, 120, 96.

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