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THE STRUCTURE OF ROHITUKINE, THE MAIN ALKALOID OF AMOORA ROHITUKA (Syn. APHANAMIXIS POLYSTACHYA) (MELIACEAE)

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Plants of the family Meliaceae have repeatedly been the object of chemical study on account of the occurrence of interesting di- and triterpenoids[1,2] and of volatile oils.[3] The presence of alkaloids has been recorded in several species[3,4a-c]; as far as we know, however, none of these bases has ever been examined in detail. We wish to report the isolation and structure determination of a novel alkaloid from Amoora rohituka[5] for which we suggest the name rohitukine.

Ethanol extraction of 6.8 kg of dried leaves and stems, followed by acid-base workup, furnished 43.6 g of a dark brown resinous material.[6] Fractionation of 2 g of this extract by column chromatography (silica gel; methylene chloride/methanol 2:1) afforded three widely separated basic fractions. Rohitukine was isolated from the most polar band and was obtained in crystalline form upon slow evaporation of the eluting solvent. Recrystallization from methanol gave 28 mg of pale yellow crystals, mp 218 - 219°C.

High-resolution mass spectrometry and elemental analysis established the molecular formula as $C_{16}H_{19}NO_5$ (M+ 305.1264; calculated 305.1264). Mass fragmentation indicated the loss of CO and H as a major pathway. The infrared spectrum of rohitukine[7] established the presence of hydroxyl and ketone groups and suggested the presence of a γ-pyrone. The nitrogen is tertiary (absence of N-H stretching bands).

^1H and ^{13}C nmr spectra[8,9] were recorded in pyridine-d_5 because of the low solubility of the alkaloid in the usual solvents. The appearance of two shielded singlets (1 H each) in the aromatic/alkene region, together with the high level of unsaturation required by the molecular formula, suggested a resorcinol- or phloroglucinol-like aromatic system.

In order to establish the structure, an x-ray analysis was carried out on crystals obtained from ethanol solution.[10] The crystals proved rapidly efflorescent but, with some difficulty, a crystal was sealed in a thin glass capillary and data were collected with this sample. The structure was solved using MULTAN.[11] Although electron density could be seen at reasonable positions in difference maps calculated toward the end of the refinement, no clearly defined solvent molecules were visible and it must be concluded that the weakly held solvent is disordered. Several models for the disorder were tried but none seemed entirely satisfactory.

It became apparent that the solvent only made significant contributions to low angle reflections; hence, the final refinement was based on data with $\sin\theta/\lambda > 0.22\text{Å}^{-1}$. Heavy atoms were refined anisotropically; hydrogen atoms were included but not refined (electron density peaks were observed for most but not all of the hydrogen atoms). The final R-factor was 7.0% and esd.s of heavier atom bond lengths and angles are <0.01Å and <0.6° respectively, when carried out by the XRAY-72[12] system of programs. From this analysis, rohitukine has the structure and relative configuration 1. The molecule in its crystal conformation is shown in Figure 1 (ORTEP[13] drawing), the crystal packing in Figure 2. Tables of bond lengths and angles and atomic coordinates may be obtained from the Cambridge Crystallographic Data Center.

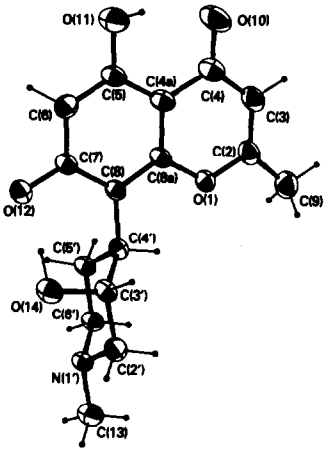


Figure 1

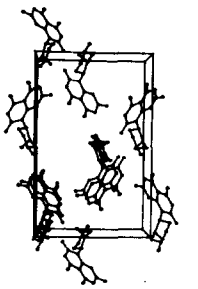
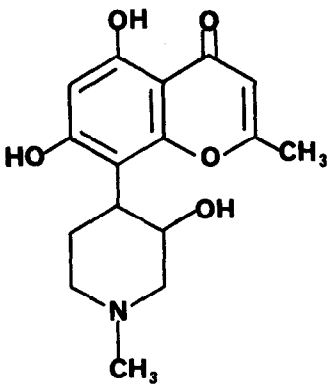


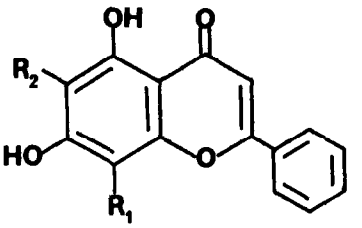
Figure 2
The unit cell viewed along the a axis.

To the best of our knowledge, rohitukine (1) is unusual in several ways: 1) it seems to be the first alkaloid from the Meliaceae whose structure has been determined;[14] 2) it is the first example of a chromone alkaloid (the flavone alkaloids ficine (2a) and isoficine (2b)[15] from Ficus pantoniana, Moraceae, appear to be the closest analogs of 1 observed so far); 3) the presence of the 4-substituted 3-hydroxypiperidine moiety in 1 is unusual in a natural compound, anatalline,[16] from Nicotiana tabacum, and gentialutin,[17] from Gentiana asclepiadea, being the only similar alkaloids which have come to our attention.

The alkaloidal extract also yielded[18] noreugenin (3),[19a] mp 296-99° (sealed capillary; lit.[19] mp 281-2°).[20] This compound has been isolated several times from higher plants but has not so far been observed in a meliaceous species.[21] Its isolation from the extract of A. rohituka does not prove that it occurs as such in the plant; it could have formed from 1 or related alkaloids during the prolonged storage of the extract;[6] (cf. the ready formation from 2 of the parent flavone, chrysin, on pyrolysis or treatment with base[15]). However, the rather large quantity of 3 isolated from our extract may suggest that at least part of it is preformed.

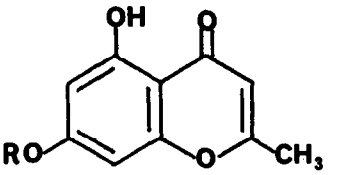


1



2a: R_1 = Me-N , R_2 = H

2b: R_1 = H, R_2 = Me-N



3: R = H

4: R = CH_3

REFERENCES AND NOTES

1. A. Chatterjee, A. B. Kundu, T. Chakrabortty, and S. Chandrasekharan, Tetrahedron, 26, 1859-67 (1970), and references cited therein.
2. W. Kraus, W. Grimminger, and G. Sawitzki, Angew. Chem. Int. Ed. Engl., 17 452-3 (1978), and references cited therein.
3. See, for example, R. Hegnauer, "Chemotaxonomy der Pflanzen," Vol. 5, pp. 64-5, Birkhäuser Verlag, Basel (1969).
4. a) J. J. Willaman and B. G. Schubert, "Alkaloid-Bearing Plants and Their Contained Alkaloids," Technical Bulletin No. 1234, Agricultural Research Service, U. S. Department of Agriculture, 1961; b) J. J. Willaman and H-L. Li, Lloydia, 33, No. 3A, 133 (1970); c) R. F. Raffauf, "A Handbook of Alkaloids and Alkaloid-Containing Plants," Wiley, New York, 1970.
5. Amoora rohituka Wight and Arn. seems to be synonymous[1] with Aphanamixis polystachya (Wall.) Parker. This question of nomenclature does not seem resolved at present; see, e.g., The Kew Record of Taxonomic Literature, Royal Botanic Gardens, Kew, p. 201, 1973. Under the circumstances, we have decided to retain the name Amoora rohituka, since we received the extract under this designation.
6. The alkaloid extract was kindly provided by Prof. Robert F. Raffauf, Northeastern University, Boston, Massachusetts, and was obtained by following a standard extraction procedure used for testing antitumor activity of plant constituents; it had been stored for several years prior to the isolation of 1 here reported. The plant material was collected and the species verified by B. C. Shah and Company of Indore (MP), India, during the spring of 1969 in Mussoorie, India.
7. Infrared spectrum ν_{max} (KBr) 3400 (broad, OH); 1660 (C=O); 1612 and 1560 (γ-pyrone) cm^{-1}.
8. NMR (220 MHz) δ in ppm from TMS (pyridine-d_5)
   1.57 (dd, J= 12 Hz, 1H), 2.14 (m, 1H), 2.21 (s, 3H), 2.27 (s, 3H), 2.36 (d, J= 12 Hz,

2H), 2.99 (d, J= 12 Hz, 2H), 3.16 (dt, J= 12 Hz, <2 Hz, 1H), 3.63 (dt, J= 12 Hz, <2 Hz, 1H), 4.44 (d, J= <2 Hz, 1H), 6.17 (s, 1H), and 6.79 (s, 1H).

9. CMR (15 MHz) δ (mult) in ppm from TMS (pyridine-d_5) 19.9 (q), 25.4(t), 38.2(d), 46.2(q), 56.8(t), 62.5(t), 69.9(d), 101.5(d), 108.5(d), 155.9(s), 156.2(s), 161.4(s), 166.8(s), 183.2(s).
10. The space group is $P2_12_12_1$ with cell dimensions a= 7.144(1), b= 13.680(2), c= 20.482(2) Å. There are four molecules in the unit cell.
11. P. Main, M. M. Woolfson, and G. Germain, "MULTAN-72. A computer program for the automatic solution of crystal structures." Universities of York, England and Louvain, Belgium, 1972.
12. J. M. Stewart, G. J. Kruger, H. L. Ammon, C. Dickinson, and S. R. Hall, XRAY-72. Tech. Report TR-192, Computer Science Center, University of Maryland, 1972.
13. C. K. Johnson, ORTEP, Oak Ridge National Laboratory, Report ORNL-3794 (1965).
14. Examination of two additional alkaloids from the Amoora extract is under way; preliminary evidence suggests that they, too, are 8-substituted 2-methyl-5,7-dihydroxychromones.
15. S. R. Johns, J. H. Russel, and M. L. Heffernan, Tetrahedron Lett., 1965, 1987.
16. T. Kisaki, S. Mizusaki, and E. Tamaki, Phytochemistry, 7, 323-27 (1968).
17. F. Rulko and K. Nadler, Diss. Pharm. Pharmacol., 22, 329 (1970).
18. Column chromatography of the crude extract on silica gel using ethyl acetate yielded crystalline 3 in several fractions. Its identification is based upon these data: molecular weight (M^+= 192); NMR (methanol-d_4) δ 2.36 (s, 3H), 6.05 (s, 1H), 6.17 (d, J= 2 Hz, 1H), 6.30 (d, J= 2 Hz, 1H), and 12.5 (s, 1H); IR (KBr) 3100, 1650, 1638, 1620, and 1560 cm^{-1}. 7-Methyl ether (eugenin, 4): mp 105-105.5° (Kofler-block, lit.[19b] 115-16°);[20] molecular weight (M^+= 206); NMR ($CDCl_3$) 2.34 (s, 3H), 3.83 (s, 3H), 6.00 (s, 1H), 6.30 (d, J= 2 Hz, 1H), 6.32 (d, J= 2 Hz, 1H), and 12.6 (s, 1H); IR (KBr) 3080, 1668, 1657, 1620, and 1590 cm^{-1}. All properties of 3 and 4 agreed completely with those of synthetic[19b] and natural[22] samples; mixed mp's were not depressed.
19. a) T. M. Meijer and H. Schmid, Helv. Chim. Acta, 31, 1603-7 (1948); b) C. B. Rao, V. K. Murty, T. V. P. Rao, and V. Venkateswarlu, Rec. Trav. Chim., 83, 1122-28 (1964).
20. The discrepancies in melting points between our values and those from the literature[19] are due to differences in technique (compound 3) and to different polymorphic forms (compound 4). Natural noreugenin[22] melted at 299-301° using our sealed capillary technique, while 4 melted at 116-117.5° when mixed with an authentic sample of natural eugenin.[22] Since 3 sublimed without melting at 245°, we found it necessary to use a sealed capillary to obtain reproducible values.
21. G. P. Ellis, in "Chromenes, Chromanones, and Chromones," Chapter 7, pp. 455-80, G. P. Ellis, ed., Wiley-Interscience, New York, 1977.
22. We thank Professor R. T. Brown, The University of Manchester, and Professor C. H. Eugster, The University of Zürich-Irchel, for authentic samples of noreugenin (3) and eugenin (4), respectively.

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