RHAZICINE — A NEW ALKALOID FROM LEAVES OF *RHAZYA STRICTA*

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Abstract — A new alkaloid, "rhazicine" has been isolated from the leaves of *Rhazya stricta* to which structure (3) has been assigned.

Rhazya stricta (Decaisne) is a small glabrous erect shrub which grows profusely in various parts of Pakistan.[1,2] It is reputed in the indigenous system of medicine as a bitter tonic and as a curative for chronic rheumatism, for sore throat and in fever.[3,4] It has been demonstrated that extracts of *R.stricta* show marked leucopenic effect in rats when given orally.[5] A number of cytotoxic alkaloids have been reported from the plant.[6]

The ethanolic extract of the fresh leaves (95 kg) of *R.stricta* was concentrated to a gum and dissolved in 10% acetic acid. The non-alkaloidal portion was removed by extraction with ethyl acetate. The acidic aqueous solution was basified with aqueous ammonia to pH 11 and extracted with ethyl acetate to afford the crude alkaloids (350 gm). The alkaloidal material was subjected to flash chromatography over silica gel for preliminary fractionation. Elution with increasing polarities of pet ether, benzene, chloroform, ethyl acetate and methanol afforded a number of fractions. The fraction obtained on elution with pet.ether-chloroform (4:5) was concentrated and again subjected to column chromatography over silica gel. Elution with MeOH-EtOAc (1:9) afforded a mixture of 4 alkaloids which were separated on precoated silica gel plates. The slower moving band was separated and again subjected to thin layer chromatography over precoated plates (aluminium oxide, Merck F254, type E) using EtOAc-EtOH (9:1) as the eluent.

The slower moving alkaloid, named rhazicine, gave an orange coloured reaction with Dragendroff's reagent and a dark pink colouration with $CeSO_4$ solution. It was obtained as white crystalline needles (hygroscopic), $|\alpha|_D = +65^{\circ}$ ($CHCl_3$). The substance afforded a characteristic indoline UV spectrum, λ_{max} MeOH 213 nm, 249 nm, and 295 nm, and λ_{min} MeOH 230 nm and 275 nm. The IR spectrum ($CHCl_3$) showed absorptions at 3500 cm^{-1} (N-H), 3400 cm^{-1} (OH), 1738 cm^{-1} (keto C=O) and 1720 cm^{-1} (ester C=O). High resolution mass spectrometry afforded the exact mass to be 368.1741 which agreed with the mass calculated for the formula $C_{21}H_{24}N_2O_4$ (368.1737), indicating the presence of eleven double bond equivalents in the molecule. Other major peaks were present at m/z 350.1630 (calc. for $C_{21}H_{22}N_2O_3$: 350.1630, M^+-H_2O), 322.1665 (calc. for $C_{20}H_{22}N_2O_2$: 322.1681,

M^+-H_2O-CO), 214.0860(a) (calc. for $C_{13}H_{12}NO_2$: 214.0868), m/z 182.0603(b)(calc. for $C_{12}H_8NO$: 182.0605),167.0779(c) (calc. for $C_{12}H_9N$: 167.0735) and m/z 122.0972(d)(100%, calc. for $C_8H_{12}N$: 122.0969). The fragmentation was found to be similar to that of rhazimine[7] previously isolated by us from the same plant.

The ^1H-NMR spectrum in $CDCl_3$ showed a three-proton double-doublet at δ 1.53 (J_1=7Hz, J_2=2.5Hz) which was assigned to the ethylidene methyl group. A three proton singlet at δ 3.56 was consistent with the presence of carbomethoxy group, while another low field singlet at δ 4.91 (1H) was assigned to the C_2-proton. A one proton quartet at δ 5.4 (J=7Hz) was assigned to the olefinic proton of the ethylidene group. The aromatic protons afforded complex multiplets in the region between δ 6.5-7.3 ppm.

The ^{13}C-NMR spectrum ($CDCl_3$) showed several interesting features. Gated spin echo measurements established that a downfield signal at δ 84.52 assigned to C_2 was a methine carbon atom. These studies also demonstrated that the two downfield signals at δ 48.32 and δ 51.64 (assigned to C_3 and C_{21} carbon atoms respectively) were both $-CH_2$ groups. These assignments strongly support the conclusion that bond between C-2 and C-3 is broken in (**3**). The ^{13}C-NMR of rhazicine was very similar to that of rhazimine (2), the major difference being the absence of the C-2 ketimine carbon resonance found in rhazimine and the appearance of a signal at δ 84.52 due C-2. The chemical shift assignments of carbon atoms of rhazicine are presented in table 1.

TABLE-1

Carbon No.	Chemical shift	Multiplicity*	Carbon No.	Chemical shift	Multiplicity*
2	84.52	-CH	13	142.55	-C-
3	48.32	$-CH_2$	14	23.09	$-CH_2$
5	57.42	-CH	15	36.91	CH
6	27.64	$-CH_2$	16	61.20	-C-
7	54.16	-C-	17	a	
8	126.16	-C-	18	12.59	$-CH_3$
9	128.43	CH	19	119.44	CH
10	118.32	CH	20	140.94	-C-
11	115.82	CH	21	51.64	CH_2
12	128.26	CH	ester C=O	170.88	
			ester CH_3	52.94	

a = signal too weak to be detected; on conversion to rhazimine[7-9] it occurred at δ 214.11.[7-9]

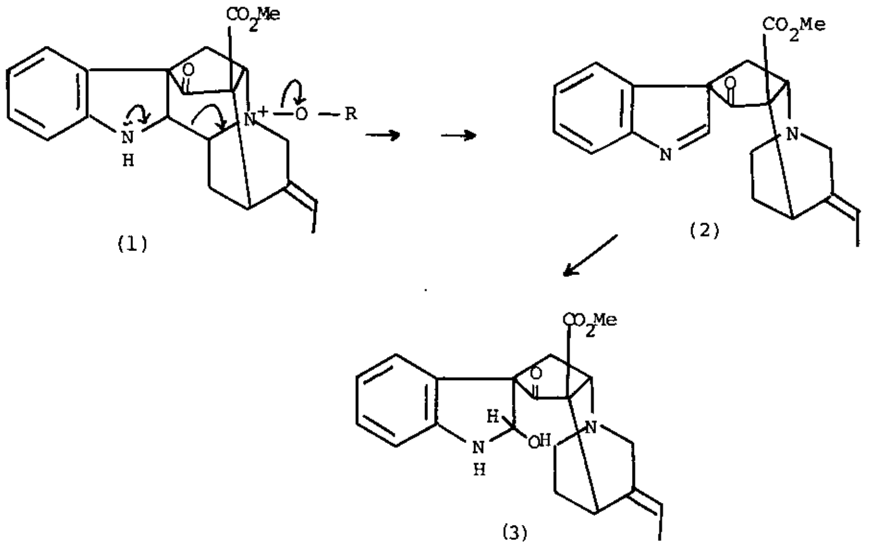
* = established by gated spin echo measurements.

Rhazicine was found to dehydrate on attempted acetylation with acetic anhydride and pyridine. The product afforded a faster moving spot which was separated by preparative layer chromatography. The substance afforded an indolenine UV spectrum and M^+ at m/z 350. The dehydration product was identified as rhazimine (2) previously isolated by us from the same plant, by direct comparison with an authentic sample.[7] Reduction of rhazicine with $NaBH_4$ in MeOH at 30°C for 12 hrs afforded dihydrorhazimine (4) which showed an indoline uv spectrum and M^+ at m/z 352. Other major peaks were present at m/z 323, 293, 263, 214 and 122 (100%). The conversion of rhazicine to rhazimine (2) and dihydrorhazimine (4) showed that rhazicine bears the same unusual *seco* structure as rhazimine with the C-2/C-3 bond cleaved. On the basis of these data structure (3) is assigned to rhazicine.

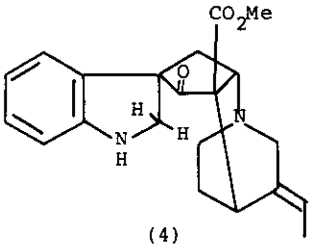
Rhazicine probably arises in the plant by a modified intramolecular Polonovski fragmentation reaction of a precursor such as (1) followed by hydration of the ketimine system (scheme 1).

REFERENCES

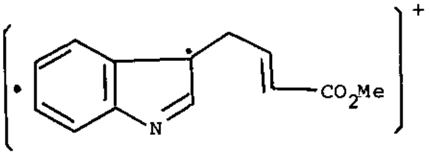
1. N.G.Bisset, *Borogiensis*, 3, 170 (1958).
2. J.D.Hooker, "Flora of British India", Reeve and Company, Vol.III, No.3, 540 (1875).
3. R.N.Chopra, S.L.Nayar and I.C.Chopra, "Glossary of Indian Medicinal Plants", CSIR, New Delhi, 212 (1956).
4. G.Watt, "A Dictionary of the Economic Products of India", Part I, W.H.Allen and Company, London, 7, 433 (1892).
5. S.Siddiqui and A.Q.S.Bukhari, *Nature*, 253, 393 (1972).
6. S.Mukhopadhyay, G.A.Handy, S.Funayama and G.A.Cordell, *J.Nat.Prod.*, 44(6), 696 (1982).
7. Atta-ur-Rahman and S.Khanum, *Tetrahedron Lett.*, (in press).
8. T.A.Broadbent and E.G.Paul, *Heterocycles*, 20(5), 863 (1982).
9. A.Chatterjee, M.Chakrabarty, A.K.Ghosh, E.W.Hagaman and E.Wenkert, *Tetrahedron Lett.*, 3879 (1978).



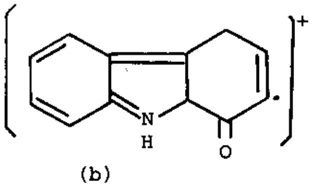
Scheme 1



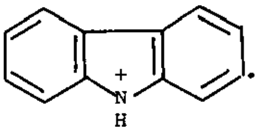
(4)



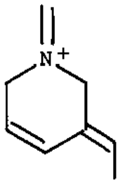
(a)



(b)



(c)



(d)

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