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RHAZIMANINE, AN INDOLE ALKALOID FROM FRUITS OF *RHAZYA STRICTA*

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Key Word Index—*Rhazya stricta*; Apocynaceae; indole alkaloid; rhazimanine.

Abstract—A new indole alkaloid, rhazimanine, has been isolated from the fruits of *Rhazya stricta*. It belongs to the Corynanthe group of alkaloids with a *cis*-quinolizidine system bearing an H-3 in the β-configuration, equatorial to ring 'D'. The stereochemistry at various asymmetric centres has been established by a series of NOED measurements.

INTRODUCTION

Rhazya stricta (Apocynaceae) is a small, glabrous, erect shrub which is widely distributed in Western Asia and abundantly found in Pakistan. It has long been used in the indigenous system of medicine for the treatment of various diseases [1–4]. The anticancer activity of some of its alkaloids is also reported [5–7]. As a result of our investigations on the fruits of *R. stricta* we report here the isolation and structure determination of a new alkaloid, 'rhazimanine' (1). It belongs to the Corynanthe group of alkaloids with the H-3 in a β-configuration. Its structure has been elucidated by extensive NMR studies including 2D NMR (COSY-45, 2D-J-resolved, NOESY), NOE difference, ^{13}C NMR and DEPT experiments [8].

RESULTS AND DISCUSSION

The crude alkaloidal extract obtained from the alcoholic extract of the fruits (without seeds) was subjected to selective extractions with chloroform at different pH values according to their differential basicities [9]. The fractions obtained at pH 6.7 and 7.3 were combined and subjected to repeated prep. TLC resulting in the isolation of rhazimanine (1) as an amorphous material, unstable to air and light. Its UV spectrum was characteristic for the indole chromophore with λ_{max}^{MeOH} 222, 268 sh, 273 sh, 282, 290 nm and λ_{min} 252, 287 nm.

The IR spectrum indicated the presence of an ester carbonyl (1728 cm^{-1}), OH and NH groups (3215–3450 cm^{-1}). The absence of characteristic Wenkert–Bohlmann bands in the region of 2700–2800 cm^{-1} indicated the presence of a *cis*-quinolizidine system [10–14]. Its high resolution mass spectrum indicated the $[M]^+$ at *m/z* 354.1938 (54%) leading to the molecular formula $C_{21}H_{26}N_2O_3$. Other significant peaks were found at *m/z* 353 (37%), 251 (100%), 223 (27%), 184 (12%), 169 (82%), 156 (42%) and 144 (36%).

The ^{1}H NMR spectrum ($CDCl_3$, 300 MHz) of **1** showed the presence of a multiplet for the ethylidine methyl at δ1.62 exhibiting a vicinal coupling with H-19 ($J_{18,19}$ = 6.87 Hz) and homoallylic couplings with H-21α ($J_{18,21\alpha}$ = 1.83 Hz) and H-21β ($J_{18,21\beta}$ = 1.80 Hz). The C-21βH resonated as a broad doublet at δ3.03 ($J_{21\beta,21\alpha}$ = 12.00 Hz, $J_{21\beta,18}$ < 2 Hz). The olefinic proton at δ5.66 (H-19) appeared as a split quartet showing vicinal coupling with H-18 ($J_{19,18}$ = 6.87 Hz) and smaller allylic couplings with H-21α, H-21β and H-15 ($J_{19,21\alpha} \simeq J_{19,21\beta} \simeq J_{19,15} \simeq$ 1 Hz). The methylene protons of the primary alcoholic group appeared as two multiplets at δ3.50 and 3.62. The ester methyl group resonated as a singlet at δ3.81. A downfield signal at δ4.36 was assigned to the H-3 in a β-configuration indicating the presence of a C/D *cis* ring junction [14–21], since the earlier reported ^{1}H NMR values of other related compounds having H-3 in a α-configuration (C/D *trans*) were upfield of the value obtained for this proton† [7, 22–26]. The signal resembled a broadened triplet in accordance with the earlier observations that in *cis*-fused indoloquinolizidines, when the H-3 in ring 'D' is equatorially disposed the resulting couplings are *ca* equal in magnitude giving rise to a triplet-like signal due to overlapping of the inner peaks of the resulting double doublet [20]. In the alternative *cis*-fused structure, a more dispersed double doublet (resembling a quartet) is observed [20]. Furthermore the bandwidth of the signal was found to be ~ 6 Hz, which was consistent with an equatorial H-3 [21]. Each proton in the ^{1}H NMR spectrum was identified by carrying out a series of homodecoupling experiments, COSY-45 and NOESY experiments.

In order to confirm the relative stereochemistry of various protons, NOE difference studies were carried out. Irradiation at δ4.36 (H-3) resulted in 5.9% NOE at δ2.25 (H-14β), 1.6% NOE at δ3.19 (H-6β), 2.7% NOE at δ2.97 (H-5β) and 1.4% NOE at δ3.81 (COOMe). This established the β-configuration of H-3; this was further supported by irradiating the chemical shifts of H-14β, H-6β, H-5β and the ester methyl protons, which resulted in corresponding NOE at H-3.

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†The only exception we could find was 16*R*,19,20*E*-isositsirikine prepared synthetically. However, no explanation was given for the low value of the chemical shift of H-3 in comparison to 16*S*,19,20*E*-isositsirikine, 16*R*,19,20*Z*-isositsirikine and 16*S*,19,20*Z*-isositsirikine all of which show H-3α below 4.0 ppm [21].

Irradiation at H-6β, besides showing a NOE at H-5α and H-3, also showed a 2.3% NOE at δ7.47 (H-9). Similarly irradiation of H-6α showed a 1.1% NOE with H-9 (δ7.47). This suggested that the C-6β and C-6α protons are proximate to H-9, and also confirmed the ^{1}H NMR assignments for C-6β and C-6α protons at δ3.19 and 3.30, respectively.

Irradiation at δ1.62 (H-18) resulted in a strong (8.2%) NOE at δ3.12 (H-15), indicating that the 19,20-double bond is in the *E*-configuration. This was further supported by the fact that when H-19 (δ5.66) was irradiated, it resulted in 4.6% NOE at δ3.03 (H-21β), which confirmed that H-19 is in close proximity to H-21β. The stereochemistry at C-16 was established by irradiating the ester methyl group at δ3.81 which resulted in 3.6% NOE at δ2.25 (H-14α) and 2.6% NOE at δ4.36 (H-3). This established that in the preferred conformation the ester methyl group points towards H-14β and H-3. Irradiation of the methylene protons of the primary alcoholic group (δ3.50/3.62) resulted in 3.9% NOE at δ1.62 (H-18) and 5.6% NOE at δ3.12 (H-15). This established the proximity of the primary alcoholic group to H-18 and H-15. These NOE interactions served to establish the '*R*' configuration at C-16 (**1a**). The NOE interactions between the ester methyl and H-3, as well as the larger interactions between H-15 and H-14β in comparison to H-15 and H-14α indicated that in the optimum conformation ring 'D' in rhazimanine exists in a boat form.

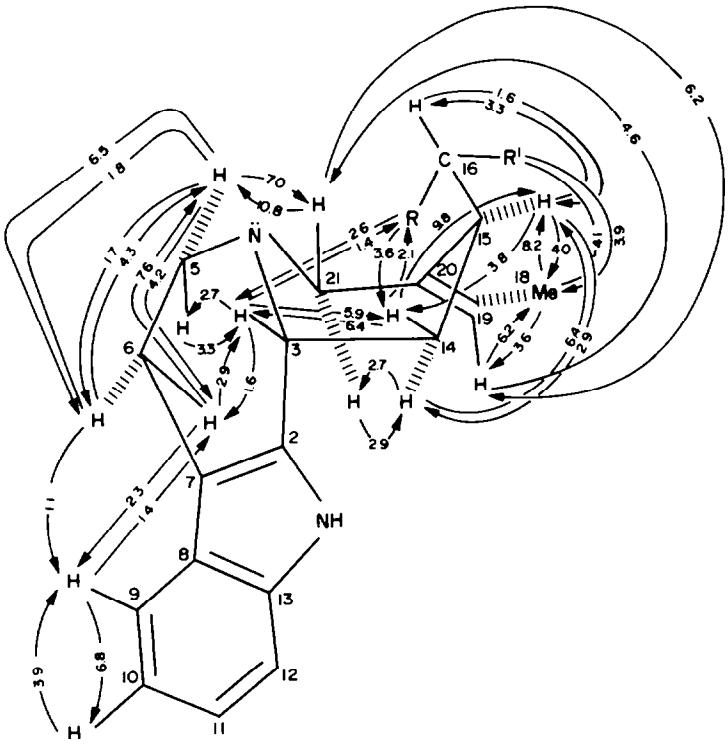
The ^{13}C NMR spectrum ($CDCl_3$, 75.4 MHz) (**1b**) indicated the presence of 21 carbon atoms. The multiplicity assignments were made by carrying out DEPT experiments with the last polarization pulse angle $\theta = 45°, 90°$ and 135°. The chemical shifts for specific carbon atoms were found to be of stereochemically diagnostic value. The upfield values for C-2 (δ131.40), C-3 (δ49.63), C-5 (δ50.21), C-6 (δ17.96) and C-7 (δ106.96) further supported the C/D *cis*-quinolizidine system as similar upfield shifts have been observed in other *cis*-quinolizidines [27–29].

The IR, ^{1}H NMR, ^{13}C NMR and NOED measurements thus served to establish that rhazimanine has C-3H in the β-configuration (occupying a pseudo-axial disposition to ring 'C' and an equatorial disposition to ring 'D') as a part of a C/D *cis*-quinolizidine ring system. Rhazimanine belongs to the Corynanthe group of alkaloids with a H-3β stereochemistry and it may arise in nature from strictosidine by a reversal of stereochemistry at C-3 during the biosynthetic process [30, 31].

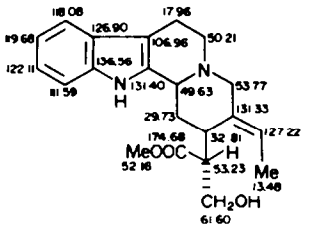
EXPERIMENTAL

Isolation of rhazimanine. Plant material was collected from a small village *ca* 90 km from Karachi and was identified by Prof. S. I. Ali at the Botany Department of Karachi University where a voucher specimen is deposited. Air dried fruits (without seeds) of *R. stricta* Decsne (10 kg) were soaked in EtOH, ground, filtered and concd. This resulted in the separation of a brown gummy material which was filtered off. The filtrate was concd, the soln acidified with 5% HCl, filtered and basified with NH_3 (conc) to pH ~ 9. The soln thus obtained was extracted with $CHCl_3$, dried (Na_2SO_4), filtered and evapd to dryness (120 g). The crude alkaloid extract thus obtained was dissolved in 10% HOAc soln. This soln was subjected to selective pH separations after stepwise basification with NH_3 (conc).

The fractions which were obtained at pH 6.7 and 7.3 were combined, basified with 10% NH_4OH soln and extracted with



1a R = COOMe
R' = CH_2OH



$CHCl_3$. The $CHCl_3$ soluble material was dried (Na_2SO_4), filtered and evapd to dryness (16 g). A portion (4 g) was subjected to prep. TLC on precoated silica gel GF-254 (2 mm) plates with $CHCl_3$–MeOH (3:2) to afford a major band (R_f 0.69) containing six alkaloids. The material thus obtained was again subjected to prep. TLC using precoated silica gel (GF-254) (0.2 mm) plates using $CHCl_3$–EtOAc–MeOH (7:1.5:1) to afford a major alkaloid (R_f 0.50) which was re-purified on silica gel plates (0.2 mm) (GF-254) with petrol–Me_2CO–Et_2NH (16:4:1) (R_f 0.19). This afforded a pure alkaloid (17.6 mg, 7.04×10^{-4}% yield) which could not be crystallized and rapidly turned yellow in air, $[\alpha]_D^{26}$ ±0° ($CHCl_3$); IR ν_{max}^{KBr} cm^{-1}: 3450–3215 (NH, OH), 2855–3000 (CH), 1728 (ester carbonyl), 1600 (C=C), 1162 (C–O) and 750 (aromatic CH); UV λ_{max}^{MeOH} nm: 222, 268 sh, 273 sh, 282 and 290; λ_{min} 252 and 287 nm; ^1H NMR (300 MHz, $CDCl_3$): δ 1.62 (*m*, 3H, $J_{18,19} = 6.87$, $J_{19,21\beta} = 1.83$, $J_{18,21\alpha} = 1.80$ Hz, H-18), 1.72 (*m*, 1H, H-14α), 2.25 (*m*, 1H, H-14β), 2.52 (*m*, 1H, H-16), 2.70 (*ddd*, 1H, $J_{5\alpha,5\beta} = 15.78$, $J_{5\alpha,6\alpha} = 4.80$, $J_{5\alpha,6\beta} \simeq 2$ Hz, H-5α), 2.97 (*m*, 1H, H-5β), 3.03 (*br d*, 1H, $J_{21\beta,21\alpha} = 12.00$, $J_{21\beta,18} \simeq 2$ Hz, H-21β) 3.12 (*m*, 1H, H-15), 3.19 (*m*, 1H, H-6β), 3.30 (*ddd*, 1H, $J_{6\alpha,6\beta} = 13.00$, $J_{6\alpha,5\alpha} = 4.80$, $J_{6\alpha,5\beta} = 1.44$ Hz, H-6α), 3.50 (*m*, 1H, H-17), 3.56 (*m*, 1H, H-21α), 3.62 (*m*, 1H, H-17). 3.81 (*s*, 3H, COOMe), 4.36 (*br s*, 1H, H-3), 5.66 (split quartet, 1H, $J_{19,18} = 6.87$, $J_{19,21\alpha} \simeq J_{19,21\beta} \simeq J_{19,15} < 1$ Hz, H-19), 7.10 (*m*, 1H, H-10), 7.16 (*m*, 1H, H-11), 7.38 (*dd*, 1H, $J_{12,11} = 7.71$, $J_{12,10} = 1.32$ Hz, H-12), 7.47 (*dd*, 1H, $J_{9,10} = 7.47$, $J_{9,11} = 1.11$ Hz, H-9), 8.74 (*s*, 1H, NH); ^{13}C NMR (75 MHz, $CDCl_3$): see **1b**; HRMS *m/z* (rel. int.): 354.1938 ([M]$^+$, calc. 354.1943 for $C_{21}H_{26}N_2O_3$, 54%), 353.1865 ($C_{21}H_{25}N_2O_3$, 37%), 339.1733 ($C_{20}H_{23}N_2O_3$, 5%), 337.1536 ($C_{20}H_{21}N_2O_3$, 3%), 323.1768 ($C_{20}H_{23}N_2O_2$, 17%), 251.1553 ($C_{17}H_{19}N_2$, 100%), 237.1386 ($C_{16}H_{17}N_2$, 18%), 223.1220 ($C_{15}H_{15}N_2$, 27%), 184.0992 ($C_{12}H_{12}N_2$, 12%), 169.0766 ($C_{11}H_9N_2$, 82%), 156.0813 ($C_{11}H_{10}N$, 42%) and 144.0817 ($C_{10}H_{10}N$, 36%).

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