Phytochemistry, Vol. 24, No. 11, pp. 2768–2770, 1985.
Printed in Great Britain.

0031–9422/85 $3.00 + 0.00

AN N-FORMYL CYCLOPEPTIDE ALKALOID FROM THE BARK OF *ZIZYPHUS SATIVA**

A. H. SHAH†, V. B. PANDEY‡, G. ECKHARDT§ and R. TSCHESCHE§

Department of Chemistry, Gomal University, D. I. Khan, Pakistan; ‡Department of Medicinal Chemistry, IMS, BHU, Varanasi, India; §Institute of Organic Chemistry and Biochemistry, Bonn University, Gerhard-Domagk Str. 1, 5300 Bonn, West Germany

(*Received* 21 *February* 1985)

Key Word Index—*Zizyphus sativa*; Rhamnaceae; bark; alkaloids; sativanine F; N-formyl cyclopeptide alkaloid.

Abstract—From the bark of *Zizyphus sativa*, previously undescribed cyclopeptide alkaloid, sativanine-F has been isolated. The structure was deduced by spectroscopic methods and chemical degradation. It is a 13-membered cyclopeptide alkaloid and provides the first example of such a naturally occurring N-formyl cyclopeptide alkaloid.

INTRODUCTION

Zizyphus sativa Gaertn is a small tree native to the Hazara district of Pakistan where it grows wild in hilly areas [2]. The dried fruits of this plant are commonly used for bronchitis while the bark is used to heal ulcers and wounds [3]. In continuation of our work on the bark of *Z. sativa* [4–7] we now report the isolation and characterisation of a new 13-membered cyclopeptic alkaloid, sativanine F. Sativanine F(**1**) provides the first example of the occurrence of a N-formyl cyclopeptide alkaloid in plants.

RESULTS AND DISCUSSION

The alkaloid was isolated by consecutive TLC and HPLC from fraction-8 of the CC [8]. It gave a very faint colour with Dragendorff's reagent. The IR spectrum showed typical absorptions for cyclopeptide alkaloids and the UV spectrum gave characteristic absorption maxima at 320 and 268 nm for 13-membered cyclopeptide alkaloids [9].

The mass spectrum of this new cyclopeptide alkaloid (Scheme 1) which carries a N-formyl group on the terminal amino acid differs fundamentally from the spectra of all other known cyclopeptide alkaloids. Since **1** contains no basic amino group, the ionized molecule is fairly stable, thus giving an intense $[M]^+$, and the usual α-cleavage products **a** and **b** are absent. Instead of this the base peak is produced by the cleavage of the peptide bond between N-formylvaline and valine yielding the ion *m/z* 128 which then eliminates 2 × CO to give *m/z* 100 and *m/z* 72. The same cleavage with a hydrogen rearrangement gives the counterpart *m/z* 506. The peaks *m/z* 68, 72, 120 and 165 indicate the presence of hydroxyproline, valine, phenylalanine and a methoxyhydroxystyrylamine group, respectively. The composition of the side chain can be deduced from the fragment **l**, while **n** and **o** prove the attachment of hydroxyproline to the side chain. Hydroxyproline is joined to *o*-methoxy-*p*-hydroxystyrylamine by an ether linkage, as can be seen from the peaks **r**, **s**, **t** and also to phenylalanine by an amide bonding as can be deduced from the fragments **p** and **q**. The whole ring system is represented by the ions **e**, **f**, **h** and **i**. The elementary composition of all fragments was substantiated by high resolution mass measurements.

In order to ensure that **1** is not an artefact produced during extraction with methanol another portion of the bark was extracted with benzene–ammonia–EtOH. **1** was isolated again which proves that it is naturally occuring. Besides **1** a homologue N-methyl sativanine F (**2**) with N-formyl-N-methyl valine as the end group was detected by mass spectrometry. This compound could not be isolated in sufficient amounts for further investigation.

In order to investigate the mass spectral behaviour of N-formyl cyclopeptide alkaloids further, the derivatives **3** from sativanine C [6], **4** from nummularine B [10] and **5** from mauritine C [11] were prepared. They gave the same characteristic fragmentation.

In the acid hydrolysate of **1** valine and phenylalanine were confirmed by PC and comparison with authentic samples.

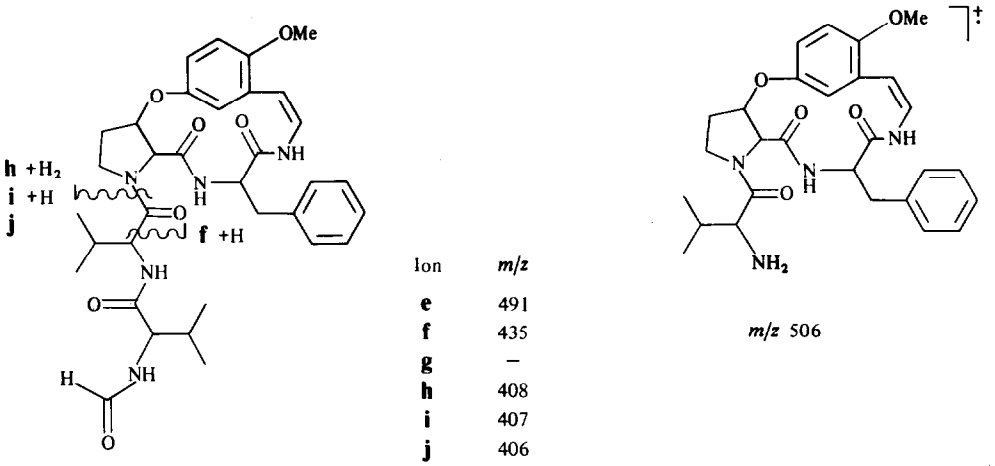
EXPERIMENTAL

Mps are uncorr. MS analyses were performed at 70 eV with evaporation of the sample in the ion source at *ca* 200°. TLC was done on silica gel Merck 60 F_{254}. For HPLC Zorbox-sil 4.6 mm × 25 cm was used.

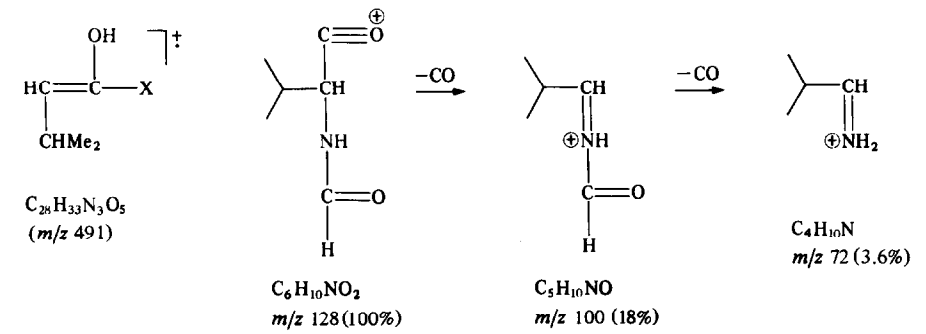
Extraction and isolation. Plant material was collected from the Hazara District of Pakistan. Crude alkaloids (6.6 g) were obtained by extraction of the powdered bark (10 kg) with MeOH in the usual manner [12]. Another (5 kg) plant material was extracted with a solvent mixture of C_6H_6–ammonia–EtOH (100:1:1). The mixture of crude alkaloids was fractionated on a 900 g silica gel M (Gebr. Herrman/Köln) column, eluting with increasingly polar CH_2Cl_2–MeOH mixtures, into 16 fractions. The chromatographic sepn was maintained using a UV detector and the collected fractions were analysed by TLC, proving in each

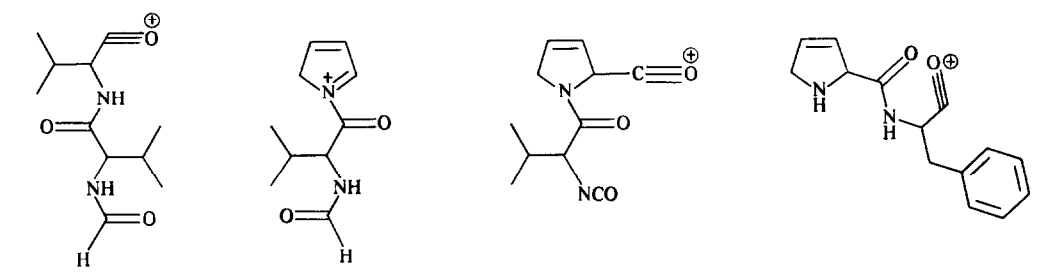
*Part 39 in the series "The Alkaloids of Rhamnaceae". For part 38, see ref. [1].

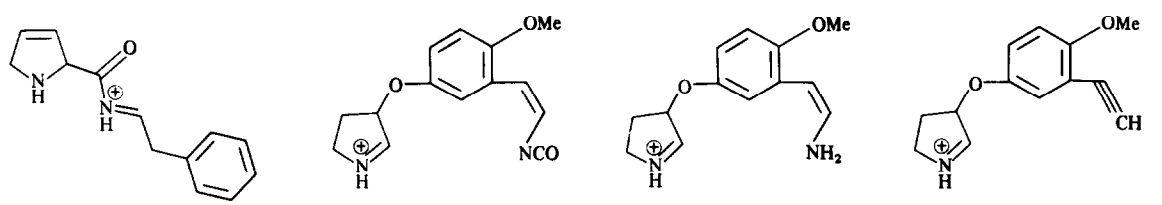
†Present address for correspondence: Research Centre, College of Pharmacy, King Saud University, P.O. Box 2457, Riyadh-11451, Kingdom of Saudi Arabia.



Ion	m/z
e	491
f	435
g	–
h	408
i	407
j	406



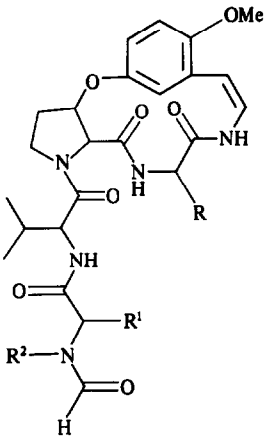


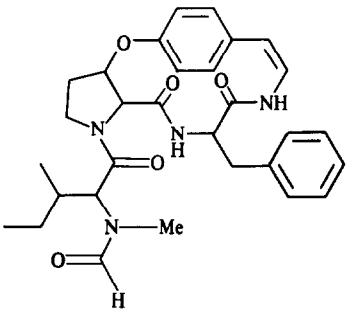


Scheme 1. Mass spectral fragmentation of sativanine F (1).

case to be a mixture of two or three main components. Sativanine F (3.5 mg) was obtained from fraction-8 by TLC in cyclohexane–Me_2CO–MeOH (35:15:1) and by HPLC in $CHCl_3$–MeOH (50:1).

Sativanine-F (1). $C_{34}H_{43}N_5O_7$ ($[M]^+$ 633.3164, calcd for: 633.3162). mp 139–141°; UV λ_{max} nm: 320 and 258; IR ν_{max} cm^{-1}: 3400, 1670, 1630 (sec. amide), 2820 (OMe), 1615 (C=C), 1190 and 1030 (aryl ether); MS: *m/z* 633 $[M]^+$, 605 $[M-CO]^+$, 506, 491, 435, 408, 407, 406, 259, 243, 233, 227, 221, 216, 215, 195, 165, 128 (base peak), 120, 72, 68. Sativanine-F (2 mg) was hydrolysed with 6 N HCl (24 hr) in a sealed tube. The hydrolysate was evapd to dryness and examined by PC (*n*-BuOH–





N – Formyl mauritine – C **(5)**

	R	R^1	R^2
Sativanine – F **(1)**	CH_2Ph	$CHMe_2$	H
N – Methyl sativanine – F **(2)**	CH_2Ph	$CHMe_2$	Me
N – Formyl sativanine – C **(3)**	CH(Me)–CH_2–Me	Me	Me
N – Formyl nummularine – B **(4)**	CH_2Ph	Me	Me

HOAc–H_2O, 4:1:5) [13] and (*n*-BuOH–H_2O–Me_2CO–ammonia, 8:6:1:1) [14] using ninhydrin as spray reagent. Valine and phenylalanine were identified by comparison with authentic compounds.

Formylation. A few mg of sativanine C, nummularine B and mauritine C were reacted separately with a mixture of HCO_2H–Ac_2O [15] and kept overnight at room temp. After solvent evapn, purification was carried out by TLC in cyclohexane–Me_2CO (3:1). N-Formyl sativanine-C (**2**): mp. 130–131°. $C_{30}H_{43}N_5O_7$ ($[M]^+$ 585.3177, calcd for: 585.3163). MS: *m/z* 114 ($C_5H_8NO_2$, base peak), 86 (C_4H_8NO) and 58 (C_3H_8N). N-Formyl nummularine-B (**3**): mp. 210°, $[\alpha]_D$ −379 (*c* = 0.2, $CHCl_3$) $C_{33}H_{41}N_5O_7$ ($[M]^+$ 619.3021, calcd for: 619.3006) MS: *m/z* 585 (M^+, base peak), 114 ($C_5H_8NO_2$), 86 (C_4H_8NO) and 58 (C_3H_8N). N-Formyl mauritine-C (**4**): $[\alpha]_D$ −311° (*c* = 0.2, $CHCl_3$) $C_{29}H_{34}N_4O_5$ ($[M]^+$ 518.2530, calcd for: 518.2530) MS: *m/z* 142 ($C_7H_{12}NO_2$, base peak), 114 ($C_6H_{12}NO$) and 86 ($C_5H_{12}N$).

Acknowledgements—The authors are grateful to M. H. Shah and Dr. Aslam Khan, A. R. I. Tarnab, Peshawar, Pakistan for the supply of plant material and to the DFG for financial support. A. H. Shah is thankful to Bonn University (GFG) and Heinrich Hertz Stiftung for scholarships and V. B. Pandey thanks Alexander von Humbodt Stiftung for a fellowship.

REFERENCES

1. Shah, A. H., Pandey, V. B., Eckhardt, G. and Tschesche, R., (communicated).
2. Nasir, E. and Ali, S. I. (1972) *Flora of West Pakistan*, p. 469. Fakhri Printing Press, Karachi.
3. Kirtikar, K. R. and Basu, B. D. (1984) *Indian Medicinal Plants*, Vol. I, p. 593. Lalit Mohan Basu, Allahabad, India.
4. Shah, A. H., Pandey, V. B., Eckhardt, G. and Tschesche, R., (communicated).
5. Shah, A. H., Pandey, V. B., Singh, J. P., Singh, K. N. and Eckhardt, G. (1984) *Phytochemistry* **23**, 2120.
6. Shah, A. H., Pandey, V. B., Eckhardt, G. and Tschesche, R. (1984) *Phytochemistry* **23**, 931.
7. Tschesche, R., Shah, A. H. and Eckhardt, G. (1979) *Phytochemistry* **18**, 702.
8. Shah, A. H. (1982) Ph.D. Dissertation, Bonn University, p. 76.
9. Tschesche, R., David, S. T., Uhlendorf, J. and Fehlhaber, H.-W. (1972) *Chem. Ber.* **105**, 3106.
10. Tschesche, R., Miana, G. A. and Eckhardt, G. (1974) *Chem. Ber.* **107**, 3180.
11. Tschesche, R., Wilhelm, H., Kaussmann, E. U. and Eckhardt, G. (1974) *Liebigs Ann. Chem.* 1694.
12. Tschesche, R., Kaussmann, E. U. and Fehlhaber, H.-W. (1974) *Chem. Ber.* **105**, 3094.
13. Partridge, S. M. (1948) *Biochem. J.* **42**, 238.
14. Shaw, K. N. F. and Fox, S. A. (1953) *J. Am. Chem. Soc.* **75**, 3421.
15. Ugi, I. (1971) *Isonitrile Chemistry*, p. 11. Academic Press, New York.