Polypeptides. Part XI.[1] Tetrazole Analogues of the *C*-Terminal Tetrapeptide Amide Sequence of the Gastrins

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The synthesis is described of two analogues of *N*-benzyloxycarbonyl-L-tryptophyl-L-methionyl-L-aspartyl-L-phenylalanine amide (a physiologically active derivative of the *C*-terminal tetrapeptide amide sequence of the gastrins) wherein the aspartyl β-carboxy-group or the terminal carboxamide group of the tetrapeptide amide are replaced by a tetrazol-5-yl residue. The optically active amino-acid analogues (carboxy replaced by tetrazol-5-yl) required in these syntheses were prepared without special difficulties, and they could be utilised in peptide synthesis without protection of the tetrazole unit.

OUR investigation of structure–function relationships in the gastrin series involved the synthesis [2] of a large number of analogues of the *C*-terminal tetrapeptide amide sequence, Trp-Met-Asp-Phe-NH_2.† Physiological activity was associated with certain analogues derived by change of the Trp, Met, and Phe positions, but analogues derived by change of the Asp position were all devoid of activity.[3,4] We concluded that the Trp, Met, and Phe residues are concerned with binding at the site of hormonal action, whereas the Asp residue fulfils a 'functional' role.[3,4] The term 'functional' is used to imply direct participation in the chemical event associated with the action of the hormone, and we were attracted to the possibility of the involvement of the aspartyl β-carboxy-group in a proton- or charge-transfer reaction. Assuming this to be so, we considered the circumstances under which physiological activity might be preserved in an analogue involving change at the Asp residue. The replacing residue would need to have dimensions similar to those of the Asp residue, and a degree of proton availability (acidity) similar to that of the β-carboxy-group. In addition there would need to be flexibility in the derived anion such that charge localisation can occur at the same site that is involved in the action of the β-carboxylate ion. These exacting requirements are met in the tetrazolyl analogue (II) which has a tetrazol-5-ylmethyl side chain in place of the aspartyl carboxymethyl side chain of the parent benzyloxycarbonyltetrapeptide amide (I). It is well known that the acidity of the tetrazole nucleus corresponds closely with that of a peptide carboxylic acid.[5] Moreover, inspection of molecular models indicated a close spatial similarity between the tetrazolyl and carboxylate ions.[6] The tetrazolyl analogue was accordingly synthesised and the present paper gives details of the synthesis. For other purposes the related analogue (III), wherein the *C*-terminal carboxamide group of the tetrapeptide amide is replaced by a tetrazol-5-yl residue was also prepared; details of this synthesis are also given.

Tetrazole analogues of glycine, DL-alanine, β-alanine, DL-phenylalanine, and DL-tryptophan have been described by McManus and Herbst,[6] and found, as expected, to have apparent pK_a values agreeing closely with those of the corresponding amino-acid. However, the incorporation of tetrazole analogues of amino-acids into peptides has not hitherto been described. Our results are of general interest in indicating, (*a*) that no special problems arise in the synthesis of optically active tetrazole analogues of amino-acids, and (*b*) that they may be incorporated into peptides without protection of the acidic tetrazole unit, in much the same way that is successful in the synthesis of peptides with unprotected carboxy-residues. This is illustrated by reference to the synthesis (Scheme 1) of the analogue (III), which has an L-5-α-aminophenethyl-1(2)*H*-tetrazole residue in place of the L-phenylalanine amide of the gastrin tetrapeptide amide.

The use of either phthaloyl or benzyloxycarbonyl groups for amino-protection was found satisfactory in the preparation of optically active tetrazole analogues of phenylalanine. Phthaloyl-L-phenylalanine amide (IV) (see Experimental section for various preparations of this compound) or benzyloxycarbonyl-D- or L-phenylalanine amide (VIII) were smoothly converted into the corresponding nitriles, (V) or (IX), by means of phosphoryl chloride in pyridine,[7] and reaction of these with sodium azide and ammonium chloride in dimethylformamide gave the L-phthaloyltetrazole (VI) and the D- and L-benzyloxycarbonyltetrazoles (X) in high yield. Treatment of the L-phthaloyltetrazole (VI) with hydrazine hydrate (2 mol.) in ethanol, or hydrogenolysis of the L-benzyloxycarbonyltetrazole (X; L-isomer) in aqueous acetic acid or in methanol and cyclohexylamine (2 mol.), gave L-5-α-aminophenethyl-1(2)*H*-tetrazole (VII; L-isomer), the tetrazole analogue of

† The abbreviations for amino-acid residues and protecting groups and their mode of use are in accordance with the suggestions of the Committee on Nomenclature which reported at the Fifth European Peptide Symposium, Peptides Proc. Fifth European Peptide Symp., ed. G. T. Young, 1963, Pergamon, London, p. 261) with the modifications adopted by I.U.P.A.C. (Inform. Bull., I.U.P.A.C., **1966**, no. 25, p. 32).

[1] Part X, H. Gregory and J. S. Morley, *J. Chem. Soc.* (*C*), 1968, 910.

[2] Described in parts VI—X of this series (see part X for references to the earlier parts).

[3] J. S. Morley, H. J. Tracy, and R. A. Gregory, *Nature*, 1965, **207**, 935.

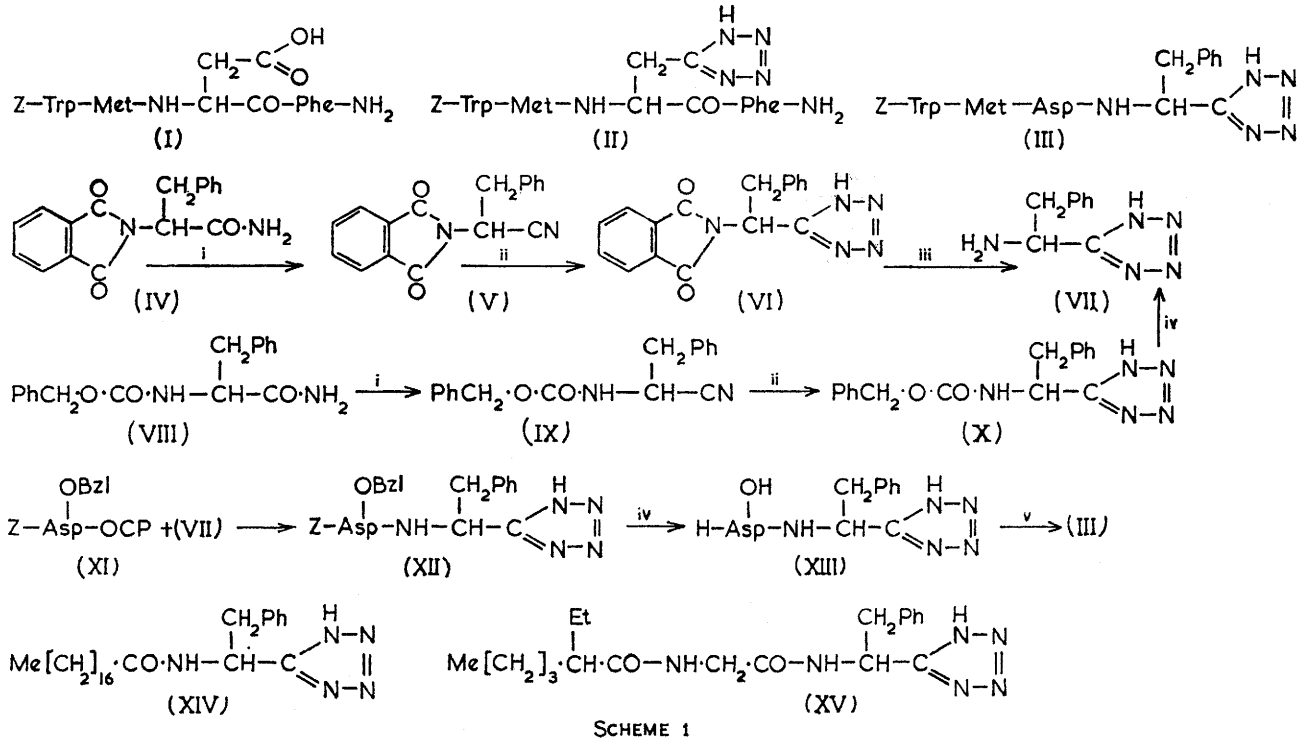
[4] J. S. Morley, *Proc. Roy. Soc.*, 1968, *B*, **170**, 97.

[5] J. M. McManus and R. M. Herbst, *J. Org. Chem.*, 1959, **24**, 1643.

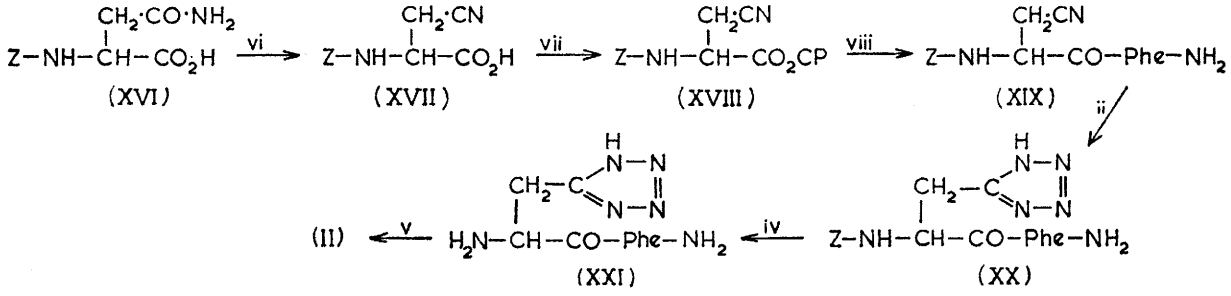
[6] J. S. Morley, *Fed. Proc.*, 1968, **27**, 1314.

[7] B. Liberek, A. Nowicka, and J. Szrek, *Roczniki Chem.*, 1965, **39**, 369.

L-phenylalanine. D-5-α-Aminophenethyl-1(2)*H*-tetrazole (VII; D-isomer), corresponding to D-phenylalanine, was similarly obtained by hydrogenolysis of the D-benzyloxycarbonyltetrazole (X; D-isomer). The optical rotation of the L-isomer (VII) prepared by the former route was identical with that of the sample prepared by hydrogenolysis, the dipeptide analogue (XIII). Coupling of this (XIII) with *N*-benzyloxycarbonyl-L-tryptophyl-L-methionine azide [8] in the presence of triethylamine gave the required benzyloxycarbonyltetrapeptide analogue (III). The n-octadecanoyl (XIV) and 2-ethylhexanoylglycyl (XV) derivatives of L-5-α-



SCHEME 1



SCHEME 2

Z = Benzyloxycarbonyl, DCC = *N,N'*-dicyclohexylcarbodi-imide, CP = 2,4,5-trichlorophenyl, DMF = dimethylformamide.
Reagents: i, $POCl_3$–pyridine; ii, NaN_3–NH_4Cl–DMF; iii, hydrazine hydrate–ethanol; iv, H_2–Pd at room temperature and pressure; v, Z-Trp-Met-N_3–Et_3N–DMF; vi, DCC–pyridine; vii, DCC or $POCl_3$ + 2,4,5-trichlorophenol; viii, Phe-NH_2–Et_3N–DMF.

by the latter, and the optical rotations of the D- and L-isomers (VII) were equal and opposite in sign.

There were no complications in the use of these tetrazoles (VII; L- and D-isomers) in peptide synthesis. Reaction of the L-isomer (VII) as its triethylammonium salt with β-benzyl *N*-benzyloxycarbonyl-L-aspartic acid 2,4,5-trichlorophenyl ester (XI) in dimethylformamide gave the protected dipeptide analogue (XII) and thence, aminophenethyl-1(2)*H*-tetrazole were also of interest; they were prepared by treatment of the L-isomer (VII) with stearoyl chloride or with *N*-2-ethylhexanoylglycine 2,4,5-trichlorophenyl ester.

The preparation of the tetrazole analogue (II), in which the aspartyl β-carboxy-group is replaced by a tetrazol-5-yl unit, is shown in Scheme 2. *N*-Benzyloxycarbonyl-L-β-cyanoalanine (XVII) prepared as previously described [8] from *N*-benzyloxycarbonyl-L-asparagine (XVI), was converted into the 2,4,5-trichlorophenyl ester (XVIII),[9] and this coupled smoothly with L-phen-

[8] H. Gregory, A. H. Laird, J. S. Morley, and J. M. Smith, *J. Chem. Soc.* (*C*), 1968, 522.
[9] C. Ressler and H. Ratzkin, *J. Org. Chem.*, 1961, **26**, 2356.

ylalanine amide to give the cyano-dipeptide derivative (XIX). The cyano-derivative (XIX) reacted sluggishly with sodium azide–ammonium chloride, but the tetrazolyldipeptide derivative (XX) was eventually obtained in good yield. Hydrogenolysis of this (XX) in aqueous acetic acid afforded [β-1(2)*H*-tetrazol-5-yl]-L-alanyl-L-phenylalanine (XXI) which, after coupling with *N*-benzyloxycarbonyl-L-tryptophyl-L-methionine azide in the presence of triethylamine, gave the required analogue (II).

When tested in the anaesthetised rat or cat, the analogue (II) was at least as active as the parent benzyloxycarbonyltetrapeptide amide (I) in stimulating gastric acid secretion. This result is in accordance with prediction, and supports our speculation concerning the role of the aspartyl β-carboxy-group in the mode of action of the gastrins.

EXPERIMENTAL

Ascending thin-layer chromatograms were run on Kieselgel G with butan-1-ol–acetic acid–water (4 : 1 : 5 v/v) (R_{FA}), butan-1-ol–acetic acid–water–pyridine (15 : 3 : 12 : 10) (R_{FB}), butan-2-ol–3% ammonium hydroxide (3 : 1) (R_{FC}), acetonitrile–water (3 : 1) (R_{FD}), acetone–chloroform (1 : 1) (R_{FE}), ethanol–chloroform (4 : 1) (R_{FF}), cyclohexane–ethyl acetate (1 : 1) (R_{GG}), cyclohexane–ethyl acetate–methanol (1 : 1 : 1) (R_{FH}), or ethyl acetate (R_{FJ}). Spots were revealed with ninhydrin, sodium hypochlorite–potassium iodide–tolidine,[10] Ehrlich's reagent, by incorporating a fluorescent indicator in the thin layer (Kieselgel GF 254, Merck), or acid potassium permanganate [prepared by dissolving potassium permanganate (100 mg.) in concentrated sulphuric acid (1 ml.) and diluting the solution to 100 ml. with water]. Acid or enzymic hydrolysates of peptide derivatives were prepared with 6N-hydrochloric acid (110°/16 hr.) or leucine aminopeptidase, and the amino-acid composition of the hydrolysates was determined with a Beckman-Spinco Amino-acid Analyser, model 120B. The hydrolysis of tetrazole containing peptides gave tetrazoyl amino-acids which appeared in the amino-acid trace at the following times (long column; elution with buffer pH 3·25 for 90 min. and then with buffer pH 4·28; buffer flow rate 68 ml./hr.; ninhydrin flow rate 34 ml./hr.): [β-1(2)*H*-tetrazol-5-yl]-alanine 45 min., 5-α-aminophenethyl-1(2)*H*-tetrazole 222 min. (Phe 180 min.). Optical rotations were determined with a Bendix NPL Automatic Polarimeter, model 143C, with Digital Converter, model 154C. Organic extracts were dried with anhydrous magnesium sulphate, and evaporations were carried out under reduced pressure in a rotary evaporator. M.ps. were determined in capillary tubes with the Tottoli m.p. apparatus (W. Buchi).

Phthaloyl-L-phenylalanine Amide.—(*a*) L-Phenylalanine amide acetate (56 g., 0·5 mole), phthalic anhydride (40·7 g., 0·55 mole), and acetic acid (125 ml.) were stirred and heated together under gentle reflux for 3 hr. Water (125 ml.) was added and the phthaloyl derivative (133 g., 90%), m.p. 223—224° (lit.,[11] m.p. 226—227·5°), $[\alpha]_D^{25}$ −206° (*c* 0·96 in dimethylformamide), R_{FD} 0·73, R_{FE} 0·57, R_{FF} 0·70, R_{FG} 0·11, R_{FH} 0·62 (Found: C, 69·0; H, 4·8; N, 9·5. Calc. for $C_{17}H_{14}N_2O_3$: C, 69·4; H, 4·8; N, 9·5%), was collected and washed with 50% aqueous acetic acid. N.m.r. [$(CD_3)_2SO$]: τ 2·26 (4H, s, phthaloyl), 2·92 (5H, s, phenyl), 5·12 (1H, 4-line m, CH), 6·6 (2H, m, CH_2).

(*b*) Phthaloyl-L-phenylalanyl chloride [12] (6·02 g) was added at 0° to conc. aqueous ammonia (30 ml.) and the mixture was stirred at 0—5° for 15 min. Water (30 ml.) was then added and stirring was continued at 0—5° for a further 15 min. The solid was collected and washed with water, and gave the product (2·82 g., 53%), m.p. 220—221° (from ethanol), $[\alpha]_D^{25}$ −200° (*c* 1·04 in dimethylformamide).

(*c*) A solution of phthaloyl-L-phenylalanine [12] (29·53 g., 0·1 mole) in dry tetrahydrofuran (200 ml.) was treated at −10° with triethylamine (14 ml., 0·1 mole) followed by pivaloyl chloride (12·3 ml., 0·1 mole). The mixture was stirred at −10 to −5° for 20 min. then saturated at −5 to 0° with dry ammonia and kept at 4° for 18 hr. The solvent was removed and the residue was collected in ice–water and crystallised from ethanol, to give *phthalamoyl-L-phenylalanine amide* (17·4 g., 56%), m.p. 221—222°, R_{FD} 0·71, R_{FE} 0·06, R_{FF} 0·55, R_{FH} 0·43 (Found: C, 65·6; H, 5·5; N, 13·2. $C_{17}H_{17}N_3O_3$ requires C, 65·5; H, 5·5; N, 13·5%), n.m.r. [$(CD_3)_2SO$]: τ 1·45 (1H, d, N*H*·CH), 2·3—3·2 (9H, complex m, aromatic), 5·52 (1H, 8-line m, NH·C*H*), 6·7 (1H, 4-line m, ·*H*CH·), 7·2 (1H, 4-line m, ·HC*H*·). A solution of this phthalamoyl derivative (5 g.) in acetic acid (15 ml.) was boiled under reflux for 23 hr.; phthaloyl-L-phenylalanine amide (3·804 g.), m.p. 214—217°, $[\alpha]_D^{25}$ −180° (*c* 1·01 in dimethylformamide), separated on cooling after the addition of water (25 ml.).

(*d*) Phthalic anhydride (8·14 g., 55 mmoles), L-phenylalanine amide acetate [13] (11·2 g., 50 mmoles), and dioxan (150 ml.) were stirred and heated under gentle reflux for 1·5 hr. N-(2-*Carboxybenzoyl*)-L-*phenylalanine amide* (14·7 g., 91%), m.p. 206—207° (from ethanol), R_{FA} 0·51, R_{FB} 0·72, R_{FC} 0·56, R_{FD} 0·57, R_{FE} 0·0, R_{FF} 0·29, R_{FG} 0·0, R_{FH} 0·19, was collected (Found: C, 65·3; H, 5·0; N, 9·3. $C_{17}H_{16}N_2O_4$ requires C, 65·4; H, 5·16; N, 9·0%), n.m.r. [$(CD_3)_2SO$] similar to that of the phthalamoyl derivative described in (*c*). A solution of this 2-carboxybenzoyl derivative (5·5 g.) in acetic acid (16·5 ml.) was boiled under reflux for 21 hr.; phthaloyl-L-phenylalanine amide (4·4 g., 85%), $[\alpha]_D^{25}$ −168° (*c* 0·91 in dimethylformamide), m.p. 222—223°, separated on cooling after the addition of water (16 ml.).

L-2-*Phenyl*-1-*phthalimidopropionitrile.*—Phosphoryl chloride (58 ml.) in dry methylene dichloride (116 ml.) was added at −5° during 10 min. to a stirred suspension of phthaloyl-L-phenylalanine amide (133 g., 0·45 mole) in dry pyridine (580 ml.). The resulting solution was stirred at −5 to −10° for 1 hr. and then treated with ice–water (1·2 l.). The solid was collected, washed with water, and crystallised from acetic acid (600 ml.), to give the nitrile (104 g., 84%), m.p. 151—152°, R_{FE} 0·87, R_{FF} 0·77, R_{FH} 0·84, $\nu_{max.}$ (mull) 2250w (C≡N) and 1780 and 1720 (phthaloyl) cm.$^{-1}$, $[\alpha]_D^{25}$ −102° (*c* 0·98 in chloroform), −153° (*c* 1·0 in dimethylformamide) {lit.,[11] m.p. 150—153·2°, $[\alpha]_D^{25}$ −103° ± 1° (*c* 2 in chloroform); lit.,[7] m.p. 150—152°, $[\alpha]_D^{21}$ −101° (*c* 1·0 in chloroform)}.

L-5-α-*Phthalimidophenethyl*-1(2)H-*tetrazole.*—A stirred mixture of L-2-phenyl-1-phthalimidopropionitrile (138 g.,

[10] S. C. Pan and J. D. Dutcher, *Analyt. Chem.*, 1956, **28**, 836.
[11] P. E. Peterson and C. Niemann, *J. Amer. Chem. Soc.*, 1957, **79**, 1389.
[12] J. C. Sheehan, D. W. Chapman, and R. W. Roth, *J. Amer. Chem. Soc.*, 1952, **74**, 3822.
[13] J. M. Davey, A. H. Laird, and J. S. Morley, *J. Chem. Soc.* (*C*), 1966, 555.

0·5 mole), sodium azide (36 g., 0·55 mole), ammonium chloride (29·5 g., 0·55 mole), and dimethylformamide (375 ml.) was heated at 92—95° for 6 hr., and then filtered and evaporated (at 0·1 mm.). The residue was stirred for 20 min. with ice–water (500 ml.) and 5N-hydrochloric acid (sufficient to give pH 1), and the solid was then collected, washed with water, and dried *in vacuo* (H_2SO_4). Recrystallisation from ethyl acetate–light petroleum (b.p. 60—80°) gave the *tetrazole* derivative (137 g., 86%), m.p. 181—182°, R_{FD} 0·62, R_{FF} 0·46, R_{FH} 0·23, $[\alpha]_D^{27}$ −219° (*c* 1·04 in dimethylformamide) (Found: C, 64·0; H, 4·1; N, 21·9. $C_{17}H_{13}N_5O_2$ requires C, 64·0; H, 4·1; N, 22·0%).

L- *and* D-1-*Benzyloxycarbonylamino-2-phenylpropionitrile*.—*N*-Benzyloxycarbonyl-L-phenylalanine amide [13] (14·9 g., 50 mmoles) in dry pyridine (62·5 ml.) was treated at −5° during 10 min. with phosphoryl chloride (6·25 ml.) in methylene dichloride (12·5 ml.). The solution was stirred at −10 to −5° for 1 hr., then ice–water (140 ml.) was added. The solid was collected, washed well with water, dried *in vacuo*, and gave the L-*isomer* (11·85 g., 85%), m.p. 132—133° [from propan-2-ol (75 ml.)], R_{FD} 0·77, R_{FE} 0·76, R_{FF} 0·79, R_{FG} 0·62, R_{FH} 0·74, $[\alpha]_D^{27}$ −66·5° (*c* 1·03 in dimethylformamide) (Found: C, 72·4; H, 5·65; N, 10·0. $C_{17}H_{16}N_2O_2$ requires C, 72·8; H, 5·75; N, 10·0%). The D-*isomer* (84% yield), prepared similarly from *N*-benzyloxycarbonyl-D-phenylalanine amide,[13] had m.p. 132—133°, $[\alpha]_D^{27}$ +66° (*c* 0·95 in dimethylformamide (Found: C, 72·7; H, 5·7; N, 10·1%).

L- *and* D-5-α-*Benzyloxycarbonylaminophenethyl*-1(2)H-*tetrazole*.— L-1-Benzyloxycarbonylamino-2-phenylpropionitrile (1·54 g., 5 mmoles), sodium azide (0·36 g., 5·2 mmoles), ammonium chloride (0·295 g., 5·5 mmoles), and dimethylformamide (3·75 ml.) were stirred together at 90—95° for 6 hr. The filtered solution was evaporated at 0·1 mm., and the residue was dissolved in methanol (5—10 ml.). Acidification of the solution to pH 2 with 2N-hydrochloric acid, followed by the addition of water (5—10 ml.), gave the L-*isomer* (1·59 g., 98%), which gave needles, m.p. 181—182° (from methanol), $[\alpha]_D^{25}$ −48° (*c* 0·96 in dimethylformamide), R_{FD} 0·74 (0·67), R_{FE} 0·59 (0·3 tailed to origin), R_{FF} 0·75 (0·60), R_{FH} 0·69 (0·36) (figures in parenthesis represent values obtained when the compound was applied to the plates as its ammonium salt) (Found: C, 63·4; H, 5·35; N, 21·3. $C_{17}H_{17}N_5O_2$ requires C, 63·1; H, 5·3; N, 21·7%). The D-*isomer*, prepared similarly from D-1-benzyloxycarbonylamino-2-phenylpropionitrile, had m.p. 181—182°, $[\alpha]_D^{25}$ +48·4° (*c* 0·99 in dimethylformamide) (Found: C, 63·1; H, 5·4; N, 21·6%).

L- *and* D-5-α-*Aminophenethyl*-1(2)H-*tetrazole*.—(*a*) L-5-α-Phthalimidophenethyl-1(2)*H*-tetrazole (98·8 g., 0·31 mole), ethanol (850 ml.), and 100% hydrazine hydrate (15·1 ml., 0·31 mole) were heated together under reflux for 1 hr. The solid was cooled, collected, washed with ethanol, and then digested at 15—20° with N-hydrochloric acid (1 × 350 ml.; 3 × 80 ml.). The combined filtered digests were neutralised to pH 5 at 5—10° with conc. aqueous ammonia to give the L-*isomer* (35·1 g., 60%), m.p. 291—293° (decomp.), $[\alpha]_D^{27}$ +57·3 (*c* 0·74 in dimethylformamide), +36·7° (*c* 0·8 in N-hydrochloric acid), R_{FA} 0·56, R_{FB} 0·70, R_{FC} 0·37, R_{FD} 0·55, R_{FE} 0·0, R_{FF} 0·39, R_{FG} 0·0, R_{FH} 0·19 (tailed to origin) (Found: C, 57·2; H, 5·85; N, 36·9. $C_9H_{11}N_5$ requires C, 57·1; H, 5·85; N, 37·0%).

(*b*) L-5-α-Benzyloxycarbonylaminophenethyl-1(2)*H*-tetrazole (100 mg.) in 90% aqueous acetic acid (5 ml.) was hydrogenolysed for 4 hr. at room temperature and pressure over 5% palladised charcoal (20 mg.). The L-isomer (48 mg.), m.p. 291—293° (decomp.), $[\alpha]_D^{27}$ +57·5° (*c* 0·79 in dimethylformamide), was obtained after evaporation and collection of the residue in methanol. The D-*isomer*, obtained similarly from D-5-α-benzyloxycarbonylaminophenethyl-1(2)*H*-tetrazole, had m.p. 290—293° (decomp.), $[\alpha]_D^{27}$ −57·2° (*c* 0·74 in dimethylformamide) (Found: C, 56·9; H, 5·7; N, 36·7%).

(*c*) L-5-α-Benzyloxycarbonylaminophenethyl-1(2)*H*-tetrazole (100 mg., 0·31 mmole) in methanol (5 ml.) and cyclohexylamine (35 μl., 0·31 mmole) was hydrogenolysed for 4·5 hr. at room temperature and pressure over 5% palladised charcoal. The residue, obtained by evaporation of the filtered solution, was dissolved in water (0·5 ml.) and the solution was carefully neutralised to pH 5 with acetic acid, to give the L-isomer, m.p. 291—293° (decomp.).

L,L-5-[α-(O-*Benzyl*-N-*benzyloxycarbonylaspartylamino*)-*phenethyl*]-1(2)H-*tetrazole*.— L-5-α-Aminophenethyl-1(2)*H*-tetrazole (3·78 g., 20 mmoles), β-benzyl *N*-benzyloxycarbonyl-L-aspartic acid 2,4,5-trichlorophenyl ester (11·8 g., 22 mmoles), triethylamine (2·8 ml., 20 mmoles), and dimethylformamide (40 ml.) were stirred together at 20—22° for 18 hr. The solution was added to a stirred mixture of N-hydrochloric acid (40 ml.), water (260 ml.), and ether (300 ml.), and the solid was collected and washed with ether (4 times) and water (6 times) to give the LL-*isomer* (8·62 g., 81%), m.p. 183—184° [from methanol (*ca.* 150 ml.)], $[\alpha]_D^{27}$ −25·1° (*c* 1·08 in dimethylformamide), R_{FA} 0·80, R_{FB} 0·86, R_{FC} 0·76, R_{FD} 0·76, R_{FE} 0·61, R_{FF} 0·72, R_{FG} 0·0, R_{FH} 0·68 (Found: C, 63·2; H, 5·5; N, 15·9. $C_{28}H_{28}N_6O_5$ requires C, 63·6; H, 5·3; N, 15·9%).

L-5-(α-L-*Aspartylaminophenethyl*)-1(2)H-*tetrazole*.— The protected derivative just described (5·28 g., 10 mmoles) in acetic acid (150 ml.) and water (15 ml.) was hydrogenolysed for 1 hr. at room temperature and pressure over 5% palladised charcoal (1 g.). Water (15 ml.) was then added and the hydrogenolysis was continued at room temperature and pressure for 2 hr. The filtered (kieselguhr) solution was evaporated, and the residue was shaken with distilled water (100 ml.). The resulting suspension was neutralised to pH 4 with aqueous ammonia and the solid was collected, washed with water and dried, to give the L,L-*isomer* (2·596 g., 85%), m.p. 247—248° (decomp.) (unchanged after recrystallisation from water), $[\alpha]_D^{27}$ −26·2° (*c* 1·01 in dimethylformamide), R_{FA} 0·42, R_{FB} 0·61, R_{FC} 0·11, R_{FD} 0·40, R_{FE}—R_{FH} 0·0 (Found: C, 51·3; H, 5·4; N, 27·5. $C_{13}H_{16}N_6O_3$ requires C, 51·4; H, 5·3; N, 27·6%).

L-5-[α-(N-*Benzyloxycarbonyl*-L-*tryptophyl*-L-*methionyl*-L-*aspartylamino*)*phenethyl*]-1(2)H-*tetrazole*.—A solution of *N*-benzyloxycarbonyl-L-tryptophyl-L-methionine azide [8] [from the hydrazide (4 mmoles) in tetrahydrofuran (8 ml.)] was added at 0° with stirring to a solution of L-5-(α-L-aspartylaminophenethyl)-1-(2)*H*-tetrazole (608 mg., 2 mmoles) in dimethylformamide (5 ml.), triethylamine (1·12 ml., 8 mmoles), and water (0·5 ml.). The mixture was stirred at 4° for 18 hr. and then water (1 ml.) was added. The resulting solution was kept at 20—24° for 3 days, and then acidified at 0° to pH 1 with 2N-hydrochloric acid, and added to a mixture of water (80 ml.) and ether (50 ml.). The solid was collected, washed with ether and water, and digested with warm ethanol (40 ml.). The filtered digests were treated with water (40 ml.) to give the L,L,L,L-*isomer* (1·261 g., 42%), m.p. 183—185° (decomp.), $[\alpha]_D^{27}$ −36·1° (*c* 0·96 in dimethylformamide), R_{FA} 0·84, R_{FB} 0·72, R_{FC} 0·39, R_{FD} 0·72 (Found: C, 58·3; H, 5·6; N,

16·5. $C_{37}H_{41}N_9O_7S$ requires C, 58·7; H, 5·5; N, 16·7%). The product was readily soluble in water at pH 8; the m.p. and optical rotation were unaffected by reprecipitation or further recrystallisation. Amino-acid ratios in acid hydrolysate: Asp 1·02, Met 0·98, 5-α-aminophenethyl-1(2)*H*-tetrazole 1·00.

L-5-[α-N-(D- *and* L-2-*Ethylhexanoylglycyl*)*aminophenethyl*]-1(2)H-*tetrazole*.—*N*-(DL-2-Ethylhexanoyl)glycine *p*-nitrophenyl ester[14] (386 mg., 1·2 mmoles), L-5-α-aminophenethyl-1(2)*H*-tetrazole (189 mg., 1 mmole), triethylamine (0·14 ml., 1 mmole), and dimethylformamide (2 ml.) were stirred together at 22—24° for 16 hr. The solution was acidified to pH 1 at 0° with 2N-hydrochloric acid, diluted with ice–water, and extracted with ethyl acetate (3 × 15 ml.). The extracts were washed with 2N-hydrochloric acid and water (3 times), dried, and evaporated, to give a mixture of the L,L- and L,D-isomers (257 mg., 69%), m.p. 162—164°, R_{FA} 0·83, R_{FB} 0·82, R_{FC} 0·57, R_{FD} 0·72, R_{FE} 0·60, R_{FF} 0·67, R_{FH} 0·67 (Found: C, 61·5; H, 7·8; N, 22·3. $C_{19}H_{28}N_6O_2$ requires C, 61·3; H, 7·6; N, 22·6%).

L-5-(α-*n-Octadecanamidophenethyl*)-1(2)H-*tetrazole*.—Stearoyl chloride (364 mg., 1·2 mmoles) in acetone (2 ml.) together with 0·5N-sodium hydroxide (sufficient to maintain the reaction mixture at pH 9·5), were added during 40 min. at 20—22° to a stirred solution of L-5-α-aminophenethyl-1(2)*H*-tetrazole (189 mg., 1 mmole) in 0·5N-sodium hydroxide. The acetone was removed *in vacuo*, the mixture was filtered, and the filtrate was acidified to pH 2 at 0° with 2N-hydrochloric acid, to give the *tetrazole derivative* (290 mg., 64%), m.p. 130—132° (from ethanol), R_{FA} 0·87, R_{FB} 0·85, R_{FC} 0·53, R_{FE} 0·58, R_{FF} 0·78, R_{FG} 0·0, R_{FH} 0·68 (Found: C, 71·2; H, 10·0; N, 15·0. $C_{27}H_{45}N_5O$ requires C, 71·2; H, 9·95; N, 15·4%).

N-*Benzyloxycarbonyl*-L-β-*cyanoalanine* 2,4,5-*Trichlorophenyl Ester*.—(*a*) This (73% yield), m.p. 135—136° (from ethanol), R_{FF} 0·66, R_{FG} 0·50, R_{FH} 0·64, was prepared from *N*-benzyloxycarbonyl-L-β-cyanoalanine and 2,4,5-trichlorophenol by the general method described by Liberek[15] (Found: C, 50·4; H, 3·1; Cl, 24·5; N, 6·5. $C_{18}H_{13}Cl_3N_2O_4$ requires C, 50·5; H, 3·1; Cl, 24·9; N, 6·55%). It was recovered unchanged after being boiled in ethanol for 1 hr.

(*b*) *N*-Benzyloxycarbonyl-L-β-cyanoalanine (24·8 g., 0·1 mole), 2,4,5-trichlorophenol (21·7 g., 0·11 mole), *NN'*-dicyclohexylcarbodi-imide (21·6 g., 0·105 mole), and pyridine (150 ml.) was stirred together at 4° for 18 hr. The filtered solution was evaporated, and the residue gave the trichlorophenyl ester (25·7 g., 60%), m.p. 133—135° (from ethanol).

N-*Benzyloxycarbonyl*-L-β-*cyanoalanyl*-L-*phenylalanine Amide*.—A solution of *N*-benzyloxycarbonyl-L-β-cyanoalanine 2,4,5-trichlorophenyl ester (6·4 g., 15 mmoles) and L-phenylalanine amide[13] (2·96 g., 16·5 mmoles) in dimethylformamide (15 ml.) and acetic acid (3 drops) was kept at 4° for 2 days. Ether (150 ml.) and ice–water (50 ml.) were added, and the solid was collected and washed with water and ether to give the *dipeptide derivative* (100%), m.p. 205—206° (from ethanol), $[\alpha]_D^{27}$ −17·5° (*c* 1·04 in dimethylformamide), R_{FD} 0·71, R_{FE} 0·39, R_{FF} 0·64 (Found: C, 63·8; H, 5·6; N, 14·2. $C_{21}H_{22}N_4O_4$ requires C, 63·9; H, 5·6; N, 14·2%).

N-*Benzyloxycarbonyl*-[β-1(2)H-*tetrazol*-5-*yl*]-L-*alanyl*-L-*phenylalanine Amide*.—*N*-Benzyloxycarbonyl-L-β-cyanoalanyl-L-phenylalanine amide (3·94 g., 10 mmoles), sodium azide (1·44 g., 22 mmoles), ammonium chloride (1·18 g., 22 mmoles), and dimethylformamide (7·5 ml.) were stirred together at 90—95° for 24 hr. The filtered solution was evaporated at 0·1 mm. and the residue was shaken with ice–water (20 ml.) and N-hydrochloric acid (20 ml.), to give a solid. This was collected, washed with water, and digested with water (50 ml.) containing ammonia (sufficient to give pH 9—10). Acidification of the filtered (kieselguhr) digests gave the *tetrazolyl derivative* (needles from ethanol) (2·42 g., 55%), m.p. 246—247° (decomp.), R_{FA} 0·74, R_{FB} 0·72, R_{FC} 0·39, R_{FD} 0·61, R_{FE} 0·0, R_{FF} 0·36, R_{FH} 0·23 (Found: C, 57·75; H, 5·35; N, 22·2. $C_{21}H_{23}N_7O_4$ requires C, 57·7; H, 5·3; N, 22·4%).

[β-1(2)H-*Tetrazol*-5-*yl*]-L-*alanyl*-L-*phenylalanine Amide*.—The benzyloxycarbonyl derivative just described (437 mg., 1 mmole) in acetic acid (50 ml.) and water (5 ml.) was hydrogenolysed over 5% palladised charcoal (100 mg.) for 6 hr. at room temperature and pressure. The filtered solution was evaporated and the residue was dried by azeotropic distillation with benzene and then collected in acetone. Further drying of the product at 60°/10 mm. gave the *dipeptide derivative* (258 mg., 85%), m.p. 176—178° (sintering at 130°), R_{FA} 0·49, R_{FB} 0·65, R_{FC} 0·27, R_{FD} 0·53, R_{FF} 0·24, R_{FH} 0·11 (Found: C, 51·4; H, 5·8; N, 32·2. $C_{13}H_{17}N_7O_2$ requires C, 51·5; H, 5·65; N, 32·4%).

N-*Benzyloxycarbonyl*-L-*tryptophyl*-L-*methionyl*-[β-1(2)H-*tetrazol*-5-*yl*]-L-*alanyl*-L-*phenylalanine Amide*.—A solution of *N*-benzyloxycarbonyl-L-tryptophyl-L-methionine azide[8] [from the hydrazide (1 mmole) in tetrahydrofuran (2 ml.)] was added at 0° to a solution of [β-1(2)*H*-tetrazol-5-yl]-L-alanyl-L-phenylalanine amide (151 mg., 0·5 mmole) and triethylamine (0·42 ml., 3 mmoles) in dimethylformamide (5 ml.). The mixture was stirred at 4° for 18 hr., then water (0·5 ml.) was added. The resulting solution was kept at 20—24° for 2 days, and then acidified at 0° to pH 1 with 2N-hydrochloric acid and added to a mixture of ice–water (20 ml.) and ether (15 ml.). The solid was collected, washed with ether and water, digested with warm ethanol, and crystallised from aqueous dimethylformamide, to give the L,L,L,L-*isomer* (198 mg., 54%), m.p. 234° (decomp.), $[\alpha]_D^{27}$ −28·0° (*c* 0·97 in dimethylformamide), R_{FA} 0·88, R_{FB} 0·85, R_{FC} 0·61, R_{FD} 0·68 (Found: C, 58·5; H, 5·7; N, 18·4. $C_{37}H_{42}N_{10}O_6S$ requires C, 58·8; H, 5·6; N, 18·55%). Amino-acid ratios in acid hydrolysate: [β-1(2)*H*-tetrazol-5-yl]-L-alanine 1·00, Met 0·96, Phe 1·00. In order to check the optical purity of this product, a small sample was hydrogenolysed at room temperature and pressure in methanol and cyclohexylamine over 5% palladised charcoal, the filtered solution was evaporated, and the residue was digested with leucine aminopeptidase;[16] the amino-acid ratios in the digest were [β-1(2)*H*-tetrazol-5-yl]alanine 0·98, Met 0·99, Phe 1·00.

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