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PEPTIDE ALKALOIDS OF *SCUTIA BUXIFOLIA*

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Key Word Index—*Scutia buxifolia*, Rhamnaceae, peptide alkaloids, amino acid configurations

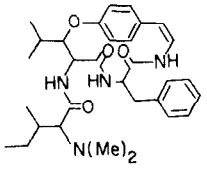
Abstract—Two new components, the peptide alkaloid scutianine D and scutianene C have been isolated from *Scutia buxifolia* and their structures elucidated The configuration of some of the asymmetric centers of scutianine A has been determined by gas chromatography

INTRODUCTION

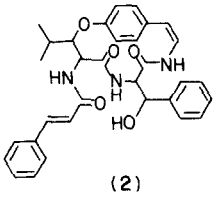
FROM the root extract of *Scutia buxifolia* Reiss. from Brazil, Tschesche *et al.*[1,2] have isolated scutianine A and scutianine B. Recently we have described the isolation of scutianine C, the major alkaloid, and scutianine B from plants collected in Argentina.[3] We report in this communication the isolation and structure elucidation of scutianine D (**1**), one of the minor alkaloids and scutianene C (**2**) a neutral component which was detected in only one of the plants and also some of the stereochemical features of scutianine A (**3**), determined using only minute quantities of alkaloid

RESULTS AND DISCUSSION

By preparative TLC two crystalline products were obtained from the root extract of *Scutia buxifolia* Reiss., scutianine D (**1**) and scutianene C (**2**).



(1)



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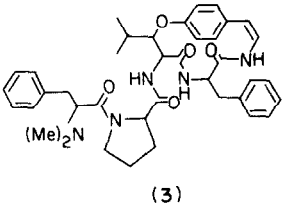


(3)

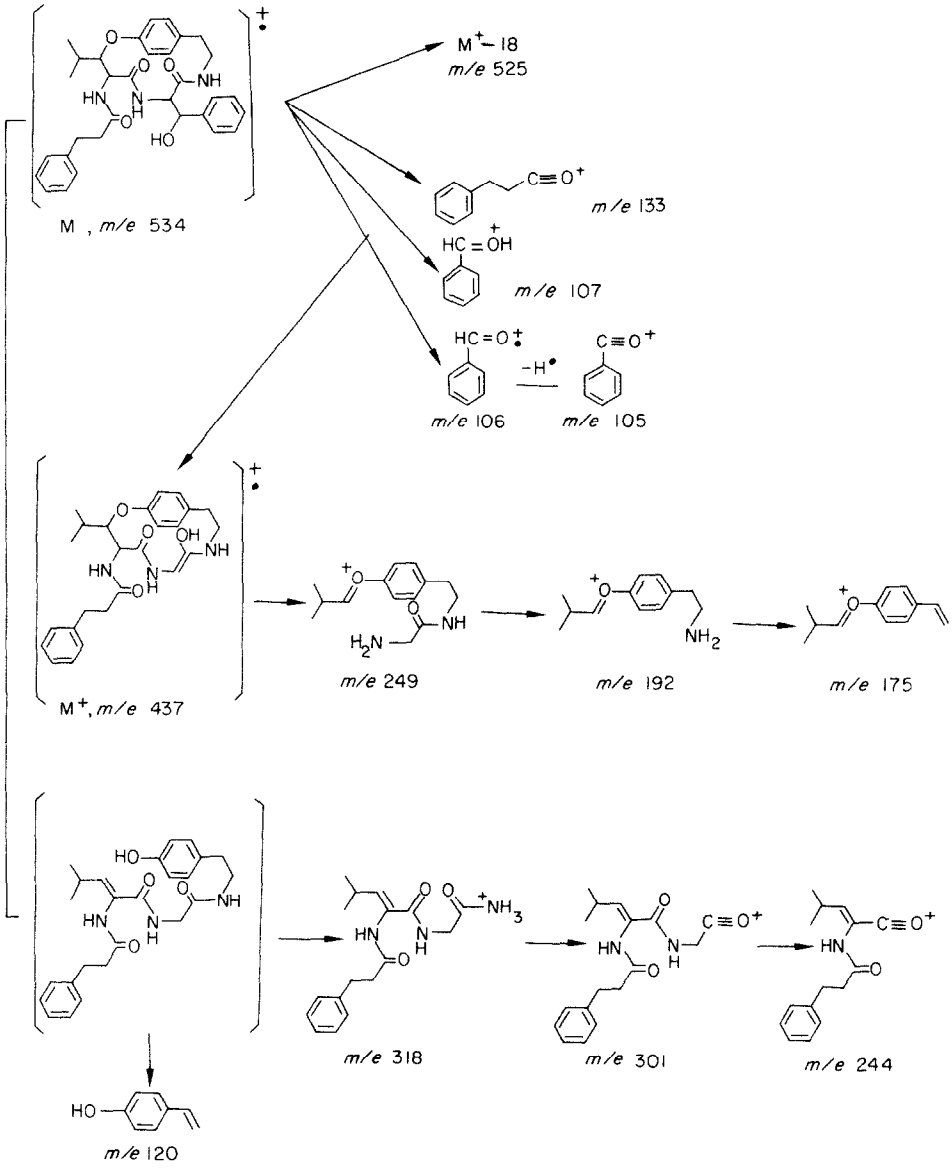
Scutianine D, m.p. 255–256°; $C_{31}H_{42}N_4O_4$, showed the IR bands characteristic of NH and CO groups and the typical UV absorption of peptide alkaloids with the 14-membered

[1] TSCHESCHE, R., WELTERS, R and FEHLHABER, H-W (1967) *Chem. Ber* **100,** 323

[2] TSCHESCHE, R., AMMERMANN, E and FELHABER, H-W (1971) *Tetrahedron Letters*, 4405

[3] MERKUZA, V M., GONZALEZ SIERRA, M., MASCARETTI, O A., RUVEDA, E A., CHANG, C-J., WENKERT, E (1974) *Phytochemistry*, In press

cyclic system. Interpretation of the low resolution mass spectrum[4] together with the information obtained by inspection of the aliphatic region of the 220 MHz PMR spectrum in d_6-DMSO and d_5-pyridine solution, according to previous experience,[5] indicated structure **1** for scutianine D.



SCHEME 1 *

* The assignment of all fragment ions, shown in this Scheme, was confirmed by extensive high resolution mass measurements

[4] FELHABER, H -W , *Z analyt Chem* (1968) **235,** 91

[5] CHANG, C -J , HAGAMAN, E W WENKERT, E , GONZALEZ SIERRA, M MASCARELLI, O A MERKLEN, V M and RUVEDA, E A (1974) *Phytochemistry* In press

Scutianene C, m.p 232–234°, was shown to be a $C_{32}H_{33}N_3O_5$ compound whose infrared CO and NH bands and UV spectrum revealed it to posses peptide bonds and the cinnamamide chromophore, respectively. The low resolution MS exhibited among its few peaks those characteristic of *p*-alkoxystyrylamine, *p*-hydroxystyrylamine, cinnamic acid derivatives and β-phenylserine units, i.e. *m/e* 190, 189, 135, 103, 105 and 106 respectively. These facts together with the analysis of the high resolution MS of tetrahydroscutianene C (Scheme 1)[1] were consonant with structure **2** for scutianene C. In agreement with this, hydrolysis of tetrahydro-**2** in acid yielded *p*-tyramine, *threo*-β-hydroxyleucine, β-phenylpropionic acid, *threo*-β-phenylserine and β-phenylnaphthalene, produced by acid decomposition of β-phenylserine [6]

Since scutianene C could be an artifact produced by Hofmann degradation[7] during the extraction procedure, it will be interesting to look for quaternary peptide alkaloids, which have not so far been in the literature.

Although the configurations of the asymmetric centers of several peptide alkaloids have been determined by total acid hydrolysis and examination of the optical rotation of the isolated dipeptides or free amino acids[8] (except of the β-hydroxyamino acid unit, which requires a special procedure of degradation[9,10]), these determinations have not been carried out on the microscale. Procedures for the precise determination of the D and L isomers of amino acids based on the separation of the diastereoisomeric dipeptides or esters and on enzymatic action have been reported;[11–15] the successful use of GLC for this purpose prompted us to adopt it, since *N,N*-dimethylamino acids could, in principle, be resolved in this way Scutianine A (**3**), a representative member among the peptide alkaloids, was selected for these determinations. The approach was the coupling of racemic phenylalanine and proline methyl esters with *N*-trifluoracetyl-L-prolyl chloride by the procedure of Halpern and Westley[16] and the separation by GLC of the resulting diastereoisomeric dipeptide derivatives The dipeptides from the L-amino acid methyl esters were prepared separately and their retention times determined When the mixtures of amino acids obtained by acid hydrolysis of dihydroscutianine A was submitted to the reactions described above and analyzed by GLC, the presence of L-phenylalanine and L-proline could be established.

A similar approach was used for the *N,N*-dimethylamino acid residue. Racemic *N,N*-dimethylphenylalanine was converted, by the mixed anhydride method, into a diastereoisomeric mixture of *N,N*-dimethyl-L-phenylalanyl-L-leucine and *N,N*-dimethyl-D-phenylalanyl-L-leucine methyl esters which was resolved by GLC, optically pure *N,N*-dimethylphenylalanine was used to determine the retention time of the L,L-dipeptide derivatives

[6] BETTZIECHE, F (1925) *Z Physiol Chem*, **150**, 177
[7] BOULVIN, G, OTTINGER, R, PAIS, M and CHIURDOGLU, G (1969) *Bull Soc Chim Belges*, **78**, 583
[8] PAIS, M and JARREAU F-X (1971) in *Chemistry and Biochemistry of Amino Acids, Peptides and Proteins* (WEINSTEIN, B ed) p 127 Marcel Dekker New York
[9] MARCHAND, J, PAIS M and JARREAU, F-X *Bull Soc Chim Fr*, 1971, 3742, MARCHAND, J, ROCCHIOCCIOLI, F, PAIS, M and JARREAU, F-X (1972) *Bull Soc Chim Fr*, 4699
[10] GONZALEZ SIERRA, M, MASCARETTI, O A DIAZ, F J, RUVEDA, E A, CHANG, C-J, HAGAMAN E W and WENKERT, E (1972) *J Chem Soc, Chem Commun* 915
[11] CLAYTON, D W, FARRINGTON, J A, KENNER, G W and TURNER, J M (1957) *J Chem Soc* 1398
[12] WEYGAND, F, PROX, A, SCHMIDHAMMER, L and KONIG, W (1963) *Angew Chem* (Intern edn) **2**, 183
[13] MANNING, J M and MOORE, S (1968) *J Biol Chem* **243**, 5591
[14] WESTLEY, J W (1971) in *Chemistry and Biochemistry of Amino Acids, Peptides and Proteins* (WEINSTEIN, B ed) p 1 Marcel Dekker, New York
[15] GRUNSTEIN, J P and WINITZ, M (1961) *Chemistry of the Amino Acids*, p 1735 John Wiley, New York
[16] HALPERN, B and WESTLEY J W (1965) *Biochem Biophys Res Commun* **19**, 361

The fact that *N,N*-dimethylamino acids do not afford methyl esters under usual conditions, probably due to steric hindrance, offered a convenient means for the analysis of such a unit. For this purpose the hydrolysate of dihydroscutianine A was treated with dimethyl sulfite in MeOH saturated with HCl, the reaction mixture, in which the only carboxylic component is the *N,N*-dimethylamino acid, was purified through an exchange resin and converted into *N,N*-dimethylphenylalanylleucine methyl ester under usual conditions of the mixed anhydride method of formation of peptide bonds. GLC analysis of this product showed the presence of *N,N*-dimethyl-L-phenylalanine in the hydrolysate of dihydroscutianine A. This procedure has the advantages of rapidity and sensitivity and supplements the methods for the determination of the optical purity of amino acids based on GLC.

EXPERIMENTAL

Scutianine B. Crystallization of the solid residue (140 mg), R_f 0.50 of the second TLC of Scutia buxifolia alkaloids,[3] from $CHCl_3$–MeOH, yielded **1**, m.p. 255–256°, homogeneous on TLC (silica gel, 5 solvents). $[\alpha]_D$ −210° (*c*,*a* 0.5, $CHCl_3$). IR cm^{-1}: 3280, 1650; no UV absorption maxima or minima. Mass (low resolution): (M^+) 534 (15 eV), 477, 378, 308, 244, 216, 195, 190, 167, 135, 114 (base ion peak), 97 (70 eV). Mass (high resolution): (M^+) of low intensity, $C_{31}H_{42}N_4O_4 = C_{27}H_{33}N_4O_4$ (M^+ 477.2494, required 477.2499) + C_4H_9O (M^+ 57.0693, required 57.0704). PMR (d_6-DMSO): δ 0.53 (3H, *d*, 7); 0.75 (3H, *t*, 7); 0.90 (3H, *d*, 7); 1.09 (3H, *d*, 7); (d_5-pyridine): δ 0.84 (3H, *t*, 7); 0.94 (3H, *d*, 7); 1.22 (3H, *d*, 7); 1.29 (3H, *d*, 7). On acid hydrolysis phenylalanine was identified.

Extraction of Scutia buxifolia. The plant material was collected at Federal (Provincia de Entre Rios, Argentina) in Sept. 1970. The powdered roots (3.7 Kg) were extracted[3] yielding a solid residue (3.6 g).

Isolation of the alkaloids. The alkaloid mixture was separated on preparative TLC in the same conditions as described before; the developed plates showed apart of the three major bands of R_f 0.67, 0.55 and 0.45, from which scutianine B, C and D were isolated; a fourth one of R_f 0.30.

Scutianine C. Crystallization of the resultant solid (106 mg) of the R_f 0.30 band from MeOH gave **2**, m.p. 232–234°, homogeneous on TLC (silica gel, 5 solvents). $[\alpha]_D$ +203° (*c*,*a* 0.12, $CHCl_3$–MeOH (3:2)). IR cm^{-1}: 3410, 3380–3350, 1670, 1630. UV: 207 nm (log ε 4.43); 218 (4.39); 275 (4.32). PMR (d_6-DMSO): δ 0.88 (3H, *d*, 7); 1.15 (3H, *d*, 7); 1.83 (1H, *m*); 4.00 (1H, *t*, 9); 4.10–4.15 (3H, *m*); 5.51 (1H, *d*, 4.5, OH); 5.94 (1H, *d*, 7); 6.43 (1H, *d*, 16); 6.63 (1H, *dd*, 7.2); 6.57–7.13 (9H, *m*); 7.23 (1H, *d*, 9, NH); 7.41 (2H, *t*, 7); 7.44 (1H, *t*, 7); 7.69 (2H, *d*, 7); 8.01 (1H, *d*, 10, NH); and 8.52 (1H, *s*, NH). Mass: *m/e* M$^+$ 190, 189, 135, 131, 106, 105, 103, 77.

*Tetrahydroscutianamine C. The hydrogenation of scutianine C under the conditions described for peptide alkaloids yielded tetrahydro-***2**, m.p. 240–242°. (Found: C, 70.63; H, 6.81; N, 7.81. Calc. for $C_{32}H_{36}N_2O_{6}$: C, 70.70; H, 6.86; N, 7.73%). Mass (high resolution): *m/e* of low intensity: $C_{32}H_{36}N_2O_6$ (M^+ − H_2O): 525.2632, required 525.2628. *Hydrolysis of tetrahydro-***2** was performed in a sealed tube at 110° with 6N HCl for 12 hr. The soln was extracted exhaustively with an eq. vol. of C_6H_6. The extract was washed with H_2O, dried and evaporated; the residue in Et_2O was treated with an ethereal CHN_2 and submitted of the resultant product to GLC (OV-1 column) showed the presence of methyl-β-phenylpropionate and β-phenylnaphthalene identified by comparison with authentic samples. The aq. acid solution was concn. in a desiccator over solid KOH and the residue treated by the GLC procedure of Moss *et al.*[17] *threo*-β-hydroxyleucine and *threo*-β-phenylserine were identified as their N-TFA *n*-propyl ester derivatives and β-cyclamine as N-TFA derivative by comparison with authentic samples.

N-TFA L-t-prolyl-L-phenylalanine methyl ester. L-phenylalanine by treatment with dimethyl sulfite in MeOH saturated with HCl[18] afforded the corresponding methyl ester hydrochloride which by coupling with N-TFA-L-prolyl chloride[19] yielded N-TFA-L-prolyl-L-phenylalanine methyl ester, m.p. 133–134° (Found: N, 7.60. Calc. for $C_{17}H_{19}N_2O_4F_3$: N, 7.53%). Mass (high resolution): $C_{17}H_{19}N_2O_4F_3$ (M^+) 372.1275, required 372.1296, low resolution: (M^+) 372, 313, 210, 194, 178, 166 (base ion peak), 162, 131.

N-TFA-L-prolyl-L-phenylalanine and N-TFA-L-prolyl-DL-phenylalanine methyl esters. By using racemic phenylalanine and following the technique described above a diastereoisomeric mixture of dipeptides derivatives was obtained and submitted to GLC analysis.

N-TFA-L-prolyl-L-proline methyl ester. The procedure used to prepare this derivative was the same as described above for N-TFA-L-prolyl-L-phenylalanine methyl ester. The product is an oil which by sublimation yields a low melting solid. Mass: M$^+$ 322 (calc. for $C_{13}H_{17}N_2O_4F_3$ 322), 263, 194, 166 (base ion peak), 128, 97, 96, 70, 69, 43, 42, 41.

N,N-dimethyl-L-phenylalanyl-L-leucine methyl ester. N,N-dimethyl-L-phenylalanine in $CHCl_3$–CH_2Cl_2 (1:1) was treated with ethyl chloroformate and NEt_3 at 0°, after being at room temp. for 15 min, L-leucine methyl ester as free base was added and stirred for another 15 min at room temperature followed by 15 min reflux. The

[17] MOSS, C. W., LAMBERT, M. A. and DIAZ, F. I. (1971) *J. Chromatog.* **60**, 134.
[18] CRUICKSHANK, P. A. and SHUTTLEWORTH, I. C. (1964) *Analyt. Chem.* **36**, 1191.

organic solution was then thoroughly extracted with 0·5 N HCl, the combined aqueous extracts were washed twice with Et_2O and then were made alkaline at 0° with solid K_2CO_3 and extracted with Et_2O. The combined Et_2O extracts were washed, dried and evaporated. The product, a low melting solid, was purified by sublimation. Mass M^+ 320 (calc for $C_{18}H_{28}N_2O_3$, 320), 289, 261, 229 (base ion peak), 169, 148, 91, 77, 57.

*N,N-dimethyl-*L*-phenylalanyl-*L*-leucine and N,N-dimethyl-*D*-phenylanyl-*L*-leucine methyl esters.* By using racemic *N,N*-dimethylphenylalanine and following the technique described above, a diastereoisomeric mixture of *N,N*-dimethylamino dipeptides was obtained and submitted to GLC analysis.

GLC analysis of the amino acids obtained from scutianine A. The mixture of amino acids obtained by acid hydrolysis of dihydroscutianine A (10 mg) under usual conditions was treated with dimethyl sulfite in MeOH saturated with HCl and divided in two fractions, one of them was treated with *N*-TFA-L-prolyl chloride and the dipeptide derivatives submitted to GLC analysis. The second fraction was purified through and exchange resin (Dowex 50 X8, H^+) and treated as described above for *N,N*-dimethyl-phenylalanine and submitted to GLC analysis.

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