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2-METHOXY-1,2-DIHYDRORHAZIMINE, AN ALKALOID FROM LEAVES OF *RHAZYA STRICTA*

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Key Word Index—*Rhazya stricta*; Apocynaceae; alkaloids; rhazimine; NMR

Abstract—Studies on the alkaloids from the leaves of *Rhazya stricta* have afforded a new alkaloid to which the structure 2-methoxy-1,2-dihydrorhazimine has been assigned.

INTRODUCTION

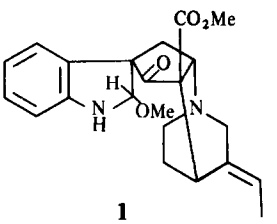
Rhazya stricta belongs to the family Apocynaceae. It is abundantly distributed in various parts of Pakistan [1–3] and is widely used in the treatment of several diseases [4, 5]. It is specially reputed for its anti-tumour activities. A number of cytotoxic alkaloids have previously been reported from the plant [6, 7].

RESULTS AND DISCUSSION

The crude alkaloidal material isolated by conventional procedures [8, 9] was subjected to chromatographic separation to afford a substance which possessed a typical indoline UV spectrum. The IR spectrum (chloroform) showed the presence of ester and ketonic carbonyl groups and an imine group. High resolution mass spectrometry afforded $[M]^+$ at m/z 382.1895 which agreed with the mass calculated for the formula $C_{22}H_{26}N_2O_4$ (382.1893) indicating the presence of 11 double bond equivalents in the molecule. The base peak at m/z 122.0965 corresponded to the formula $C_8H_{12}N$ attributed to the ion (*d*) [10] which could arise by cleavage between the C-5 and C-16 bonds. The facile loss of methanol from the $[M]^+$ suggested the presence of a methoxy group. The ^{1}H NMR spectrum (deuterochloroform) showed a three-proton doublet at δ1.52 (J_1 = 7 Hz, J_2 = 2.5 Hz) which was assigned to the ethylidine methyl group. A three-proton singlet at δ3.14 was assigned to the methyl protons of the methoxy group while another three-proton singlet at δ3.53 was assigned to the ester methyl group. A downfield one-proton singlet at δ4.93 was attributed to the C-2 proton. The olefinic proton of the ethylidene group resonated as a quartet at δ5.5 (J = 7 Hz). The aromatic protons appeared as complex multiplets in the region δ6.5–7.5.

The substance was found to be highly labile, being readily transformed to a faster moving substance when kept in chloroform at 30° for 2–3 hr. The ^{1}H NMR spectrum of the transformation product showed the disappearance of the one-proton singlet for the C-2 proton at δ4.93 and the appearance of a low field singlet at δ7.70 due to the olefinic proton of the ketimine system in rhazimine [10]. This transformation product was identified as rhazimine [10], previously reported by us from the same plant, by direct comparison with an authentic sample (co-chromatography, mp, IR, UV, ^{1}H NMR, mass spectrum). The ready transformation of the indoline to rhazimine bearing an indolenine chromophore strongly supported the conclusion that the methoxy group was located at C-2. The lack of a bond between C-2 and C-3 in rhazimine had previously been confirmed by gated spin-echo measurements which had established the presence of a proton on C-2 (ketimine), and showed that C-3 and C-21 were both CH_2 groups [10–12]. The stereochemistry of **1** is not known.

The ^{13}C NMR (deuterochloroform) of the alkaloid and its comparison with rhazimine is shown in Table 1. On the basis of these data, structure **1** is assigned to the alkaloid. The presence of **1** in the crude plant extract before contact with methanol was ascertained by TLC. This showed that **1** is a genuine natural product and not an artefact of isolation. The substance probably arises in the plant by hydration and subsequent methylation of rhazimine.



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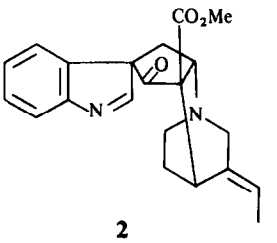

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Table 1. ^{13}C-NMR spectral data of 2-methoxy-1,2-dihydrorhazimine (1) and rhazimine (2)

Carbon No.	2-Methoxy-1,2-dihydro-rhazimine	Rhazimine
2	85.93	60.55
3	48.41	51.55
5	57.02	61.01
6	27.30	30.25
7	53.02	63.07
8	127.10	137.46
9	128.07	128.23
10	119.75	125.00
11	115.34	127.88
12	127.75	128.69
13	143.01	142.70
14	22.25	31.93
15	36.93	37.24
16	54.02	58.07
17	*	214.11
18	12.75	12.86
19	119.38	120.84
20	130.89	137.32
21	51.74	52.92
OCH_3	50.74	—
Ester C=O	†	168.33
Ester OMe	51.81	52.01

*,†Signals too weak to be detected. In the transformation product, rhazimine, the corresponding signals 'a' and 'b' appeared at δ214.71 (ketone) and 168.33 (ester carbonyl), respectively

EXPERIMENTAL

Isolation of 2-methoxy-1,2-dihydrorhazimine. The crude alkaloidal material (170 g) isolated by the previously reported procedure [8, 9] from leaves of *R. stricta* Decaisne (45 kg) was subjected to flash chromatography over silica gel. The fraction obtained on elution with petrol–$CHCl_3$ (4:5) was concd to a gum (18 g) and again subjected to flash chromatography over silica gel. The fraction obtained on elution with $CHCl_3$–MeOH (25:3) afforded a mixture of five alkaloids, which was again loaded onto another silica gel column. Elution with mixtures of $CHCl_3$–MeOH of increasing polarity afforded a number of fractions. The fraction obtained on elution with $CHCl_3$–MeOH (10:1) afforded an alkaloid which was purified by prep. TLC on Al_2O_3 (Merck, Type E) to afford 52 mg of a white crystalline (hygroscopic) material which gave a dark pink colouration with $CeSO_4$, $[\alpha]_D$ ($CHCl_3$) +85°. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3400 (NH), 1745 (keto C=O) and 1720 (ester C=O); UV λ_{max}^{MeOH} nm: 210, 249 and 295; λ_{min}^{MeOH} nm: 232 and 275; ^{1}H NMR ($CDCl_3$): δ1.52 (3H, *dd*, J_1 = 7 Hz, J_2 = 2.5 Hz, C<u>H</u>$_3$–HC=C), 3.14 (3H, *s*, –OC<u>H</u>$_3$), 3.53 (3H, *s*, C–OC<u>H</u>$_3$), 4.97 (1H, *s*, H-2), 5.5 (1H, *q*, J = 7 Hz >C=C<u>H</u>–CH$_3$), 5.5–7.5 (4H, *m*, ArH); high resolution MS: 382.1895 ([M]$^+$, 48% calc. for $C_{22}H_{26}N_2O_4$: 382.1893); 367.1672 (9.8%, calc. for $C_{21}H_{23}N_2O_4$: 367.1658); 350.1629 (15%, calc. for $C_{21}H_{22}N_2O_3$: 350.1630 [M – MeOH]$^+$); 323.1759 (15%, calc. for $C_{20}H_{23}N_2O_2$: 323.1759); 263.1546 (16%, calc. for $C_{18}H_{19}N_2$: 163.1548); 218.1175 (48%, calc for $C_{13}H_{16}NO_2$: 218.1181); 182.0603 (24%, calc. for $C_{12}H_8NO$: 182.0606) [13]; 167.0693 (18%, calc. for $C_{12}H_9N$: 167.0734) [14]; 122.0966 (100%, calc. for $C_8H_{12}N$: 122.0969).

Conversion of 2-methoxy-1,2-dihydro-rhazimine (1) to rhazimine (2). 2-Methoxy-1,2-dihydrorhazimine (13 mg), was dissolved in $CHCl_3$ and allowed to stand for 2 hr at 30°. TLC on silica gel $CHCl_3$–MeOH (17:3) showed the formation of a faster moving spot which was separated by prep. TLC and crystallized from $CHCl_3$–MeOH (4:1) as colourless needles. The product was identified as rhazimine by direct chromatographic and spectral comparison (IR, UV, ^{1}H NMR, ^{13}C NMR, MS) with an authentic sample.

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