428. *Some Derivatives of* 1-*Aminocyclopentanecarboxylic Acid and Related Compounds.*

By T. A. Connors and W. C. J. Ross.

Some derivatives of 1-aminocyclopentanecarboxylic acid have been prepared for examination as tumour-growth inhibitors. These include several esters and acyl derivatives, and dipeptides derived from glycine and DL-phenylalanine. Substituted derivatives of the parent amino-acid include the 2- and 3-carboxy- and the 2,3-benzo-compounds. 1-Amino-cyclopropane-, -cyclobutane-, -cyclohexane-, -cycloheptane-, and -cyclo-octane-carboxylic acid have also been prepared and some new derivatives are described. Two isomers of 2-aminocyclopentane-1-carboxylic acid have been prepared and characterised. α-Cyclopropyl- and αβ-diphenyl-alanine have also been obtained.

The observation that 1-aminocyclopentanecarboxylic acid inhibits tumour-growth [1] has led to the preparation and testing of a number of derivatives of this amino-acid and some closely related compounds. The parent amino-acid is not well tolerated and it was hoped that derivatives would retain the anti-tumour activity whilst having lower overall toxicities.

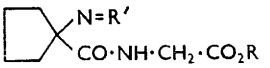
Of the methods described for the preparation of 1-aminocyclopentanecarboxylic acid,[2,3]

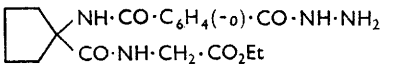
[1] Connors, Elson, and Ross, *Biochem. Pharm.*, 1958, **1**, 239.

[2] Zelinsky, Annenkov, and Kulikov, *J. Russ. phys. Chem. Ges.*, 1911, **43**, 1097; McDonald, U.S.P. 2,560,584; Henze and Speer, *J. Amer. Chem. Soc.*, 1942, **64**, 522; Connors and Ross, *Biochem. Pharm.*, 1959, **1**, 93.

[3] Zelinsky and Stadnikov, *Z. physiol. Chem.*, 1911, **75**, 350.

hydrolysis of the corresponding hydantoin by aqueous barium hydroxide at 150—160° proved the most satisfactory. This acid has not been well characterised, only a liquid ethyl ester [4] and an infusible copper salt [3] being described apart from a casual reference to the hydrochloride.[5] * In the present investigation the hydrochlorides of the methyl, ethyl, and isopropyl ester, the acetyl, benzoyl, and phthaloyl derivative, the amide and phthaloylamide, the phthaloyl anhydride, the *N*-carbamoyl derivative, and the benzoyl derivative of the ethyl ester have been prepared.

(I) 

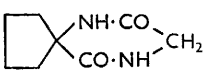


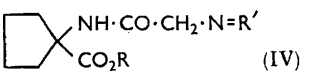
(II)

Thionyl chloride in the presence of pyridine converted the phthaloyl derivative into the acid chloride which with glycine ethyl ester gave 1-phthalimido-*N*-(ethoxycarbonylmethyl)-cyclopentanecarboxyamide [I; R = Et, R′ = *o*-$C_6H_4(CO)_2$]. This dipeptide was also obtained by the interaction of 1-phthalimidocyclopentanecarboxylic acid and glycine ethyl ester by the mixed anhydride method with isobutyl chloroformate as reagent. On addition of hydrazine to an ethanolic solution of the phthaloyl compound [I; R = Et, R′ = *o*-$C_6H_4(CO)_2$] a crystalline precipitate was formed within a few minutes, which gave no ninhydrin reaction but dissolved readily in 2N-hydrochloric acid and was identified as the hydrazide (II); if its solution in hydrochloric acid was heated phthalhydrazide separated and the hydrochloride of the peptide (I; R = H, R′ = H_2) was obtained by evaporating the filtered solution. A similar hydrazide had been obtained from 1-phthalimidocyclopentanecarboxyamide but in this case treatment with dilute hydrochloric acid resulted in complete hydrolysis. The free peptide was obtained by passing a solution of the hydrochloride through an ion-exchange column. It was obtained as a hydrate and when an attempt was made to remove the water of crystallisation by sublimation in a high vacuum the dioxopiperazine (III) was formed.

When this cyclic dipeptide (III) was heated with concentrated hydrochloric acid ring fission occurred with the re-formation of the original dipeptide (I; R = H, R′ = H_2).

Ethyl 1-aminocyclopentanecarboxylate reacted with phthaloylglycyl chloride, to give the dipeptide derivative [IV; R = Et, R′ = *o*-$C_6H_4(CO)_2$]. Treatment of this compound with hydrazine hydrate under mild conditions gave the acid [IV; R = H, R′ = *o*-$C_6H_4(CO)_2$]

(III) 



(IV)

which was also obtained in low yield by the action of phthaloylglycyl chloride on 1-aminocyclopentanecarboxylic acid in the presence of an aqueous suspension of magnesium hydroxide. More vigorous treatment with hydrazine led to the formation of the free peptide (IV; R = H, R′ = H_2) which was characterised as the hydrochloride.

Ethyl 1-aminocyclopentanecarboxylate condensed with *N*-phthaloyl-DL-phenylalanine in the presence of cyclohexylcarbodi-imide, giving the dipeptide [V; R = Et, R′ = *o*-$C_6H_4(CO)_2$] which was readily converted into the free dipeptide (V; R = H, R′ = H_2) by treatment with hydrazine and acid hydrolysis.

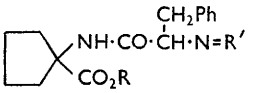
Attempts to prepare the phenylalanyl dipeptide derivative (VI) by the acid chloride

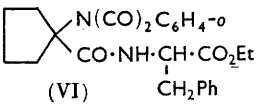
* Since this work was completed our attention has been drawn to B.P. 752,692 in which the preparation of 1-aminocyclopentanecarboxylic acid by alkaline hydrolysis under pressure at an unspecified temperature has been described. The phthalimido-acid, amide, and benzamido-acid are mentioned but adequate analytical data are not given. Neelakantan and Hartung [5] describe a hydrochloride, m. p. 222—224°; we have prepared a *hydrochloride*, m. p. 286—290°, prisms from dilute hydrochloric acid (Found, for a specimen dried at 100°/0·05 mm.: C, 44·0; H, 7·5; N, 8·3. $C_6H_{11}O_2N$,HCl requires C, 43·5; H, 7·3; N, 8·4%).

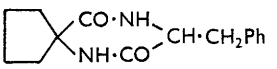
[4] Zelinsky, Annenkov, and Kulikov, *Z. physiol. Chem.*, 1910, **73**, 465.
[5] Neelakantan and Hartung, *J. Org. Chem.*, 1958, **23**, 964.

or the carbodi-imide method led to a gum which on reaction with hydrazine was converted in good yeld into the dioxopiperazine (VII).

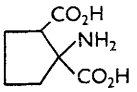
The 2- (VIII) and 3-carboxylic acid (IX) derivatives of 1-aminocyclopentanecarboxylic acid have been prepared by alkaline hydrolysis of the corresponding hydantoins which

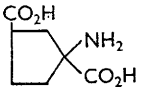

(V)

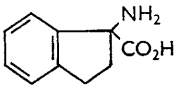

(VI)


(VII)

were obtained by the Bucherer–Bergs method from the known oxocyclopentanecarboxylic esters. 1-Aminoindane-1-carboxylic acid (X)—the 2,3-benzo-derivative of the tumour-growth inhibitory amino-acid—was similarly obtained from indan-1-one.


(VIII)


(IX)


(X)

In connection with studies on the mode of action of 1-aminocyclopentanecarboxylic acid it was desirable to prepare α-aminoadipic acid and 2-aminocyclopentane-1-carboxylic acid. The former would be produced *in vivo* by β-oxidation followed by ring fission, and the latter would be rather more readily converted into the same toxic cyclopent-1-ene-carboxylic acid that would result from deamination of the cyclic α-amino-acid.

α-Aminoadipic acid has been obtained by a number of methods [6] and it has now been found convenient to obtain this aminodicarboxylic acid by the following new route:

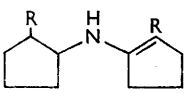
$$EtO_2C{\cdot}CH{:}CH{\cdot}CH_2Br + NHAc{\cdot}CH(CO_2Et)_2 \longrightarrow \underset{(XI)}{EtO_2C{\cdot}CH{:}CH{\cdot}CH_2{\cdot}C{\cdot}(NHAc)(CO_2Et)_2} \longrightarrow$$

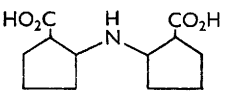
$$\underset{(XII)}{EtO_2C{\cdot}[CH_2]_3{\cdot}C(NHAc)(CO_2Et)_2} \longrightarrow \underset{(XIII)}{HO_2C{\cdot}[CH_2]_3{\cdot}CH(NH_2){\cdot}CO_2H}$$

The low-melting unsaturated ester (XI) obtained by condensing ethyl γ-bromocrotonate with diethyl acetamidomalonate was readily converted into the crystalline acetamido-triester (XII) on hydrogenation over a platinum catalyst. α-Aminoadipic acid (XIII) was obtained by acid-hydrolysis of this triester.

Several methods for the preparation of 2-aminocyclopentane-1-carboxylic acid were explored. Catalytic reduction of ethyl 2-aminocyclopent-1-enecarboxylate (XIV; R = CO_2Et) gave a small amount of an amino-acid which formed a hydrochloride, m. p. 210°, together with a compound, $C_{12}H_{19}O_4N$. Titration curves and the behaviour on ion-exchange columns suggested that the latter compound was an aminodicarboxylic acid and the negative ninhydrin reaction indicated that a primary amino-group was absent.


(XIV)


(XV)


(XVI)

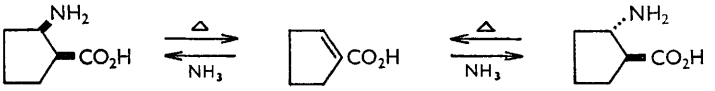
Thompson [7] has observed that 1-amino-2-cyanocyclopent-1-ene (XIV; R = CN) undergoes self-condensation to the secondary amine (XV; R = CN). Similar condensation of the unsaturated ester (XIV; R = CO_2Et) would give the diester (XV; R = CO_2Et).

[6] Sorensen, *Compt. rend. Trav. Lab. Carlsberg*, 1903, **6**, 1; Dieckmann, *Ber.*, 1905, **38**, 1656; Linstead and Wang, *J.*, 1937, 807; Gaudry, *Canad. J. Res.*, 1949, *B*, **27**, 21; Waalkes, Fones, and White, *J. Amer. Chem. Soc.*, 1950, **72**, 5760; Rothstein and Claus, *ibid.*, 1953, **75**, 2981.

[7] Thompson, *J. Amer. Chem. Soc.*, 1958, **80**, 5483.

Reduction of this diester (XV) and hydrolysis of the product would lead to di-(2-carboxycyclopentyl)amine (XVI) which would have the composition and properties of the second product obtained from the unsaturated ester (XIV; R = CO_3Et). Its formation is also consistent with the production of ammonium salts which were isolated during the working up of the reaction mixture. Pyrolysis of the diacid (XVI) gave ammonia and cyclopent-1-enecarboxylic acid.

α-Amino-acids have been prepared by the action of ammonia under pressure and at elevated temperature on αβ-unsaturated acids.[8] Under these conditions cyclopent-1-enecarboxylic acid gave two, isomeric, amino-acids $C_6H_{11}O_2N$, the separation of which was achieved by way of the copper salts.* The amino-acid, m. p. 242—243°, which gave a sparingly soluble copper salt formed a hydrochloride, m. p. 165—170°, and an *N*-benzoyl ethyl ester, m. p. 99°. The mother-liquors from the copper salts of the original reaction mixture contained unchanged cyclopentenecarboxylic acid and an amino-acid which gave a hydrochloride, m. p. 212°, and an *N*-benzoyl ethyl ester, m. p. 93°. This amino-acid proved identical with the product obtained in low yield by the method described above. It appears that the two amino-acids are *cis*- (XVIIa) and *trans*-isomers (XVIIb) of 2-aminocyclopentanecarboxylic acid. The possibility that one compound might have been produced by α-addition of the amino-group forming 1-aminocyclopentanecarboxylic acid was excluded since this acid was shown to give an *N*-benzoyl ethyl ester, m. p. 77°, depressed on admixture with either of the products obtained by the reaction of ammonia with cyclopent-1-enecarboxylic acid (XVIII).



(XVIIa) (XVIII) (XVIIb)

Initial results [1] indicated that whilst 1-aminocyclopentanecarboxylic acid inhibited tumour growth 1-aminocyclohexanecarboxylic acid was ineffective. In connection with structure–activity studies it was therefore of interest to examine other alicyclic α-amino-acids.

Of the methods available for the preparation of 1-aminocyclopropane-1-carboxylic acid [9] hydrolysis of hydantoin-5-spirocyclopropane for a short time with aqueous barium hydroxide at 155° has given a good yield of the amino-acid. Under more vigorous conditions the yield was lower and there was evidence of ring fission. 1-Aminocyclobutanecarboxylic acid was similarly prepared from the cyclobutane spiran which had been first obtained by Ingold *et al.*; [9] this amino-acid was prepared by Dem'yanov and Tel'nov [10] by alkaline hydrolysis of the spirohydantoin but the product does not appear to have been characterised.

1-Aminocyclohexane-, 1-amino-3-methylcyclohexane-, 1-aminocycloheptane-, and 1-aminocyclo-octane-carboxylic acid, as well as 1-amino-1,2,3,4-tetrahydro-1- and 2-amino-1,2,3,4-tetrahydro-2-naphthoic acid, were obtained from the appropriate ketone by way of the hydantoin which was hydrolysed by barium hydroxide. The hydantoin from 2-methylcyclohexanone was resistant to alkaline hydrolysis but readily yielded 1-amino-2-methylcyclohexanecarboxylic acid when hydrolysed with dilute sulphuric acid under pressure. Similar resistance to hydrolysis imposed by the 2-methyl group was observed

* Our attention has been drawn to a very recent paper by Plieninger and Schneider (*Chem. Ber.*, 1959, **62**, 1594) in which the addition of ammonia to cyclopent-1-enecarboxylic acid is stated to give exclusively the amino-acid, m. p. 240°, to which they assign the *trans*-configuration.

[8] Slimmer, *Ber.*, 1902, **35**, 400.

[9] Ingold, Sako, and Thorpe, *J.*, 1922, **121**, 1177; Dem'yanov and Feofilaktov, *J. Gen. Chem.* (*U.S.S.R.*), 1939, **9**, 340; Burroughs, *Nature*, 1957, **179**, 360; Rinderknecht and Niemann, *J. Amer. Chem. Soc.*, 1951, **73**, 4259.

[10] Dem'yanov and Tel'nov, *Bull. Acad. Sci. U.R.S.S. Classe Sci. Math. nat., Ser. chim.*, 1937, p. 529.

by Skita and Levi [11] who prepared 1-amino-2-methylcyclohexanecarboxylic acid by acid-hydrolysis of the corresponding amino-nitrile. In the course of this work some minor discrepancies with published constants for known compounds were encountered; where this occurred the *N*-benzoyl derivatives of the amino-acids were prepared to characterise our products.

Two isosteres of the active 1-aminocyclopentanecarboxylic acid examined were α-methylnorvaline and α-cyclopropylalanine. These were made from the relevant ketones, and the latter amino-acid, which showed growth-inhibitory activity of a low order, was converted into its glycyl dipeptide.

αβ-Diphenylalanine was synthesised by the hydantoin method and characterised as its *N*-benzoyl and *N*-carbamoyl derivative.

Experimental

M. p.s, which are corrected, were determined in sealed capillaries.

1-*Aminocyclopentanecarboxylic Acid.*—Hydantoin-5-spirocyclopentane (cf. Henze and Speer [2]) (14·9 g.), barium hydroxide octahydrate (53·3 g.), and water (325 ml.) were heated for 2 hr. at 160°. After cooling, the precipitated barium carbonate was removed and ammonium carbonate (12 g.) was added to the filtrate to remove the excess of barium. Concentration of the filtered solution gave the amino-acid (11·7 g.) as prisms, m. p. 260—285°. After drying at 120° for 3 hr. the m. p. rose to 328—329° (Zelinsky and Stadnikov [3] give m. p. 320° for the monohydrate) (Found: C, 55·8; H, 8·6; N, 11·1. Calc. for $C_6H_{11}O_2N$: C, 55·8; H, 8·6; N, 10·9%).

A suspension of the amino-acid (8 g.) in methanol (200 ml.) was saturated with dry hydrogen chloride, and the mixture was heated under reflux for 3 hr. After removal of most of the methanol under reduced pressure addition of ether caused the separation of an oil which gradually solidified. This, the *methyl ester hydrochloride*, formed needles, m. p. 207—208° (decomp.) when a mixture of acetone and ether was added to a methanolic solution of it at 0° [Found: C, 47·0; H, 8·0%; equiv. (Volhard), 173·6. $C_7H_{13}O_2N$,HCl requires C, 46·8; H, 7·9%; equiv., 179·7].

The *ethyl ester hydrochloride*, needles, m. p. 228° (decomp.), from ether–ethanol (Found: C, 49·3; H, 8·2; N, 6·8. $C_8H_{15}O_2N$,HCl requires C, 49·6; H, 8·3; N, 7·2%), and the *isopropyl ester hydrochloride*, needles, m. p. 180°, from ether–propan-2-ol [Found: C, 51·6; H, 8·5; N, 7·2%; equiv. (Volhard), 208. $C_9H_{17}O_2N$,NCl requires C, 52·0; H, 8·7; N, 6·8%; equiv., 207·5], were similarly prepared.

The N-*acetyl derivative* was obtained by heating the amino-acid (10 g.) with acetic anhydride (9·5 ml.) and acetic acid (25 ml.) at 100° for 2 hr. It formed needles, m. p. 195—197°, from ethanol–light petroleum (b. p. 40—60°) (Found: C, 56·4; H, 7·5; N, 7·8. $C_8H_{13}O_3N$ requires C, 56·1; H, 7·7; N, 8·2%).

To a stirred solution of the amino-acid (2 g.) in 2N-aqueous sodium carbonate (28 ml.) was added benzoyl chloride (3·2 ml.). The 1-*benzamidocyclopentanecarboxylic acid* thus obtained formed needles, m. p. 214—215°, from aqueous ethanol (Found: C, 66·7; H, 6·5; N, 6·0%; equiv. by titration, 236. $C_{13}H_{15}O_3N$ requires C, 66·9; H, 6·5; N, 6·0%; equiv. 233).

1-*Phthalimidocyclopentanecarboxylic Acid, Anhydride, Chloride, and Amide.*—1-Aminocyclopentanecarboxylic acid (3 g.) and phthalic anhydride (4·5 g.) were heated in a sealed tube at 170° for 1 hr. The methanol-soluble fraction of the product formed fine needles, m. p. 156—158°, from water. This material was almost certainly the *phthalimido-acid dihydrate* (equiv. by titration, 291. $C_{14}H_{13}O_4N,2H_2O$ requires equiv., 295) since drying for several hours at 100°/2 mm. raised the m. p. to 163° and analysis indicated that the *acid* was then anhydrous (Found: C, 64·7; H, 5·4; N, 5·4. $C_{14}H_{13}O_4N$ requires C, 64·9; H, 5·1; N, 5·4%). The methanol-insoluble fraction formed prismatic needles, m. p. >360°, from hot ethanol; this was probably 3,6-*dioxopiperazine*-2,5-*bis*(*spirocyclopentane*) derived from the free amino-acid (Found: N, 12·2. $C_{12}H_{18}O_2N_2$ requires N, 12·6%).

The phthalimido-acid (1·17 g.) and dicyclohexylcarbodi-imide (0·94 g.) were dissolved in tetrahydrofuran (10 ml.); dicyclohexylurea soon separated. Next day the solid (450 mg.) was

[11] Skita and Levi, *Ber.*, 1908, **41**, 2925.

filtered off and washed with a little ether. The solution contained the *anhydride* of the phthalimido-acid; this derivative formed prisms, m. p. 118°, from ether–pentane (Found: C, 67·1; H, 5·0; N, 5·6. $C_{28}H_{24}O_7N_2$ requires C, 67·3; H, 4·8; N, 5·6%) and when heated in water for 2 hr. was reconverted into the phthalimido-acid, m. p. 158°.

A mixture of the phthalimido-acid (9 g.) and thionyl chloride (30 ml.) containing pyridine (0·5 ml.) was stirred at room temperature for 1 hr. and then at 40° for ½ hr. After removal of the excess of thionyl chloride under reduced pressure at 40° the residual *acid chloride* was extracted with benzene–pentane and crystallised from pentane as prisms, m. p. 44° (Found: C, 60·5; H, 4·7. $C_{14}H_{12}O_3NCl$ requires C, 60·6; H, 4·4%).

Shaking an ethereal solution of this chloride with concentrated aqueous ammonia afforded 1-*phthalimidocyclopentanecarboxyamide*. It formed prisms, m. p. 215°, from ethanol (Found: C, 64·9; H, 5·4; N, 10·5. $C_{14}H_{14}O_3N_2$ requires C, 65·1; H, 5·5; N, 10·9%). An attempt to remove the phthaloyl group from this amide by the action of one equivalent of ethanolic hydrazine led to a sparingly soluble *hydrazide*, m. p. 210°, needles from ethanol (Found: C, 57·9; H, 6·3; N, 19·6. $C_{14}H_{18}O_3N_4$ requires C, 57·9; H, 6·3; N, 19·3%). When this hydrazide was heated with dilute hydrochloric acid 1-aminocyclopentanecarboxylic acid was formed: it has not proved possible to obtain 1-aminocyclopentanecarboxyamide from the hydrazide, complete hydrolysis occurring under all the conditions employed.

1-*Aminocyclopentanecarboxyamide*.—Ethyl 1-aminocyclopentanecarboxylate hydrochloride (10 g.) was suspended in dry ether and after the addition of triethylamine (7·2 ml.) the mixture was stirred for 2 hr. After removal of the precipitated triethylamine hydrochloride the ethereal solution was evaporated and the residual oil was washed into a pressure bottle with concentrated aqueous ammonia (75 ml.). The contents of the sealed bottle were stirred at 30° for 3 days by which time a single phase had formed. Evaporation of the solution gave a residue which was extracted with acetone. Addition of light petroleum to this extract caused separation of the *amide* (2·2 g.). On recrystallisation from acetone–pentane it formed needles, m. p. 95—96° (Found: C, 56·3; H, 9·5; N, 21·6. $C_6H_{12}ON_2$ requires C, 56·2; H, 9·4; N, 21·9%). Heating this amide (125 mg.) with phthalic anhydride (125 mg.) at 90° for 1 hr. gave the phthalimido-derivative, m. p. 212°, not depressed by admixture with the compound of m. p. 215° described above.

1-*Ureidocyclopentanecarboxylic Acid*.—1-Aminocyclopentanecarboxylic acid (12 g.) and potassium cyanate (16·2 g.) in sufficient water for complete dissolution were heated on a steam-bath for 1 hr. When the pH of the cooled solution was adjusted to 5 by hydrochloric acid the *ureido-compound* separated. The acid formed small prisms, m. p. 203° (decomp.), from water (Found: C, 48·9; H, 6·7; N, 16·2. $C_7H_{12}O_3N_2$ requires C, 48·8; H, 7·0; N, 16·3%).

Ethyl 1-*Benzamidocyclopentanecarboxylate*.—A solution of ethyl 1-aminocyclopentanecarboxylate hydrochloride (500 mg.) in pyridine (2 ml.) containing benzoyl chloride (500 mg.) was heated at 100° for 15 min. After dilution with water the mixture was extracted with ether, and this extract washed successively with 2N-hydrochloric acid, water, 2N-sodium carbonate, and water, dried (Na_2SO_4), and evaporated, giving a colourless gum. This was resolved by passing a solution in benzene through a column of activated alumina; further washing with benzene slowly eluted the *benzamido-ester*, prismatic needles, m. p. 76—77°, from light petroleum (b. p. 40—60°) (Found: C, 68·8; H, 7·1; N, 5·4. $C_{15}H_{19}O_3N$ requires C, 68·9; H, 7·3; N, 5·4%).

N-(*Ethoxycarbonylmethyl*)-1-*phthalimidocyclopentanecarboxyamide*.—(1) 1-Phthalimidocyclopentane-1-carbonyl chloride (23·5 g.) was added during 30 min. to a stirred solution of glycine ester hydrochloride (11·2 g.) and triethylamine (24·8 ml.) in dioxan (80 ml.) at 5—10°. After stirring of the cooled solution for a further 4 hr. water (1½ l.) was added and most of the dioxan was removed at 50—60° under reduced pressure. The resinous product was extracted with ether (1 l.), and the washed extract was dried ($CaCl_2$). Adding pentane to the concentrated ether extract precipitated the *peptide derivative* (16·3 g.). Recrystallisation from ether–pentane gave needles, m. p. 90° (Found: C, 62·7; H, 5·9; N, 8·1. $C_{18}H_{20}O_5N_2$ requires C, 62·8; H, 5·9; N, 8·1%).

(2) Isobutyl chloroformate (1·3 ml.) was added slowly to a cooled (5°) and stirred solution of 1-phthalimidocyclopentanecarboxylic acid (2·6 g.) and triethylamine (1·4 ml.) in dry chloroform (25 ml.). After this mixture had been stirred for a total time of 2 hr. at 5° a solution of glycine ester hydrochloride (1·4 g.) and triethylamine (1·4 ml.) in chloroform (10 ml.) was added during 30 min. Stirring was continued for a further 3 hr. and the solution was then washed successively with water, saturated aqueous sodium hydrogen carbonate, and water,

and dried (Na_2SO_4). Evaporation of the chloroform gave a brown oil, an ethereal solution of which gave needles, m. p. 88—89° undepressed by admixture with the product obtained by method 1, when pentane was slowly added.

Action of Hydrazine on the Phthalimido-ester.—80% w/w Hydrazine hydrate (0·12 ml.) was added to a solution of the ester (680 mg.) in ethanol (10 ml.). Within a few minutes crystals separated; these were sparingly soluble in ethanol but dissolved in warm aqueous ethanol and crystallised on cooling as plates, m. p. 205° (decomp.) (Found: C, 57·9; H, 6·6; N, 14·8. $C_{18}H_{24}O_5N_4$ requires C, 57·6; H, 6·4; N, 14·9%). The *hydrazide* which was sparingly soluble in water dissolved readily in 2N-hydrochloric acid; when this solution was heated phthalhydrazide separated. When no further precipitate was formed the solution was filtered and evaporated to dryness. The gum obtained was dissolved in ethanol; gradual addition of ether to this solution precipitated a microcrystalline powder, m. p. 197—199°. 1-*Amino*-N-*carboxymethylcyclopentanecarboxyamide hydrochloride* formed prisms, m. p. 200—201°, from ethanol–ether (Found: C, 43·2; H, 7·0; N, 13·1. $C_8H_{14}O_3N_2$,HCl requires C, 43·2; H, 6·8; N, 12·6%). In larger-scale preparations it was advantageous to collect the sparingly soluble hydrazide and to heat this with dilute acid—its insolubility rendered the normal method of hydrazinolysis inapplicable. In a typical experiment 13 g. of the phthalimido-ester gave 11·3 g. of hydrazide and 4·5 g. of peptide hydrochloride.

An aqueous solution of the hydrochloride was passed slowly through a column of Amberlite resin IR-4B which had been previously washed with ammonia and water. The column was washed with water until only a faint ninhydrin reaction was given by the eluates. Evaporation of these eluates gave the *hemihydrate* of the free peptide which formed plates, m. p. 269—270°, from aqueous ethanol (Found: C, 49·3; H, 8·0; N, 14·3. $C_8H_{14}O_3N_2,\frac{1}{2}H_2O$ requires C, 49·3; H, 7·8; N, 14·4%).

In an attempt to obtain the anhydrous peptide the hemihydrate was sublimed at 180°/0·02 mm. The product, which formed prisms, m. p. 275—280° (decomp.) from water, was apparently 3,6-*dioxopiperazine*-2-*spirocyclopentane* (III) (Found: C, 57·3; H, 7·2; N, 16·6. $C_8H_{12}O_2N_2$ requires C, 57·1; H, 7·2; N, 16·7%). Evaporation of a solution of the dioxopiperazine in concentrated hydrochloric acid gave a product, m. p. 200°, not depressed on admixture with the hydrochloride of the dipeptide described above.

Ethyl 1-*Phthaloylglycylamidocyclopentanecarboxylate.*—A solution of phthaloylglycyl chloride (8·06 g.) in dioxan (35 ml.) was slowly added to a cooled (10°), stirred solution of ethyl 1-aminocyclopentanecarboxylate hydrochloride (7·5 g.) in triethylamine (10·8 ml.) and dioxan (40 ml.). After stirring of the cooled mixture for 3 hr. water was added and the solid which remained was collected and washed successively with water, saturated aqueous sodium hydrogen carbonate, and water (yield, 9·1 g.). The *phthaloyl-ester* of the dipeptide formed needles, m. p. 146—147°, from aqueous ethanol (Found: C, 62·8; H, 6·2; N, 8·3. $C_{18}H_{20}O_5N_2$ requires C, 62·8; H, 5·9; N, 8·1%).

1-*Phthaloylglycylamidocyclopentanecarboxylic Acid.*—(1) Phthaloylglycyl chloride (4·47 g.) in dry dioxan was added dropwise during 35 min. to a stirred ice-cooled suspension of 1-aminocyclopentanecarboxylic acid (2·58 g.) and magnesium hydroxide (1·21 g.) in water (75 ml.). The whole was stirred for a further 20 min. at 5° and then allowed to reach room temperature during the following 20 min. After acidification with hydrochloric acid the solution was evaporated to dryness and the residue was extracted with hot ethyl acetate to remove phthaloylglycine. The insoluble residue (900 mg.) was crystallised repeatedly from water, giving 1-*phthaloylglycylamidocyclopentanecarboxylic acid* (500 mg.) as needles, m. p. 218—220° (Found: C, 60·7; H, 5·2; N, 8·8. $C_{16}H_{16}O_5N_2$ requires C, 60·7; H, 5·1; N, 8·8%).

(2) A solution of ethyl phthaloylglycylamidocyclopentanecarboxylate (1·73 g.) and 80% w/w hydrazine hydrate (0·26 ml.) in ethanol (500 ml.) was heated under reflux for 2 hr. and the ethanol was then removed under reduced pressure. The residue was digested for 10 min. at 40° with 2N-hydrochloric acid which left phthalhydrazide undissolved. Evaporation of the solution under reduced pressure gave a solid which after several crystallisations from water had m. p. 212° not depressed on admixture with the acid prepared by method 1 (Found: C, 60·8; H, 5·4; N, 8·5%).

1-*Glycylamidocyclopentanecarboxylic Acid.*—A solution of ethyl phthaloylglycylcyclopentanecarboxylate (40·65 g.) and hydrazine hydrate (7·65 ml.) in ethanol (1½ l.) was heated under reflux for 3 hr. and the product isolated as described under method 2 above. The solid (23·07 g.) had m. p. 186° (gassing at 195°), raised to 202—204° by washing with hot acetone and ether.

After several crystallisations by pouring of a concentrated aqueous solution into an excess of acetone the *hydrochloride* of the *glycyl-peptide* was obtained as needles, m. p. 216° (Found: C, 43·1; H, 6·9; N, 12·6; Cl, 15·9. $C_8H_{14}O_3N_2$,HCl requires C, 43·2; H, 6·8; N, 12·6; Cl, 15·9%).

Ethyl 1-(N-*Phthaloyl*-DL-*phenylalanylamido*)*cyclopentanecarboxylate*.—A mixture of ethyl 1-aminocyclopentanecarboxylate hydrochloride (11·6 g.), triethylamine (8·28 ml.), and tetrahydrofuran (150 ml.) was stirred vigorously for ½ hr. and then the triethylamine hydrochloride was filtered off and washed with a little tetrahydrofuran. To the filtrate was added *N*-phthaloyl-DL-phenylalanine (17·6 g.) followed by dicyclohexylcarbodi-imide (12·36 g.), and the clear solution was stirred at room temperature for 5 hr. The dicyclohexylurea (5·4 g., 83%) which separated was removed. After addition of a little acetic acid to remove unchanged di-imide the solution was again filtered and the filtrate was evaporated to dryness. An ethyl acetate solution of the residue was washed successively with 2N-hydrochloric acid, water, saturated aqueous sodium hydrogen carbonate, and water, dried (Na_2SO_4) and concentrated. Addition of pentane precipitated crystals (17·6 g.). When crystallised from benzene–pentane the *phthaloyl dipeptide ester* formed prismatic needles, m. p. 142—144° (Found: C, 69·0; H, 6·1; N, 6·4. $C_{25}H_{26}O_5N_2$ requires C, 69·2; H, 6·1; N, 6·5%).

1-(DL-*Phenylalanylamido*)*cyclopentanecarboxylic Acid*.—A mixture of the phthaloyl ester (4·34 g., 0·01 mole), 80% w/w hydrazine hydrate (0·65 ml., 0·011 mole), and ethanol (50 ml.) was heated under reflux for 2 hr. The solid which separated was removed and the filtrate evaporated to dryness under reduced pressure. The residue was extracted with 2N-hydrochloric acid (50 ml.), and the extract was evaporated. Adding ether to an ethanolic solution of the residue afforded fluffy needles (1·5 g.) of the *phenylalanyl dipeptide hydrochloride*, m. p. 200° (decomp.) (Found: C, 58·0; H, 7·0; N, 9·1. $C_{15}H_{20}O_3N_2$,HCl requires C, 57·6; H, 6·8; N, 8·9%). The free *dipeptide*, obtained as described above, formed needles, m. p. 268—270° (slight decomp.), from aqueous alcohol (Found: C, 65·1; H, 7·2; N, 10·1. $C_{15}H_{20}O_3N_2$ requires C, 65·2; H, 7·2; N, 10·1%).

Attempted Preparation of N-α-*Ethoxycarbonylphenethyl*-1-*phthalimidocyclopentanecarboxyamide*.—(1) A mixture of DL-phenylalanine ethyl ester hydrochloride (5·16 g.) and triethylamine (6·3 g.) in tetrahydrofuran (50 ml.) was vigorously stirred for ½ hr. and then filtered. A solution of 1-phthalimidocyclopentane-1-carbonyl chloride (from 5·85 g. of acid) in dry tetrahydrofuran (20 ml.) was slowly added with stirring to the cooled (0°) filtrate. After the addition was complete, cooling was discontinued. Next day water was added and most of the tetrahydrofuran removed by distillation. An ether extract of the residue was washed with dilute acid and then with sodium hydrogen carbonate solution, dried (Na_2SO_4), and evaporated. A colourless gum (8·5 g.) was obtained.

(2) To a solution of DL-phenylalanine ester in tetrahydrofuran prepared as described above were added 1-phthalimidocyclopentanecarboxylic acid (5·85 g.) and dicyclohexylcarbodi-imide (4·7 g.) in tetrahydrofuran (25 ml.). Cyclohexylurea soon separated. The whole was stirred overnight at room temperature and the product (9·0 g.) then isolated in the usual manner: it did not crystallise.

5-*Benzyl*-3,6-*dioxopiperazine*-2-*spirocyclopentane*.—The product (13·2 g.) obtained by method 1 or 2 above was heated under reflux for 24 hr. with hydrazine hydrate (2 ml.) in ethanol (250 ml.). Solid separated at first but this eventually dissolved. The residue obtained by evaporation to dryness was extracted with dilute hydrochloric acid. This extract yielded only a trace of deliquescent solid but crystallising the acid-insoluble material from methanol gave prismatic needles, m. p. 262—264°, of the *spiran* (VII) (Found: C, 69·5; H, 7·1; N, 10·4. $C_{15}H_{18}O_2N_2$ requires C, 69·7; H, 7·0; N, 10·8%).

Hydantoin-5-*spirocyclopentane*-2′-*carboxylic Acid and its Ethyl Ester*.—(*a*) A mixture of ethyl 2-oxocyclopentanecarboxylate (5 g., prepared [12] from diethyl adipate), sodium cyanide (3·2 g.), and ammonium carbonate in 1 : 1 v/v aqueous ethanol (200 ml.) was heated at 58—60° for 16 hr. After removal of the ethanol under reduced pressure the clear aqueous solution was rendered acid to Congo Red by concentrated hydrochloric acid. Evaporation of this solution gave a sticky brown solid, an acetone extract of which was passed through a column of activated alumina. The eluates obtained on washing with fresh acetone contained the *hydantoin-spiran ester* (2 g.) which formed rosettes of needles, m. p. 145°, from ether–light petroleum (b. p. 40—60°) (Found: C, 53·0; H, 6·1; N, 12·3. $C_{10}H_{14}O_4N_2$ requires C, 53·1; H, 6·2; N, 12·4%).

[12] Pinkney, *Org. Synth.*, Coll. Vol. II, p. 116.

(*b*) Ethyl 2-oxocyclopentanecarboxylate (5 g.), ammonium chloride (6 g.), ammonium carbonate (10 g.), sodium cyanide (6 g.), ethanol (100 ml.), and water (80 ml.) were heated in a pressure bottle at 60—70° for 26 hr. The product was worked up as under (*a*), but as the acetone extract did not yield a solid product it was heated under reflux with 4N-hydrochloric acid (150 ml.). The filtrate obtained on evaporation of the solution to half volume and treatment with charcoal deposited prisms (2·7 g.), m. p. 214—216°. On repeated crystallisation from water the *hydantoin-spiran acid* formed needles, m. p. 225—226° (Found: C, 48·4; H, 5·2; N, 14·0. $C_8H_{10}O_4N_2$ requires C, 48·5; H, 5·1; N, 14·1%).

1-*Aminocyclopentane*-1,2-*dicarboxylic Acid.*—A solution of the hydantoincarboxylic acid (10·9 g.) and barium hydroxide octahydrate (15·5 g.) in water (100 ml.) was heated in a pressure bottle at 170—180° for 90 min. Ammonium carbonate (16·3 g.) was added to the hot filtered solution, and the precipitated barium carbonate was removed. The residue obtained on evaporation was rubbed with methanol, yielding a granular solid, charring at 218° and melting with decomposition at 223°. When methanol or acetone was added to a concentrated aqueous solution *ammonium hydrogen* 1-*aminocyclopentane*-1,2-*dicarboxylate* separated as plates, m. p. 249° (decomp. from 226°) (Found: C, 44·3; H, 7·2. $C_7H_{14}O_4N_2$ requires C, 44·2; H, 7·4%).

This ammonium salt (500 mg.), acetic anhydride (2 ml.), and acetic acid (50 ml.) were heated under reflux for 2 hr. and then the solution was evaporated to dryness. The residue was dissolved in acetone and on addition of a large volume of dry ether the N-*acetyl derivative* of the dicarboxylic acid slowly separated as prisms, m. p. 226—228° [Found: C, 50·1; H, 6·0; N, 6·4%; equiv. (by titration), 111·7, 107·0. $C_9H_{13}O_5N$ requires C, 50·2; H, 6·1; N, 6·5%; equiv., 107·6].

Hydantoin-5-*spirocyclopentane*-3′-*carboxylic Acid.*—3-Oxocyclopentanecarboxylic acid (6·4 g., prepared essentially by the method of Ruzicka, Almeida, and Brack [13]), sodium cyanide (9·8 g.), and ammonium carbonate (19·2 g.) in 1 : 1 v/v aqueous ethanol (200 ml.) were heated in a pressure bottle at 65—70° for 25 hr. The product (6·3 g.) obtained by treatment as under (*b*) formed prisms, 223—226°. Recrystallisation from water afforded 4·7 g. of the *spiran acid*, m. p. 228—230°. The product is evidently a mixture of isomers for repeated crystallisation gradually raised the m. p. to 243—245° (Found: C, 48·2; H, 5·2; N, 14·5. $C_8H_{10}O_4N_2$ requires C, 48·5; H, 5·0; N, 14·1%).

1-*Aminocyclopentane*-1,3-*dicarboxylic Acid.*—The preceding hydantoin (5·5 g.) was hydrolysed as described for the 2-carboxy-isomer, and the product obtained after removal of the excess of barium was a deliquescent solid (3·9 g.; m. p. 160—185°). On repeated treatment with hot methanol this material yielded a high-melting non-deliquescent solid which on crystallisation from water gave 1-*aminocyclopentane*-1,3-*dicarboxylic acid hydrate*, prisms, m. p. 264° (Found, for a specimen dried at 100°/0·5 mm. for 1 hr.: C, 44·2; H, 7·1; N, 7·4. $C_7H_{11}O_4N,H_2O$ requires C, 44·0; H, 6·9; N, 7·3%). After drying at 130°/0·5 mm. for 6 hr. the m. p. rose to 277° and a *hemihydrate* was produced (Found: C, 46·1; H, 6·7; N, 8·0. $C_7H_{11}O_4N,\frac{1}{2}H_2O$ requires C, 46·2; H, 6·6; N, 7·7%). Clearly the treatment with methanol completed the removal of the ammonia from the acid. Refluxing the deliquescent solid (1 g.) with acetic anhydride (2 ml.) and acetic acid (20 ml.) for 2 hr. afforded the N-*acetyl derivative*, prisms, m. p. 225—226° (from ether–ethanol) [Found: C, 50·5; H, 6·4; N, 6·3%; equiv. (by titration), 113. $C_9H_{13}O_5N$ requires C, 50·2; H, 6·1; N, 6·5%; equiv., 107·6].

1-*Aminoindane*-1-*carboxylic Acid.*—The hydantoin (8·5 g.; m. p. 242—243°; Henze and Speer [2] give m. p. 240°) derived from indan-1-one was heated with barium hydroxide octahydrate (20·8 g.) and water (150 ml.) at 180° for 2 hr. and the product was worked up in the usual manner. When acetone was added to a concentrated aqueous solution of the *amino-acid* a gelatinous precipitate formed but on standing overnight at 0° this became transformed into large prisms, m. p. 269—270° (dependent on the rate of heating with a tendency to sublime above 260°) (Found: C, 68·0; H, 6·5; N, 7·9. $C_{10}H_{11}O_2N$ requires C, 67·8; H, 6·3; N, 7·9%).

The amino-acid (1 g.), acetic anhydride (1 ml.), and acetic acid (20 ml.) were heated under reflux for 2 hr. On dilution with water needles separated. Recrystallisation from aqueous ethanol gave the N-*acetyl derivative* as needles, m. p. 259—260° (decomp.) (Found: C, 66·0; H, 6·0; N, 6·1. $C_{12}H_{13}O_3N$ requires C, 65·7; H, 6·0; N, 6·4%).

Triethyl 1-*Acetamidobutane*-1,1,4-*tricarboxylate.*—Diethyl acetamidomalonate (21·7 g.) was dissolved in a solution from sodium (2·5 g.) in ethanol (200 ml.) and after the addition of ethyl γ-bromocrotonate (25·7 g., b. p. 96°/9 mm., prepared essentially by the method of Ziegler

[13] Ruzicka, Almeida, and Brack, *Helv. Chim. Acta*, 1934, **17**, 183.

et al.[14]), the mixture was heated under reflux until neutral to litmus (about 5 hr.). Most of the ethanol was removed under reduced pressure and then water was added and the solution was extracted with ether. The washed extract was dried ($CaCl_2$) and evaporated, giving a low-melting solid which was difficult to purify. 18·1 g. of this solid were dissolved in ethanol (25 ml.) and shaken in hydrogen over Adams platinum catalyst. The theoretical uptake of hydrogen was complete in 2 days. Evaporating the filtered solution yielded a solid. Crystallisation from ether gave the saturated *triester* (6·5 g.) as plates, m. p. 98—99° (Found: C, 54·4; H, 7·7; N, 3·8. $C_{15}H_{25}O_7N$ requires C, 54·4; H, 7·6; N, 4·2%).

α-Aminoadipic Acid.—The saturated triester (4·4 g.) was heated under reflux for 3 hr. with concentrated hydrochloric acid (25 ml.) and water (25 ml.), and then the solution was evaporated to dryness, giving a brown solid (2·4 g.), m. p. 138—140°. A slight excess of pyridine was added to a warm solution of this hydrochloride in water (12 ml.) and ethanol (15 ml.). On cooling, crystals were formed and after recrystallisation from aqueous ethanol the α-amino-adipic acid (1 g.) had m. p. 199—201° (lit.,[15] m. p. 206° for the anhydrous form).

Ethyl 2-Aminocyclopent-1-enecarboxylate.—Dry ammonia was passed through a mixture of ethyl 2-oxocyclopentanecarboxylate (20 g.) and ammonium nitrate (11 g.). At first the fluid suspension gradually set to a stiff paste but during the next 2 days the mass liquefied. Shaking the whole with ether gave two layers; the upper layer was dried (Na_2SO_4) and concentrated. Addition of pentane caused separation of needles (13·5 g.), m. p. 55—57°, of the unsaturated amino-ester (cf. Dieckmann [16] and Prelog and Szpilfogel [17]).

A solution of this ester and a slight excess of benzoyl chloride in pyridine was left for 1 hr., after which water was added and the precipitated solid was collected. *Ethyl 2-benzamido-cyclopent-1-enecarboxylate* formed prismatic needles, m. p. 110—111°, from ether–methanol (Found: C, 69·6; H, 6·5; N, 5·4. $C_{15}H_{17}O_3N$ requires C, 69·5; H, 6·6; N, 5·4%).

Reduction of Ethyl 2-Aminocyclopent-1-enecarboxylate.—The amino-ester (7·75 g.) in glacial acetic acid (30 ml.) containing Adams platinum catalyst (200 mg.) absorbed 1 mol. of hydrogen during 19 hr. When the filtered solution was evaporated under reduced pressure crystals of ammonium acetate were formed in the condenser. The product did not crystallise and so was hydrolysed under reflux for 1 hr. with concentrated hydrochloric acid (25 ml.) and water (25 ml.). Concentration then gave a gum which yielded some ammonium chloride immediately when rubbed with methanol. When the methanol extract was kept for several days a solid, m. p. 210°, separated. Heating this hydrochloride gave a sublimate of cyclopent-1-enecarboxylic acid, m. p. 120°. The mother-liquors from the hydrochloride were diluted with water and passed through the acid form of an Amberlite resin column (grade IR-120). The absorbed material was eluted with dilute aqueous ammonia and evaporation of these eluates gave an amino-acid which redissolved in ammonia but separated as prisms when the ammonia was boiled off or acetic acid was added. The *product*, which did not give a positive ninhydrin reaction or a solid hydrochloride, melted at 227—235° (decomp.; dependent on the rate of heating) and at the m. p. a crystalline sublimate formed and ammonia was evolved. This had m. p. 119°, not depressed on admixture with cyclopentenecarboxylic acid. The compound was almost certainly *di-(2-carboxycyclopentyl)amine* (Found: C, 59·9; H, 7·9; N, 6·0. $C_{12}H_{19}O_4N$ requires C, 59·7; H, 7·9; N, 5·8%).

Isomers of 2-Aminocyclopentanecarboxylic Acid.—Cyclopent-1-enecarboxylic acid was prepared essentially by the method of Cook and Linstead [18] except that in agreement with McElvain and Starn [19] we find the dehydration of 1-cyano-1-hydroxycyclopentane by thionyl chloride in pyridine gives higher yields of 1-cyanocyclopent-1-ene. A solution of the acid (20 g.) in aqueous ammonia (250 ml.; *d* 0·880) was heated in a stirring autoclave at 150° for 2 days. Basic copper carbonate (10 g.) was then added to the cooled mixture, and the clear solution was heated to remove ammonia. At first some copper oxide separated but further evaporation of the decanted liquor led to a crystalline copper salt. When the volume had been reduced to about 50 ml. the light blue plates were collected. The copper salt was purified by redissolving it in an excess of dilute ammonia and then boiling off the ammonia. It was a

[14] Ziegler, Spath, Schaaf, Schumann, and Winkelmann, *Annalen*, 1942, **551**, 80.
[15] Behal and Tiffeneau, *Bull. Soc. chim. France*, 1908, **4**, 301.
[16] Dieckmann, *Annalen*, 1901, **317**, 27.
[17] Prelog and Szpilfogel, *Helv. Chim. Acta*, 1945, **28**, 1684.
[18] Cook and Linstead, *J.*, 1934, 956.
[19] McElvain and Starn, *J. Amer. Chem. Soc.*, 1955, **77**, 4571.

dihydrate (Found: C, 40·6; H, 6·7; N, 8·5; Cu, 17·9. $C_{12}H_{20}O_4N_2Cu,2H_2O$ requires C, 40·5; H, 6·8; N, 7·9; Cu, 17·9%). On drying at 130°/0·5 mm. the crystals become deep blue with a metallic sheen, a *monohydrate* apparently being formed (Found: C, 42·0; H, 6·7; N, 8·5; Cu, 18·6. $C_{12}H_{20}O_4N_2Cu,H_2O$ requires C, 42·6; H, 6·5; N, 8·3; Cu, 18·8%).

Hydrogen sulphide was passed into a solution of the copper salt in dilute hydrochloric acid, and the precipitated copper sulphide was removed. Evaporation of the colourless solution gave a crystalline residue of the hydrochloride which was dried by rubbing it with acetone (yield, 5·7 g.). The *2-aminocyclopentanecarboxylic acid hydrochloride* formed prisms when acetone was added to a methanol solution. The m. p., 165—170°, was not well defined since decomposition with the formation of cyclopent-1-enecarboxylic acid occurs at the point of fusion (Found: C, 42·7; H, 7·3; N, 8·5. $C_6H_{11}O_2N,HCl$ requires C, 43·5; H, 7·3; N, 8·5%. The low carbon value was due to the difficulty in removing the last traces of ammonium chloride).

An aqueous solution of the hydrochloride (1 g.) was digested with silver oxide (from 1 g. of silver nitrate) and after removal of the silver chloride the colourless solution was evaporated and the residue was crystallised by repeatedly boiling a concentrated aqueous solution to which ethanol was added from time to time. This give plates of the *amino-acid monohydrate*, m. p. 242—243° (decomp.) (Found: C, 49·4; H, 8·6; N, 9·8. $C_6H_{11}O_2N,H_2O$ requires C, 49·0; H, 8·9; N, 9·5%). The amino-acid gave a weak ninhydrin reaction in aqueous solution but gave a strong blue colour when the test was carried out on filter paper. *Ethyl 2-benzamidocyclopentanecarboxylate* formed flattened needles, m. p. 98—99° (Found, after drying at 55°/0·5 mm.: C, 69·1; H, 7·1; N, 5·4. $C_{15}H_{19}O_3N$ requires C, 68·9; H, 7·3; N, 5·4%).

The mother-liquors from the sparingly soluble copper salt were acidified with hydrochloric acid, and the unchanged cyclopentenecarboxylic acid was extracted with ether. The aqueous layer was saturated with hydrogen sulphide, filtered, and evaporated. After determination of the chloride-ion content of the residue by a Volhard titration an exact equivalent of silver oxide was added to an aqueous solution. Filtration and concentration gave a solution which was adjusted to pH 4 with dilute sulphuric acid and evaporated to dryness. Extraction with methanol left some ammonium sulphate undissolved and when the extract was evaporated with dilute hydrochloric acid a solid was obtained. This isomeric *hydrochloride* (1 g.) which formed prismatic needles, m. p. 210—212°, from methanol–acetone was identical with that prepared in low yield by the two methods described above (Found: C, 43·1; H, 7·2; N, 8·2%). The isomeric amino-acid was also characterised as its *N-benzoyl ethyl ester*, prismatic needles, m. p. 91·5—93° [from ether–light petroleum (b. p. 40—60°)] depressed to 72° on admixture with the isomer of m. p. 98—99° (Found: C, 68·8; H, 7·2; N, 5·3%).

1-Aminocyclopropanecarboxylic Acid.—Diethyl cyclopropane-1,1-dicarboxylate (125 ml.; b. p. 116—119°/12 mm., prepared essentially by the method of Dox and Yoder [20]) and aqueous ammonia (625 ml.; *d* 0·880) were stirred in a pressure bottle at room temperature for 2 days. The crystals which began to separate after 10 hr. were collected (49·9 g. of material, m. p. 199°; Dox and Yoder [20] give m. p. 198°). The diamide was converted into hydantoin-5-spirocyclopropane (40·1 g.; m. p. 220—222°) by the method of Ingold *et al.*[9] who report m. p. 214°. A mixture of the hydantoin (10 g.) and barium hydroxide octahydrate (25 g.) in water (300 ml.) was heated in a pressure bottle at 155° for 40 min. The amino-acid (4·5 g.), isolated in the manner already described, formed needles, m. p. 248—249° (decomp.), when acetone was added to a concentrated aqueous solution and the mixture kept at 0° for several hr. (Found: C, 47·5; H, 7·2; N, 13·8. Calc. for $C_4H_7O_2N$: C, 47·5; H, 7·0; N, 13·9%). Burroughs [9] gives decomp. >200° and Vahatalo and Virtanen [21] give decomp. ~234—236°. It formed a hydrochloride, plates, m. p. 220—223° (decomp.), from ethanol–acetone; Ingold *et al.*[9] give m. p. 222° (decomp.).

1-Aminocyclobutanecarboxylic Acid.—Diethyl cyclobutane-1,1-dicarboxylate (84 g.) was similarly converted into the diamide (reaction time 5 days; m. p. 280—283°; yield 16·9 g.; Ingold *et al.*[9] give m. p. 275—277°) and then into the spirohydantoin (m. p. 224°; 13 g.; Ingold *et al.*[9] give m. p. 225°) which on hydrolysis as described above gave the *amino-acid* (8·3 g.), m. p. 290° (decomp.), prismatic needles from aqueous ethanol (Found: C, 52·1; H, 7·6; N, 12·1. $C_5H_9O_2N$ requires C, 52·1; H, 7·9; N, 12·2%). The *hydrochloride* formed plates, m. p. 260—265° (rapid heating), from acetone (Found: Cl^-, 23·2. $C_5H_9O_2N,HCl$ requires Cl, 23·4%). The amino-acid formed an *N-acetyl derivative*, m. p. 179°, needles from acetone–light petroleum (b. p. 40—60°) (Found: C, 53·7; H, 7·4; N, 8·7. $C_7H_{11}O_3N$ requires C, 53·5; H, 7·1; N, 8·9%),

[20] Dox and Yoder, *J. Amer. Chem. Soc.*, 1921, **43**, 2097.
[21] Vahatalo and Virtanen, *Acta Chem. Scand.*, 1957, **11**, 741.

and an N-*benzoyl derivative*, m. p. 204—205°, needles from ether [Found: C, 66·1; H, 6·0; N, 6·7%; equiv. (by titration), 220. $C_{12}H_{13}O_3N$ requires C, 65·7; H, 6·0; N, 6·4%; equiv., 219·2].

1-*Aminocyclohexanecarboxylic Acid*.—Hydantoin-5-spirocyclohexane, needles, m. p. 221—225° (from ethanol) (Found: C, 57·1; H, 7·4; N, 17·3. Calc. for $C_8H_{12}O_2N_2$: C, 57·1; H, 7·2; N, 16·7%) (Adkins and Billica [22] give m. p. 213—215°; Tiffeneau *et al.*[23] give m. p. 222°), was hydrolysed by barium hydroxide at 160°, giving 1-aminocyclohexanecarboxylic acid, plates, m. p. 330—340° (from water) (Adkins and Billica [22] give m. p. 318—319°, Zelinsky and Stadnikov [24] m. p. 334—335°, and Cocker *et al.*[25] m. p. 350°), which formed an *N*-benzoyl derivative, plates, m. p. 198—200°, from aqueous ethanol [Found: C, 68·0; H, 6·5; N, 5·3%; equiv. (by titration), 251. Calc. for $C_{14}H_{17}O_3N$: C, 68·0; H, 6·9; N, 5·7%; equiv., 247]. Upham and Dermer [26] give m. p. 190—191°.

1-*Amino*-3-*methylcyclohexanecarboxylic Acid*.—This amino-acid similarly prepared from hydantoin-5-spiro-(3-methylcyclohexane) (Henze and Speer [2]) formed prisms from water which sublimed at 305—310° without melting (Zelinsky and Stadnikov [24] give m. p. 330°, and Bucherer and Brandt [27] give m. p. 260°). The N-*benzoyl derivative* formed plates, m. p. 220—221°, from aqueous ethanol [Found: C, 69·1; H, 7·4; N, 5·2%; equiv. (by titration), 264. $C_{15}H_{19}O_3N$ requires C, 69·0; H, 7·3; N, 5·4%; equiv., 261].

1-*Amino*-1,2,3,4-*tetrahydro*-1-*naphthoic Acid*.—The hydantoin from α-tetralone had m. p. 248° (needles from ethanol; Novelli [28] gives m. p. 237·5—239·5° and Rothman and Day [29] give m. p. 237·2—238·2°); it yielded 1-*amino*-1,2,3,4-*tetrahydro*-1-*naphthoic acid*, m. p. 258—260°, prisms from water, which effloresce (Found: C, 68·9; H, 7·2; N, 7·3. $C_{11}H_{13}O_2N$ requires C, 69·1; H, 6·9; N, 7·3%) [N-*benzoyl derivative*, m. p. 174—178° (from ethanol) (Found: C, 73·5; H, 6·0; N, 5·1%; equiv. (by titration), 292. $C_{18}H_{17}O_3N$ requires C, 73·1; H, 5·8; N, 4·8%; equiv., 295].

2-*Amino*-1,2,3,4-*tetrahydro*-2-*naphthoic Acid*.—The hydantoin from β-tetralone had m. p. 268—269° (prisms from aqueous ethanol; Novelli [30] gives m. p. 250—260°; Jules *et al.*[31] give m. p. 267—268°); it yielded 2-*amino*-1,2,3,4-*tetrahydro*-2-*naphthoic acid*, m. p. 297—303°, prisms from water (Found: C, 69·4; H, 7·2; N, 7·1. $C_{11}H_{13}O_2N$ requires C, 69·1; H, 6·9; N, 7·3%), which forms a *benzoyl derivative*, prisms, m. p. 213—215°, from ethanol [Found: C, 72·7; H, 6·0; N, 4·6%; equiv. (by titration), 297. $C_{18}H_{17}O_3N$ requires C, 73·1; H, 5·8; N, 4·8%; equiv., 295].

1-*Aminocycloheptanecarboxylic Acid*.—This amino-acid, prepared in the usual manner from the spirohydantoin, formed plates, m. p. 320° (decomp.), from water (Zelinsky and Stadnikov [24] give m. p. 306—309°). The N-*benzoyl derivative* formed prismatic needles, m. p. 202—203°, from ether (Found: C, 69·0; H, 7·3; N, 6·4. $C_{15}H_{19}O_3N$ requires C, 68·9; H, 7·3; N, 5·4%).

1-*Aminocyclo-octane*-1-*carboxylic Acid*.—The appropriate hydantoin,[23] m. p. 246°, similarly afforded 1-*aminocyclo-octane*-1-*carboxylic acid*, m. p. 321° (decomp.), plates from aqueous acetone (Found: C, 63·2; H, 9·8; N, 8·5. $C_9H_{17}O_2N$ requires C, 63·1; H, 10·0; N, 8·2%) [N-*benzoyl derivative*, m. p. 226°, needles from aqueous acetone (Found: C, 69·7; H, 7·8; N, 5·2. $C_{16}H_{21}O_3N$ requires C, 69·8; H, 7·7; N, 5·1%)].

α-*Methylnorvaline*.—5-Methyl-5-propylhydantoin, m. p. 123—124° (Henze and Speer [2] give 123—124·5°), on hydrolysis with barium hydroxide affords α-methylnorvaline, sublimes above 312° (Kurono [32] gives m. p. 295°), which yields a *benzoyl derivative*, prisms, m. p. 160—161°, from ether–light petroleum (b. p. 40—60°) (Found: C, 66·0; H, 7·2; N, 6·7. $C_{13}H_{17}O_3N$ requires C, 66·4; H, 7·3; N, 6·0%).

1-*Amino*-2-*methylcyclohexanecarboxylic Acid*.—Hydantoin-5-spiro-(2-methylcyclohexane) (10 g.; m. p. 223—225°; Henze and Speer [2] give m. p. 215—216°, Tiffeneau *et al.*[23] give m. p. 228°) in water (35 ml.) containing concentrated sulphuric acid (15 ml.) was heated at 160—180°

[22] Adkins and Billica, *J. Amer. Chem. Soc.*, 1948, **70**, 3121.
[23] Tiffeneau, Tchoubar, Saiaslambert, and Dupré, *Bull. Soc. chim. France*, 1947, 445.
[24] Zelinsky and Stadnikov, *Ber.*, 1906, **39**, 1722.
[25] Cocker, Lapworth, and Peters, *J.*, 1931, 1382.
[26] Upham and Dermer, *J. Org. Chem.*, 1957, **22**, 799.
[27] Bucherer and Brandt, *J. prakt. Chem.*, 1934, **140**, 129.
[28] Novelli, *Anales Asoc. quím. Argentina*, 1941, **29**, 83.
[29] Rothman and Day, *J. Amer. Chem. Soc.*, 1954, **76**, 111.
[30] Novelli, *Anales Farm. Bioquím. Buenos Aires*, 1954, [2], **21**, 81.
[31] Jules, Faust, and Sahyun, U.S.P. 2,716,648.
[32] Kurono, *Biochem. Z.*, 1922, **134**, 424.

for 4 hr. Barium hydroxide octahydrate (85 g.) was added to the cooled solution; the precipitate was centrifuged off, then an excess of ammonium carbonate was added to the supernatant liquid and the warm solution was filtered and concentrated. The amino-acid was obtained as needles, m. p. 330° (Skita and Levi [11] give m. p. 300° for material produced by acid-hydrolysis of the corresponding amino-nitrile). The N-*benzoyl derivative* formed prisms, m. p. 196°, from aqueous ethanol [Found: C, 69·0; H, 7·3%; equiv. (by titration), 266. $C_{15}H_{19}O_3N$ requires C, 68·9; H, 7·3; equiv., 261].

5-*Cyclopropyl*-5-*methylhydantoin.**—Cyclopropyl methyl ketone (30 g.), sodium cyanide (42 g.), and ammonium carbonate (165 g.) in water (200 ml.) and ethanol (200 ml.) were heated at 58—60° for 5 hr. Next day the solution was evaporated to low bulk and then acidified with concentrated hydrochloric acid, a heavy white precipitate being formed. This was collected and more product was obtained by evaporating the liquors to dryness and exhaustively extracting the residue with ether. The combined yield of *hydantoin* was 32 g. It formed small prisms, m. p. 94—96°, of a hydrate on crystallisation from water. On drying at 100° the m. p. rose to 148—151°; material with this m. p. was obtained directly when the product was crystallised from ether (Found: C, 54·6; H, 6·5; N, 18·7. $C_7H_{10}O_2N_2$ requires C, 54·5; H, 6·5; N, 18·2%).

α-*Cyclopropylalanine.*—The preceding hydantoin (13·8 g.), barium hydroxide octahydrate (27 g.), and water (200 ml.) were heated at 160° for 1 hr. Amonium carbonate (13·8 g.) was added to the cooled solution which was filtered and then evaporated to low bulk. Needles, m. p. 290—292°, of the amino-acid separated (Zelinsky and Dengin [33] give m. p. 273—275° and report that the compound sublimes at about 110°; however, we find that the material loses solvent and becomes opaque at 120—130° but does not sublime though charring occurs above 200°). The amino-acid formed a hydrochloride, prisms, m. p. 264—266°, from water (Zelinsky and Dengin [32] give m. p. 256°). As it proved impossible to obtain satisfactory analyses for the amino-acid or its hydrochloride, possibly owing to the difficulty of removing solvent of crystallisation, the compound was characterised as the more readily purified hydrobromide and *N*-benzoyl derivative. The *hydrobromide* formed prisms, m. p. 254—257°, from water [Found: C, 34·8; H, 6·1; N, 6·7; Br, 37·6%; *M* (by titration of Br^-), 209. $C_6H_{11}O_2N$,HBr requires C, 34·3; N, 5·8; N, 6·7; Br, 38·1%; *M*, 210]. N-*Benzoyl*-α-*cyclopropylalanine* * formed plates, m. p. 195—196°, from water [Found: C, 66·5; H, 6·7; N, 6·1%; equiv. (by titration), 238. $C_{13}H_{15}O_3N$ requires C, 66·9; H, 6·5; N, 6·0%; equiv., 233].

N-*Phthaloylglycyl*-α-*cyclopropylalanine Ethyl Ester.*—α-Cyclopropylalanine (10 g.) in ethanol (30 ml.) was saturated with dry hydrogen chloride and then heated under reflux for 2 hr. Next day evaporation gave the ester hydrochloride (13·3 g.) which was used directly without purification. It was treated in tetrahydrofuran (70 ml.) with triethylamine (9·6 ml.) and then vigorous stirring was continued for 2—3 hr. Phthaloylglycine (7·93 g.) was next added to the filtered solution, followed by dicyclohexylcarbodi-imide (8·1 g.) in tetrahydrofuran (10 ml.). The initially clear solution deposited cyclohexylurea (7·9 g., 96%) during stirring for 16 hr. An ethyl acetate solution of the residue obtained after removal of the tetrahydrofuran was washed successively with dilute hydrochloric acid, water, aqueous sodium hydrogen carbonate, and water. Gradual addition of light petroleum (b. p. 40—60°) to the dried (Na_2SO_4) solution gave the *peptide derivative* (9·3 g.), m. p. 145—146°, recrystallising from ethyl acetate–pentane as needles, m. p. 146° (Found: C, 62·5; H, 5·6; N, 7·9. $C_{18}H_{20}O_5N_2$ requires C, 62·8; H, 5·9; N, 8·1%).

Glycyl-α-*cyclopropylalanine.*—Phthaloylglycylcyclopropylalanine ethyl ester (9·51 g., 0·3 mole), and 80% w/w hydrazine hydrate (1·75 ml., 0·3 mole) in ethanol (200 ml.) were heated under reflux for 2 hr. After removal of the ethanol, 2N-hydrochloric acid (150 ml.) was added and the solution was heated on a steam-bath for 5 min. The cooled solution was filtered and evaporated to dryness. Chromatography revealed a mixture of peptide and ester and so the whole was dissolved in a solution of barium hydroxide octahydrate (10 g.) in water (30 ml.) and set aside. Next day an excess of ammonium carbonate was added and the filtered solution was evaporated to low bulk. Addition of ethanol and then ether precipitated the *peptide* (2 g.), m. p. 249—250°. Recrystallisation from aqueous ethanol gave needles, m. p. 251—252° (Found, for a specimen dried at 120°/1 mm.: C, 51·8; H, 7·4; N, 15·2. $C_8H_{14}O_3N_2$ requires C, 51·6; H, 7·6; N, 15·0%). The *ethyl ester hydrochloride* formed prisms, m. p. 152—153°, from aqueous ethanol (Found: C, 48·3; H, 7·6. $C_{10}H_{18}O_3N_2$,HCl requires C, 47·9; H, 7·6%).

* These compounds are referred to in B.P. 752,692 but adequate analytical data are not given.

[33] Zelinsky and Dengin, *Ber.*, 1922, **55**, 3354.

αβ-*Diphenylalanine*.—5-Benzyl-5-phenylhydantoin (18 g.; m. p. 215°; Slotta *et al.*[34] give m. p. 210°) and barium hydroxide octahydrate (34·4 g.) in water (600 ml.) were heated at 160—180° for 10 hr. The *amino-acid* (12·0 g.) isolated in the usual manner formed needles, m. p. 274—275°, from water (Found: C, 74·9; H, 6·3; N, 5·8. $C_{15}H_{15}O_2N$ requires C, 74·7; H, 6·3; N, 5·8%). N-*Benzoyl*-αβ-*diphenylalanine* formed prisms, m. p. 84°, from aqueous ethanol (Found: C, 76·5; H, 6·1; N, 3·9. $C_{22}H_{19}O_3N$ requires C, 76·5; H, 5·6; N, 4·0%), and the N-*carbamoyl derivative* was obtained as prisms, m. p. 190—191°, from aqueous ethanol (Found: C, 67·3; H, 5·9; N, 9·7. $C_{16}H_{16}O_3N_2$ requires C, 67·6; H, 5·7; N, 9·9%).

This investigation was supported by the British Empire Cancer Campaign, the Jane Coffin Childs Fund for Medical Research, the Anna Fuller Fund, and the National Cancer Institute of the National Institutes of Health, U.S. Public Health Service.

THE CHESTER BEATTY RESEARCH INSTITUTE,
INSTITUTE OF CANCER RESEARCH: ROYAL CANCER HOSPITAL,
FULHAM ROAD, LONDON, S.W.3. [*Received, October 30th*, 1959.]

[34] Slotta, Behnisch, and Szyszka, *Ber.*, 1934, **67**, 1529.