122.1 (C-1'), 111.8 (C-2'), 148.5 (C-3'), 151.4 (C-4'), 110.9 (C-5'), 122.0 (C-6'), 59.8 (C-3-OMe), 60.6 (C-6-OMe), 61.9 (C-7-OMe), 61.5 (C-8-OMe), 55.7 (C-3'-OMe), 55.4 (C-4'-OMe).

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NUMMULARINE-S: A CYCLOPEPTIDE ALKALOID FROM STEM BARK OF *ZIZYPHUS NUMMULARIA*

A. H. SHAH, R. M. A. KHAN, S. K. MAURYA* and V. P. SINGH*

Research Centre, College of Pharmacy, P.O. Box 2457, King Saud University, Riyadh, Saudi Arabia; *Department of Medicinal Chemistry, Institute of Medical Sciences, B.H.U., Varanasi 221005, India

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Key Word Index—*Zizyphus nummularia*; Rhamnaceae; nummularine-S; 13-membered cyclopeptide alkaloid.

Abstract—From the stem bark of *Zizyphus nummularia* a 13-membered cyclopeptide alkaloid containing a short side chain has been isolated. The structure was determined by spectroscopic methods and chemical degradation. *Zizyphus nummularia* seems to be closely related to *Z. jujuba*, *Z. sativa* and *Z. amphibia*.

INTRODUCTION

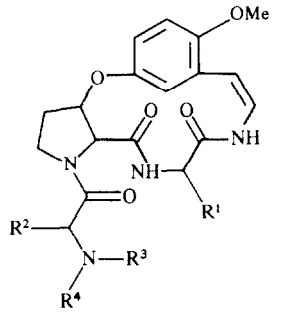
The bark, leaves and fruit of *Zizyphus* species (Rhamnaceae) have been used as folk medicines in tropical and subtropical countries [1–3]. Chemical investigation of different *Zizyphus* species has led to the isolation of several cyclopeptide alkaloids [4, 5]. Such alkaloids are polyamide plant bases containing a styrylamine unit as an integral part of 13-, 14-, or 15-membered macrocyclic ring; the size of the rings has been adopted as the basis for classification of the compounds [4]. *Zizyphus juazeiro* [6], *Z. spina-christi* [7] and *Z. mauritiana* [8] possess only 14-membered cyclopeptide alkaloids, while *Z. abyssinica* [9] contains only 15-membered alkaloids of this class. 13- and 15-membered cyclopeptide alkaloids were isolated from *Z. mucronata* [10] and *Z. oenoplia* [11]. However, from *Z. nummularia* [12–16], just like *Z. sativa* [17–22], *Z. jujuba* [23] and *Z. amphibia* [24, 25] only 13- and 14-membered cyclopeptide alkaloids were isolated.

In continuation of our work on cyclopeptide alkaloids from the Rhamnaceae, we recently reported the isolation of several alkaloids from the stem bark of *Z. nummularia* [26]. We report herein the isolation and characterization of a further previously undescribed cyclopeptide alkaloid, nummularine-S (**1**) from the stem bark of this plant.

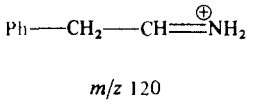
RESULTS AND DISCUSSION

The alkaloid (**1**) was isolated by consecutive TLC and chromatotron from the polar fractions of the CC. The IR spectrum showed typical absorptions for cyclopeptide alkaloids and the UV spectrum showed absorption maxima at 318 and 268 nm characteristic of the styrylamine moiety in 13-membered cyclopeptide alkaloids [27].

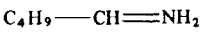
The molecular formula of (**1**) was determined by high resolution mass spectrometry as $C_{29}H_{36}N_4O_5$ ([M] + *m/z* 520.2693). The spectrum closely resembled that of nummularine-C (**2**), a 13-membered cyclopeptide alkaloid with a short side chain [12]. The main fragments observed in the mass spectrum are listed in Table 1 and the assignments are depicted in Scheme 1. The identity of each fragment was substantiated by high resolution mass spectrometry; the various fragments are represented as described in ref. [19]. The α-cleavage product **a** (*m/z* 86) of the terminal amino acid leucine or isoleucine formed the base peak of the spectrum and fragment **b** was found at *m/z* 463. Due to the short side chain, the ions **c**, **d**, **e** and **m** were absent [20]. The characteristic fragments for the methoxy styrylamine unit *m/z* 165, phenylalanine *m/z* 120 and hydroxyproline *m/z* 96 revealed the identity of the units forming the 13-membered heterocyclic ring of the



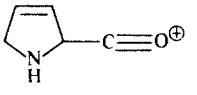
	R^1	R^2	R^3	R^4
Nummularine-S (**1**)	$-CH_2-Ph$	$-CH_2-CH(Me)_2$	—H	—H
Nummularine-C (**2**)	$-CH_2-CH(Me)_2$	$-CH_2-Ph$	—Me	—Me



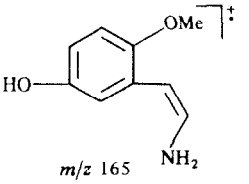
m/z 120



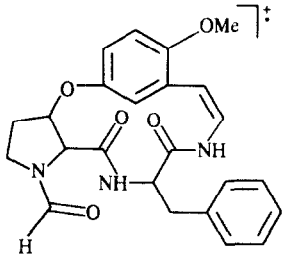
a *m/z* 86



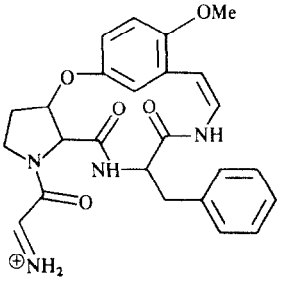
m/z 96



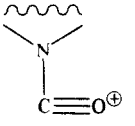
m/z 165



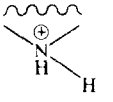
f *m/z* 435



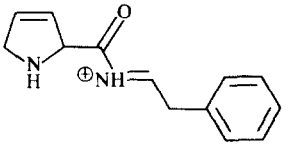
b *m/z* 463



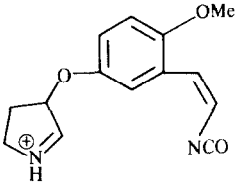
g *m/z* 434



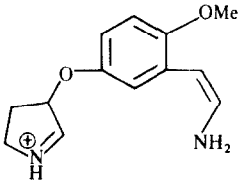
h *m/z* 408
i *m/z* 407
j *m/z* 406



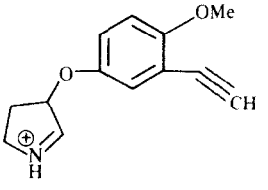
q *m/z* 215



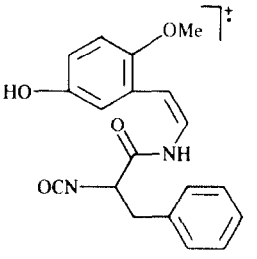
r *m/z* 259



s *m/z* 233



t *m/z* 216



u *m/z* 338

Scheme 1.

Table 1. High resolution mass spectrometry of nummularine-R

Peak	Formula	Div.	Found	Intensity (%)
$[M]^+$	$C_{29}H_{36}N_4O_5$	+0.4	520.2693	11.7
a	$C_5H_{12}N$	+0.1	86.0971	100
b	$C_{25}H_{27}N_4O_5$	—	463	<0.5
f	$C_{24}H_{25}N_3O_5$	−1.4	435.1780	7.1
g	$C_{24}H_{24}N_3O_5$	—	434	<0.5
h	$C_{23}H_{26}N_3O_4$	−1.4	408.1909	7.0
i	$C_{23}H_{25}N_3O_4$	−2.1	407.1824	15.1
j	$C_{23}H_{24}N_3O_4$	−2.3	406.1744	2.0
q	$C_{13}H_{15}N_2O$	−0.2	215.1182	4.0
r	$C_{14}H_{15}N_2O_3$	+0.7	259.1089	0.7
s	$C_{13}H_{17}N_2O_2$	−0.7	233.1283	6.4
t	$C_{13}H_{14}NO_2$	−0.8	216.1017	2.5
u	$C_{19}H_{18}N_2O_4$	—	338	<0.5
	$C_9H_{11}NO_2$	−0.7	165.0782	70.9
	$C_8H_{10}N$	+0.1	120.0815	10.5
	C_5H_6NO	+1.4	96.0463	8.7
	C_4H_6N	+0.5	68.0505	9.5

molecule. Fragment ions **f**, **g**, **h**, **i**, **j** represent the whole ring system while **q**, **r**, **s**, **t**, and **u** show the linkage of different units. The end amino acid was confirmed to be leucine by PC where in the acid hydrolysate of (**1**) leucine and phenylalanine were confirmed by direct comparison with authentic compounds. Based on these findings nummularine-S was proved to have the structure (**1**) which differed from (**2**) in having leucine instead N,N-dimethyl phenylalanine as the end amino acid and phenylalanine instead of leucine as the ring bound amino acid. The co-occurrence of 13- and 14-membered cyclopeptide alkaloids in *Z. nummularia* is very interesting and may be of chemotaxonomic significance. It may indicate a close relationship of this plant to *Z. jujuba*, *Z. sativa* and *Z. amphibia*.

EXPERIMENTAL

Mps: 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 $60F_{254}$) and PC on Whatman No. 1.

Extraction and isolation. Stem bark of *Z. nummularia* (5 kg) was collected and extd as described in ref. [15], semi-solid crude alkaloids (2.8 kg) were obtained. The alkaloid mixt. was fractionated on a silica gel (100 g) column eluting with increasingly polar $CHCl_3$–MeOH mixts into 8 main fractions. The collected fractions were analysed by TLC proving in every case to be mixts of two or three components. Nummularine-S (2.9 mg) was obtained by prep. TLC, using chromatotron-2974 for the polar fractions. For TLC $CHCl_3$–EtOAc–MeOH (15:1:1) and for chromatotron cyclohexane–$CHCl_3$–MeOH (10:1:1) were used.

Nummularine-S. $C_{29}H_{36}N_4O_5$ ($[M]^+$ *m/z* 520.2693), mp 210–211°, IR ν_{max} cm^{-1}: 3340 (−NH), 2820 (−OMe), 1670 and 1640 (amide), 2995–2830 (CH), 1610 (C=C), 1220–1030 (aryl ether); UV λ_{max} nm: 318 and 268. About 2 mg of (**1**) were hydrolysed with 6 M HCl (24 hr) in a sealed tube. The hydrolysate was evapd to dryness and examined by PC (*n*-BuOH–H_2O–Me_2CO–NH_3, 8:6:1:1) and (*n*-BuOH–HOAc–H_2O, 4:1:5) [13, 14] using ninhydrin as detection reagent.

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