

887. Some Potential Amino-acid Antagonists and Related Compounds.

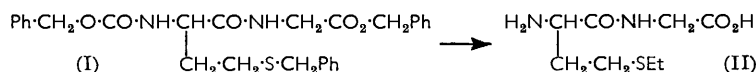
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2-Chloroethionine, glycylethionine, ethionylglycine, glycylethionylglycine, and some dipeptides containing the tumour-growth-inhibitory 1-aminocyclopentanecarbonyl residue with sarcosine, leucine, and tyrosine have been prepared. Unsuccessful attempts were made to prepare α -fluoroglycine and β -fluoroaspartic acid.

THE reactive alkylating agent S-2-chloroethylcysteine has unusual mutagenic properties.¹ Its homologue 2-chloroethionine (S-2-chloroethylhomocysteine) is also of interest since it is a reactive isostere of ethionine which could be an irreversible antagonist of the essential amino-acid, methionine.² DL-Homocysteine was generated from its S-benzyl derivative by the action of sodium in liquid ammonia and treated with ethylene bromohydrin. The resulting S-2-hydroxyethyl-DL-homocysteine with concentrated hydrochloric acid gave S-2-chloroethyl-DL-homocysteine hydrochloride.

A few potential amino-acid antagonists, notably ethionine³ and 1-aminocyclopentanecarboxylic acid,⁴ are inhibitors of tumour growth. Harris and Work⁵ showed that peptides from amino-acid antagonists can be more effective than the simple antagonist, and so it was of interest to prepare peptides of these two growth-inhibitory amino-acids.

Difficulty was experienced in the saponification of the crystalline product formed when benzyloxycarbonyl-DL-ethionine was coupled with glycine ethyl ester by the mixed anhydride method; difficulties in the hydrolysis of similar esters are recorded in the literature.⁶ However, when the product (I) obtained from S-benzyl-N-benzyloxycarbonyl-DL-homocysteine and glycine benzyl ester was treated successively with sodium and ethyl bromide in liquid ammonia DL-ethionylglycine (II) was readily obtained. Similar treatment of the product obtained by coupling S-benzyl-DL-homocysteine benzyl ester with benzyloxycarbonylglycine afforded glycyl-DL-ethionine.



Mild alkaline hydrolysis of ester (I) gave the corresponding acid which reacted with glycine benzyl ester (mixed anhydride method), giving a crystalline product which on treatment with sodium and ethyl bromide in liquid ammonia gave glycyl-DL-ethionylglycine.

Two peptides from 1-aminocyclopentanecarboxylic acid and glycine have advantages over the aminocycloalkane-acid as tumour-growth-inhibitors,^{4,7} and since N-methylation stabilises a peptide linkage towards enzymic attack it was decided to prepare the sarcosine peptides. First, ammonia and chloroacetamidocyclopentanecarboxylic acid (III) gave the known glycyl peptide;⁸ then methylamine yielded 1-sarcosylamidocyclopentanecarboxylic acid, although in a different experiment the dioxopiperazine derivative (IV) was obtained whose structure was confirmed when it was also obtained during an attempted

¹ Connors and Ross, *Biochem. Pharm.*, 1958, **1**, 93.

² Cf. Baker, *Cancer Chemotherapy Reports*, 1959, **4**, 1.

³ Levy, Montanez, Murphy, and Dunn, *Cancer Res.*, 1953, **13**, 507; Dunn and Murphy, *ibid.*, 1958, **18**, 569; White and Shimkim, *Cancer*, 1954, **7**, 867.

⁴ Connors, Elson, and Ross, *Biochem. Pharm.*, 1958, **1**, 239.

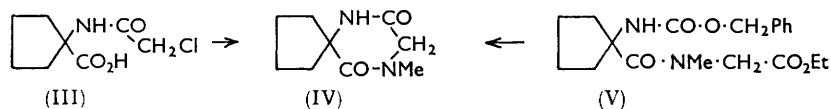
⁵ Harris and Work, *Biochem. J.*, 1950, **46**, 190.

⁶ MacClaren, *Austral. J. Chem.*, 1958, **11**, 360.

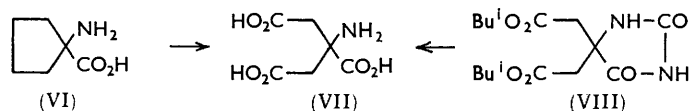
⁷ Connors, Elson, Haddow, and Ross, *Biochem. Pharm.*, 1960, **5**, 108.

⁸ Connors and Ross, *J.*, 1960, 2119

synthesis of 1-amino-*N*-carboxymethyl-*N*-methylcyclopentanecarboxamide by way of its *N*-benzyloxycarbonyl ethyl ester (V). Hydrogenation of the product obtained by condensing *N*-benzyloxycarbonyl-L-leucine with ethyl 1-aminocyclopentanecarboxylate



afforded ethyl 1-L-leucylamidocyclopentanecarboxylate; the ethyl ester of *N*-1-aminocyclopentanecarboxyl-L-tyrosine was similarly obtained. Reaction of 1-aminocyclopentanecarboxylic acid with benzyl chloroformate by the conventional procedure gave only poor yields of 1-benzyloxycarbonylamino-cyclopentanecarboxylic acid; however, the method used by Leplawy *et al.*⁹ for α -amino- α -methylpropionic acid gave excellent yields. 1-Aminocyclopentanecarboxylic acid (VI) is the only member of a series of homologous aminocycloalkane-amino-acids which exhibits antitumour activity and it is of interest to investigate whether a metabolite is responsible for this effect. α -Amino adipic acid, which would be formed by β -oxidation followed by ring fission, has already been shown to be ineffective as a tumour-growth-inhibitor. Symmetrical oxidative ring fission of 1-aminocyclopentanecarboxylic acid would give 2-aminopropane-1,2,3-tricarboxylic acid (VII), an isostere of citric acid to which it may be an antagonist. The amino-acid (VII) has been prepared by way of the hydantoin (VIII) derived from the di-isobutyl acetonedicarboxylate. 1-Methylaminocyclopentanecarboxylic acid was prepared for test by way of the toluene-*p*-sulphonamido-derivative.



Fluorinated derivatives of naturally occurring substances often act as effective antagonists, *e.g.*, fluoroacetic acid and fluorouracil, so we attempted to synthesise fluoroglycine which might be expected to interfere with the production of nucleic acid bases *in vivo*. A possible route was indicated by the work of Maguire and Shaw¹⁰ who showed that the chlorine atom of ethyl chlorofluoroacetate could be selectively replaced by the 2,3-dichlorophenoxy-radical: $\text{C}_6\text{H}_3\text{Cl}_2\text{ONa} + \text{CHClF} \cdot \text{CO}_2\text{Et} \rightarrow \text{C}_6\text{H}_3\text{Cl}_2\text{O} \cdot \text{CHF} \cdot \text{CO}_2\text{Et} + \text{NaCl}$. An analogous reaction with ammonia would give fluoroglycine. However, chlorofluoroacetic acid and an excess of aqueous ammonia at room temperature gave chloride and fluoride ions at practically identical rates and glyoxylic acid was formed in at least 60% yield. This indicates that if fluoroglycine is a primary product it rapidly reacts further, giving iminoacetic acid (directly or by way of diaminoacetic acid), which would then be hydrolysed. The direct route is reminiscent of the behaviour of an α -aminoperfluoroalkylcarbamate:¹¹ $\text{NH}_2 \cdot \text{CRF} \cdot \text{NH} \cdot \text{CO}_2\text{Et} \rightarrow \text{NH}_2 \cdot \text{CR} \cdot \text{N} \cdot \text{CO}_2\text{Et}$.

Attempts to prepare fluoroglycine derivatives by reaction of ethyl chlorofluoroacetate with dimethylamine, aniline, or methylaniline in non-aqueous solvents were also unsuccessful, titration of aqueous extracts of the reaction mixtures again showing simultaneous liberation of chloride and fluoride ions. Reaction of ethyl chlorofluoroacetate with potassium phthalimide gave ethyl diphthalimidoacetate as the only identifiable product. Our results indicate that α -fluorinated amino-acids, like primary α -fluoroamines,¹¹ are labile. Since it seemed likely that the lability of the fluorine atom was due to the nitrogen atom in an α -position an attempt was made to prepare a

⁹ Leplawy, Jones, Kenner, and Sheppard, *Tetrahedron*, 1960, **11**, 39.

¹⁰ Maguire and Shaw, *J.*, 1957, 2713.

¹¹ Barr and Haszeldine, *J.*, 1956, 3428.

fluorinated aspartic acid in which these atoms are separated by two carbon atoms. However, when ethyl chlorofluoroacetate reacted with diethyl sodioacetamidomalonate considerable amounts of ionic fluoride were again produced and the desired intermediate could not be isolated.

Preliminary biological tests indicate that *S*-2-chloroethylhomocysteine causes a 40% inhibition of the growth of the transplanted Walker rat carcinoma when given as a single intraperitoneal dose of 25 mg./kg. The other new compounds described in this paper have no significant effect on the growth of this tumour.

EXPERIMENTAL

S-2-Hydroxyethyl-DL-homocysteine.—Sodium was added to a briskly stirred solution of *S*-benzyl-DL-homocysteine (6.75 g.) in liquid ammonia (150 ml.) until a blue colour persisted and then ethylene bromohydrin (4.5 ml.) was slowly run in. After 5 min. Amberlite IR-120 resin (NH₄⁺ form) was introduced and the ammonia was allowed to evaporate. The resin was extracted with water (500 ml.) and after being washed with ether (100 ml.) this extract was evaporated to dryness. When kept at 0° a solution of the residue in water (70 ml.) and acetone (300 ml.) deposited *S*-2-hydroxyethyl-DL-homocysteine (3.69 g.) which formed needles, m. p. 230° (decomp.), from aqueous acetone (Found: C, 40.3; H, 6.9; N, 7.8; S, 18.0%; equiv., by formol titration, 185. C₆H₁₃NO₂S requires C, 40.2; H, 7.3; N, 7.8; S, 17.9%; equiv., 179). Snyder *et al.*¹² gave m. p. 275—280° (decomp.), for *S*-2-hydroxyethyl-DL-homocysteine prepared by hydrolysis of 3,6-bis-[2-(2-hydroxyethylthio)ethyl]-2,5-dioxopiperazine.

S-2-Chloroethyl-DL-homocysteine Hydrochloride.—2-Hydroxyethylhomocysteine (1.39 g.) in concentrated hydrochloric acid (9 ml.) was heated on a steam-bath for 30 min., then evaporated under reduced pressure. The crystals of *S*-2-chloroethyl-DL-homocysteine hydrochloride, m. p. 137°, which separated were washed with acetone and dried *in vacuo* (Found: C, 31.3; H, 5.6; Cl, 30.6; N, 6.4; S, 14.0. C₆H₁₃Cl₂NO₂S requires C, 30.8; H, 5.6; Cl, 30.3; N, 6.0; S, 13.7%).

Benzyloxycarbonyl-DL-ethionine.—DL-Ethionine (1.99 g.) in 2*N*-sodium hydroxide (15 ml.) was shaken with benzyl chloroformate (1.6 ml.) for $\frac{3}{4}$ hr. with ice-cooling. After dilution with water (50 ml.) the solution was washed with ethyl acetate (3 × 50 ml.), then acidified and extracted with ethyl acetate (3 × 50 ml.). The washed and dried (Na₂SO₄) ethyl acetate extracts were evaporated and crystallisation of the residue from ethyl acetate–light petroleum (b. p. 60—80°) gave *benzyloxycarbonyl-DL-ethionine* (2.52 g.) as plates, m. p. 92—93° (Found: C, 56.7; H, 6.5; N, 4.8; S, 10.8. C₁₄H₁₉NO₄S requires C, 56.5; H, 6.4; N, 4.7; S, 10.8%).

Benzyloxycarbonyl-DL-ethionylglycine Ethyl Ester.—A solution of the above compound (2.1 g.) in chloroform (20 ml.) containing triethylamine (0.95 ml.) was cooled to <5° and isobutyl chloroformate (0.87 ml.) was added. After 15 minutes' stirring at <5° a solution of glycine ethyl ester hydrochloride (0.79 g.) in chloroform (10 ml.) containing triethylamine (0.95 ml.) was added and the whole was kept for 2 hr. at 20°. Chloroform (30 ml.) was then added and the solution was washed with 2*N*-hydrochloric acid (2 × 50 ml.), saturated aqueous sodium hydrogen carbonate (2 × 50 ml.), and water (50 ml.). The peptide ester (1.83 g.), obtained after evaporating the dried chloroform solution, crystallised from ethyl acetate–light petroleum (b. p. 60—80°) giving prisms, m. p. 74° (Found: C, 56.4; H, 6.7; N, 7.2; S, 8.1. C₁₈H₂₆N₂O₅S requires C, 56.5; H, 6.9; N, 7.3; S, 8.4%). No useful product was obtained on attempted hydrolysis of this ester.

DL-Ethionylglycine.—*S*-Benzyl-*N*-benzyloxycarbonyl-DL-homocysteine (35.9 g.) was dissolved in chloroform (350 ml.) containing triethylamine (15 ml.) and the cooled solution was treated with isobutyl chloroformate (13 ml.) and then with a solution of glycine benzyl ester toluene-*p*-sulphonate (33.7 g.) in chloroform (150 ml.) containing triethylamine (14 ml.). The mixture was kept at 20° for 16 hr. The solvent was removed and the residue was dissolved in ethyl acetate (400 ml.), washed with dilute hydrochloric acid, saturated aqueous sodium hydrogen carbonate, and water, dried (Na₂SO₄), and evaporated under reduced pressure to a non-crystalline product (46.3 g.). Sodium was added to a stirred solution of this (unpurified) *S*-benzyl-*N*-benzyloxycarbonyl-DL-homocysteinyglycine benzyl ester (1.41 g.) in liquid ammonia (100 ml.) until a persistent blue colour appeared. A nitroprusside test for thiol groups was then positive. Ethyl bromide (1 ml.) was added and shortly afterwards the

¹² Snyder, Howe, Cannon, and Nyman, *J. Amer. Chem. Soc.*, 1943, **65**, 2211.

nitroprusside test was negative. After the addition of Amberlite IR-120 (H^+) resin (50 g.) the ammonia was allowed to evaporate and the vessel was evacuated. The ion-exchange resin was then washed with water (3×75 ml.) and the extracts were concentrated, treated with charcoal, and filtered. Addition of acetone caused separation of DL-ethionylglycine (220 mg.), which formed needles, m. p. 195° (decomp.), from aqueous acetone (Found: C, 43.4; H, 7.2; N, 12.3; S, 14.3. $C_8H_{16}N_2O_3S$ requires C, 43.6; H, 7.3; N, 12.7; S, 14.6%).

S-Benzyl-DL-homocysteine Benzyl Ester Toluene-p-sulphonate.—*S-Benzyl-DL-homocysteine* (14.1 g.), toluene-*p*-sulphonic acid monohydrate (12.1 g.), benzyl alcohol (45 ml.), and benzene (35 ml.) were heated under reflux in a Stark and Dean apparatus until no more water was removed (2.5 ml. collected). After benzene (60 ml.) and then ether (150 ml.) had been added to the cooled mixture it was kept in a cold room. Next day the precipitate was collected and washed with ether (yield 25.8 g.). The *toluene-p-sulphonate* formed needles, m. p. $118-119^\circ$, from methanol-ether (Found: C, 61.0; H, 5.7; N, 2.7; S, 13.0. $C_{25}H_{29}NO_5S_2$ requires C, 61.6; H, 6.0; N, 2.9; S, 13.1%).

Benzylloxycarbonylglycyl-S-benzyl-DL-homocysteine Benzyl Ester.—Isobutyl chloroformate (12.2 ml.) was added to a cooled (2°), stirred solution of benzylloxycarbonylglycine (19.63 g.) in chloroform (350 ml.) containing triethylamine (15 ml.) and after 20 min. a solution of *S*-benzyl-DL-homocysteine benzyl ester toluene-*p*-sulphonate (45.73 g.) in chloroform (350 ml.) containing triethylamine (13 ml.) was added. After $3\frac{1}{2}$ hr. at 20° the mixture was evaporated and the residue extracted with ethyl acetate. The substituted dipeptide (37 g.), isolated as already described, did not crystallise and was used without purification in the next stage.

Glycyl-DL-ethionine.—Sodium was added to a stirred solution of the above dipeptide derivative (37.0 g.) in liquid ammonia (1 l.) until a persistent blue colour appeared. This mixture was treated with ethyl bromide (8 ml.), and the product (5.5 g.) was isolated as described above. *Glycyl-DL-ethionine* formed needles, m. p. 195° (decomp.), from aqueous acetone (Found: C, 43.3; H, 7.3; N, 12.7; S, 14.7%).

Benzylloxycarbonylglycyl-S-benzyl-DL-homocysteine.—Benzylloxycarbonylglycyl-*S*-benzyl-DL-homocysteine benzyl ester (6.65 g.) was dissolved in dioxan (70 ml.), and 2*N*-sodium hydroxide (15 ml.) was added. After 1 hr. at 20° the solution was just acidified with hydrochloric acid, and the solvent removed. The residue was dissolved in ethyl acetate (100 ml.), washed with water (100 ml.), and extracted with saturated aqueous sodium hydrogen carbonate (2×75 ml.) followed by 2*N*-aqueous ammonia (2×75 ml.). The combined aqueous extracts were acidified and extracted with chloroform (3×75 ml.). The washed and dried (Na_2SO_4) chloroform extracts yielded *benzylloxycarbonylglycyl-S-benzyl-DL-homocysteine* (3.65 g.), m. p. 138° , when crystallised from chloroform-light petroleum (b. p. $40-60^\circ$) and from benzene (Found: C, 60.6; H, 6.0; N, 6.8; S, 7.8. $C_{21}H_{24}N_2O_5S$ requires C, 60.5; H, 5.8; N, 6.7; S, 7.7%).

Benzylloxycarbonylglycyl-S-benzyl-DL-homocysteinyglycine Benzyl Ester.—Isobutyl chloroformate (10 ml.) was added to a cooled (2°) stirred solution of benzylloxycarbonylglycyl-*S*-benzyl-DL-homocysteine (30.0 g.) in chloroform (200 ml.) containing triethylamine (11.5 ml.) and after 15 min. a solution of glycine benzyl ester toluene-*p*-sulphonate (24.3 g.) in chloroform (150 ml.) containing triethylamine (10 ml.) was added. After 16 hr. at 20° the mixture was evaporated and extracted with ethyl acetate. The product, isolated in the usual manner, was obtained as a yellow syrup which was purified by chromatography through activated alumina in ethyl acetate solution. The *tripeptide derivative*, m. p. $110-114^\circ$, crystallised from ethyl acetate-light petroleum (b. p. $40-60^\circ$) (yield 32.1 g.) (Found: C, 64.0; H, 5.9; N, 7.3; S, 5.6. $C_{30}H_{33}N_3O_6S$ requires C, 63.9; H, 5.9; N, 7.5; S, 5.7%).

Glycyl-DL-ethionylglycine.—Sodium was added to a stirred solution of the above tripeptide derivative (3.0 g.) in liquid ammonia (120 ml.) until a persistent blue colour appeared. The mixture was treated with ethyl bromide (1.5 ml.), and the product (1.1 g.) was isolated as before. *Glycyl-DL-ethionylglycine* formed needles, m. p. 220° (decomp.), from aqueous acetone (Found: C, 43.2; H, 7.0; N, 15.0; S, 11.1. $C_{10}H_{19}N_3O_4S$ requires C, 43.5; H, 6.9; N, 15.1; S, 11.5%).

1-Toluene-p-sulphonamidocyclopentanecarboxylic Acid.—Solutions of 1-aminocyclopentanecarboxylic acid (1.29 g.) in *N*-aqueous sodium hydroxide (20 ml.) and toluene-*p*-sulphonyl chloride (2 g.) in ether (10 ml.) were mixed and stirred at room temperature for 16 hr. After removal of the ether the *sulphonamido-acid* was precipitated by acidification with dilute hydrochloric acid. The product, sparingly soluble in water but very soluble in ethanol, formed prismatic needles, m. p. $210-214^\circ$, from aqueous ethanol (Found: C, 54.9; H, 6.2; N, 4.8; S, 11.5. $C_{13}H_{17}NO_4S$ requires C, 55.1; H, 6.1; N, 4.9; S, 11.3%).

1-(*N*-Methyltoluene-*p*-sulphonamido)cyclopentanecarboxylic Acid.—A mixture of the above sulphonamido-acid (2.83 g.), methyl iodide (2.5 ml.), and 2*N*-aqueous sodium hydroxide (25 ml.) was heated in a sealed tube at 100° for 2½ hr. The product obtained by acidifying the cooled mixture formed prismatic needles, m. p. 135—137°, from aqueous methanol or benzene-light petroleum (b. p. 60—80°) (Found: C, 56.2; H, 6.4; N, 5.1; S, 10.9. C₁₄H₁₉NO₄S requires C, 56.5; H, 6.4; N, 4.7; S, 10.8%).

1-Methylaminocyclopentanecarboxylic Acid.—A solution of the methylated sulphonamido-acid (8.7 g.) in concentrated hydrochloric acid (90 ml.) was heated in a pressure bottle at 140° for 7 hr. and then evaporated to dryness. An aqueous solution of the residue was treated with charcoal and passed through the base form of Amberlite IR-45 resin. Evaporation of the eluates gave the methylamino-acid (3.7 g.) which was crystallised by dissolving it in a little water, adding ethanol, and evaporating the mixture to low bulk; prismatic needles were obtained. These did not melt but sublimed above 300°. The amino-acid did not give a positive ninhydrin reaction in solution and only gave a yellow colour on paper (Found: C, 58.6; H, 9.0; N, 9.6. C₇H₁₃NO₂ requires C, 58.7; H, 9.1; N, 9.8%).

1-Chloroacetamidocyclopentanecarboxylic Acid.—Chloroacetyl chloride (16 ml.) was added slowly with stirring to an ice-cooled solution of 1-aminocyclopentanecarboxylic acid (12.9 g.) in 2*N*-aqueous sodium hydroxide (200 ml.). Next day the solution was acidified with hydrochloric acid. The precipitated chloroacetamido-acid crystallised from acetone-light petroleum (b. p. 60—80°) as needles, m. p. 161—163° (7.6 g.) (Found: C, 46.8; H, 6.0; Cl, 17.2; N, 7.1%; equiv., 206.5. C₈H₁₂ClNO₃ requires C, 46.7; H, 5.9; Cl, 17.2; N, 6.8%; equiv., 205.6).

Reaction of Chloroacetamidocyclopentanecarboxylic Acid with Ammonia and with Methylamine.—(1) A solution of the chloroacetamido-acid in a large excess of aqueous ammonia (*d* 0.88) was left at room temperature for 24 hr.; titration of chloride ion then indicated that reaction was complete. The material obtained on evaporation was dissolved in a small amount of water and treated with the amount of silver oxide required to react with the chloride present. Evaporation of the filtered solution gave glycylamidocyclopentanecarboxylic acid, prisms, m. p. 273—274° (from aqueous ethanol) (Found: C, 51.8; H, 7.4. C₈H₁₄N₂O₃ requires C, 51.6; H, 7.6%). The dipeptide formed a hydrochloride, m. p. 210° alone or mixed with another sample.⁸

(2) A solution of 1-chloroacetamidocyclopentanecarboxylic acid (4.11 g.) in 8*N*-aqueous methylamine (200 ml.) was kept at room temperature for 48 hr. The residue obtained by evaporation under reduced pressure was redissolved in water and stirred with silver oxide (1.96 g.) for 1 hr. Filtration through "Hyflo Supercel" yielded 1-sarcosylamidocyclopentanecarboxylic acid monohydrate (3.45 g.), m. p. 181—182°, that formed needles from aqueous acetone (Found: C, 49.2; H, 8.5; N, 12.9. C₉H₁₆N₂O₃·H₂O requires C, 49.5; H, 8.3; N, 12.8%).

(3) When the solution obtained as in (2) was evaporated on a steam-bath and the residue heated there for a further 2 hr. and then extracted with acetone, a 50% yield of the 4-methyl-3,6-dioxopiperazine-2-spirocyclopentane hydrate, m. p. 87—89°, needles [from acetone-light petroleum (b. p. 40—60°)], was obtained (Found: C, 54.2; H, 8.1; N, 14.0. C₉H₁₄N₂O₂·H₂O requires C, 54.0; H, 8.1; N, 14.0%). Sublimation at 150°/0.3 mm. afforded the anhydrous dioxopiperazine, m. p. 125—126° (Found: C, 59.2; H, 7.6; N, 15.2. C₉H₁₄N₂O₂ requires C, 59.3; H, 7.7; N, 15.4%). Crystallisation from moist acetone-ether regenerated the monohydrate.

1-Benzylloxycarbonylamino-cyclopentanecarboxylic Acid.—1-Aminocyclopentanecarboxylic acid (24.8 g.) was dissolved in 1.67*N*-sodium hydroxide (120 ml.), and acetone (100 ml.) was added. After adjustment of the pH to 11.5 by hydrochloric acid and cooling at 0° a solution of benzyl chloroformate (79 g.) in acetone (100 ml.) was added dropwise with stirring during 70 min. The pH was maintained at 11.5—12.0 by addition of 2*N*-sodium hydroxide (Pye automatic titrator). After a further 90 min. at room temperature the solution was concentrated, diluted with water (500 ml.), washed with ethyl acetate (2 × 350 ml.), acidified with hydrochloric acid, and extracted with ethyl acetate (2 × 500 ml.). The washed and dried extracts were evaporated and the residue (47.6 g.) was crystallised from ethyl acetate-light petroleum (b. p. 60—80°), forming needles, m. p. 95° (Found: C, 63.9; H, 6.5; N, 5.4. Calc. for C₁₄H₁₇NO₄: C, 63.9; H, 6.5; N, 5.3%). Tailleux and Berlinguet¹³ give m. p. 92—93°.

Condensation of 1-Benzylloxycarbonylamino-cyclopentanecarboxylic Acid with Sarcosine Ethyl Ester.—Sarcosine ethyl ester hydrochloride (1.37 g.), triethylamine (1.4 ml.), and tetrahydrofuran (25 ml.) were stirred together for ½ hr. and the triethylamine hydrochloride was filtered

¹³ Tailleux and Berlinguet, *Canad. J. Chem.*, 1961, **39**, 1309.

off and washed with tetrahydrofuran (10 ml.). The above benzyloxycarbonyl derivative (2.36 g.) was dissolved in the filtrate and then a solution of dicyclohexylcarbodi-imide (1.86 g.) in tetrahydrofuran (10 ml.) was added with stirring. Next day the filtered solution was evaporated to dryness and the residue dissolved in ethyl acetate, washed with water (40 ml.), 2N-hydrochloric acid (40 ml.), and saturated sodium hydrogen carbonate solution (40 ml.), and dried (Na_2SO_4). The concentrated ethyl acetate solution was passed through a column of activated alumina and on evaporation of the eluates a syrup (3.08 g.) was obtained. Hydrolysis of this product with sodium hydroxide in aqueous dioxan gave a white amorphous powder which after hydrogenation in ethanol over palladium-charcoal gave the dioxopiperazine derivative, m. p. and mixed m. p. 86—87°.

Ethyl 1-(Benzyloxycarbonyl-L-leucylamido)cyclopentanecarboxylate.—Isobutyl chloroformate (2.16 ml.) was added to a cooled (5°), stirred solution of benzyloxycarbonyl-L-leucine (4.4 g.) in tetrahydrofuran (15 ml.) containing triethylamine (2.3 ml.), and after 20 min. a solution of ethyl 1-aminocyclopentanecarboxylate hydrochloride (3.33 g.) in chloroform (10 ml.) containing triethylamine (2.3 ml.) was added. After 16 hr. at 20° the mixture was evaporated and extracted with ethyl acetate. The extract afforded *ethyl 1-(benzyloxycarbonyl-L-leucylamido)cyclopentanecarboxylate*, m. p. 75—78°, $[\alpha]_D -22^\circ$ (c 2 in EtOH), which crystallised from aqueous ethanol (yield 3.7 g.) (Found: C, 65.1; H, 8.0; N, 7.2. $\text{C}_{22}\text{H}_{32}\text{N}_2\text{O}_5$ requires C, 65.3; H, 8.0; N, 6.9%).

Ethyl 1-(L-Leucylamido)cyclopentanecarboxylate Hydrochloride.—The benzyloxycarbonyl derivative (3.7 g.) was hydrogenated at atmospheric pressure in ethanol (50 ml.) containing ethanolic 2N-hydrogen chloride (1.5 ml.), glacial acetic acid (2 drops), and 5% palladium-charcoal. Evaporating the filtered solution gave a solid (2.1 g.). *Ethyl 1-(L-leucylamido)cyclopentanecarboxylate hydrochloride hemihydrate*, m. p. 129—130°, $[\alpha]_D -5^\circ$ (c 2 in EtOH), crystallised from ethanol-ether (Found: C, 53.2; H, 8.4; Cl, 11.5; N, 9.4. $\text{C}_{14}\text{H}_{27}\text{ClN}_2\text{O}_3 \cdot \frac{1}{2}\text{H}_2\text{O}$ requires C, 53.2; H, 8.9; Cl, 11.2; N, 8.9%).

N-(1-Benzyloxycarbonylamidocyclopentanecarbonyl)-L-tyrosine Ethyl Ester.—Solutions of benzyloxycarbonyl-1-aminocyclopentanecarboxylic acid (5.3 g.) in tetrahydrofuran (10 ml.) and L-tyrosine ethyl ester (4.2 g.) in tetrahydrofuran (15 ml.) were mixed and dicyclohexylcarbodi-imide (4.08 g.) was then added and the mixture was stirred for 3 hr. Next day a little acetic acid was added and the solution was filtered and evaporated to dryness. An ethyl acetate extract of the residue was washed with 2N-hydrochloric acid, saturated aqueous sodium hydrogen carbonate, and water and then dried (Na_2SO_4). The *dipeptide derivative* obtained on evaporation crystallised from ethyl acetate-light petroleum (b. p. 40—60°). It (5.9 g.) had m. p. 102°, $[\alpha]_D +5^\circ$ (c 2 in EtOH) (Found: C, 65.1; H, 7.1; N, 6.2. $\text{C}_{25}\text{H}_{30}\text{N}_2\text{O}_6 \cdot \text{H}_2\text{O}$ requires C, 65.0; H, 7.0; N, 6.2%).

N-(1-Aminocyclopentanecarbonyl)-L-tyrosine Ethyl Ester Hydrochloride.—The preceding derivative (5.9 g.) was hydrogenated as described above, affording a gum which solidified in contact with ether. *N-(1-Aminocyclopentanecarbonyl)-L-tyrosine ethyl ester hydrochloride hydrate*, m. p. 128—130° (2.8 g.), $[\alpha]_D -13^\circ$ (c 2 in EtOH), was obtained (Found: C, 55.0; H, 6.9; Cl, 9.8; N, 7.3. $\text{C}_{17}\text{H}_{25}\text{ClN}_2\text{O}_4 \cdot \text{H}_2\text{O}$ requires C, 54.5; H, 7.3; Cl, 9.5; N, 7.5%).

5,5-Di-(isobutyloxycarbonylmethyl)hydantoin.—A solution of di-isobutyl acetonedicarboxylate (66 ml., b. p. 170°/6 mm.), sodium cyanide (20 g.), and ammonium carbonate (76 g.) in 1 : 1 v/v aqueous ethanol (1.2 l.) was heated at 60° for 16 hr., then concentrated to low bulk under reduced pressure and after the pH had been adjusted to 7 with hydrochloric acid was extracted with ether. On evaporation the dried ether extract gave a product (22 g.) which crystallised on slow cooling of a solution in a large volume of boiling water. The *hydantoin* formed needles, m. p. 116—117°, from ether-light petroleum (b. p. 40—60°) (Found: C, 54.8; H, 7.2; N, 8.6. $\text{C}_{15}\text{H}_{24}\text{N}_2\text{O}_6$ requires C, 54.9; H, 7.4; N, 8.5%).

Diammonium Hydrogen 2-Aminopropane-1,2,3-tricarboxylate.—A solution of the above hydantoin (5 g.) and barium hydroxide octahydrate (15 g.) in water (200 ml.) was heated in a pressure bottle at 160° for 1 hr. The solution was then saturated with carbon dioxide and the precipitate which contained the amino-acid as an insoluble barium salt was collected. Heating the precipitate with an excess of aqueous ammonium carbonate and evaporating the filtered solution gave a glass (2.2 g.). *Diammonium hydrogen 2-aminopropane-1,2,3-tricarboxylate* formed fine needles, m. p. 220—230° (decomp.; closed capillary), from aqueous ethanol (Found: C, 31.9; H, 6.3; N, 18.9. $\text{C}_6\text{H}_{15}\text{N}_3\text{O}_8$ requires C, 32.0; H, 6.7; N, 18.6%).

Reaction of Chlorofluoroacetic Acid with Ammonia.—A solution of chlorofluoroacetic acid

(8 g.; prepared by hydrolysis of ester that had been prepared by the method of Bacon *et al.*¹⁴ or Englund¹⁵) in water (200 ml.) was saturated with ammonia at 8°. At intervals 25-ml. portions were removed and the excess of ammonia removed by drawing air through at reduced pressure. Chloride ion was then determined by direct Volhard titration and fluoride ion by the lead chlorofluoride method.¹⁶ The following figures were obtained in a typical run:

Time (hr.)	0	4.5	16	24	56	200
Cl ⁻ (%)	0.8	4.7	15.1	22.9	55.5	65.0
F ⁻ (%)	0.5	4.4	14.0	25.7	57.9	66.0

Addition of aminoguanidine in dilute acetic acid after 200 hr. gave a precipitate of aminoguanidineglyoxylic acid (60%).

Reaction of Ethyl Chlorofluoroacetate with Dimethylamine and with Aniline.—(1) The ester (5 ml.) was heated in a sealed tube with a 1.4*N*-solution of dimethylamine in benzene (10 ml.) at 180° for 2 hr. An aqueous extract of the product contained chloride ion (13%) and fluoride ion (14%). (2) A solution of the ester (5.5 ml.) and aniline (5.5 ml.) in benzene (20 ml.) was heated under reflux for 26 hr. Titration of an aqueous extract indicated 4% release of chloride ion and 3.5% release of fluoride ion.

Reaction of Ethyl Chlorofluoroacetate with Potassium Phthalimide.—The ester (2 g.) and potassium phthalimide (2.62 g.) were heated in a sealed tube at 160° for 1½ hr. A benzene-light petroleum (b. p. 60—80°) extract of the product was passed through a column of activated alumina. Elution of the column with benzene gave *ethyl diphthalimidoacetate*, m. p. 146—147°, prisms [from chloroform-light petroleum (b. p. 60—80°) or methanol] (Found: C, 63.5; H, 4.2; N, 7.4. C₂₀H₁₄N₂O₆ requires C, 63.5; H, 3.7; N, 7.4%).

Reaction of Ethyl Chlorofluoroacetate with Diethyl Sodioacetamidomalonate.—Sodium (2.3 g.) was dissolved in dry ethanol (70 ml.) and to this solution was added diethyl acetamidomalonate (21.7 g.) followed by ethyl chlorofluoroacetate (11.6 g.) in ethanol (20 ml.). After being heated for about 9 hr. at 70° the mixture was neutral to phenolphthalein. About one-half of the solvent was removed and water (500 ml.) was added. Titration of an aliquot part of the filtered solution indicated that 54% of the chlorine and 44% of the fluorine were in the ionic form.

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¹⁴ Bacon, Bradley, Hoegberg, Tarrant, and Cassaday, *J. Amer. Chem. Soc.*, 1948, **70**, 2653.

¹⁵ Englund, *Org. Synth.*, 1954, **34**, 16, 49.

¹⁶ Simons, "Fluorine Chemistry," Academic Press, Inc., New York, 1950.