EXPERIMENTAL

The plant material, *Lilium candidum* L., was collected in South Slovakia and the herbarium specimen is deposited in the Department of Pharmacognosy and Botanics of the Pharmaceutical Faculty, Comenius University, in Bratislava. Mps: uncorr.

Extraction and isolation of 8-(3-methylsuccinoyl)-kaempferol. Dry flowers (1500 g) were macerated several times at room temp. with 96 and 70% EtOH. The extract was filtered and evapd. The residue (699 g) was dissolved in 5% HCl and substances present in acidic soln were extracted successively with petrol, Et_2O and $CHCl_3$. Chromatography of the crude ether fraction (30 g) after removal of the solvent over silica gel using benzene–acetone gave 440 fractions. Rechromatography of fractions 64–101 over silica gel using benzene–acetone afforded 3,5,7,4′-tetrahydroxy-8-(3″-methylsuccinoyl)-flavone (27.5 mg). Mp 221–222°.

UV λ_{max}^{MeOH} nm: 242, 249, 322, 371. IR: 840, 1615, 1650, 1715, 2000–3100, 3410 cm^{-1}. Mass: M^+ 400 $C_{20}H_{16}O_9$ *m/z* 382, 367, 355 $C_{19}H_{15}O_7$, 338 $C_{19}H_{14}O_6$, 337, 323, 313, 286, 258, 257, 229, 213. ^1H NMR (300, 13 MHz, CD_3OD, TMS has been used as int. standard): δ8.01 (2H, *d*, $J_{2',3'}$ = 8,9 Hz, H-2′, H-6′), 6.92 (2H, *d*, H-3′, H-5′), 6.21 (1H, *s*, H-6), 3.68 (1H, *dd*, $J_{2a'',2b''}$ = 18.7 Hz, $J_{2a'',3''}$ = 9.6 Hz, H-2a″), 3.37 (1H, *dd*, $J_{2b'',3''}$ = 4.5 Hz, H-2b″), 3.07 (1H, *dqd*, $J_{3'',5''}$ = 7.3 Hz, H-3″), 1.27 (3H, *d*, H-5″). ^{13}C NMR δ203.1 (C-1″, *s*), 180.0 (C-4″), 177.2 (C-4), 171.6 (C-7), 167.3 (C-8a), 160.9 (C-4′), 158.9 (C-5), 150.2 (C-2), 138.1 (C-3), 131.5 (C-2′, C-6′), 116.4 (C-3′, C-5′), 122.8 (C-1′), 105.2 (C-8), 104.5 (can be exchanged) (C-4a), 100.3 (C-6), 48.8 (solvent Me_2O in MeOH, overlapped by the solvent) (C-2″), 36.2 (C-3″), 17.9 (C-5″).

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A CYCLOPEPTIDE ALKALOID FROM THE BARK OF *ZIZYPHUS RUGOSA*

V. B. PANDEY, Y. C. TRIPATHI, S. DEVI, J. P. SINGH* and A. H. SHAH†

Department of Medicinal Chemistry, Institute of Medical Sciences, Banaras Hindu University, Varanasi 221 005, India; †Research Centre, College of Pharmacy, King Saud University, Riyadh 11451, Saudi Arabia

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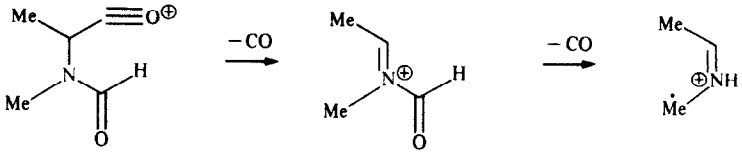
Key Word Index—*Zizyphus rugosa*; Rhamnaceae; bark; alkaloids; rugosanine-A; *N*-formylcyclopeptide alkaloid; uncharacterised norcorypalline.

Abstract—Rugosanine-A has been isolated from the stem bark of *Zizyphus rugosa*. The structure was deduced by spectroscopic methods and chemical degradation. It is a 13-membered *N*-formylcyclopeptide alkaloid and provides the third example of such a naturally occurring *N*-formylcyclopeptide alkaloid.

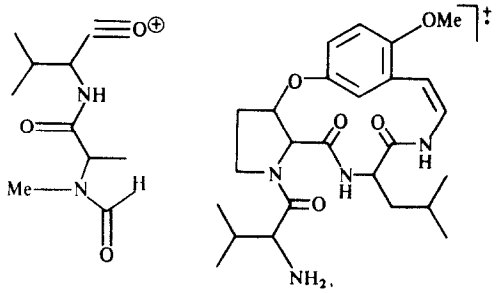
INTRODUCTION

Zizyphus rugosa Lam. (Family: Rhamnaceae) is a large shrub distributed throughout India. The bark of the plant is commonly used in the Indian medicine for the treatment of diarrhoea while the flowers, together with leaves, are used in menorrhagia [1]. In continuation of our search for the peptide alkaloids from the bark of *Z. rugosa* [2], we now report here the isolation and characterisation of a new 13-membered *N*-formyl cyclopeptide alkaloid, rugosanine-A (**1**) together with an uncharacter-

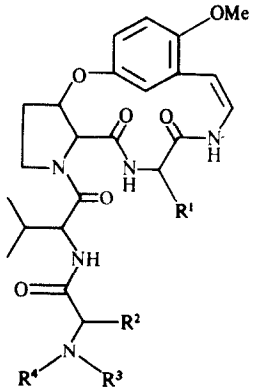
*Present address: Department of Chemistry, S.G.R.(P.G.) College, Dobhi, Jaunpur, India.



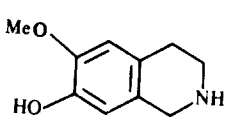
m/z 114 (ion *i*) *m/z* 86 (ion *ii*) *m/z* 58 (ion *iii*)



m/z 213 (ion *iv*) *m/z* 472 (ion *v*)



	R¹	R²	R³	R⁴
1	$CH_2—CH(Me)_2$	Me	—C(=O)H	Me
2	CH_2Ph	$CH(Me)_2$	—C(=O)H	H
3	$CH(Me)—CH_2—Me$	Me	H	Me
4	$CH_2—CH(Me)_2$	Me	H	Me
5	$CH_2—CH(Me)_2$	Me	Me	Me



6

ized simple isoquinoline alkaloid-A (**6**). Only two *N*-formyl cyclopeptide alkaloids sativanine-F [3] and sativanine-K [4] have earlier been reported, rugosanine-A provides the third example of the occurrence of a *N*-formyl cyclopeptide alkaloid in plants.

RESULTS AND DISCUSSION

The alkaloid, rugosanine-A mp 237–240° was isolated from the crude base fraction by column chromatography and repeated preparative TLC of chloroform–methanol (9:1) eluants on silica gel. It gave a very faint colour with Dragendorff's reagent. The molecular formula was determined by high resolution mass spectroscopy as $C_{30}H_{43}N_5O_7$. The IR spectrum showed typical absorptions for cyclopeptide alkaloids and the UV spectrum gave characteristic absorption maxima at 320 and 260 nm for a 13-membered cyclopeptide alkaloids [5].

Like the *N*-formyl cyclopeptide alkaloid sativanine-F (**2**) [3], the mass spectrum of rugosanine-A (**1**) which contains *N*-formyl group on the terminal amino acid exhibits an intense [M^+] peak at *m/z* 585 (25%) and the usual α-cleavage products *a–d* are absent. Instead of this, the base peak at *m/z* 114 (ion-*i*, Scheme 1) is produced by the cleavage of the peptide bond between *N*-formyl monomethylalanine and valine, which then eliminates 2 × carbonyl units to give *m/z* 86 (ion-*ii*) and *m/z* 58 (ion-*iii*), respectively. Fragment *m/z* 213 (ion-*iv*) formed by the total breakage of the side chain indicated the linkage of the side chain intermediate amino acid valine with *N*-formyl monomethylalanine. The counterpart of the fragment *m/z* 114 is the ion *m/z* 472 (ion-*v*) which further gets fragmented into ions *e–j*, *l–u*, identical to sativanine-C (**3**) [6]. This data demonstrated the structure of the macrocyclic ring system consisting of hydroxy proline, methoxyhydroxy styrylamine and ring bound leucine or isoleucine. From this it could be concluded that the end amino acid *N*-formylmonomethylalanine is attached to valine and which is further attached to a proline unit of the macrocyclic ring system. The elementary composition of all fragments was substantiated by high resolution mass measurements.

The identity of the ring bound intermediate and end amino acids was proved to be leucine, valine and *N*-monomethyl alanine, respectively, by paper chromatographic comparison of the hydrolysate of **1** with authentic samples. Thus structure **1** is proposed for rugosanine-A.

The structure **1** was further supported by deformylation of **1** with 0.5 N HCl which gave compound **4**. Compound **4** was identical with nummularine-P [7] (mmp, co-TLC and superimposable IR). Compound **4** on methylation with methanal and sodium borohydride and purification by prep. TLC and crystallization, furnished a compound **5**. Compound **5** was identical with sativanine-H [8] (mmp, co-TLC and superimposable IR). In order to ensure that **1** is not an artifact produced during extraction with methanol, another portion of the bark was extracted with benzene–ammonia–ethanol. Compound **1** was isolated again which proves that it is naturally occuring.

Crystallization of the benzene–chloroform (1:4) eluants furnished alkaloid-A, mp 283–285°, $C_{10}H_{13}NO_2$ (M^+ at *m/z* 179.090). Its UV spectrum resembled that of a 6,7-substituted simple tetrahydroisoquinaline [9]. The mass spectrum exhibited a molecular ion peak at *m/z* 179 and a base peak due to loss of hydrogen at *m/z* 178 which further eliminates Me and carbonyl units to give ions *m/z* 163 and 135, respectively. The identity of each fragment was proved by high resolution mass spectrometry. Thus, the structure A may be norcorypalline (**6**) [10] or its isomer.

EXPERIMENTAL

Mps. uncorr. IR were run in KBr pellets. UV spectra: MeOH. MS analysis was performed at 70 eV with evaporation of sample in the ion source at *ca* 200°. CC were carried out on silica gel refers to BDH (60–120 mesh) and paper chromatography (PC) on Whatman paper No. 1. TLC plates were prepared with silica gel G (Merck). Solvents used for TLC and prep. TLC were $CHCl_3$–MeOH (2:1) (solvent A), $CHCl_3$–EtOAc–MeOH (10:5:1) (solvent B), $CHCl_3$–EtOAc–MeOH (1:1:1:5) (solvent C) and for PC, *n*-BuOH–HOAc–H_2O (4:1:5) (solvent D). Dragendorff's reagent and ninhydrin were used as spraying reagents for alkaloids and amino acids respectively.

Zizyphys rugosa Lam. was collected from Mirzapur District, U.P., India and identified by the Department of Botany, Banaras Hindu University and a specimen sample is kept in the Department.

Extraction. Z. rugosa stem bark (4 kg) was repeatedly extracted with a mixture of C_6H_6–MeOH–NH_3 (100:1:1) at room temp. [11]. The combined extract was concd to small vol. and further extracted with 7% aq. citric acid. The crude alkaloids (6.5 g) were obtained from the aq. acidic soln by basification, extraction with $CHCl_3$ and dist. on the water bath. It was chromatographed over a silica gel column eluting with solvents of increasing polarity. Crystallization of the C_6H_6–$CHCl_3$ (1:4) eluants furnished an alkaloid A (9 mg). Eluants from $CHCl_3$–MeOH (9:1) were mixed according to TLC (two spot) and repeated prep. TLC (solvent C) furnished an alkaloid rugosanine-A (15 mg).

Alkaloid-A. Crystallized from MeOH as colourless granules, mp 283–285°; UV λ_{max}^{MeOH} nm: 235 sh, 285 ($\log\varepsilon$ 3.90, 3.62); MS *m/z*: 179.0907 [M^+] ($C_{10}H_{13}NO_2$), 178 [$M-1$]$^+$ ($C_{10}H_{12}NO_2$), 163.0634 [$M-1$-Me]$^+$ ($C_9H_9NO_2$), 135.0694 [$M-1$-Me$-$CO]$^+$ (C_8H_9NO), 134.0614 [$M-1$-Me$-$CO-1]$^+$ (C_8H_8NO).

Rugosanine-A. Crystallized from MeOH as colourless granules, mp 237–240° (dec.), $C_{30}H_{43}N_5O_7$ (M^+ at *m/z* 585.3177), R_f 0.25 (solvent A), 0.38 (solvent B), 0.52 (solvent C). It showed IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3385 (NH), 2785 (NCH_3), 2860 (OMe), 1635, 1690 (sec. amido group), 1610 (C=C), 1205–1240 (phenol ether), UV λ_{max}^{MeOH} nm; 260, 320; ($\log\varepsilon$ 3.68, 3.64) MS *m/z* (rel. int.): 585.3187 [M]$^+$ (28), 557.3238 ($C_{29}H_{43}N_5O_6$, 16), 472.2693 ($C_{25}H_{36}N_4O_5$, 1.2), 457.2587 ($C_{25}H_{35}N_3O_5$, 18), 401 (1.2), 400 (1), 374.2353 ($C_{20}H_{28}N_3O_4$, 1), 373.2012 ($C_{20}H_{27}N_3O_4$, 2), 372 ($C_{20}H_{26}N_3O_4$, 1), 304 (2), 259.1096 ($C_{14}H_{15}N_2O_3$, 1.3), 233.1294 ($C_{13}H_{17}N_2O_2$, 1), 221.0881 ($C_{11}H_{13}N_2O_4$, 1), 216.1017 ($C_{13}H_{14}NO_2$, 4), 213 (2), 211 (30), 209.1303 ($C_{11}H_{17}N_2O_2$, 1), 195.1143 ($C_{10}H_{15}N_2O_2$, 2), 185 (1), 181 (2), 165.0791 ($C_9H_{11}NO_2$, 24), 114.0560 ($C_5H_8NO_2$, 100), 86.0612 (C_4H_8NO, 50), 58.0674 (C_3H_8N, 37). Compound **1** (3 mg) was hydrolysed with 6 N HCl (24 hr) in a sealed tube. The hydrolysate was evaporated to dryness and examined by PC (solvent D) using

ninhydrin as spray reagent. Leucine, valine and *N*-monomethylalanine were identified by comparison with authentic samples.

Compound **1** (7 mg) was deformylated by treatment with 0.5 N HCl in MeOH at room temp. for 45 hr. It was purified by prep. TLC and crystallization from MeOH furnished compound **4**, mp 179–180°, which was identified as nummularine-P (mmp, co-TLC and superimposable IR). Compound **4** (4.5 mg) was treated with HCHO and $NaBH_4$ and the reaction product purified by prep. TLC and crystallisation from MeOH gave the *N*-methylated product, compound **5**, mp 91–92°. Compound **5** was identified as sativanine-H by direct comparison with an authentic sample (mmp, co-TLC and superimposable IR).

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A *SECO*-PHTHALIDEISOQUINOLINE ALKALOID FROM *FUMARIA INDICA* SEEDS

Y. C. TRIPATHI, V. B. PANDEY, N. K. R. PATHAK and M. BISWAS

Department of Medicinal Chemistry, Institute of Medical Sciences, Banaras Hindu University, Varanasi 221005, India

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Key Word Index—*Fumaria indica*; Fumariaceae; seed; *seco*-phthalideisoquinoline alkaloid; narceimicine.

Abstract—From the seeds of *Fumaria indica*, a previously undescribed *seco*-phthalideisoquinoline alkaloid, narceimicine, has been isolated and its structure established by spectroscopic methods.

INTRODUCTION

In continuation of our work on the alkaloids of *Fumaria indica* seeds [1–5], we report here the isolation and characterization of a new alkaloid, designated narceimicine.

RESULTS AND DISCUSSION

Chromatographic resolution of the crude base fraction of the defatted seeds of *F. indica* furnished pale yellow granules of narceimicine, mp 242–246° (MeOH–H_2O), $C_{21}H_{21}NO_8$ ($[M]^+$, *m/z* 415). It showed characteristic UV absorption maxima comparable with those of *trans*-stilbene [6]. The presence of a carboxylic group in the molecule was indicated by a band at 1670 cm^{-1} in the IR spectrum and a positive colour test with Bromothymol Blue. The 90 MHz ^1H NMR (CF_3CO_2D) spectrum of narceimicine is comparable with that of narceimine (**2**) [7] and showed signals for two methylenedioxy groups as a pair of 2H singlets at δ6.12 and 6.31, two *N*-methyl groups centred at δ3.05, two isolated aromatic hydrogens as singlets at δ7.49 and 6.96 and two vicinal aromatic hydrogens as an AB quartet at δ7.47 and 7.16 (*J*