ISOLATION AND STRUCTURAL STUDIES ON THE ALKALOIDS OF RHAZYA STRICTA

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Abstract-A new alkaloid isorhazicine (2) has been isolated from the leaves of Rhazya stricta. The structure and relative stereochemistry have been determined by spectroscopic studies, particularly by NOE difference, COSY-45° and DEPT experiments. Isorhazicine (2) was shown to differ from rhazicine (1) in that it bears a 'Z' configuration of the ethylidene group.

INTRODUCTION

Rhazya stricta (Decaisne) is well reputed in the indigenous system of medicine for the treatment of several diseases. It is a rich source of indole alkaloids[1,2]. A number of alkaloids having important biological activities have been reported from the plant[3-6]. Continuing studies carried out on R.stricta in our laboratories have resulted in the isolation and structure elucidation of several new alkaloids which include a series of novel seco ajmalinoid - type alkaloids[7-14].

This paper describes the isolation and structure of isorhazicine (2), as well as the relative stereochemistry of rhazicine (1) the gross structure of which was presented by us in our earlier communication[12].

RESULTS AND DISCUSSION

The crude alkaloidal material isolated by conventional procedures[11,12] was subjected to repeated column chromatography to afford a fraction containing the alkaloids rhazicine (1)[12] and isorhazicine (2). The pure alkaloids were isolated by a combination of column chromatography over silica gel and thin layer chromatography on alumina plates. They afforded identical uv spectra characteristic for the indoline nucleus and very similar IR spectra showing absorptions at 3500 cm^{-1} (NH), 3400 cm^{-1} (OH), 1738 cm^{-1} (ester C=O) and 1720 cm^{-1} (keto C=O). Rhazicine (1) and isorhazicine (2) afforded virtually identical mass spectra with molecular ions at m/z 368.172 corresponding to the

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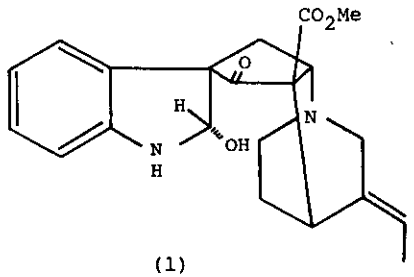
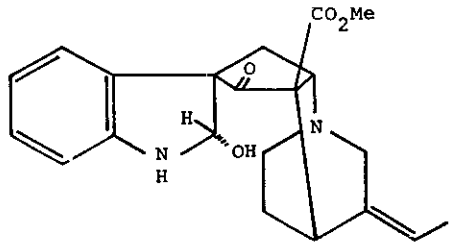


(1)



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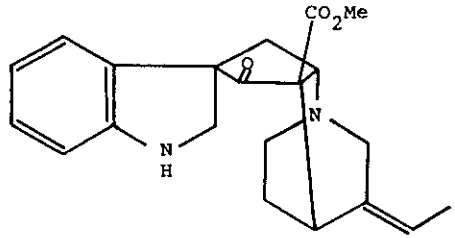

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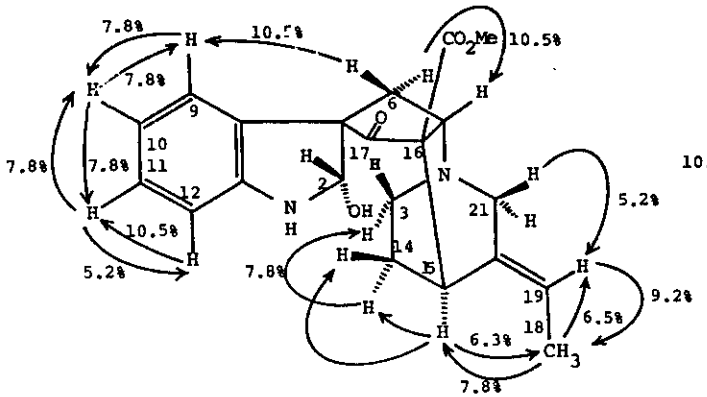

Fig.1 Rhazicine

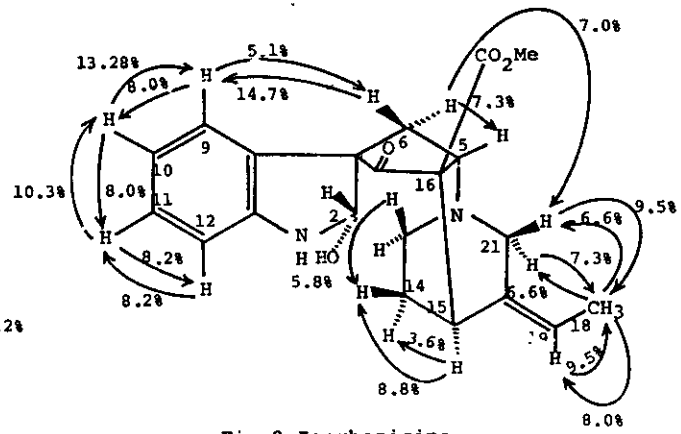

Fig.2 Isorhazicine

formula $C_{21}H_{24}N_2O_4$ indicating the presence of eleven double bond equivalents in the molecule. Other major fragments appeared at m/z 350.1630 (calcd. for $C_{21}H_{22}N_2O_2$: 350.1630), 322.1665 (calcd. for $C_{20}H_{22}N_2O_2$: 322.1681), 214.0860 (calcd. for $C_{13}H_{12}NO_2$: 214.0868), 182.0602 (calcd. for $C_{12}H_8NO$: 182.0605), 167.0779 (calcd. for $C_{12}H_9N$: 167.0739) and 122.0972 (calcd. for $C_8H_{12}N$: 122.0969). The fragmentation pattern was found to be similar to that of rhazicine[13]. Both isomers readily dehydrated on attempted acetylation with acetic anhydride in pyridine to faster moving materials. Both the dehydration products were found to be inseparable from each other or from rhazimine by tlc in several solvent systems and possessed identical mass spectra to rhazimine[13]. Reduction of isorhazicine with $NaBH_4$ in MeOH at 30°C for 12 h afforded (3) which showed an indoline UV spectrum. The M^+ of the reduction product appeared at m/z 352 while other fragments were found at m/z 323, 293, 214, 182, 167, 122 (100%). The above transformation strongly supports the conclusion that both rhazicine and isorhazicine have the seco ajmalinoid structure with the C-2/C-3 bonds cleaved.

The ^{13}C-NMR spectra of both isomers are shown in Table II. The multiplicities of carbons were established by using polarization transfer (DEPT) [17] and gated spin echo (GASPE) experiments. Isorhazicine showed a downfield signal at δ 84.44, which was assigned to the C-2 methine carbon atom. Two other downfield signals at δ 48.08 and δ 49.52 were assigned to C-3 and C-21 carbon atoms respectively and both were shown to be CH_2 groups by DEPT measurements. The C-21 carbon atom appeared at δ 51.64 in rhazicine. The upfield shift of δ 2.12 at this carbon in isorhazicine suggested that the ethylidene group in it may occupy a 'Z' configuration which would force the methyl hydrogen atoms and the hydrogen atoms at C-21 close enough to cause a shielding effect due to the polarisation of the C-H bond[16]. The presence of CH_2 group at C-3 and CH group at C-2 was established by polarisation transfer studies and this supported the conclusion that the bond between C_2 and C_3 is broken.

The relative stereochemisty of isorhazicine was established by carrying out extensive NOE difference studies. Irradiation at δ 1.53 (18-Me) resulted in 6.6% NOE at δ 3.78 (21-Hα) and 6.6 NOE at 3.56% (21-Hβ). Irradiation at δ3.78 (21-Hα) on the other hand resulted in 9.5% NOE at δ1.53 (18-Me) while irradiation at δ3.56 (21-Hβ) resulted in 7.3% NOE at δ 1.53 (18-Me). These NOE effects conclusively establish the configuration of the ethylidene group in isorhazicine (2) as 'Z'. This was further confirmed by irradiation at δ 2.82 (15-Hα) which resulted in 8.8% NOE at δ2.29 (14-Hα) and 3.6% NOE at δ 2.40 (14-Hβ), but no NOE effect was discernible at the chemical shifts of the methyl or olefinic protons. Irradiation at δ 5.49 (19-H) showed 9.5% NOE at δ 1.53 (18-Me). Irradiation at δ 2.31 (6-Hα) resulted in 7% NOE at δ3.56 (21-Hβ), 7.3% NOE at δ3.67 (5-H) and 14.7% NOE at δ7.27 (9-H). Irradiation at δ 4.10 (3-Hα) resulted in 4% NOE at δ2.40

Table-1: ^{1}H-NMR ($CDCl_3$)

Proton	Rhazicine Chemical shift in ppm (J in Hz)	Isorhazicine Chemical shift in ppm (J in Hz)
N-H	7.7 (s, 1H)	7.8 (s, 1H)
C_2-H	4.91 (s, 1H)	4.93 (s, 1H)
C_3-Hβ	2.96 (d, $J_{3\beta,3\alpha}$=15.0Hz)	3.08 (d, $J_{3\beta,3\alpha}$= 16.0Hz)
C_3-Hα	3.95 (d, $J_{3\alpha,3\beta}$=15Hz)	4.10 (d, $J_{3\alpha,3\beta}$=16Hz)
C_5-H	3.64 (m, 1H)	3.67 (m, 1H)
C_6-Hβ	2.07 (d, $J_{6\beta,6\alpha}$=13Hz)	2.03 (d, $J_{6\beta,6\alpha}$=14Hz)
C_6-Hα	2.39 (d, $J_{6\alpha,6\beta}$=13Hz)	2.31 (m)
C_9-H	7.25 (d,1H, $J_{9,10}$=7.7Hz)	7.27 (d, 1H, $J_{9,10}$=7.5Hz)
C_{10}-H	6.84 (m, 1H)	6.86 (m, 1H)
C_{11}-H	7.09 (m, 1H)	7.08 (m, 1H)
C_{12}-H	6.56 (d, 1H, $J_{12,11}$=7.8Hz)	6.58 (d, 1H, $J_{12,11}$=7.4Hz)
C_{14}-Hα	2.60 (m)	2.40 (m)
C_{14}-Hβ	2.29 (m)	2.29 (m)
C_{15}-Hα	2.86 (dd, $J_{15\alpha,14\alpha}$=12.8Hz) $J_{15\alpha,14\beta}$=5.8Hz)	2.82 (dd, $J_{15\alpha,14\alpha}$=12.1Hz) $J_{15\alpha,14\beta}$=3.6Hz
C_{18}-H	1.51 (dd, $J_{18,19}$=7.0Hz) $J_{18,21\alpha}$=2.4Hz	1.53 (dd, $J_{18,19}$=7.0Hz) $J_{18,21\alpha}$=2.4Hz
C_{19}-H	5.45 (q, $J_{19,18}$=7.1Hz)	5.49 (q, $J_{19,18}$=7Hz)
C_{21}-Hβ	3.03 (d, $J_{21\beta,21\alpha}$=16.5Hz)	3.56 (d, $J_{21\beta,21\alpha}$=18Hz)
C_{21}-Hα	4.11 (d, $J_{21\alpha,21\beta}$=16.5Hz	3.78 (d, $J_{21\alpha,21\beta}$=18Hz)
ester CH_3	3.57 (s, 3H)	3.58 (s, 3H).

Table-II: ^{13}C-NMR ($CDCl_3$)

Carbon	Rhazicine Chemical shift (ppm)	Isorhazicine Chemical shift (ppm)	Multiplicity
2	84.52	84.44	-CH
3	48.32	48.08	$-CH_2$
5	57.42	57.54	-CH
6	27.64	27.28	$-CH_2$
7	54.16	53.79	-C-
8	126.16	126.10	-C-
9	128.43	128.85	CH
10	118.32	118.75	-CH
11	115.82	115.60	-CH
12	128.69	128.26	-CH
13	142.55	142.38	-C-
14	23.09	22.63	$-CH_2$
15	36.91	36.57	-CH
16	61.20	57.16	-C-
17	a	a	
18	12.59	12.50	CH_3
19	119.44	119.14	-CH
20	140.94	139.76	-C-
21	51.64	49.52	CH_2
CO_2CH_3	168.33	170.88	C=O
CH_3	52.01	52.49	CH_3

a = signal too weak to be detected.

(14-H β) while irradiation at δ 3.09 (3-H β) resulted in 6% NOE at δ 4.10 (3-Hα). Other NOE difference measurements for isorhazicine are presented in Fig. 2. Similarly the NOE difference studies on rhazicine (1) confirmed that the stereochemistry of the ethylidene group was 'E'. Thus in contrast to isorhazicine, irradiations at δ 1.51 (18-Me) in rhazicine did not show any effect at the C-21Hα or Hβ protons but gave 7.8% NOE at δ 2.86 (15-Hα) and 6.5% NOE at δ 5.45 (19-H). Irradiation at δ 2.86 (15-H) correspondingly resulted in 7.8% NOE at δ1.51 (18-Me) and 6.5% NOE at δ 2.29 (14-H β). Irradiation at δ 5.45 (19-H) resulted in 9.2% NOE at δ 1.51 (18-Me) while irradiation at δ 3.03 (21-Hβ) resulted in 5.2% NOE at δ 5.45 (19-H). Irradiation at δ2.07 (6-Hβ) gave 10.5% NOE δ 3.03 (21-Hβ), 10.5% at δ 7.25 (9-H) and 8% NOE at δ 3.64 (5-H). Irradiation at 2.60 (14-H β) resulted in 7.89% NOE at δ 2.96 (3-H β) while 6.5% NOE was observed at δ 2.60 (14Hα) on irradiation at δ 2.29 (14-Hβ).The NOE difference measurements for rhazicine are presented in Fig.1.

EXPERIMENTAL

The ethanolic extract of the fresh leaves (95 kg) of Rhazya stricta was concentrated to a gum and the material was dissolved in 10% acetic acid. The non-alkaloidal portion was removed by extraction with EtOAc. The aqueous acidic solution was basified with aq. ammonia to pH 11 and extracted with EtOAc to afford the crude alkaloids (350 g). This alkaloidal material was subjected to flash chromatography (alkaloid : silica gel, 1:40) for preliminary fractionation. Elution with benzene, pet. ether, $CHCl_3$, EtoAc and finally with MeOH afforded several fractions. The fraction obtained on elution with pet.ether-$CHCl_3$ (4:5) was concentrated and again subjected to column chromatography over silica gel (Merck neutral F_{254}). Elution with MeOH-EtOAc (1:9) afforded a mixture of four alkaloids which were separated by preparative TLC (aluminium oxide, Merck, F_{254} Type E) by using EtOAc-EtOH (9:1) as eluent. The two slower moving spots, rhazicine (1) and isorhazicine (2) gave dark pink colouration with $CeSO_4$ solution. Both were obtained as hygroscopic white crystalline needles. Spectral data for rhazicine (1) ($[\alpha]_D$, UV, IR, HRMS, ^{1}H-NMR ^{13}C-NMR) were presented in our earlier report[12]. The ^{1}H-NMR spectra (300 MHz) and ^{13}C-NMR spectra (75 MHz, broad band and DEPT) of both substances were recorded on Bruker AM-300 NMR spectrometer. The chemical shifts for each proton were assigned after 2D J-resolved, COSY 45° and NOE difference measurements and are summarized in Table 1. Chemical shift assignments for each

carbon atom are tabulated in Table II.

Spectral data of isorhazicine (2)

$[\alpha]_D^{CHCl_3}$ = + 61.4°, UV: λ_{max}^{MeOH} 213 nm, 249 nm, 295 nm λ_{min}MeOH 230 nm, 275 nm. IR $\nu_{max}CHCl_3$: 3500 cm^{-1} (N-H), 3400 cm^{-1} (OH), 1738 cm^{-1} (ester C=O) and 1720 cm^{-1} (keto C=O), HRMS: 368.1741, M^+, 8% (calcd. for $C_{21}H_{24}N_2O_4$: 368.1737), other major fragments were present at m/z 350.1630, 15% (calcd. for $C_{21}H_{22}N_2O_3$: 350.1630, M^+-H_2O), 322.1665, 6% (calcd. for $C_{20}H_{22}N_2O_2$: 322.1681, M^+ - H_2O-CO), 214.0860, 4% (calcd. for $C_{13}H_{12}NO_2$: 214.0868), 182.0602, 2% (calcd. for $C_{12}H_8NO$: 182.0605), 167.0779, 1.7% (calcd. for $C_{12}H_9N$: 167.0739) and 122.0972, 100% (calcd. for $C_8H_{12}N$: 122.0969).

Determination of configuration at C-2 of rhazicine and isorhazicine.

Isorhazicine (2.8 mg, 7.6 x 10^{-6}M) was dissolved in anhydrous pyridine (3 ml) and 2-phenyl butanoic anhydride (0.0047 ml) was added. The solution was kept at 25°C for 6-8 hrs. Water (0.3 ml) was added and the solution was allowed to stand for further 30 minutes to effect the hydrolysis. The mixture was then basified with 1M NaOH to pH 9 and extracted with $CHCl_3$. The aqueous layer was acidified with 0.1 M HCl to pH 3 and extracted with benzene (10 ml). The volume of benzene was reduced to 0.5 ml and the optical rotation was measured in micro-polarimetric tube. A negative optical rotation was recorded.

An identical procedure was adopted for rhazicine and it also resulted in a negative optical rotation establishing that both possess 'S' configuration at C-2.[15]

Acetylation of isorhazicine (1)

A mixture of isorhazicine (2 mg) in THF (50 ml), pyridine (0.2 ml) and acetic anhydride (10 ml) was stirred at 30°C for 12 hrs. The mixture was basified with 1M NaOH solution to pH 9 and extracted with EtOAc. The reaction product afforded MS : m/z = 350, 322, 214, 182, 167 and 122 (100%). UV λ_{max}MeOH: 222 nm, 265 nm, λ_{min}MeOH: 250 nm.

Reduction of isorhazicine (1).

Isorhazicine (2.5 mg) was dissolved in MeOH (0.3 ml) in a reaction flask. $NaBH_4$ (5 mg) in MeOH (0.5 ml) was added. The mixture was stirred at 30°C for 6 hrs and then extracted with EtOAc. The product afforded MS: m/z = 352, 323, 293, 214, 182, 167 and 122 (100%); UV: λ_{max}MeOH 213

nm, 249 nm 295 nm; λ_{min}^{MeOH} 230 nm, 275 nm.

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