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Research Article

Cyclopeptide Alkaloids of *Zizyphus Rugosa*

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Abstract: Threecyclopeptidealkaloids,Nummularine-M, Nummularine-N and Rugosanine-C has been isolated from the root bark *Zizyphusrugosa* and their structures were established by spectral avidences. This is the first report of Nummularine-M and Nummularine-N in *Z.rugosa*. Rugosanine-C is a new 14-membered cyclopeptide alkaloid.

Keywords:*Zizyphusrugosa*, Cyclopeptidealkaloid.

INTRODUCTION

The plant Zizyphusrugosa Lam (Family: Rhamnacea) is a large straggling armed shrub sometimes climbing; young branches clothed with fulvous tomentum. Leaves 5-12.5 cm, brodlyelliptocal, denticulate, glaborus above; peteds 6-12cm long, tomentose, prickles from a broad base solitary, short and recurved. Flowers in long-pedunculedtomentoes cymes, buds globose, calyx pubscentout side; drupes 6-8 mm diameter globose, when ripe[1]. This plant finds medicinal values in the Indian system of medicine. Powdered bark mixed with ghee is applied to the swelling in the cheek caused by infection of teeth. The bark also considered as astringent to stop diarrhoea. Flowers with an equal quantity of the petiols of the betel and half as much time are given in four grain pills twice a day for menorrhagea[2] and of nutritive value Review of the literature revealed the report of ten peptied alkaloids[3,4,5,6] from the bark of this plant. We report have with three cyclopeptide alkaloids from the bark of *Z.rugosa* which has not been reported from this plant.

EXPERIMENTAL

The powderedstem bark (4kg) of *Zizyphusrugosa* was extracted with three times its volume of a mixture of benzene, concentratedaqueous ammonia and methanol in the ratio 100:1:1, respectively. The five

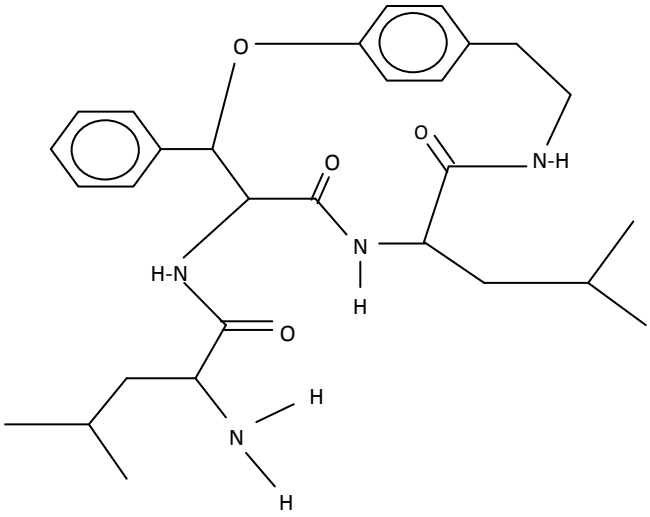
successive extraction were adequate. The total extract was concentrated and stirred mechonically with 5% aqueous citric acid two times (250 ml, 100 ml) for 6 hours. The aqueous acidic phase was separated from benzene layer by a separatory funnel. The aqueous acidic phase was basified with NH_4OH(PH~2) and exacted with $CHCl_3$ in liquid-liquid extractor. Evaporation of the chloroform extract after washing with water and drying afforded a mixture of crude alkaloids (4.5 Kg). The crude alkaloid fraction was chromatographed over silica gel column eluting with a mixture of chloroform-methanol of increasing polarity and the collected elvants were monitored by this layer chromatography and evaporate to dryness. The chloroform-methanol (2.4:2) elvants were mixed together and crystallized from acetone as colorless crystals (8.5 mg) a cyclopeptide alkaloid, nummularine-M[7](1), m.p. 263-65^{0}c (uncorr) and chloroform-methanol (24:4) elvants were mixed and crystallised from methanol as colourless crystals (20 mg) a peptide alkaloid nummularine-N [2], m.p. 243-45C (uncow). Moreover, chloroform-methanol (32:40) eluantes were collected together crystallised from methanol as amorphous solid (18mg), another cyclopeptide alkaloid Rugosanine-C[3], a new cyclopeptide alkaloid.

Nummularine-M[1]: It exhibited UV(MeOH) strong end absorption at about 200 and shoulders at 250 and 280 nm. IR ν_{max} cm^{-1}: 3300(-NH), 2790 (-NCH_3) 1685, 1635 (-NH-CO), 1240 (Ar-O-C). Mol. Wt. (High Resolution MS)534. 3190; calcd. for $C_{31}H_{42}N_4O_4$:534. 3174, MS: m/z 534 (M^+), 519.2987, 505.2804, 477.2479, 421.1984, 419.1830, 378.1912, 337.1712, 274.1310, 244.1325, 229.0956, 224.1073, 216.1369, 201.1044, 135.0688, 131.0501, 114.1267, 103.0936, 85.0887, 86.0959. Hydrolysis of [1] with 6N HCl for 10 hours in a sealed tube at 100-120^{0}c furnished two ninhydrin positive spots corresponding to N, N-dimethylisoleucine and isoleucine by co-PCcomparison with authentic sample. The spectral data of all the compound tallies with the reported data. The structure of the alkaloid was further confirmed by direct comparison with authentic sample (m, m.p., co-TLC and superimposable IR)

Nummularine – N [2]: It exhibited UV(MeOH) λ max 267 (log ε 4.00)and 320 nm (log ε 3.8). IR v max cm-1:3300 (-NH), 2821(-OCH_3), 2775 (-NCH_3), 1688 and 1640 (secondary amido group), 1610 (styryl double bond), 1220 and 1020 (phenol ether). 90 MHz, 1H-NMR ($CDCl_3$):δ0.68 (dd, J=5Hz, 26 x methyl groups), 1.72 (m, H-C), 2.48 (s), 2 x NCH_3), 260-3.70 complex pattern, three CH_2), 3.80 (s, Ar-0-CH3), 4.00-4.8 (complex pattern, methylene and methineH flanked between corbnyl and introgen function), 5.61 (dt H_2C—C—H)' 6.00 (d, J=7.5 Hz, one cisolefinicH),MS:m/z 591 (M+), 548, 534, 532, 491, 435, 434, 408, 406, 338, 259, 243, 233, 216, 165, 157, 96, 68, 58 (base peak). Compound [2] on hydrolysis with 6N HCl in a sealed tube for 10 hours at 100-1200c furnished three ninhydrine positive spots corresponding to phenylalanine, valine and N,N-dimethylglycine. The results of hydrolysis experiment of the alkaloid [2] coupled with the knowledge of its 1H-NMR and mass spectral data lead to conclusion that compound [2] is nummularine-N. It was further confirmed by direct-comparision with anthentic sample (by IR, UV and m.mp).

Rugosonine-C[3]: It exhibited IR (KBr) λmax (cm^{-1}): 3400(-NH), 2960 –CH), 1645 (amide), 1635 (C=C), 1550 (ramatic), 1220 and 1030 (aryl ether); UV (MeOH) λ max(nm): typical strong end absorption at 204; **90MHz 1HNMR, (**$CDCl_3$(δ):0.71 (12H, dd, J=6.5 Hz), 1.73 (2H,m), 2.60-3.65 (4H, complex pattern), 4.45-5.35 (4H, complex pattern), 6.05 (1H, d, J=9 Hz), 6.70-8.60 (15H, complex pattern); **100MHz ^{13}CNMR** ($CDCl_3$+CD_3OD) 18:122.8 (C-1), 124.4 (C-2), 167.0 (C-4), 52.1 (C-5), 170.4 (C-7), 52.8 (C-8), 81.8 (C-9), 156(c-11), 114.5 (C-12),121.9 (C-12), 132.2 (C-13), 130.4 (C-13), 132.0 (C-14), 39.0 (C-15), 25.3 (C-16), 22.2 (C-17), 22.0 (C-18), 172.0 (C-20), 66.1 (C-21), 34.1 (C-22), 24.8 (C-23), 20.5 (C-24), 23.1 (C-25), 140.0 (C-26), 127.0 (C-27), 127 (C-27), 128.4 (C-28), 128.4(C-28), 125.0 (C-29); **HRMS** m/z (relative intensity, %):506.2892. [M^+] (18), 491.2658 (25), 449.2188 (24), 421.1998 (33), 419.1833 (12), 378.1945 (15), 337.1916 (20), 274.1313(32), 244.1330 (9), 201.0664 (21), 224.1073 (12), 216.1388 (10), 173.0715(22), 135.0682 (33), 131.0496 (14), 103.0546 (46)m 86.0969 (90), 85.0891. Hydrolysis of [3] with 6N HCl in a sealed tube at 120^{0}c for 24 hours furnished one ninhydrin positive spot corresponding to

leucine by co-Pc comparison with authentic sample the structure [1] fits will with for compound [3] on the basis of all spectral data.



RESULT

Compound [1] Colourless crystals, m.p. 263-650c $[\alpha]_D$ 46.66^0 (c,0^0, $CHCl_3$) was recognised to be a 14-membered cyclopeptide alkaloid from its UV spectrum. The IR spectrum exhibited bands for –NH, -NMe, -NHCO and Ar-O-C. It is isomeric with integerrenine[9] and both molecules show identical mass fragmentation patterns indicating their gross structural similarity. However, acid hydrolysis revealed the assential difference between the two molecules. Thus, while integerrinine provides N, N-dimethylisoleucine and **leucine**[1] gives N, N-dimethylisoleucine and isoleucine. Based on these findings the structure of the **Compound [1]** was proved to have the **structure[1]** which differs from that of integerrenine in having an isoleucine unit instead of leucine as an amino acid bound to the nitrogen of styrylamine function. **Compound [1]** is therefore, an isomer of integerrinine. Obviously, the structure of **Compound [1]** is estabilished as **nummularine-M[7],**Although, **nummularine-M** has been previouly isolated from *Z. nummularia* but this is the first report of the occurrence of this alkaloid in Z. rugosa.

Compound [2]bright colourless crystals, m.p. 243-450c, showed the presence of a 13-membered ring cyclopeptide alkaloid system in its UV spectrum. The IR exhibited bands for-NH,-OMe, -NMe, amide, styryl double bond and phenol ether. On acid hydrolysis it gave phenylalanine, valine and N, N-dimethylglycine. The 1H-NMR spectrum showed a close similarity to that of nummularine-B10, with the exception that the former exhibited a signal for an additional-NMe group and the absence of secondary-CMe group. The mass spectrum was dentical to that of nummularine-B. Examination of spectral data and hydrolysis experiment shows that the Compound[2]possessess a structure which differs from nummularine-B. The foregoing data suggested beyond doubt the structure of compound [2] as reported for nummularine-N.

Compound [3]: amorphous solid, $[\alpha]_D$ $- 138^0$ (c,0.20, $CHCl_3$), was recognised as a 14-membered ring cyclopeptide alkaloid as indicated by its UV spectrum. The IR spectrum exhibited band for –NH, -CH, phenol, ether, styryl double bond, aromatic and secondary amido group. On acid hydrolysis, it gave leucine. ^{1}HNMR spectrum revealed the presence of four methyl, two methylene, six methine, five-NH, nine Ar-H and cis-olefinic protons. ^{13}CNMR of **Compound [3]** was comparable with that of **franganine[11]**. The difference between two was observed with the chemical shift of carbon signals due to ring bound

amino acids attached to ether function. In **compound [3]** phenylalanine unit is attached. A scrutiny of IR, UV, ^{1}HNMR, ^{13}CNMR,, hydrolysis and partial hydrolysis experiments and high resolution mass spectrometric analysis revealed that **compound [3]** has gross structural similarity with that of nummularine-M and integrennine. Compound [3] differs from **integrennine** in only having leucine as terminal amino acid instead of N, N-dimethylisoleucine. **Compund [3]** on N-methylation furnished **discarine-C.** Obviously, the foregoing finding leave on doubt about the structure of **Compound [3]**. This is a new 14-membered ring cyclopeptide alkaloid designated as **rugosanine-C.**

DISCUSSION

The spectral data of the compound [1] and compound [2] tallies with the reported spectral data. However, compound {3} differs from intetrennine in only having liucine as terminal amino acid instead of N, N-dimethylisoleucine and that was confirmed by hydrolysis and mass spectroscopic analisps.

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REFERENCES

1. K.R. Kitikar and B.D. Basu, "Indian Medicinal Plants", 1984, 592, 1
2. R. Tschesche, Ch. Spilles and G. Eckhardt, Ber. , 1974, **107**, 686.
3. V.B.Pandey, J.P.Singh and et al., Phytochemistry, 1988, **276,** 1915.
4. R. Tchesche, J.P.Singh et al; *Pharmazie, 1981,* 36, 511,
5. Y. C. Tripathi, S. K. Maurya and et al. *Phyotochemistry, 1989,* **28(5),** 1563,
6. A. singh; M.B.Pandey and et al; *Journal*of theIndian Chemical Society,2008, **85(6)** 658
7. V.B.Pandey; J.P.Singh, and etal, Phyotochemistry, 1984, **23(9),** 2118.
8. K.S.Vinayaka; P. Ravindra; H. G. Kumar Swamy. Research and Reviews in Biomedicine and Biotechnology, 2004, **2(4-2),** 20.
9. R. Tschesche, J. Rheingans, H-W. Fehlhaber and G Legler, *Chem. Br.***,** 1967**,100,** 3924
10. V.B.Pandey; J.P.Singh et al; Phytochemistry, 1984, **23(9),** 2118.
11. A. H. Shah; A. M. Agect; and et al.; Fitoterapia, 1986, **57(6),** 452.

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