

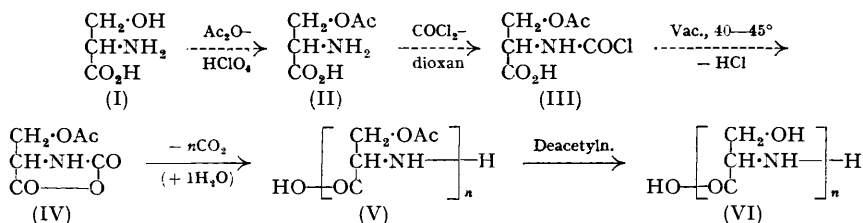
405. Synthesis of Poly-(*O*-acetyl-DL-serine) and of Poly-DL-serine.

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O-Acetyl-*N*-carboxy-DL-serine anhydride (IV) has been prepared by direct action of carbonyl chloride on *O*-acetyl-DL-serine. Polymerisation of the anhydride by heat in a high vacuum, or at room temperature in solution and in the presence of initiators, gives poly-(*O*-acetyl-DL-serine) (V), and by subsequent treatment with ammonia poly-DL-serine (VI). The average chain length of the polymers described varied from 10 to 45 units.

THE preparation of the *N*-carboxy-anhydrides of *O*-acetyl- and *O*-carbobenzyloxy-DL-serine, through *O*-acetyl-*N*-carbobenzyloxy- and *ON*-dicarbobenzyloxy-DL-serine respectively, was described by Frankel and Halman (*J.*, 1952, 2735). With the aid of these anhydrides the synthesis of poly-(*O*-acetyl-DL-serine) and poly-DL-serine is possible in principle, but some of the features of the preparative procedure made a different route desirable (cf. Frankel, Breuer, and Cordova, *Experientia*, 1952, **8**, 299; Breuer, Thesis, Hebrew University, Jerusalem, 1950—51).

Frankel and Halman's preparation of *O*-acetyl-*N*-carboxy-DL-serine anhydride involves isolation of *N*-carbobenzyloxy-DL-serine, its acetylation, chlorination, and subsequent ring closure. This procedure can be greatly simplified. Acetylation of only the hydroxyl group of DL-serine (I) can be realized by using perchloric acid and acetic anhydride in glacial acetic acid (cf. Sakami and Toennies, *J. Biol. Chem.*, 1942, **144**, 203), which leads to *O*-acetyl-DL-serine (II). By direct action of carbonyl chloride on a suspension of (II) in dioxan under the conditions described in the Experimental section, *O*-acetyl-*N*-carboxy-DL-serine anhydride (IV) was obtained directly. The intermediate *N*-substituted chloride (III) is decomposed during the treatment, with loss of hydrogen chloride. This procedure is an extension to a polyfunctional α -amino-acid of the findings of previous authors (Fuchs, *Ber.*, 1922, **55**, 2943; Levy, *Nature*, 1950, **165**, 152; Farthing and Reynolds, *ibid.*, p. 647, who cite patent literature; Farthing, *J.*, 1950, 3213) who have shown that anhydro-*N*-carboxy-derivatives of the type (IV) can be obtained directly by the action of carbonyl chloride on certain simple bifunctional α -amino-acids.



Polymerisation of the anhydride (IV) was effected by heat in a high vacuum, near the decomposition temperature, as in previous cases (cf. Frankel and Berger *J. Org. Chem.*, 1951, **16**, 1513 for earlier references), or in solution. Dioxan, nitrobenzene, or pyridine served as solvents; in the first case, at room temperature, water or sodium hydroxide was used as initiator (Coleman and Farthing, *J.*, 1950, 3218; Coleman, *ibid.*, p. 3222), or the dioxan solutions were heated. With nitrobenzene as solvent, glycine dimethylamide was the initiator (Hanby, Waley, and Watson, *J.*, 1950, 3009; Coleman, *loc. cit.*).

Poly-(*O*-acetyl-DL-serine) (V) thus obtained was soluble in hot glacial acetic acid and precipitated from solution on addition of dry ether. The biuret reaction was strongly and the ninhydrin reaction weakly positive. The average chain length varied, depending on the conditions of the polymerisation, from about 10 to about 45 units, as deduced from analytical data, including amino-nitrogen values determined by van Slyke's manometric method.

Complete removal of the acetyl groups from the poly-(*O*-acetyl-DL-serine) without hydrolysis of peptide bonds presented difficulties. On the one hand, the quantitative

removal of the acetyl group was not easily accomplished and, on the other, it became apparent in our experiments, that the peptide bonds in the poly-DL-serine preparations are very readily hydrolysed even under mild conditions. Thus, *e.g.*, an aqueous solution of poly-DL-serine which was brought to pH 8—9 by addition of sodium hydroxide and then kept at 30° for 2 hr., showed an increase in amino-nitrogen content from 0.59 to 1.75%, corresponding to a degradation from an average chain length of 27 to one of 9 units. After a further hour, the amino-nitrogen content was 2%. Similar observations were frequently made in our experiments, and are in agreement with recent findings that a peptide linkage adjacent to a hydroxyl group undergoes hydrolysis in acid or alkaline medium much more easily than in absence of such hydroxyl groups (Desnuelle and Casal, *Biochem. Biophys. Acta*, 1948, 2, 64; *Chem. Abs.*, 1949, 43, 2943; Sanger, *Adv. Protein Chem.*, 1952, 7, 18—24). Removal of the acetyl groups from poly-(*O*-acetyl-DL-serine) was attempted with barium or lithium hydroxide, with alkoxide solutions, and with ammonia. Finally, concentrated aqueous ammonia was found completely effective, practically without hydrolysis of the peptide bonds. The acetamide and ammonium acetate formed were removed by extraction with alcohol, followed by vacuum-sublimation.

Poly-DL-serine is hygroscopic, easily soluble in water, and gives strong ninhydrin and biuret reactions. From its aqueous solutions, on evaporation, hard films are obtained.

Hydrolysis of poly-(*O*-acetyl-DL-serine) and of poly-DL-serine by boiling hydrochloric acid (18 hr.) was practically quantitative, yielding DL-serine. In the former case the product sometimes contained *O*-acetyl-DL-serine which was detected by paper chromatography.

Infra-red absorption spectra of poly-DL-serine and of poly-(*O*-acetyl-DL-serine) were kindly measured out by Dr. Sadtler and are consistent with the constitutions assigned. They will appear in Sadtler's "Catalog of Infra-red Spectrograms," Philadelphia.

EXPERIMENTAL

M. p.s were determined in a Fisher-Johns apparatus.

DL-Serine (Merck, Rahway, U.S.A.), used in these experiments, showed m. p. 228° (decomp.) (Found: amino-N, 13.2. Calc. for C₃H₇O₃N: amino-N, 13.3%).

O-Acetyl-DL-serine (cf. Sakami and Toennies *loc. cit.*).—The acetylation mixture was prepared from perchloric acid (60.4%; 4.99 g.) and acetic anhydride (98.7%; 2.52 g.), made up to 50 ml. with acetic acid (100%; prepared from glacial acetic acid and acetic anhydride). To a solution of DL-serine (2.5 g.) in this mixture, at 0°, acetic anhydride (98.7%, 12 g.) was added. After 1½ hr. water (1 ml.) and then amylamine (3.48 g.) were added. *O*-Acetyl-DL-serine (3.1 g., 91%) was precipitated by ether (300 ml.). It was dried for 2 days in a vacuum-desiccator over solid sodium hydroxide and had m. p. 144° (decomp.) [Sakami and Toennies, *loc. cit.*, give m. p. 143—144° (decomp.)].

O-Acetyl-N-carboxy-DL-serine Anhydride.—A stream of dry carbonyl chloride was passed through a suspension of finely ground *O*-acetyl-DL-serine (7.5 g.) in dried dioxan (150 ml.), with exclusion of water, in a ground-glass apparatus, under a reflux condenser, and with stirring, at 40—48° (bath). Within 1½ hr. the *O*-acetyl-DL-serine dissolved entirely. Passage of carbonyl chloride was continued for a further ½ hr.; the solution was then filtered, excess of carbonyl chloride removed *in vacuo* at room temperature, and the dioxan distilled off at the water-pump at 40—45° (bath). The remaining oil was left overnight in a vacuum-desiccator over phosphoric oxide. It was used directly for the following polymerisation reactions.

Polymerisation Experiments.—Dioxan was dried over sodium. Where necessary, moisture was excluded by calcium chloride.

(a) *Polymerisation in a high vacuum.* *O*-Acetyl-N-carboxy-DL-serine anhydride (1.5 g.) was heated gradually at an initial pressure of 0.04 mm. Within 1 hr., the bath temperature reaching 50°, a slight evolution of gas occurred, causing a rise in pressure to 1 mm. The temperature was gradually raised as gas evolution ceased; after another 45 min., at 86°, a strong evolution of gas began which lasted for 1 hr., the temperature reaching 108°. At this point the substance hardened at once. The pressure, which during the strong evolution of gas increased considerably, dropped to the initial value, and heating to 125° caused no visible change. The substance was kept overnight in the high-vacuum system. It was soluble in cold dichloroacetic acid and formamide, and only slightly in hot glacial acetic acid. It was dissolved in formamide,

precipitated by absolute alcohol, and washed three times with absolute alcohol, being separated each time by centrifugation.

Poly-(O-acetyl-DL-serine) thus obtained (700 mg.) was yellowish, slightly soluble in hot glacial acetic acid, giving a strong biuret and a weak ninhydrin reaction. The average chain length was 45 units [Found: C, 45.9; H, 5.5; N, 10.4; amino-N, 0.24. $(C_5H_7O_3N)_{45}, H_2O$ requires C, 46.4; H, 5.4; N, 10.8; amino-N, 0.24%].

(b) *Polymerisation in solution.* With dioxan as solvent small amounts of water or sodium hydroxide solution were used as initiators at room temperature, or, alternatively, the solution was heated without initiator. Precipitates of poly-(O-acetyl-DL-serine) were filtered off and submitted to ether-extraction (Soxhlet) or first dissolved in hot glacial acetic acid, reprecipitated by ether, and freed from acetic acid by ether-extraction (Soxhlet). Glacial acetic acid was also used to fractionate some of the polymer preparations (cf. Table).

In the case of nitrobenzene, glycine dimethylamide was the initiator, and the polymer was precipitated by absolute alcohol.

When pyridine was used, water present in traces might have acted as initiator. In this case the polymerisation led to gel formation. Glacial acetic acid was added and the polymer precipitated by dry ether.

The poly-(O-acetyl-DL-serine) preparations thus obtained constituted white, in some instances (when obtained by heat-polymerisation) yellowish to brown, substances, with a tendency to form films from solution; they gave weak ninhydrin and strong biuret reactions. As in other cases with polymeric polypeptides (cf., e.g., Hanby, Waley, and Watson, *J.*, 1950, 3009), C and H determinations were not always satisfactory.

The polymerisation experiments in solution are summarised in the Table.

Expt.	Anhydride (g.)	Solvent (ml.)	Mode of polymern.	Time of polymern. (days)	Average degree of polymern. (<i>n</i>)
1	4.5	Dioxan (45)	One drop of water as initiator at room temp.*	10	9
2	11.4	Dioxan (60)	Three drops of 0.5% sodium hydroxide as initiator at room temp. ^b	15	17 35 ^c
3	3.1	Dioxan (20)	Heat on water-bath under reflux	35 hr.	35
4	2.0	Pyridine (6)	Room temp.	2	17
5	3.0	PhNO ₂ (20)	0.01 ml. of glycine dimethylamide as initiator	14	? ^d

Expt.	Found (%):				Reqd. for $(C_5H_7O_3N)_n, H_2O$:			
	C	H	Total N	Amino-N	C	H	Total N	Amino-N
1	43.5	6.3	10.6	1.2	45.8	5.5	10.7	1.18
2	—	—	9.1	0.63	—	—	10.8	0.63
	43.9	5.3	10.0	0.31	46.3	5.4	10.8	0.31
3	—	—	10.2	0.31	—	—	10.8	0.31
4	—	—	10.1	0.64	—	—	10.8	0.63
5	44.3	5.6	10.0	—	46.5	5.4	10.8	—

* The small amount of ppt. formed on addition of water was removed by centrifugation.

^b One drop of the sodium hydroxide solution was added in the first instance. Since for 14 days no precipitate was formed, two additional drops were added.

^c By dissolving the initial ppt. in hot glacial acetic acid and reprecipitating it by ether, a polymer having an average degree of polymerisation $n = 35$ was obtained.

^d This polymer was not soluble in any suitable solvent and amino-nitrogen analyses could therefore not be carried out.

Deacetylation of Poly-(O-Acetyl-DL-serine) by Ammonium Hydroxide Solution.—Poly-(O-acetyl-DL-serine) ($n = 35$; 0.5 g.) was added to a solution of aqueous ammonia (28%; 5 ml.). Within 2 hr. a clear solution was obtained which was left at room temperature for 3 days. The ammonia was then removed in a stream of air at room temperature, and then the water entirely evaporated in a vacuum-desiccator over concentrated sulphuric acid. In order to remove quantitatively acetamide and the ammonium acetate formed, the remaining residue was extracted with hot alcohol (Soxhlet) and then sublimed at 75°/3—5 mm. The residual poly-DL-serine constituted a white hygroscopic substance, soluble in water, acetic acid, or dilute aqueous alkali. The last solvent causes considerable hydrolysis of the peptide linkages even in the cold. The average degree of polymerisation was 35 [Found: C, 40.7; H, 6.3; N, 15.8; amino-N, 0.45. $(C_3H_5O_2N)_{35}, H_2O$ requires C, 41.1; H, 5.8; N, 16.0; amino-N, 0.45%].

Complete Hydrolysis of Poly-(O-acetyl-DL-serine) and of Poly-DL-serine.—Solutions of poly-(O-acetyl-DL-serine) (obtained by polymerisation in dioxan with sodium hydroxide as initiator; $n = 35$; 55.3 mg.), of poly-(O-acetyl-DL-serine) (obtained by polymerisation in hot dioxan; $n = 35$; 57.0 mg.), and of poly-DL-serine ($n = 27$; 43.6 mg.) in hydrochloric acid (17.5%:

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each in 4 ml.) were refluxed for 18 hr., then neutralised by lithium hydroxide (*ca.* 5N) (methyl-orange) and brought to 25 ml. with water. In aliquot parts of the solutions total and amino-nitrogen were determined (they were practically identical and indicated complete hydrolysis). Parts of the solutions were evaporated to dryness *in vacuo* and the remaining solids extracted by ether or acetone, to remove lithium chloride. The residue which still gave lithium- and chloride-ion reactions was submitted to short-path distillation. In the sublimate, DL-serine was identified by the m. p. and mixed m. p.

The above hydrolysates were submitted to paper partition chromatography, along with the DL-serine, O-acetyl-DL-serine, and poly-DL-serine, by the ascending method with phenol-water as mobile phase. On development with ninhydrin, the three hydrolysates yielded spots identical with that found for the DL-serine. The second hydrolysate gave an additional spot identical with that given by O-acetyl-DL-serine. Poly-DL-serine yielded no spot. Poly-(O-acetyl-DL-serine) could not be tested owing to its insolubility in water.

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