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A 13-MEMBERED CYCLOPEPTIDE ALKALOID FROM *ZIZYPHUS SATIVA**

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Key Word Index—*Zizyphus sativa*; Rhamnaceae; cyclopeptide alkaloid; sativanine-D.

Abstract—From the bark of *Zizyphus sativa* a new type of 13-membered cyclopeptide alkaloid, sativanine-D (**1**) has been isolated. Its structure was proved mainly by mass spectrometry and chemical degradation.

INTRODUCTION

Zizyphus sativa Gaertn (Rhamnaceae) is a 5–6 m tall tree commonly found in the Hazara district of Pakistan. Various medicinal properties are attributed to this plant in the local system of medicine [2–4]. We have recently reported the isolation and characterization of six cyclopeptide alkaloids from this plant [5, 6]. Extensive chromatography of the crude bases furnished a further previously unknown cyclopeptide alkaloid (**1**). Sativanine-D (**1**) is the first 13-membered cyclopeptide alkaloid containing an additional ring in the side chain. Its structure has been determined mainly by mass spectrometry and corroborated by other physical and chemical methods.

RESULTS AND DISCUSSION

The alkaloid was isolated from the polar fraction by TLC on silica gel. By high resolution mass spectrometry the elementary composition of **1** was determined as $C_{30}H_{43}N_5O_6$. The IR spectrum showed the usual secondary amide and conjugated double bond absorption, plus bands atrributed to phenolic ether and N–Me groups. The UV spectrum revealed the presence of a 13-membered ring system containing cyclopeptide alkaloid [7].

The mass spectrum of **1** differed greatly from those of other 13-membered cyclopeptide alkaloids but is very similar to the spectra of the 14-membered cyclopeptide alkaloids nummularine-G [8] and sativanine-B [5] which contain an additional ring in the side chain. The $[M]^+$ (m/z 569) appears as a peak of high intensity. The usual α-cleavage product **a** and the fragments **b**, **c** and **d** are missing. The base peak results from the resonance stabilized fragment m/z 113 of the imidazolidinone ring. Fragments **l** and **m** show the linkage of the imidazolidinone ring with the intermediate amino acid valine (whose nitrogen is a part of the imidazolidinone ring). Fragment **n** provides the evidence that valine is bound with hydroxyproline. The fragments **e**, **f**, **g** and **h** show the complete ring system while **p**, **s**, **t** and **u** demonstrate the bonding among the substituted styrylamine, hydroxyproline and a C_6 amino acid (Scheme 1). The identity of each fragment was proved by high resolution mass spectrometry (Table 1).

Compound **1** is the first representative of its type in the 13-membered cyclopeptide alkaloids. To provide further support to its mass spectrometric fragmentation nummularine-B [9] was treated with formalin [10]. The derivative thus formed nummularine-B-cycl. (**2**) gave an analogous fragmentation pattern upon electron impact.

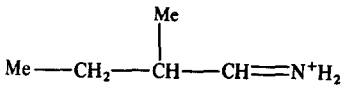
In the acid hydrolysate of **1** isoleucine, valine and N-methylalanine were confirmed by PC and comparison with authentic samples, thus proving structure **1** for sativanine-D.

Table 1. High resolution mass spectrometry of sativanine-D (**1**)

Ion*	Formula	Div.	Found	Intensity (%)
$[M]^+$	$C_{30}H_{43}N_5O_6$	+0.7	569.3220	19.4
e	$C_{25}H_{35}N_3O_5$	−1.3	457.2563	2.8
f	$C_{21}H_{27}N_3O_5$	0.4	401.1954	0.5
g	$C_{21}H_{26}N_3O_5$	—	400	<0.5
h	$C_{20}H_{28}N_3O_4$	−1.9	374.2061	0.5
i	$C_{20}H_{27}N_3O_4$	−2.7	373.1975	0.7
l	$C_{10}H_{17}N_2O_2$	−1.9	197.1271	0.8
m	$C_9H_{17}N_2O$	−1.3	169.1327	0.6
n	$C_{10}H_{15}N_2O_2$	1.4	195.1147	2.1
p	$C_{11}H_{17}N_2O_2$	±0	209.1290	2.2
s	$C_{13}H_{17}N_2O_2$	—	233	<0.5
t	$C_{13}H_{14}NO_2$	−0.1	216.1023	2.2
u	$C_{16}H_{20}N_2O_4$	—	304	<0.5
	$C_9H_{11}NO_2$	−0.1	165.0789	9.2
	$C_5H_9N_2O$	−0.1	113.0714	100
	C_5H_6NO	−0.2	96.0447	1.2
	$C_5H_{12}N$	−0.3	86.0967	1.9
	$C_4H_{10}N$	−0.1	72.0812	4.3
	C_4H_6N	0.4	68.0504	2.8

*For nomenclature of different fragments see ref. [6].

*Part 37 in the series "The Alkaloids of Rhamnaceae". For Part 36 see ref. [1].



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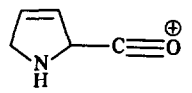

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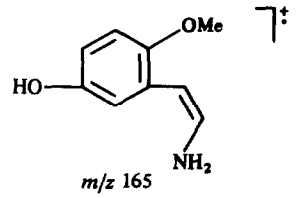

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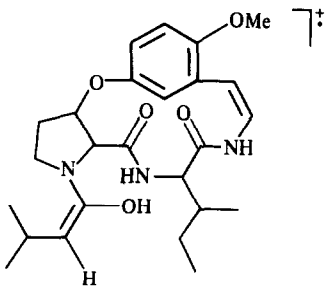

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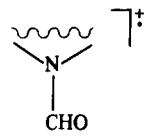

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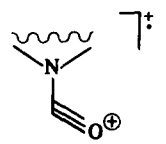
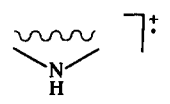
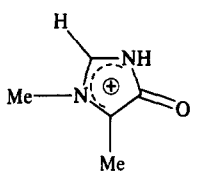


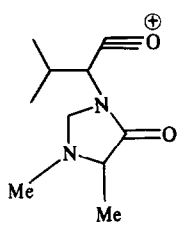
g *m/z* 400



h i + H
i *m/z* 373



m/z 113



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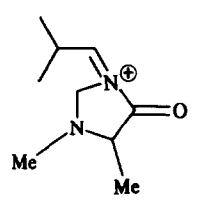

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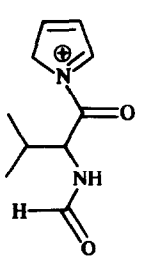

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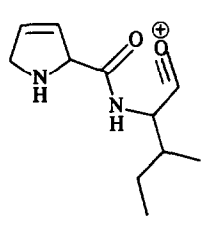

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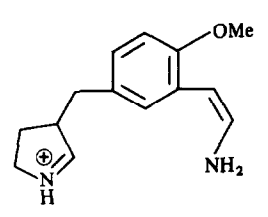

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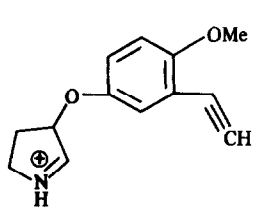

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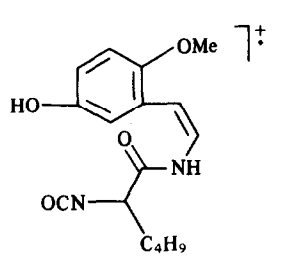
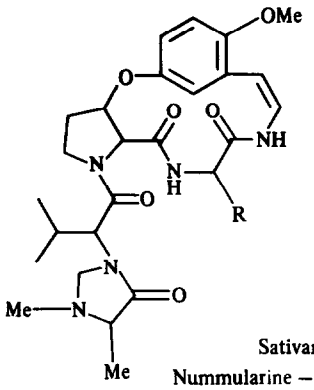


u *m/z* 304

Scheme 1. Characteristic fragments in the mass spectrum of **1**.



Sativanine-D **1** R = Me—CH_2—CH(Me)—

Nummularine-B-cycl **2** R = $PhCH_2$—

EXPERIMENTAL

Plant material was collected from Hazara district, Pakistan. MS were measured at 70 eV with evapn of the samples in the ion source at *ca* 200°. TLC was carried out on silica gel HF_{254} (Merck) and for PC Whatman No. 1 and 2043b (Scheicher & Schull) papers were used.

Extraction. Crude alkaloids (6.6 g) were obtained by extraction of powdered bark (10 kg) with MeOH in the usual manner [11]. Another 5 kg of plant material was extracted with a solvent mixture of C_6H_6–NH_3–EtOH (100:1:1) [12]. Compound **1** was also isolated using this method. The crude alkaloid mixture was fractionated on a column of 900 *g* silica gel (Gebr. Herrmann/Koln), eluting with increasingly polar CH_2Cl_2–MeOH mixtures, into 15 fractions. The chromatographic separation was followed using UV monitor and the collected fractions analysed by TLC, proving in every case to be a mixture of two or three main components. The fractions were separated into individual components using prep. TLC or CC.

Sativanine-D (**1**). Compound **1** (3.4 mg) was separated from fraction 10 by TLC using C_6H_6–Me_2CO–MeOH (35:15:1) and CH_2Cl_2–Me_2CO–MeOH (30:15:1), mp 119–121°; $\nu_{max}^{CHCl_3}$ cm^{-1}: 1680, 1635 (amide), 2830 (OMe), 1615 (C=C), 1220 and 1025 (aryl ether); λ_{max}^{MeOH} nm: 320 and 265. M_r (MS): 569.3220; calc. for $C_{30}H_{43}N_5O_6$, 569.3213.

Nummularine-B-cycl. (**2**). Nummularine-B (20 mg) was dissolved in 5 ml EtOH and treated with 37% HCHO. The soln was refluxed for 4 hr, the solvent evapd and the residue treated with 7% NH_3. The residue was then washed with H_2O and crystallized from EtOH [10], mp 148–150°, $[\alpha]_D^{20}$ −406 (*c* 0.2, $CHCl_3$), M_r (MS): 603.3067; calc. for $C_{33}H_{41}N_5O_6$, 603.3056. MS *m/z*: 603 ($C_{33}H_{41}N_5O_6$) $[M]^+$ (9.7%), 113 ($C_5H_9N_2O$, 100%).

Hydrolysis. Compound **1** was heated in a sealed tube with 1 ml of 6 N HCl for 24 hr at 120°. Excess reagent was evapd *in vacuo* and the residue dissolved in H_2O for PC. The amino acids were identified by comparison with authentic samples using *n*-BuOH–HOAc–H_2O (4:1:5) [13] and *n*-BuOH–H_2O–Me_2CO–NH_3 (8:6:1:1) [14], and ninhydrin as spray reagent.

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