(ion **a**) (45), 205 (ion **e**) (55), 202 (ion **b**) (30), 187 (ion **f**) (25), 165 (ion **c**) (52), 147 (ion **d**) (30) and 69 $[C_5H_9]^+$ (100).

*Tetrahydronimbaflavone* (**4**). Compound **2** (50 mg) in MeOH (20 ml) was stirred with Pd–C (10 mg) in presence of $H_2$ gas at room temp. for 6 hr, filtered and processed to yield **4** (50 mg), mp 130°, $C_{26}H_{34}O_5$ ($M^+$ 426 *m/z*); IR $\nu_{max}$ $cm^{-1}$: 3338, 3000, 1650, 1620, 1510, 1460, 1380, 1276, 1220, 1140, 1090 and 840. $^1H$ NMR: $\delta$0.8, 0.85, 0.9, 0.95 (4 × Me), 1.2–1.4 (4H, *m*, side chain methylenes), 2.5 (2H, *m*, methine), 3.7 (*s*, OMe), 5.2 (1H, *d*, *d*, *J* = 10 Hz and 4 Hz H-2), 5.85 (1H, *s*, Ar-H-6), 6.7 (1H, *d*, *J* = 10 Hz, H-5'), 7.1–7.25 (2H, *m*, H-2' and H-6'). MS *m/z* (rel. int.): 426 $[M]^+$ (55), 369 $[M - C_4H_9]^+$ (50), 222 (ion **a**) (~ 5), 204 (ion **b**) (45), 165 (ion **c**) (80), 147 (ion **d**) (30).

3-*Deacetyl salannin* (**3**). The fractions eluted with $C_6H_6$–$CHCl_3$ (1:2) and $CHCl_3$ (monitored TLC) on removal of solvent yielded a tetranortriterpenoid (250 mg), mp 195–200°; which was found to be identical (mp, IR, $^1H$ NMR and MS [6] with 3-deacetyl salannin (**3**).

*Acknowledgements*—Thanks are due to Dr. K. P. Madhusudanan for the mass spectra and to Mrs. K. Kapoor for $^1H$ NMR and decoupling experiments. We are also grateful to Dr. S. P. Popli for his interest in the work.

## REFERENCES

1. Ekong, D. E. U. (1967) *Chem. Commun.* 808.
2. Shirataki, Y., Komatsu, M., Yokoe, I. and Manaka, A. (1981) *Chem. Pharm. Bull.* **29**, 3033.
3. Sharif, E. A., Gupta, R. K. and Krishnamurti, M. (1980) *Tetrahedron Letters* 641.
4. Bohm, B. A. (1975) *The Flavonoids* (Harborne, J. B., Mabry, T. J. and Mabry, H., eds.) pp. 561–631. Chapman & Hall, London.
5. Basak, S. P. and Chakraborty, D. P. (1968) *J. Ind. Chem. Soc.* 466.
6. Kraus, W. and Cramer, R. J. (1981) *Liebigs Ann. Chem.* 181.

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# CYCLOPEPTIDE ALKALOIDS FROM *ZIZYPHUS NUMMULARIA*

V. B. PANDEY, J. P. SINGH, K. K. SETH, A. H. SHAH* and G. ECKHARDT†

Department of Medicinal Chemistry, Institute of Medical Sciences, Banaras Hindu University, Varanasi, India; *Department of Chemistry, Gomal University, D. I. Khan, Pakistan; †Institut für Organische Chemie und Biochemie der Universität Bonn, Gerhard Domagk Str.-1, W. Germany

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**Key Word Index**—*Zizyphus nummularia*; Rhamnaceae; peptide alkaloids; nummularine-M; nummularine-N, nummularine-B.

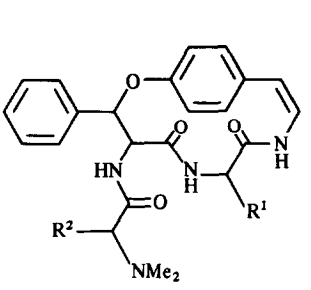
**Abstract**—In addition to the known alkaloid nummularine-B, two new peptide alkaloids nummularine-M and nummularine-N have been isolated from *Zizyphus nummularia* and their structures elucidated. Nummularine-M is a 14-membered cyclopeptide and belongs to the integerrinine type, whereas nummularine-N is a 13-membered cyclopeptide like nummularine-B.

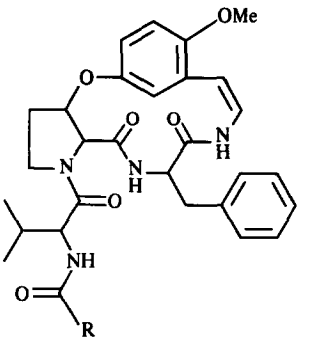
## INTRODUCTION

In continuation of our work on cyclopeptide alkaloids from the plants belonging to the Rhamnaceae we now report the isolation of two new cyclopeptide alkaloids from *Zizyphus nummularia*. About a dozen cyclopeptide alkaloids have so far been reported from the root bark of *Z. nummularia* [1–3]. We report here the alkaloids of the stem bark of this plant which has not yet been investigated. Repeated column chromatography of the alkaloid fraction on silica gel followed by prep. TLC gave small amounts of two new cyclopeptide alkaloids, nummularine-M [4] and nummularine-N along with a known peptide alkaloid, nummularine-B [1].

## RESULTS AND DISCUSSION

Nummularine-M, $C_{31}H_{42}N_4O_4$ ($[M]^+$ *m/z* 534.3190) was recognised to be a 14-membered cyclopeptide alkaloid from its UV spectrum [5]. The IR spectrum exhibited bands for –NH, –NMe, –NH–CO and Ar–O–C. It is isomeric with integerrinine (**4**) [5] and both molecules show identical mass fragmentation patterns indicating their gross structural similarity. However, acid hydrolysis revealed the essential difference between the two molecules. Thus, while integerrinine yields *N*,*N*-dimethylisoleucine and leucine on acid hydrolysis, nummularine-M gives *N*,*N*-dimethylisoleucine and isoleucine. Based on these findings the structure of





**1** $R^1=R^2=$ Me—$CH_2$—CH(Me)—

**4** $R^1=$ CH(Me)—$CH_2$—

$R^2=$ Me—$CH_2$—CH(Me)—

**2** R = N(Me)—$CH_2$—

**3** R = N(H)—CH(Me)—

nummularine-M was proved to have the structure **1**, which differs from that of integerrinine in having an isoleucine unit instead of leucine as an amino acid bound to the nitrogen of the styrylamine function.

Nummularine-N, $C_{31}H_{41}N_5O_6$ ([M]$^+$ *m/z* 591), showed the presence of a 13-membered ring cyclopeptide alkaloid system in its UV spectrum [1]. The IR spectrum exhibited bands for –NH, –OMe, –NMe, amide, >C=C< and phenol ether. On acid hydrolysis it gave phenylalanine, valine and *N,N*-dimethylglycine. The $^1$H NMR spectrum showed a close similarity to that of nummularine-B (**3**), with the exception that the former exhibited a signal for an additional N–Me group and the absence of a *sec.* C–Me group. The mass spectrum was identical to that of nummularine-B. Examination of spectral data and hydrolysis experiment shows that nummularine-N possesses structure **2** which differs from nummularine-B only in having a *N,N*-dimethylglycine instead of a N-methylalanine as the end amino acid.

This is the first report of the presence of the two new peptide alkaloids, nummularine-M, nummularine-N and the known peptide, nummularine-B, in stem bark of *Z. nummularia.*

## EXPERIMENTAL

Stem bark of *Z. nummularia* (5 kg) collected from Mirzapur district, U.P., India was repeatedly extracted with a mixture of $C_6H_6$, $NH_4OH$ and MeOH in the ratio of 100:1:1, respectively. The bases were separated from the combined $C_6H_6$ extracts by shaking with 5% aq. citric acid. The aq. layer was basified with $NH_4OH$ and extracted with $CHCl_3$ and the crude alkaloids (2.8 g) obtained. The mixture of crude alkaloids was chromatographed on a silica gel (100 g) column, eluting with increasingly polar $CHCl_3$–MeOH mixtures into eight fractions. The collected fractions were analysed by TLC proving in every case to be mixtures of two or three components. The pure compounds were separated by repeated prep. TLC of the above fractions using $CHCl_3$–EtOAc–MeOH (1:1:1.5, solvent A) and $C_6H_6$–EtOAc–MeOH (30:15:4).

*Nummularine-M.* Amorphous powder (8.5 mg), mp 263–265° (uncorr.); $[\alpha]_D$-46.66° (*c* 0.1, $CHCl_3$), $R_f$ 0.85 (solvent A); UV (MeOH) strong end absorption and shoulders at 250 and 280 nm; IR $\nu_{max}^{KBr}$ cm$^{-1}$: 3300 (–NH), 2790 (–NMe), 1685, 1635 (NH–CO), 1240 (Ar–O–C); MW: high resolution MS *m/z* 534.3190; Calcd. for $C_{31}H_{42}N_4O_4$: 534.3174; MS: *m/z* 534 [M]$^+$, 477, 378, 337, 274, 244, 229, 224, 216, 201, 135, 131, 114 (base peak). The elementary composition of all fragments were substantiated by high resolution measurements. The alkaloid (3.5 mg) was hydrolysed with 6 N HCl (10 hr) in a sealed tube. The hydrolysate was evapd to dryness and examined by PC (*n*-BuOH–HOAc–$H_2O$, 4:1:5). *N,N*-Dimethylisoleucine and isoleucine were identified by comparison with authentic materials.

*Nummularine-N.* Bright colorless crystals (15 mg), mp 243–245° (uncorr.); $R_f$ 0.73 (solvent A); UV $\lambda_{max}^{MeOH}$ nm (log $\varepsilon$): 267 (4.00) and 320 (3.8); IR $\nu_{max}^{KBr}$ cm$^{-1}$: 3300 (–NH), 2821 (–OMe), 2775 (–NMe), 1688 and 1640 (amide), 1610 (>C=C<), 1200 and 1020 (phenol ether); $^1$H NMR ($CDCl_3$): $\delta$0.68 (*dd*, *J* = 5.0 Hz, 2C–Me groups), 2.48 (*s*, 2–NMe), 3.80 (*s*, 1–OMe), 6.00 (*d*, *J* = 7.5 Hz, 1 olefinic H), 6.7–8.6 (*m*, aromatic protons, –NH and 1 olefinic H); MS: *m/z* 591 [M]$^+$, 548, 534, 532, 491, 435, 434, 408, 406, 338, 259, 243, 233, 216, 165, 157, 96, 68, 58 (base peak). The alkaloid (4 mg) was hydrolysed with 6 N HCl (10 hr) in a sealed tube and the hydrolysate was examined by PC (*n*-BuOH–HOAc–$H_2O$, 4:1:5). Phenylalanine, valine and *N,N*-dimethylglycine were identified by comparison with authentic materials.

*Nummularine-B* (8 mg) was also obtained and identified by spectroscopic methods and chromatographic comparison with an authentic sample in several solvent systems.

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## REFERENCES

1. Tschesche, R., Miana, G. A. and Eckhardt, G. (1974) *Chem. Ber.* **107**, 3180 and references cited therein.
2. Tschesche, R., Elgamal, M, Miana, G. A. and Eckhardt, G. (1975) *Tetrahedron* **31**, 2944.

3. Tschesche, R., Elgamal, M. and Eckhardt, G. (1977) *Chem. Ber* **110**, 2649.
4. Presented in the conference 'Erster Gesamt-kongress der Pharmazeutischen Wissenschaften' in the name of nummularidine (now designated as nummularine-M) held at Munich (1983). Abstract p. 121.
5. Tschesche, R., Rheingans, J., Fehlhaber, H.-W. and Legler, G. (1967) *Ber.* **100**, 3924.

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# SATIVANINE-G, A CYCLOPEPTIDE ALKALOID FROM *ZIZYPHUS SATIVA*

A. H. SHAH, V. B. PANDEY*, J. P. SINGH*, K. N. SINGH* and G. ECKHARDT†

Department of Chemistry, Gomal University, D.I. Khan, Pakistan; *Department of Medicinal Chemistry, Institute of Medical Sciences, Banaras Hindu University, Varanasi, India; †Institut für Organische Chemie und Biochemie der Universität, Bonn, Gerhard Domagk Str.-1, W. Germany

(*Received* 7 *December* 1983)

**Key Word Index**—*Zizyphus sativa*; Rhamnaceae; peptide alkaloid; sativanine-G.

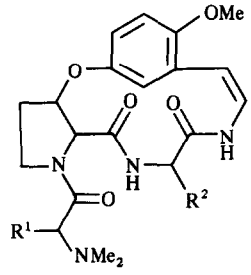
**Abstract**—In addition to the already described peptide alkaloids from the bark of *Zizyphus sativa*, a new compound of this class, sativanine-G, has been isolated and its structure elucidated. This alkaloid contains a 13-membered ring system and belongs to the nummularine-C class.

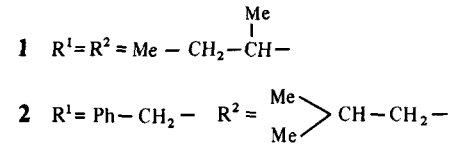
## INTRODUCTION

In continuation of our work on cyclopeptide alkaloids from the family Rhamnaceae we now report a new alkaloid from *Zizyphus sativa*. The alkaloids frangulanine [1], nummularine-B [2], mucronine-D [3], sativanines-A, -B [4], -C [5], -D, -E and -F [6] have earlier been reported from this plant. We have now isolated a new alkaloid, sativanine-G, by repeated chromatography and prep. TLC of the alkaloid fraction isolated from the stem bark.

## RESULTS AND DISCUSSION

Sativanine-G, mp 92°, $C_{28}H_{42}N_4O_5$ ([M]$^+$ *m/z* 514.3168) was recognised to be a 13-membered cyclopeptide alkaloid from its UV spectrum [3]. The IR spectrum exhibited bands for –NH, *sec.* amide, –OMe, –NMe, >C=C< and aryl ether. On acid hydrolysis it gave *N,N*-dimethylisoleucine and isoleucine. Mass spectral peaks of sativanine-G correspond with those of nummularine-C [2] with the only exception that the base peak of the former is 34 mu lower than that of the latter. These data reveal that sativanine-G possesses the structure **1** which differs from nummularine-C (**2**) by having *N,N*-dimethylisoleucine instead of *N,N*-dimethylphenylalanine as the end amino acid and isoleucine instead of leucine as the amino acid residue bound to the styrylamine moiety. Sativanine-G is a new addition to the growing list of 13-membered cyclopeptide alkaloids which belong to the nummularine-C type.





**1** $R^1 = R^2 =$ Me – $CH_2$–CH(Me)–

**2** $R^1$ = Ph–$CH_2$– $R^2$ = (Me)(Me)CH–$CH_2$–

## EXPERIMENTAL

Mps are uncorr IR and UV were determined in KBr and MeOH, respectively. MS analysis was performed at 70 eV with evapn of the sample in the ion source at *ca* 200°. TLC was done on silica gel Merck $60F_{254}$

*Extraction and isolation.* Bark of *Z. sativa* Gaertn [7, 8] was collected in Hazara District, Pakistan. Extraction of plant material (10 kg) was carried out in the usual manner [9] and semi-solid crude alkaloids (6.6 g.) were obtained. The alkaloid mixture was fractionated on a silica gel M (900 g, Geb. Herrmann/Köln) column, eluting with increasingly polar $CH_2Cl_2$–MeOH mixtures into 20 fractions. The chromatographic separation was followed by UV monitoring and collected fractions were analysed by TLC proving in every case to be a mixture of two or three main components The fractions were separated into individual components by prep TLC and repeated CC Sativanine-G (6 mg)