Polypeptides. Part 17.[1] Aminoxy-analogues of Aspartame and Gastrin *C*-Terminal Tetrapeptide Amide †

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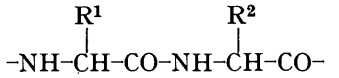
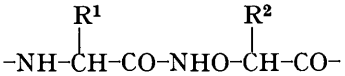
L- and D-2-Aminoxy-3-phenylpropionic acid have been prepared from D- and L-phenylalanine by a route which is generally applicable to other optically active amino-acids. Appropriate methodology enabled incorporation of the L-isomer in place of L-phenylalanine in Asp-Phe-OMe (to provide an aminoxy-analogue which was not sweet) and Trp-Met-Asp-Phe-NH_2 (to provide an aminoxy-analogue which did not stimulate gastric acid secretion).

OUR general interest in ' backbone ' modifications of biologically active peptides [2,3] led us to synthesise aminoxy analogues wherein the amino-group involved in the peptide linkage is replaced by aminoxy. In these analogues there is a lengthening of the peptide backbone, and modification of the hydrogen bond donor or acceptor properties of adjacent amide groups, consequent of the insertion of an oxygen atom.



normal peptide



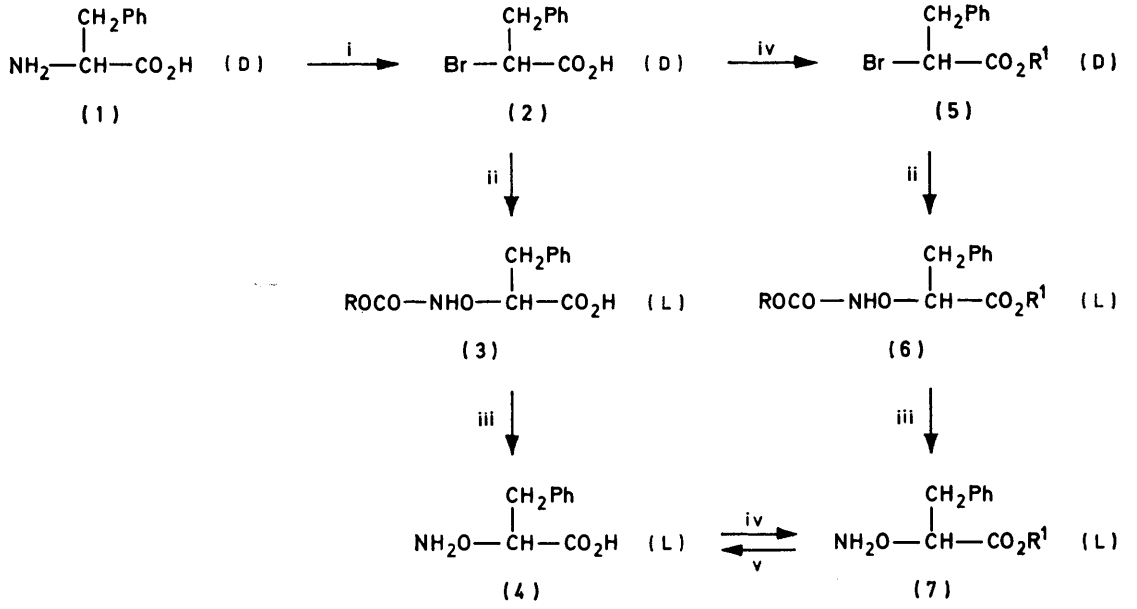
aminoxy-analogue

Specifically, in the present paper, we describe the preparation of aminoxy-analogues of L-aspartyl-L-phenylalanine methyl ester (the sweetening agent aspartame) and L-tryptophyl-L-methionyl-L-aspartyl-L-phenylalanine amide (the *C*-terminal tetrapeptide amide common to all the gastrins), derived by replacing L-phenylalanyl by L-2-aminoxy-3-phenylpropionyl. However, the methodology involved in the synthesis of L-2-aminoxy-3-phenylpropionic acid and the incorporation of this acid into peptides has been applied successfully in other cases, and we believe it to be generally applicable.

The route adopted for the synthesis of L-2-aminoxy-3-phenylpropionic acid (4) is shown in Scheme 1. D-Phenylalanine (1) was converted into D-2-bromo-3-phenylpropionic acid (2), which after reaction with *N*-benzyloxycarbonylhydroxylamine and subsequent deprotection of the product (3; R = $PhCH_2$) gave the L-aminoxy-acid (4). At the outset of this work a similar route [Scheme 1, (1) ⟶ (2) ⟶ (5) ⟶ (6; R = R^1 = Et) ⟶ (7) ⟶ (4)] using α-bromo-acid ethyl esters [*e.g.* (5; R = Et)] and ethoxycarbonyl for *N*-protection of the hydroxylamine had been used by Testa *et al.*[4] in the synthesis of DL-2-aminoxy-3-phenylpropionic and other racemic 2-aminoxy-acids; these authors also applied their method to L-leucine and obtained D-2-aminoxy-4-methylvaleric acid, though the configuration of the product was incorrectly assigned at the time (see ref. 5). Our modified route arose from a need to explore the use of Z and Boc for *N*-protection, so as to provide intermediate *N*-protected acids (3; R = $PhCH_2$ or Bu^t) more suited to subsequent synthetic work. Reaction of *N*-benzyloxycarbonylhydroxylamine with D-2-bromo-3-phenylpropionic acid (2) and sodium hydroxide (2 equiv.) in refluxing ethanol, under the general conditions described by Testa *et al.*,[4] resulted in appreciable ester interchange, and the product was a mixture of the benzyloxy- and ethoxy-carbonyl acids (3; R = $PhCH_2$ and Et). However, use in this reaction of sodium hydride (2 equiv.) in dimethylformamide at 0—5 °C gave the required product (3; R = $PhCH_2$) in good yield as its crystalline dicyclohexylammonium salt. Cleavage of the protecting group by means of hydrogen bromide in acetic acid gave L-2-aminoxy-3-phenylpropionic acid hydrobromide and thence, after neutralisation, the free acid (4). A similar series of reactions starting from L-phenylalanine gave D-2-aminoxy-3-phenylpropionic acid, and the use of *N*-t-butoxycarbonylhydroxylamine in place of *N*-benzyloxycarbonylhydroxylamine gave equally satisfactory results *via* the intermediate (3; R = Bu^t). There has been great interest in L-2-aminoxy-3-phenylpropionic acid since it was shown to be a specific and powerful inhibitor of the plant enzyme phenylalanine ammonia lyase.[6] We, therefore, give full details of its synthesis in the Experimental section. After our work was completed, the extensive studies of Kisfaludy and his colleagues [7] on 2-aminoxy-acids were reported in the patent literature. Their route to L-2-aminoxy-3-phenylpropionic acid (4) follows the scheme outlined above but, contrary to our experience, the general conditions of Testa *et al.*[4] were apparently applied successfully in the conversion of (2) ⟶ (3; R = $PhCH_2$).

The stereochemical course of these reactions was anticipated from the studies of Ingold [8] and Neuberger.[9] Conversion of 2-amino- to 2-bromo-acids proceeds with high retention of configuration at the α-carbon atom, whereas nucleophilic displacement of bromine generally follows an S_N2 mechanism with inversion of the configuration. The correctness of our assignments was established after conversion of both products to 2-hydroxy-3-phenylpropionic acids of known configuration by catalytic hydrogenolysis. The aminoxy-acid derived from D-phenylalanine gave L-2-hydroxy-3-phenylpro-

† Abbreviations used: Z = benzyloxycarbonyl, Boc = t-butoxycarbonyl, DCCI = dicyclohexylcarbodi-imide, DMF = dimethylformamide, TFA = trifluoroacetic acid, HOBt = 1-hydroxybenzotriazole.

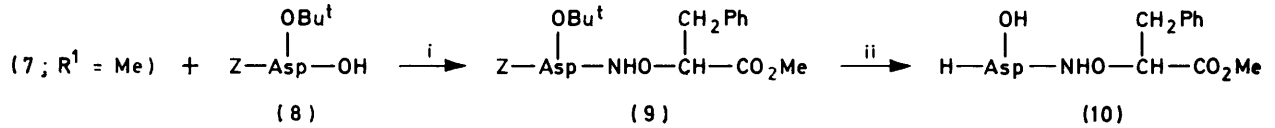


SCHEME 1 Synthesis of L-2-aminoxy-3-phenylpropionic acid. Reagents: i, $NaNO_2$–KBr–H_2SO_4 at 0—5°; ii, ROCONHOH–NaH in DMF at 0—5°; iii, HBr in AcOH; iv, ROH–$SOCl_2$; v, conc. HCl, reflux

pionic acid and is therefore of the L-configuration. The aminoxy-acid derived from L-phenylalanine gave D-2-hydroxy-3-phenylpropionic acid and is therefore of the D-configuration.

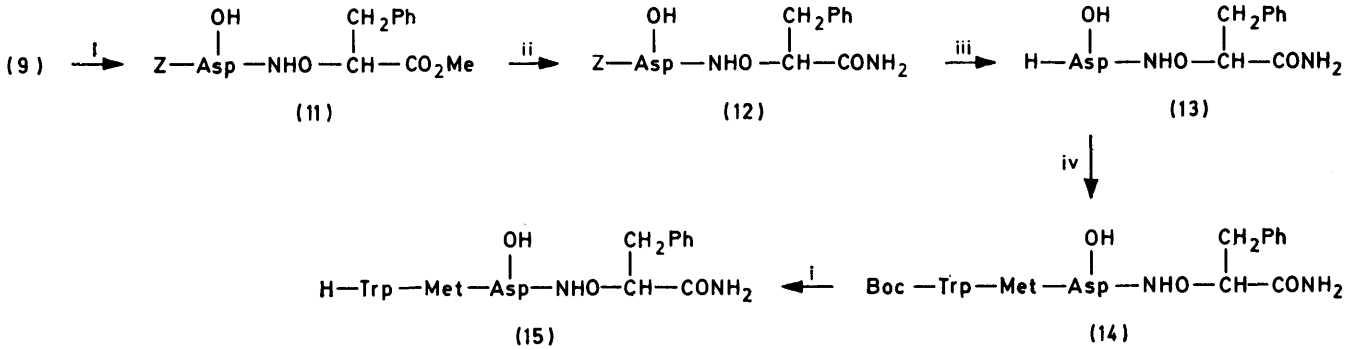
In the preparation of the aminoxy-analogue of aspartame (Scheme 2), L-2-aminoxy-3-phenylpropionic acid (4) was converted to its methyl ester (7; R^1 = Me) hydrochloride in high yield by means of thionyl chloride in methanol [an alternative preparation of the ester (see Scheme 1) involved reaction of the bromo-ester (5; R^1 = Me) with *N*-benzyloxycarbonylhydroxylamine (in dimethylformamide with sodium hydride as base) followed by acidolytic cleavage of the product (6; R = $PhCH_2$, R^1 = Me)]. Coupling of the methyl ester (7; R^1 = Me) with β-t-butyl-*N*-benzyloxycarbonyl-L-aspartic acid (8) proceeded smoothly by means of DCCI and HOBt to give *N*-benzyloxycarbonyl-(β-t-butyl)-L-aspartyl-L-2-aminoxy-3-phenylpropionic acid methyl ester (9). Treatment of this with hydrogen bromide in acetic acid gave L-aspartyl-L-2-aminoxy-3-phenylpropionic acid methyl ester (10) as its hydrobromide. In contrast to aspartame (*ca.* 200 times more sweet than sucrose), neither this salt, nor the free base released by brief treatment of the salt with Amberlite 1RA 400 (acetate form) was sweet. To explain this result it might be argued that the shift in the relative positions of the $CH_2CO_2^-$ and CH_2Ph side chains consequent of the presence of oxygen in the aminoxy-analogue is incompatible with the requirements for binding at receptor sites in the taste bud, and consequently no sweet sensation is generated. However Mazur *et al.*[10] have shown that, in aspartame analogues, a degree of flexibility in the nature and arrangements of atoms attached to the nitrogen of the amide group is permissible without loss of sweetening ability. These results, and the results with L-aspartyl-aminomalonic diesters[11] (some of which are much sweeter than aspartame) suggest that binding at the receptor is concerned mainly with the isoasparagine residue. The amide end of this residue is clearly involved in the interaction (as well as the flat zwitterionic features). We therefore suggest that the loss in sweetness in changing from aspartame to the aminoxy-analogue is related mainly to a weakening of the potential for the amidic carbonyl to participate in hydrogen bonding consequent upon the introduction of adjacent oxygen. L-Aspartyl-D-2-aminoxy-3-phenylpropionic acid methyl ester, similarly prepared from D-aminoxy-3-phenylpropionic acid, was also not sweet.

In the preparation of the aminoxy-analogue of gastrin tetrapeptide amide (Scheme 3), *N*-benzyloxycarbonyl-(β-t-butyl)-L-aspartyl-L-2-aminoxy-3-phenylpropionic acid methyl ester (9) was treated successively with aqueous 90% TFA, saturated methanolic ammonia, and 40% hydrogen bromide in acetic acid. The intermediate products (11) and (12) were characterised by t.l.c. and n.m.r., and the resulting hydrobromide was passed through an ion-exchange resin (AcO^- and part Cl^- form).



SCHEME 2 Synthesis of the aminoxy-analogue of aspartame. Reagents: i, DCCI–HOBt; ii, HBr in AcOH

After silica chromatography of the lyophilised residue, pure L-aspartyl-L-2-aminoxy-3-phenylpropionamide (13) was isolated in good overall yield as its hemi-hydrochloride. Coupling of the amide (13) with t-butoxycarbonyl-L-tryptophyl-L-methionine azide, following the general conditions of Honzl and Rudinger,[12] gave *N*-t-butoxycarbonyl-L-tryptophyl-L-methionyl-L-aspartyl-L-2-aminoxy-3-phenylpropionamide (14) in 75% yield. Finally, treatment of the t-butoxycarbonyl derivative (14) with TFA gave L-tryptophyl-L-methionyl-L-2-aminoxy-3-phenylpropionamide (15). The stability of the aminoxy-linkage in this analogue towards aminopeptidase M was found to be high. Thus 24 h digests of the analogue with this enzyme contained Trp and Met in equimolar amounts but very little Asp (in contrast the ratio Met : Asp in acid hydrolysates was close to unity). This result agrees with those of Kisfaludy *et al.*[13] who found that the resistance towards proteases of model tripeptide analogues containing 2-aminoxy-acid residues approximated to that of tripeptides similarly substituted by D-amino-acid residues.



SCHEME 3 Synthesis of the aminoxy-analogue of gastrin *C*-terminal tetrapeptide amide. Reagents: i, aqueous 90% TFA: ii, NH_3–MeOH; iii, HBr in AcOH; iv, Boc-Trp-Met-N_3 in DMF

There was no increase in gastric acid secretion following intravenous injection of 500 μg of the aminoxy-analogue (15) into conscious, Heidenhain pouched dogs (*ca.* 20 kg) under conditions previously described;[14] 50 μg of gastrin tetrapeptide amide caused a marked response. The same dose did not inhibit responses to histamine. Either or both of the speculations discussed in relation to the aminoxy-analogue of aspartame may account for the inactivity of this analogue.

EXPERIMENTAL

Ascending thin-layer chromatographs were run on Kieselgel G plates. Details of solvent mixtures and spray reagents are given in Part 13.[15] Optical rotations were determined with a Bendix NPL 143C automatic polarimeter with 154C digital converter. Acid or enzymic hydrolysates of peptides or analogues were prepared with 6N-hydrochloric acid (110° for 16 h) or aminopeptidase M (Rohm, GMBH Chemische Fabrik), and the amino-acid composition of the hydrolysates was determined with a Locarte automatic amino-acid analyser. Evaporations were carried out below 40° under reduced pressure in a rotary evaporator.

N-Benzyloxycarbonylhydroxylamine.—The methods previously described[16,17] gave products contaminated with *NO*-bisbenzyloxycarbonylhydroxylamine, and the separation of these compounds was tedious. The following method proved satisfactory. A stirred solution of hydroxylamine hydrochloride (84 g, 1.2 mol) in water (350 ml) was neutralised at 0—10 °C with 4N-sodium hydroxide (300 ml, 1.2 mol) and ice. Solutions of benzyl chloroformate (52 g, 0.304 mol) in ether (50 ml) and 4N-sodium hydroxide were then added simultaneously at 0—10° over 30 min (pH >9 throughout) adding ice as necessary. The mixture was further stirred at 23—25° for 3 h then added to ice (*ca.* 200 g) and light petroleum (b.p. 40—60°) (500 ml). The mixture was neutralised with concentrated hydrochloric acid (*ca.* 90 ml), and the crystalline solid, m.p. 66—68 °C (31 g, 61% based on benzyl chloroformate) was collected, washed thrice with light petroleum and thrice with water, and dried at 0.1 mmHg over P_2O_5. Homogeneity was established by t.l.c. in systems D, H, K, and Q (the mono- and di-acylated hydroxylamines are visualised by u.v. light, whereas only the mono-derivative gives an immediate blue-purple colour with $FeCl_3$ spray). The products from several batches were combined and crystallised from ethyl acetate–light petroleum.

L- *and* D-2-*Bromo*-3-*phenylpropionic Acid.*[4]—Sodium nitrite (96 g, 1.40 mol) was added over 45 min to a stirred solution of L-phenylalanine (165 g, 1 mol) and potassium bromide (391 g, 3.28 mol) in 3N-sulphuric acid (1.65 l). Stirring was continued for 1 h at 0—5° and for 1.5 h at 23—25 °C, then the mixture was extracted with ether (4 × 500 ml). The extracts were washed with water (2 × 500 ml), dried ($MgSO_4$), and evaporated. The residue was dissolved in cyclohexane (150 ml) and the solution was kept at 4 °C overnight. The crystals of L-3-phenyl-lactic acid (16.4 g), m.p. 123—125 °C, were filtered off and the filtrate was evaporated, to give the bromo-acid (182 g). The D-isomer (188.5 g) was prepared likewise from D-phenylalanine (1 mol).

N-Benzyloxycarbonyl-L- *and* -D-*aminoxy*-3-*phenylpropionic Acid Dicyclohexylammonium Salt.*—*N*-Benzyloxycarbonylhydroxylamine (16.7 g, 0.1 mol) in freshly distilled dimethylformamide (20 ml) was stirred at 0—5 °C and 80% sodium hydride oil dispersion (B.D.H.) (6.0 g, 0.2 mol) was added portionwise over 30 min at 0—5 °C. Stirring was continued at 0—5 °C for 10 min, then a solution of D-2-bromo-2-phenylpropionic acid (22.9 g, 0.1 mol) in dimethylformamide (20 ml) was added dropwise over 15 min at 0—5 °C. The mixture was left at 20—25 °C overnight then diluted with ice–water and extracted with ether (2 × 150 ml) (backwashing with water). The aqueous phase and water

backwashings were combined, acidified with concentrated hydrochloric acid and ice, then extracted with ether (3 × 200 ml). The combined extracts were washed twice with water, dried ($MgSO_4$) and evaporated to *ca.* 200 ml. Dicyclohexylamine (20 ml) was added and the solid (31.8 g, 64%) was collected and washed well with ether; recrystallisation from ethyl acetate (300 ml) gave the pure L-*isomer dicyclohexylammonium salt* (19.9 g), m.p. 161—162 °C (sharp with effervescence) (Found: C, 70.5; H, 7.7; N, 5.6. $C_{29}H_{40}N_2O_5$ requires C, 70.1; H, 8.1; N, 5.6%). The acid, liberated from the salt as described in the next section, had R_{FA} 0.83, R_{FB} 0.89, R_{FC} 0.72, R_{FD} 0.66, R_{FF} 0.69, R_{FG} 0.45, R_{FK} 0.92 (visualised with permanganate); τ($CDCl_3$) 2.7 (10 H, m, ArH), 4.85 (2 H, s, CH_2O), 5.4 (1 H, m, CH), and 6.85 (2 H, m, CH_2). The D-isomer dicyclohexylammonium salt, m.p. 161—162 °C, was obtained likewise in similar yield using L-2-bromo-3-phenylpropionic acid (lit.,[7] 157—159 °C).

L- *and* D-2-*Aminoxy*-3-*phenylpropionic Acid and Hydrobromide*.— *N*-Benzyloxycarbonyl-L-2-aminoxy-3-phenylpropionic acid dicyclohexylammonium salt (17.4 g, 35 mmol), ethyl acetate (250 ml), ice–water (50 ml), and aqueous 1N-citric acid (37.5 ml) was shaken together until all the solid had dissolved. The organic layer was separated, washed thrice with water, dried ($MgSO_4$), and evaporated. The residual oil was cooled in ice, treated with 45% w/v hydrogen bromide in acetic acid (15 ml), and the resulting solution was kept at ambient temperature for 1 h. Dry ether (100 ml) was added and the L-*isomer hydrobromide* (7.14 g, 78%), m.p. 165—167 °C, $[\alpha]_D^{25}$ −53.9° (*c* 1 in methanol), $[\alpha]_D^{26}$ −46.6° (*c* 1 in DMF), R_{FA} 0.71, R_{FB} 0.65, R_{FC} 0.33, R_{FD} 0.58, R_{FK} 0.75 (visualised by u.v. light and permanganate), was collected, washed well with ether, and dried at 0.1 mmHg (Found: C, 41.4; H, 5.0; N, 5.4. $C_9H_{11}NO_3$,HBr requires C, 41.2; H, 5.6; N, 5.3%); τ[$(CD_3)_2SO$] 9.77 (5 H, s, phenyl), 5.1 (1 H, m, CH), and 6.9 (2 H, m, CH_2). The D-*isomer hydrobromide*, prepared likewise from *N*-benzyloxycarbonyl-D-2-aminoxy-3-phenylpropionic acid dicyclohexylammonium salt, had $[\alpha]_D^{25}$ +53.1° (*c* 1 in methanol) (Found: C, 41.5; H, 4.8; N, 5.3%). The L-isomer hydrobromide (2.62 g, 10 mmol) in propan-2-ol (30 ml) was treated with pyridine (0.81 ml, 10 mmol) at 4 °C. After 1 h the L-acid (0.96 g, 50%), m.p. 143—144 °C, $[\alpha]_D^{25}$ −66.3° (*c* 1 in methanol), $[\alpha]_D^{27}$ −53.6° (*c* 1 in DMF), R_{FC} 0.50, R_{FD} 0.49, R_{FK} 0.58, was collected and washed with small quantities of ice-cold propan-2-ol (Found: C, 59.4; H, 6.0; N, 7.7. Calc. for $C_9H_{11}NO_3$: C, 59.7; H, 6.1; N, 7.7%) {lit.,[7] m.p. 145—146 °C, $[\alpha]_D^{27}$ −88° (*c* 0.4 in water), −57° (*c* 0.4 in 5N-hydrochloric acid}. The D-acid, prepared likewise, had m.p. 143—144 °C, $[\alpha]_D^{25}$ +65.6° (*c* 1 in methanol) (Found: C, 59.6; H, 6.1; N, 7.7%) {lit.,[7] m.p. 147—148 °C, $[\alpha]_D^{27}$ +82° (*c* 0.4 in water), +60 ° (*c* 0.4 in 5N-hydrochloric acid}.

Confirmation of Configuration.—L-2-Aminoxy-3-phenylpropionic acid hydrobromide (500 mg, 1.9 mmol), prepared from D-phenylalanine in methanol (20 ml), was shaken with hydrogen at room temperature and pressure for 5 h in the presence of 5% palladium on charcoal (200 mg). The mixture was filtered (kieselguhr) and the filtrate was evaporated to give L-2-hydroxy-3-phenylpropionic acid (300 mg, 95%), m.p. 123—124 °C (from water), $[\alpha]_D^{25}$ −21.0° (*c* 1 in DMF). Reference L-2-hydroxy-3-phenyl propionic acid, prepared by direct deamination of L-phenylalanine, had the same m.p. and optical rotation. D-2-Aminoxy-3-phenylpropionic acid hydrobromide (from L-phenylalanine) was likewise converted into D-2-hydroxy-3-phenylpropionic acid, $[\alpha]_D^{25}$ +20.6° (*c* 1 in DMF).

Methyl L- *and* D-2-*Aminoxy*-3-*phenylpropionate Hydrochloride*.—(*a*) The above L-aminoxy-acid (4.62 g, 20 mmol) was added to a stirred solution of thionyl chloride (2.44 ml, 34 mmol) and methanol (20 ml) at −5 °C. The mixture was stirred for 16 h at room temperature, and then evaporated to a crystalline residue which was collected and washed with ether. Recrystallisation from methanol–ether gave the L-*methyl ester hydrochloride* (4.15 g, 90%), m.p. 148—152 °C (decomp.), R_{FA} 0.76, R_{FB} 0.80, R_{FC} 0.74, R_{FD} 0.79, R_{FF} 0.70, R_{FK} 0.89, $[\alpha]_D^{26}$ −32.2° (*c* 1 in DMF) (Found: C, 5.1; H, 6.1; N, 5.6; Cl, 15.5. $C_{10}H_{13}NO_3$·HCl requires C, 51.9; H, 6.1; N, 6.0; Cl, 15.3%), τ[$(CD_3)_2SO$] 2.7 (5 H, s, ArH), 5.0 (1 H, m, α-CH), 6.28 (3 H, s, methyl ester), and 6.85 (2 H, s, benzylic). The D-*methyl ester hydrochloride* (93%), prepared similarly from the D-aminoxy-acid, had m.p. 146—148 °C (decomp.), $[\alpha]_D^{26}$ +31.8° (*c* 1 in DMF) (Found: C, 52.2; H, 5.9; N, 5.8%).

(*b*) A solution of *N*-benzyloxycarbonylhydroxylamine (920 mg, 5.5 mmol) in dry DMF (2 ml) was stirred at 0° while a 50% sodium hydride oil dispersion (240 mg, 5 mmol) was added. After 40 min, when effervescence had ceased, methyl D-2-bromo-3-phenylpropionate * (1.1 g, 4.5 mmol) was added dropwise, and the mixture was stirred at room temperature for 16 h. Evaporation of the reaction mixture gave an oily residue which was shaken with N-potassium hydrogencarbonate (15 ml) and ether (20 ml). The organic layer was separated, washed with 1N-potassium hydrogencarbonate (15 ml) and water (until neutral), and then dried and evaporated. The residue was triturated repeatedly with light petroleum, and then dried *in vacuo*, to give a gelatinous solid (1.45 g). This crude methyl *N*-benzyloxycarbonyl-L-2-aminoxy-3-phenylpropionate (725 mg, 2.2 mmol) (n.m.r. indicated contamination with 15—20% *N*-benzyloxycarbonylhydroxylamine) was dissolved in 45% hydrogen bromide in acetic acid (2.5 ml) and the solution was kept at room temperature for 1 h. The crude hydrobromide obtained by evaporation was collected, dissolved in water (10 ml), and the solution was neutralised at 0—5 °C with aqueous 2N-sodium hydrogencarbonate. The free aminoxy-ester was extracted into ether (2 × 10 ml), and the dried extracts were treated at 0 °C with 2*N*-hydrogen chloride in ethyl acetate (2 ml). Excess of solvent was removed by evaporation, affording the L-methyl ester hydrochloride, which crystallised on trituration with ether (266 mg, 52%), m.p. 154—156 °C (decomp.), $[\alpha]_D^{26}$ −31.5° (*c* 1 in DMF). The behaviour of this sample in t.l.c. was identical to that of the sample prepared as described in (*a*). The two samples had identical n.m.r. and i.r. spectra.

N-*Benzyloxycarbonyl*-(β-*t*-*butyl*)-L-*aspartyl*-L- *and* -D-2-*aminoxy*-3-*phenylpropionic Acid Methyl Ester*.—Methyl L-2-aminoxy-3-phenylpropionate (3.35 g, 17.2 mmol) (prepared as an oil by treatment of the hydrochloride with saturated potassium carbonate solution, and isolated by extraction into dichloromethane) was dissolved in DMF (30 ml) and *N*-benzyloxycarbonyl-(β-t-butyl)-L-aspartic acid [18] (6.15 g, 19 mmol) was added. The clear solution was stirred at 5 °C while HOBt (2.56 g, 19 mmol) was added, followed immediately by DCCI (3.92 g, 19 mmol). The mixture was stirred at 4° for 16 h, then filtered and evaporated. The residue was dissolved in ether (100 ml), and the solution was washed successively with 1N-sodium carbonate solution (3 × 40 ml), water (40 ml), aqueous 10% citric

* Prepared from the bromo-acid and methanol following ref. 4.

acid (3 × 40 ml), water (until neutral), and then dried. Evaporation gave an oily residue which gave crystals of the L,L-*isomer* slowly from its solution in ether (5.20 g, 60.5%), m.p. 91—92 °C, R_{FA} 0.94, R_{FB} 0.97, R_{FD} 0.82, R_{FE} 0.66, R_{FF} 0.71, R_{FG} 0.50, R_{FH} 0.71, $[\alpha]_D^{27}$ −42.5° (*c* 1 in DMF) (Found: C, 62.5; H, 6.3; N, 5.7. $C_{26}H_{32}N_2O_8$ requires C, 62.3; H, 6.4; N, 5.6%), τ($CDCl_3$) 2.65 (10 H, m, ArH), 4.85 (2 H, s, benzylic urethane), 5.3 and 5.65 (1 H, m, α-CH), 6.3 (3 H, s, methyl ester), 6.8 (2 H, m, phenylalanyl side chain CH_2), 7.3 (2 H, m, aspartyl CH_2), and 8.6 (9 H, s, t-butyl ester). The L,D-isomer, prepared similarly using methyl D-2-aminoxy-3-phenylpropionate, was obtained as an oil with similar t.l.c. and n.m.r. characteristics.

L-*Aspartyl*-L- *and* -D-2-*aminoxy*-3-*phenylpropionic Acid Methyl Ester and Hydrobromide*.—The preceding LL-benzyloxycarbonyl derivative (2.0 g, 4 mmol) was dissolved in 40% hydrogen bromide in acetic acid (10 ml) and kept at room temperature for 1 h. The solution was evaporated and the residue was collected with ether. Recrystallisation from acetic acid gave the L,L-*methyl ester hydrobromide* (925 mg, 59%), m.p. 173—175 °C (decomp.), $[\alpha]_D^{27}$ −8.3° (*c* 1 in dimethylformamide) (Found: C, 43.3; H, 4.9; N, 6.6; Br, 19.9. $C_{14}H_{18}N_2O_6$,HBr requires C, 43.0; H, 4.9; N, 7.1; Br, 20.4%), τ[$(CD_3)_2SO$] 2.75 (5 H, s, ArH), 5.1 and 5.5 (1 H, m, α-CH), 6.45 (3 H, s, methyl ester), and 6.7—7.2 (4 H, m, β-CH_2). The hydrobromide (150 mg, 0.382 mmol) in water (10 ml) was stirred with Amberlite resin (IRA 400; AcO^- form; 10 ml) for 30 min, at room temperature. The mixture was filtered and the filtrate lyophilised, to give L-*aspartyl*-L-2-*aminoxy*-3-*phenylpropionic acid methyl ester* as a tasteless solid (118 mg, 100%), R_{FA} 0.51, R_{FB} 0.70, R_{FC} 0.32, R_{FD} 0.55, R_{FK} 0.48. The L,D-*isomer hydrobromide*, prepared similarly from the L,D-benzyloxycarbonyl derivative, had m.p. 179—181 °C (decomp.), $[\alpha]_D^{27}$ −37.5° (*c* 1 in DMF) and was indistinguishable from the L,L-isomer on t.l.c. (Found: C, 43.2; H, 4.7; N, 7.2%).

N-*Benzyloxycarbonyl*-L-*aspartyl*-L-2-*aminoxy*-3-*phenylpropionamide*.— *N*-Benzyloxycarbonyl-(β-t-butyl)-L-aspartyl-L-2-aminoxy-3-phenylpropionic acid methyl ester (1.0 g, 2.0 mmol) was dissolved in aqueous 90% TFA (5 ml), and the solution was kept at room temperature for 1 h. The mixture was evaporated, and the residue in water was filtered, and lyophilised. The resulting crude *N*-benzyloxycarbonyl-L-aspartyl-L-2-aminoxy-3-phenylpropionic acid methyl ester, R_{FA} 0.83, R_{FB} 0.80, R_{FC} 0.45, R_{FD} 0.59, R_{FK} 0.82; τ($CDCl_3$) 2.6—2.8 (10 H, m, ArH), 4.81 (2 H, s, benzylic urethane), and 6.20 (3 H, s, methyl ester), no t-butyl ester proton signal, was dissolved in dry methanol, and the solution was saturated with dry ammonia at 0 °C and stored in a sealed flask for 16 h at room temperature. The residue after evaporation was dissolved in water (15 ml) and washed with ether (10 ml). The aqueous solution was acidified to pH 2—3 with 1N-hydrochloric acid, and the cloudy mixture was extracted with ethyl acetate (3 × 10 ml). The combined extracts were washed with water until neutral, dried, and evaporated to give the benzyloxycarbonyl amide as an amorphous gum (540 mg, 62.5%), R_{FA} 0.80, R_{FB} 0.78, R_{FC} 0.39, R_{FD} 0.55, R_{FK} 0.77, τ($CDCl_3$) 2.65 (10 H, m, ArH), 4.9 (2 H, s, benzylic urethane), and 6.8—7.5 (4 H, m, side chain CH_2).

L-*Aspartyl*-L-2-*aminoxy*-3-*phenylpropionamide*.—A solution of the preceding crude benzyloxycarbonyl amide (540 mg, 1.12 mmol) in 40% hydrogen bromide in acetic acid (10 ml), was kept at room temperature for 1 h, and then evaporated. The residue, in water, was shaken for 5 min with Amberlite anion exchange resin (IRA 400; AcO^- form; 10 ml), after which the solution was filtered from the resin and lyophilised. The product was purified by column chromatography on silica gel using chloroform–methanol–water (55 : 40 : 10) for elution, giving the pure *amide* (265 mg, 72%), R_{FA} 0.63, R_{FB} 0.90, R_{FC} 0.37, R_{FD} 0.43, R_{FK} 0.44 (Found: C, 47.0; H, 5.6; N, 13.2; Cl, 5.0. $C_{13}H_{17}N_3O_5 \cdot 0.5HCl \cdot H_2O$ requires C, 47.0; H, 5.9; N, 12.7; Cl, 5.35%). The source of chloride ion in the product was probably unexchanged chloride ion in the Amberlite resin.

N-*t-Butoxycarbonyl*-L-*tryptophyl*-L-*methionyl*-L-*aspartyl*-L-2-*aminoxy*-3-*phenylpropionamide*.— *N*-t-Butoxycarbonyl-L-tryptophyl-L-methionyl hydrazide [19] (0.148 mg, 0.33 mmol) in DMF (2 ml) was stirred and cooled to −25 °C and treated with 3.5N-hydrogen chloride in dioxan (0.376 ml, 1.32 mmol) followed immediately by t-butyl nitrite (0.043 ml, 0.36 mmol). The mixture was stirred at −25 °C for 6 min, then cooled to −50 °C, and neutralised to pH 7.0 with triethylamine (0.231 ml, 1.65 mmol). L-Aspartyl-L-2-aminoxy-3-phenylpropionamide (100 mg, 0.30 mmol) in DMF (1 ml) was added, and the pH of the solution adjusted to 7—8 by addition of triethylamine. The mixture was stirred at 4 °C for 16 h and evaporated. The residue was shaken with ethyl acetate (20 ml) and aqueous 10% citric acid (8 ml) and the aqueous phase was extracted with ethyl acetate (2 × 10 ml). The combined extracts were washed with brine until neutral, then dried and evaporated to give the *butoxycarbonyl derivative* which was collected with ether (160 mg, 75%), m.p. 138—140 °C (decomp.), R_{FA} 0.82, R_{FB} 0.83, R_{FC} 0.52, R_{FD} 0.62, R_{FK} 0.67, $[\alpha]_D^{27}$ −23.3° (*c* 1 in DMF) (Found: C, 55.6; H, 6.0; N, 11.6. $C_{34}H_{44}N_6O_9S \cdot H_2O$ requires C, 55.8; H, 6.3; N, 11.5%).

L-*Tryptophyl*-L-*methionyl*-L-*aspartyl*-L-2-*aminoxy*-3-*phenylpropionamide Trifluoroacetate*.—The above butoxycarbonyl derivative (50 mg) was dissolved in aqueous 90% TFA (2 ml). The solution was kept under nitrogen for 30 min at 20—22 °C and then evaporated. The residue, in water, was lyophilised to give the *trifluoroacetate* (50 mg), R_{FA} 0.80, R_{FB} 0.77, R_{FC} 0.37, R_{FD} 0.54, R_{FK} 0.64. Amino-acid radios after 24 h aminopeptidase M digest: Trp 0.95, Met 1.00, Asp 2.07; after 16 h acid hydrolysate, Met 0.97, Asp 1.03. Gastrin tetrapeptide amide trifluoroacetate (Try-Met-Asp-Phe-NH_2, TFA) treated similarly gave Trp 1.04, Met 1.02, Asp 1.00, Phe 0.98 (aminopeptidase M digest) and Met 0.99, Asp 1.07, Phe 0.95 (acid hydrolysate).

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