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FURTHER ALKALOIDAL CONSTITUENTS OF THE LEAVES OF *RHAZYA STRICTA**

YUSUF AHMAD, KANIZ FATIMA, PHILIP W. LE QUESNE† and ATTA-UR-RAHMAN‡

P.C.S.I.R. Laboratories, Karachi-39, Pakistan; †Department of Chemistry, Northeastern University, Boston, MA 02115, U.S.A.; ‡H.E.J. Research Institute of Chemistry, University of Karachi, Karachi-32, Pakistan

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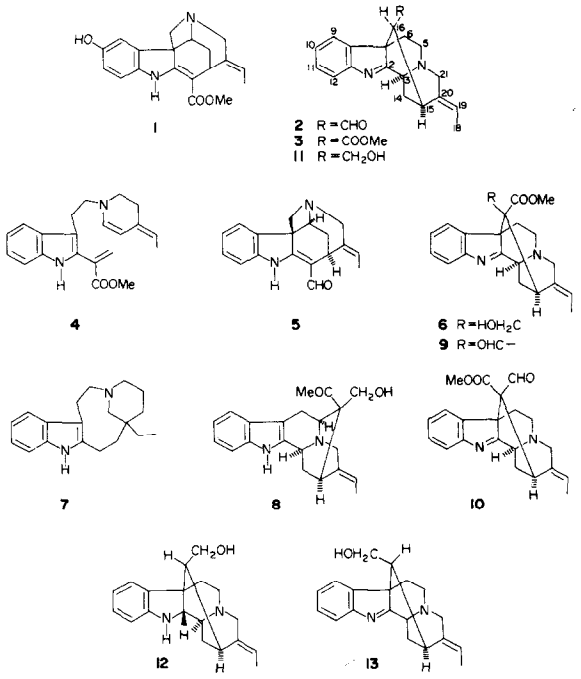
Key Word Index—*Rhazya stricta*; Apocynaceae; leaves; alkaloids; rhazimal; rhazimol; rhazinol; stricticine; strictine.

Abstract—Studies on the alkaloidal constituents of the leaves of *Rhazya stricta* have resulted in the isolation and structure elucidation of rhazimal (16-formylstrictamine), rhazimol (deacetylakuammiline), rhazinol (a hydroxymethyl analogue of strictamine). Two more new alkaloids stricticine and strictine have also been isolated.

INTRODUCTION

In previous publications we have reported the isolation and structure determination of sewarine (**1**) [1–3] and strictalamine **2** [4] from *Rhazya stricta*, and in our most recent paper [4] we described the determination of the complete structures and absolute configurations of strictalamine (**2**), strictamine (**3**) and, by extension, the *Picralima* series of alkaloids.

The isolation of sewarine (**1**) from the alkaloidal fraction of *R. stricta* is facilitated by its relative insolubility in, and ease of crystallization from, organic solvents. These characteristics are probably due to a degree of zwitterionic character. Isolation of other alkaloids is rendered more difficult because the total alkaloidal material remaining after removal of sewarine ('asewarine') contains a substantial amount of apparently polymeric material, which may arise from polymerization reactions of secamine (**4**) [5, 6] and related compounds.



In the present work several methods were explored to facilitate isolation of alkaloids. In one, the 'asewarine' fraction was dissolved in 2% tartaric acid solution (pH 3.0) and the alkaloids separated by extraction into organic solvents at different pH values.

RESULTS AND DISCUSSION

Adjustment of the pH of the aqueous tartaric acid solution of alkaloids to 3.5, followed by extraction successively with petrol, benzene, chloroform and ethyl acetate, gave alkaloid-containing fractions. The chloroform-soluble fraction, which was much the most abundant, was chromatographed over neutral alumina (activity III). Elution with petrol and benzene gave no products, but with chloroform a mixture of alkaloids was obtained, which was separated by prep. TLC. One of the two main compounds was identified as (−)-nor-*C*-fluorocurarine (**5**) by direct comparison with authentic material. The formation of **5** from strictalamine (**2**) by base-mediated rearrangement has been previously reported [4]. The second alkaloid was non-crystalline, but gave a M^+ at m/z 352, which suggests the molecular formula $C_{21}H_{24}N_2O_3$. The UV spectrum was indoleninic and the IR spectrum showed the presence of a probably hydrogen-bonded ester group at 1725 cm^{-1}. These data are consistent with those of deacetylakuammiline (**6**), previously obtained from *Picralima nitida* [7].

Several other extractive techniques were tested for their suitability for the isolation of pure alkaloids. Direct ethanol extraction of the air-dried leaves of *R. stricta* gave, after removal of solvent, a green viscous mass, which on Soxhlet extraction with petrol, benzene and ethyl acetate, respectively, gave alkaloid-rich fractions. Column chromatography of these fractions gave (−)-quebrachamine (**7**), strictamine (**3**), akuammidine (**8**) and the previously reported 16-formylstrictamine (**9**) [8, 9] (in our preliminary communication called 'rhazimal'). Reduction of **9** with sodium borohydride gave deacetylakuammiline (**6**).

*Preliminary communication see ref. [8].

It is noteworthy that the ^{1}H NMR spectrum of **9** allows it to be easily distinguished from the 16-epimer (**10**) of rhazinaline [10]. In **9** the carbomethoxy-methyl signal appears at δ 3.8 and the aldehyde proton signal at 8.5, whereas the respective values reported for rhazinaline (**10**) are 3.16 and 9.93. These values reflect the shielding influence of the indolenine ring on the substituents directly above it.

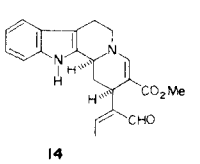
Further chromatography of the above benzene extract gave a new alkaloid, stricticine, mp 68–70° and molecular formula $C_{19}H_{20}N_2O$. The substance possessed an indolenine chromophore, UV λ_{max}^{MeOH} nm: 222, 266. Further studies are being carried out on its structure.

Soxhlet extraction of 'asewarine' with benzene gave, after column chromatography and removal of strictamine, a new alkaloid of molecular formula $C_{19}H_{22}N_2O$, named rhazinol. The possession of an indoleninic UV spectrum, and its IR and mass spectra suggested that the alkaloid might be the hydroxymethyl analogue (**11**) of strictamine (**3**). In order to test this hypothesis, strictamine (**3**) was reduced with lithium aluminum hydride in ether–THF to give the dihydroindolenine alcohol (**12**), which on oxidation with activated manganese dioxide gave **11**, spectrally almost identical with, but chromatographically distinguishable from, rhazinol. We, therefore, conclude that rhazinol has structure **13**, which is epimeric with sewarine at C-16.

A feature of the ^{1}H NMR spectrum of strictamine (**3**) which was discussed earlier [4] is a one-proton signal at δ 4.78. This signal was assigned [4, 11] to the C-16 hydrogen atom, its low field position being ascribed to the effect of the indolenine nucleus [12] or the adjacent carbomethoxy group [4]. The fact that alcohol **11**, prepared above during the determination of the structure of rhazinol, also gives rise to a ^{1}H signal in this region, at δ 4.6, rules out the carbomethoxy group as the origin of the deshielding effect. Further, the ^{1}H NMR spectrum of rhazinol also shows a similar signal, at δ 4.6, from which it may be inferred that the aromatic system as such is also not responsible for the deshielding. Examination of Dreiding models reveals that the C-3 protons in strictamine (**3**), rhazinol (**13**) and the synthetic alcohol (**11**) all lie within the deshielding influence of the indolenine ketimine systems as well as being adjacent to the basic nitrogens. We, therefore, ascribe these signals to the C-3 protons in these compounds. Confirmation of the assignment is afforded by the ^{1}H NMR spectrum of alcohol **12**, which has no signals between δ 4.0 and 5.0.

Further chromatography of the benzene-soluble portion of 'asewarine' from which rhazinol was obtained gave a new alkaloid named strictine, $C_{22}H_{26}N_2O_4$. The substance exhibited an indolenine chromophore UV λ_{max}^{MeOH} nm: 225, 264. Further work on the structure of this compound is in progress.

It should be noted that alkaloids vallesiachotamine (**14**) and polyneuridine (**15**) have recently been reported from *R. stricta* by other workers [12].



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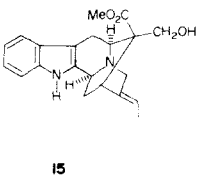

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EXPERIMENTAL

NMR spectra were measured at 60 MHz using TMS as int. standard. CC was generally carried out on Al_2O_3 Woelm activity III (Merck.) and neutral (BDH). Mps are uncorr. TLC was performed on Si gel PF-254 and GF-254 plates (Merck) in EtOH–C_6H_6. TLC plates were viewed under UV (254 nm).

Preliminary preparation. Air-dried fr. leaves of *R. stricta* Decaisne (20 kg) were percolated × 3 with 95% EtOH (100 l.) at room temp. during 6 days. The dark green EtOH extract was filtered and concd under red. pres. below 25° until a dark green solid began to separate out. This solid, which was non-alkaloidal, was filtered off and washed with a little EtOH. The clear filtrate on concn under red. pres. yielded a dark green viscous mass (1.3 kg), which gave strong alkaloidal reactions.

Preparation of asewarine. The above crude alkaloid-bearing extract (800 g) was digested with warm H_2O in which most of it dissolved. The soln was decanted, treated with charcoal and filtered. The brown filtrate on cooling and basification with NH_3 yielded a heavy yellow ppt which was exhaustively extracted with EtOAc (3 × 1 l.) The extract was washed with H_2O, dried (Na_2SO_4) and concd under red. pres. to 25% vol. On leaving this soln overnight at 0°, sewarine (**1**) (1.99 g) separated out as yellow microneedles, mp 244–245°. The solvent from the filtrate after removal of sewarine was removed under red. pres. and the residue, which swelled up as a foam, was scraped out and powdered. This alkaloidal fraction (150 g) was named 'asewarine'.

Isolation of deacetylakuammiline (**6**). Asewarine (20 g) was dissolved in 2% aq. tartaric acid (250 ml). After filtration of a very small amount of an insoluble material, the pH of the soln was adjusted from 3 to 3.5 with dilute NH_3 and separation of alkaloids was effected by extraction successively with petrol (bp 60–80°; 3 × 500 ml), C_6H_6 (3 × 500 ml), $CHCl_3$ (3 × 500 ml) and EtOAc (3 × 500 ml). Removal of each solvent under red. pres. gave 0.11, 0.57, 5.44 and 0.57 g of alkaloidal material, respectively, from their extracts. Material obtained from evaporation of the $CHCl_3$ extracts (5 g) was chromatographed over a column of neutral Al_2O_3 (activity III; 300 g). The column was eluted successively with petrol (1 l.), C_6H_6 (1 l.) and C_6H_6–$CHCl_3$ (1:1) 500 ml). The C_6H_6–$CHCl_3$ eluate afforded a brownish yellow product (200 mg), which gave a positive Dragendorff test. TLC on Si gel PF-254 with C_6H_6–EtOH (4:1) indicated this to be a mixture of alkaloids with one component predominating. Trituration of this product with EtOH gave strictamine (**3**) (100 mg).

Further elution of the column with $CHCl_3$ (2.5 l.) gave a product (150 mg), which TLC (EtOH–C_6H_6, 3:7) indicated to be a mixture of two alkaloids. This mixture was subjected to prep. TLC in the same solvent. One component was found to be (−)-nor-C-fluorocurarine (**5**) by comparison of its physical and spectral data with those of an authentic sample. The second component (30 mg) proved to be deacetylakuammiline (**6**); $[\alpha]_D$ (MeOH) + 15°; UV λ_{max}^{MeOH} nm (ε): 229 (61 270), 264 (5739); IR ν_{max}^{KBr} cm^{-1}: 1725–1730 (ester); MS [M]$^+$ 352.178 (calcd for $C_{21}H_{24}N_2O_3$ 352.1781); *m/z* 352 [M]$^+$ (100%), 321 (98), 293 (24), 292 (48), 263 (20), 249 (10) and 121 (48). Comparison with ref. [7]: UV λ_{max}^{EtOH} nm (ε): 220 (61 170), 628 (5539); IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1745, 1625, 1595.

Isolation of 16-formylstrictamine (**9**). The crude EtOH extract before removal of sewarine (500 g) was extracted (Soxhlet) successively with petrol (bp 60–80°) (3 × 500 ml), C_6H_6 (4 × 500 ml) and EtOAc (3 × 500 ml), the fractions affording, after evaporation, 10.22, 6.5 and 10.56 g, respectively, of materials. CC of petrol extracts afforded the known alkaloids quebrachamine (**7**), mp 141–143°, strictamine (**3**), mp 110–112°, akuammidine (**8**), mp 234–235° and a complex mixture of alkaloids. TLC of the C_6H_6 extract also indicated that it was a

complex mixture of alkaloids, two of which were identified as quebrachamine (**7**) and strictamine (**3**) by chromatographic comparison with authentic samples. On subjecting the C_6H_6 extract (1.5 g) to repeated prep. TLC (EtOH–C_6H_6, 4:1) on Si gel-60 PF-254, it easily separated into three bands, the extraction of which yielded strictamine (**3**) (150 mg, R_f 0.6), (−)-quebrachamine (**7**) (200 mg, R_f 0.85) and 16-formylstrictamine (**9**) (100 mg, R_f 0.95); $[\alpha]_D$ (MeOH) + 57°; UV λ_{max}^{MeOH} nm (ε): 222 (14 105), 265 (3663); IR ν_{max}^{KBr} cm^{-1}: 1725–1730 (ester); NMR ($CDCl_3$); δ 8.53 (1H, *s*, –CHO), 5.5 (1H, *m*, olefinic proton), 3.8 (3H, *s*, Me) 1.8 (3H, *d*, J = 8 Hz, ethylidene methyl); MS $[M]^+$ 350.1630 (calcd. for $C_{21}H_{22}N_2O_3$, 350.162); *m/z* 350 $[M]^+$ (100%), 321 (52), 292 (25), 263 (27), 249 (10), 180 (7), 167 (8), 149 (48). Lit. [10] UV λ_{max}^{EtOH} nm (ε): 266 (5786); 243 (5563). IR ν_{max}^{Nujol} cm^{-1}: 1751 ν(–CO_2Me), 1724 (–CHO), 1621 (>C = C<), 1592 (aromatic) NMR ($CDCl_3$): δ 9.93 (1H, *s*, –CHO), 3.16 (3H, *s*, CO_2Me), 4.71 (1H, *m*, C-3, C-4), 1.78 (3H, *d* and *d*, J = 8.5 Hz); MS: *m/z* 350 $[M]^+$ (100), 321, 291.

Another crystalline alkaloid, 'stricticine', mp 68–70° isolated by chromatography of the above C_6H_6 extract afforded $[M]^+$ at *m/z* 292 and possessed an indoleninic UV λ_{max}^{MeOH} nm (ε): 222 (15 398), 266 (5639). MS: *m/z* 292 $[M]^+$ (100); 279 (25), 265 (28).

Reduction of 16-formylstrictamine (**9**). To a soln of 16-formylstrictamine (**9**) (5 mg, 0.011 mmol) in MeOH (0.5 ml) was added $NaBH_4$ (20 mg) and the reaction mixture stirred at 30° for 2 hr. H_2O (10 ml) was added and the soln extracted with $CHCl_3$. The $CHCl_3$ layer was separated, dried (Na_2SO_4) and solvent removed to give a product identical with deacetylakuammiline (**6**) (see above).

Isolation of rhazinol (**13**). 'Asewarine' (200 g) was subjected to Soxhlet extraction successively with petrol (500 ml × 3), C_6H_6 (500 ml × 4), $CHCl_3$ (500 ml × 4) and EtOAc (500 ml × 3). The fractions on evaporation gave 25.23, 20.03, 50 and 15.24 g of gummy materials, respectively.

From the petrol extract on CC, quebrachamine (**7**), strictamine (**3**), akuammidine (**8**) and a further complex alkaloidal mixture were obtained.

The C_6H_6 extract (20 g) was subjected to CC, using neutral activity III Al_2O_3 (600 g). Elution of the column, first with petrol (1 l.) and then petrol–C_6H_6 mixtures with increasing proportions of C_6H_6, resulted in the elution of a gummy alkaloidal material (200 mg) which on TLC was found to be a mixture of two alkaloids. On elution with C_6H_6–petrol a crystalline alkaloid (100 mg), mp 187°, was obtained, which was identical with (−)-nor-*C*-fluorocurarine (**5**) by TLC and spectral comparison. On further elution with C_6H_6 (2 l.), rhazinol (**13**) [3] (200 mg) was obtained as a glass. Final purification by prep. TLC on Si gel-60 PF-254 using EtOH–C_6H_6 (4:1) gave non-crystalline material: UV λ_{max}^{MeOH} nm (ε): 225 IR ν_{max} (13 609), 266 (5439); IR ν_{max} (ester peak absent); NMR ($CDCl_3$): δ 7.1–7.8 (4H, *m*, aromatic protons), 5.5 (1H, *m*, olefinic proton), 4.6 (1H, *d*, J = 5 Hz), 4.0 (1H, *m*, H-16), 1.75 (3H, *d*, J = 6 Hz, ethylidene methyl); MS $[M]^+$ 294.173 (calcd. for $C_{19}H_{22}N_2O$, 294.173); *m/z* 294 $[M]^+$ (100%), 279 (15), 266 (41), 265 (46), 264 (48), 263 (75), 224 (13), 194 (10).

Reduction of strictamine (**3**). Strictamine (**3**) (0.5 g) was dissolved in a dry Et_2O–THF (1:1, 500 ml) and $LiAlH_4$ was added slowly to the reaction mixture. After 5–6 hr reflux, the cooled reaction mixture was worked-up by careful dropwise addition of H_2O followed by aq. NaOH soln (2 N, 300 ml). The insoluble salts were filtered off and the residue thoroughly washed with Et_2O. The aq. layer was extracted × 3 with Et_2O (500 ml) and the Et_2O layers combined and dried (K_2CO_3), filtered and dried under vacuum to afford 0.45 g of alcohol **12**. TLC in C_6H_6–EtOH (9:1) showed complete conversion to the reduced product **5**. UV λ_{max}^{MeOH} nm (ε): 242 (6240), 289 (3050); IR ν_{max} (ester peak absent); NMR ($CDCl_3$): δ 7.1–7.8 (4H, aromatic protons), 5.3 (1H, *d*, J = 7 Hz, olefinic proton) no peak between 4 and 5 (C-3 proton is upfield due to reduction of indolenine double bond), 1.75 (3H, *d*, J = 4 Hz, ethylidene methyl). MS: *m/z* 296 $[M]^+$ (100%), 289 (40), 265 (30) and 167 (15).

Oxidation of alcohol **12**. Alcohol **12** (0.44 g; 1.5 mmol) was dissolved in $CHCl_3$ (30 ml) and freshly prepared activated MnO_2 (1 g) was added. The reaction mixture was stirred at room temp. for 5 hr and filtered. The residue was washed thoroughly with $CHCl_3$ and the solvent removed under vacuum to give **11**: UV λ_{max}^{MeOH} nm: 225, 266; IR ν_{max} (carbonyl absent). NMR ($CDCl_3$): δ 7.1–7.8 (4H, *m*, aromatic protons), 5.5 (1H, *d*, J = 8 Hz, olefinic proton), 4.6 (1H, *d*, J = 4 Hz, C-3 proton), 1.75 (3H, *d*, J = 4 Hz, ethylidene methyl). MS: *m/z* 294 $[M]^+$ (100%), 279 (15), 266 (41), 265 (46), 264 (48), 168 (20), 149 (10). This could readily be distinguished chromatographically from rhazinol by TLC on Si gel G PF-254 and development with C_6H_6–EtOH (4:1).

Isolation of stricticine. Asewarine (200 g) was Soxhlet extracted with various solvent as described under the isolation of rhazinol above. The C_6H_6 extract (20 g) was subjected to CC over neutral Al_2O_3 (Activity III). On elution with C_6H_6–petrol (1:1) a mixture of two alkaloids was obtained, one of which was found to be identical with strictamine. This mixture (200 mg) was subjected to prep. TLC (EtOH–C_6H_6, 1:4) on Si gel PF-254, to afford a new alkaloid 'strictine' (15 mg). UV λ_{max}^{MeOH} nm (ε): 225 (13 500), 264 (5519); MS: *m/z* 382 $[M]^+$ (100%), 368 (26), 353 (36), 338 (24), 322 (100), 295 (20), 279 (24), 263 (48), 229 (15), 209 (12.5), 194 (23), 180 (48), 168 (12.2), 167 (48).

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