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SHORT COMMUNICATION

OCCURRENCE OF GEISSOSCHIZINE AND OTHER MINOR BIOGENETICALLY RELATED ALKALOIDS IN *RHAZYA STRICTA*

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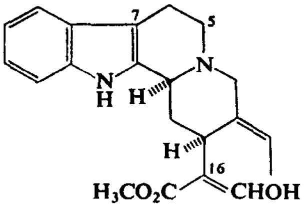
Abstract—Chemical investigation of *Rhazya stricta* Decaisne has led to the isolation of more than a dozen indole alkaloids. Two more indolic bases, rhazinine and R1, obtained from this plant, were shown to be identical with antirhine and geissoschizine respectively. The biogenetic significance of the occurrence of the latter is discussed. Four other minor indole alkaloids, viz. R2, R3, rhazinilam and rhazinaline are also present.

INTRODUCTION

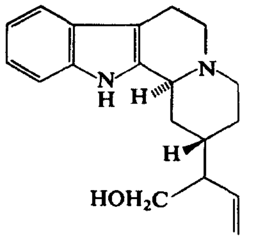
The chemical investigation of *Rhazya stricta* Decaisne (Apocynaceae) has led to the isolation of more than a dozen alkaloids of different structural patterns, viz. those of the rhazine (akuammidine),[1] strictamine,[2] eburnamine[3] and aspidospermine[1,3,4] types. Recently the occurrence of a group of novel indolic bases has been reported.[5,6] One of these, strictosidine,[6] may be regarded as the precursor of all C_{19}–C_{20} indole alkaloids. In the present communication, the isolation of several minor alkaloids, *rhazinilam*, *rhazinaline*, *R*2 and *R*3 and the structure proof of two alkaloids, rhazinine and R1 from the leaves, are reported.

RESULTS AND DISCUSSION

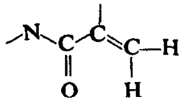
Alkaloid R1, $C_{21}H_{24}N_2O_3$ (M^+ 352), m.p. 187° (dec.) $[\alpha]_D^{25}$ $+72{\cdot}2°$ ($CHCl_3$) (0·001 per cent yield) is identical with geissoschizine[7,8] (I), one of the acid-catalyzed cleavage products of the "dimeric" alkaloid geissospermine, from TLC comparison, mixed m.p. determination,



(I)



Antirhine (II)



(III)

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superimposable i.r. spectra and X-ray powder pattern with an authentic sample, m.p. 189° (dec.) $[\alpha]_D^{27}$ + 100° ($CHCl_3$). The occurrence of geissoschizine in *R. stricta* is of biogenetic interest, since it can be regarded as the immediate precursor of both the rhazine and the strictamine group of alkaloids and also as the intermediate which upon subsequent rearrangement followed by double-bond isomerization and vinylogous retro-Mannich transformation leads to the secamines and the aspidosperma and eburnamine type of alkaloids occurring in the same plant. This provides a strong phylogenic evidence for the biosynthetic sequence for indole alkaloids postulated by Scott *et al.*[9] It is interesting that Battersby *et al.*[10] have recently found geissoschizine in another species in the Apocynaceae, in *Vinca rosea*, and have shown that geissoschizine is indeed one of the key intermediates in the biosynthesis of the indole alkaloids.

The second alkaloid, rhazinine, occurs in 0·01 per cent yield.[11] Rhazinine, $C_{19}H_{24}N_2O$ (M^+ 296), m.p. 118° $[\alpha]_D$ + 1·3° (MeOH) exhibits the characteristic color reactions and u.v. spectrum of tetrahydro-β-carboline alkaloids and comparison of the physical and spectral (i.r., NMR and mass) properties of rhazinine with antirhine[12] (II) and of their dihydro derivatives suggested that the two compounds are identical. This was finally confirmed by mixed m.p. determination, TLC comparison and superimposable i.r. spectra of rhazinine with an authentic sample of antirhine. Certain observations about this compound, not reported by Johns *et al.*,[12] will be communicated in a forthcoming publication.

The third alkaloid, rhazinilam, $C_{19}H_{22}N_2O$ (M^+ 294) occurs in 0·003 per cent yield in the leaves of *R. stricta.* It lacks a characteristic u.v. spectrum, showing only end absorption (213 nm, log ϵ 4·37) and inflection points at 220 and 275 nm (log ϵ 4·28 and 3·33). The presence of a —NH— group and an amide moiety is indicated from the presence of absorption bands at 3225 cm^{-1} and 1672 cm^{-1} respectively in the i.r. spectrum. The NMR spectrum shows the presence of four aromatic protons (multiplet 7·10–7·50 δ) and ethyl side chain (methyl triplet around 0·72 δ, $J = 7{\cdot}5$ c/s) and two vinylic protons appearing as doublets ($J = 2$ c/s in each case) at 5·72 δ and 6·45 δ respectively. The positions of the signals of these vinylic protons and the coupling constant suggest that both of them are attached to an exocyclic methylene group which forms part of the grouping (III). The mass spectrum of rhazinilam confirms the presence of an ethyl group and an amide moiety in the molecule. A significant peak at *m/e* 265 (M–29) is due to the loss of the ethyl side chain. Another intense peak at *m/e* 237 (M–29–28) may be due to the loss of CO from the fragment of mass 265, which is typical of an amide. These preliminary studies show that rhazinilam possesses a novel structure and further work on it is in progress.

Rhazinaline*, $C_{21}H_{22}N_2O_3$ (M^+ 350), m.p. 137° (0·002 per cent yield) and two other minor bases—R2, $C_{21}H_{22}N_2O_3$ (M^+ 350), m.p. 176° (0·001 per cent yield) and R3 (M^+ 350), m.p. 217° (0·0003 per cent yield) have also been isolated.

EXPERIMENTAL

The m.ps were determined on the Kofler block and are uncorrected. The u.v. spectra were measured in 95 per cent ethanol (aldehyde-free), the i.r. spectra in nujol mull or KBr disc.

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* Isolation work on rhazinaline has been done in collaboration with Dr. R. Majumder (née Raychaudhuri).

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Isolation of the Alkaloids

In a typical extraction 1 kg of the crushed and dried leaves of *R. stricta* were extracted successively with petroleum ether (60–80°) and $CHCl_3$ in a Soxhlet. The marc was then extracted with ethanol. The petroleum ether extract was concentrated and then poured into 1·5 l. of 5 per cent aq. citric acid. The mixture was stirred vigorously for 10 hr and the aqueous layer was separated. The aqueous solution was then extracted successively with benzene (1 l.) and $CHCl_3$ (1 l.). The $CHCl_3$ extract was washed with dilute ammonia solution and then with water. The extract was then dried, concentrated and chromatographed on Brockmann alumina. The total solid obtained from the benzene eluate on further chromatography yielded in addition to the known alkaloid strictamine 20 mg of rhazinaline, which crystallized from petroleum ether–benzene (4:1) in stout needles, m.p. 137°. The combined mother liquor left after the crystallization of rhazinaline (obtained from 10 kg of plant material) on concentration deposited an amorphous white solid R3, which crystallized as granules, m.p. 217° (30 mg yield) from 30% methanolic $CHCl_3$. (Found: C, 71·50; H, 6·00; N, 7·94. $C_{21}H_{22}N_2O_3$ required C, 72·00; H, 6·29; N, 8·00%.)

The $CHCl_3$ extract was concentrated and then churned with 5 per cent aq. citric acid (1 l.) for 10 hr. The aqueous acid layer was separated, basified with NH_3 (pH 10) and extracted with $CHCl_3$ (5 × 200 ml). The $CHCl_3$ extract was dried, concentrated and chromatographed over Brockmann alumina. The benzene–$CHCl_3$ (3:1) eluates furnished a mixture of bases which on further chromatography over silica gel afforded rhazinilam (30 mg), crystallizing as colorless plates and R3, m.p. 176° (10 mg) crystallizing from a 1:1 mixture of cyclohexane and benzene. Rhazinine (100 mg) crystallized as needles, m.p. 118°, when the benzene–$CHCl_3$ (1:3) eluates were concentrated. In addition to these the known alkaloids rhazine, rhazidine and quebrachamine were also isolated. (Rhazinilam; found: C, 77·49; H, 7·41; N, 9·48. $C_{19}H_{22}N_2O$ required C, 77·45; H, 7·48; N, 9·53%. R2 found: C, 72·21, H, 6·12; N, 7·82. $C_{21}H_{22}N_2O_3$ required C, 72·00; H, 6·29; N, 8·00%.)

The ethanol extract of the leaves was concentrated and then churned with 5 per cent aq. citric acid (1·5 l.). The aqueous solution was exhaustively extracted with $CHCl_3$ (1 l.). The aqueous portion remaining after this processing was just basified with NH_3 (pH 8) and extracted successively with ether (1 l.) and $CHCl_3$ (1·5 l.). The ether solution was shaken with 2 per cent NaOH (3 × 150 ml) to remove acidic components. The alkaline solution was neutralized and extracted with ether. Concentration of this extract afforded crystals of geissoschizine, which was recrystallized from EtOAc to give colorless plates, m.p. 187° (dec.) (yield—0·001 per cent). (Found: C, 71·62; H, 6·70; N, 8·12. Calc. for $C_{21}H_{24}N_2O_3$: C, 71·59, H, 6·82; N, 7·95%.)

Hydrogenation of Rhazinine

100 mg rhazinine was hydrogenated in presence of 80 mg PtO_2 in ethanolic medium. The reaction mixture was filtered and the filtrate was concentrated. Dihydrorhazinine, $C_{19}H_{26}ON_2$, m.p. 108°, (yield—80 mg) crystallized from the concentrate.

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