505. *Synthetic Polypeptides. Part IV.* Preparation and Polymerisation of Oxazolid-2 : 5-diones derived from α-Amino-dicarboxylic Acids.*

By D. Coleman.

An improved method of converting α-amino-dicarboxylic acids into the hydrochlorides of their ω-half-esters has been developed, and applied to the production of several new half-esters of L-glutamic and DL-aspartic acids. Some of these with carbonyl chloride give crystalline oxazolid-2 : 5-diones, including the hitherto undescribed 4-carbomethoxymethyl-DL-oxazolid-2 : 5-dione. Polymers and co-polymers of methyl and ethyl L-glutamate and of methyl DL-aspartate were not of high molecular weight and readily formed gels. This has been ascribed to an intermolecular reaction of terminal amino-groups with ester groups, and the resulting cessation of chain-growth, accompanied by the formation of cross-linkages.

It has been shown (Part III *) that a simple route is available for the preparation of 4-2′-carbethoxyethyl-L-oxazolid-2 : 5-dione by reaction of carbonyl chloride with γ-ethyl glutamate hydrochloride. It has now been found that a modification of the method used in the synthesis of the latter (previously described) results in higher yields and in the isolation of other ω-half-esters of L-glutamic and DL-aspartic acids. The amino-acid is added to the alcohol which contains its equivalent of dry hydrogen chloride (and not the large excess of acid used by most previous workers). The solution obtained after a few minutes' shaking contains a mixture of the hydrochlorides of the amino-acid and of the ω-half-ester. These are readily separated by fractional precipitation with ether. One recrystallisation of the ω-half-ester hydrochloride is usually sufficient. For dissolution of the amino-acids in the acidified alcohols it is absolutely essential that the latter be rigorously anhydrous. The methyl and ethyl glutamate hydrochlorides and methyl DL-aspartate hydrochloride reacted with carbonyl chloride, to give the oxazolid-2 : 5-diones as crystalline compounds. The higher alkyl esters, however, with carbonyl chloride gave oils which were uncrystallisable, doubtless owing to their lower melting points. γ-Ethyl L-glutamate hydrochloride was the only one of the half-esters that was hygroscopic, and in this case it was found convenient to prepare the free ester from the hydrochloride before treatment with carbonyl chloride.

The co-polymers and polymers obtained, such as poly(methyl DL-aspartate) and poly(methyl and ethyl L-glutamate), were of low molecular weight (films could not be cast from dioxan or chloroform). Moreover, the solution when kept for a few days formed irreversible gels. It is probable that the observed gelation was the result of the formation of cross-linkages produced by intermolecular reaction of ester with terminal amino-groups. Loss of amino-groups has been shown quantitatively in the case of poly(benzyl L-glutamate) ester by Hanby and Waley (*J.*, 1950, 3239), who ascribe the disappearance of amino-groups to cyclisation at the chain-ends, with formation of terminal pyrrolidone rings. However, intermolecular reaction probably also occurs, and which reaction predominates will depend on the nature of the solvent [poly(methyl DL-aspartate) will undergo intermolecular reaction only, since it cannot undergo ring closure].

The effect of the solvent on this type of reaction may be illustrated by the methyl esters of glycine and alanine. If these are left in ether or toluene for a few days at room temperature (*i.e.*, under conditions similar to those of the polymerisation) a high yield of the corresponding polypeptides is obtained (Frankel and Katchalski, *J. Amer. Chem. Soc.*, 1942, **64**, 2264), whereas in methanol a similarly high yield but of cyclic products is obtained (diketopiperazines).

The insolubility (and probably, as a consequence, the lack of haptenic activity in biological tests) of synthetic poly-L-glutamic acid contrasted with the solubility of the natural product thus finds a more acceptable explanation than that put forward by Ambrose (*J.*, 1950, 3246). The former is cross-linked and, as is well known, even a small amount of cross-linking is sufficient to modify profoundly the solubility of high polymers (Flory, *J. Amer. Chem. Soc.*, 1941, **63**, 3091). On the other hand, the natural product, although slightly branched, is essentially a linear molecule (Hanby and Rydon, *Biochem. J.*, 1946, **40**, 297) and its solubility is to be expected in view of the irregularities introduced by a slight degree of branching.

Attempts were made to prepare the methyl half-ester of aminomalonic acid as a first step in the preparation of polypeptides containing no side chains but having pendent carboxyl groups.

* Part III, *J.*, 1950, 3222.

Although these do not occur naturally, dipeptides of this type have been prepared (Schneider, *Biochem. Z.*, 1937, **291**, 328).

Experimental.

γ-Ethyl Glutamate Hydrochloride.—L-Glutamic acid (80·0 g.) was added, with shaking, to dry ethanol (330 c.c.) containing hydrogen chloride (24·0 g.); after 20 minutes it had dissolved. Two volumes of ether were added, and after 1 hour the solution was filtered from glutamic acid hydrochloride. More ether (2·5 l.) was added and the crystalline precipitate (m. p. 131°) that separated overnight was recrystallised from ethanol–ether, giving γ-ethyl glutamate hydrochloride (55·0 g.; m. p. 134°). (*Note.* In this and the following experiments it is essential that the esterifying alcohol be rigorously anhydrous. The alcohols in every case were first refluxed for 2 hours with sodium and the corresponding phthalate ester and then distilled just before use.)

Conversion into free ester. The above hydrochloride (5·0 g.) was dissolved in methanol (30·0 c.c.) and cooled to 0°. 10N-Ammonia (2·3 c.c.) was added, and the solution left overnight in the refrigerator, the free ester crystallised (3·0 g.; m. p. 187°). On treatment with carbonyl chloride the corresponding oxazolid-2 : 5-dione was obtained (m. p. 71°).

γ-Methyl Glutamate Hydrochloride.—L-Glutamic acid (18·6 g.) was added to dry methanol (75·0 c.c.) containing the equivalent of hydrogen chloride (4·8 g.). Ether (600 c.c.) was added, and the solution left overnight to crystallise, giving *γ-methyl glutamate hydrochloride* (m. p. 154°; 13·0 g.) (Found: C, 36·25; H, 6·0; N, 7·1; Cl, 17·9. $C_6H_{12}O_4NCl$ requires C, 36·25; H, 6·1; N, 7·1; Cl, 18·3%).

4-2′-Carbomethoxyethyl-L-oxazolid-2 : 5-dione.—γ-Methyl glutamate hydrochloride (8·0 g.), suspended in dioxan (300 c.c.), was treated with carbonyl chloride for 4 hours at 30° and then 1 hour at 40°. A rapid stream of air was passed through the solution for 16 hours to remove excess of carbonyl chloride, and the dioxan was then removed *in vacuo* at 40°. The 4-2′-carbomethoxyethyl-L-oxazolid-2 : 5-dione, recrystallised from chloroform–light petroleum, had m. p. 99° (6·5 g.).

β-Methyl Aspartate Hydrochloride.—Aspartic acid hydrochloride (11·0 g.) was added to dry methanol (70·0 c.c.) containing hydrogen chloride (3·80 g.). After 10 minutes ether (300 c.c.) was added, and the precipitate, recrystallised from ethanol–ether, was *β-methyl aspartate hydrochloride* (m. p. 190°; 8·0 g.) (Found: C, 32·6; H, 5·4; N, 7·6; Cl, 19·4. $C_5H_{10}O_4NCl$ requires C, 32·7; H, 5·45; N, 7·6; Cl, 19·35%).

4-Carbomethoxymethyl-DL-oxazolid-2 : 5-dione.—The foregoing hydrochloride (7·0 g.) in dioxan (250 c.c.) was treated with carbonyl chloride. The *diketone* (5·5 g.), recrystallised from chloroform–light petroleum, had m. p. 84° (Found: C, 41·4; H, 4·6; N, 8·2. $C_6H_7O_5N$ requires C, 41·6; H, 4·5; N, 8·2%).

γ-Propyl L-Glutamate Hydrochloride.—L-Glutamic acid (12·0 g.) was added to *n*-propanol (100 c.c.) containing hydrogen chloride (10·2 g.). After 30 minutes ether (200 c.c.) was added, and the precipitated *hydrochloride*, recrystallised from propanol–ether, had m. p. 152° (6·0 g.) (Found: C, 42·65; H, 7·1; N, 6·3; Cl, 15·7. $C_8H_{16}O_4NCl$ requires C, 42·6; H, 7·1; N, 6·2; Cl, 15·7%).

γ-2-Methoxyethyl Glutamate Hydrochloride.—To L-glutamic acid (14·7 g.), 2-methoxyethanol (60 c.c.), and hydrogen chloride (3·70 g.), was added ether (100 c.c.), which precipitated glutamic acid hydrochloride (6·50 g.). Further addition of ether (100 c.c.) gave *γ-2-methoxyethyl glutamate hydrochloride*, m. p. 174° (7·6 g.). Recrystallised from ethanol–ether, it had m. p. 176° (Found: C, 41·45; H, 6·9; N, 6·1. $C_8H_{16}O_5NCl$ requires C, 41·5; H, 7·0; N, 6·1%).

Attempted Preparation of Ethyl Hydrogen Aminomalonate.—(*a*) Aminomalonic acid was shaken with ethanol containing dry hydrogen chloride, but addition of ether or acetone to the solution gave no precipitate of the required half-ester. (*b*) Ethyl aminomalonate was cooled to 0° and ½ equiv. of ethanolic potassium hydroxide (also cooled to 0°) was added with stirring. Methylamine was evolved but no half-ester was isolated.

DL-Aspartic Acid.—After unsuccessful efforts to induce reaction between maleic anhydride and fumaric acid (Tutiya, *Bull. Agric. Chem. Soc. Japan*, 1941, **17**, 87) with ammonia, the method finally adopted (a modification of that of Dunn and Fox, *J. Biol. Chem.*, 1933, **101**, 493) was to treat methyl fumarate (60 g.) with methanol (200 c.c.) containing ammonia (70·0 g.) at 110° for 24 hours in an autoclave. The precipitate obtained (diketopiperazine-diacetamide) was hydrolysed for 6 hours with 6N-sodium hydroxide. After acidification with 6N-hydrochloric and separation of sodium chloride, aspartic acid was isolated by adjusting the pH to 4·0. Recrystallisation from water (300 c.c.) gave DL-aspartic acid (21·0 g.) (Found: N, 10·5. Calc. for $C_4H_7O_4N$: N, 10·5%).

Pyrrolidonecarboxylic Acid.—γ-Ethyl glutamate hydrochloride (9·0 g.) in methanol (63·0 c.c.) was treated with concentrated ammonia solution (10·0 c.c.). After 2 hours the solution was concentrated *in vacuo*, and pyrrolidonecarboxylic acid (4·0 g., m. p. 162°) collected

Polymerisations.—*With sodium salt of phenylalanine as initiator* (see Part III). 0·5-g. samples of the two carbomethoxyalkyloxazolid-2 : 5-diones were allowed to polymerise singly and together in dry pyridine, dioxan, ethyl acetate, or chloroform (7·0 c.c.). The solutions were isolated from atmospheric moisture by means of drying agents contained in a side-tube attached to the reaction vessel as described previously. The solutions rapidly increased in viscosity for the first two days, but within the next few days they invariably formed irreversible gels which did not disperse on the addition of more solvent. When copolymerised in chloroform with the oxazolid-2 : 5-diones of DL-valine, α-amino*iso*-butyric acid, or hexylglycine, a similar increase in viscosity culminating in gelation was observed. Thin layers of the solutions when allowed to evaporate did not give coherent films, indicating that the molecular weights were lower than those obtained previously (15,000) with the L-leucine–DL-phenylalanine co-polymer.

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