628. *Synthetic Polypeptides. Part II. Properties of Oxazolid-2 : 5-diones and an Initial Study of the Preparation of Polypeptides therefrom.*

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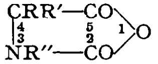
The findings of Woodward and Schramm (*J. Amer. Chem. Soc.*, 1947, **69**, 1551) are confirmed, but the resulting polypeptides had molecular weights of 15,000, rather than of " several million " as claimed. Several other soluble polypeptides, similar to the DL-phenylalanine–L-leucine copolymer described by Woodward and Schramm, are prepared, and the general nature of the formation of polypeptides from solutions of 4-substituted oxazolid-2 : 5-diones is established. Apparatus and technique for a quantitative study of the formation of polymers are described. The reaction is found to be difficult to reproduce exactly when water and aniline are used as initiator-catalysts. The synthetic polypeptides have some properties different from those of natural proteins, *e.g.*, they are soluble only in non-polar solvents and are very stable to hydrolytic agents.

In this investigation the substances (I)—(VIII) were studied.



	R.	R′.	R″.		R.	R′.	R.″
(I.)	H	H	H	DL-(VI.)	Pr^i	H	H
(II.)	Me	Me	H	DL-(VII.)	*cyclo*hexyl	H	H
DL-(III.)	H	CH_2Ph	H	L-(VIII.)	Bu^i	H	H
(IV.)	H	H	Ph	(IX).	H	H	Me
DL-(V.)	Ph	H	H				

When suitably treated, these substances, either alone or in pairs, form polypeptides of high molecular weight; this process is often referred to as " polymerisation " and that term will be used with that meaning in this paper. First, the solution-polymerisation of the individual compounds, and then that of pairs, were investigated to find systems which gave soluble polymers. Generally it was found that the polypeptide was precipitated and this limited the molecular weight. Only two soluble polymers were found : poly-DL-phenylalanine, which was soluble in dioxan, tetrahydrofuran, or chloroform, and insoluble in benzene, dimethylformamide, or acetone–benzene; and poly-DL-valine, which was soluble in chloroform and insoluble in benzene. Several new soluble copolymers were found, namely, pairs from α-amino*iso*butyric acid, DL-phenylalanine, DL-*C*-phenylglycine; these polymers were soluble in lipophilic solvents such as benzene and chloroform, and in one instance in dioxan. With the exception of valine the polymers from one amino-acid were insoluble in chloroform, whereas the copolymers were soluble, as is the case with other polymer systems. The solubility in lipophilic solvents is no doubt due to the irregularly distributed hydrocarbon side chains; in the absence of these, the hydrogen bonding between the amide groups gives higher intermolecular attraction and renders the polymers insoluble. Thus polyglycine was insoluble in dimethylformamide, a powerful solvent for strongly hydrogen-bonded compounds.

The work of Woodward and Schramm (*J. Amer. Chem. Soc.*, 1947, **69**, 1551) was repeated under controlled conditions. In the absence of water, polymerisation was absent or negligible. When traces of water were added, these were adsorbed by the glass and polymerisation largely took place at the glass surface. Traces of the polymer were not completely dissolved and were finally removed by centrifuging. The solution was viscous and could be cast into films which gave an *X*-ray diffraction pattern very similar to that of α-keratin. Molecular weights determined by the end-group method showed values of 15,000, corresponding to approximately 115 residues (two methods were used : chemical determination of amino-nitrogen, and physical determination of carboxyl by methylene-blue absorption; similar results were obtained by each method, by independent observers). Woodward and Schramm (*loc. cit.*) claimed values of " several million." However, traces of polymer were removed by us; these may have been of higher molecular weight, but their amount was hardly sufficient largely to increase the mean molecular weight. Further the 4-*iso*butyloxazolid-2 : 5-dione was not completely pure (Part I).

From the results of these initial experiments it was decided to study the formation of the α-amino*iso*butyric acid–DL-phenylalanine polypeptide in benzene. This was preferred to the Woodward and Schramm polypeptide because of the inaccessibility of pure 4-*iso*butyloxazolid-2 : 5-dione at that time. Benzene was preferred to chloroform because of its greater stability. The problem then arose of devising techniques necessary to study the polymerisation quantitatively (cf. Waley and Watson, *Rec. Trav. chim.*, 1950, **69**, 27, who have developed an elegant alternative method).

Oxazolid-2 : 5-diones as a class are sensitive to traces of moisture, bases, etc., and so these had to be controlled. Provision has to be made for escape of carbon dioxide. The starting materials were not very soluble in cold benzene and so agitation had to be provided. Because of the instability of the oxazolid-2 : 5-diones and their low solubility, periodical sampling of the reaction mixture was not possible. Eventually a simple apparatus was designed; this served as reaction vessel and viscometer and allowed carbon dioxide to escape, and agitation was accomplished by putting the reactor-viscometer (Fig. 1) on a rocker device. The viscosity was measured by timing a ball rolling down the inclined reactor.

The viscometers were calibrated with solutions of known viscosity. The viscosity was directly proportional to the time of roll of the ball, so each viscometer was readily calibrated.

The reactor-viscometer was dried and oxazolid-2 : 5-diones, solvent, and initiator (1% of water or aniline in anhydrous dioxan) introduced with the ball. The mixture was agitated at room temperature and the viscosity measured periodically.

Fig. 1.

Viscometer tube.

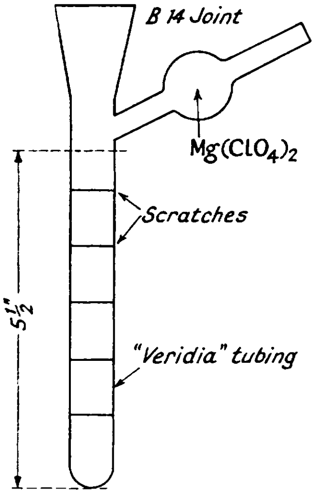
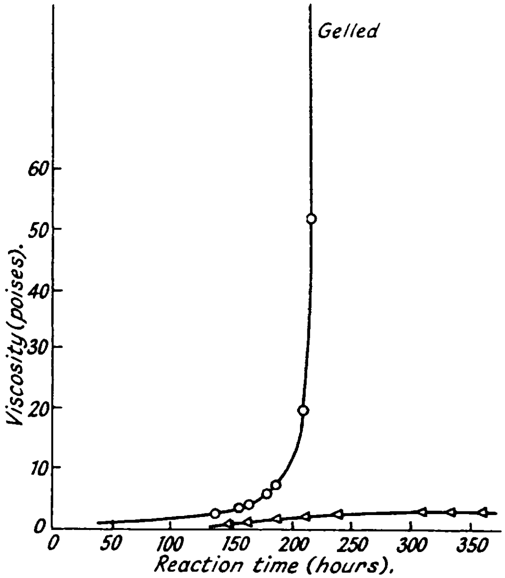


Fig. 2.



When equimolecular proportions of oxazolid-2 : 5-diones were used, the solution increased in viscosity and finally gelled (Fig. 2). However, the results were not reproducible and in some experiments the initiation appeared to be partly or completely inhibited. When water was used as initiator this effect was more pronounced than when aniline was used. It may be that adsorption of the water at the glass surface was a complicating factor. Whatever the cause, aniline gave better results and it appeared that other initiators and/or catalysts should be sought.

No further viscosity measurements could be made after the gel point, and whilst the addition of *m*-cresol prevented gelation this substance was not used because it has an active hydrogen atom.

It was shown, however, that gelation was avoided by varying the ratios of the oxazolid-2 : 5-diones in the phenylalanine–α-amino*iso*butyric acid system (cf. lower curve of Fig. 2), and this may prove a useful way of overcoming the gelation in other systems.

The molecular weight of 15,000 obtained for the L-leucine–DL-phenylalanine polypeptide implies about 115 residues per molecule, the bulk of the molecular weight being in the side chains. This molecular weight is low compared with that of silk and wool. Further, the bulky side chains would restrict hydrogen bonding between the –CO– groups of one chain and the –NH– groups of another.

The low moisture regain of the film (2·9%) is a reflection of the non-polar nature of the side chains. Linked with this is the remarkable resistance to boiling mineral acids and alkalis. Twenty hours' treatment had no apparent effect, whereas natural proteins are invariably degraded, usually to the constituent α-amino-acids. However, natural proteins contain a considerable number of acidic and basic side chains owing to α-amino-acids such as glutamic acid, lysine, arginine, and these facilitate the approach of hydrolytic agents. A similar reason may be advanced to account for the insolubility in cupriethylenediamine reagent and 6M-urea, and the solubility in benzene and chloroform.

The ionic nature of the polymerisation of oxazolid-2 : 5-diones was confirmed by observing that polymerisation was more rapid in mixtures of *m*-cresol and pyridine than in the separate solvents. This was confirmed by the absence of poly(methyl methylacrylate) when (I) decomposed in pure methyl methylacrylate, which rapidly polymerises in presence of free radicals. The work described in Part III also supports an ionic mechanism.

EXPERIMENTAL.

Initial Examination of Oxazolid-2 : 5-dione–Solvent Systems.—Approximately 0·05 g. of mixed oxazolid-2 : 5-diones, or 0·03 g. of each oxazolid-2 : 5-dione, was put in small test-tubes with approximately 2 c.c. of solvent and set aside, corked, at room temperature. The results are summarised in the table. Where the name of the solvent is not qualified the technical grade was used.

Oxazolid-2 : 5-dione.	Solvent.	Results.
(III)	C_6H_6–$COMe_2$ (50 : 50)	Ppt. after 9 days
(III)	Dioxan	Soln. viscous after 1 day
(III)	Tetrahydrofuran, redistilled	,, ,, ,,
(III)	C_6H_6 (dried over $CaCl_2$)	Ppt. after 12 days
(III)	$CHCl_3$	Soln. viscous after 2 days
(III)	Dioxan (dried over Na)	No change
(I + III)	H·CO·NMe_2	Viscous after 2 hrs. Ppt. after 5 hrs.
(I)	H·CO·NMe_2	Ppt. after 5 hrs.
(III)	H·CO·NMe_2	Viscous after 10 mins. Ppt. after 1 hr.
(IX)	H·CO·NMe_2	No change after 14 days
(II)	C_6H_6 (dried over $CaCl_2$	No change after 18 days
(II)	Dioxan	Ppt. after 5 days
(II)	Tetrahydrofuran	,, ,,
(II)	H·CO·NMe_2	,, ,,
(II)	EtOAc	,, ,,
(II + III)	C_6H_6	Soln. viscous after 6 days
(I)	H·CO·NMe_2	Ppt. after 5 days
(II)	$CHCl_3$	Ppt. after 13 days
(II + III)	$CHCl_3$	Soln. viscous after 3 days
(II + V)	C_6H_6	Ppt. after 13 days
(II + V)	$CHCl_3$	Thioxtropic gel after 2 hrs.
(V)	EtOAc + trace of NH_2Ph	Ppt. after 1 hr.
(V)	Dioxan	No change
(III + V)	$CHCl_3$	Thixotropic gel after 1 hr.
(V)	$CHCl_3$	,, ,, ,,
(III + VI)	$CHCl_3$	Soln. viscous after 24 hrs.
(IV + VI)	$CHCl_3$	No change
(IV)	C_6H_6–$CHCl_3$	,,
(I + III)	Dioxan	Ppt. after 1 day. Filtered. More ppt. after a further 3 days
(VI)	Dioxan	Ppt. after 5 days
(II + VI)	$CHCl_3$	Soln. viscous after 1 day
(VI)	C_6H_6	Ppt. after 5 days
(VI)	$CHCl_3$	Soln. viscous after 1 day
(V + VI)	$CHCl_3$	,, ,, ,,
(V + VI)	C_6H_6	Ppt. after 4 days
(III + VI)	C_6H_6	,, ,,
(II + VI)	Dioxan	Soln. viscous after 5 days *
(V + VI)	Dioxan	,, ,, ,, *
(III + V)	Dioxan	Soln. viscous after 1 day *
(III + VI)	Dioxan	Soln. viscous after 5 days
(I + VII)	C_6H_6	Ppt. after 2 days
(VII + VIII)	C_6H_6	Soln. viscous, gelling after 5 days
(VI + VII)	C_6H_6	Ppt. after 2 days
(II + VII)	C_6H_6	Soln. viscous, gelling after 7 days
(IV + VII)	C_6H_6	No change
(V + VII)	C_6H_6	Ppt. after 2 days
(III + VII)	C_6H_6	,, ,,
(II + IV)	C_6H_6	No change
(VI + VIII)	C_6H_6	Gel pptd. after 2 days
(IV + V)	C_6H_6	No change
(V + VIII)	C_6H_6	Traces of ppt. after 2 days
(III + V)	C_6H_6	,, ,, ,,

* These solutions gelled after a total of 12 days.

Preparation of L-*Leucine*–DL-*Phenylalanine Copolymer.*—A 1-l. three-necked flask, equipped with " Quickfit " joints, air-tight stirrer, gas-delivery tube reaching to the bottom of the flask, and exit tube, was dried at 110° for 1 week. Benzene, refluxed for 6 days over sodium wire, was distilled into the dried flask through the condenser used for the refluxing; 433 g. were collected, and (III) (3·49 g.) and (VIII) (2·87 g.; equimolar amounts) rapidly added. On stirring, all of (VIII) dissolved, but only some of the (III). Dry, carbon dioxide-free nitrogen was passed in to displace the air. After 8 days there was no change. It was concluded that no polymerisation-initiator was present. Accordingly distilled water (0·05 g.) was shaken with sodium-dried benzene (340 c.c.) and the whole rapidly added to the reaction mixture. After 5 days the reaction mixture became cloudy and the gas-lead choked with polymer. All the glass in contact with the reaction mixture was coated with a film of polymer, and the solution was viscous. Stirring did not cause the film to dissolve. When no more reaction was apparent dry chloroform was added. The bulk of the polymer film dissolved and left a little suspended gelatinous matter. The mixture was centrifuged to separate the undissolved matter (0·1 g. when dry). The clear solution possessed non-Newtonian viscosity.

Properties. The polymer solution on evaporation on water, glass, or mercury gave films of moderate strength (Found : N, 10·85%. Calc. for a 1 : 1 molecular copolymer : N, 10·8%). When heated in a capillary tube the material discoloured at 220° and slowly charred up to 300° without melting. When heated on a copper block the solid became less tough at 110° and at 210° stuck to the glass probe; at 220° it became brown and finally at 230° began to smell of burnt malt.

The clear benzene–chloroform solution of the copolymer was miscible with carbon tetrachloride and dioxan. Addition of ethanol, ethyl acetate, or light petroleum gave a white " ropy " solid, which redissolved on addition of more benzene or chloroform.

The solution was dialysed for 3 days at ~0·5% concentration against benzene with a Cellophane membrane, whereafter it no longer gave a ninhydrin reaction. It was then concentrated to 4·8% w/v. Aliquots of 2·00 c.c. were examined for amino-nitrogen in the Van Slyke apparatus. Because of the viscosity of the polymer solution the burette had to be filled by suction; otherwise the apparatus was used in the conventional manner. Values of 14,000—17,000 were obtained, on the assumption of one amino-group per molecule. The carboxyl groups were estimated by measuring the absorption of methylene-blue by the polymer; on the assumption of one carboxyl group per molecule, values of 14,000—15,000 were obtained.

Design and Calibration of Reactor-viscometer.—The vessel was a tube, of ⅜" internal diameter, fitted with a " Quickfit " B14 socket and stopper and a side-arm near the joint. The side-arm was slightly blown out to accommodate glass wool and a drying agent [$Mg(ClO_4)_2$]. The side-arm was further protected by a tube packed with " Drierite " ($CaSO_4$). The overall length of the tube was 8½"; the lower 5½" consisted of " Veridia " tubing of uniform ⅜" internal diameter. The outside was scratched at 1" intervals. To measure viscosity the tube was filled with the solution and held in a brass frame in a thermostat (at 20°) and left for 20 minutes. The time required for a steel ball of known diameter to roll a fixed distance was measured within 0·2 second. The tube was held at 20° to the horizontal, referring to the water-level in the thermostat tank by two pointed lugs on the brass frame.

To derive the constants of the instrument glycerol–water mixtures of known abolute viscosity (Sheeley, *Ind. Eng. Chem.*, 1932, **24**, 1060) were used. " Dynamite grade " glycerol was distilled (b. p. 173°/13 mm.) and diluted with distilled water. The solution were tested with balls of different diameters (1/32", 3/64", 3/32"). The plot of absolute viscosity (poises) against time of roll was sensibly linear from 0·1 to 15·0 poises. The constant of the instrument depended on the ball diameter and, although the constants were inversely proportional (±2%) to the square of the ball diameter, each ball diameter and tube were calibrated individually. For a 3/64"-diameter ball the instrument constants were all *ca.* 0·0341 for a 3" roll.

Thus $n = kt$, where n = viscosity of solution in poises, k = instrument constant, and t = time of roll of ball in seconds.

Use of the Reactor-viscometer.—The side-arm of the viscometer was plugged with glass wool, filled with anhydrous magnesium perchlorate, and plugged again with glass wool, and then a drying tube containing Drierite was fitted on the end. The tube and stopper were dried at 110° overnight, and allowed to cool with the stopper in place. Oxazolid-2 : 5-dione(s), initiator, and ball were added and a known amount of sodium-dried benzene was distilled directly into the tube, the only exit to the atmosphere being that through the viscometer side-arm. The viscometer was stoppered and transferred to a shaking machine, designed to give gentle end-to-end agitation. The viscosity of the system was measured periodically as described above.

After (II) (0·23 g.), (III) (0·38 g.; equimolar quantity), and a 1% solution of water in sodium-dried dioxan (0·05 c.c.) in benzene (7·0 c.c.) had been kept for 6 days, the viscosity had perceptibly increased and was measured, with a 2" roll and 3/64" ball. The results are summarised in Fig. 2. The solvent was removed from the gel (Found : N, 12·0. Calc. for 1 : 1 molecular copolymer, N, 12·1%). A sample was removed quickly after 216 hours and 5% of *m*-cresol was added. This viscosity fell, the time of roll decreasing from 1020 to 110 seconds. The gelation was probably caused by formation of molecular aggregates, not to the formation of a three-dimensional polymer.

This experiment was repeated but with half the above concentration and amounts of monomers and initiators. After 12 days there was no change. On addition of 0·05 c.c. of a 1% solution of aniline in dry benzene the viscosity increased in 24 hours, and after a further 14 days the solution gelled.

The first experiment was repeated, but this time there was no reaction after 3 weeks. On another occasion there was no reaction after 8 weeks. When 0·05 c.c. of a 1% solution of aniline in dry benzene was used instead of the water as initiator the viscosity increased only slightly after 3 weeks. Pyridine and quinoline similarly showed slight catalytic activity, but it appeared that the reaction was much more rapid in ordinary benzene.

Polymerisation of (II) and (III) in 1 : 2 molar ratio was then examined. (II) (0·12 g.), (III) (0·38 g.), dry dioxan (0·025 c.c.) containing 1% of water, and benzene (7·0 c.c.) were placed in the viscometer. There was no reaction after 12 days, and a 1% solution of aniline in dry benzene (0·05 c.c.) was added. Polymerisation then started (see Fig. 2). The solution was clear and cast to clear films. The films appeared to be amorphous when examined in polarised light, with slight indications of crystallinity on stretching ($\not> 10\%$).

Solubility of Polymers.—Films of the L-leucine–DL-phenylalanine copolymer (1 : 1 molar ratio), and the DL-phenylalanine–α-amino*iso*butyric acid (1 : 1 molar ratio) became insoluble in the original solvent (benzene) after a few days' exposure to the air. If moisture was rigidly excluded during casting of the films the polymer would re-dissolve. Refluxing for 20 hours with 6N-hydrochloric acid, 50% sulphuric acid, or 5N-sodium hydroxide had no effect.

(II) readily dissolved in cold water and after 1 hour gave a clear mobile solution with a positive biuret reaction. The low peptide is therefore water-soluble, as would be expected from the bulky nature of the *gem*-dimethyl group.

Structure of Polymers.—*X*-Ray diffraction photgraphs of the polymers were taken by Dr. C. J. Brown of these laboratories; some details have already been published (Astbury *et al.*, *Nature*, 1948, **162**, 596; Brown, Coleman, and Farthing, *ibid.*, 1949, **163**, 834).

" α "-Structures were found in the L-leucine–DL-phenylalanine copolymer when precipitated by varying amounts of ethanol and light petroleum, and also when cast into films on glass, mercury, or water, and similarly with the DL-phenylalanine–α-amino*iso*butyric acid copolymer. " β "-Structures were found in other polymers as originally shown by Astbury *et al.*

Moisture Regain of DL-*Phenylalanine–α-Amino*iso*butyric Acid* (1 : 1) *Copolymer.*—A film of polymer was exposed to the atmosphere for a month, between two Petri dishes. The film (0·284 g.) was heated at 110° for 20 hours and allowed to cool in a desiccator and weighed (0·276 g.) (Found : H_2O, 2·9%).

Mechanism of Polymerisation.—A mixture of (III) and (VIII) slowly effervesced in *m*-cresol and in pyridine separately; but in a mixture of these the reaction was much more vigorous and the solution rapidly gelled. (I) was decomposed in pure warm methyl methylacrylate, in which it was soluble; polyglycine was precipitated; after filtration the filtrates gave no precipitate when poured into methanol, indicating the absence of poly(methyl methylacrylate).

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