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# PEPTIDE ALKALOIDS OF *SCUTIA BUXIFOLIA*

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**Key Word Index**—*Scutia buxifolia*; Rhamnaceae; peptide alkaloids; scutianine H; MIKES; $^{13}C$ NMR spectra; scutianine D; scutianine E.

**Abstract**—From the bark extract of *S. buxifolia*, the known alkaloids scutianines B, C, D and E, and the new one, scutianine H, have been isolated. The structure of scutianine H, based mainly on its MS fragmentation and on the MIKES (mass-analysed ion kinetic energy spectrum) of the base ion peak, is suggested. From the $^{13}C$ NMR spectral analysis of the diastereoisomeric scutianines D and E, information for the assignment of the stereochemistry of the β-hydroxyleucine unit in related alkaloids was also obtained.

Continuing our chemical study of the constituents of *Scutia buxifolia* Reiss., we report on work which is being carried out in our laboratories and, in view of the recent publication of Tschesche and Hillebrand [1], we also make some explanatory comments about the designations of the cyclopeptide alkaloids isolated from this interesting plant.

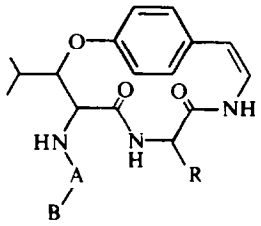
The first member of the scutianines, isolated by Tschesche *et al.* in 1967 [2], was named scutianine A, **1a**, and its structure elucidation represented a pioneer contribution to the field. Later on, in 1971, the same group also isolated scutianine B, **2** [3].

In 1974 we determined the stereochemistry of all the chiral centres of scutianine A, **1a** [4], with the exception of those of the β-hydroxyleucine unit, which had been previously determined on other alkaloids through special degradations [5, 6]. We also reported the isolation and the structure of two new alkaloids, scutianine D, **3**, and scutianine C, **4**, as well as a neutral product, called scutianene C, **5**, because of its structural similarity with **4** [4, 8]. By analysis of the 220 MHz $^1H$ NMR spectrum, the *erythro* form of the β-hydroxyleucine unit was shown to be present in **4** and the *threo* form of the β-phenylserine moiety was identified amongst the hydrolysis products of dihydro-**4** [8]. The 220 MHz $^1H$ NMR spectrum of **5** showed that the signals corresponding to the α and β protons of the hydroxyleucine unit had δ values different than those observed for the corresponding protons of **4**, indicating some change in the stereochemistry of this unit in **5** in relation to alkaloid **4**. Thus, **5**—whether or not an artefact—should be related to an alkaloid stereoisomeric with **4**.

Simultaneously, Tschesche *et al.* [7] published the isolation, from the same source, of three alkaloids named scutianine C, **3**, and two diastereoisomers, scutianine D and scutianine E, **4**. The stereochemistry of the β-hydroxyamino acid units of the latter compounds was shown to be L-*erythro*-β-hydroxyleucine, L-*threo*-β-phenylserine and D-*erythro*-β-hydroxyleucine and D-*threo*-β-phenylserine, respectively. By comparison of the spectral properties it can be deduced that the previously reported scutianines C and D [8, 4] are identical to the scutianines D, **4**, and C, **3**, reported by the Bonn group [7]. Consequently, we suggest that these latter designations be adopted, leaving the scutianene **5** undetermined, until a correlation with an alkaloid of known stereochemistry is carried out.

More recently, the isolation of scutianine F, **1b**, a desmethylderivative of scutianine A, and of scutianine G, another diastereoisomer of **4** was reported [9, 1]. Further, by re-examination of the bark extract of *S. buxifolia*, scutianine B, **2**, the now-named scutianines C, **3**, D and E, **4**, and the new cyclopeptide alkaloid, scutianine H were isolated.

Scutianine H, **6**, mp 242–243°, showed $M^+$ at *m/e* 550 indicating a formula $C_{31}H_{42}N_4O_5$. The IR spectrum exhibited bands corresponding to peptide linkages and the UV spectrum and the MS fragmentation were




**1a** A = Pro; B = Dimethyl Phe; R = $CH_2C_6H_5$. Scutianine A [2].
**1b** A = Pro; B = Methyl Phe; R = $CH_2C_6H_5$. Scutianine F [9].
**2** A = Dimethyl Phe; R = $CH_2C_6H_5$. Scutianine B [3].
**3** A = Dimethyl Ileu; R = $CH_2C_6H_5$. Scutianine D [4]; Scutianine C [7].
**4** A = Dimethyl Phe; R = $CH(OH)C_6H_5$. Scutianine C [8]; Scutianine D* [7]; Scutianine E† [7]; Scutianine G [1].
**5** A = $C_6H_5CH{=}CH{-}CO$; R = $CH(OH)C_6H_5$. Scutianene C [4].
**6** A = Dimethyl Ileu; R = $CH(OH)C_6H_5$. Scutianine H (This work).
* L-*erythro*-β-hydroxyleucine; L-*threo*-β-phenylserine.
† D-*erythro*-β-hydroxyleucine; D-*threo*-β-phenylserine.

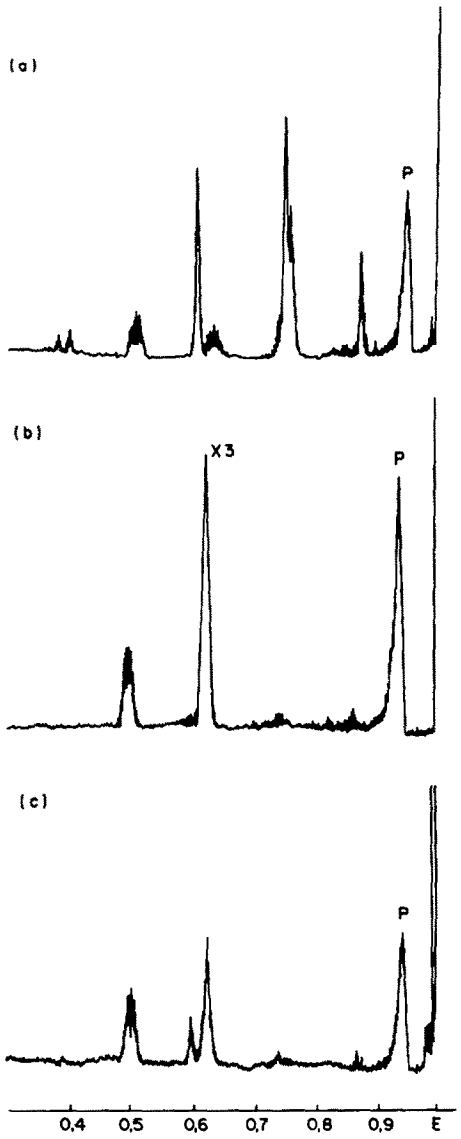



Fig. 1. (a) MIKES of *m/e* 114, $(CH_3)_2\overset{+}{N}=CH-CH(CH_3)-CH_2CH_3$, from *N,N*-dimethylisoleucine. (b) MIKES of *m/e* 114, $(CH_3)_2\overset{+}{N}=CH-CH_2-CH(CH_3)_2$, from *N,N*-dimethylleucine. The peak at 0·63 E should be multiplied by a factor of three to obtain the actual height. (c) MIKES of *m/e* 114, $(CH_3)_2\overset{+}{N}=CH-CH_2-CH_2CH_2CH_3$, from *N,N*-dimethylnorleucine. P is a phanthom peak typical of the instrument.

typical of a 14-membered peptide alkaloid. The base peak at *m/e* 114 and the fragments at *m/e* 507, 493, 444, 394, 190, 135, 107, 106, 105 and 97 indicated that the basic terminal, the β-hydroxy and the ring amino acids were *N,N*-dimethylleucine or *N,N*-dimethylisoleucine, β-hydroxyleucine and β-phenylserine, respectively. The peak at *m/e* 105, typical of the benzoyl group, in the MS of oxodihydroscutianine H and the identification of *p*-tyramine amongst the hydrolysis products of dihydroscutianine H, confirm the presence of the β-phenylserine and styrylamine units in **6**.

Although differentiation between *N,N*-dimethylleucine and *N,N*-dimethylisoleucine has been successfully carried out by high resolution $^1H$ NMR [10, 4],

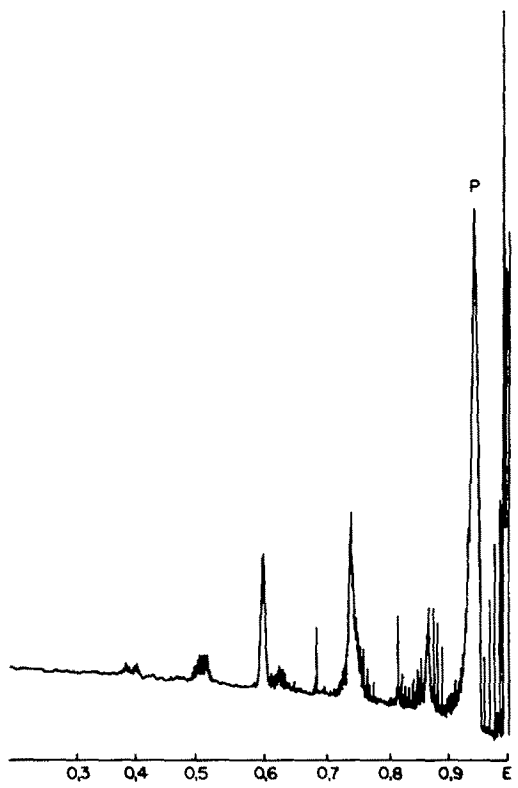



Fig. 2. MIKES of *m/e* 114, $(CH_3)_2\overset{+}{N}=CH-CH(CH_3)-CH_2CH_3$, from the EI fragmentation of scutianine C.

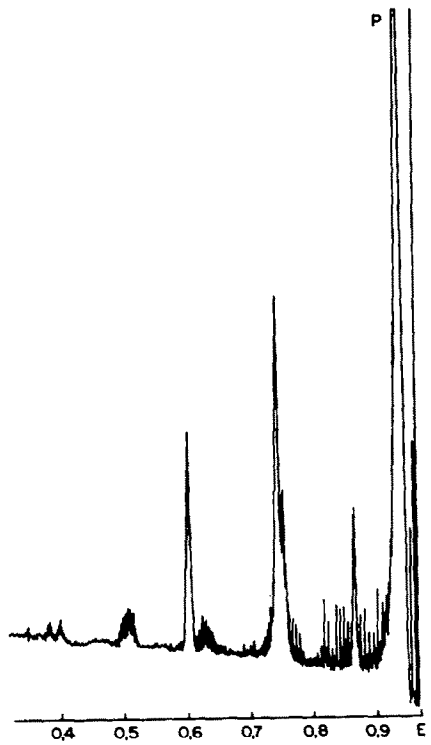



Fig. 3. MIKES of *m/e* 114, $(CH_3)_2\overset{+}{N}=CH-C_4H_9$ from the EI fragmentation of scutianine H.

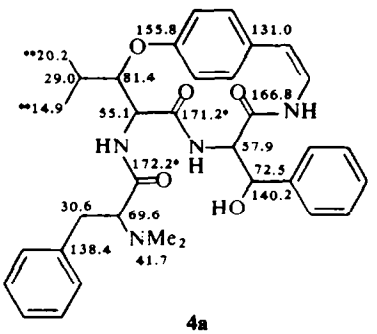
it was interesting to find a method which can differentiate unambiguously the possible isomers of *m/e* 114, including *N,N*-dimethylnorleucine, using only minute quantities of natural product.

In 1974, McLafferty *et al.* reported the application of metastable ion (MI) and collisional activation (CA) spectra to the determination of leucine and isoleucine residues in peptides [11]. Therefore, we felt that perhaps the MI spectra or more correctly the mass-analysed ion kinetic energy spectra (MIKES) could provide fingerprints of the various isomers of *m/e* 114 permitting the unequivocal differentiation between the isomers. Indeed, MIKES of *m/e* 114 from *N,N*-dimethylisoleucine (Fig. 1a), from *N,N*-dimethylleucine (Fig. 1b) and from *N,N*-dimethylnorleucine (Fig. 1c) are dramatically different, showing that there is no equilibration of ion structures and that the fragmentation of these ions is very structure dependent. Since the basic terminal amino acid of scutianine C has been previously determined as *N,N*-dimethylisoleucine [4, 7], we used it as a model alkaloid, and the MIKES of its *m/e* 114 ion is shown in Fig. 2. Except for the appearance of spikes in the spectrum due to thermal effects and interfering fragments from the first field free region, the aspect of this spectrum is the same shown in Fig. 1a and is significantly different than those shown in Figs. 1b and 1c. Thus, it seems that, independent of its interpretation, the MIKES can be very useful to the natural products chemist as a fingerprint tool, much like IR spectroscopy. Indeed, we used this technique to establish the structure of the basic terminal amino acid of scutianine H (Fig. 3).

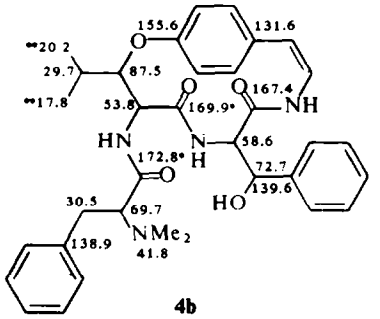
The availability of the diastereoisomeric cyclopeptide alkaloids, scutianines D and E, with β-hydroxyamino acid units of known stereochemistry [7], prompted us to study them by $^{13}C$ NMR spectroscopy, hoping to detect features characteristic of a given configuration, since that information could be used as a complement of previous $^{1}H$ NMR studies [5], for the configuration assignment of other members of this group of natural products.

For the analysis of the $^{13}C$ NMR spectra we focused our attention mainly on the $sp^3$ carbon shifts, and their assignments, based on previously reported data for related alkaloids [12, 13], on the multiplicity of signals in SFORD spectra and on the analysis of model compounds, are shown in **4a** and **4b**, for scutianines D and E, respectively. The shifts of the methyl groups, their non-equivalence ($\Delta\delta$ = 5.3 ppm) and the shifts of the α, β and γ carbons of the β-hydroxyleucine unit of **4a**, are in good agreement with the reported ones for frangulanine, discarines A and B, and lasiodine B [12, 13]. The α and β carbons of the phenylserine unit and those of the *N,N*-dimethylamino acid residue of **4a**, were assigned by comparison with model compounds **7** and **8**. The difference in δ value between the $CH_2$ group of the model compound **7**, and the same carbon of **4a**, unambiguously assigned on the basis of its multiplicity in the SFORD spectrum, can be attributed to a γ effect, probably as the result of a preferred conformation of the *N,N*-dimethylphenylalanine moiety in the alkaloid.

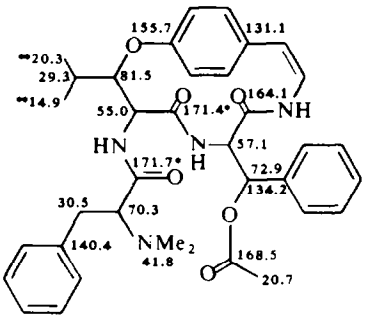
Scutianine E, **4b**, shows shifts similar to the ones observed in **4a**, except for the β-hydroxyleucine unit. The difference between the methyl groups is clearly less ($\Delta\delta$ = 2.4 ppm), indicating that they are less hindered than in **4a**, and further, the α and β carbons are shielded



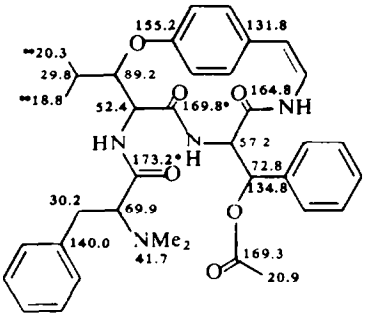

**4a**




**4b**




**4c**




**4d**

($\Delta\delta$ = −1.2 ppm) and deshielded ($\Delta\delta$ = 6.1 ppm), respectively. The difference in configuration of the phenylserine residue in **4a** and **4b** does not significantly affect the shifts of the α and β carbons of this unit.

Acetylation of scutianine E, **4d**, apart from producing shielding effects on the non-protonated aromatic carbon and weak effects on the α and β carbons of the phenylserine unit, and shielding of one of the CO groups, as in scutianine D, **4c**, induces shielding and deshielding of

the $\alpha$ and $\beta$ carbons, respectively, of the hydroxyleucine.

The clear difference in the $\delta$ values of the $\beta$ carbons and the different $\Delta\delta$ between the methyl shifts of the hydroxyleucine residues of both alkaloids, **4a** and **4b**, together with previously reported $^1$H NMR data [5], could be used as an indication of the configuration of this unit. Discarines A and B, the major bases of *D. longispina* [14] for example, show signals at 82.4 ppm and $\Delta\delta$ = 5.7 ppm, and at 80.8 ppm and $\Delta\delta$ = 5.6 ppm, for the $\beta$ carbons and for the difference between the chemical shifts of the methyl groups respectively [12, 13] indicating that the hydroxyleucine moiety of these alkaloids possess the L-*erythro* configuration.

## EXPERIMENTAL

A double-focusing mass spectrometer with a reversed Nier–Johnson geometry was used to obtain the mass spectra and the MIKES. Measurements were carried out with an electron energy of 70 eV and an ion source temp. of 100–120°. The accelerating potential was 3 kV. All samples were introduced via a direct inlet system. The MIKES were obtained by focusing the precursor ion (*m/e* 114 in this case) on the collector with the magnetic field and scanning the electric sector potential. The $^1$H NMR were recorded at 100 MHz and the $^{13}$C NMR spectra at 25.2 MHz in the Fourier transform mode and in $CDCl_3$ solutions. Chemical shifts are expressed on the TMS scale according to: $\delta$TMS = $\delta CDCl_3$ + 76.9 ppm. The asterisks on the formulae indicate possible signal reversal. The acetates and Me esters were all prepared by standard methods. $Ac_2O$ and $C_5H_5N$ and ethereal $CH_2N_2$ respectively, and the physical data were compared, when available, with those of the literature.

*Extraction of* Scutia buxifolia. The plant material was collected at Livramento (Rio Grande do Sul, Brazil) in July 1976. The powdered bark (5 kg) was extracted as before [8], yielding a mixture of alkaloids as a solid residue (4.1 g).

*Isolation of the alkaloids.* The alkaloid mixture was fractionated on a $SiO_2$ (H type) chromatographic column using mixtures of $CHCl_3$–MeOH as solvent and on PLC, as described before [8], if further purification was required.

*Scutianine D* (**4a**). Crystallization of the resultant solid (320 mg) from $CHCl_3$–$Et_2O$ gave **4a**, mp 217–218°; $[\alpha]_D$ − 202° ($CHCl_3$, *c* = 0.1). (Lit. [7] mp 219–220°; $[\alpha]_D$ −196° ($CHCl_3$, *c* = 0.1)). No UV absorption. IR $\nu$ cm$^{-1}$: 3600; 3285; 2795; 1650; 1625; 1250. MS *m/e* (rel. int.) 584 (0.02); 493 (4.5); 478 (0.2); 435 (0.1); 387 (1.4); 342 (4.2); 331 (1.4); 190 (0.6); 148 (100); 135 (2.1); 120 (0.6); 107 (1.0); 106 (4.6); 105 (4.8); 97 (1.1); 91 (2.42); 77 (4.1). $^1$H NMR ($CDCl_3$): $\delta$ 0.98 (3H, *d*, *J* = 6 Hz); 1.24 (3H, *d*, *J* = 6 Hz); 2.23 (6H, *s*). $^{13}$C NMR $\delta$ [sp$^2$] CH: 120.7; 121.2; 122.4; 125.0; 126.0; 126.4; 127.9; 128.2; 129.0; 129.6; 130.7.

*Scutianine D acetate* (**4c**). Mp 236°, from EtOH–$H_2O$; $[\alpha]_D$ − 163° ($CHCl_3$, *c* = 0.1). (Lit. [7] amorphous; $[\alpha]_D$ − 140° ($CHCl_3$, *c* = 0.1)). IR $\nu$ cm$^{-1}$: 3320; 2780; 1760; 1650; 1620; 1230. $^{13}$C NMR $\delta$ [sp$^2$] CH: 118.9; 121.4; 122.6; 124.9; 126.1; 126.4; 128.4; 129.0; 129.8.

*Scutianine E* (**4b**). Crystallization of the resultant yellowish oil (450 mg) from MeOH–$H_2O$ gave **4b**, mp 110–110.2°; $[\alpha]_D$ − 21° ($CHCl_3$, *c* = 0.1). (Lit. [7] mp 121°; $[\alpha]_D$ − 22.2° ($CHCl_3$, *c* = 0.1)). No UV absorption. IR $\nu$ cm$^{-1}$: 3600; 3280; 2800; 1650; 1625; 1250. MS *m/e* (rel. int.) 584 (0.05); 493 (4.1); 478 (1.2); 387 (1.3); 190 (1.0); 148 (100); 135 (4.1); 120 (1.5); 107 (3.2); 106 (2.2); 105 (21); 97 (2.7); 91 (5.1); 77 (18.4). $^1$H NMR ($CDCl_3$): $\delta$ 1.11 (6H, *d*, *J* = 6 Hz); 2.34 (6H, *s*). $^{13}$C NMR $\delta$ [sp$^2$] CH: 121.0; 122.5; 123.1; 125.2; 126.2; 127.7; 128.1; 128.2; 129.0; 129.9; 130.3.

*Scutianine E acetate* (**4d**). Mp 105°; from EtOH–$H_2O$; $[\alpha]_D$ + 58° ($CHCl_3$, *c* = 0.1). (Lit. [7] mp 178–180°, $[\alpha]_D$ +52.1° ($CHCl_3$, *c* = 0.1)). IR $\nu$ cm$^{-1}$: 3320; 2780; 1750; 1680; 1620; 1240. MS *m/e* (rel. int.) 626 (0.1); 583 (0.3); 567 (0.2); 535 (58); 475 (3.7); 190 (2.6); 148 (100); 135 (10.5); 120 (5.3); 107 (5.3); 106 (4.1); 105 (9.8); 97 (8.3); 91 (18.9); 77 (7.4). $^1$H NMR ($CDCl_3$): $\delta$ 1.2 (6H, *d*, *J* = 6 Hz); 2.04 (3H, *s*); 2.38 (6H, *s*). $^{13}$C NMR $\delta$ [sp$^2$] CH: 119.4; 121.5; 123.0; 125.2; 126.1; 126.6; 127.8; 128.3; 129.2; 130.0; 131.1.

*Scutianine B* (**2**). Crystallization of the resultant solid (50 mg) from $CHCl_3$–hexane gave **2**, mp 248°; $[\alpha]_D$ − 290° ($CHCl_3$, *c* = 0.1). Identical with an authentic sample (TLC, 3 solvents). MS *m/e* 148 (base ion peak).

*Scutianine C* (**3**). Crystallization of the resultant solid (70 mg) from $CHCl_3$–$Et_2O$ gave **3**, mp 263–265°; $[\alpha]_D$ − 182° ($CHCl_3$, *c* = 0.1). MS *m/e* 534 ($M^+$); 114 (base ion peak). Identical with an authentic sample (TLC, 3 solvents).

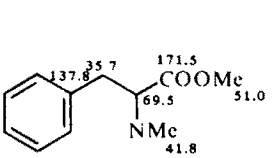
*Scutianine H* (**6**). The crystalline residue (50 mg), recrystallized from $CHCl_3$–$Et_2O$ gave **6**, mp 242–243°; homogeneous on TLC; MS (high resolution), found: 550.3184, calc. for $C_{31}H_{42}N_4O_5$; 550.3155; $[\alpha]_D$ −233° ($CHCl_3$, *c* = 0.1). No UV absorption. IR $\nu$ cm$^{-1}$: 3580; 3285; 2780; 1640; 1620; 1500; 1250. MS *m/e* (rel. int.) 550 (0.2); 535 (0.2); 507 (0.04); 493 (1.5); 444 (1.0); 401 (0.3); 394 (0.42); 353 (0.1); 342 (0.2); 331 (0.3); 329 (0.4); 190 (1.8); 177 (0.8); 135 (10.4); 120 (3.15); 114 (100); 107 (3.2); 106 (5.7); 105 (6.3); 97 (10.3); 85 (16.8); 77 (5.6). $^1$H NMR ($CDCl_3$): $\delta$ 0.74–0.98 (9H, *m*); 1.22 (3H, *d*, *J* = 8 Hz); 2.00 (6H, *s*); ($d_5$-pyridine): $\delta$ 0.84 (3H, *t*, *J* = 6 Hz); 0.94 (3H, *d*, *J* = 6 Hz); 1.20 (3H, *d*, *J* = 8 Hz); 1.28 (3H, *d*, *J* = 6 Hz); 2.32 (6H, *s*).

*Dihydroscutianine H.* The hydrogenation of scutianine H under the conditions described for peptide alkaloids yielded dihydro-**6**, mp 276–277° (from $Me_2CO$). IR $\nu$ cm$^{-1}$: 3580; 3290; 1640; 1500; 1250. MS *m/e* (rel. int.) 552 (0.1); 495 (0.2); 446 (0.1); 403 (0.3); 344 (0.3); 333 (0.3); 192 (0.2); 175 (0.7); 137 (0.2); 114 (100); 107 (2.5); 106 (3.1); 105 (3.3); 97 (2.2); 85 (3.9); 77 (3.3). Hydrolysis of dihydro-**6** was performed in a sealed tube at 110° with 6N HCl for 12 hr, the aq. acidic soln was concd and the residue was used to identify *p*-tyramine by a previously described procedure [14].

*Oxodihydroscutianine H.* A soln of dihydro-**6** (10 mg) was oxidized as described before [8]. Crystallization of the residue from $Me_2CO$ gave oxodihydroscutianine H, mp 288–290°. MS *m/e* (rel. int.) 550 (1.2); 535 (0.14); 521 (0.12); 507 (0.4); 493 (1.1); 446 (0.2); 353 (0.4); 218 (0.6); 195 (1.1); 190 (0.3); 175 (2.3); 155 (3.5); 135 (1.7); 114 (100); 107 (9.2); 105 (12.4); 97 (5.8); 85 (12.3); 77 (6.2).

N,N-*Dimethyl-norleucine.* It was prepared by reductive methylation of the corresponding amino acid [15], mp 165.6–166.5°. IR *v* cm$^{-1}$: 2800; 1645. $^1$H NMR 60 MHz ($CDCl_3$): $\delta$ 0.95 (3H, unresolved *t*); 1.2 (4H, *m*); 2.95 (6H, *s*); 3.5 (1H, *m*); 8.92 (1H, *br s*).

N,N-*Dimethylphenylalanine methyl ester* (**7**). Viscous oil. $^1$H NMR 60 MHz ($CDCl_3$): $\delta$ 2.36 (6H, *s*); 2.8–3.5 (3H, *m*); 3.55 (3H, *s*); 7.35 (5H, *s*).

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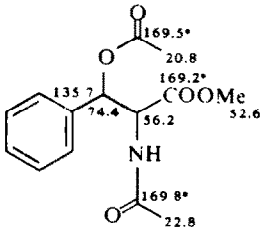


8

O,*N-Diacetyl-β-phenylserine methyl ester* (**8**). From a commercial sample of $\beta$-phenylserine, mainly in the *threo* form—from $^1$H NMR analysis according to [6]—compound **8** was prepared. Mp 145°. IR $\nu$ cm$^{-1}$: 3280; 1760; 1750; 1680. $^1$H NMR 60 MHz ($CDCl_3$): $\delta$ 1.9 (3H, *s*); 2.1 (3H, *s*); 3.6 (3H, *s*); 5.1 (2H, *m*); 6.2 (1H, *d*); 7.3 (5H, *s*). MS *m/e* (rel. int.) 279 (5 3); 220 (2.6); 173 (25); 160 (21); 149 (43.4); 130 (50); 113 (38.2); 107 (51); 89 (100); 79 (20); 43 (22).

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