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PEPTIDE ALKALOIDS OF *DISCARIA LONGISPINA* AND *SCUTIA BUXIFOLIA*

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Key Word Index—*Discaria longispina*; *Scutia buxifolia*; Rhamnaceae; peptide alkaloids; adouetine Y′; frangufoline; scutianine B and C.

Abstract—The minor alkaloids of *Discaria longispina* have been shown to be the known compounds adouetine Y′ and frangufoline. Scutianine B and the new alkaloid scutianine C have been isolated from *Scutia buxifolia*.

INTRODUCTION

RECENTLY five alkaloids have been isolated from the roots of the Argentinian plant *Discaria longispina* (Hook and Arn.) Miers.[1] The major products were shown to be frangulanine and the new bases discarine A and B whose structures were established.[1] The minor products now have been identified as the known alkaloids adouetine Y′ (**1**)[2,3] and frangufoline (**2**)[3,4] on the basis of mass spectral analysis and the isolation of isoleucine and leucine, respectively, on hydrolysis. This constitutes the first discovery of adouetine Y′ in a rhamnaceous plant.

The roots of *Scutia buxifolia* Reiss. have yielded recently the alkaloids scutianine A and scutianine B (**3**) and some indication of the presence of other bases.[5,6] In an attempt to isolate the latter components of the plant, common to the Mesopotamian region of Argentina, the roots were reinvestigated. Surprisingly, scutianine A was not detected and a new alkaloid, scutianine C (**4**), was the major basic constituent. However scutianine A proved to be the major alkaloid of a Brazilian *S. buxifolia*.[7] The structure analysis of scutianine C (**4**) is presented herewith.

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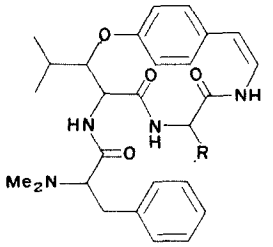
[7] Collected at Julio Castilhos, Rio Grande do Sul Brazil; the alkaloid was identified by m.p., high resolution mass-spectral analysis and characteristic acid hydrolysis products.

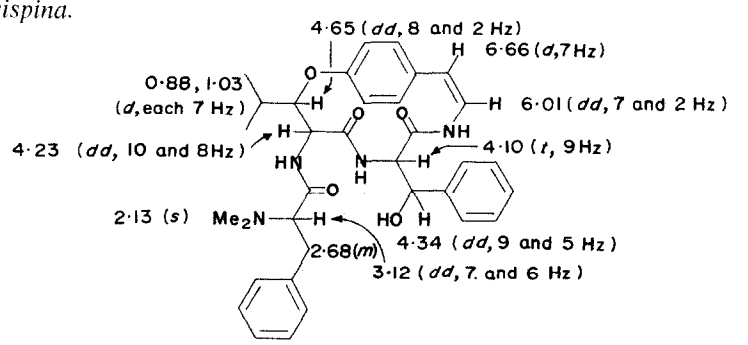


(1) R = *sec*-Bu
(2) R = *i*-Bu
(3) R = $CH_2C_6H_5$
(4) R = $CH(OH)C_6H_5$

RESULTS AND DISCUSSION

Two crystalline alkaloids, identified as scutianine B (**3**) and the new substance scutianine C (**4**), m.p. 202–204°, were isolated by preparative TLC from extracts of *S. buxifolia* roots. Scutianine C is a $C_{34}H_{40}N_4O_5$ compound whose NH and COIR bands revealed it to possess peptide linkages, whose 3300 cm^{-1} strong IR band and 5·48 ppm (*d*, 1, *J* 5 Hz) PMR signal (disappearing on the addition of 1 drop of D_2O) showed the presence of an OH group and whose low-resolution MS exhibited among its few peaks those characteristic of *N*,*N*-dimethylphenylalanine, *p*-hydroxystyrylamine and β-phenylserine[8] units, i.e. *m/e* 148, 135 and the group of 105, 106 and 107, respectively.

Inspection of the low-resolution mass spectra of dihydroscutianine C, the product of hydrogenation of **4**, and of oxodihydroscutianine C, the product of chromic acid oxidation[9] of dihydro-**4**, and interpretation of their fragmentation patterns according to previous experience[1,5,6,8,10] indicated structure **4** for scutianine C. In agreement with this structure hydrolysis of dihydroscutianine C in acid yielded *p*-tyramine, β-phenylserine and β-phenylnaphthalene, a known product of acid decomposition of β-phenylserine,[11] acid hydrolysis of oxodihydroscutianine C produced *inter alia* ω-aminoacetophenone[8] and base hydrolysis of oxodihydroscutianine C led to benzoic acid.[8] Exhaustive analysis of the 220 M Hz PMR spectrum of a DMSO solution of scutianine C (**4**) (cf. **5**)[12] confirmed the structural assignment. The β-hydroxyleucine moiety of the alkaloid possesses the same *erythro* configuration assigned earlier[13] to this subunit of the major bases of *Discaria longispina*.



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EXPERIMENTAL

Adouetine Y'. The solid (170 mg), R_f 0·83 on the preparative TLC of an isolation mixture of *D. longispina* alkaloids,[1] was chromatographed on silica gel plates with $CHCl_3$–Et_2O–MeOH (90:30:2).[3] Crystallization of the solid residue (80 mg), R_f 0·35, from $CHCl_3$–Et_2O yielded 1; m.p. 295–297°; homogeneous on TLC (silica gel, 3 solvents); $[\alpha]_D$–390° (*ca* 0·1, $CHCl_3$). IR cm^{-1} 3330, 1640; no UV absorption. MS: (M^+) 534, 491, 443 → 398 (*m** 357·8), 387, 385, 303, 274, 210, 195, 190, 189, 182, 165, 148 (base ion peak) → 133 (*m** 119·5), 135, 97, 86. Hydrolysis of dihydro-**1** in aqueous acid[1] yielded isoleucine, *N,N*-dimethylphenylalanine and *p*-tyramine.

Frangufoline. The solid (100 mg), R_f 0·88 on the preparative TLC of an isolation mixture of *D. longispina* alkaloids,[1] was chromatographed as above. Crystallization of the solid residue (60 mg), R_f 0·44, from $CHCl_3$–MeOH–Et_2O yielded **2**, m.p. 234–236°; homogenous on TLC (silica gel, 3 solvents). IR cm^{-1} 3350, 1645; no UV absorption. MS: (M^+) 534, 491, 443 → 398 (*m** 357·8), 387, 385, 344, 303, 274, 210, 195, 190, 189, 182, 165, 148 (base ion peak) → 133 (*m** 119·5), 135, 97, 86. On acid hydrolysis leucine was identified. Frangufoline was identical (TLC) with an authentic sample.

Extraction of Scutia buxifolia. The plant material was collected at Federal (Provincia de Entre Rios, Argentina) in May 1972. The powdered roots (7 kg) were extracted with EtOH for 24 hr × 5 at the end of which period the solvent was drained. Evaporation of the combined extracts yielded an oily residue whose suspension in H_2O was acidified to pH 1·5 with 2 N HCl and extracted exhaustively with Et_2O. The remaining aqueous phase was basified to pH 9 with NH_3 and extracted with Et_2O. The extract was washed with H_2O, dried (Na_2SO_4) and evaporated to dryness yielding a solid residue (5·81 g).

Isolation of the alkaloids. The alkaloid mixture was separated on preparative TLC with silica gel GF 254 (0·75 mm) by EtOAc–Et_2O–$CHCl_3$ (10:10:1). The developed plates showed three major bands of R_f 0·67, 0·55 and 0·45. Each band was scraped off the plate and eluted with CH_2Cl_2–MeOH (97:3).

Scutianine B. The solid (692 mg) of R_f 0·67 was purified by a second TLC with silica gel GF 254 (0·75 mm) and $CHCl_3$–MeOH (19:1). The developed plates exhibited three bands of R_f 0·75, 0·50 and 0·27 the first of which gave 152 mg of a solid. Crystallization of the latter from $CHCl_3$–Et_2O yielded **3**; m.p. 235–236°; homogeneous on TLC (silica gel, 5 solvents); $[\alpha]_D$–308° (*ca* 0·1, $CHCl_3$). IR cm^{-1} 3300, 1650; no UV absorption. MS: (M^+) 568, 477, 190, 189, 148 (base ion peak), 135, 120, 91; identical with the published mass spectrum.[6] Hydrolysis of dihydro-**3** in aqueous acid[1] yielded *p*-tyramine and phenylalanine.

Scutianine C. The solid (661 mg) of R_f 0·55 was submitted to second preparative TLC with silica gel GF 254 (0·75 mm) and $CHCl_3$–MeOH (19:1). The major band, R_f 0·50, was scraped off the plate and eluted with CH_2Cl_2–MeOH (97:3). Crystallization of the resultant solid (344 mg) from Et_2O–MeOH gave **4**; m.p. 202–204°; homogeneous on TLC (silica gel, 5 solvents); $[\alpha]_D$ −188° (*ca* 0·15, $CHCl_3$). IR cm^{-1} 3820, 1650; no UV absorption.

Dihydroscutianine C. The hydrogenation of scutianine C under conditions described for the discarines[1] yielded dihydro-**4**; m.p. 206–207°; homogeneous on TLC (silica gel, 5 solvents). UV shoulder 230 nm (log ε 3·97), max 275 (2·94). MS (low resolution): (M^+) 586, 495, 480, 192, 175, 148 (base ion peak), 120, 107, 106, 105, 91. MS (high resolution): (M^+) of low intensity; $C_{34}H_{42}N_4O_5 = C_{27}H_{35}N_4O_5$ (M^+ 495·2605, required 495·2607) + C_7H_7 (M^+ 91·0547, required 91·0547). Hydrolysis of dihydro-**4** was performed in a sealed tube at 110° with 6 N HCl for 12 hr. The soln was extracted exhaustively with an equal vol. of C_6H_6.[8] The extract was washed with H_2O, dried and evaporated and the residue identified as β-phenylnaphthalene on comparison with an authentic sample by GLC (OV-1 column). The aq. acidic soln was concentrated in a desiccator over solid KOH and a part of the residue used to identify *p*-tyramine by a previously described procedure.[1] The remaining part was treated by the GLC procedure of Moss *et al.*[14] (OV-1 column) and β-phenylserine mostly in the *threo* form identified as its N-TFA *n*-propyl ester derivative by comparison with an authentic sample.

Oxodihydroscutianine C. A solution of dihydro-**4** (10 mg) and oxidizing agent (0·03 ml) (prepared from 26·7 g CrO_3 and 23 ml H_2SO_4 in 100 ml H_2O) in acetone (3·4 ml) was left at room temp. for 10 min.[9] Water was added and the mixture made alkaline with NH_3 and extracted with Et_2O. The extract was washed (H_2O), dried (Na_2SO_4) and evaporated. Crystallization of the residue (9·1 mg) from absolute EtOH–EtOAc yielded oxodihydro-**4**; m.p. 253–254°; homogeneous on TLC (silica gel, 3 solvents). MS: (M^+) 584, 493, 421, 330, 195, 192, 175, 167, 163, 148 (base ion peak), 120, 107, 105, 77. Acid hydrolysis was performed as on dihydro-**4** above. A mixture of the residue from the aqueous acid phase and TFA (0·5 ml) was heated at 100° for 10 min and thereafter the excess TFA removed by a stream of N_2. GLC analysis of the residue (OV-1 column) led to the identification of ω-aminoacetophenone, as N-TFA derivative, by comparison with an authentic specimen. Alkaline hydrolysis of oxodihydro-**4** (4 mg) in MeOH (4 ml) according to the procedure of Tschesche,[8] esterification of the acidic product with CH_2N_2 and submittal of the resultant ester to GLC showed the presence of methyl benzoate identified by comparison with an authentic sample.

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Reference compounds. Leucine, isoleucine, phenylalanine, *p*-tyramine and *threo*-β-phenylserine were commerical samples. The preparation of β-phenylnaphthalene, *N*,*N*-dimethylphenylalanine and ω-aminoacetophenone followed the procedures of Bettzieche,[11] Bowman[15] and Sheehan,[16] respectively.

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