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AN *N*-FORMYL CYCLOPEPTIDE ALKALOID FROM *ZIZYPHUS NUMMULARIA* BARK

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Key Word Index—*Zizyphus nummularia*; Rhamnaceae; bark; mauritine-A; scutianine-C; frangufoline; nummularine-T; *N*-formyl cyclopeptide alkaloid.

Abstract—A 13-membered *N*-formylcyclopeptide alkaloid, nummularine-T, has been isolated from the bark of *Zizyphus nummularia* and its structure established by spectroscopic and chemical methods. It provides the fourth example of a naturally occurring *N*-formyl cyclopeptide alkaloid.

INTRODUCTION

Zizyphus nummularia is a small, thorny bush distributed in north western India, various medicinal properties are attributed to it in the Indian system of medicine [1, 2]. In our continuing search for cyclopeptide alkaloids from the bark of *Z. nummularia* [3–6], we report the isolation and characterization of mauritine-A, scutianine-C, frangufoline and a new 13-membered *N*-formyl cyclopeptide alkaloid, designated nummularine-T (**1**).

Column chromatography of the methanol fraction of the crude bases and repeated preparative TLC furnished alkaloid, nummularine-T (**1**) as granules. It gave a very faint colour with Dragendorff's reagent. The molecular formula was determined by high resolution mass spectrometry as $C_{33}H_{41}N_5O_7$. The IR spectrum exhibited bands for –NH, OMe, –NH–CO, C=C and Ar–O–C. The UV spectrum showed *A* maxima at 318 and 267 nm typical for a styrylamine chromophore of 13-membered cyclopeptide alkaloids [7]. The ^{1}H NMR spectrum showed the presence of three Me (δ0.56, 0.68, 1.23), one NMe (δ2.88) and one Me (δ3.78) groups together with one olefinic H(δ5.98) and eight Ar-H, three NH (δ3.78), one *N*-formyl H and one olefinic H (δ6.65–7.75).

Like the *N*-formyl cyclopeptide alkaloid sativanine-F (**2**) [8], the mass spectrum of **1**, which contains an *N*-formyl group on the terminal amino acid, exhibited an intense molecular ion peak at *m/z* 619, and the usual α-cleavage of products were absent. The base peak was produced at *m/z* 114 (ion a) by the cleavage of a peptide bond between *N*-formyl monomethylalanine and valine which further eliminates two carbonyl groups to give ions at *m/z* 86 (ion b) and *m/z* 58 (ion c), respectively. Fragment *m/z* 213 (ion d) formed by the total breakage of the side chain indicated the linkages of the side chain intermediate amino acid valine with *N*-formyl monomethylalanine. The counterpart of the fragment *m/z* 114 is *m/z* 506 (ion e) which is further fragmented into ions identical to sativanine-F (**2**). These data demonstrated the structure of the macrocyclic ring system consisting of hydroxyproline, methoxyhydroxystyrylamine and ring-bound phenylalanine. They further indicate that the terminal amino acid, *N*-formyl monomethylalanine, is attached to valine which is further attached to the proline unit of the macrocyclic ring system. The elementary composition of major fragments was substantiated by high resolution mass measurements.

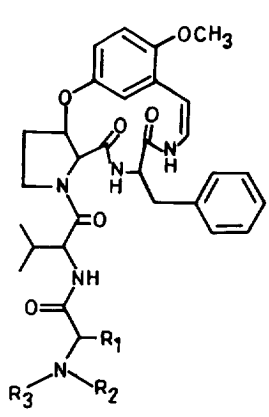
The attachment of phenylalanine bound to the nitrogen of the styrylamine function of **1** was achieved by partial hydrolysis. On heating with HCl–HOAc–H_2O (1:1:1) at 100° for 5 hr, **1** furnished a major compound **5** [9] which on further hydrolysis with 6 M HCl gave phenylalanine. Total hydrolysis of **1** furnished phenylalanine, valine and *N*-monomethylalanine identified, by co-PC with authentic samples.

The structure **1** was further supported by deformylation of **1** with 0.5 M HCl in methanol when it gave alkaloid **3**. Alkaloid **3** on methylation with formaldehyde and sodium borohydride furnished alkaloid **4**. Alkaloids **3** and **4** were identified as nummularine-B [3] and amphibine-H [10], respectively. In order to ensure that **1** was not an artifact produced during extraction with methanol, another sample of the bark was extracted with benzene–ammonia–ethanol, and **1** was again isolated, proving that it is naturally occurring.

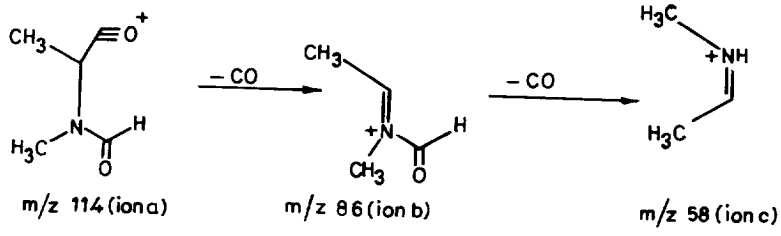
Column chromatography of the chloroform–methanol (4:1) fraction and preparative TLC furnished 14-membered cyclopeptide alkaloids mauritine-A [11], scutianine-C [12] and frangufoline [13] which were identified by spectral analysis and comparison with authentic samples.

EXPERIMENTAL

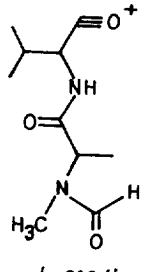
TLC plates were prepd with silica gel G (Merck). Solvents used for TLC and prep. TLC were $CHCl_3$–MeOH (3:1) (solvent A), $CHCl_3$–EtOAc–



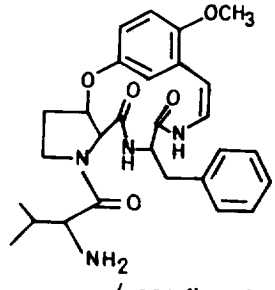
	R_1	R_2	R_3
1.	CH_3	$-C(=O)H$	CH_3
2.	$CH(CH_3)_2$	$-C(=O)H$	H
3.	CH_3	H	CH_3
4.	CH_3	CH_3	CH_3



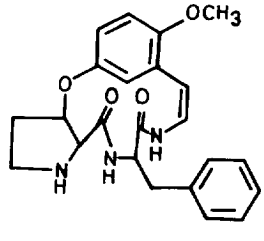
m/z 114 (ion a) m/z 86 (ion b) m/z 58 (ion c)



m/z 213 (ion d)



m/z 506 (ion e)



(5)

MeOH (10:8:1) (solvent B), $CHCl_3$–EtOAc–MeOH (1:1:1) (solvent C) and for PC: *n*-BuOH–HOAc–H_2O (4:1:5) (solvent D). Dragendorff's reagent and ninhydrin were used as developing reagents for alkaloids and amino acids, respectively.

Zizyphus nummularia was collected from Mirzapur District, U.P., India and its authenticity was verified by the Department of Botany, Banaras Hindu University, Varanasi. A specimen sample is kept in the Department.

Extraction. The crude alkaloids (15 g) were extracted from the dried bark of *Z. nummularia* (12 kg) by usual methods [11] and then subjected to systematic fractionation on a silica gel column eluted with solvents of increasing polarity into four major frs, namely C_6H_6, $CHCl_3$, $CHCl_3$–MeOH (4:1) and MeOH frs. The $CHCl_3$–MeOH (4:1) fr. was chromatographed on a silica gel column, and prep. TLC of the eluted fractions furnished mauritine-A (22 mg), mp 102–104°, scutianine-

C (18 mg), mp 264–266° and frangufoline (20 mg), mp 242–244°. These alkaloids were identified by spectral analysis (IR, UV, ^{1}H NMR, HRMS) and direct comparison with authentic samples (mmp, co-TLC and superimposable IR).

Nummularine-T (**1**). Chromatography of the MeOH fraction over silica gel column followed by repeated prep. TLC of the eluted fractions gave nummularine-T (28 mg). It crystallized from MeOH as granules, mp 188–190°, $C_{33}H_{41}N_5O_7$ ([M]$^+$, *m/z* 619.2988), R_f 0.25 (solvent A), 0.40 (solvent B), 0.52 (solvent C); IR ν_{max}^{KBr} cm^{-1}: 3400 (–NH), 2865 (–OMe), 2775 (–NMe), 1680, 1655 (sec amide group), 1620 (C=C), 1220, 1050 (phenol ether); UV λ_{max}^{MeOH} nm: 267, 318 (log ε 3.60, 3.40); ^{1}H NMR (90 MHz $CDCl_3$): δ0.56 (3H, *d*, *J* = 7 Hz, C-Me), 0.68 (3H, *d*, *J* = 7 Hz, C-Me), 1.23 (3H, *d*, *J* = 7 Hz, C-Me), 1.38–1.70 (1H, *m*, C-H), 2.15–2.95 (3H, *m*, CH_2, C-H), 2.88 (3H, *s*, N-Me), 3.18–3.66 (2H, *m*, $-CH_2$) 3.78 (3H, *s*, Ar-OMe), 4.22 (2H, *m*, CH_2), 4.44–5.12 (3H, *m*, CH), 5.37 (1H, *m*, CH), 5.98 (1H, *d*, *J* = 9 Hz, olefinic-H), 6.65–8.92 (13H, *m*, 8 Ar-H, 3NH, 1 formyl H, 1 olefinic H); HRMS: 619.2988 (76%, $C_{33}H_{41}N_5O_7$), 591.2925 (2, $C_{32}H_{41}N_5O_6$), 506 (1.5) 491 (1.5), 435 (1.5), 434 (1), 408.1888 (3, $C_{23}H_{26}N_3O_4$), 407.1829 (10, $C_{23}H_{25}N_3O_4$), 406 (2), 259.1280 (15, $C_{14}H_{15}N_2O_3$), 243.1142 (3, $C_{14}H_{15}N_2O_2$), 233.1290 (4, $C_{13}H_{17}N_2O_2$), 221.0923 (3, $C_{11}H_{13}N_2O_3$), 216 (6), 215.1184 (5, $C_{13}H_{15}N_2O$), 213.1236 (25, $C_{10}H_{17}N_2O_3$), 195.1125 (7, $C_{10}H_{15}N_2O_2$), 185.1290 (17, $C_9H_{17}N_2O_2$), 114.0557 (100, base peak, $C_5H_8NO_2$), 96.04444 (5, C_5H_6NO), 86.0606 (75, C_4H_8NO), 58.0673 (48, C_3H_8N).

Compound **1** (12 mg) was heated at 100° with 4 ml of a mixt. of conc HCl–HOAc–H_2O (1:1:1) for 4 hr. The hydrolysed product was extracted with $CHCl_3$ and the major compound (**5**) was separated by prep. TLC (solvent A); as amorphous solid, R_f 0.45 (solvent A); MS: *m/z* 407 ([M]$^+$, $C_{23}H_{25}N_3O_4$) 338, 259, 233, 232, 216, 215, 165, 120, 96. Acid hydrolysis of **5** with 6 M HCl in a sealed tube for 18 hr at 125° gave phenylalanine (co-PC). Compound **1** (4 mg) was hydrolysed with 6 M HCl in a sealed tube for 20 hr and the hydrolysate was examined by PC (solvent D) using ninhydrin as spray reagent. Phenylalanine, valine and *N*-monomethylalanine were identified by comparison with authentic samples.

Alkaloid **1** (10 mg) was deformylated by treatment with 0.5 M HCl in MeOH at room temp. for 40 hr. It was purified by prep. TLC (solvent C) and crystallization from MeOH furnished alkaloid **3**, mp 226–228° which was identified as nummularine-B (mmp, co-TLC and superimposable IR). Alkaloid **3** (6 mg) was treated with HCHO and $NaBH_4$ and the reduction product was purified by prep. TLC and crystallized from MeOH which gave the *N*-methylated product **4**, mp 201–203°. Alkaloid **4** was identified as amphibine-H by direct comparison with authentic sample (mmp, co-TLC and superimposable IR).

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REFERENCES

1. Chopra, R. N., Nayar, S. L. and Chopra, I. C. (1956) *Glossary of Indian Medicinal Plants*, p. 261. C.S.I.R., New Delhi.
2. Kirtikar, K. R. and Basu, B. D. (1975) *Indian Medicinal Plants*, Vol. 1, p. 592. (Blatter, E., Caisus, J. F. and Mhaskar, K. S., eds).
3. Pandey, V. B., Singh, J. P., Seth, K. K., Shah, A. H. and Eckhardt, G. (1984) *Phytochemistry* **23**. 2118.
4. Pandey, V. B., Dwivedi, S. P. D., Shah, A. H. and Eckhardt, G. (1986) *Phytochemsitry* **25**, 2690.
5. Dwivedi, S. P. D., Pandey, V. B., Shah, A. H. and Eckhardt, G. (1987) *J. Nat. Prod.* **50**, 235.
6. Shah, A. H., Khan, R. M. A., Maurya, S. K., Singh, V. P. and Pandey, V. B. (1989) *Phytochemsitry* **28**, 305; Corrigendum (1989) *Phytochemistry* **28**, 3582.
7. Tschesche, R., David, S. T., Uhlendorf, J. and Fehlhaber, H. W. (1972) *Chem. Ber.* **105**, 3106.
8. Shah, A. H., Pandey, V. B., Eckhardt, G. and Tschesche, R. (1985) *Phytochemistry* **24**, 2768.
9. Tripathi, Y. C., Maurya, S. K., Singh, V. P. and Pandey, V. B. (1989) *Phytochemsitry* **28**, 1563.
10. Devi, S., Pandey, V. B., Singh, J. P. and Shah, A. H. (1987) *Phytochemsitry* **26**, 3374.
11. Tschesche, R., Wilhelm, H. and Fehlhaber, H. W. (1972) *Tetrahedron Letters* 2609.
12. Tschesche, R. and Ammermann, R. (1974) *Ber. Chem.* **107**, 2274.
13. Tschesche, R., Elgamal, M., Miana, G. A. and Eckhardt, G. (1975) *Tetrahedron* **31**, 2944.