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SATIVANINE-E, A NEW 13-MEMBERED CYCLOPEPTIDE ALKALOID CONTAINING A SHORT SIDE-CHAIN, FROM *ZIZYPHUS SATIVA*[1]

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ABSTRACT.—A new 13-membered cyclopeptide alkaloid, sativanine-E (**1**), carrying a short side chain, has been isolated from *Zizyphus sativa* (Rhamnaceae). The structure was obtained by spectral evidence and hydrolysis.

Zizyphus sativa Gaertn. (Rhamnaceae) is a small tree native to the Hazara District of Pakistan, where it grows wild in the hilly areas (1). The bark of this plant is used to heal ulcers and wounds. The gum is used to treat eye disease while the syrup of the dried fruit is used for bronchitis (2). In our earlier communications, we reported the isolation and characterization of several cyclopeptide alkaloids from the bark of this plant (3-6). This paper describes the isolation and structure determination of a new alkaloid, sativanine-E (**1**), from the same source. Sativanine-E (**1**) is a 13-membered cyclopeptide alkaloid containing a short side chain like nummularine-C (**2**) (7).

The ir spectrum of **1** exhibited bands for -NH, secondary amide, $-OCH_3$, -NMe, $>C=C<$, and aryl ether, typical for cyclopeptide alkaloids. The uv spectrum showed absorption maxima at 321 and 268 nm characteristic of the styrylamine chromophore in the 13-membered cyclopeptide alkaloids (8), and shoulders observed at 289, 282, and 272 nm were typical for a tryptophan unit (9). The ^{1}H-nmr spectrum confirmed the presence of an *N,N*-dimethyl group, a -OMe group, and a *cis*-styrylamine moiety. The two (D_2O exchangeable) NH signals were recognized at 8.07 and 8.43 ppm. The *cis*-olefin proton, adjacent to the aromatic ring of the 13-membered ring system, appeared as a doublet at 5.92 ppm (J=9 Hz). The two doublets at 0.91 and 0.97 ppm (J=7 Hz each) were assigned to the two methyl groups of leucine. The presence of leucine was confirmed by paper chromatography of the hydrolysate of **1** in comparison with an authentic sample of leucine.

The molecular formula of **1** was determined by high resolution ms to be $C_{33}H_{41}N_5O_5$. The ms of **1** resembled that of the 13-membered cyclopeptide alkaloid, nummularine-C (**2**), and the 14-membered cyclopeptide alkaloid, mauritine-C (**3**) (10), containing a short side chain. The principal fragmentations observed are listed in Table 1, and the assignments are depicted in Scheme 1. The identity of each fragment was substantiated by high resolution ms, and various fragments are represented as usual (6).

α-Cleavage yielded the two most intense ions, **a** at *m/z* 187 and **b** at *m/z* 457. The fragment **a** disintegrated further into ions *m/z* 144 and *m/z* 130, typical for tryptophan derivatives (11). Due to the short side chain, the ions **c, d, e,** and **m** are absent (6). The

[1]Part 38 in the series "The Alkaloids of Rhamnaceae." For Part 37, see Shah, *et al.* (3).

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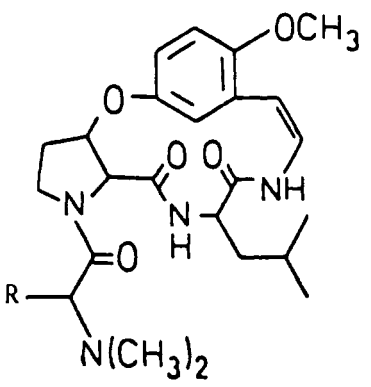

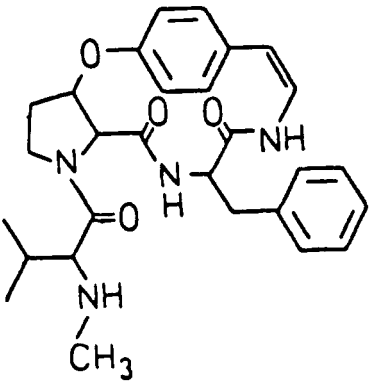

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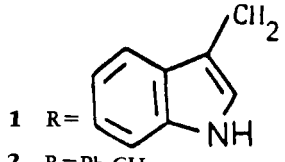
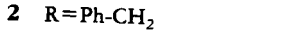


1 R=



2 R=Ph-CH_2

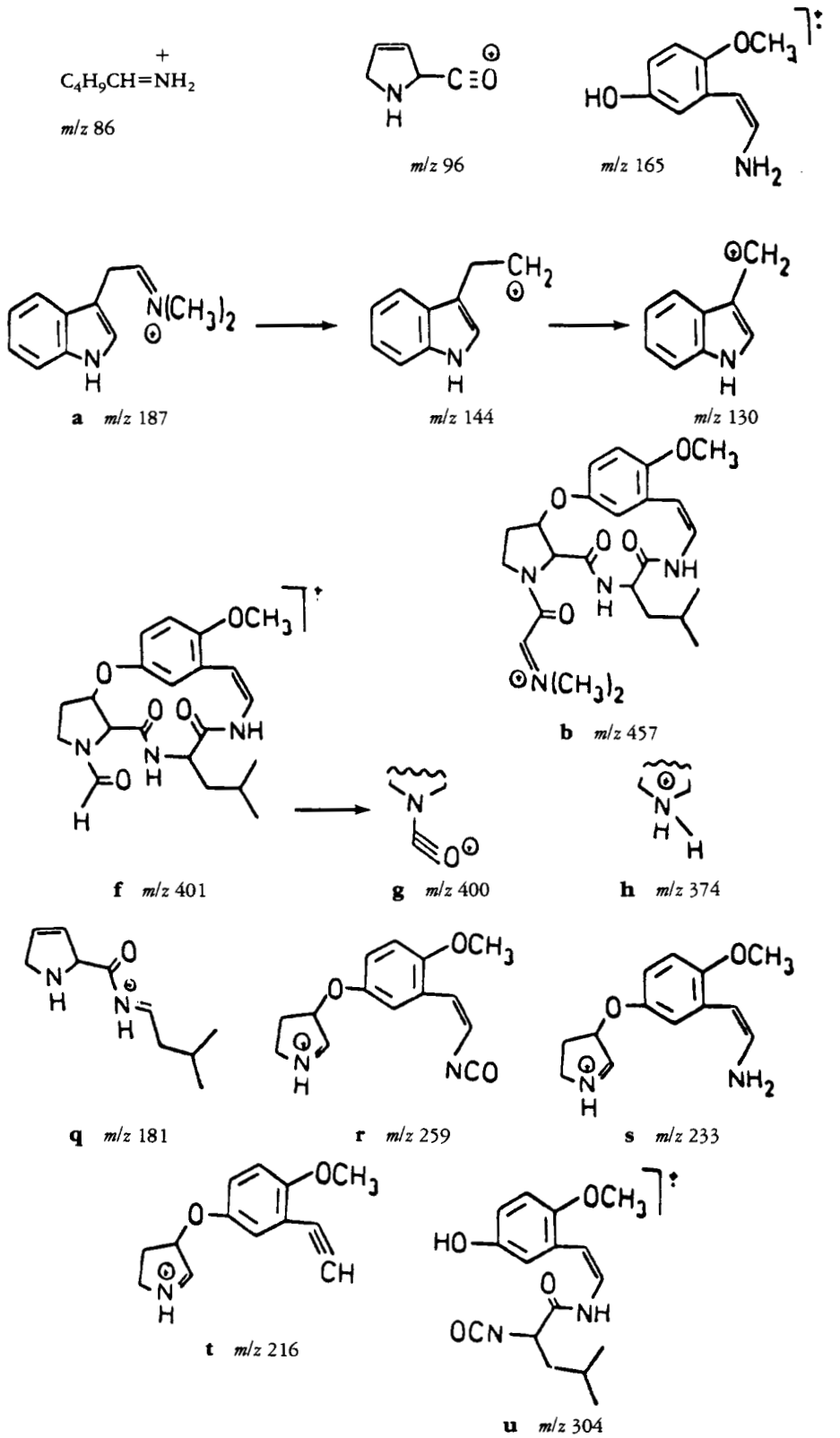
typical fragments for hydroxyproline *m/z* 96 and 68, leucine *m/z* 86 and a methoxy styrylamine group *m/z* 165 revealed the identity of the units that formed the 13-membered ring; and **f** *m/z* 401, **g** *m/z* 400, **h** *m/z*374, **i** *m/z* 373, **q** *m/z* 181, **r** *m/z* 259, **s** *m/z* 233, and **u** *m/z* 304 suggest their linkage (Scheme 1). From these data, structure **1** was established for sativanine-E.

EXPERIMENTAL

GENERAL.—The optical rotation was obtained on a Perkin-Elmer 141 photoelectric polarimeter, and melting points were determined on a Weigand microscope stage and are uncorrected. Cary-14 (uv), Perkin-Elmer 221 (ir) and Bruker-HX 90 (nmr) spectrometers were used. Mass spectral analyses were performed on Kratos MS-30 and MS-50 mass spectrometers operating at 70 eV with the evaporation of the sample in the ion source at about 200°. Tlc was carried out on silica gel HF_{254} (Merck), and paper chromatography was performed on Whatman No. 1 and 2043_b (Schleicher and Schull) paper.

TABLE 1. High Resolution Mass Spectrometry of Sativanine-E (**1**)

Ion	Formula	Div.	Found	Intensity (%)
$M^{\oplus}$	$C_{33}H_{41}N_5O_5$	−0.6	587.3114	5.7
a	$C_{12}H_{15}N_2$	1.5	187.1251	57.59
b	$C_{24}H_{33}N_4O_5$	2.7	457.2478	100
f	$C_{21}H_{27}N_3O_5$	0.4	401.1954	0.54
g	$C_{21}H_{26}N_3O_5$	—	400	0.5
h	$C_{20}H_{28}N_3O_4$	−1.9	374.2061	0.95
i	$C_{20}H_{27}N_3O_4$	0.5	373.2007	2.17
j	$C_{20}H_{26}N_3O_4$	−2.3	372.1900	0.65
q	$C_{10}H_{17}N_2O$	—	181	0.5
r	$C_{14}H_{15}N_2O_3$	—	259	0.5
s	$C_{13}N_{17}N_2O_2$	−2.8	233.1262	0.61
t	$C_{13}H_{14}NO_2$	−0.8	216.1017	1.77
u	$C_{16}H_{20}N_2O_4$	—	304	0.5
	$C_9H_{11}NO_2$	−0.9	165.0781	5.62
	C_9H_8N	0.3	130.0660	15.98
	C_5H_6NO	−0.2	96.0448	1.73
	$C_5H_{12}N$	0.5	86.0974	3.32
	C_4H_6N	0.9	68.0509	5.74



SCHEME 1. Important fragments in the mass spectrum of sativanine-E (**1**).

PLANT MATERIAL.—The plant material was collected from the Hazara District, Pakistan. The herbarium specimen of the plant is on file in PCSIR Laboratories, Peshawar, Pakistan.

EXTRACTION AND ISOLATION.—The crude alkaloids (6.6 g) were obtained by extraction of powdered bark (10 kg) with MeOH in the usual manner (12). The crude bases were fractionated on a 900 g silica gel (Gebr. Herrmann/Köln) chromatography column, eluting with an increasingly polar CH_2Cl_2-MeOH mixture which was subdivided into 16 fractions (13). The chromatographic separation was followed using an LKB Uvicord, and the collected fractions were analyzed by tlc. In every case, the fractions proved to be a mixture of two or three main components. The fractions were separated into individual components using plc or column chromatography.

SATIVANINE-E (**1**).—From fraction 12 of the chromatography column, 12 mg of **1** was separated by tlc, using $CHCl_3$-Me_2CO-MeOH (10:6:1) and CH_2Cl_2-MeOH (20:1) as solvent systems; mp 127-128°, $[\alpha]^{20}D$ −99 (c=0.2, $CHCl_3$); in ν max ($CHCl_3$) 3385, 3250 (NH), 2775 (NCH_3), 1670 (amide), 1630 (C=C), 1590, 1490 (aromat), 1040, and 1195 (arylether) cm^{-1}; uv λ max (MeOH) 321, 268, 289, 282, and 272 nm; ^1H nmr ($CDCl_3$/TMS) δ 0.91 d and 0.97 d (3H each, J=7 Hz, CH-Me_2), 2.48 s (6H, N-Me_2), 3.79 s (3H, OCH_3), 4.22 t (1H, J=4.4 Hz, CH), 4.42 d (1H, J=3.2 Hz, 3-HyPro-2-H), 5.32 sextet (1H, J_1=6.4 Hz, J_2=3.2 Hz, 3-HyPro-3-H), 5.92 d (1H, J=9 Hz, vinylamine), 6.94 q (1H, J_1=9 Hz, J_2=12 Hz, vinylamine), 8.07 and 8.43 (1H each, D_2O exchangeable, NH), 6.5-7.8 ppm signal complex (5H indole, 3H Ar., 1NH, 1 vinyl H). Mol. wt (ms): 587.3114; calcd for $C_{33}H_{41}N_5O_5$ 587.3120.

HYDROLYSIS.—Compound **1** was heated in a sealed tube with 1 ml of 6 N HCl for 24 h at 120°. The excess reagent was evaporated in vacuo, and the residue was dissolved in H_2O for paper chromatography. The presence of leucine was confirmed by comparison with an authentic sample using *n*-BuOH-HOAc-H_2O (4:1:5) (14) and *n*-BuOH-H_2O-Me_2CO-NH_3 (8:6:1:1) (15) as solvent systems with ninhydrin as the spray reagent.

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REFERENCES CITED

1. E. Nasir and S.I. Ali, "Flora of West Pakistan," Fakhri Printing Press, Karachi, 1972, p. 469.
2. K.R. Kirtihar and B.D. Basu, "Indian Medicinal Plants," Lalit Mohan Basu, Allahabad, India, 1984, p. 593.
3. A.H. Shah, V.B. Pandey, G. Eckhardt, and R. Tschesche, *Phytochemistry*, 1984 (in press).
4. A.H. Shah, V.B. Pandey, G. Eckhardt, and R. Tschesche, *Phytochemistry*, **23**, 931 (1984).
5. A.H. Shah, V.B. Pandey, J.P. Singh, K.N. Singh, and G. Eckhardt, *Phytochemistry*, **23**, 2120 (1984).
6. R. Tschesche, A.H. Shah, and G. Eckhardt, *Phytochemistry*, **18**, 702 (1979).
7. R. Tschesche, G.A. Miana, and G. Eckhardt, *Chem. Ber.*, **107**, 3180 (1974).
8. R. Tschesche, S.T. David, J. Uhlendorf, and H.W. Fehlhaber, *Chem. Ber.*, **105**, 3106 (1972).
9. R. Tschesche, C. Spilles, and G. Eckhardt, *Chem. Ber.*, **107**, 686 (1974).
10. R. Tschesche, H. Wilhelm, E.U. Kaussmann, and G. Eckhardt, *Liebigs Ann. Chem.*, 1694 (1974).
11. R. Tschesche, E.U. Kaussmann, and H.W. Fehlhaber, *Tetrahedron Letters*, **9**, 865 (1972).
12. R. Tschesche, E.U. Kaussmann, and H.W. Fehlhaber, *Chem. Ber.*, **105**, 3094 (1974).
13. A.H. Shah, "The alkaloids of Rhamnaceae. New cyclopeptide Alkaloids from *Zizyphus sativa* Gaertn;" Ph.D. Dissertation, Bonn University, 1982, p. 76.
14. S.M. Partridge, *Biochem. J.*, **42**, 238 (1948).
15. K.N.F. Shaw and S.A. Fox, *J. Am. Chem. Soc.*, **75**, 3421 (1953).

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