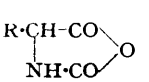
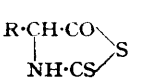
679. *The Synthesis of Simple Peptides from Anhydro-N-carboxy-amino-acids.*

By J. Leggett Bailey.

Various simple peptides of DL- and L-alanine, L-leucine, L-cystine, L-tyrosine, and glycine have been prepared by reaction of the corresponding anhydro-*N*-carboxyamino-acids with amino-acid and peptide esters in anhydrous solvents at low temperatures. The intermediate carbamates have in some cases been isolated and characterised. The use of 2-thio-oxazolid-5-one for the addition of a glycyl residue to an α-amino-acid ester is described.

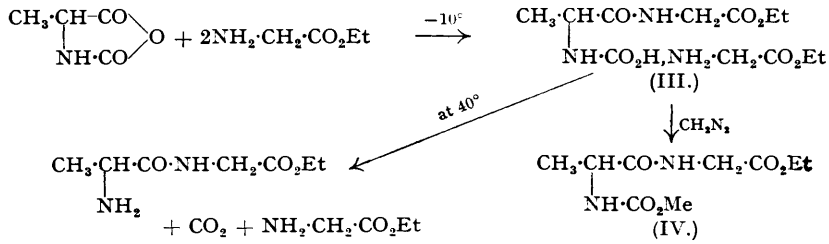
Considerable attention has been focused on the polymerisation potentialities of the anhydro-*N*-carboxyamino-acids (I) (Astbury, Dalgliesh, Darmon, and Sutherland, *Nature*, 1948, **162**, 596; Woodward and Schramm, *J. Amer. Chem. Soc.*, 1947, **69**, 1551). The more limited use of these compounds in simple peptide synthesis has been recently reviewed (Fruton, "Advances in Protein Chemistry," Vol. V; Cook and Levy, *J.*, 1950, 646), but it is pertinent to recall the following impotant contributions to this field. Phenylalanylglycine, phenylalanylglycylglycine, and phenylalanyltyrosyl anhydride have been prepared from anhydro-*N*-carboxy-β-phenylalanine (Wessely, *Z. physiol. Chem.*, 1926, **157**, 91). Condensation of anhydro-*N*-carboxy-D-alanine with two equivalents of histidine ester in chloroform afforded D-alanyl-L-histidine (31%) after hydrolysis (Hunt and Du Vigneaud, *J. Biol. Chem.*, 1938, **125**, 699). Glycylglycine ester and glycine morpholide were produced in low yield by reaction of anhydro-*N*-carboxyglycine at 0° with glycine ester and morpholine, respectively (Billimoria and Cook, *J.*, 1949, 2323).

(I.) 

 (II.)

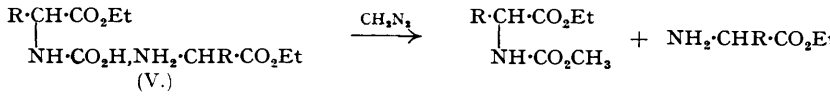
Glycine peptides up to pentaglycine and various dipeptides have recently been prepared from the analogous 2-thio-5-thiazolidones (II) (Cook and Levy, *loc. cit.*). The successful use of these compounds and the fact that substitution in the 4-position greatly enhances their stability prompted the present re-examination of the properties of anhydro-*N*-carboxyamino-acids (other than the glycine derivative) at low temperatures. As already briefly reported (Leggett Bailey, *Nature*, **1949**, **164**, 889), reaction of the latter with two equivalents of an α-amino-ester or with one equivalent each of an α-amino-ester and a tertiary base has provided a ready means of synthesising simple peptides.

Preliminary experiments were carried out with anhydro-*N*-carboxy-DL-alanine, which is already considerably more stable than the corresponding glycine derivative. Reaction of the former in ethyl acetate with two equivalents of glycine ester at −10° produced fine needles of the carbamate (III) in 80% yield. A solution of (III) in chloroform evolved carbon dioxide slowly

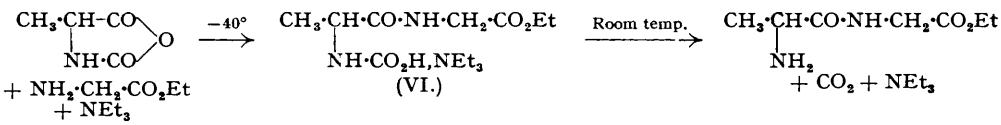


at room temperature but more rapidly at 40°. The dry material could be stored at room temperature under carbon dioxide without appreciable decomposition. Treatment of (III) with ethereal diazomethane at 0° gave the carbomethoxy-derivative (IV) and glycine ester.

In general, the properties of (III) resembled those of the carbamates (V) formed when carbon dioxide is passed into solutions of α-amino-acid esters at 0° (Frankel and Katchalski, *J. Amer. Chem. Soc.*, **1943**, **65**, 1670). For instance, the compounds (V) reacted analogously with diazomethane:



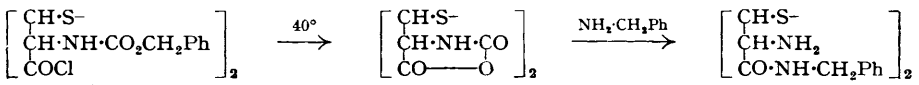
When (III) was dissolved in a small volume of water, carbon dioxide was evolved and crystals of 2 : 5-diketo-3-methylpiperazine separated. Attempts to obtain a pure specimen of DL-alanylglycine from (III) were unsuccessful, and the possibility of substituting a tertiary base for one equivalent of glycine ester was next explored. Whereas anhydro-*N*-carboxy-DL-alanine in the presence of triethylamine polymerised almost immediately at room temperature, the rate of polymerisation appeared to be much slower at −40°, and at −70° no polyalanine was deposited after two hours. When one equivalent of glycine ester, precooled to −40°, was added to a solution of the anhydride and one equivalent of triethylamine in ethyl acetate at −40°, reaction was slow at first, but after an induction period of about 10 minutes the temperature rose suddenly. The new carbamate (VI) could then be precipitated by means of anhydrous ether. It proved to be more deliquescent than (III) and decomposed rapidly at room temperature yielding DL-alanylglycine ester, triethylamine, and carbon dioxide:



Hydrolysis of (VI) with barium hydroxide yielded DL-alanylglycine in 62% yield, with some 2 : 5-diketo-3-methylpiperazine. The DL-alanylglycine obtained was an amorphous powder but when coupling was effected with anhydro-*N*-carboxy-L-alanine crystalline L-alanylglycine was obtained (68%). Diketopiperazine formation would not be expected to occur during the hydrolysis of a tripeptide ester, and accordingly crystalline L-alanylglycylglycine was obtained in 88% yield from the condensation of anhydro-*N*-carboxy-L-alanine with glycylglycine ester in the presence of methyldioctylamine. This base was more easily rendered anhydrous than triethylamine and could be recovered from the aqueous solutions by extraction with solvent.

When (VI) (prepared from L-alanine) was dissolved in chloroform at room temperature and coupled at −65° with a second equivalent of the anhydride, an 85% yield of L-alanyl-L-alanylglycine was obtained after hydrolysis.

In view of the important role played by cysteine in biological processes, it was desirable to attempt the synthesis of cysteine or cystine peptides by this method. The use of biscarbobenzyloxy-L-cystyl chloride (VII) in the preparation of L-cystylbisglycine is well known (Loring and Du Vigneaud, *J. Biol. Chem.*, 1935, **111**, 385). When a solution of (VII) in dioxan was warmed to 40°, fine needles slowly separated and proved to be the new di(anhydro-*N*-carboxy)-L-cystine (VIII) combined with a molecule of solvent. Reaction of (VIII) with two equivalents of benzylamine in tetrahydrofuran at −10° and subsequent decomposition of the mixture with water gave the crystalline di(benzylamide) (IX). Treatment of (VIII) with two equivalents of glycine ester at −10° gave a highly insoluble carbamate which decomposed at room temperature to give the crystalline ester of L-cystylbisglycine, which after hydrolysis afforded L-cystylbisglycine (78%) of optical activity in good agreement with that found by Du Vigneaud (*loc. cit.*). Further reaction of this ester with anhydro-*N*-carboxy-DL-alanine in the presence of methyldioctylamine at −65° yielded di-DL-alanyl-L-cystylbisglycine (48%).



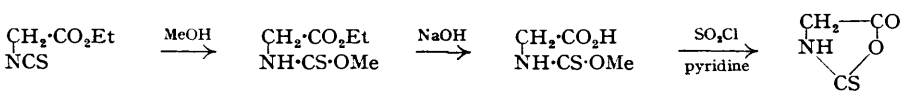
(VII.) (VIII.) (IX.)

In a similar way L-leucyl and L-tyrosyl peptides were readily available from anhydro-*N*-carboxy-L-leucine and *O*-acetylanhydro-*N*-carboxy-L-tyrosine respectively. Thus L-tyrosylglycine (62%), L-tyrosylglycylglycine (77%), L-tyrosyl-L-tyrosine (60%), L-alanyl-L-tyrosylglycine (70%), and L-leucylglycylglycine (88%) were all obtained crystalline and had optical rotation data in good agreement with the values given in the literature.

All the peptides prepared by the present method ran as single spots on unidimensional chromatograms with butanol–acetic acid as the mobile phase.

The failure, reported by other workers, of anhydro-*N*-carboxyglycine to couple satisfactorily with α-amino-acid esters has been confirmed. It is possible, however, to couple this anhydride with glycylglycine ester to give a 40% yield of tripeptide ester hydrochloride. When carbon dioxide is passed into chloroform solutions of glycylglycine ester and glycylglycylglycine ester, very stable, non-hygroscopic, crystalline salts are obtained. In the light of this knowledge it is surprising that better success has not attended coupling experiments with anhydro-*N*-carboxyglycine.

The stability of 2-thio-oxazolid-5-one (XIII) is intermediate between those of anhydro-*N*-carboxyglycine and of 2-thiothiazolid-5-one. Thus polymerisation in an aqueous medium under the most favourable conditions produces polyglycine of molecular weight 300—450.



(X.) (XI.) (XII.) (XIII.)

Brief mention of this new compound has been made in another connection (Alexander, Leggett Bailey, and Carter, *Text. Res. J.*, 1950, **20**, 385) but a fuller account of its preparation *via* carbethoxymethyl *iso*thiocyanate (X), *N*-thioncarbomethoxyglycine ethyl ester (XI), and *N*-thioncarbomethoxyglycine (XII) is now reported. Reaction of (XIII) with glycine ethyl ester and triethylamine at 0° and subsequent addition of ethanolic hydrogen chloride made possible a synthesis of glycylglycine ester hydrochloride in 52% yield.

Application of the general method now outlined to the production of higher peptides containing amino-acid residues in a definite sequence will be described in a further communication.

Experimental.

The peptide coupling experiments were carried out under strictly anhydrous conditions. All solvents were stored over suitable drying agents for 2—3 weeks before use. Tetrahydrofuran, which was found to be an excellent solvent for anhydro-*N*-carboxyamino-acids at low temperatures, was purified by refluxing it with potassium and was stored over anhydrous calcium sulphate.

Anhydro-N-*carboxy*-DL-*alanine*.—This was prepared by reaction of carbonyl chloride with DL-alanine or its hydrochloride in dioxan at 40—50°. On removal of the solvent *in vacuo*, the residue crystallised (cf. Levy, *Nature*, 1950, **165**, 152; Farthing and Reynolds, *ibid.*, p. 647). Recrystallisation several times

from ether–light petroleum (b. p. 40—60°) gave wart-like crystals, m. p. 45—46° (decomp.), in 75% yield.

Anhydro-N-carboxy-L-alanine.—Similarly prepared (80% yield) from L-alanine in tetrahydrofuran at 50°, and recrystallised from tetrahydrofuran–ether, this compound had m. p. 90° (decomp.). A sample was decomposed with N-hydrochloric acid at 35°; the L-alanine so produced had $[\alpha]_D^{23}$ +14·0° compared with $[\alpha]_D^{23}$ +14·1° for the starting material.

Di(anhydro-N-carboxy)-L-cystine.—Di(carbobenzyloxy)-L-cystine (5 g.) (Bergmann and Zervas, *Ber.*, 1932, **65**, 1192) in dioxan (30 c.c.) was shaken with phosphorus pentachloride (11 g.) with cooling during 15 minutes. The solution was filtered and warmed to 40° during one hour. *Di(anhydro-N-carboxy)-L-cystine* separated in fine needles (2·8 g., 74%). Recrystallised from dioxan, it had no melting point (Found: C, 38·1; H, 4·25; N, 7·1; S, 16·2. $C_8H_8O_6N_2S_2,C_4H_8O_2$ requires C, 37·9; H, 4·21; N, 7·4; S, 16·8%). Recrystallised from tetrahydrofuran, the compound melted at 128° (decomp.).

Anhydro-N-carboxy-L-leucine.—Prepared in 85% yield by the action of carbonyl chloride on L-leucine in tetrahydrofuran at 45°, and recrystallised from ether–light petroleum (b. p. 40—60°), the compound melted at 77°.

O-Acetylanhydro-N-carboxy-L-tyrosine.—*O*-Acetyl-*N*-carbobenzyloxy-L-tyrosine (3·5 g.) (Bergmann *et al.*, *Z. physiol. Chem.*, 1934, **224**, 17) in dioxan (20 c.c.) was shaken with phosphorus pentachloride (3·2 g.), with cooling. The filtered solution was warmed to 40° during 20 minutes, and the *L-tyrosine* derivative (m. p. 99—100°) precipitated with light petroleum (b. p. 40—60°). Recrystallisation from ethyl acetate gave a product, m. p. 120° (Found: C, 57·6; H, 4·6; N, 5·5. $C_{12}H_{11}O_5N$ requires C, 57·9; H, 4·4; N, 5·6%).

Salt of N-Carboxy-DL-alanylglycine Ethyl Ester with Glycine Ethyl Ester.—Glycine ethyl ester hydrochloride (0·28 g., 2 equivs.) was stirred with a 2% solution of ammonia in chloroform (1·2 c.c.) and the solution filtered from the precipitated ammonium chloride (cf. Hillman, *Z. Naturforsch.*, 1946, **1**, 682). After removal of the slight excess of ammonia by concentration of the solvent *in vacuo*, ethyl acetate (1 c.c.) was added, and the solution cooled to −10°. A solution of anhydro-*N*-carboxy-DL-alanine (0·11 g.) in ethyl acetate (1 c.c.) was precooled to −10° and added slowly to the glycine ester. Fine needles of the *carbamate* (III) separated rapidly, and after one hour were washed with anhydrous ether and stored in an atmosphere of dry carbon dioxide (0·25 g., 80%; m. p. 71—72°, decomp.). A carbon dioxide determination was carried out according to Van Slyke and Neill (Peters and Van Slyke, "Quantitative Clinical Chemistry," 1932, Vol. II, 283) (Found: N, 13·4; CO_2, 13·6. $C_{12}H_{23}O_7N_3$ requires N, 13·1; CO_2, 13·7%). The salt (III) (0·3 g.) was dissolved in water (2 c.c.) at room temperature; long needles of 2 : 5-diketo-3-methylpiperazine crystallised out (0·11 g., 92%; m. p. 239—240°, decomp.). When an ethereal solution of diazomethane was added to (III), brisk evolution of nitrogen was observed. When the reaction was complete, ethereal hydrogen chloride was added to precipitate glycine ethyl ester hydrochloride. Concentration of the filtered solution yielded an oil, which was carbomethoxy-DL-alanylglycine ethyl ester (Found: C, 46·3; H, 6·4; N, 12·15. Calc. for $C_9H_{16}O_5N_2$: C, 46·6; H, 6·9; N, 12·08%).

Salt of N-Carboxy-DL-alanylglycine Ethyl Ester with Triethylamine.—Glycine ester hydrochloride (0·14 g., 1 equiv.) was treated with ammonia in chloroform as above, and the free ester transferred to ethyl acetate (1·5 c.c.). The solution was cooled to −40°, and anhydrous triethylamine added (0·14 c.c., 1 equiv.). Anhydro-*N*-carboxy-DL-alanine (0·11 g.) in ethyl acetate (1·5 c.c.) was cooled to −40° (carbon dioxide–acetone bath) and added slowly to the mixture of bases. After an induction period of about 10 minutes an exothermic reaction took place. The reaction mixture was left for two hours at the same temperature, and the carbamate (IV) precipitated with precooled ether; it decomposed fairly rapidly at room temperature. Addition of ethereal diazomethane at −40° to (IV) gave a colourless solution after 12 hours. In this case the liberated triethylamine could be removed *in vacuo* to leave the oily carbomethoxy-derivative which crystallised after 1 week.

DL-*Alanylglycine.*—The carbamate (IV) (0·3 g.) was hydrolysed with 0·37N-barium hydroxide (2·7 c.c.) at room temperature during 15 minutes. The solution was neutralised with 0·37N-sulphuric acid, filtered, and concentrated *in vacuo* to remove the triethylamine. The residue was taken up in a few drops of water, and DL-alanylglycine (95 mg., 62%) precipitated with acetone.

L-*Alanylglycine.*—A solution of glycine ethyl ester in chloroform (2 c.c.) was prepared from the hydrochloride (0·28 g.) and cooled to −65° in a solid carbon dioxide–acetone bath. After addition of methyldioctylamine (0·40 c.c.), a solution of anhydro-*N*-carboxy-L-alanine (0·23 g.) in tetrahydrofuran (1·5 c.c.) was precooled to −65° and slowly run into the mixture. Reaction was complete after three hours, and the mixture was then allowed to warm to room temperature, and the solvent removed *in vacuo*. The L-alanylglycine ethyl ester was hydrolysed, neutralised, and concentrated. Addition of a few drops of absolute alcohol yielded crystalline L-alanylglycine (m. p. 232—235°, decomp.; 0·22 g., 68%) (Found: N, 17·0; amino-N, 8·45. Calc. for $C_5H_{10}O_3N_2$: N, 17·0; amino-N, 8·5%), $[\alpha]_D^{22}$ +49·1° (*c*, 10 in water). A small quantity of 2 : 5-diketo-3-methylpiperazine crystallised from the mother-liquor after removal of the alcohol.

L-*Alanylglycylglycine.*—Glycylglycine ethyl ester was prepared from the hydrochloride (0·25 g.) by means of 2% chloroformic ammonia (0·6 c.c.) and caused to react with methyldioctylamine (0·4 c.c.) in chloroform (2 c.c.) and anhydro-*N*-carboxy-L-alanine (0·17 g.) in tetrahydrofuran (2 c.c.) at −65° during three hours. When the clear solution was warmed to −20° it set solid and cleared again at +10°. The solvent was concentrated *in vacuo*, and L-alanylglycylglycine ethyl ester, m. p. 103°, crystallised out. Hydrolysis as above gave long needles of L-alanylglycylglycine (m. p. 218°, decomp.; 0·22 g., 88%) (Found: N, 20·8; amino-N, 6·9. Calc. for $C_7H_{13}O_4N_3$: N, 20·7; amino-N, 6·9%), $[\alpha]_D^{22}$ +31·8° (*c*, 10 in water).

L-*Alanyl*-L-*alanylglycine.*—Anhydro-*N*-carboxy-L-alanine (0·23 g.) was treated as above with glycine ester in the presence of methyldioctylamine at −65°. The solution was allowed to warm to room temperature, and a vacuum applied. The mixture of L-alanylglycine ethyl ester and methyldioctylamine was then cooled again to −65° and treated with a second equivalent of anhydro-*N*-carboxy-L-alanine. When worked up in the usual way crystalline L-*alanyl*-L-*alanylglycine* (m. p. 236—238°, decomp.; 0·37 g., 85%) was obtained (Found: N, 19·5; amino-N, 6·4. $C_8H_{15}O_4N_3$ requires N, 19·4; amino-N, 6·5%); $[\alpha]_D^{22}$ −48·1° (*c*, 2 in water).

L-*Cystylbisbenzylamide.*—*Di*(anhydro-*N*-carboxy)-L-cystine (0·19 g.), dissolved in tetrahydrofuran (1 c.c.), was treated with benzylamine (0·22 c.c.; 2 equivs.) diluted with the same solvent (1 c.c.) at −40° and left for one hour. The gelatinous precipitate was decomposed with warm water, and the solid filtered off. Recrystallisation of the latter from ethyl acetate gave L-cystylbisbenzylamide in 93% yield (rectangular prisms, m. p. 122°) (Found: C, 57·3; H, 6·18; N, 13·35; S, 15·05. $C_{20}H_{26}O_2N_4S_2$ requires C, 57·4; H, 6·21; N, 13·38; S, 15·29%).

L-*Cystylbisglycine.*—Di(anhydro-*N*-carboxy)-L-cystine (0·76 g.), dissolved in tetrahydrofuran (3 c.c.), was added to glycine ethyl ester (from 1·2 g. of hydrochloride; 4 equivs.) in the same solvent (2 c.c.) at −10°. After two hours the mixture was allowed to warm to room temperature, and anhydrous ether (0·5 c.c.) added. The solution was filtered, and a further quantity of ether (1 c.c.) added. The *diethyl* ester of L-cystinylbisglycine crystallised on storage in the ice-box. Recrystallisation from tetrahydrofuran–light petroleum (b. p. 40—60°) gave tufts of crystals, m. p. 72—73° (Found: N, 13·7. $C_{14}H_{26}O_6N_4S_2$ requires N, 13·65%). The substance was hygroscopic and its solution in tetrahydrofuran became yellow overnight at room temperature. Hydrolysis of this ester was effected with 0·37N-barium hydroxide during 2 minutes at 10°. After treatment of the aqueous solution (neutralised in the usual way) with charcoal, absolute alcohol was added until a slight turbidity was produced. The solution was then warmed to 50° and kept at room temperature overnight. L-Cystylbisglycine crystallised in rectangular and square prisms and the total yield of peptide obtained after further addition of alcohol to the mother-liquor amounted to 78% based on the anhydride (Found: N, 15·95; S, 17·95. Calc. for $C_{10}H_{18}O_6N_4S_2$: N, 15·8; S, 18·1%); it had $[\alpha]_D^{21}$ −72·5°, $[\alpha]_D^{26·5}$ −68·5° (*c*, 1 in water) (Loring and Du Vigneaud recorded $[\alpha]_D^{27}$ −67·5°). The peptide moved very slowly on a paper chromatogram and the colour with ninhydrin was very faint. Best results were obtained by applying the nitroprusside test after a run using butanol–acetic acid of composition BuOH : AcOH : H_2O = 10 : 3 : 8 (R_F = 0·088).

Di-DL-*alanyl*-L-*cystylbisglycine.*—Anhydro-*N*-carboxy-DL-alanine (0·11 g.) in ethyl acetate (1 c.c.) was treated with the diethyl ester (0·2 g.; obtained as above) and triethylamine (0·14 c.c.) in ethyl acetate (2 c.c.) at −40° during two hours. The solvent was then removed *in vacuo* at room temperature, and the residue hydrolysed with baryta during two minutes at 10°. *Di*-DL-*alanyl*-L-*cystylbisglycine* was precipitated from a neutralised aqueous solution by means of absolute alcohol (0·12 g., 48%); it was very hygroscopic, and an analysis was carried out on material dried over phosphoric oxide at room temperature (Found: C, 36·7; H, 6·1; N, 15·8; S, 11·5. $C_{16}H_{28}O_8N_6S_2,2H_2O$ requires C, 36·1; H, 6·0; N, 15·79; S, 12·0%). The peptide gave a somewhat elongated spot on a paper chromatogram, and with the medium quoted above the approximate R_F value was 0·15.

L-*Tyrosylglycine.*—A solution of *O*-acetylanhydro-*N*-carboxy-L-tyrosine (0·25 g.) in ethyl acetate (2·5 c.c.) was cooled to −65° and added slowly to glycine ethyl ester (from 0·14 g. of hydrochloride) and methyldioctylamine (0·32 c.c.) in chloroform (2 c.c.) at the same temperature. After six hours the solvent was removed *in vacuo*, and the residue hydrolysed with 0·37N-barium hydroxide (10 c.c.) at room temperature during 30 minutes. Neutralisation in the usual way gave crystalline L-tyrosylglycine (m. p. 260—264°, decomp.) in 62% yield (Found: N, 11·7; amino-N, 5·9. Calc. for $C_{11}H_{14}O_4N_2$: N, 11·8; amino-N, 5·9%), $[\alpha]_D^{22}$ +82·6° (*c*, 2 in water). A second condensation was carried out with the same quantities of reagents, and the intermediate carbamate was decomposed with diazomethane in chloroform. *O-Acetylcarbomethoxy*-L-*tyrosylglycine ethyl ester* which had separated was crystallised from ethyl acetate (needles, m. p. 145—146°; 0·3 g.) (Found: C, 55·7; H, 6·0; N, 7·75. $C_{17}H_{22}O_7N_2$ requires C, 55·6; H, 6·0; N, 7·65%).

L-*Tyrosylglycylglycine.*—*O*-Acetylanhydro-*N*-carboxy-L-tyrosine (0·25 g.) in tetrahydrofuran (5 c.c.) was added to glycylglycine ethyl ester (from 0·20 g. of hydrochloride) and methyldioctylamine (0·32 c.c.) in chloroform (2 c.c.). After 6 hours' reaction at −65°, the intermediate carbamate had formed a heavy white precipitate. By working up in the usual way crystalline L-tyrosylglycylglycine was obtained (m. p. 193—195°, decomp.; 0·23 g., 77%) (Found: N, 14·2; amino-N, 4·55. Calc. for $C_{13}H_{17}O_5N_3$: N, 14·2; amino-N, 4·7%). A 2·7% solution of the dried material in 20% hydrochloric acid showed $[\alpha]_D^{22}$ +43·1°.

L-*Alanyl*-L-*tyrosylglycine.*—A solution of L-tyrosylglycine ethyl ester in chloroform was prepared as above from *O*-acetylanhydro-*N*-carboxy-L-tyrosine (0·25 g.) and glycine ethyl ester in the presence of methyldioctylamine. After decomposition of the carbamate by warming it to 40° *in vacuo*, the solution was cooled to −65° and allowed to react with anhydro-*N*-carboxy-L-alanine (0·11 g.) in tetrahydrofuran (1·5 c.c.) during three hours. Hydrolysis of the peptide ester afforded L-alanyl-L-tyrosylglycine as fine needles (Found: C, 54·1; H, 6·3; N, 13·5. $C_{14}H_{14}O_5N_3$ requires C, 54·4; H, 6·16; N, 13·60%), $[\alpha]_D^{22}$ +18·5° (*c*, 2 in water).

L-*Tyrosyl*-L-*tyrosine.*—*O*-Acetylanhydro-*N*-carboxy-L-tyrosine (0·25 g.) in chloroform (3 c.c.) was coupled with tyrosine ethyl ester (0·21 g.) in the presence of methyldioctylamine (0·32 c.c.) in chloroform (2 c.c.) at −65° during six hours. Removal of the solvent and hydrolysis as above gave crystalline L-tyrosyl-L-tyrosine in 60% yield (Found: N, 8·3; amino-N, 4·0. Calc. for $C_{18}H_{20}O_5N_2$: N, 8·1; amino-N, 4·1%). A 4% solution of the dried peptide in water containing one equivalent of hydrochloric acid gave a rotation of $[\alpha]_D^{22}$ +29·4°.

L-*Leucylglycylglycine.*—Anhydro-*N*-carboxy-L-leucine (0·20 g.) in tetrahydrofuran (1·5 c.c.) was coupled with glycylglycine ethyl ester (from 0·25 g. of hydrochloride) in the presence of methyldioctyl-

amine (0·40 c.c.) in chloroform (1 c.c.) at −40° during five hours. Removal of solvent and hydrolysis as above gave the finely crystalline L-leucylglycylglycine (m. p. 217—219°, decomp.; 0·33 g., 88%) (Found: N, 17·0; amino-N, 5·8. Calc. for $C_{10}H_{19}O_4N_3$: N, 17·1; amino-N, 5·7%), $[\alpha]_D^{21}$ +54·8° (*c*, 5 in water).

Diglycylglycine Ethyl Ester Hydrochloride.—Anhydro-*N*-carboxyglycine (0·2 g.), dissolved in tetrahydrofuran (3·5 c.c.), was coupled with glycylglycine ethyl ester (from 0·4 g. of hydrochloride) in the same solvent (2 c.c.) in the presence of trimethylamine (0·12 g.) during three hours at −65°; 2 equivs. of ethanolic hydrogen chloride were then added, and the solution kept in the ice-box overnight. The crystals which had deposited were recrystallised from water–acetone, yielding diglycylglycine ethyl ester hydrochloride, m. p. 214—216° (0·20 g., 40%).

Salt of N-*Carboxyglycylglycine Ethyl Ester with Glycylglycine Ethyl Ester.*—Dry carbon dioxide was passed into a solution of glycylglycine ethyl ester in chloroform at 0°. An almost quantitative yield of the *carbamate* was obtained as a finely crystalline powder which decomposed at 88—90° with evolution of carbon dioxide (Found: N, 15·47; CO_2, 12·0. $C_{13}H_{24}O_8N_4$ requires N, 15·4; CO_2, 12·1%). Treatment of this salt (0·36 g.) with excess of an ethereal solution of diazomethane at room temperature during 24 hours caused clusters of needles of N-*carbomethoxyglycylglycine ethyl ester* to form. Recrystallised from ethyl acetate, the compound had m. p. 104° (0·18 g., 82%) (Found: N, 12·9. $C_8H_{14}O_5N_2$ requires N, 12·8%).

Salt of N-*Carboxydiglycylglycine Ethyl Ester with Diglycylglycine Ethyl Ester.*—Prepared similarly by passing dry carbon dioxide into a solution of the tripeptide ester in chloroform at 0°, the *carbamate* had m. p. 115° (decomp.) (Found: N, 22·5; CO_2, 11·3. $C_{17}H_{30}O_{10}N_6$ requires N, 22·1; CO_2, 11·6%).

Carbethoxymethyl iso*Thiocyanate* (X).—Prepared according to Johnson and Hemingway (*J. Amer. Chem. Soc.*, 1916, **38**, 1550), the compound was obtained as an oil, b. p. 104—106°/7 mm.

N-*Thioncarbomethoxyglycine Ethyl Ester* (XI).—The *iso*thiocyanate (X) (10 g.) was refluxed with anhydrous methanol (50 c.c.) and triethylamine (0·5 c.c.) during 3 hours. Fractionation gave the di-ester, b. p. 101—102°/0·1 mm. (13·5 g.).

N-*Thioncarbomethoxyglycine.*—The ester (XI) (33 g.) was treated with N-sodium hydroxide (18·5 c.c.) during one hour at room temperature and then neutralised with N-hydrochloric acid. The solution was shaken with decolorising charcoal and concentrated *in vacuo*. An ether extract yielded the *half-ester* (XII) which, after crystallisation from chloroform–light petroleum (b. p. 40—60°), had m. p. 79° (20 g.) (Found: N, 9·3; S, 20·9. $C_4H_7O_3NS$ requires N, 9·4; S, 21·2%).

2-*Thio-oxazolid-5-one.*—The half-ester (XII) (20 g.) was dissolved in anhydrous ether (600 c.c.), anhydrous pyridine (11·5 c.c.) was added, and the mixture cooled to 0°. Thionyl chloride (10 c.c.) in ether (150 c.c.) was added slowly down the jacket of an efficient stirrer during 75 minutes. The solvent was decanted and concentrated *in vacuo*, and the residue taken up in chloroform and reprecipitated with light petroleum (b. p. 40—60°). 2-*Thio-oxazolid-5-one* crystallised from ether in hexagonal plates, m. p. 108° (5·2 g.) (Found: C, 31·0; H, 21·7; N, 11·8; S, 26·9. $C_3H_3O_2NS$ requires C, 30·8; H, 2·6; N, 12·0; S, 27·4%).

Reaction of 2-*Thio-oxazolid-5-one with Glycine Ethyl Ester.*—2-Thio-oxazolid-5-one (0·12 g.), in dioxan (2 c.c.) was added to a solution of glycine ethyl ester (from 0·14 g. of hydrochloride) and triethylamine (0·1 c.c.), also in dioxan. After 30 minutes at room temperature 2 equivs. of ethanolic hydrogen chloride were added. Glycylglycine ethyl ester hydrochloride, m. p. 182°, separated overnight (0·1 g.).

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