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Two new 14-membered cyclopeptide alkaloids from *Zizyphus xylopyra*

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The phytochemical investigation of the bark of *Zizyphus xylopyra* resulted in the isolation of two new 14-membered ring cyclopeptide alkaloids, xylopyrine-G and xylopyrine H. Their structures have been established by chemical and spectral evidences.

Keywords: *Zizyphus xylopyra*; rhamnaceae; cyclopeptide alkaloids; xylopyrine-G; xylopyrine-H

1. Introduction

Zizyphus xylopyra Willd. (Rhamnaceae) is a large straggling shrub, distributed throughout India (Council of Scientific and Industrial Research, 1976). In our previous communication, we reported the isolation of cyclopeptide alkaloids, xylopyrine-A, xylopyrine-B (A.K. Singh, M.B. Pandey, V.P. Singh, & V.B. Pandey, 2007), xylopyrine-C, scuitianine-C (A.K. Singh, M.B. Pandey, V.P. Singh, & V.B. Pandey, 2008), xylopyrine-D, xylopyrine-E (M.B. Pandey, A.K. Singh, J.P. Singh, V.P. Singh, & V.B. Pandey, 2008b), xylopyrine-F, nummularine-P and sativanine-H (Pandey, J.P. Singh, A.K. Singh, & V.P. Singh, 2008a) from the above plant. Here, we report the isolation and characterisation of two new cyclopeptide alkaloids, xylopyrine-G and xylopyrine-H from the bark of *Z. xylopyra*.

2. Results and discussion

Chromatographic separation of crude base fraction of the bark of *Z. xylopyra* followed by preparative TLC furnished the alkaloids, xylopyrine-G (**1**) and xylopyrine-H (**2**).

Xylopyrine-G (**1**), $C_{34}H_{38}N_4O_5$ ([M^+], 582.2848) and xylopyrine-H (**2**), $C_{32}H_{36}N_4O_4$ ([M^+], 540.2740) gave positive Dragendorff test for alkaloids (Shah, Pandey, Eckhardt, & Miana, 1988). The IR spectra of **1** and **2** were typical for

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peptide alkaloids and showed strong bands characteristic of N–CH_3 group, secondary amido group of peptide linkage, styryl double bond and arylether (Tschesche and Kaussmann, 1975) at ν_{max} values 2790, 1685 and 1635, 1609, 1235 and 1040 cm^{-1}, respectively. The UV spectrum exhibited typical strong end absorption bands at λ_{max} 210 nm and shoulder at 250 nm characteristic of styrylamine chromophore in the 14-membered ring containing cyclopeptide alkaloid (Tschesche and Kaussmann, 1975).

The structure of the majority of the peptide alkaloids can largely be determined by their High-Resolution Mass Spectrum (HRMS) analysis (Fehlhaber, 1968). In view of this fact, the HRMS analysis of compounds **1** and **2** was applied to elucidate their structures.

Like N-formylcyclopeptide alkaloid sativanine-K (Shah, Al-yahya, Devi, & Pandey, 1987), alkaloid **1**, which carried a N-formyl group on terminal amino acid, showed an intense molecular ion peak in the mass spectrum at *m/z* 582. The cleavage of the peptide bond took place between N-formylmonomethylleucine and phenylalanine in **1**, and the 14-membered ring system yielding the ion at *m/z* 427 and the base peak at *m/z* 156. The ion *m/z* 156 further eliminates 2 × CO to give ions *m/z* 128 and *m/z* 100. The linkage of the side chain in **1** as N-formylmonomethylleucine with a 14-membered ring bound phenylalanine can be deduced by the fragment at *m/z* 156 forming the base peak and ions *m/z* 243 and 215. The α-cleavage products of **2** gave ion peaks at *m/z* 455 and the base peak at *m/z* 86. The ion *m/z* 455 further fragments to give the ion *m/z* 427. The linkage of the side chain in **2** as N-monomethylvaline with 14-membered ring-bound phenylalanine was established by the elementary composition of ions *m/z* 427, 455, 215, 187 and 86. Further fragmentation of ion *m/z* 427 obtained from compounds **1** and **2** were identical to that of the reported alkaloid, xylopyrine-C (**3**) (Singh et al., 2008) which fragments into ions *m/z* 412, 371, 308, 278, 250, 224, 135 (styrylamine), 131, 120 (phenylalanine) and 102. The elementary composition of all the fragment ions was substantiated by HRMS.

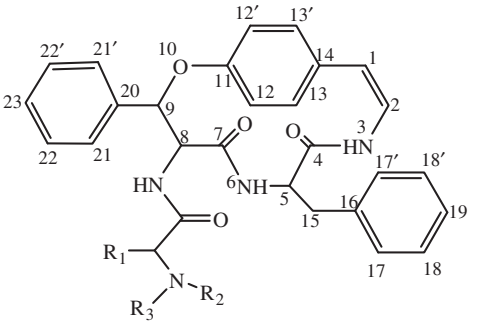
Thus, compounds **1** and **2** differ from each other only in their terminal amino acids. The identity of the ring-bound and terminal amino acids were proved to be phenylalanine and N-formylmonomethylleucine in **1** and phenylalanine and N-monomethylvaline in **2**, respectively, by acid hydrolysis of **1** and **2** and co-paper chromatographic comparison of the hydrolysate. Partial hydrolysis of compounds **1** and **2** furnished identical compound **4**. Deformylation of compound **1** furnished compound **5**. Compound **5** on methylation gave methylated product as compound **6**, which was identical to the reported alkaloid, crenatine-A (Silva, Bhakuni, Sammes, Pais, & Jarreau, 1974). Compound **2** on methylation gave a methylated compound identical to integerrissine (**7**) (Lagarias, Goff, Klein, & Rapoport, 1979).

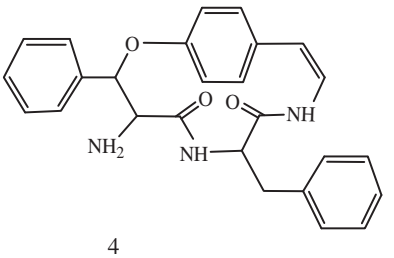
The structures of xylopyrine-G and xylopyrine-H were thus settled, respectively, as **1** and **2** (Figure 1), which was further supported by ^{13}C-NMR data.

3. Experimental

3.1. *General*

Melting points are uncorrected. UV spectra were measured on a Carry-14 spectrometer using spectral methanol. IR was recorded on Perkin–Elmer spectrophotometer model 221 in KBr pellet. MS analyses were performed on Kratos MS-50





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	R_1	R_2	R_3
1:	$CH_2CH(CH_3)_2$	CH_3	CHO
2:	$CH(CH_3)_2$	CH_3	H
3:	CH_2Ph	CH_3	H
5:	$CH_2CH(CH_3)_2$	CH_3	H
6:	$CH_2CH(CH_3)_2$	CH_3	CH_3
7:	$CH(CH_3)_2$	CH_3	CH_3

Figure 1. Structure of compounds **1**–**7**.

mass spectrometer operating at 70 eV with the evaporation of sample in the ion source at 200°C. ^{13}C-NMR spectra were taken on 125 MHz Brucker WH90 spectrometer. TLC was performed on silica gel G (Merck); paper chromatography was carried out on Whatman No. 1 paper; column chromatography was performed on silica gel columns (BDH, 60–120 mesh); solvents used for TLC: $CHCl_3$–MeOH (8 : 1) (solvent A), (2 : 1) (solvent B) and for paper chromatography: *n*-BuOH–HOAc–H_2O (4 : 1 : 5) (solvent C) and spots on paper chromatograms were visualised by ninhydrin reagent.

3.2. *Plant material*

The plant *Z. xylopyra* was collected from Mirzapur District, UP, India and identified by Prof. N.K. Dubey, Department of Botany, Banaras Hindu University, Varanasi. A specimen sample no. 222 is preserved in the department. The aerial bark was used in this study.

3.3. *Extraction and isolation*

Dried aerial bark (4 kg) was powdered and extracted repeatedly with a mixture of C_6H_6–NH_4OH–MeOH (100 : 1 : 1). The combined extract was concentrated under reduced pressure and extracted thoroughly with 7% aqueous citric acid. The acidic

solution was basified with ammonia and extracted with $CHCl_3$. The $CHCl_3$ extract was evaporated to dryness which gave a mixture of crude alkaloids (4 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 (25 : 1) and (20 : 1) were purified separately by preparative TLC with solvents A and B, which furnished the compounds xylopyrine-G (31 mg) (**1**) and xylopyrine-H (26 mg) (**2**), respectively.

3.3.1. *Xylopyrine-G (**1**)*

Compound **1** crystallised from MeOH as colourless granules, m.p. 231–233°C, R_f 0.25 (solvent A), 0.35 (solvent B), $[\alpha]_D^{25} = 230$ (c, 0.32, $CHCl_3$). It showed UV (MeOH) λ_{max}, nm ($\log \varepsilon$): 210 (4.30), 250 (3.10); IR (KBr) ν_{max} cm^{-1}: 3410 (–NH), 2965–2860 (−CH), 2790 (–OCH_3), 1680, 1635 (sec. amide), 1610 (–C=C–), 1240, 1040 (aryl ether); 125 MHz ^{13}C-NMR ($CDCl_3 + CD_3OD$) δ: 121.4 (C-1), 125-5 (C-2), 169.7 (C-4), 53.9 (C-5), 171.7 (C-7), 55.2 (C-8), 80.8 (C-9), 155.8 (C-11), 121.5 (C-12), 121.5 (C-12′), 130.4 (C-13), 130.4 (C-13′), 133.3 (C-14), 38.9 (C-15), 135.6 (C-16), 127.8 (C-17), 127.8 (C-17′), 128.7 (C-18), 128.7 (C-18′), 126.1 (C-19), 134.6 (C-20), 129.0 (C-21), 129.0 (C-21′), 130.2 (C-22), 130.2 (C-22′), 127.5 (C-23), terminal amino acid N-formylmonomethylleucine [172.4, 74.6, 25.6, 30.3, 19.2, 20.2, 171.8, 41.6]; HRMS, *m*/*z*: 582.2848 ($C_{34}H_{38}N_4O_5$), 427.1898 ($C_{26}H_{25}N_3O_3$), 412.1782 ($C_{26}H_{24}N_2O_3$), 371.1764 ($C_{24}H_{23}N_2O_2$), 308.1160 ($C_{18}H_{16}N_2O_2$), 278.1178 ($C_{18}H_{16}NO_2$), 250.1233 ($C_{17}H_{16}NO$), 243.0770 ($C_{13}H_{11}N_2O_3$), 224.1078 ($C_{15}H_{14}NO$), 215.0836 ($C_{12}H_{11}N_2O_2$), 156.1026 ($C_8H_{14}NO_2$), 135.0684 (C_8H_9NO), 131.0498 (C_9H_7O), 128.1078 ($C_7H_{14}NO$), 120.0814 ($C_8H_{10}N$), 102.0548 (C_8H_6) and 100.1128 ($C_6H_{14}N$).

3.3.2. *Deformylation of xylopyrine-G (**1**)*

Compound **1** (7 mg) was deformylated by treatment with 0.5 N HCl in MeOH at room temperature for a period of 48 h. Usual work-up and crystallisation from MeOH gave a colourless crystalline solid of compound **5** (4 mg) m.p. 185–187°C; MS, *m*/*z*: 554.2896 ($[M]^+$ $C_{33}H_{38}N_4O_4$).

3.3.3. *Methylation of compound **5***

Compound **5** (8 mg) was treated with HCHO and $NaBH_4$ by slow addition and checking the reaction product by TLC. On usual work-up and crystallisation from MeOH, it furnished the N-methylated product **6** as colourless granules (6 mg), m.p. 221–223°C; MS, *m*/*z*: 568.3052 ($[M]^+$, $C_{34}H_{40}N_4O_4$). It was identical to crenatine-A (Silva et al., 1974) (mmp, co-TLC and super-imposable IR) on comparison with authentic sample.

3.3.4. *Xylopyrine-H (**2**)*

Compound **2**, crystallised from MeOH as colourless granules, m.p. 216–218°C, R_f 0.45 (solvent A), 0.62 (solvent B); $[\alpha]^{25} = 280$ (c, 0.28, $CHCl_3$). It showed UV (MeOH) λ_{max}, nm ($\log \varepsilon$): 215 (4.10), 248 (3.20); IR (KBr) ν_{max}, cm^{-1}: 3440 (–NH), 2955–2840 (–CH), 2785 (–OCH_3), 1678, 1630 (sec. amide), 1610 (–C=C–), 1240, 1040

(aryl ether); 125 MHz ^{13}C-NMR ($CDCl_3$ + CD_3OD) δ: 121.8 (C-1), 125.8 (C-2), 170.0 (C-4), 54.1 (C-5), 172.0 (C-7), 55.1 (C-8), 80.3 (C-9), 156.0 (C-11), 121.8 (C-12), 121.8 (C-12′), 130.7 (C-13), 130.7 (C-13′), 134.0 (C-14), 39.0 (C-15), 135.0 (C-16), 128.0 (C-17), 128.0 (C-17′), 128.3 (C-18), 128.3 (C-18′), 126.5 (C-19), 133.9 (C-20), 130.2 (C-21), 130.2 (C-21′), 129.8 (C-22), 129.8 (C-22′), 127.1 (C-23), terminal amino acid N-monomethylvaline [171.9, 56.6, 30.6, 18.8, 18.6, 41.8]; HRMS, *m/z*: 540.2740 ($C_{32}H_{36}N_4O_4$), 455.1850 ($C_{27}H_{25}N_3O_4$), 427.1896 ($C_{26}H_{25}N_3O_3$), 412.1784 ($C_{26}H_{24}N_2O_3$), 371.1776 ($C_{24}H_{23}N_2O_2$), 308.1159 ($C_{18}H_{16}N_2O_3$), 278.1176 ($C_{18}H_{16}NO_2$), 250.1235 ($C_{17}H_{16}NO$), 224.1076 ($C_{15}H_{14}NO$), 215.0824 ($C_{12}H_{11}N_2O_2$), 187.0875 ($C_{11}H_{11}N_2O$), 135.0682 (C_8H_9NO), 131.0496 (C_9H_7O), 120.0812 ($C_8H_{10}N$), 102.0842 (C_8H_6), and 86.0970 ($C_5H_{12}N$).

3.3.5. *Methylation of xylopyrine-H (**2**)*

Compound **2** (8 mg) was treated with HCHO and $NaBH_4$ by slow addition and checking the reaction mixture by TLC. On usual work-up and crystallisation from MeOH, it furnished the N-methylated product **7** as colourless granules, m.p. 282–284°C; MS, *m/z*: 554.2898 ($[M]^+$, $C_{33}H_{35}N_4O_4$). It was identical to alkaloid integerrissine (Lagarias et al., 1979) (mmp, co-TLC and super-imposable IR) on comparison with an authentic sample.

3.3.6. *Hydrolysis of xylopyrine-G (**1**) and xylopyrine-H (**2**)*

Compounds **1** and **2** (6 mg each) were hydrolysed separately with 6 N HCl (1 mL) by heating in a sealed tube on oil bath at 120°C for 24 h. The hydrolysates were examined by paper chromatography (solvent C) and the spots visualised by spraying with ninhydrin. Compound **1** showed two spots on paper chromatogram for phenylalanine and monomethylleucine, whereas compound **2** exhibited spots for phenylalanine and monomethylvaline, identified by comparison with authentic samples.

3.3.7. *Partial hydrolysis of xylopyrine-A (**1**), xylopyrine-B (**2**) and xylopyrine-C (**3**)*

The partial hydrolysis of compounds **1** (6 mg), **2** (7 mg) and **3** (7 mg) was done by heating with 4 mL of a mixture of conc. HCl–HOAc–H_2O (1 : 1 : 1) separately on water bath for 5 h. On usual work-up, they furnished identical compound **4** as a colourless amorphous solid; MS, *m/z*: 407.1824 ($[M]^+$, $C_{23}H_{25}N_3O_4$). Compound **4** on hydrolysis with 6 N HCl in a sealed tube for 20 h a 120°C on oil bath gave phenylalanine (co-PC with authentic sample).

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