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Rhazizine : A Novel Alkaloid from the leaves of Rhazya stricta

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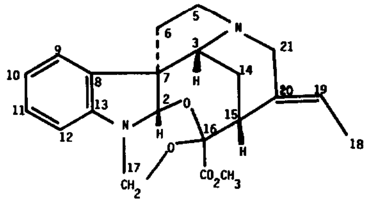
Abstract:- A novel alkaloid rhazizine (1) has been isolated from the leaves of R.stricta. Its structure has been investigated by extensive spectroscopic studies including 2D NMR experiments and its stereochemistry has been determined by NOE difference measurements.

Rhazya stricta Decaisne (Apocynaceae) is a small glabrous erect shrub, abundantly distributed in Pakistan[1-3]. The plant is well reputed in the indigenous system of medicine for the treatment of various diseases[4-6]. Extracts of R. stricta showed anticancer[7] and anti-neoplastic activity[4]. We have previously reported a number of new alkaloids from R. stricta[4,11-17]. In continuation of our studies on the isolation and structure elucidation of the constituents of the plant, we have isolated a new alkaloid rhazizine (1) the structure of which has been assigned by spectroscopic studies.

The crude alkaloidal material obtained from the ethanolic extracts of the fresh leaves of R. stricta by conventional procedure[4,5] were subjected to column chromatography for preliminary fractionation. The fraction obtained on elution with chloroform-methanol (4:1) afforded a mixture of alkaloids. On repeated preparative layer chromatographic purification of the mixture on silica gel (GF-254) in chloroform-methanol-diethylamine (8.5:1.5: vapours), a new alkaloid (25 mg) was obtained. It gave a pink coloured reaction with ceric sulphate solution and dark orange colouration with Dragendorff's reagent, $[\alpha]_D$ MeOH = + 84°.

The UV spectrum of rhazizine (MeOH) was characteristic for the dihydroindole chromophore, having absorption maxima at 209, 249 and 290 nm and minima at 235 and 274 nm. The IR spectrum showed absorption bands at 1730 cm^{-1} (ester C=O) and 1480, 1320, 1225 cm^{-1} (ether linkage). HRMS afforded the molecular ion at m/z 368.1686 (20%) in agreement with the formula $C_{21}H_{24}N_2O_4$, indicating eleven double bond equivalents in the molecule. Other fragment ions appeared at m/z 350.1630 (15%, $C_{21}H_{22}N_2O_3$), 322.1665 (10%, $C_{20}H_{22}N_2O_2$), 214.0860 (10%, $C_{13}H_{12}NO_2$), 182.0602 (20%, $C_{12}H_8NO$), 167.0779 (9%, $C_{12}H_9N$) and 122.0972 (60%, $C_8H_{12}N$).

The ^1H-NMR spectrum (CD_3OD, 400 MHz) indicated the presence of 24 protons, each of which was identified by COSY-45, hetero-COSY and NOE difference[18-20] measurements. The methyl proton of the ethylidene chain appeared as a double doublet at δ 1.61, showing vicinal coupling



(1)

with the C-19 proton at δ5.78 ($J_{18,19}$ = 7.89Hz) and homoallylic coupling with C-21β H at δ 4.51 ($J_{18,21\beta}$= 1.97Hz). The C-21α H was found to resonate as a multiplet at δ 4.17. A 2H multiplet at δ5.33 was assigned to the C-17 protons, their downfield chemical shift suggesting the presence of α-hetero atoms[23]. A sharp singlet at δ 5.1 was assigned to indolic C-2 proton, its downfield chemical shift being indicative of the presence of an oxygen α to it. Assignments for other protons are presented in Table-1.

Two dimensional NMR measurements were performed to gain a deeper insight into the structure. The COSY-45 spectrum allowed the $^1H/^1H$ coupling interactions to be unravelled. The C-18 methyl proton at δ 1.61 showed a cross peak with the C-19 proton (δ 5.78) and with C-21βH (δ4.51) respectively. Similarly C-21β H (δ4.51) showed coupling with C-18H (δ1.61) and C-21βH at δ4.17. The C-6β proton at δ 2.78 showed coupling with C-6 αH (δ3.23) as well as with the C-5α and β protons at δ4.17 and δ3.72 respectively. Similarly the C-15β and C-3β protons showed coupling with C-14αH (δ2.72) and C-14βH (δ2.23) respectively. The two C-14 protons also showed coupling interactions with each other. The COSY-45 spectrum is presented in Figure 1.

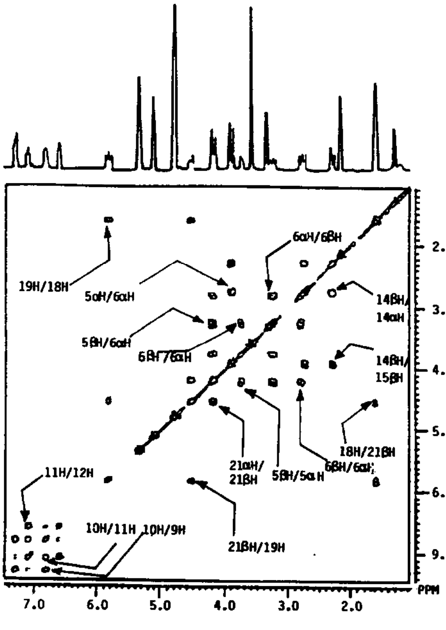


Fig.1: COSY-45 spectrum of rhazizine (1)

able-1: $^1H/^1H$ Connectivities from COSY-45 spectrum of rhazizine (1)

Hydrogen	Chemical shift (δ)	Coupled to H (δ) (COSY-45)	Hydrogen	Chemical shift (δ)	Coupled to H (δ) (COSY-45)
C-2βH	5.1		C-12H	6.57	11-H (7.02)
C-3βH	3.90	14αH (2.72)	C-14αH	2.72	3βH (3.90)
C-5αH	4.17	5βH(3.73), 6αH(3.23)	C-14βH	2.23	15βH (3.89)
		6βH (2.78)	C-15βH	3.89	14βH (2.23)
C-5βH	3.73	5αH (4.17), 6αH (3.23)	C-17H	5.33	
C-6αH	3.23	6βH (2.78), 5βH (3.72)	C-18H	1.61	9-H(5.78), 21βH (4.51)
		5αH (4.17)	C-19H	5.78	C-18H (1.61)
C-6βH	2.78	6αH (3.23), 5αH (4.17)	C-21αH	4.17	21βH (4.51)
C-9H	7.28	10-H (6.80)	C-21βH	H4.51	21α H (4.17), 18H (1.62)
C-10H	6.80	9-H (7.28), 11-H (7.02)	$CO_2\underline{CH}_3$	3.57	
C-11H	7.02	10-H (6.80), 12-H (6.57)			

Table-II: ^{13}C-NMR, DEPT multiplicities and $^1H/^{13}C$ connectivities (hetero COSY) .

Carbon No.	Chemical shift (δ)	Multiplicity (DEPT)	Connected to H (δ)	Carbon No.	Chemical shift (δ)	Multiplicity (DEPT)	Connected to H (δ)
2	86.47	CH	5.1	13	144.06	-C-	
3	71.36	CH	3.90	14	26.36	CH_2	αH (2.72),βH (2.23)
5	57.81	CH_2	αH (4.17),βH (3.73)	15	35.20	CH	3.89
6	20.93	CH_2	αH (3.23),βH (2.78)	16	110.50	-C-	
7	57.18	-C-		17	73.36	CH_2	5.33
8	129.93	-C-		18	13.52	CH_3	1.61
9.	128.48	CH	7.28	19	126.66	CH	5.78
10	119.90	CH	6.80	20	130.56	-C-	
11	129.17	CH	7.02	21	62.80	CH_2	αH (4.17),βH (4.51)
12	116.34	CH	6.58	$CO_2\underline{CH}_3$	51.91	CH_3	3.57
				$\underline{C}O_2CH_3$	171.40	-C-	

The ^{13}C-NMR spectrum (CD_3OD, 100MHz) indicated the presence of 21 carbon atoms in the molecule. DEPT multiplicity assignments[21,22] established that there were two CH_3, five CH_2, eight CH and six quaternary carbon atoms. The C-2 carbon α- to N_a resonated at δ 86.47[24]. The C-3 methine carbon resonated at δ 71.36 due to the absence of the anomeric effect, C-3 being <u>cis</u> to N_b lone pair. The other methylene carbons α- to the nitrogen appeared at δ 57.81 (C-5) and δ62.80 (C-21) respectively. The methyl of the ester carbonyl group was found to resonate at δ51.91 whereas the carbonyl carbon appeared at δ 171.40. The ^{13}C-NMR chemical shift assignments are presented in Table-II.

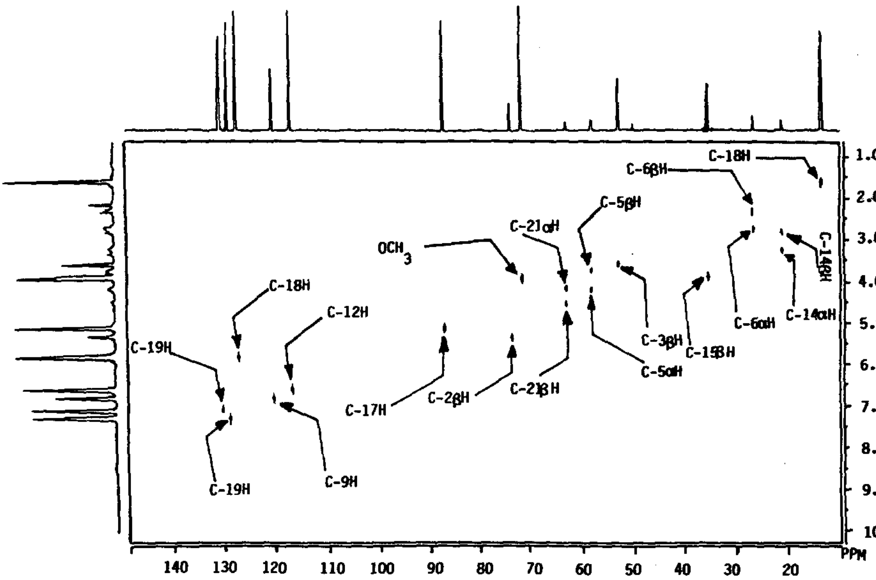


Figure 2: Direct (one bond) hetero-COSY spectrum of rhazizine (1)

The direct (one-bond) ^1H-^{13}C shift correlation (hetero COSY) spectrum[22] showed that the methylenic carbons resonating at δ57.18 and at δ62.80 was coupled to the protons at δ4.17 (C-5αH, C-21αH). Similarly C-3βH and C-15βH appearing at δ 3.89 showed connectivities with the carbons at δ 71.36 and δ35.20 respectively. Another methylenic carbon at δ 73.36 showed connectivity with its protons at δ 5.33 thus indicating that both the protons at this centre (C-17) have the same chemical shift. The ^1H-^{13}C correlations obtained are presented in Table.II.

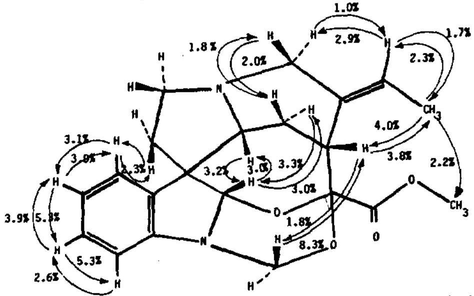
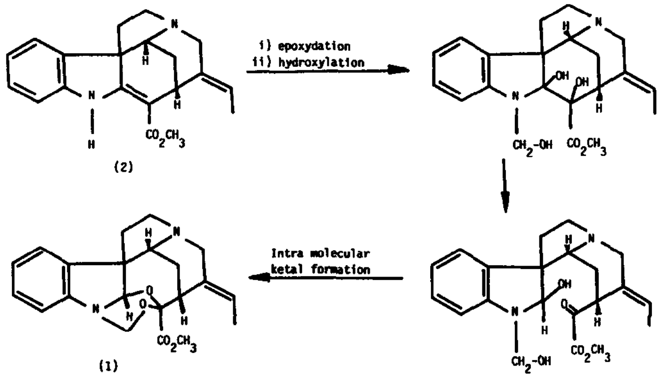


Fig. 3: NOE difference interactions of rhazizine (1)

NOE difference studies served to establish the stereochemistry at various asymmetric centres. Irradiation at δ1.61 (C-18H) resulted in 4% NOE at δ3.89 (C-15βH) thus establishing the "E" configuration of the ethylidene chain. Similarly on irradiation at δ 3.89 (C-15βH) NOE was evident at δ5.33 (C-17H) indicating that the C-17 protons lie close in space to the C-15 proton. Irradiation at δ 5.1 (C-2β H) resulted in 3.3% NOE at δ3.89 (C-3βH) thus establishing the βstereochemistry at C-3. Irradiation at δ 2.78 (C-6 α H) showed 3.7% NOE at δ 7.28 (C-9H). Other important NOE interactions of other protons are presented in Figure 3.

On the basis of these spectral studies structure (1) has been assigned for rhazizine.

Rhazizine may arise in nature from a strychnos alkaloid, such as (2) by its oxidation to the diol (3) followed by cleavage of the C-C bond and ketal formation (Scheme-1).



Scheme-1

Experimental

UV and IR spectra were recorded on a Shimadzu UV-240 spectrophotometer JASCO A-302 spectrophotometer respectively. HRMS were recorded on Finnigan MAT-312 mass spectrometer connected to PDP 11/34 (DEC) computer system. The ^{1}H-NMR spectra were recorded at 400 MHz on Varian XL-400 NMR spectrometer. The ^{13}C-NMR spectra were recorded at 100 MHz on the same instrument. The optical rotation was recorded on Polartronic Universal Australian Standard K-157 digital polarimeter. TLC experiments were performed on silica gel (GF-254, 0.2 mm) plates (E. Merck).

Isolation of rhazizine (1)

The ethanolic extract of the fresh leaves (95 kg) of R. stricta was concentrated, acidified with 10% acetic acid (1L), filtered and basified with aqueous ammonia (conc) to pH 11. The basic solution was extracted with ethyl acetate (25 L) to afford the crude alkaloids (350 gm). The alkaloidal material was subjected to flash chromatography for preliminary fractionation and eluted with increasing polarities of mixtures of benzene, pet.ether, chloroform, ethyl acetate, methanol and methanol-triethylamine. The fraction obtained on elution with ethyl acetate-methanol (70 gm) was subjected to repeated chromatographic purification on silica gel (GF-254) in chloroform-methanol - diethylamine (8.5:1.5: vapours) to afford a new alkaloid 25 mg $[\alpha]_D$ (MeOH) + 84° (MeOH). It gave a pink coloured reaction with ceric sulphate solution and dark orange colouration with Dragendorff's reagent.

UV (MeOH) λ_{max} : 209, 249 and 290 nm λ_{min} 235 and 274 nm.

IR (KBr) : ν_{max} cm^{-1} 1738 (ester C=O), 1480, 1320, 1225 (ether linkage)

MS m/z (rel.int.%): 368 (20), 350 (15), 322 (10), 214 (10) 182 (20) 167 (9) and 122 (60).

^{1}H-NMR (CD_3OD, 400MHz) : 1.60 (dd, 3H, $J_{18,19}$ = 7.14, $J_{18,21\beta}$ = 1.97 Hz, 18H) 2.23 (d 1H, $J_{14\beta,14\alpha}$ = 12.20 Hz, 14βH), 2.72 (m, 1H, 14αH), 2.78 (m, 1H, 6βH), 3.30 (d, 1H, $J_{6\alpha,6\beta}$ = 11.94H$_2$, 6αH), 3.51(s, 3H, $OC\underline{H}_3$), 3.72 (d, 1H, $J_{5\beta,5\alpha}$ = 11.56Hz, 5βH), 3.89 (m, 2H, 3βH, 15β H), 4.16 (m, 2H, 5αH, 21αH), 4.51 (dd, 1H, $J_{21\beta,21\alpha}$ = 15.11Hz, $J_{21\beta,18}$ = 1.97 Hz, 21βH), 5.10 (s, 1H, 2βH), 5.33 d.2H, $J_{17\alpha,17\beta}$ = $J_{17\beta,17\alpha}$ =12.09, 17 H), 5.79 (q, 2H, $J_{19,18}$ = 7.10Hz, 19H), 6.57 (d, 1H, $J_{12,11}$ = 7.61Hz, 12H), 6.80 (dd, 1H, $J_{10,11}$ = $J_{10,9}$ = 7.20Hz, 10H), 7.08 (dd, 1H, $J_{11,10}$ = $J_{11,12}$ = 7.6 Hz, 11H), 7.28 (d, 1H, $J_{9,10}$ = 7.54Hz, 9H).

^{13}C-NMR (CD_3OD, 100 MHz) : Table-II.

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