Absolute Configuration of Tylophorine

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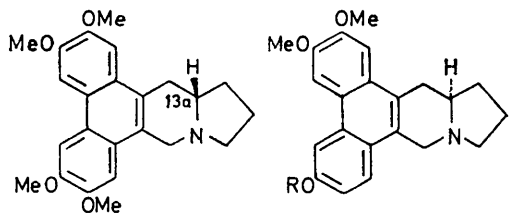
The configuration at C-13a in tylophorine (I) has been established as *S*. Ozonolysis of tylophorine gave (*S*)-pyrrolidine-2-acetic acid identical with a sample synthesized from (*S*)-proline. The absolute configuration of analogous alkaloids of the group can be deduced by comparison of their o.r.d. spectra with that of tylophorine.

THE phenanthroindolizidine group of alkaloids consists of *ca.* fifteen bases isolated from *Tylophora, Cynanchum,* and *Ficus* species.[1] The gross structure of tylophorine, the earliest known member of this group, has been established as (I) by degradation [2,3] and by syntheses.[4-6] The only alkaloids of this class for which the absolute configurations have been determined are the base (IIa) and its methyl ether (IIb) (antofine) isolated from *Cynanchum vincetoxicum* L. Pers.[7] Ozonolysis of antofine yielded D-proline thus establishing the *R*-configuration at C-13a.

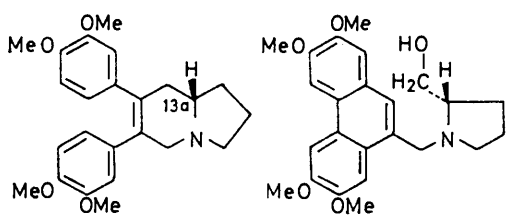
The secophenanthridine alkaloid L-septicine (III) has been shown to have the *S*-configuration at C-13a by synthesis from (*S*)-pyrrolidine-2-methanol.[8] Photo-oxidation of L-septicine is known to give tylophorine but the optical rotation of the product was not determined.[9] An attempt to synthesize tylophorine having the *S*-configuration at C-13a from compound (IV) [4] by treatment with methanesulphonyl chloride and subsequently with sodium hydride was unsuccessful.

We now present evidence for the *S*-configuration at C-13a of tylophorine. Exhaustive ozonolysis of tylophorine and purification of the resultant mixture of amino-acids by ion-exchange chromatography [10,11] gave,



(I) (II)

a, R = H
b, R = Me



(III) (IV)

besides glycine, β-alanine, and γ-aminobutyric acid, an amino-acid corresponding in R_F to the expected proline.

[1] For a recent review, see T. R. Govindachari, *J. Indian Chem. Soc.*, 1973, **50**, 1.
[2] T. R. Govindachari, M. V. Lakshmikantham, K. Nagarajan, and B. R. Pai, *Tetrahedron*, 1958, **4**, 311.
[3] T. R. Govindachari, M. V. Lakshmikantham, B. R. Pai, and S. Rajappa, *Tetrahedron*, 1960, **9**, 53.
[4] T. R. Govindachari, M. V. Lakshmikantham, and S. Rajadurai, *Tetrahedron*, 1961, **14**, 284.
[5] R. B. Herbert and C. J. Moody, *Chem. Comm.*, 1970, 121.
[6] B. Chauncy and E. Gellert, *Austral. J. Chem.*, 1970, **23**, 2503.
[7] W. Wiegrebe, L. Faber, and Th. Breyhan, *Arch. Pharm.*, 1971, **304**, 188.
[8] J. H. Russel and H. Hunziker, *Tetrahedron Letters*, 1969, 4035.
[9] J. H. Russel, *Naturwiss.*, 1963, **50**, 443.
[10] C. H. W. Hirs, S. Moore, and W. H. Stein, *J. Amer. Chem. Soc.*, 1954, **76**, 6063.
[11] W. H. Stein and S. Moore, *Cold Spring Harbor Symposia, Quant. Biol.*, 1950, **14**, 179.

Because of the paucity of material (approximate yield of the amino-acid on the basis of g.l.c. studies, 20 mg from 5·1 g of tylophorine), direct comparison with proline could not be effected. The acid was converted into the methyl ester and coupled with *N*-trifluoracetyl-(—)-(*S*)-prolyl chloride so that g.l.c. comparison with authentic an authentic synthetic sample of (V), the most significant mass spectral fragments being (*a*)—(*h*).[14]

DL-Pyrrolidine-2-acetic acid [15] and its ethyl ester [16] are known synthetically, and the acid has also been isolated from cured tobacco leaves.[17] For comparison with the product obtained from tylophorine, racemic methyl

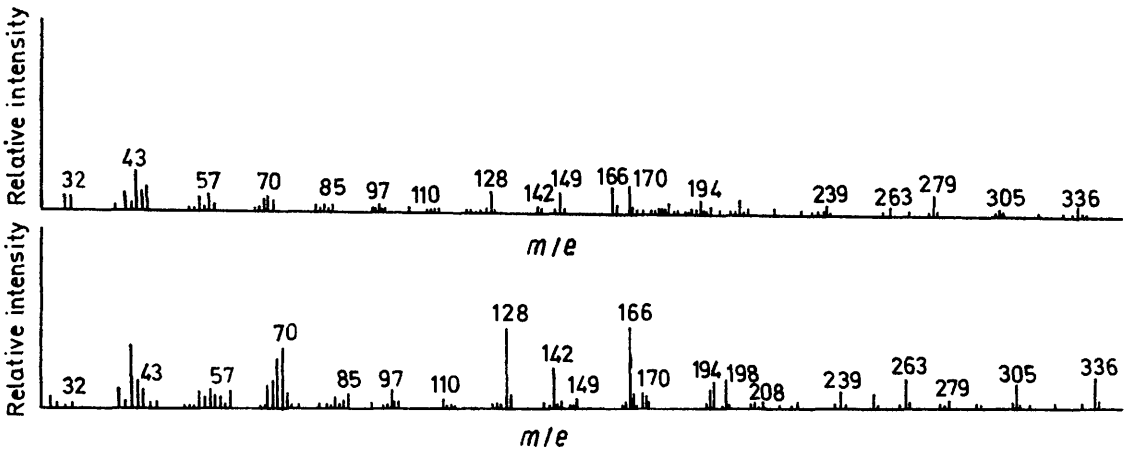
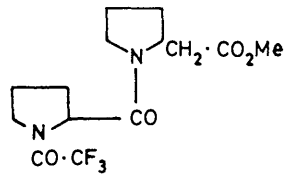
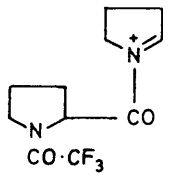


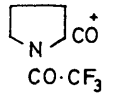
FIGURE 1 Mass spectra. Top: synthetic *N*-TFA-(—)-(*S*)- prolyl-(*RS*)-pyrrolidine-2-acetic acid methyl ester. Bottom: *N*-TFA-(—)-(*S*)-prolyl-(*S*)-pyrrolidine-2-acetic acid methyl ester from ozonolysis



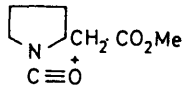
(V) M^+, *m/e* 336 (4%)



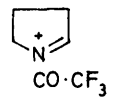
(*a*) *m/e* 263 (5%)



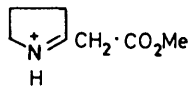
(*b*) *m/e* 194 (3%)



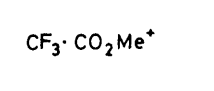
(*c*) *m/e* 170 (15%)



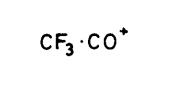
(*d*) *m/e* 166 (100%)



(*e*) *m/e* 142 (45%)



(*f*) *m/e* 128 (95%)

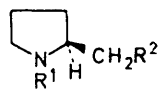


(*g*) *m/e* 97 (5%)

(*h*) *m/e* 70 (70%)

dipeptides obtained from (*S*)-proline and (*RS*)-proline could be used to ascertain the chirality of the amino-acid.[12,13] The mass spectrum of the product showed that it was not the expected *N*-TFA-(—)-(*S*)-prolylproline methyl ester but its higher homologue, *N*-TFA-(—)-(*S*)-prolylpyrrolidine-2-acetic acid methyl ester (V) (M^+ 336). Its mass spectrum (Figure 1) was identical with that of pyrrolidine-2-acetate was prepared from ethyl pyrrole-2-acetate by transesterification with methanol followed by catalytic reduction. A reference sample of methyl (*S*)-pyrrolidine-2-acetate (VIf) was prepared from (*S*)-pyrrolidine-2-methanol (VIa) through the intermediates (VIb—e).

[12] B. Halpern and J. W. Westley, *Biochem. Biophys. Res. Comm.*, 1965, **19**, 361; 1965, **20**, 710.
[13] W. A. Bonner, *J. Chromat. Sci.*, 1972, **10**, 159.
[14] Q. N. Porter and J. Baldas, 'Mass Spectrometry of Heterocyclic Compounds,' Wiley-Interscience, New York, 1971, p. 311.
[15] B. R. Baker, R. E. Schaub, and J. H. Williams, *J. Org. Chem.*, 1952, **17**, 116.
[16] R. Adams, S. Miyano, and M. D. Nair, *J. Amer. Chem. Soc.*, 1961, **83**, 3323.
[17] H. Tomita, S. Mizusaki, and E. Tamaki, *Agric. and Biol. Chem.* (*Japan*), 1964, **28**, 451 (*Chem. Abs.*, 1964, **61**, 16,456f).



(VI)

a; R^1 = H, R^2 = OH
b; R^1 = $PhCH_2$, R^2 = OH
c; R^1 = $PhCH_2$, R^2 = Cl
d; R^1 = $PhCH_2$, R^2 = CN
e; R^1 = $PhCH_2$, R^2 = CO_2Me
f; R^1 = H, R^2 = CO_2Me
g; R^1 = H, R^2 = CO_2H

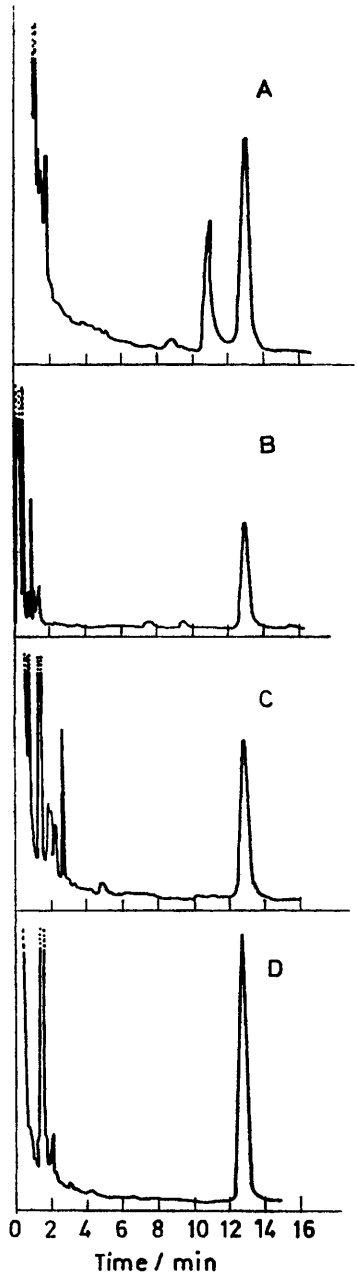


FIGURE 2 G.l.c. traces of *N*-TFA-(−)-(*S*)-prolylpyrrolidine-acetic acid methyl ester. A: Dipeptide from synthetic methyl (*RS*)-pyrrolidine-2-acetate. B: Dipeptide from synthetic methyl (*S*)-pyrrolidine-2-acetate. C: Dipeptide from tylophorine, before purification by t.l.c. D: Dipeptide from tylophorine, after purification by t.l.c

Coupling of racemic methyl pyrrolidine-2-acetate with *N*-TFA-(−)-(*S*)-prolyl chloride gave a mixture of two diastereoisomers having retention times of 10·8 and 12·9 min in g.l.c. Coupling of the (*S*)-ester (VIe) gave a dipeptide with retention time 12·9 min, identical with that of the product obtained from tylophorine (Figure 2). Tylophorine hence possesses the *S*-configuration at C-13a.

As expected the o.r.d. of tylophorine (I) and antofine (IIb) show multiple Cotton effects, the extrema in the region 220—280 nm being opposite in sign. The indolizidine ring in all alkaloids of the group lacking functionality at C-14 is most likely to be in the stable *trans*-configuration,[18] and their o.r.d. hence provides an easy method of determining the absolute configuration at C-13a. As an example, the o.r.d. of (+)-isotylocrebrine (VII) [19] (Figure 3) shows it to have the *R*-configuration.

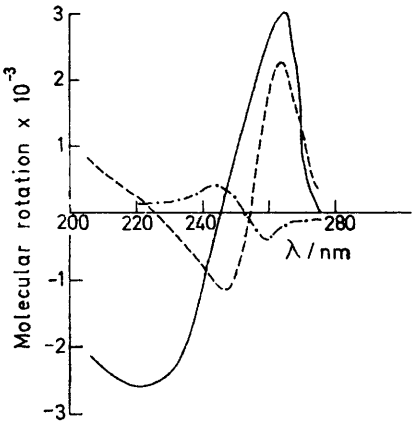
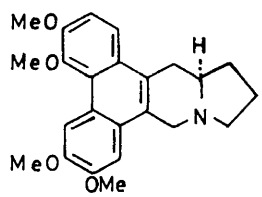


FIGURE 3 O.r.d. in 0·01N-hydrochloric acid: — · — · — tylophorine; —— antofine; – – – – isotylocrebrine



(VII)

EXPERIMENTAL

I.r. spectra were obtained on a Perkin-Elmer Infracord and n.m.r. spectra on a Varian Associates A-60 instrument. Optical rotations were taken in 2% chloroform solution at 25° unless otherwise stated. Mass spectra were obtained at 70 eV with a Varian MAT CH7 mass spectrometer. The sample to be determined was dissolved in methylene chloride, a small amount of acid-washed Chromosorb W (60—80 mesh) was added, and the contents were mixed thoroughly in a Vortex mixer. Excess of solvent was removed under a gentle stream of nitrogen at 37 °C. The resulting dry powder was packed in a glass capillary and introduced into the mass spectrometer *via* the direct inlet. G.l.c. analyses

[18] T. A. Crabb, R. F. Newton, and D. Jackson, *Chem. Rev.*, 1971, **71**, 109.

[19] T. R. Govindachari, N. Viswanathan, J. Radhakrishnan, B. R. Pai, S. Natarajan, and P. S. Subramaniam, *Tetrahedron*, 1973, **29**, 891.

were carried out with a Varian Aerograph model 2700 instrument equipped with a flame ionisation detector, using a 6 ft × 2 mm i.d. glass column packed with 3% OV-17 on 80—100 mesh GasChrom-Q (Applied Science Labs, State College, Pa., U.S.A.). The temperature settings were as follows: column oven 190, injection port 250, and detector 300 °C. Typical gas pressures were: nitrogen 24 (column head pressure), air 25, and hydrogen 15 lb in^{-2}. O.r.d. curves were determined in 0·01N-hydrochloric acid on a JASCO J-20 spectropolarimeter.

Ozonolysis of Tylophorine.—A solution of tylophorine (I) (isolated and purified by the method described earlier [20]) in 10% aqueous formic acid (100 ml) was treated with ozone at room temperature for 24 h at the rate of 200 ml min^{-1}. The solution was heated with 30% hydrogen peroxide (15 ml) and 98% formic acid (15 ml) for 3 h on a boiling water-bath. The solution was treated with platinum black (0·4 g) at 60° for 1 h to remove excess of peroxide, filtered, and the filtrate extracted with chloroform to remove neutral impurities. The aqueous solution was evaporated *in vacuo* (5 mmHg), the final traces of formic acid being removed by repeated additions of water (3 × 5 ml) and evaporation. The product from three such batches (3 × 1·7 g tylophorine) was subjected to ion-exchange chromatography: it was dissolved in 0·1N-hydrochloric acid (15 ml) and aliquot portions (2 ml) were chromatographed on 1 cm × 110 cm jacketed columns of Dowex 50 × 8, 200—400 mesh, in the H^+ form. The column was operated at 30 ± 1 °C and eluted with 1N-hydrochloric acid at the rate of 25 ml h^{-1}. After eluting the column with 250 ml of 1N-hydrochloric acid, the acid concentration of the eluant was gradually increased by adding 4N-hydrochloric acid to a 250 ml mixing chamber containing 200 ml of the 1N-acid. The column was then warmed from 30 to 50 °C and 100 fractions (2 ml each) were collected. The amino-acids were located by t.l.c. of a 10—20 μl aliquot portion of each fraction on silica in phenol–water (75 : 25, w/w) followed by spraying with ninhydrin. Comparison with authentic samples showed that glycine was eluted between effluent volume 220—230 ml, β-alanine at 240—255 ml, γ-aminobutyric acid at 255—280 ml, and pyrrolidine-2-acetic acid at 310—340 ml. The pyrrolidine-2-acetic acid fractions from six 2 ml aliquot chromatograms of the ozonolysis product were pooled and evaporated to dryness *in vacuo* at 1 mmHg at 30—35°. The residue was redissolved in 0·1N-hydrochloric acid (4 ml) and rechromatographed on a Dowex 50 × 8 column as described above. The pyrrolidine-2-acetic acid fractions were pooled and lyophilised to dryness.

Methanol (3 ml) was chilled to −5° and treated dropwise with freshly distilled thionyl chloride (0·5 ml). After keeping at 0° for 15 min, the lyophilised amino-acid was added, and the solution was kept at 40° for 2 h, then left at 30° overnight. The volatile material was evaporated off *in vacuo*, flushing with 3 ml portions of methanol and finally with benzene. The residue was dissolved in water (8 ml) and washed with ether. The aqueous solution was basified with ammonia to pH 9 and extracted with methylene chloride. The organic layer was washed with water, dried, and evaporated. The residual gum was taken up in methylene chloride (10 ml) and treated with *N*-TFA-(−)-(*S*)-prolyl chloride [13] (0·7 g). The solution was cooled to 0°, treated with triethylamine to neutral pH, and then left overnight at 30°. The solution was washed successively with dilute hydrochloric acid, water, dilute sodium hydrogen carbonate solution, and again with water, dried, and evaporated. The crude dipeptide was dissolved in methylene chloride (1 ml) and 0·3 ml of the solution was applied to half of a 20 cm × 20 cm × 0·5 mm silica gel G t.l.c. plate. Authentic *N*-TFA-(−)-(*S*)-prolyl-(*RS*)-pyrrolidine-2-acetic acid methyl ester (see below) was spotted on the other separate half. After development in benzene–methanol (9 : 1, v/v), the reference half of the plate was divided into 1 cm sections from the origin to the solvent front. The combined methanolic extract (3 × 1 ml) of each 1 cm section was dried at 37° in a current of nitrogen and dissolved in 100 μl of methanol. A 2 μl aliquot portion from each tube was subjected to g.l.c. as described above. G.l.c. analysis showed that the R_F values in t.l.c. of *N*-TFA-(−)-(*S*)-prolyl-(*R*)-pyrrolidine-2-acetic acid methyl ester and *N*-TFA-(−)-(*S*)-prolyl-(*S*)-pyrrolidine-2-acetic acid methyl ester were 0·31 and 0·43 respectively. The diastereoisomeric mixture gave two peaks in g.l.c. with retention times of 10·8 and 12·9 min. Authentic *N*-TFA-(−)-(*S*)-prolyl-(*S*)-pyrrolidine 2-acetic acid methyl ester gave a single peak having a retention time of 12·9 min. The dipeptide obtained from tylophorine gave a single peak in g.l.c. both before and after t.l.c. purification with a retention time of 12·9 min (Figure 2). The mass spectrum of the latter [*m/e* 336 (M^+, 4%), 305 (3), 279 (1), 263 (5), 239 (3), 198 (3), 194 (3), 170 (15), 167 (12), 166 (100), 149 (10), 142 (45), 129 (14), 128 (95), 110 (10), 98 (8), 97 (5), 96 (25), 85 (15), and 70 (70)] was identical with that of synthetic *N*-TFA-(−)-(*S*)-prolylpyrrolidine-2-acetic acid methyl ester (Figure 1).

1-[N-*Trifluoroacetyl*-(−)-(S)-*prolyl*]-(RS)-*pyrrolidine*-2-*acetic Acid Methyl Esters.*—(a) *Methyl pyrrole-2-acetate.* A solution of ethyl pyrrole-2-acetate [21] (2 g) in methanol (150 ml) was refluxed for 16 h with concentrated sulphuric acid (0·2 ml). The solution was evaporated *in vacuo*, ice was added, and the product extracted with ether. The ether layer was washed with sodium hydrogen carbonate solution and water, dried, and evaporated to yield the methyl ester (0·8 g), b.p. 100—110° (bath temperature) at 0·6 mmHg, δ (CCl_4) 8·8br (1H, NH), 6·5 (1H, q, α-H), 5·9 (2H, m, β-H), 3·55 (3H, s, OMe), and 3·45 (2H, s, CH_2). The ester could also be prepared by alkaline hydrolysis of ethyl pyrrole-2-acetate followed by esterification with diazomethane.

(b) *Methyl* (RS)-*pyrrolidine-2-acetate.* A solution of methyl pyrrole-2-acetate (3 g) in glacial acetic acid (15 ml) was shaken in a Parr apparatus with hydrogen at 40 lb in^{-2} at room temperature in the presence of 5% rhodium–alumina catalyst (0·75 g) for 6 h. The solution was filtered, evaporated *in vacuo*, diluted with water, basified with potassium carbonate, and extracted with methylene chloride to yield the racemic *ester* (0·5 g), b.p. 110° (bath temperature) at 1 mmHg (Found: C, 58·2; H, 9·5. $C_7H_{13}NO_2$ requires C, 58·7; H, 9·2%).

(c) 1-[N-*Trifluoroacetyl*-(−)-(S)-*prolyl*]-(RS)-*pyrrolidine*-2-*acetic acid methyl esters* (V). A solution of the foregoing racemic ester (0·4 g) in methylene chloride (20 ml) was treated with *N*-TFA-(−)-(*S*)-prolyl chloride as described above to yield the diastereoisomeric mixture of dipeptides as a gum, *m/e* 336 (M^+, 3%), 305 (2), 279 (5), 263 (4), 239 (2), 208 (5), 198 (2), 194 (3), 170 (10), 167 (20), 166 (100), 149 (60), 142 (30), 129 (10), 128 (95), 113 (10), 111 (10), 97 (20), 95 (10), 85 (20), and 70 (50).

1-[N-*Trifluoroacetyl*-(−)-(S)-*prolyl*]-(S)-*pyrrolidine*-2-

[20] T. R. Govindachari, B. R. Pai, and K. Nagarajan, *J. Chem. Soc.*, 1954, 2801.

[21] L. Mandell and E. C. Roberts, *J. Heterocyclic Chem.*, 1965, **2**, 479.

acetic Acid Methyl Ester.—(a) (−)-(S)-1-*Benzylpyrrolidine*-2-*methanol* (VIb). A solution of (−)-(*S*)-pyrrolidine-2-methanol [22] (VIa) (10 g) and benzyl chloride (16 g) in toluene(100 ml) was refluxed under nitrogen with anhydrous potassium carbonate (10 g) with stirring for 16 h. Dilute hydrochloric acid was added till the aqueous layer was strongly acidic. The aqueous layer was separated, shaken with ether, cooled, basified with ammonium hydroxide, and extracted with methylene chloride. The product was chromatographed over silica gel in benzene–methylene chloride to yield (−)-(S)-1-*benzylpyrrolidine*-2-*methanol* (11 g) as a viscous oil, b.p. 115—120° at 0·5 mmHg, $[\alpha]_D$ −59·5° (Found: C, 75·0; H, 9·3. $C_{12}H_{17}NO$ requires C, 75·4; H, 9·0%).

(b) (S)-1-*Benzylpyrrolidine*-2-*acetonitrile* (VId). A solution of the foregoing pyrrolidinemethanol (11 g) in chloroform (100 ml) containing pyridine (0·3 ml) was treated with thionyl chloride (8 ml). The solution was heated at 45—50° for 3 h, evaporated *in vacuo*, flushing with benzene. The residual solid (VIc) was dissolved in dimethylformamide (70 ml) and heated for 5 h at 60—70° with stirring with a solution of potassium cyanide (14 g) and mercury(II) cyanide (2 g) in water (70 ml). The solution was diluted with water and extracted with methylene chloride to give a dark oil which was chromatographed over silica gel in benzene. The column was eluted with benzene and benzene–chloroform (1 : 1) to give an oil, homogeneous by t.l.c. Distillation of this oil *in vacuo* gave the *nitrile* (9 g) as an oil, b.p. 125—130° at 0·5 mmHg, $[\alpha]_D$ −73·8°, $\nu_{max.}$ (neat) 2250 cm^{-1} (C≡N) (Found: C, 77·8; H, 7·9. $C_{13}H_{16}N_2$ requires C, 78·0; H, 8·1%), *m/e* 200 (M^+), 160 ($M - CH_2CN$), and 91 ($C_6H_5{\cdot}CH_2$).

Racemic 1-benzylpyrrolidine-2-methanol and 1-benzyl-2-chloromethylpyrrolidine have been reported in the literature.[23]

(c) (S)-1-*Benzylpyrrolidine*-2-*acetic acid methyl ester* (VIe). A solution of the foregoing nitrile (9 g) in methanol (150 ml) was saturated with dry hydrogen chloride gas at 0° and then left overnight at room temperature. The solution was evaporated *in vacuo*, poured on ice, basified with ammonia, and extracted with methylene chloride to give the *ester* (8 g) which was distilled *in vacuo*, b.p. 130° at 1·5 mmHg, $[\alpha]_D$ −67·8, $\nu_{max.}$ (neat) 1740 cm^{-1} (ester) (Found: C, 71·9; H, 8·6. $C_{14}H_{19}NO_2$ requires C, 72·1; H, 8·2%).

(d) *Methyl* (S)-*pyrrolidine*-2-*acetate* (VIf). A solution of the foregoing ester (8 g) in glacial acetic acid (100 ml) was shaken with hydrogen at 35—40 lb in^{-2} for 8 h at 35—40° in a Parr apparatus in the presence of 10% palladium–charcoal catalyst (3 g). The solution was filtered, evaporated *in vacuo*, diluted with water, basified with ammonia, and extracted with methylene chloride. Chromatography of the product over silica gel in chloroform yielded some starting ester (3 g) in the earlier fractions. The later fractions, eluted by chloroform–methanol (95 : 5), gave the debenzylated *ester* (2·5 g) which was distilled at 100° (bath temperature) at 1 mmHg, $[\alpha]_D$ +3·4° (Found: C, 58·5; H, 9·4. $C_7H_{13}NO_2$ requires C, 58·7; H, 9·2%), *m/e* 143 (M^+) and 70 ($M - CH_2CO_2Me$).

(e) (S)-*Pyrrolidine*-2-*acetic acid* (VIg) *hydrochloride*. The foregoing ester (0·9 g) was refluxed for 4 h with 6N-hydrochloric acid (70 ml). The solution was evaporated *in vacuo*, and the residue was dried and crystallised from acetone–methanol–ether to give the hydrochloride (0·5 g), m.p. 175—176°, $[\alpha]_D^{28}$ +19·3° (water, *c* 1·74), $\nu_{max.}$ (KBr) 1720 cm^{-1} (Found: C, 43·8; H, 7·4. $C_{16}H_{12}ClNO_2$ requires C, 43·5; H, 7·3%).

(f) 1-[N-*Trifluoroacetyl*-(−)-(S)-*prolyl*-(S)-*pyrrolidine*-2-*acetic acid methyl ester* (V). A solution of the ester (VIf) (0·3 g) in methylene chloride (20 ml) was treated with *N*-TFA-(−)-(S)-prolyl chloride as described above to yield the dipeptide as a gum.

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