BHIMBERINE - A NEW INDOLE ALKALOID FROM *RHAZYA STRICTA*

Atta-ur-Rahman*, Habib-ur-Rehman, and Sohail Malik
H.E.J.Research Institute of Chemistry
University of Karachi, Karachi-32, Pakistan

Abstract - A new Corynanthe type indole alkaloid, bhimberine, has been isolated from Rhazya stricta. Its structure has been assigned as (1) on the basis of spectral studies. Its stereochemistry has been determined by a series of NOED measurements and ^{13}C-NMR assignments made by DEPT pulse sequences.

Rhazya stricta Decsne.(Apocynaceae) is a small, glabrous erect shrub which is widely distributed in Western Asia and abundantly found in Pakistan. It has been used as an indigenous medicine for the treatment of various ailments[1-4]. The anticancer activity of some of its alkaloids is also reported[5-7]. As a result of our continuing investigations on Rhazya stricta[8,9] we have isolated a new alkaloid, bhimberine, to which structure (1) has been assigned on the basis of extensive NMR studies including homonuclear 2D NMR (COSY-45, J-resolved, NOESY)[10-12], NOE difference measurements, ^{13}C-NMR and DEPT experiments[13,14].

RESULTS AND DISCUSSION

The crude alkaloid mixture obtained from the alcoholic extract of the fruits of R. stricta was selectively extracted with chloroform at different pH values. The fractions obtained at pH 6.7 and 7.3 were combined and subjected to repeated preparative TLC. This resulted in the isolation of two alkaloids a faster moving alkaloid named "bhimberine" (1) and the slower moving rhazimanine, which has already been reported by us[15].

Alkaloid (1) obtained as an amorphous material was sensitive to air and light. Its UV spectrum was typical of an indole chromophore. The IR spectrum indicated the presence of an ester carbonyl at 1730 cm^{-1} and O-H and N-H groups at 3460-3380 cm^{-1}. The absence of characteristic Wenkert-Bohlmann bands in the region 2700-2800 cm^{-1} indicated the presence of a cis-quinolizidine system[16-20]. Its molecular ion peak occurred at m/z 354.1940 (66%) and the molecular formula was thus established as $C_{21}H_{26}N_2O_3$ (calcd. 354.1943). This was further confirmed by FAB mass spectrometry[21]. The mass fragmentation pattern was found to be almost identical to that reported earlier for rhazimanine[15]. Its ^{1}H-NMR ($CDCl_3$, 300 MHz) indicated the presence of 26 protons each of which was identified by a series of homo-decoupling experiments and further substantiated

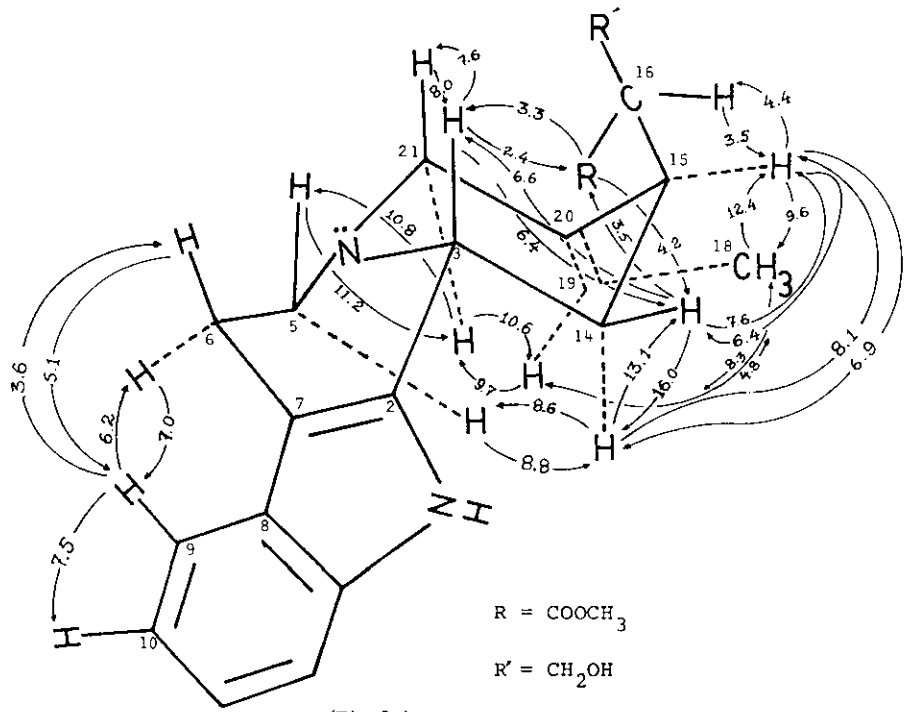
by recording COSY-45 and NOESY spectra. The ethylidine methyl (C-18H) appeared as a split doublet at δ 1.60 and showed a vicinal coupling with C-19H ($J_{18,19}$=6.89Hz) and homoallylic couplings with C-21 protons ($J_{18,21\beta}$=1.97Hz, $J_{18,21\alpha}$=1.69Hz). The split quartet resonated at δ5.78 was assigned to C-19H which showed vicinal coupling ($J_{19,18}$=6.89Hz) and allylic couplings ($J_{19,21\alpha} \simeq J_{19,21\beta} \simeq J_{19,15} <$ 1Hz). The characteristic downfield chemical shift value for C-3H (δ 4.29) indicated the presence of C/D cis ring juction[22-29] as in rhazimanine[15] and also suggested the β-configuration of C-3H since the earlier reported chemical shift values in other related compounds having C-3H in α-configuration were relatively upfield[7,30-35] to the value obtained in bhimberine.

The signal obtained for C-3H (δ 4.29) actually appeared as a dispersed double doublet ($J_{3,14\alpha}$ =11.70 Hz, $J_{3,14\beta}$=4.20Hz) resembling a 1:1:1:1 quartet. This was in accordance with the earlier observations that in cis fused indoloquinolizidines, when C-3H in ring D is axially disposed, gauche (ae) to one of the adjacent methylene protons and trans diaxial (aa) to the other proton, then the resulting couplings give rise to a 1:1:1:1 quartet with splittings J_{aa}=9-12Hz, J_{ae} = 2-5Hz[28,36,37]. A singlet resonating at δ 3.76 was assigned to the ester methyl group while the methylene protons (C-17H) of the hydroxymethylene group appeared as multiplets centred at δ3.68 and δ3.83. The C-16 proton appeared as a multiplet at δ 2.46. All the ^{1}H-NMR assignments are presented in detail in experimental section.

In order to confirm the relative stereochemistry of various protons, NOE difference studies were carried out. Irradiation of C-18H (δ 1.60) resulted in 8.3% NOE on C-19H (δ 5.78) and 12.4% NOE on C-15H (δ 3.16). Similar NOE effects were observed on C-18H when C-15H and C-19H were irradiated (Fig.1a). This indicated that C-18H is proximate to C-15H suggesting the E-configuration at 19,20 double bond. This was further supported by irradiation of C-19H (δ 5.78) which resulted in 9.7% NOE on C-21 H (δ 3.62). A similar enhancement was obtained upon irradiation of C-21α H (Fig.1a). The NOE interaction between C-3H and C-14 β H as well as of C-3H and C-14 βH with $COOCH_3$ (Fig.1a), indicated that the ester group is close to C-3H and C-14 β H. Since the stereochemistry at C-15 asymmetric centre may be fixed on biogenetic grounds[38] therefore, the only plausible explanation for the above NOE interactions is that the C-3 proton possesses a β-orientation. This was further supported by the NOE interactions between C-3H and C-21β H (Fig.1a). The NOE results suggest significant steric hinderance across C_{15} - C_{16} bond so that in the optimum conformation the ester methyl lies close to C-14 β H and C-3H while the C-16 proton lies eclipsed over the C-15 proton. In rhazimanine[15](16R) a significant interaction was seen between the 18-methyl and C-17 methylene protons. The absence of this interaction in bhimberine and the presence of NOE interactions between ester methyl and C3H/C14 βH, as well as significant

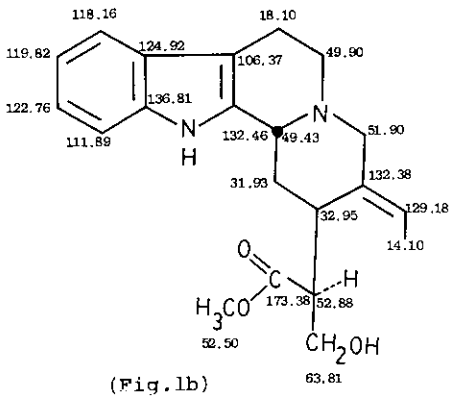
interactions between C-15H and C-16H all indicate a 16S stereochemistry in bhimberine. The NOE interactions of C-9H with C-6 α H and C-6 β H and vice versa confirmed the ^{1}H-NMR assignments for C-6 α H at δ 3.37 and C-6 β H at δ 3.28.

The NOE interactions between C-3H and C-21 β H, C-14 H and C-5 α H, and C-5 β H and C-21 α H served to establish that in the otpimum conformation ring D in bhimberine exists in a chair form (Fig.1a).



(Fig.1a)

The ^{13}C-NMR spectrum ($CDCl_3$ 75.4MHz) indicated the presence of twenty one carbon atoms. The multiplicity assignments (Fig. 1b) were made by carrying out multipulse ID, DEPT[13,14] experiments with the last polarization pulse angle 45°, 90° and 135°. The C/D cis quinolizidine system with C-3 βH was further supported by the chemical shift values of several stereochemically diagnostic carbon atoms such as C-2 (δ 132.38), C-3 (δ 49.43), C-5 (δ 49.90), C-6 (δ 18.10) and C-7 (δ 106.37). Similar upfield values have earlier been observed in rhazimaine[15] and other related cis indoloquinolizidines[39-41].



(Fig.1b)

Bhimberine may arise in the nature from strictosidine by a reversal of stereochemistry at C-3 during the biosynthetic process[42,43].

EXPERIMENTAL

Infrared (IR) spectrum was recorded on JASCO A-302 spectrophotometer, ultraviolet (UV) spectrum was recorded on Shimadzu UV-240 spectrophotometer, mass spectra were recorded on Finnigan MAT 312 mass spectrometer connected to PDP 11/34 (DEC) computer system, NMR spectra were recorded on Bruker AM-300 NMR spectrometer. TLC experiments were performed on silica gel GF-254 precoated plates (E.Merck).

Isolation of Bhimberine. The plant material was collected from a small village about 90 km from Karachi and was identified by Prof. S.I.Ali at the Botany Department of Karachi University.

The air dried fruits of Rhazya stricta (10 kg) were soaked in ethanol, ground, filtered and concentrated. This resulted in the separation of a brown gummy material which was filtered off. The filterate was concentrated and the solution was acidified with 5% HCl, filtered and basified with NH_3 (conc.) to pH $\sim$ 9. The solution thus obtained was extracted with $CHCl_3$ dried with Na_2SO_4 (anhydrous) filtered and evaporated to dryness (120 g). The crude alkaloidal extract thus obtained was dissolved in 10% acetic acid solution. This solution was subjected to selective pH-separations. The stepwise basification with NH_3 (conc.) and extraction with chloroform resulted in a number of fractions which were concentrated basified with 10% NH_4OH solution , dried with Na_2SO_4 (anhydrous) and evaporated to dryness. The fractions obtained at pH 6.7 and 7.3 were combined (16 g) and a portion (4g) of this material was subjected to preparative TLC on 2 mm silica gel plates with $CHCl_3$:MeOH (6:4) as a solvent system. This afforded a major band (R_f = 0.69) containing six alkaloids. The material thus obtained was again subjected to preparative TLC

using 0.2 mm silica gel plates with $CHCl_3$:EtOAc:MeOH (7:1.5:1). This afforded a mixture of two alkaloid which were repurified on 0.2 mm silica gel plates using petroleum ether (40°-60°): acetone:diethylamine (8:2:0.5). This resulted in the isolation of two alkaloids the slower moving (R_f=0.19) rhazimanine[15] and the faster moving (R_f=0.20) bhimberine (1). Bhimberine (7.2 mg, 2.88 x 10^{-4} % yield) thus obtained was amorphous, and sensitive to light and air. The alkaloid (1) was also isolated by us from the leaves of this plant by the same procedure.

UV: (CH_3OH) λ_{max} 222 (log ε 4.20), 268 sh(log ε 3.61), 273 sh (log ε3.61), 282 (log ε3.61) and 290 nm (log ε 3.55), λ_{min} 252 (log ε 3.55) and 2.87 nm (log ε 3.55).

IR: ($CHCl_3$) 3460-3380 cm^{-1} (N-H, O-H), 2920-2850 cm^{-1} (C-H), 1730 cm^{-1} (ester carbonyl), 1602 cm^{-1} (C=C), 1160 cm^{-1} (C-O) and 754 cm^{-1} (aromatic C-H).

MS: M^+ 354 (66%), 353 (67%), 339 (10%), 337 (9%), 323 (28%), 251 (100%), 237 (40%), 223 (20%), 184 (12%), 169 (43%), 156 (24%) and 144 (19%); HRMS: observed 354.1940, calculated 354.1943 ($C_{21}H_{26}N_2O_3$).

^{1}H-NMR: ($CDCl_3$, 300 MHz, δ ppm), 1.53 (1H, m, C-14αH), 1.60 (1H, split doublet, $J_{18,19}$=6.89Hz, $J_{18,21\beta}$ =1.97Hz, $J_{18,21\alpha}$ =1.69Hz, C-18H), 2.32 (1H, m, C-14βH), 2.46 (1H, m, C-16 H), 2.62 (1H, m, C-5 α H), 2.94 (1H, m, C-5 β H), 2.99 (1H, m, C-21 β H), 3.16 (1H, m, C-15 H), 3.28 (1H, m, C-6 β H), 3.37 (1H, m, C-6 α H), 3.62 (1H, m, C-21 α H), 3.68 (1H, m, C-17H), 3.76 (3H, s, $COOCH_3$), 3.83 (1H, m, C-17H), 4.29 (1H, dd, $J_{3,14\alpha}$=11.70Hz, $J_{3,14\beta}$=4.20Hz, C-3H), 5.78 (1H, split quartet, $J_{19,18}$=6.89Hz, $J_{19,21\alpha} \simeq J_{19,21\beta} \simeq J_{19,15}$<1Hz, C-19 H), 7.09 (1H, ddd, $J_{10,9}$=7.93Hz, $J_{10,11}$=7.77Hz, $J_{10,12} \simeq$1Hz, C-10 H), 7.17 (1H, ddd, $J_{11,12}$=7.93Hz, $J_{11,10}$=7.77Hz, $J_{11,9}$=1.42Hz, C-11 H), 7.73 (1H, dd, $J_{12,11}$=7.93Hz, $J_{12,10} \simeq$ 1Hz, C-12 H), 7.43 (1H, dd, $J_{9,10}$=7.93Hz, $J_{9,11}$=1.42 Hz, C-9H) and 9.40 (1H, s, N-H).

^{13}C-NMR: ($CDCl_3$, 75.4 MHz, δ ppm) presented in Fig. 1b.

REFERENCES AND FOOTNOTES

1. J.D.Hooker, "Flora of British India", Vol. 3, p.640 Reeve and Co., London (1875).

2. A.K.Nadkarni, "Indian Materia Medica", Vol. 1,p. 1056, Popular Prakashan, Bombay (1976).

3. W.Dymock, C.J.H.Warden and D.Hooper, "Pharmacographia Indica", Vol.2, p.391, Kegan Paul, Trench, Trubner and Co., London (1893).

4. R.N.Chopra, S.L.Nayar and I.C.Chopra, "Glossary of Indian Medicinal Plants", p.212, C.S.I.R. Publication, New Delhi (1956).

5. S.Siddiqui and A.Q.S.Bukhari, Nature, 235, 393 (1972).

6. S.Mukhobpadhyay, G.A.Handy, S.Funayama and G.A.Cordell, J.Nat.Prod., 44, 696 (1981).

7. S.Mukhopadhyay, A.El-Sayed, G.A.Handy and G.A.Cordell, J.Nat.Prod., 46, 409 (1983).

8. Atta-ur-Rahman and S.Malik, J.Nat.Prod., 47, 388 (1984).

9. Atta-ur-Rahman and S.Malik, J.Nat.Prod., 48, 153 (1985).

10. A.Bax and R.Freeman, J.Magn.Reson., 44, 542 (1981).

11. W.P.Aue, J.Karhan and R.R. Ernst, J.Chem.Phys., 64, 4226 (1976).

12. G.Wagner, A.Kumar and K.Wuthrich, Eur.J.Biochem., 114, 375 (1981).

13. D.M.Doddrell, D.T.Pegg and M.R.Bendall, J.Magn.Reson., 48, 323 (1982).

14. R.Benn and H.Gunther, Angew Chem.Int.Edi.Engl., 22, 350 (1983).

15. Atta-ur-Rahman, S.Malik and H.Rehman, Phytochemistry, (accepted).

16. E.Wenkert, D.K.Roychaudhari, J.Am.Chem.Soc., 78, 6417 (1956).

17. F.Bohlmann, Angew.Chem., 69, 641 (1957)

18. F.Bohlmann, Ber., 91, 2157 (1958).

19. E.Wenkert, D.K.Roychaudhari, J.Am.Chem.Soc., 80, 1613 (1958).

20. W.E.Rosen, Tetrahedron Lett., 481 (1961).

21. K.L.Rinehart. Jr., Science, 218 254 (1982).

22. W.E.Rosen, Tetrahedron Lett., 481 (1961).

23. W.E.Rosen and J.N.Soolery, J.Am.Chem.Soc., 83, 4861 (1961).

24. E.Wenkert and B.Wickberg, J.Am.Chem.Soc.., 84, 4914 (1962).

25. M.M.Robinson, W.G.Pierson, R.A.Lucas, I.Hsu, and R.L.Dziemian, J.Org.Chem., 28, 768 (1963).

26. E.Wenkert, B.Wickberg and C.L.Leicht, J.Am.Chem.Soc., 83, 5037 (1961).

27. E.Wenkert, B.Wickberg and C.L.Leicht, Tetrahedron Lett., 822 (1961).

28. M.Uskokovic, H.Bruderer, C.von Planta, T.Williams, and A.Brossi, J.Am.Chem.Soc., 86, 3364 (1964).

29. C.M.Lee, W.F.Trager and A.H.Beckett, Tetrahedron, 23, 375 (1967).

30. The only exception we could find was 16R-19,20-E-isositsirikine prepared synthetically. No explanation was however given for the low value of the chemical shift of C-3H in comparison to 16S-19,20-E-isositsirikine, 16R-19 20-Z-isositsirikine and 16S-19,20-Z-isositsirikine all of which show C-3H (α) below 4.0 ppm [34].

31. J.P.Kutney and R.T.Brown, Tetrahedron, 22, 321 (1966).

32. J.C.Simoes, B.Gilbert, W.J.Cretney, M.Hearn and J.P.Kutney, Phytochemistry, 15, 543 (1976).

33. W.Kohl, B.Witte and G.Hofle, Z.Naturforsch., 37b, 1346 (1982).

34. C.Kan, S.-K.Kan, M.Lounasmaa and H.-P. Husson, Acta Chem.Scand., B35, 269 (1981).

35. T.Hirata, S.L.Lee and A.I.Scott, J.Chem.Soc.Chem.Commun., 1081 (1979).

36. A.C.Huitric, J.B.Carr, W.F.Trager and B.J.Nist, Tetrahedron, 19, 2145 (1963).

37. In the alternative cis fused indoloquinolizidine structure in which C-3H in ring D is equatorially disposed i.e. gauche to both the adjacent methylene protons, the resulting couplings are about equal in magnitude and give rise to a triplet - like (1:2:1) signal due to the overlapping of the inner peaks of the resulting double-doublet[15,28].

38. Atta-ur-Rahman and A.Basha, "Biosynthesis of Indole Alkaloids", Clarendon Press, Oxford (1983).

39. E.Wenkert, J.S.Bindra, C.-J.Chang, D.W.Cochran and F.M.Schell, Acc.Chem.Res., 7, 46 (1974).

40. M.C.Koch, M.M.Plant, N.Preaux, H.E.Gottlieb, E.W.Hagaman, F.M.Schell and E.Wenkert, J.Org.Chem., 40, 2836 (1975).

41. E.Wenkert, C.-J.Chang, H.P.S. Chawala, D.W.Cochran, E.W.Hagaman, J.C.King and K.Orito, J.Am.Chem.Soc., 98, 3645 (1976).

42. M.Rueffer, N.Nagakura and M.H.Zenk, Tetrahedron Lett., 1593 (1978).

43. N.Nagakura, M.Rueffer and M.H.Zenk, J.Chem.Soc.Perkin Trans.I, 2308 (1979).

Received, 14th October, 1985