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Mauritine-K, A new antifungal cyclopeptide alkaloid from *Zizyphus mauritiana*

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Abstract : The cyclopeptide alkaloids, mauritine-K and sativanine-K have been isolated from the root bark of *Zizyphus mauritiana* and their structures established by spectral and chemical evidences. Mauritine-K is a new cyclopeptide alkaloid and sativanine-K is the first report from *Z. mauritiana*. Mauritine-K exhibited significant antifungal activity.

Keywords : *Zizyphus mauritiana*, rhamnaceae, mauritine-K, sativanine-K.

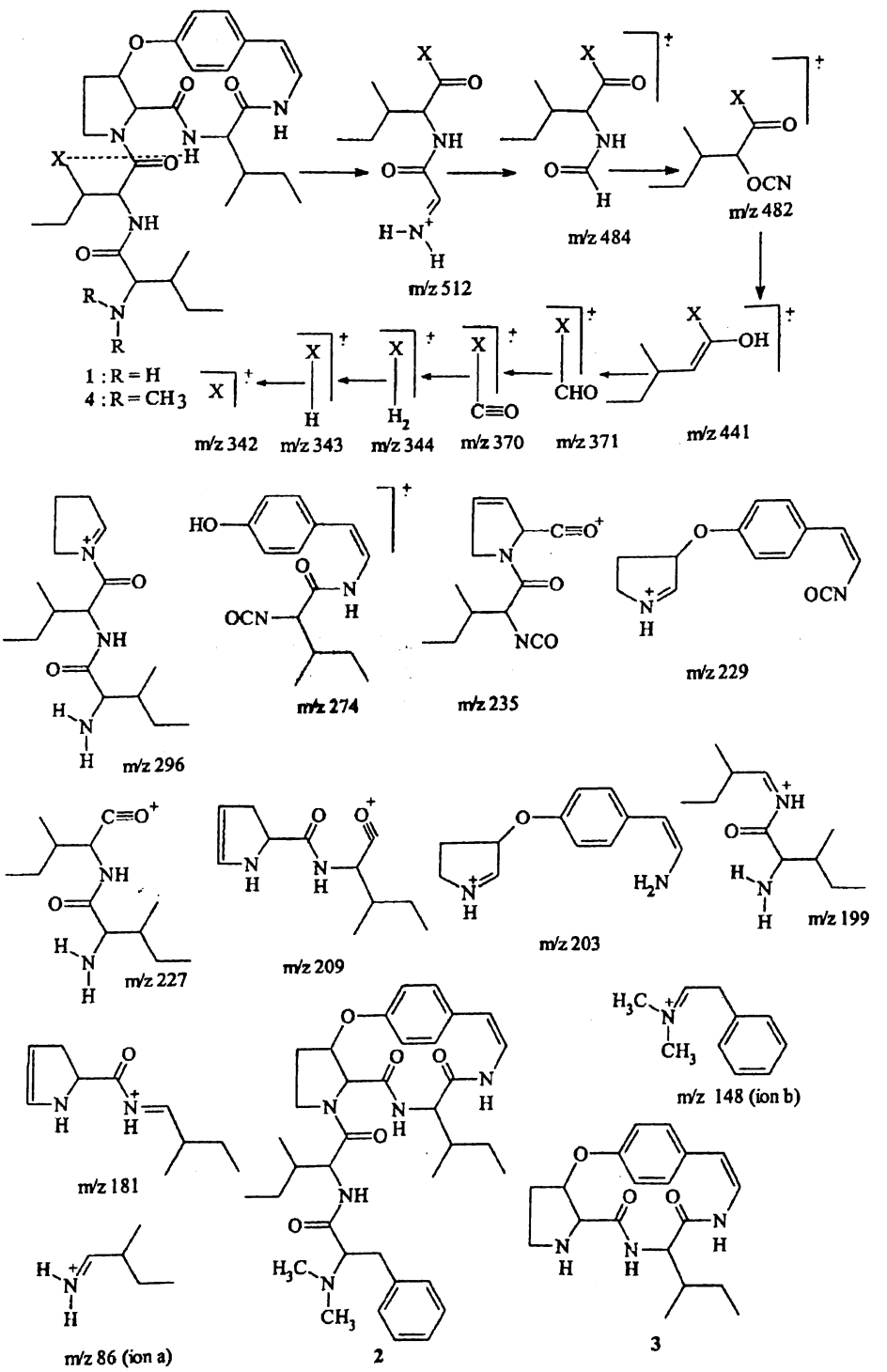
Zizyphus mauritiana Lam. (Rhamnaceae) is indigenous and naturalized throughout India, Burma and Nepal and used in Indian System of Medicine for the treatment of ulcers, stomach diseases, fever and eye complaints[1]. A number of cyclopeptide alkaloids, amphibine-A, -B, -C; mauritine-A, -B, C, -D, -E, -F, -H, -J and frangulanine have earlier been reported from this plant[2]. In view of antifungal and antibacterial activities reported for cyclopeptide alkaloids[3], the chemical studies on *Z. mauritiana* was conducted which resulted in the isolation of cyclopeptide alkaloids, mauritine-K (**1**) and sativanine-K[4].

Results and discussion

Mauritine-K, $C_{31}H_{47}N_5O_5$ ([M]$^+$, 569.3577) gave Dragendorff reaction for alkaloids. The IR spectrum of **1** was typical for peptide alkaloids and showed strong bands characteristic of secondary amide, styryl double bond, arylether and -NH groups. The UV spectrum exhibited typical strong end absorption at 203 nm and shoulder at 280 nm, characteristic of styrylamine chromophore in the 14-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[5]. In view of this fact, the HRMS of **1** was applied to elucidate the structure.

The mass fragmentation pattern of mauritine-K (**1**) closely resembled that of amphibine-D[6] (**2**). The α-cleavage product of the terminal amino acid, isoleucine in **1** and *N,N*-dimethylphenylalanine in **2** formed the base peaks respectively at *m/z* 86 (ion a) and *m/z* 148 (ion b). Like amphibine-D (**2**) the characteristic fragment for *p*-hydroxystyrylamine unit at *m/z* 135, hydroxyproline at *m/z* 96 and 68 and isoleucine at *m/z* 86 revealed the identity of the units forming the 14-membered heterocyclic ring of compound **1**. The fragment ions at *m/z* 512, 484, 482, 441, 371, 370, 344, 343, 342 respresented the whole ring system and the ions at *m/z* 296, 274, 235, 229, 227, 209, 203, 199, 181 showed the linkage of the different unit in **1**. The elementary composition of all the fragments were substantiated by HRMS. Compound **1** thus differs from **2** only in their terminal amino acids. The identity of the amino acids were proved to be isoleucine and hydroxyproline in **1** and isoleucine, hydroxyproline and *N,N*-dimethylphenylalanine in **2** by acid hydrolysis of **1** and **2** and PC comparison of the hydrolysate. Compounds **1** and **2** furnished identical compound **3** on partial hydrolysis. Methylation of **1** gave *N*-methylated product **4**, $C_{33}H_{51}N_5O_5$ ([M]$^+$, 597.3890). The structure of mauritine-K is thus settled as **1**. ^{13}C NMR data of **1** tallies with the reported data of amphibine-D[7] **2** with only difference in the attachment of terminal amino acid, thus supported the structure **1** for mauritine-K.

Antifungal activity with some plant pathogenic fungi indicated maximum spore germination inhibition (100%) in *Botrytis cinerea* at 1000 μg ml^{-1} concentration of mauritine-K, although 88.0% and 98.2% inhibition were also discerned at 200 and 500 μg ml^{-1} in the same fungus. The spore germination inhibition in *Alternaria solani* was observed by



95.5% at 1000 μg ml^{-1} dose of the chemical. More than 70% germination inhibition was observed in spores of *Alternaria mali, A. tenuissima, A. carthami, Fusarium navali* and *F. lini* at 500 μg ml^{-1}. Other concentrations were only mildly effective in inhibiting spore germination by mauritine-K. The compound sativanine-K did not show any activity.

Experimental

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 were performed on a Kratos MS-50 mass spectrometer operating at 70 eV with evaporation of sample in the ion source at 200 °C. ^{13}C NMR spectra was taken on 100 MHz NMR spectrophotometer. Column chromatography was carried out on silica gel columns (B.D.H., 60–120 mesh), TLC was performed on silica gel G (Merck). Paper chromatography was carried out on Whatmann 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); on paper chromatograms the spots were detected with ninhydrin reagent.

Table 1. Antifungal activity of mauritine-K

Fungi	Control		% Inhibition for spore germination (μg ml^{-1})		
	Water	Water + methanol	200	500	1000
Alternaria mali	22.5	20.4	44.0	75.2	90.0
A. tenuissima	24.1	20.0	40.5	70.3	86.2
A. melongena	15.5	23.0	35.2	65.7	86.5
A. carthami	18.4	20.0	38.2	72.5	88.6
A. solani	15.0	17.0	19.0	52.0	95.5
Botrytis cinerea	11.0	–	88.0	98.2	100.0
Fusarium navali	24.0	23.0	35.0	74.0	88.0
F. lini	18.2	18.2	35.2	72.4	91.0
F. oxysporum	15.0	15.0	33.0	66.2	84.0

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

Dried root 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. The acidic solution was basified with ammonia and extracted with $CHCl_3$ which furnished 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 from $CHCl_3$-MeOH (8 : 1) and (2 : 1) followed by preparative TLC with solvent A and B furnished the compounds, mauritine-K (14 mg) (**1**) and sativanine-K (20 mg).

Mauritine-K (**1**) : Alkaloid **1** crystallised from MeOH as colourless granules, m.p. 218–220 °C, R_f 0.42 (solvent A), 0.62 (solvent B). It showed UV (MeOH) λ_{max} nm : 203 (strong end absorption), 280 sh; IR (KBr) ν_{max} cm^{-1} : 3400 (-NH), 2960 (-CH), 1665 (secondary amide), 1640 (-C=C-), 1510 (aromatic), 1230, 1030 (arylether); ^{13}C NMR ($CDCl_3$ + CD_3OD) δ : 122.4 (C-1), 125.5 (C-2), 167.0 (C-4), 58.4 (C-5), 171.6 (C-7), 64.5 (C-8), 84.0 (C-9), 156.5 (C-11), 121.9 (C-12), 114.3 (C-12′), 132.2 (C-13), 132.8 (C-13′), 130.1 (C-14), 37.4 (C-15), 25.0 (C-16), 10.1 (C-17), 15.8 (C-18), 33.2 (C-19), 46.4 (C-20), 172.0 (C-22), 54.0 (C-23), 35.2 (C-24), 24.6 (C-25), 12.1 (C-26), 15.3 (C-27), 169.8 (C-29), 70.4 (C-30), 34.8 (C-31), 24.2 (C-32), 12.0 (C-33), 15.2 (C-34); HRMS, *m/z* : 569.3577 ([M]$^+$, $C_{31}H_{47}N_5O_5$), 512.2873 ($C_{27}H_{38}N_5O_5$), 484.3142 ($C_{26}H_{36}N_4O_5$), 482.2529 ($C_{26}H_{34}N_4O_5$), 441.2627 ($C_{25}H_{35}N_3O_4$), 371.1845 ($C_{20}H_{25}N_3O_4$), 370.1767 ($C_{20}H_{24}N_3O_4$), 344.1974 ($C_{19}H_{26}N_3O_3$), 343.1896 ($C_{19}H_{25}N_3O_3$), 342.1817 ($C_{19}H_{24}N_3O_3$), 296.2338 ($C_{16}H_{30}N_3O_2$), 227.1759 ($C_{12}H_{23}N_2O_2$), 235.1083 ($C_{12}H_{15}N_2O_3$), 229.0977 ($C_{13}H_{13}N_2O_2$), 199.1810 ($C_{11}H_{23}N_2O$), 209.1290 ($C_{11}H_{17}N_2O_2$), 203.1184 ($C_{12}H_{15}N_2O$), 274.1317 ($C_{15}H_{18}N_2O_2$), 186.0919 ($C_{12}H_{12}NO$), 181.1341 ($C_{10}H_{17}N_2O$), 135.0684 (C_8H_9NO), 96.0449 (C_5H_6NO), 86.0970 ($C_5H_{12}N$, base peak), 68.0500 (C_4H_6N).

Hydrolysis of mauritine-K (**1**) *and amphibine-D* (**2**) : Alkaloids **1** (6 mg) and **2** (5 mg) were heated separately in a sealed tube with 6 *N* HCl (1 ml) for 24 h at 120 °C. The hydrolysates were examined by PC (solvent C) which showed two ninhydrin positive spots identified as isoleucine and hydroxyproline in **1** and three spots identified as isoleucine, hydroxyproline and *N,N*-dimethylphenylalanine in **2** by comparison with authentic samples.

Partial hydrolysis of mauritine-K (**1**) *and amphibine-D* (**2**) : Alkaloids **1** (6 mg) and **2** (6 mg) were heated separately on water bath with 5 ml of a mixture of conc. HCl-AcOH-H_2O (1 : 1 : 1) and on usual work up they furnished identical compound **3** as colourless amorphous powder, MS, *m/z* : 343 ([M])$^+$, 274, 229, 209, 203, 181, 135, 96, 86, 68. Compound **3** on hydrolysis with 6 *N* HCl in a sealed tube for 18 h at 120 °C gave isoleucine (co-PC with authentic sample).

Methylation of mauritine-K (**1**) : Mauritine-K (11 mg) was treated with HCHO and $NaBH_4$ by adding slowly and checking the reaction product by TLC. On usual work up it furnished the *N*-methylated product **4**, $C_{33}H_{51}N_5O_5$ ([M]$^+$, 597.3890).

Sativanine-K : Sativanine-K crystallised from MeOH as colourless granules, m.p. 161–162 °C. It showed UV (MeOH) λ_{max} nm : 260 (log ε 4.01), 320 (log ε 3.77); IR (KBr) ν_{max} cm^{-1} : 3400 (-NH), 2995–2920 (-CH), 2860 (-OMe), 1635, 1690 (sec. amide), 1610 (-C=C-), 1220 (arylether); MS, *m/z* : 514.2778 ([M]$^+$, $C_{27}H_{38}N_4O_6$), 486, 401, 400, 374, 373, 372, 259, 233, 216, 209, 181, 165, 142 (base peak), 114, 96, 86, 68. It was identical as sativanine-K by comparison with authentic sample (m.m.p., co-TLC and superimposable IR).

Antifungal activity of mauritine-K :

The fungi viz. *Alternaria mali, A. tenuissima, A. melongena, A. carthami, Botrytis cinera, Fusarium navali, F. lini* and *F. oxysporum* were isolated from their respective hosts and purified by single spore isolation on Potato Dextrose Agar (peeled potato 25 g + dextrose 20 g + agar 15 g and distilled water 1000 ml). Sporulating cultures 7–9 days old were used for the experiment. Mauritine-K was dissolved in a few drops of methanol, then required quantity of sterile distilled water was added to give the desired concentrations (200, 500 and 1000 μg ml^{-1}) and methanol was evaporated by placing the vials on a boiling water bath. About 200 spores of the fungi were placed in greese free glass slides with a drop of the chemical solution. The slides were placed in moist chambers and incubated at 25 ± 2 °C for 24 h. Germinated spores were counted after staining with lactophenol prepared in cotton blue and percent inhibition of different fungi was calculated. The results are mentioned in Table 1.

References

1. K. R. Kirtikar and B. D. Basu, "Indian Medicinal Plants", eds. E. Blatter, J. F. Caisus and K. S. Mahasker, 1984, Vol. I, p. 589.
2. A. K. Singh, M. Tripathi, V. P. Singh and V. B. Pandey, *Oriental J. Chem.*, 2002, **18**, 399.
3. R. Tschesche and E. U. Kaussmann, "The Alkaloids", ed. R. H. F. Manske, Academic Press, New York, 1975, p. 165.
4. A. H. Shah, M. A. Al-Yahya, S. Devi and V. B. Pandey, *Phytochemistry*, 1987, **26**, 1230.
5. H.-W. Fehlhaber, *Zeitschrift fur Analytische Chemie*, 1968, **235** (Band 1), 91.
6. R. Tschesche, E. U. Kaussmann and H.-W. Fehlhaber, *Chem. Ber.*, 1972, **105**, 3094.
7. B. H. Han, M. H. Park and Y. N. Han, *Phytochemistry*, 1990, **29**, 3315.