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CYCLOPEPTIDE ALKALOIDS OF *SCUTIA BUXIFOLIA*

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Key Word Index—*Scutia buxifolia*; Rhamnaceae; peptide alkaloids; scutianines-K and -L; chiral phase gas chromatography.

Abstract—Two new peptide alkaloids, scutianines-K and -L were isolated from *Scutia buxifolia*, a plant growing in Brazil, Argentina and Uruguay. Their structures have been determined on the basis of spectroscopic studies. The stereochemistry of the *N*,*N*-dimethyl amino acid side-chain and the ring amino acid residues in both alkaloids have been assigned by gas chromatography employing modified cyclodextrins as chiral stationary phases. © 1997 Elsevier Science Ltd

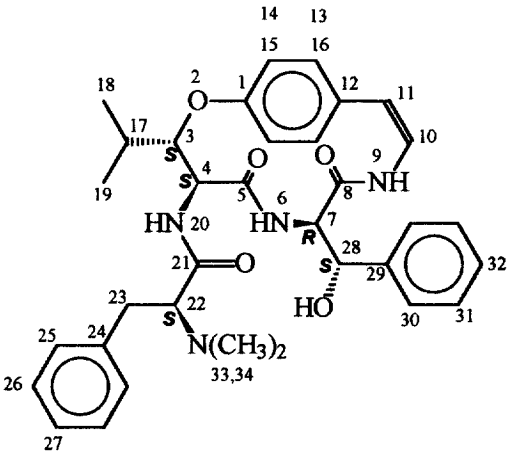
INTRODUCTION

In continuation of our chemical studies on *Scutia buxifolia* [1, 2], we now report on the isolation and structural elucidation of two new peptide alkaloids (**1** and **2**) found together with six known peptide alkaloids [2]. Elucidation of the structures of **1** and **2** was largely achieved through the use of a combination of FAB mass, ^{1}H and ^{13}C NMR spectroscopy and some chemical transformations.

RESULTS AND DISCUSSION

Scutianine-K (**1**) was obtained as colourless crystalline material. Positive ion FAB-mass spectroscopy gave a quasi-molecular ion peak $[M+H]^+$ at *m/z* 585, corresponding to $C_{34}H_{40}N_4O_5$. The base peak appeared at *m/z* 148, corresponding to $C_{10}H_{14}N$, suggesting the presence of a *N*,*N*-dimethyl phenylalanine unit. The peaks at *m/z* 135 (C_8H_9NO) and 190 ($C_{12}H_{16}NO$) indicate the presence of styrylamine and hydroxyleucine units, respectively. The fragment ions at *m/z* 107 (C_7H_7O), 106 (C_7H_6O) and 105 (C_7H_5O) confirmed the presence of a *β*-phenylserine unit in **1**. The ^{1}H and ^{13}C NMR spectral data of scutianine-K strongly suggest it to have a structure similar to those of scutianines D, E [3] and G [4].

The ^{1}H NMR spectrum ($CDCl_3$, 400 MHz) of **1** showed two sets of three doublets. The first set at *δ* 0.96 ($J_{17,19}$ = 6.6 Hz) and 1.23 ($J_{17,18}$ = 6.6 Hz) was assigned to the C-19 and C-18 methyl protons, respectively. The double doublet at *δ* 4.55 ($J_{6,7}$ = 8.3 Hz, $J_{7,28}$ = 1.2 Hz) was assigned to the C-7 methine proton. The C-3 and C-4 methine protons appeared as double doublets at *δ* 4.94 ($J_{3,17}$ = 2.0 Hz; $J_{3,4}$ = 7.0 Hz) and 4.46 ($J_{3,4}$ = 7.0 Hz; $J_{4,20}$ = 10.0 Hz), respectively. The C-22 methine proton appeared as a double doublet at *δ* 2.63 ($J_{22,23\alpha}$ = 6.7 Hz; $J_{22,23\beta}$ = 5.7 Hz). The C-11 olefinic proton appeared as a doublet at *δ* 6.73 ($J_{11,10}$ = 7.6 Hz), whereas the other olefinic proton at C-10 showed a double-doublet at *δ* 6.68 ($J_{10,11}$ = 7.6 Hz; $J_{10,9}$ = 9.0 Hz).



(1)

The NMR spectrum also permitted the assignment of all amide protons at *δ* 6.51 (NH-6), 6.45 (NH-9) and 7.52 (NH-20), as doublets with *J* = 8.3, 7.6 and 10.0 Hz, respectively. A singlet at *δ* 2.09 was assigned

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Table 1. ^1H and ^{13}C NMR assignments for scutianine-K (**1**) in $CDCl_3$ at 400/100 MHz

Position	Assignment	δ ^1H* J(Hz)	δ ^{13}C†
1	-C=(Ar)		156.0
3	-CH-O	4.94 (*dd*) $J_{3,4} = 7.0$, $J_{3,17} = 2.0$	82.1
4	-NH-CH-CO	4.46 (*dd*) $J_{3,4} = 7.0$, $J_{4,20} = 10.0$	55.0
5	-CO-		171.9
6	-OC-NH-CH-	6.51 (*d*), $J_{6,7} = 8.3$	
7	-NH-CH-CO-	4.55 (*dd*) $J_{6,7} = 8.3$, $J_{7,23} = 1.2$	58.0
8	-CO-		167.0
9	-CO-NH-CH-	6.45 (*d*) $J_{9,10} = 7.6$	
10	-NH-CH=CH-	6.68 (*dd*) $J_{9,10} = 9.0$, $J_{10,11} = 7.6$	122.8
11	-CH=CH-Ar-	6.73 (*d*), $J_{10,11} = 7.6$	118.5
12	-C-(Ar)		140.5
13–16	-CH=(Ar)	7.0–7.4‡	120.0–132.0‡
17	Me-CH-Me	1.84 (*m*)	29.0
18	Me-CH	1.23 (*d*), $J_{17,18} = 6.6$	15.0
19	Me-CH	0.96 (*d*), $J_{17,19} = 6.6$	20.5
20	-CH-NH-CO	7.52 (*d*), $J_{4,20} = 10.0$	
21	-CO-		172.0
22	Me_2N-CH-CO-	2.63 (*dd*) $J_{22,23\alpha} = 6.7$, $J_{22,23\beta} = 5.7$	68.5
23	$-CH_2$-Ph	2.71 (*dd*, α), 3.12 (*dd*, β) $J_{22,23\alpha} = 6.7$, $J_{22,23\beta} = 5.7$, $J_{23\alpha,23\beta} = 14.0$	30.9
24	-C=(Ar)		139.6
25–27	-CH-(Ar)	7.0–7.4‡	120.0–132.0‡
28	-CH(OH)-Ph	5.39 (*d*), $J_{7,28} = 1.2$	71.0
29	-C-(Ar)		140.4
30–32	-CH=(Ar)	7.0–7.4‡	120.0–132.0‡
33–34	$-NMe_2$	2.09 (*s*)	42.0

* Assignments confirmed by ^1H-^1H COSY and NOESY.
† Assignments confirmed by DEPT and HETCOR.
‡ Peaks occur in the given range, no assignment.

to the protons of the *N,N*-dimethyl group (H-33 and H-34) of the *N,N*-dimethyl phenylalanine unit.

The assignments of the protons and couplings presented in Table 1 were further confirmed by 2D NMR experiments (COSY and NOESY) [5], which showed prominent cross-peaks at the expected positions; Fig. 1 shows NOE-relationships for **1**. H-3 correlates with H-17 and H-18, but not H-4, which in turn showed correlation with H-19 and NH-6, indicating the stereochemistry at C-3/C-4 as described previously [6]. A cross-peak was observed between H-7 and NH-6, and between H-7 and H-28, indicating a 7-*R*,28-*S*-stereochemistry, which was finally proved by enantioselective gas chromatography (see below).

The ^{13}C NMR spectrum (100 MHz, $CDCl_3$) of scutianine-K also provided strong support for the proposed structure **1**. The data (Table 1) were interpreted on the basis of DEPT and HETCOR experiments, together with previous assignments for similar compounds [1], which allowed the assignment of all carbons present.

Compound **2**, designated as scutianine-L, was also

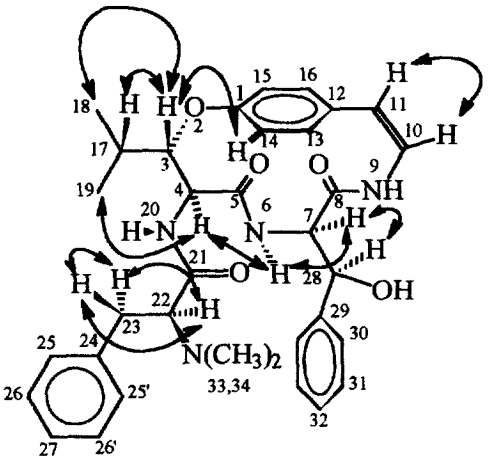
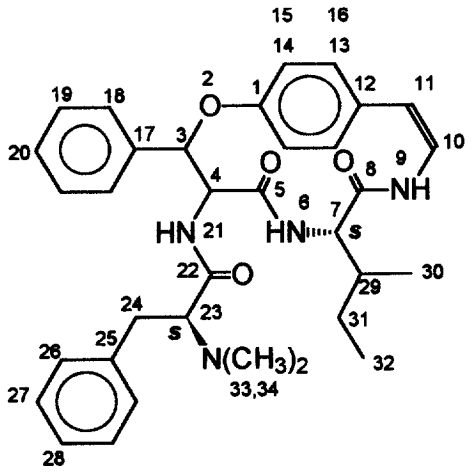


Fig. 1. NOE-correlations (from NOESY) for scutianine-K (**1**).

obtained as colourless crystalline material. It was determined to have a molecular formula of $C_{34}H_{40}N_4O_4$ by analysis of mass spectral data. Com-



(2)

pound **2** showed a $[M+H]^+$ peak at *m/z* 569 and the fragment base peak at *m/z* 148 corresponding to $C_{10}H_{14}N$, again indicating the presence of a *N,N*-dimethyl phenylalanine unit in the positive FAB-mass spectra.

The ^1H NMR spectrum ($CDCl_3$, 400 MHz) of **2** showed resonances at δ 0.90 (3H, *t*, 32-Me) and 0.98 (3H, *d*, 30-Me). In the COSY-(^1H-^1H) spectrum, the triplet at δ 0.90 showed a cross-peak with the signals at δ 1.40 (2H, *m*), which correspond to H-31, whereas the doublets at δ 0.95 showed a cross-peak with the signals at δ 2.30 (1H, *m*), which corresponds to H-29. The latter had a cross-peak with H-7 (δ 4.55) and this with H-6 (NH) at δ 6.30. This spin-system confirms isoleucine as the α-amino acid of the ring.

β-Phenylserine, which is the hydroxylated amino acid of the macrocyclic ring, was identified from cross-peaks between H-3, H-4 and H-21 (NH). H-3 resonated at δ 6.38 (1H, *d*, $J_{3,4}$ = 14.6 Hz) and showed cross-peaks with H-4 which resonated at δ 7.35 (1H, *dd*, $J_{3,4}$ = 14.6 and $J_{4,21}$ = 10.2 Hz). H-4 exhibited another cross-peak with H-21 (NH), which resonated at δ 9.08. The vicinal coupling constant of *ca* 14 Hz (ϕ *ca* 180°) of the methine proton of the β-phenylserine (H-3 and H-4) indicates an *erythro*-configuration for this residue [7].

The side-chain amino acid *N,N*-dimethyl phenylalanine was characterized by the occurrence of a double doublet at δ 3.62, which was assigned to the H-23 methinic proton. It showed cross-peaks with H-24α at 2.94 ($J_{23,24\alpha}$ = 4.6 Hz) and with H-24β at 3.26 ppm ($J_{23,24\beta}$ = 8.2 Hz). The diastereotopic methylene protons H-24, in addition to these couplings, showed a geminal one ($J_{24\alpha,24\beta}$ = 14.2 Hz).

The C-10 and C-11 protons of the styrylamine moiety and the amidic proton (NH-6 and NH-9) were difficult to assign, due to superimposition of these resonances with the aromatic protons (Table 2). The ^{13}C NMR spectral data (Table 2) of scutianine-L, however, are in good agreement with structure **2**.

The absolute stereochemistry of the side-chain *N,N*-dimethyl phenylalanines and of the C-7 amino acids of alkaloids **1** and **2** was determined by chiral phase gas chromatography (CPGC) using 3-pentyl-2,6-dimethyl-β-cyclodextrin (3-Pe-2,6-Me-β-CD) and 3-butyl-2,6-pentyl-γ-cyclodextrin (3-Bu-2,6-Pe-γ-CD) [8] as stationary phases. The *N*-trifluoroacetylated methyl esters of the amino acids, isoleucine, phenylalanine and *N,N*-dimethyl phenylalanine [9] in the enantiomerically pure L-form and racemic D,L-mixture were used as CPGC standards. Racemic phenylserine was used in both diastereomeric forms, which were resolved using L-amino acid oxidase [6, 10]. By comparison of the *R,S* of these standards with those of corresponding amino acid derivatives obtained from hydrolysates of the alkaloids, it was possible to assign the absolute configurations unambiguously.

In scutianine-K (**1**), *N,N*-dimethyl phenylalanine and β-phenylserine have L(*S*) and D-*threo* (α-*R*/β-*S*) configurations, respectively; scutianine-L (**2**) possesses *N,N*-dimethyl phenylalanine and isoleucine in the L(*S*)-form. Assignments were verified by co-injection and subsequent CPGC mass spectrometry.

EXPERIMENTAL

General. Mps are uncorr. ^1H and ^{13}C NMR were recorded at 400 and 100.6 MHz, respectively. Chiral phase GC analyses (FID) were carried out using 0.25 mm id × 25 m fused-silica capillaries coated with 2,6-dimethyl-3-pentyl-β-cyclodextrin and 2,6-dipentyl-3-butyryl-γ-cyclodextrin, each diluted with the polysiloxane OV 1701 (20 and 50%, respectively), run with 65 kPa H_2 carrier. TLC was performed on Merck silica gel 60 F_{254}.

Plant material. Scutia buxifolia Reiss was collected in March 1993 in a suburb of Santana do Livramento, in the state of Rio Grande do Sul, Brazil. A voucher specimen is deposited at the Herbarium of the University of Santa Maria.

Extraction and isolation. Dried material (10 kg) was extracted with 5 l MeOH in a soxlhet apparatus to give, after removal of solvent, 10 g of a mixt. of alkaloids. The mixt. was fractionated on silica as described previously [1, 2].

Isolation of scutianin-K (**1**). Recrystallization of the resultant solid (35 mg) of a fr. eluted at R_f 0.20 ($CHCl_3$–MeOH, 19:1), homogeneous on TLC, from MeOH–Et_2O gave compound **1**, mp 215–217° $[\alpha]_D^{25}$ −20.9° ($CHCl_3$, *c* = 0.1). EIMS (*m/z*): 584 $[M]^+$, 493, 387, 353, 260, 190, 135, 107, 106, 105. ^1H and ^{13}C NMR: Table 1.

Isolation of scutianine-L (**2**). Recrystallization of the resultant solid (20 mg) of a fr. at R_f 0.10 ($CHCl_3$–MeOH, 19:1), homogeneous on TLC, from MeOH–di-isopropyl ether gave compound **2**, mp 122–123°. $[\alpha]_D^{25}$ −72° ($CHCl_3$, *c* = 2.4). EIMS (*m/z*): 568 $[M]^+$, 477, 421, 229, 148, 135, 91, 86. ^1H and ^{13}C NMR: Table 2.

Table 2. ^1H and ^{13}C NMR assignments for scutianine-L (**2**) in $CDCl_3$ at 400/100 MHz

Position	Assignment	δ ^1H* J(Hz)	δ ^{13}C†
1	-C=(Ar)		154.8
3	-CH-O	6.38 (*d*), $J_{3,4} = 14.6$	77.8
4	-NH-CH-CO	7.35 (*dd*) $J_{3,4} = 14.6$, $J_{4,21} = 10.2$	58.4
5	-CO-		168.2
6	-OC-NH-CH-	6.30 (*d*), $J_{6,7} = 8.2$	
7	-NH-CH-CO-	4.55 (*dd*) $J_{6,7} = 8.2$, $J_{7,29} = 4.2$	68.2
8	-CO-		165.6
9	-CO-NH-CH-	7.10–7.30‡	
10	-NH-CH=CH-	7.10–7.30‡	126.3
11	-CH=CH-Ar	6.73 (*d*), $J_{10,11} = 8.5$	124.9
12	-C=(Ar)		139.4
13–16	-CH=(Ar)	7.2–7.4‡	126.0–129.0‡
17	-C=(Ar)		133.1
18–20	-CH=(Ar)	7.2–7.4‡	126.0–129.0‡
21	-CH-NH-CO	9.08 (*d*), $J_{4,21} = 10.2$	
22	-CO-		172.8
23	Me_2-N-CH-CO-	3.62 (*dd*) $J_{23,24\alpha} = 4.6$, $J_{23,24\beta} = 8.2$	71.0
24	-CH_2-Ph	2.94 (*dd*, α), 3.26 (*dd*, β) $J_{23,24\alpha} = 4.6$, $J_{23,24\beta} = 8.2$, $J_{24\alpha,24\beta} = 14.2$	30.0
25	-C=(Ar)		129.5
26–28	-CH=(Ar)	7.2–7.4‡	126.0–129.0‡
29	CH_3-CH-CH_2	2.30 (*m*)	35.6
30	CH_3-CH-	0.98 (*d*), $J_{29,30} = 6.8$	16.2
31	CH_3-CH_2-CH-	1.40 (*m*)	24.40
32	CH_3-CH_2-	0.90 (*t*), $J_{31,32} = 7.0$	11.80
33, 34	-NMe_2	2.40 (*s*)	42.0

* Assignments confirmed by ^1H-^1H COSY and NOESY.
† Assignments confirmed by DEPT and HETCOR.
‡ Peaks occur in the given range, no assignment.

Dihydroalkaloids. Hydrogenation of compounds **1** and **2** (5 mg each), under the condition described for peptide alkaloids [6], yielded the corresponding dihydroalkaloids **3** and **4** (*ca* 4 mg each).

Hydrolysis. Hydrolysis of dihydro derivatives **3** and **4** was performed in a sealed tube at 90–110° with 6N HCl for 24 hr. The acidic soln was concd and the residue treated as described for amino acids [10, 11].

GC analysis of N,N-*dimethyl phenylalanine and ring amino acids.* Derivatized amino acids were analysed by enantioselective capillary CPGC. The stereochemistry of the *N,N*-dimethyl phenylalanine and C-7 of the ring amino acids (phenylserine in **1** and isoleucine in **2**) were unambiguously established. Methyl *N,N*-dimethyl phenylalanine: 3-Pe-2,6-Me-β-CD, isothermal 105°, α (separation factor) = 1.16. Methyl *N*-trifluoroacetyl phenylserine: 3-Pe-2,6-Me-β-CD, isothermal 105°, $\alpha = 1.04$. Methyl *N*-trifluoroacetyl isoleucine: 3-Bu-2,6-Pe-γ-CD, 80 → 160° at 2° min^{-1}, $\alpha = 1.11$.

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