TSCHESCHAMINE - A NEW CYCLOPEPTIDE ALKALOID FROM THE BARK OF ZIZYPHUS SATIVA GAERTN

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Abstract - A new minor 13-membered cyclopeptide alkaloid tscheschamine has been isolated from the bark of Zizyphus sativa and its structure was determined on the basis of spectral studies and hydrolysis.

Zizyphus species (Rhamnaceae) are used in traditional medicine for treating insomnia[1], healing of wounds and ulcers[2] and bronchitis[3]. They are a rich source of cyclopeptide alkaloids[4]. Cyclopeptide alkaloids are polyamide plant bases containing a styrylamine unit in a 13-, 14-, or 15-membered macrocyclic ring system[5]. It is interesting to note that from Z. sativa[6] just like Z. amphibia[7,8], Z. nummularia[9-12] and Z. jujuba[13] only 13- and 14-membered cyclopeptide alkaloids were isolated. The 13- and 15-membered cyclopeptide alkaloids were found in Z. oenoplia[14] and Z. mucronata[15]. Z. abyssinica[16] possessed only 15-membered cyclopeptide alkaloids while Z. mauritiana[17] and Z. spina-christi[18,19] were found to contain only 14-membered alkaloids of this class. Recently we reported the isolation of nine new alkaloids from Z. sativa[20-25]. In this communication we report the isolation and structure determination of a new minor alkaloid, tscheschamine (1), from the bark of this plant.

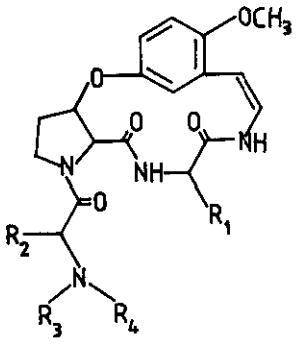
EXPERIMENTAL

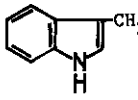
Zizyphus sativa Gaertn bark was collected from Hazara district of Pakistan. The stem bark (5 kg) was repeatedly extracted with a mixture of C_6H_6-EtOH-conc.NH_4OH aq. (100:2:1) and solvent evaporated. The residue was dissolved in 7% citric acid and filtered. The aq. layer was basified with NH_4OH to pH 10, and extracted with $CHCl_3$ to give crude alkaloids (2.1 g). The mixture of crude bases was chromatographed on silica gel (90 g) column, eluting with increasingly polar CH_2Cl_2-MeOH mixtures into 10 main fractions. The collected fractions were analysed by TLC proving in every case to be mixture of two or three compounds. The pure compounds were separated by prep. TLC using chromatotron-7924. JEOL JNM-FX 100 (100 MHz, nmr), Pye Unicam SP8-100 (uv)

and Perkin-Elmer 580 B (ir) instruments were used. Mass spectra were measured on MS-50 (Kratos) at 70 eV with evaporation of the sample in the ion source at ca. 200°C.

Tscheschamine (1): Amorphous powder (3.1 mg), mp 197-198°C (uncorr.) was isolated from fraction-VII. Uv (MeOH) λ_{max} nm: 260 and 320; Ir ν^{KBr} 3300 (NH), 2820 (OMe), 1680 and 1640 (amide), 1615 (C=C), 1230 and 1040 (aryl ether). ^{1}H-Nmr ($CDCl_3$/TMS): δ = 0.8-0.95 signal complex (6H, 2C-Me), 3.8 s (3H, OMe), 4.3 d (1H, J = 5.8 Hz, 3-HyPro-2-H), 5.5 m (1H, 3-HyPro-3-H), 5.9 d (1H, J = 9 Hz, vinyl-H), 6.95 dd (1H, J_1 = 9 Hz, J_2 = 12 Hz, vinyl-H), 6.7-8.6 m (12 H, 8 ArH, 2 olefin protons, 2 amide protons), 8.25 and 7.3 ppm (1H each, D_2O exchangeable). MW(Ms): 520.2692; calcd for $C_{29}H_{36}N_4O_5$: 520.2688. Ms 70 eV, m/z (rel. int.): 520 $[M]^+$ (12), 86[a] (100), 463 [b] (< 0.5), 435 [f] (24), 434 [g] (0.2), 408 [h] (1.5), 407 [i] (5.9), 406 [j] (0.6), 243 [p] (8.2), 259 [r] (3.2), 233 [s] (2.5), 216 [t] (1.9), 165 ($C_9H_{11}NO_2$, 11.2), 120 ($C_8H_{10}N$, 8.6), 96 (C_5H_6NO, 8.5), 68 (C_4H_6N, 9.1).

Hydrolysis: Compound(1) (1.5 mg) was heated in a sealed tube with 1 ml of 6N HCl for 24 h at 120°. Excess reagent was evaporated in vacuo and the residue taken up in H_2O for paper chromatography. The amino acids were identified by comparison with authentic samples using n-BuOH-HOAc-H_2O (4:1:5)[10] and n-BuOH-H_2O-Me_2CO-conc. NH_4OH aq. (8:6:1:1)[11] as solvent systems and ninhydrin as spray reagent.

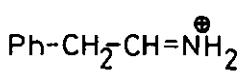


	R_1	R_2	R_3	R_4
Tscheschamine (1):	$Ph-CH_2-$	$CH_3-CH_2-CH(CH_3)-$	H-	H-
Nummularine-C (2):	$(CH_3)_2CH-CH_2-$	$Ph-CH_2-$	CH_3-	CH_3-
Sativanine-E (3):	$(CH_3)_2CH-CH_2-$		CH_3-	CH_3-
Sativanine-G (4):	$CH_3-CH_2-CH(CH_3)-$	$CH_3-CH_2-CH(CH_3)-$	CH_3-	CH_3-

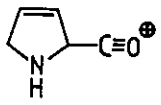
RESULTS AND DISCUSSION

Tscheschamine ($[M]^+$ m/z 520.2692) was recognized to be a 13-membered cyclopeptide alkaloid from its uv spectrum.[15] The ir spectrum exhibited bands characteristic for cyclopeptide alkaloids.[12] The ^{1}H-nmr spectrum of (1) confirmed the presence of OMe and cis-styrylamine moiety. The two (D_2O exchangeable) NH signals were recognized at δ 8.2 and 7.3 ppm. The cis-olefin proton

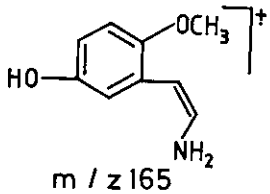
Scheme_1: Important fragments in the mass spectrum of (1).



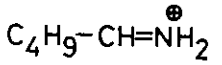
m / z 120



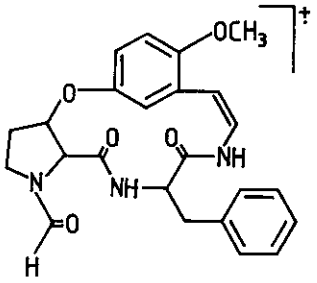
m / z 96



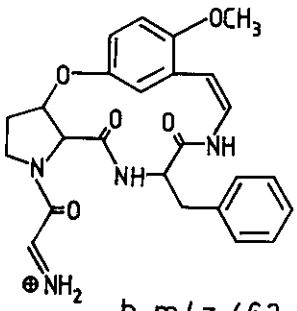
m / z 165



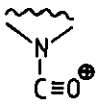
a m/z 86



f m / z 435

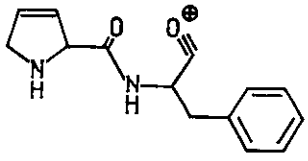


b m / z 463

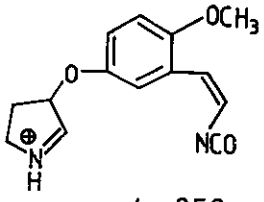


g m / z 434

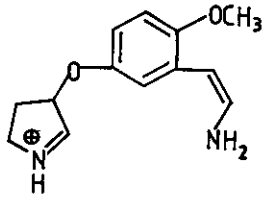
h m / z 408
i m / z 407
j m / z 406



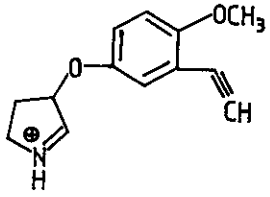
p m / z 243



r m / z 259



s m / z 233



t m / z 216

adjacent to the substituted benzene of the 13-membered ring system appeared as doublet at δ 5.9 ppm while the second olefin proton was found at 6.9 ppm. On acid hydrolysis of (1) isoleucine and phenylalanine were confirmed by paper chromatography. The molecular formula of (1) was determined by high resolution mass spectroscopy as $C_{29}H_{36}N_4O_5$. The mass spectrum of (1) [Scheme-1] was most revealing. The molecular ion appeared at m/z 520. The α-cleavage product a (m/z 86) of the terminal amino acid isoleucine formed the base peak of the spectrum. The fragment b was observed at m/z 463. The typical fragments for hydroxyproline m/z 96 and 68, phenylalanine group m/z 120 and methoxystyrylamine group m/z 165 reveal the identity of the units that form 13-membered ring system; f m/z 435, g m/z 434, h m/z 408, i m/z 407 represent the whole macrocyclic ring while p m/z 243, r m/z 259, s m/z 233, and t m/z 216 show their linkage. The elementary composition of all fragments was substantiated by high resolution mass spectroscopy. All of spectral data prove the structure (1) for tscheschamine.

The mass spectrum of (1) is closely related to that of [nummularine-C (2)[9] sativanine-E (3)[20] and sativanine-G (4)[22]] 13-membered cyclopeptide alkaloids containing a short side chain. Tscheschamine (1) differs in structure from (2) in having isoleucine unit instead of N,N-dimethylphenylalanine as terminal amino acid and phenylalanine instead of leucine as an amino acid bound to the nitrogen of the styrylamine function. Tscheschamine[26] is a new addition to the growing list of nummularine-C type of 13-membered cyclopeptide alkaloids.

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REFERENCES

1. S.S. Kang, S.H. Shim, H. Wagner, V. Mohanchari, O. Seligmann, and G. Ober-Maier, **Phytochemistry**, 1979, 18, 353.
2. K.R. Kirtikar and B.D. Basu, "Indian Medicinal Plants", Periodical Expert Book Agency, Delhi, 1984, Vol. 1, p. 593.
3. R.N . Chopra, S.L. Nayer, and I.C. Chopra, "Glossary of Indian Medicinal Plants", CSIR, New Delhi, 1956, p. 262.
4. R. Tschesche and E.U. Kaussmann, "The Alkaloids" (Ed. R.H.F. Manske), Academic Press, New York, 1975, Vol. XV, p. 165.

5. A.H. Shah and V.B. Pandey, **J. Chem. Soc. Pak.**, 1985, 7, 363.

6. A.H. Shah, V.B. Pandey, G. Eckhardt, and R. Tschesche, **Phytochemistry**, 1984, 23, 931.

7. R. Tschesche, E.U. Kaussmann, and H.-W. Fehlhaber, **Chem. Ber.**, 1972, 105, 3094.

8. R. Tschesche, Ch. Spilles, and G. Eckhardt, **Chem. Ber.**, 1974, 107, 686.

9. R. Tschesche, G.A. Miana, and G. Eckhardt, **Chem. Ber.**, 1974, 107, 3180.

10. R. Tschesche, M. Elgamal, G.A. Miana, and G. Eckhardt, **Tetrahedron**, 1975, 31,2944.

11. R. Tschesche, M. Elgamal, and G. Eckhardt, **Chem. Ber.**, 1977, 110, 2649.

12. V.B. Pandey, J.P. Singh, K.K. Seth, A.H. Shah, and G. Eckhardt, **Phytochemistry**, 1984, 23, 2118.

13. R. Tschesche, I. Khokhar, H. Wilhelm, and G. Eckhardt, **Phytochemistry**, 1976, 15, 541.

14. B.K. Cassels, G. Eckhardt, E.U. Kaussmann, and R. Tschesche, **Tetrahedron**, 1974, 30, 2461.

15. R. Tschesche, S.T. David, J. Uhlendorf, and H.-W. Fehlhaber, **Chem. Ber.**, 1972, 105, 3106.

16. R. Tschesche, S.T. David, R. Zerbes, G. Eckhardt, and E.U. Kaussmann, **Liebigs Ann. Chem.**, 1974, 1915.

17. R. Tschesche, H.Wilhelm, E.U. Kaussmann, and G. Eckhardt, **Liebigs Ann. Chem.**, 1974, 1694.

18. R. Tschesche, I. Khokhar, Ch. Spilles, and M. von Radloff, **Phytochemistry**, 1974, 13, 1633.

19. A.H. Shah, A.M. Ageel, M. Tariq, J.S. Mossa, and M.A. Al-Yahya, **Fitoterapia**, 1986, 6, 452.

20. A. H. Shah, V. B. Pandey, G. Eckhardt, and R. Tschesche, J. **Nat. Prod.**, 1985, 48, 555.

21. A.H. Shah, V.B. Pandey, G. Eckhardt, and R. Tschesche, **Phytochemistry**, 1985, 24, 2765, and 2768.

22. A.H. Shah, V.B. Pandey, J.P. Singh, K.N. Singh, and G. Eckhardt, **Phytochemistry**, 1984, 23, 2120.

23. R. Tschesche, A.H. Shah, and G. Eckhardt, **Phytochemistry**, 1979, 18, 702.

24. A.H. Shah, G.A. Miana, S. Devi, and V.B. Pandey, **Planta Medica**, 1986, 6, 500.

25. A.H. Shah, M.A. Al-Yahya, S. Devi, and V.B. Pandey, **Phytochemistry**, 1987, 26, 1230.

26. This name was given in the honour of late Professor Rudolf Tschesche, Institute of Organic Chemistry and Biochemistry, Bonn University, W. Germany.

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