629. *Synthetic Polypeptides. Part III. Initiators for the Co-polymerisation of Oxazolid-2 : 5-diones.*

By D. Coleman.

The formation of polypeptide co-polymers from substituted oxazolid-2 : 5-diones has been studied further. The conditions of the reaction are clarified and a controlled comparison has been made of various initiators. In general there were long and variable induction periods (5—8 weeks). However, the alkali-metal salt of an α-amino-acid starts the reaction immediately and provides a means of controlling the molecular weight of the co-polymer. It may be used with completely dry solvents or may conveniently be produced *in situ* by using sodium carbonate in conjunction with the moist solvent. By its use co-polymers have been obtained of sufficiently high molecular weights to produce fibres, from DL-phenylalanine–L-leucine and DL-phenylalanine–α-amino*iso*butyric acid. A comparison of the *X*-ray diagrams of the synthetic fibres with those of natural proteins reveals a striking similarity in an α-keratin pattern. A simple preparation of L-4-2′-carbethoxyethyloxazolid-2 : 5-dione which could be applied to half-esters of other amino-acids is described. Water-soluble polypeptides have been made from L-lysine and L-glutamic acid.

The preparation of polypeptides of high molecular weight from oxazolid-2 : 5-diones requires the presence of a catalyst as " initiator." Coleman and Farthing (preceding paper) have shown that when water or aniline was used for this purpose consistent results could not be obtained from DL-4-benzyl- and 4 : 4-dimethyl-oxazolid-2 : 5-diones. Since it was later found possible to prepare pure L-leucine the system used by Woodward and Schramm (*J. Amer. Chem. Soc.*, 1947, **69**, 1551) has also been examined. With alcohols, amines, tertiary bases, or water as initiator a similar lack of consistency has been observed. Generally there was no or very little reaction even after the mixture had been shaken for two months at room temperature under controlled conditions.

An initiator was therefore needed that would react quickly and completely with the monomer. Before Woodward and Schramm's work no attempts had been made to use small amounts of initiator with a view to obtaining high molecular weights. Wesseley (*Z. physiol. chem.*, 1927, **170**, 78) had polymerised oxazolid-2 : 5-dione in boiling absolute ethanol, and Go and Tani (*Bull. Chem. Soc. Japan*, 1939, **14**, 510) had allowed the same compound to polymerise in moist air and in pyridine. The last reagent has been often used (Hanby, Waley, and Watson, *Nature*, 1948, **161**, 132), apparently with no very clear idea of its mechanism. It is in fact an apparent exception to the necessity for the initiator to possess an " active hydrogen." The mechanism of the initation reaction put forward by Woodward and Schramm was :

$$HX + \begin{matrix} CHR—CO \\ NH—CO \end{matrix}\!\!>\!O \longrightarrow X\cdot CO\cdot CHR\cdot NH\cdot CO_2H$$

The product of this reaction would be decarboxylated immediately to $X{\cdot}CO{\cdot}CHR{\cdot}NH_2$, which since it possesses an " active hydrogen " initiates the next step in the reaction.

The behaviour of pyridine would be explained if it had contained a trace of water. This would react with the monomer to form the free amino-acid, and in the presence of a large excess of pyridine the amino-group of the amino-acid would be free to initiate reaction. If this were so, then a stronger base than pyridine might be more effective in neutralising the carboxyl group of the amino-acid and thus freeing the amino-group. These considerations prompted the use of sodium carbonate in moist benzene and the alkali-metal salts of amino-acids in dry benzene. In both cases it was found that reaction started immediately and that, as shown by the absence of an inflexion in the plots of viscosity with time, the propagation and initiation reactions were identical. Furthermore, since the same curve could be obtained on using either the sodium salt of the amino-acid or sodium carbonate, the former salt was the actual initiator. This was confirmed by the complete absence of activity when sodium carbonate was used in conjunction with dry, rather than moist, benzene. The reaction in moist benzene occurred readily with the oxides, hydroxides, and carbonates of potassium and rubidium, but did not occur with other salts of these metals or with metals of other groups. Perhaps not surprisingly, lithium carbonate was completely inactive : probably lithium carbonate, being insoluble, was unable to form even the small amount of salt required.

The alkali-metal salts are effective for a variety of α-amino-acids, but exceptions are the sodium salts of glycine and cystine which significantly are insoluble in benzene, in contrast to the others which are slightly soluble. The superior effectiveness of potassium over sodium carbonate is caused by its greater solubility.

The sodium salt of phenylalanine in dry benzene containing the oxazolid-diones from L-leucine and DL-phenylalanine gave a co-polymer of molecular weight agreeing with that calculated on the assumption that each molecule of initiator reacts. In moist benzene the rate of reaction is reduced, probably because of the hydrolysis of the sodium salt and the consequent reduction in the number of reacting chains. A similar explanation applies to the behaviour of sodium carbonate in moist benzene. Here the molecular weight of the product increases as the amount of sodium carbonate decreases, but the relation is non-linear because of the effect of water on the salt equilibrium.

As shown by a comparison of the activities of octadecylamine and aniline, the stronger the amine the more effective it is as initiator. Independent evidence that the basicity of the amine affects the reactivity is provided by the differing behaviour of oxazolid-2 : 5-dione and 3-phenyl-oxazolid-2 : 5-dione. When allowed to react with equimolecular amounts of aniline the former gives exclusively the polymer whilst the latter gives a nearly quantitative yield of amide : in the first case, reaction of a small amount of aniline gives a strong base which immediately reacts with fresh monomer; in the second, the product is a weak base comparable with aniline itself and thus little polymer is formed.

These considerations suggest that the still greater activity of the α-amino-acid salts is caused by the enhanced basicity of the amino-group brought about by the inductive effect of the carbonyl group. Where the carbonyl and amino-groups are not connected to the same carbon atom as in sodium 6-aminohexanoate the activity is much reduced and becomes comparable in magnitude to that of octadecylamine. This accounts for the absence of an initiation period, because the initiation reaction with the sodium salt of an α-amino-acid is essentially the same as the propagation reaction, since here also there is a carbonyl group on the same carbon atom as the reacting amino-group. The initiator and propagator of the reaction may both be expressed as $NaO{\cdot}[CO{\cdot}CHR{\cdot}NH]_n{\cdot}H$, where $n = 1$ in the case of the initiator.

The influence of carbonyl groups on the reactivity of amino-groups is well illustrated by the reaction of esters of α-amino-acids with nitrous acid to give diazo-compounds. A similar reaction occurs with some α-amino-ketones, *e.g.*, aminocamphor and ω-aminoacetophenone (Angeli, *Ber.*, 1893, **26**, 1715; 1904, **37**, 2080). According to Angeli the essential condition for the formation of diazo-compounds from aliphatic amines is the presence of the grouping $X{:}C{\cdot}CH{\cdot}NH_2$ (where X may be C, O, or N) or $O{:}S{\cdot}CH{\cdot}NH_2$. Thus aminoacetonitrile and aminomethanedisulphonic acid yield diazo-compounds.

Reaction at High Temperatures.—The co-polymerisation of the components used by Woodward and Schramm has been found to occur at a much increased rate at higher temperatures, and provided that dry benzene is used in conjunction with small amounts of initiator (the sodium salt of phenylalanine) products of high molecular weight are obtained which are similar to those obtained at room temperature. However, the other co-polymer system studied does not behave in this way, for when the L-4-*iso*butyloxazolid-2 : 5-dione is replaced by 4 : 4-dimethyloxazolid-

2 : 5-dione no increase in viscosity occurs in boiling benzene. This is no doubt related to the greater stability of the latter compound at higher temperatures.

The properties of synthetic polypeptide films made from certain monoaminocarboxylic acids have already been described (Part II). By use of the new initiators polymers of higher molecular weight are obtained which afford films with better ability to orient and thus give a clearer *X*-ray diagram. Solubility remains much the same, but films from polymers with a molecular weight above 40,000 are even more insoluble, *e.g.*, insoluble in *o*-chlorophenol. These co-polymers have also fibre-forming properties.

The molecular weights were determined by estimating the weights of methylene-blue that combined with the terminal carboxyl groups (Davidson, *J. Textile Inst.*, 1948, **39**, 65). On attempting to confirm the results by estimating the terminal amino-groups it was found that Sanger's method (*Biochem. J.*, 1945, **39**, 507) failed because of the impossibility of hydrolysing the product of the reaction of dinitrofluorobenzene with the polypeptide. However, by using the Van Slyke method for the determination of amino-groups agreement was obtained for a polypeptide of molecular weight 15,000 made from DL-4-benzyl- and L-4-*iso*butyl-oxazolid-2 : 5-dione.

The *X*-ray fibre photograph of the co-polymer from L-4-*iso*butyl- and DL-4-benzyl-oxazolid-2 : 5-dione shows one marked difference from the film-photograph, in that the side-chain spacing orients along the equator in place of the 10·4-A. reflexion. One interpretation of this (Brown, Coleman, and Farthing, *Nature*, 1949, **163**, 834) is that the molecules in the fibre lie transversely to the fibre axis, the structure being held together along the fibre by hydrogen bonds and the interlocking of the side-chains. Further *X*-ray results, supported by infra-red evidence, are obviously needed before a definite conclusion can be reached.

A further thirty combinations (two at a time) of various oxazolid-2 : 5-diones have now been studied, but only two were of interest, the majority again giving precipitates of low molecular weight. The two exceptions were the derivatives of L-glutamic acid and L-lysine.

Water-soluble Polypeptides.—Globular proteins dissolve in aqueous solutions, and most of the fibrous proteins are also soluble after suitable treatment, *e.g.*, silk fibroin by dissolution in cupriethylenediamine and neutralisation, followed by dialysis of the inorganic salts (Coleman and Howitt, *Proc. Roy. Soc.*, *A*, 1947, **190**, 145). It follows therefore that synthetic water-soluble polypeptides may be of more fundamental interest than the insoluble type. This does not apply to polypeptides that owe their solubility to the absence of intermolecular hydrogen bonds, such as polysarcosine, but to polymers and co-polymers of L-glutamic acid and L-lysine, since like the proteins these possess polar side-chains. Polylysine has been prepared (cf. Frankel *et al.*, *J. Amer. Chem. Soc.*, 1948, **70**, 2094) and a co-polymer with valine has been obtained in chloroform. The removal of the protecting carbobenzyloxy-group by reduction proved somewhat troublesome, and from the practical point of view poly(glutamic acid) was more attractive. This was made by forming the half ester (Abderhalden and Nienburg, *Z. physiol. Chem.*, 1933, **219**, 155) from L-glutamic acid, the method being improved so that it could be used on a larger scale. The ester was then converted directly into the oxazolid-2 : 5-dione; the reaction was more rapid than with the neutral amino-acids, doubtless because of the greater solubility of the glutamic ester in dioxan. The product polymerised readily in moist dioxan, and the polymer was readily hydrolysed to poly(glutamic acid) by mild treatment with alkali.

Experimental.

L-4-iso*Butyloxazolid*-2 : 5-*dione* (I).—Considerable difficulty was experienced in preparing this compound owing to impurities in the L-leucine available. Low-melting products were obtained which polymerised spontaneously. This was caused by the presence of about 10% of tyrosine, the phenolic hydroxyl group initating the polymerisation.

L-Leucine (methionine-free; B.D.H.) was twice recrystallised from water until a negative test for tyrosine was shown by the absence of a red colour with Millon's reagent. The purified L-leucine (32·0 g.) was then agitated with sodium-dried dioxan (400 c.c.) in a three-necked 2-l. flask. Carbonyl chloride was passed in for ¾ 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 then removed *in vacuo* at 40°. The product crystallised almost immediately. Recrystallised from ether–light petroleum, the substance (87·0%) had m. p. 76—77°.

DL-4-*Benzyloxazolid*-2 : 5-*dione* (II).—DL-Phenylalanine (50 g.) in sodium-dried dioxan (350 c.c.) was treated with carbonyl chloride for 1 hour at 20° and then 4 hours at 40°. (II) was isolated as above, but from ethyl acetate–light petroleum, as colourless needles (88·0%), m. p. 125°.

DL-4-iso*Butyloxazolid*-2 : 5-*dione* (III).—DL-*iso*Butylglycine (10·0 g.) in dioxan (300 c.c.), treated as above for 5 hours at 20°, gave a colourless oil which, crystallised from ether–light petroleum, had m. p. 47—50° (75%).

4-*Dimethyloxazolid*-2 : 5-*dione* (IV).—α-Amino*iso*butyric acid (25·0 g.) in dioxan (350 c.c.), treated for 1 hour at 20° and then 14 hours at 50°, gave (IV) (52·0%), m. p. 95° (from chloroform–light petroleum).

L-4-2′-*Carbethoxyethyloxazolid*-2 : 5-*dione* (V).—(*a*) Finely powdered L-glutamic acid (5·0 g.) was shaken with dry ethanol (50 c.c.) containing hydrogen chloride (3·0 g.). In 5 minutes the glutamic acid had dissolved and the solution was evaporated to dryness as quickly as possible *in vacuo* below 30°. It was recrystallised from ethanol–ether, to give γ-ethyl glutamic ester hydrochloride (4·5 g.), m. p. 130. This (4·0 g.) was then treated with carbonyl chloride for 1 hour at 20° and then 1 hour at 40° in dioxan (150 c.c.). Recrystallised from chloroform–light petroleum the *dione* (3·1 g.) had m. p. 66° (Found : C, 47·95; H, 5·3; N, 7·0. $C_8H_4O_5N$ requires C, 47·8; H, 5·5; N, 7·0%).

(*b*) The method used above is only applicable to small amounts (about 5·0 g.), because conversion into the di-ester occurs during removal of the alcoholic hydrochloric acid. The scale could be increased at least 10 times by cooling the mixture quickly to 0° and passing in dry ammonia to neutralise the excess of hydrochloric acid. The mixture was then allowed to come to room temperature and filtered from ammonium chloride, and the ester isolated in the usual way. The yield of hydrochloride was 88% (cf. 62·0% by the small-scale method). Various alternative methods of making the ethyl or methyl ester were tried without success, including the reaction of alkyl halides with silver glutamate and the action of diazomethane on a suspension of glutamic acid in ether.

DL-4-sec.-*Butyloxazolid*-2 : 5-*dione* (VI).—DL-*iso*Leucine (5·0 g.) in dioxan (200 c.c.) was treated with carbonyl chloride for ½ hour at 20°, and then for ½ hour at 40°. (VI) crystallised on removal of the dioxan and, recrystallised from ether–light petroleum, had m. p. 76—77° (5·2 g., 90%).

L-4-4′-*Carbobenzyloxyaminobutyloxazolid*-2 : 5-*dione* (VII).—L-Lysine monohydrochloride (20·9 g.), dissolved in sodium hydroxide (113 c.c.; 2N.), was cooled to 0°. Benzyl chloroformate (60·0 g.) and 4N-sodium hydroxide (142 c.c.), both cooled to 0°, were added in 4 portions with vigorous shaking, the temperature of the mixture being kept below 10°. 5N-Hydrochloric acid (200 c.c.) was added and the mixture extracted with ether. The ethereal extract was purified by extraction with potassium hydrogen carbonate solution from which it was transferred to fresh ether by acidification. On removal of the ether a colourless syrup (28·0 g.) of ε-carbobenzyloxylysine was left. This was dissolved in dry ether (110 c.c.), and cooled to 0°, and powdered phosphorus pentachloride (16·2 g.) was added. After 30 minutes' vigorous shaking most of the solid had disappeared. The solution was filtered and concentrated *in vacuo* at 40°. Ethyl acetate was twice added and the solution evaporated again each time *in vacuo* at 50°, to give a dark-coloured oil. This was twice recrystallised from ether–light petroleum to give (VI) (8·5 g.), m. p. 100° (decomp.).

L-4-*Methyloxazolid*-2 : 5-*dione* (VIII).—DL-Alanine, recrystallised from aqueous alcohol, was treated as above for 1 hour at 20° and 2¼ hours at 40°; a yellow oil (VIII) was obtained.

DL-4-iso*Propyloxazolid*-2 : 5-*dione* (IX).—DL-Valine (50 g.) in dioxan (400 c.c.) was treated as above for 3 hours at 50°. Crystallised three times from chloroform–light petroleum, the product (84%) had m. p. 81°.

3-*Methyloxazolid*-2 : 5-*dione* (X).—*N*-Carbomethoxysarcosine (18·0 g.) was heated at 40° with redistilled thionyl chloride (25·0 g.) for 1½ hours. After evaporation the orange residue was kept at 40° *in vacuo* for 3 hours and then washed with ether. Recrystallised three times from chloroform it gave (X) as large needle-shaped prisms, m. p. 105°.

Comparison of Various Initiators in the Co-polymerisation of (II) *and* (IV).—0·002 G.-mol. of (II) and 0·001 g.-mol. of (IV) in 7·0 c.c. of sodium-dried benzene were used [these (Part II) gave clear, viscous solutions] in the rolling-ball viscometer (Part II). The progress of the reaction was followed viscosimetrically in the way previously described. Results are given in the table and are summarised in the figure.

With the same reaction mixture (but moist benzene) the oxides, hydroxides, and carbonates of lithium, magnesium, calcium, lead, Cu^{II}, and silver were completely inactive. Sodium stearate, silicate, borate, and chloride, and disodium phosphate were also ineffective. Trisodium phosphate gave a slight initial effervescence, but reaction was slight (initial surface layer of carbonate). Benzyltrimethylammonium hydroxide, sodium ethoxide, and ammonium carbonate caused effervescence but gave no polymer.

Sodium Salts of the Amino-acids used as Initiators.—A known weight of amino-acid was neutralised with N-sodium hydroxide (carbonate-free) and the solution evaporated in a vacuum-desiccator over concentrated sulphuric acid. For lysine, sarcosine, and 6-aminohexanoic acid the hydrochlorides were used, with 2 moles of sodium hydroxide. The presence of sodium chloride was shown to have no effect on the polymerisation.

Properties. On equilibration with dry benzene overnight, with shaking, the sodium salts were found to be very slightly soluble, except for the salts of glycine and cystine. The solubilities were qualitatively assessed as follows: a drop of the filtered solution on filter paper was treated with a drop of 1% solution of ninhydrin in butanol; the paper was then heated for 3 minutes at 110°, a positive result being shown by the red colour peculiar to α-amino-acids.

Solubility of the Free Amino-acids in Benzene.—L-Leucine (0·5 g.) was treated in boiling anhydrous benzene (100 c.c.) for 3 hours and the mixture filtered. The filtrate gave a negative test with ninhydrin. Glycine and α-amino*iso*butyric acid were also insoluble, but phenylalanine, although insoluble in cold benzene, was very slightly soluble at 80°.

Further Co-polymerisations.—Low-molecular-weight co-polymers of (VII) with (I), (II), and (III) were obtained in benzene or chloroform. On the other hand, when (I) was polymerised alone in

Initiator.	(Mg.-mol.).	Reaction time (days).	Roll-time over 2 in. (secs.).	Viscosity (poises).
NH_2Ph	0·05	10·0	10·0	0·51
		15·0	10·0	0·51
	0·30	7·0	12·0	0·61
	0·60	8·0	20·0	1·02
$C_{18}H_{37}·NH_2$	0·055	2·0	42·0	2·14
		3·0	82·0	4·18
		4·0	95·0	4·84
		7·0	127·0	6·47
		8·0	127·0	6·47
	0·011	1·0	Soln. just viscous but faintly cloudy	
		2·0	Gel on sides of tube	
		3·0	7·0	0·36
			Further separation of gel	
C_5H_5N.....	0·05	35·0	} Viscosity of solutions too low for measurement	
	0·10	42·0		
Quinoline	0·05	37·0		
	0·10	37·0		
NEt_3	2·0	6·0	No change	
	20·0	13·0	} Viscosity too low for measurement	
	40·0	19·0		
Piperidine	2·0	3·0	Slight turbidity	{ Viscosity stationary at 1·02
	6·0	8·0	20·0	
	20·0	0·12	Gel precipitated	
H_2O	0·014		{ Incomplete dissolution of monomers; no change on storage	
	0·100	50·0		
	0·20			
EtOH	0·08	7·0	,, ,,	
	0·22	7·0	,, ,,	
$CH_2Ph·OH$	0·04	8·0	Incomplete dissolution of the monomers; no change on storage	
	0·24	21·0	15·0	0·76
$(CH_2·OH)_2$	0·05	1·0	7·0	0·36
		2·0	11·5	0·58
		3·0	14·0	0·72
		6·0	19·0	0·87
p-$C_6H_4(CH_2·OH)_2$	0·04	5·0	7·0	0·36
		6·0	90·0	4·60
		7·0	272·0	13·80
		8·0	272·0	13·80
		9·0	280·0	14·20
		10·0	280·0	14·20

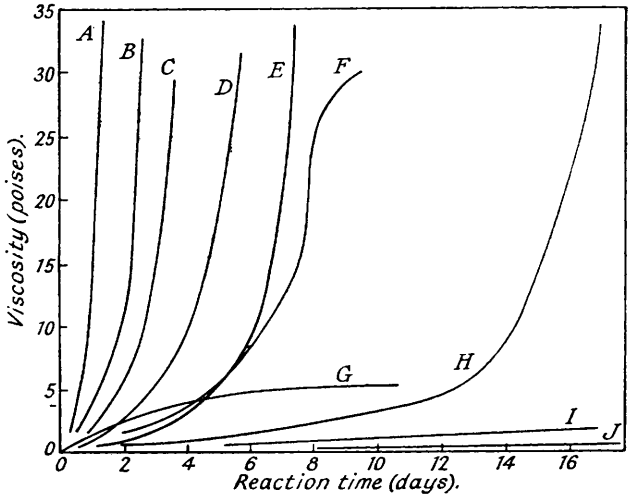
Initiator.	(Mg.-mol.).	Reaction time (days).	Roll-time over 2 in. (secs.).	Viscosity (poises).
m-$C_6H_4Me·OH$	0·10	3·0	15	0·76
		1·50	1·0	Separation of gel particles
Na_2CO_3; C_6H_6 (dry)	0·30	7·0	No change	
Na_2CO_3; C_6H_6 (+0·05% H_2O)	0·05	2·0	6·0	0·31
		7·0	16·5	0·84
		8·0	22·0	1·12
		9·0	24·0	1·22
	0·10	2·0	45·0	2·29
		7·0	620·0	31·60
		8·0	>1000	>50·0
	0·20	2·0	81·0	4·13
		6·0	>1000	>50·0
	0·35	3·0	>1000	>50·0
	0·40	2·0	160·0	8·16
		3·0	650·0	33·10
	0·50	3·0	>1000	>50·0
$NaHCO_3$ (moist C_6H_6)	0·25	1·0	No change	
		2·0	10·5	0·53
		6·0	340·0	17·30
	0·50	10·0	380·0	19·40
NaOH (moist C_6H_6) ...	0·125	3·0	6·0	0·31
		11·0	14·5	0·74
	0·40	3·0	6·0	0·31
		11·0	60·0	3·06
		14·0	90·0	4·59
		15·0	108·0	5·50
		16·0	116·0	5·90
		18·0	220·0	11·0
		22·0	>1000	>50·0
		24·0	>1000	>50·0
K_2CO_3 (moist C_6H_6) ...	0·015	3·0	34·0	1·73
		4·0	65·0	3·31
		5·0	72·0	3·67
		6·0	105·0	5·35
		7·0	178·0	9·07
		10·0	1100	56·0
		11·0	1470	75·00
	0·10	1·0	30·0	1·53
		2·0	491·0	25·0
		5·0	912·0	46·50
	0·66	0·76	>1000	>50·0
$RbCO_3$ (moist C_6H_6) ...	0·036	26·0	52·0	2·65
		27·0	53·0	2·70
	0·82	1·0	36·0	1·83
		2·0	>1000	>50·0

Initiator.	(Mg.-mol.).	Reaction time (days).	Roll-time over 2 in. (secs.).	Viscosity (poises).
		Na *salts*.		
Phenylalanine (moist C_6H_6)	0·027	3·0	50·0	2·55
		4·0	72·0	3·66
		5·0	110·0	5·60
		6·0	130·0	6·63
		7·0	171·0	8·72
		10·0	602·0	30·70
		12·0	758·0	38·65
Phenylalanine ...	0·027	1·0	6·0	0·31
		2·0	43·0	2·19
		3·0	76·0	3·87
		6·0	405·0	20·65
		7·0	758·0	38·70
		8·0	>1000	>50·0
	0·054	1·0	193·0	9·85
		2·0	1145·0	58·25
		3·0	Transparent gel	
Leucine	0·03	1·0	7·0	0·36
		3·0	88·0	4·50
		6·0	451·0	22·95
		8·0	>1000	>50·0
$NH_2 \cdot CMe_2 \cdot CO_2H$	0·03	3·0	92·0	4·70
		6·0	431·0	21·95
		9·0	1070·0	54·50

Initiator.	(Mg.-mol.).	Reaction time (days).	Roll-time over. 2 in. (secs.)	Viscosity (poises).
		Na *salts*.		
Sarcosine	0·04	1·0	64·0	3·27
		3·0	343·0	17·60
		4·0	1200·0	61·20
$NH_2 \cdot [CH_2]_5 \cdot CO_2H$ ...	0·04	2·0	17·5	0·89
		3·0	46·0	2·34
		6·0	64·0	3·26
		7·0	76·0	3·98
		10·0	76·0	3·98
Lysine	0·03	1·0	8·0	0·41
		2·0	49·0	2·49
		3·0	101·0	5·15
		4·0	175·0	8·93
		7·0	1440·0	73·40
Phenylalanine	0·06	28·0	No change	
Polyglycine	50 mg.	1·0	6·0	0·31
		5·0	6·0	0·31
Glycine	0·03	30·0	No change	
Cystine	0·05	20·0	No change	
$CH_2Ph \cdot NH \cdot CH(CO_2H)_2$	0·05	3·0	160	8·16
		5·0	>1000	>50·0
Polyglycine	10 mg.	7·0	No change	
NaCl	1·8	21·0	No change	

chloroform or pyridine highly viscous solutions were obtained but no gel separated. In benzene and ethyl acetate an insoluble precipitate separated from a non-viscous solution. With (IX) in chloroform a clear viscous solution was obtained in 2 days. (V) formed a viscous solution in 1 day in moist pyridine or dioxan, and with (IV) gave a clear, viscous co-polymer in dioxan. Insoluble precipitates were obtained from (*a*) (V) with (I), (II), or (IX) in pyridine or dioxan, with (VI) or (XI) in pyridine, or with (III), (VII), or (XII) in dioxan, (*b*) (VI) in pyridine alone or with (I), or in benzene with (I) or (II), (*c*) (III) with (II) in benzene, (*d*) (X) in moist dioxan alone or with (II), (IV), or (XI), and (*e*) (VIII) alone or with (I), (II), (IV), (VI), (XI), or (XII).

Polymerisation mixture : (II) (2 *mg.-mols.*) *and* (IV) (1 *mg.-mol.*) *in moist benzene* (7·0 *c.c.*).



Mg.-mols. of initiator : *A*, $C_6H_5{\cdot}CH_2{\cdot}CH(NH_2){\cdot}CO_2Na$, 0·054; *B*, Na_2CO_3, 0·4; *C*, K_2CO_3, 0·1; *D* Na_2CO_3, 0·1, = $C_6H_5{\cdot}CH_2{\cdot}CH(NH_2){\cdot}CO_2Na$, 0·027 (*dry benzene*); *E*, K_2CO_3, 0·015; *F*, $C_6H_5{\cdot}CH_2{\cdot}CH(NH_2){\cdot}CO_2Na$, 0·027; *G*, $C_{18}H_{37}{\cdot}NH_2$, 0·05; *H*, NaOH, 0·04; *I*, Rb_2CO_3, 0·02; *J*, H_2O, ROH, or NH_2R, 0—0·1.

Use of Sodium Salts for Preparation of Products of Higher Molecular Weights.—(II) (2 mg.-mols.), (IV) (1 mg.-mol.), and sodium carbonate (0·07 mg.-mol.) in moist benzene (7 c.c.) during 6 days gave a product of mol. wt. 25,500.

(I) (5 mg.-mols.), (II) (5 mg.-mols.), and sodium carbonate (0·05 mg.-mol.) in moist benzene (30 c.c.) during 6 days gave a product of mol. wt. 43,500.

(I) (5·0 mg.-mols.) (II) (5·0 mg.-mols.) and sodium phenylalaninate (0·08 mg.-mol.) in dry benzene (30 c.c.) gave a product of mol. wt. 15,800 (theor., based on the amount of initiator, 16,250).

Mol. wts. were determined *via* the content of end-carboxyl groups in portions of film cast from the solution. The film was dyed to saturation point by methylene-blue 2B, and the amount of dye taken up estimated by dissolving the washed film in *o*-chlorophenol and determining the amount of dye colorimetrically.

Products of still higher mol. wt. were prepared but the exact values could not be determined because the films had become insoluble in solvents such as *o*-chlorophenol, so that the small amounts of dye taken up could not be estimated.

After (I) (5 mg.-mols.), (II) (5 mg.-mols.), and sodium phenylalanine (0·01 mg.-mol.) had been kept in anhydrous benzene (30 c.c.) for 16 days, the viscosity was too high for measurement. Cast films could be cold-drawn to the extent of 50%. The mol. wt. calculated on the proportion of initiator used was 130,000.

High-temperature Polymerisations.—(I) (20 mg.-mols.), (II) (20 mg.-mols.), and sodium phenylalanine (0·027 mg.-mol.) in anhydrous benzene (60 c.c.) were heated on the steam-bath for 100 hours. (After 50 hours a sample gave good films.) As the reaction proceeded, about half of the polymer was precipitated. The precipitate dissolved slowly on the addition of more benzene, and differed in no way from the dissolved polymer. In this experiment the whole of the polymer remained colourless, but on repetition under apparently identical conditions the final product whilst of about the same viscosity was faintly yellow, probably owing to slow decomposition of the polypeptide by slight overheating. When the steam-bath was replaced by a water-bath at 85—90° no discoloration was observed.

Attempts to co-polymerise monomers (II) and (IV) at high temperatures failed.

Polymerisation at 100° *without a Solvent.*—A 2 : 1 (mol.) mixture of (II) and (IV) was heated for 1 hour at 100° (*a*) with and (*b*) without sodium carbonate. In both cases carbon dioxide was evolved and a yellow colour developed (in less than five minutes if sodium carbonate was present). The product from (*a*) was insoluble in benzene, that from (*b*) soluble (to give fluid solutions). Polymerisation had thus

proceeded further in the presence of sodium carbonate. These experiments demonstrate the limitations of polymerisation in the absence of a solvent, *i.e.*, in the fused state. Discoloration, indicating decomposition, occurs in the neighbourhood of the melting point.

Properties of the Films.—An outstanding property of the films of molecular weight greater than 40,000 was that when freshly prepared they could be extended at room temperature by 100% without breakage, as contrasted with previous films for which the limit was <10%. Within a few hours they would draw only to the extent of 20% (this value then remained constant), probably owing to gradual loss of solvent. When the films were examined by *X*-rays with the beam parallel to the surface, an oriented diffraction pattern was obtained which was strikingly similar to those given by α-proteins. The solubilities were the same as those of previous films. A few days after preparation the films became insoluble in benzene and in thiophan 1 : 1-dioxide.

The polymers were unchanged after being heated with 8N-sulphuric acid or 5N-sodium hydroxide for 12 hours at 130°. Fibres from both co-polymers gave good *X*-ray photographs with an α-keratin pattern having a meridional spacing of 5·22 A. The equatorial spacing, however, differed from that of the films in that the 11·7-A. (side-chain spacing) instead of the 10·4-A. spacing was oriented (Brown *et al.*, *loc. cit.*).

Preparation of Water-soluble Polypeptides.—Polymers of sarcosine or α-amino*iso*butyric acid were prepared by leaving the oxazolid-diones in moist dioxan for 5 days. The precipitates obtained were readily soluble in water.

*Poly-*DL-*alanine.* (VIII) did not polymerise in solution at room temperature, but an almost colourless polymer, readily soluble in water, was obtained after 30 minutes' heating to 100°.

Poly-(L-*glutamic acid*). (V) was polymerised for 2 days in moist dioxan at room temperature, to a clear transparent gel. Dried in a vacuum-desiccator over paraffin wax this gave poly(γ-ethyl L-glutamate) as a colourless powder; the polymer was insoluble in water but, when it was gently warmed with sufficient N-sodium hydroxide to remove the ethyl groups and form the sodium salt, an aqueous solution giving a strong biuret reaction resulted. On acidification, the bulk was precipitated as poly-(glutamic acid). It was washed with water, alcohol, and ether and finally dried in a vacuum-desiccator over concentrated sulphuric acid.

γ-Ethyl L-*glutamate–*α-*amino*iso*butyric acid co-polymer.* Equimolecular amounts of (IV) and (V) co-polymerised in moist dioxan to give an extremely clear and viscous solution.

L-*Lysine–*DL-*valine co-polymer.* (IX) (0·143 g.) and (VII) (0·31 g.) were co-polymerised for 20 hours in moist chloroform (10 c.c.). The product was dried *in vacuo*, suspended in acetic acid (20 c.c.) at 50° and reduced by bubbling in hydrogen and adding phosphonium iodide (4 × 0·5 g.) at 30-minutes' intervals. The solution was left overnight and then decanted from the precipitate, which after being washed with ether was dissolved in water (1·0 c.c.) and precipitated by addition of alcohol (2·5 c.c.) and ether (35·0 c.c.). The product (0·11 g.) was freely soluble in water and gave a strong biuret test.

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