Isolation of delbidine (3). The ground plant was extracted by percolation with 95% EtOH and evapd under red. pres. The EtOH extract was processed in the usual manner by acid–base extraction (pH 8.5) for the isolation of alkaloids. The crude base (11.5 g) was dissolved in $CHCl_3$ (30 ml), MeOH (1 ml) and chromatographed on a VLC column (90 g Al_2O_3, EM-1085-4) and eluted (28 fractions, 100 ml each) with hexane, Me_2CO and Me_2CO–MeOH. Fraction 26 (190 mg) when suspended in CH_2Cl_2 gave a ppt. (45 mg) which crystallized from MeOH as colourless cubes mp > 360°, $[\alpha]_D^{25°}$ + 22.3° (MeOH; *c* 0.268); MS, *m/z* 343 (M^+, 10%), 326 (5), 287 (7), 269 (20), 176 (19), 91 (49), 55 (100). IR ν_{max}^{nujol} 3508, 3360, 1685, 1660, 1460, 1370, 1350, 1332, 1315, 1295, 1280, 1265, 1220, 1200, 1180, 1085, 1065, 1040, 1030, 1000, 960, 940, 910, 880, 860, 810 cm^{-1}. ^1H NMR (DMSO-d_6): 1.36 (3H, *s*, C-18 Me), 4.5 (1H, *br s*, C-17 H), 4.7 (1H, *br s*, C-17 H).

Hydrolysis of geyeridine hydrochloride (4) to give (3). Geyeridine HCl (4; 5.1 mg) was dissolved in 3% KOH in MeOH (3 ml) and kept at room temp. for 16 hr. Usual work-up gave a gum (2.9 mg) which crystallized from MeOH to afford 1 as colourless cubes, mp > 360° TLC, CO-TLC and IR spectra showed identity with delbidine.

Acknowledgement—We are grateful to Prof. F. R. Stermitz for sending us a sample of geyeridine·HCl.

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CYCLOPEPTIDE ALKALOIDS FROM *ZIZYPHUS RUGOSA* BARK

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Key Word Index—*Zizyphus rugosa*; Rhamnaceae; alkaloids; nummularine-P; sativanine-H; rugosanine-B.

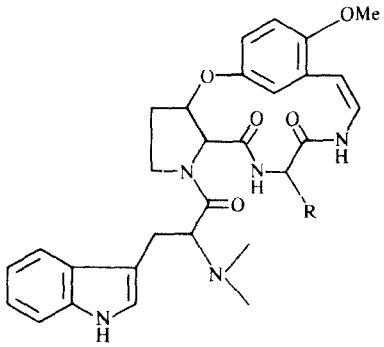
Abstract—The isolation of cyclopeptide alkaloids, nummularine-P, sativanine-H and rugosanine-B, a new 13-membered cyclopeptide alkaloid from the bark of *Zizyphus rugosa* is reported. The structure of the new alkaloid was elucidated by spectroscopic methods as well as by chemical degradation.

INTRODUCTION

Zizyphus rugosa Lam (Family: Rhamnaceae) is a large shrub distributed throughout India. In the Indian system of medicine the bark of this plant is used in the treatment of diarrhoea [1]. In continuation of our search for peptide alkaloids from the bark of *Z. rugosa* [2, 3], we now report here the isolation and characterization of a new 13-membered cyclopeptide alkaloid, rugosanine-B together with two known peptide alkaloids nummularine-P [4] and sativanine-H [5].

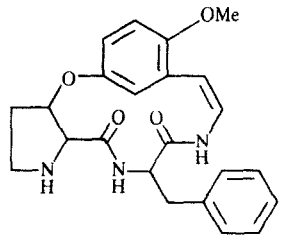
RESULTS AND DISCUSSION

Column chromatography of the basic fraction of *Z. rugosa* bark and repeated preparative TLC of chloroform–methanol (5:1) eluants on silica gel furnished



1 R = —CH_2—Ph

2 R = —CH(Me)—CH_2—Me



3

the alkaloid rugosanine-B (**1**) as colourless granules mp 216–218°. The molecular formula was determined by high resolution mass spectrum as $C_{36}H_{39}N_5O_5$ (M^+, 621.3124). The IR spectrum showed typical absorption for cyclopeptide alkaloids and the UV spectrum produce characteristic absorption maxima at 322 and 265 nm suggesting it to be a 13-membered cyclopeptide alkaloid. The IR and UV data as well as the mass spectral fragmentation pattern of **1** were exactly similar to those reported for nummularine-R (**2**) [6]. The presence of a *N*, *N*-dimethyl tryptophan unit in **1** like that of nummularine-R was indicated by its hydrolysis with barium hydroxide. The essential difference between **1** and **2** was observed by acid hydrolysis. Acid hydrolysis of **1** with 6M HCl gave phenylalanine whereas nummularine-R produced isoleucine. The attachment of phenylalanine bound to the nitrogen of a styryl function in **1** was settled by partial hydrolysis. On heating with 12 M HCl–HOAc–H_2O (1:1:1) at 100° for 5 hr, **1** furnished a major compound (**3**) which on further hydrolysis with 6M HCl gave phenylalanine. On the basis of these observations the structure of rugosanine-B is settled as **1**. It differs from that of nummularine-R in having a phenylalanine unit instead of isoleucine as the amino acid bound to the nitrogen of the styrylamine function. Rugosanine-B is thus a new addition in the lengthening chain of 13-membered cyclopeptide alkaloids.

The structure of the known cyclopeptide alkaloids were established by spectral evidence, hydrolysis experiments and direct comparison with authentic samples. These alkaloids have not earlier been reported from *Z. rugosa*.

EXPERIMENTAL

The plant material was collected from Mirzapur district, U. P. India and identified by the Department of Botany, Banaras Hindu University, Varanasi. A specimen sample is kept in the Department. The stem bark of the plant was utilized for the present investigation.

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. [7]. The combined extract was concd to small vol. and further extracted with 7% citric acid. The crude alkaloids (6.5 g) were obtained from the aq. acidic soln by basification and subsequent extraction with $CHCl_3$. Extensive chromatography of the crude base and repeated prep. TLC of the $CHCl_3$–MeOH (5:1) eluants with $CHCl_3$–EtOAc–MeOH (1:1:2) furnished rugosanine-B (20 mg).

Rugosanine-B. Crystallized from MeOH as colourless granules mp 216–218° (dec.), $C_{36}H_{39}N_5O_5$ (M^+, *m/z* at 621.3124), R_f: 0.32 ($CHCl_3$–MeOH, 2:1) and 0.48 ($CHCl_3$–EtOAc–MeOH, 10:5:1). It showed IR $\nu_{max}^{CHCl_3}$ cm^{-1}:3380, 3248 (–NH), 2782 (–NMe), 2860 (OMe), 1635, 1690 (sec. amide), 1620 (C=C), 1585, 1470 (aromatic), 1040 and 1210 (aryl ether). UV λ_{max}^{MeOH} 322 and 265 nm (characteristic of the styryl amine chromophore in the 13-membered cyclopeptide alkaloid), 292 *sh*, 280 *sh* and 270 *sh* (tryptophan unit) [8]. MS, *m/z*: 621.3124 ([M^+], 5%), 491 (100), 435 (2), 434 (1.5), 408 (2.5), 407 (4), 406 (3), 338 (2), 259 (2), 243 (4), 233 (3), 216 (4), 187 (60), 165 (10), 144 (15), 130 (25), 96 (5), 68 (10).

Hydrolysis. Compound **1** (3 mg) was heated in a sealed tube with 1 ml of 6M HCl for 20 hr at 120°. The presence of phenylalanine was confirmed by PC comparison with an authentic sample using *n*-BuOH–HOAc–H_2O (4:1:5) with ninhydrin as detection reagent. Compound **1** (3 mg.) was heated with $Ba(OH)_2$ (40 mg) in 1 ml H_2O for 24 hr at 120°. The hydrolysate was neutralised with 1M H_2SO_4, filtered, and examined on a paper chromatogram, spraying with Ehrlich's reagent [9]. *N,N*-Dimethyl tryptophan was detected in the hydrolysate by comparison with an authentic sample.

Partial hydrolysis. Compound **1** (5 mg) was heated with 3 ml of 12 M HCl–HOAc–H_2O (1:1:1) at 100° for 5 hr. The hydrolysate after prep. TLC with $CHCl_3$–MeOH (50:1) gave one major compound **3** which on hydrolysis with 6 M HCl in a sealed tube for 20 hr at 120° gave phenylalanine (co-PC).

Repeated CC and prep. TLC of the crude base fraction of *Z. rugosa* also furnished nummularine-P, mp 179–180° and sativanine-H, mp 190–192°. The structures of these alkaloids were established by a study of spectral data, hydrolysis and direct comparison with authentic sample (mmp and co-TLC).

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HAPALONAMIDES AND OTHER OXIDIZED HAPALINDOLES FROM *HAPALOSIPHON FONTINALIS*

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Key Word Index—*Hapalosiphon fontinalis*; Cyanophyta; Stigonemataceae; indole alkaloids; fungicides.

Abstract—Dechlorofontonamide, anhydrohapaloxindoles B and M, and hapalonamides G, H, and V have been isolated as minor indole alkaloids from a cultured strain of the terrestrial blue-green alga *Hapalosiphon fontinalis*.

INTRODUCTION

Fontonamide (**1**) and anhydrohapaloxindole A (**2**), two minor alkaloids from *Hapalosiphon fontinalis*, appear to be singlet oxygen oxidation products of hapalindole A (**3**), the major alkaloid in this terrestrial blue-green alga [1]. Hapalonamides A (**4**) and G (**5**), the proposed precursors of fontonamide [1], are formed along with **1** and **2** when hapalindole A is oxidized under singlet oxygen oxidation conditions. In our continuing studies on the minor constituents of *H. fontinalis*, we have now detected hapalonamide G in the lipophilic extract of the cyanophyte. Two other hapalonamides H (**6**) and V (**7**) have also been found, as well as dechlorofontonamide (**8**) and two new anhydrohapaloxindoles B (**9**) and M (**10**).

RESULTS AND DISCUSSION

Structure elucidation of the six new alkaloids was straightforward. The chromophore of each compound was ascertained by UV spectroscopy and the molecular composition and presence or absence of chlorine were determined by mass spectrometry. The gross structures and relative stereochemistries were solved using previously described methodology [1, 2]. Dechlorofontonamide and anhydrohapaloxindoles B and M showed ^{1}H NMR spectra, including NOE difference spectra, that were comparable with the ones for **1** and **2** [1].

The hapalonamide G from *H. fontinalis* was found to be identical in all respects with semisynthetic material, the latter formed when an oxygen-aerated solution of **3** in aqueous methanol buffered at pH 8 with sodium phosphate was irradiated at room temperature in the presence of a trace of rose bengal [1]. Hapalindole G (**11**) was also produced in the reaction mixture when **3** was incompletely oxidized. Hapalonamide G appeared to have formed by a free-radical induced transformation of **3** to **11**, followed by singlet oxygen cleavage of the indole Δ^2-double bond in **11**. Hapalonamide A [1] is presumably also present in the algal extract, but has not been detected.

Hapalonamide H gave essentially the same ^{1}H NMR data as hapalindole H [2] with respect to the chemical