Absolute Stereochemistry of Ancistrocladine and Ancistrocladinine †

By **Tuticorin R. Govindachari,*** **Kuppuswamy Nagarajan, Papagudi C. Parthasarathy, Tuticorin G. Rajagopalan,** and **Haridutt K. Desai,** CIBA-Geigy Research Centre, Goregaon, Bombay 400063, India
Gopinath Kartha, Center for Crystallographic Research, Roswell Park Memorial Institute, Buffalo, New York 14203, U.S.A.
Sow-mei Lai Chen and **Koji Nakanishi,** Department of Chemistry, Columbia University, New York 10027, U.S.A.

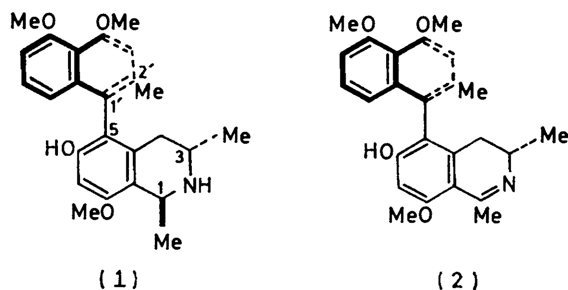
Evidence for the absolute stereochemistry of ancistrocladine [(1*S*,3*S*)-5(*R*)-(4,5-dimethoxy-2-methyl-1-naphthyl)-1,2,3,4-tetrahydro-8-methoxy-1,3-dimethylisoquinolin-6-ol] (1) and ancistrocladinine[(3*S*)-5(*R*)-(4,5-dimethoxy-2-methyl-1-naphthyl)-3,4-dihydro-8-methoxy-1,3-dimethylisoquinolin-6-ol] (2), based on a study of n.m.r., *X*-ray, c.d., and chemical data, is presented.

Ancistrocladine and ancistrocladinine are novel isoquinoline alkaloids isolated from the roots of *Ancistrocladus heyneanus* Wall. (Ancistrocladaceae), for which gross structures were assigned [1,2] on the basis of degradation experiments and spectroscopic studies of the derived products.

† Presented in part at the Third Indo–Soviet Symposium on the Chemistry of Natural Products, Tashkent, October 1973.

[1] T. R. Govindachari and P. C. Parthasarathy, *Tetrahedron*, 1971, **27**, 1013.
[2] T. R. Govindachari, P. C. Parthasarathy, and H. K. Desai, *Indian J. Chem.*, 1971, **9**, 1421.

This paper presents evidence for the absolute stereochemistry of ancistrocladine and ancistrocladinine as depicted in formulae (1) and (2), respectively. Analysis



of 100 MHz n.m.r. spectra of *O*-methylancistrocladine [1] and isoancistrocladine, prepared [2] by reduction of ancistrocladinine with $NaBH_4$, enabled the assignment of *trans*-stereochemistry for the 1- and 3-methyl substituents in ancistrocladine and *cis* in isoancistrocladine with the 3-methyl groups possessing equatorial conformation in both compounds. In isoancistrocladine, 1-H showed a long-range coupling (1 Hz) with 4-H besides a vicinal coupling (7 Hz) with 1-Me at δ 4·32. This would be expected only when the 1-methyl group in isoancistrocladine is equatorial since the axial 1-H will be ideally placed [3] for homoallylic coupling with the 4-axial proton. However, in *O*-methylancistrocladine, 1-H showed only a vicinal coupling [δ 4·42 (q, *J* 7 Hz)] and no long-range coupling with 4-H. On irradiation of the 3-methyl group [δ 0·93 (d, *J* 7 Hz)] in *O*-methylancistrocladine, the multiplet at δ 3·12 due to 3-H simplified to a doublet of doublets with *J* 4·5 and 10 Hz. Likewise in isoancistrocladine the multiplet at δ 2·70 of 3-H became a doublet of doublets (*J* 4·5 and 10 Hz) on irradiation of the 3-methyl group at δ 0·93 (d, *J* 7 Hz). This clearly suggests that 3-H in both *O*-methylancistrocladine and isoancistrocladine must be axial and hence the 3-methyl group is equatorial in both. Therefore in *O*-methylancistrocladine and hence in ancistrocladine the 1- and 3-methyl substituents are *trans*. The foregoing assignment is also in accord with the spectral analysis of the *cis*- and *trans*-isomers of 1,3-dimethyl-6,7-methylenedioxytetrahydroisoquinoline (see Experimental section).

X-Ray diffraction techniques on the hydrobromide of ancistrocladine confirmed the gross structure assigned earlier [1] to ancistrocladine and also the relative stereochemistry of the two methyl groups at C(1) and C(3) deduced from n.m.r. data. More important, the *X*-ray work established the relative stereochemistry of the methyl groups at C(2′) and C(1). The hydrobromide of ancistrocladine formed monoclinic crystals from methanol, space group C_2 with $a = 41{\cdot}06$, $b = 7{\cdot}03$, $c = 8{\cdot}9$ Å, and $\beta = 93{\cdot}4°$. The unit cell contained four molecules. Three dimensional *X*-ray diffraction data were collected to a Bragg angle of 75° using Cu-K_α radiation on a G.E. 490 automatic diffractometer by the stationary crystal, stationary counter method. 2460 Non-equivalent reflections were measured of which 54% had intensities considered observed. The fall-off in scattered intensity in the Bragg angle was rather large corresponding to an overall value of 6·5 for the thermal parameter *B*. The diffraction intensities were placed on an absolute scale by the usual methods and the structure was solved by direct phasing methods.[4] Phases were assigned to a number of ($h0l$) type reflections using SIGMA 1 relations. In addition, the phase of (30 0 5) and (1 1 2) reflections were fixed to define the origin. The reflections (27 1 $\bar{8}$) and (1 3 7) were given permuting starting phases ±45 and ±135°. These phases were used as starting phases to develop eight phase sets for 353 reflections of *E* greater than 1·4 using the tangent refinement method.[5] The electron density map corresponding to the largest consistent set was computed and this showed possible positions for the bromine atoms as well as 25 carbon atoms in the molecule of ancistrocladine. Iterative cycles of structure factor calculation followed by least squares refinement and electron density calculation revealed all the non-hydrogen atoms in the molecule.

During the refinement process, it became evident that the bromine atom was disordered with two possible sites, the occupancy of one site being twice the other. The agreement index at this stage (for 1729 reflections) was 0·16. The molecule is shown in Figure 1, and the

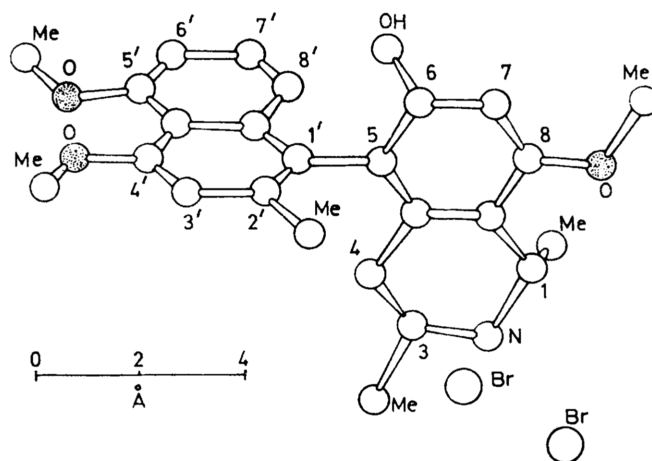


FIGURE 1 The molecular structure of ancistrocladine hydrobromide

observed and calculated structure factors are listed in Supplementary Publication No. SUP 20995 (3 pp.).* The planes of the phenyl and naphthalene rings are at an angle of 87° and the exciton chirality method [6] using split-type Cotton effects was particularly useful in defining the absolute configuration at this chiral site. The shape and amplitude of the u.v. spectrum (Figure 2)

* For details of Supplementary Publications see Notice to Authors No. 7 in *J.C.S. Perkin, I* 1972, Index issue.

[3] D. W. Cameron, D. G. I. Kingston, N. Sheppard, and A. Todd, *J. Chem. Soc.*, 1964, 98.

[4] G. Germain, P. Main, and M. M. Woolfson, *Acta Cryst.*, 1971, **A27**, 368.

[5] J. Karle, *Acta Cryst.*, 1968, **9**, 635.

[6] N. Harada and K. Nakanishi, *Accounts Chem. Res.*, 1972, **5**, 257.

of the isoquinoline (Figure 3), derived [1] from *O*-methylancistrocladine by dehydrogenation, indicate that it consists of overlapping naphthalene and isoquinoline chromophores. The intense shorter wave-length absorptions at 232 and 245 nm with transition moments along the long axes of the nuclei ($^1A \longrightarrow {}^1B_b$) interact to give an exciton-split c.d. spectrum (Figure 2) with extrema of $\Delta\varepsilon_{245} - 91{\cdot}0$ and $\Delta\varepsilon_{225} + 25{\cdot}1$ (in EtOH). This is similar to the bianthryl case treated by Grinter and Mason.[7] The negative first Cotton effect indicates that the chirality of the long axes is negative as shown in Figure 3, and this leads to the absolute configuration (1) for ancistrocladine, since the relative stereochemistry of the methyl groups at C(1) and C(2′) and at C(1) and C(3) have been established from n.m.r. and *X*-ray studies.

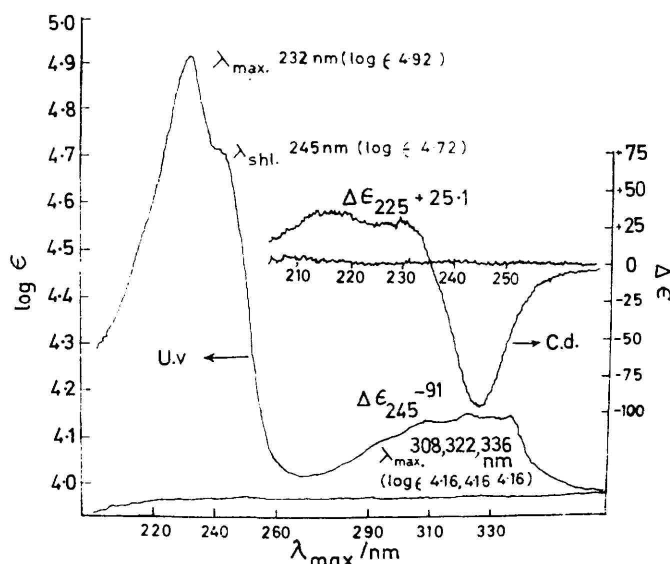


FIGURE 2 U.v. and c.d. spectra of the isoquinoline derived from *O*-methylancistrocladine by dehydrogenation

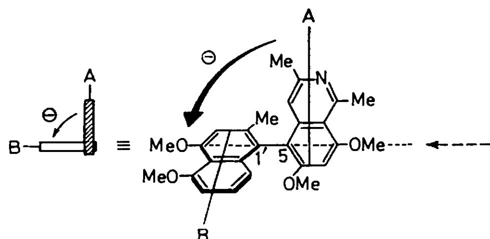
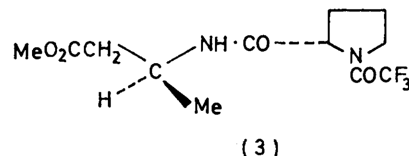


FIGURE 3 The isoquinoline derived from *O*-methylancistrocladine; chirality is viewed from the direction of the dotted arrow

The correctness of the exciton chirality method [6] for derivation of the absolute configuration of ancistrocladine in respect of the dissymmetry arising from the restricted rotation around the C(5)–C(1′) bond was unambiguously established in the following manner. Extensive ozonolysis of ancistrocladine in 10% aqueous formic acid followed by exhaustive purification of the mixture of amino-acids gave (+)-L-β-amino-n-butyric acid * hydrochloride, $[\alpha]_D$ +28·86° (*c* 1·76 in H_2O). The identity of the amino-acid was further established by preparation [9] of the *N*-trifluoroacetyl-L-prolyl peptide methyl ester (3) which had the same retention time



(20·1 min) on g.l.c. as the peptide methyl ester obtained from (+)-L-β-amino-n-butyric acid. It is pertinent to note that the corresponding peptide methyl ester derived from (±)-β-amino-n-butyric acid on g.l.c. had retention times of 17·1 and 20·1 min for the -(−)D- and (+)-L-isomers, respectively. This proves the absolute configuration at C(3) is *S* in ancistrocladine. The *X*-ray determination of structure had established the relative configuration of the molecule with the methyl groups at C(2′) and C(3) on the same side of the general plane of the isoquinoline ring and the C(1)-methyl group on the other side. It therefore follows that the chirality of C(1) is *S*. The total absolute assignment of the structure of ancistrocladine can therefore be depicted as in (1).

Since ancistrocladinine, on reduction with zinc and aqueous sulphuric acid, gave ancistrocladine together with isoancistrocladine, ancistrocladinine, and isoancistrocladine must be represented by structure (2) and [1 (with C-1 methyl α)], respectively.

EXPERIMENTAL

U.v. spectra were taken in 95% ethanol on a Beckman DK 2A spectrophotometer. Rotations were taken in $CHCl_3$ at 25° unless otherwise noted. I.r. spectra were recorded on a Perkin-Elmer model 421 spectrophotometer. N.m.r. measurements were made for $CDCl_3$ solutions on a Varian A-60 or HA-100-D spectrometer with Me_4Si as internal standard. Merck silica gel was used for t.l.c. and column chromatography, and for visualisation the developed t.l.c. plates were sprayed with a 1% solution of vanillin in aq. H_2SO_4 (1 : 1) and heated to 110° for 5 min. G.l.c. analyses were carried out on a 183 × 0·2 cm (i.d.) glass column with a Varian Aerograph model 2740 instrument equipped with a flame ionisation detector and using nitrogen as the carrier gas. The stationary phase was 3% OV-17 with a column support of GasChrom-Q (mesh 80—100, Applied Science Laboratories, State College, Penn., U.S.A.). The temperature settings were column oven 146°, injection port 260°, and detector 305°. Typical gas pressures were nitrogen 21·6 (column head pressure), air 25, and hydrogen 25 lb in^{-2}.

cis-*1,3-Dimethyl-6,7-methylenedioxy-1,2,3,4-tetrahydroisoquinoline*.— 1,3-Dimethyl-6,7-methylenedioxy-3,4-dihydroisoquinoline (0·2 g) dissolved in methanol (5 ml) was

* An authentic sample of -(+)L-β-amino-n-butyric acid made [8] from (+)-L-α-alanine had $[\alpha]_D$ + 25·05° (*c* 2·03 in 1N-HCl).

[7] R. Grinter and S. F. Mason, *Trans. Faraday Soc.*, 1964, **60**, 274.

[8] K. Balenović, D. Cerar, and Z. Fuks, *J. Chem. Soc.*, 1952, 3316.

[9] B. Halpern and J. W. Westley, *Biochem. and Biophys. Res. Comm.*, 1965, **19**, 361.

treated with sodium borohydride (0·2 g) during 0·5 h to afford the *tetrahydroisoquinoline* as white needles (0·15 g) (from hexane), m.p. 60—62°, δ (100 MHz), 1·21 (3H, d, *J* 6 Hz, 3-Me), 1·40 (3H, d, *J* 7 Hz, 1-Me), 1·80 (1H, s, NH), 2·50 (2H, m, 4-H_2), 2·98 (1H, m, 3-H), 4·08 (1H, oct, $J_{1\text{-H},4\text{-H}ax} \cong 1$, $J_{1\text{-H},1\text{-Me}}$ 7 Hz, 1-H), 5·85 (2H, s, OCH_2O), 6·51 (1H, s, 5- or 8-H), and 6·66 (1H, s, 5- or 8-H). Irradiation of 3-Me resulted in the appearance of 3-H as a doublet of doublets with *J* 4·5 and 10 Hz (Found: C, 70·25; H, 7·35. $C_{12}H_{15}NO_2$ requires C, 70·25; H, 7·3%).

trans-1,3-*Dimethyl*-6,7-*methylenedioxy*-1,2,3,4-*tetrahydroisoquinoline*.— 1,3-Dimethyl-6,7-methylenedioxy-3,4-dihydroisoquinoline (5 g) was dissolved in aq. H_2SO_4 (2N; 130 ml). To the well-stirred solution at 60—70°, zinc dust (4 g) was added in portions. After 1 h, more aq. H_2SO_4 (2N; 130 ml) was added followed by zinc dust (8 g) added in small quantities during 3 h. The mixture was left overnight and basified with aq. NaOH and extracted repeatedly with ether. The residue from the ether extract on examination by t.l.c. showed two spots of which one had the same R_F value as the *cis*-isomer. The residue was chromatographed on alumina (250 g) and eluted with benzene. Initial fractions contained the *cis*-isomer (2·45 g) and later fractions gave, on crystallisation from hexane, the trans-*tetrahydroisoquinoline* (1·55 g) as needles, m.p. 75—78°, δ (100 MHz) 1·17 (3H, d, *J* 6·5 Hz, 3-Me), 1·38 (3H, d, *J* 7 Hz, 1-Me), 1·52 (1H, s, NH), 2·25 (1H, dd, *J* 10 and 17 Hz, 4-H_{ax}), 2·67 (1H, dd, *J* 4·5 and 17 Hz, 4-H_{eq}), 3·25 (1H, m, 3-H), 4·08 (1H, q, *J* 7 Hz, 1-H), 5·85 (2H, s, OCH_2O), 6·50 (1H, s, 5- or 8-H), and 6·55 (1H, s, 5- or 8-H). Irradiation of 3-Me resulted in the emergence of the 3-H multiplet as a doublet of doublets with *J* 4·5 and 10 Hz (Found: C, 69·9; H, 7·5. $C_{12}H_{15}NO_2$ requires C, 70·25; H, 7·3%).

Isoancistrocladine.—Ancistrocladinine (0·5 g) dissolved in methanol (40 ml) was treated slowly at room temperature with $NaBH_4$ (0·5 g) to yield isoancistrocladine (0·3 g), as pale yellow needles from methanol, m.p. 235—236°, $[\alpha]_D$ +61·83° (*c* 1·76), $\lambda_{max.}$ 230, 293, 306, 320, and 335 nm (log ε 4·79, 4·01, 4·04, 3·94, and 3·87), δ (100 MHz) 0·93 (3H, d, *J* 7 Hz, 3-Me), 1·48 (3H, d, *J* 7 Hz, 1-Me), 1·85 (2H, m, 4-H_2), 2·17 (3H, s, 2′-Me), 2·70 (1H, m, 3-H), 4·32 (1H, oct, $J_{1\text{-H},4\text{-H}ax}$ 1, $J_{1\text{-H},1\text{-Me}}$ 7 Hz, 1-H), 6·50 (1H, s, 3′- or 7-H), 6·76 (1H, dd, *J* 2·5 and 9 Hz, 6′- or 8′-H), 6·80 (1H, s, 3′- or 7-H), and 7·20 (1H, dd, *J* 8 and 9 Hz, 7′-H). Upon irradiation of 3-Me, the 3-H multiplet became a doublet of doublets, *J* 4·5 and 10 Hz (Found: C, 74·05; H, 7·55. $C_{25}H_{29}NO_4$ requires C, 73·7; H, 7·15%).

Reduction of Ancistrocladinine with Zinc and Sulphuric Acid.—Ancistrocladinine (1·5 g) dissolved in aq. H_2SO_4 (2N; 50 ml) was heated to 70° on a water-bath and treated slowly with zinc dust (5 g) added during 3 h. More zinc (4 g) was added after addition of aq. H_2SO_4 (50 ml). The mixture was left overnight, basified in the cold with aq. sodium hydroxide, and extracted repeatedly with chloroform. Fractional crystallisation of the residue from methanol gave isoancistrocladine, m.p. 234°, undepressed on admixture with the previous sample, and ancistrocladine, m.p. 264—267° (decomp.), identical with an authentic specimen of ancistrocladine.

(+)-L-β-*Amino-n-butyric Acid from Ancistrocladine*.— Ancistrocladine (14 g) dissolved in aq. formic acid (300 ml; 10%) was cooled in ice and dry ozonised oxygen was rapidly bubbled in for 20 h. To this solution was added hydrogen peroxide (30%; 75 ml) and formic acid (98%; 75 ml) and the mixture was heated on a boiling water-bath for 3 h. Platinum black (2 g) was then added and the mixture was heated on a water-bath at 60° for 1 h to destroy the excess of peracid. The mixture was cooled, filtered, and the filtrate extracted with chloroform. The aqueous layer was evaporated to dryness *in vacuo* when a dark brown gummy residue A (4·75 g) was obtained. The residue A (2 g) dissolved in distilled water (20 ml) was passed over a column of Dowex 50W-X8 (1·5 × 30 cm). The column was washed with water until the washings gave no residue on evaporation. The column was then washed with aq. ammonia (1N) and the washings were evaporated to dryness *in vacuo*. This gave a dark brown residue B (0·5 g). Residue B was treated with glacial acetic acid (2 ml) and dried *in vacuo*, to yield the crude residue C, which on t.l.c. (phenol–water 3 : 1) showed two spots (sprayed with ninhydrin) corresponding to α-alanine and β-amino-n-butyric acid. The latter was isolated by ion-exchange chromatography according to the procedure [10] of Stein and Moore. The foregoing residue C (0·5 g) was dissolved in aq. hydrochloric acid (0·1N; 2 ml) and a 1 ml aliquot portion was applied to a column (1 × 110 cm) of Dowex 50-XB (200—400 mesh) in the H^+ form. The column was maintained at 50° and eluted with 1N-HCl at the rate of 25 ml h^{-1}. Fractions (3 ml) were collected and the amino-acids were located by t.l.c. of a small aliquot portion in phenol–water (3 : 1; w/w) followed by spraying with ninhydrin. Fractions 100—110 were homogeneous on t.l.c. and had the same R_F as authentic β-amino-n-butyric acid; these were combined and lyophilised to dryness. The residue (49 mg) in water had $[\alpha]_D$ +28·86° (*c* 1·76 in H_2O). The remaining 1 ml aliquot part was processed as above and the combined residue was used for liberation of the free amino-acid.

The foregoing residue (82 mg) dissolved in water (10 ml) was passed through a short column (2 × 6 cm) of Amberlite IR-4B(OH) resin and the washings (25 ml) were evaporated to dryness *in vacuo* at 20°. The residue (48 mg) was crystallised from dry methanol when prisms of (+)-L-β-amino-n-butyric acid separated, m.p. 218—219° (decomp.), undepressed on admixture with an authentic sample [8] of -(+)L-β-amino-n-butyric acid. The i.r. and mass spectra of the two samples were identical (Found: C, 46·5; H, 9·0. Calc. for $C_4H_9NO_2$: C, 46·6; H, 8·8%).

N-*Trifluoroacetyl*-L-*prolyl Peptide Methyl Ester of* (±)-β-*Amino-n-butyric Acid*.—Thionyl chloride (0·4 ml) in a 50 ml flask provided with a guard tube was cooled to −5° and was then treated with methanol (2 ml) added in three portions during 15 min. After cooling the mixture for 15—20 min at −5°, (±)-β-amino-n-butyric acid (0·7 g) was added slowly during 20 min. The mixture was left at −5° for 15 min and then at 40° for 2 h. Methanol was removed under reduced pressure and water (8 ml) was added to the residue. The aqueous solution was extracted with ether, basified with ammonia to pH 9, saturated with common salt, and extracted with ether (3 × 30 ml). The ether extract was dried (Na_2SO_4) and evaporated at low temperature under reduced pressure. A yellow syrupy residue (0·6 g) was obtained. To this residue a solution of *N*-trifluoroacetyl-L-prolyl chloride [11] (0·45 g) was added in methylene chloride (4·5 ml) cooled in ice. The mixture was left for 10 min and carefully basified with triethylamine.

[10] W. H. Stein and S. Moore, Cold Spring Harbor Symposia, *Quant. Biol.*, 1950, **14**, 179.
[11] W. A. Bonner, *J. Chromat. Sci.*, 1972, **10**, 159.

The solvent was evaporated off *in vacuo* and the residue was washed with water after extraction with ether. Evaporation of the dried ether layer gave a gummy residue (0·25 g) which slowly crystallised from ether as needles, m.p. 128—132°, $\nu_{max.}$ (KBr) 1645, 1700 (amide CO), and 1728 cm^{-1} (CO_2Me). On g.l.c. this compound exhibited two peaks having retention times of 17·1 and 20·1 min corresponding to the (−)-D- and(+)-L-isomers, respectively.

N-*TFA*-L-*Pro*-L-β-*amino-n-butyric Acid Methyl Ester.*—This was made by the same procedure as described above for the (±)-isomer from authentic[8] (+)-L-β-amino-n-butyric acid (200 mg). The product on g.l.c. showed a peak having a retention time of 20·1 min.

N-*TFA*-L-*Pro*-L-β-*amino-n-butyric Acid Methyl Ester from Ancistrocladine.*—The crude product obtained from the amino-acid (39 mg) derived from ancistrocladine was purified in the following manner: a 20 × 20 cm t.l.c. plate was divided into halves. The foregoing gum was dissolved in methylene chloride (1 ml) and 0·3 ml of this solution was applied to one half of the plate. Authentic *N*-TFA-L-Pro-DL-β-amino-n-butyric acid methyl ester (0·3 mg) dissolved in methylene chloride (0·3 ml) was spotted on the other half. The plate was developed in benzene–methanol (90 : 10, v/v) and dried in a current of air at room temperature. The two halves of the plate were divided into 1 cm bands from the origin to the solvent front and the silica gel from each band was scraped off and transferred to a 15 ml conical centrifuge tube. The silica gel was then extracted three times using methanol (1 ml). The combined methanol extract from each 1 cm section was dried at 37° in a current of N_2 and was analysed on g.l.c. The compound extracted from the 1 cm section from the reference side of the plate having R_F 0·3—0·4 on analysis by g.l.c. exhibited two peaks with retention times 17·1 and 20·1 min corresponding to the (−)-D- and (+)-L-isomers, respectively. The corresponding section taken from the other half of the plate having R_F 0·3, on analysis by g.l.c. showed only one peak at 20·1 min, having the same retention time as the (+)-L-isomer.

We thank Dr. H. Fuhrer for the 100 MHz n.m.r. spectra and decoupling experiments, Mr. C. T. Lu for help in collecting the intensity data, the National Institute of Health and the National Science Foundation for grants in support, Dr. S. Selvavinayakam and his associates for the analytical and spectral data, and Mr. K. S. Ramachandran for stimulating discussions.

[3/2408 *Received, 26th November*, 1973]