[Contribution from the Laboratory of High Molecular Chemistry and the Department of Organic Chemistry, the Hebrew University]

SYNTHESIS OF POLYASPARTIC ACID

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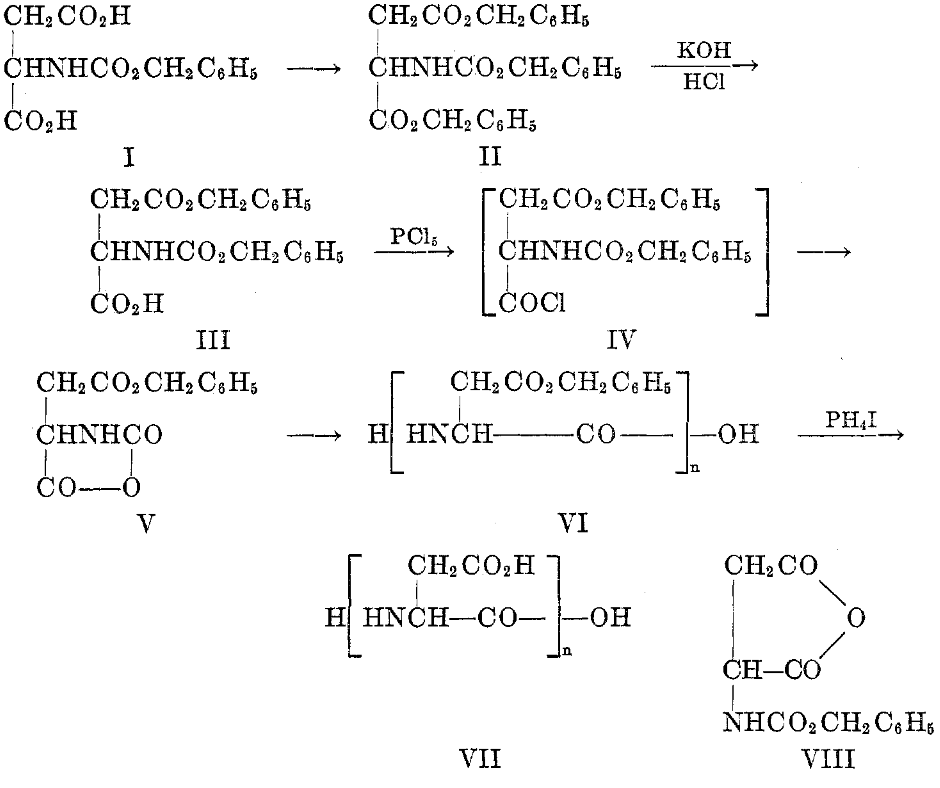
In continuation of previous work on the polymerization of representative normal and basic amino acids [glycine (1); DL-alanine (2); DL-serine (3); L-lysine (3, 4)], the present paper describes the synthesis of polyaspartic acid as an example of the polymerization of a trifunctional acidic acid [*c.f.* Frankel and Berger (5)]. In the meantime the preparation of polyglutamic acid was reported [Hanby, Waley, and Watson (6)].

Out of several ways tried (5) the following, as indicated by formulae I–VII,



will be described in detail. N-carbobenzyloxy-L-aspartic acid (I) (7) was converted into its disilver salt which on reaction with benzyl iodide in ether yielded dibenzyl N-carbobenzyloxy-L-aspartate (II). Partial hydrolysis of II, affecting the benzyl group attached to the α-carboxyl group only, was accomplished by the calculated amount of potassium hydroxide in benzyl alcohol. The potassium salt of β-benzyl N-carbobenzyloxy-L-aspartate rendered, on acidification of its aqueous solution, the monoester, N-carbobenzyloxyaspartic acid β-benzyl ester (III). It differs from the corresponding α-benzyl ester which has been prepared by Max Bergmann (8) by its melting point (101–103° as against 84–85°). The fact that only the ester group in the α-position is hydrolyzed in our procedure is in accordance with the findings of Pauli and Weir (9) who obtained N-benzoylaspartic acid β-methyl ester on partial hydrolysis by alkali of dimethyl N-benzoylaspartate: we found that the influence of the carbobenzyloxy group on the acidic dissociation constant of glycine is similar to that of the benzoyl group.

Under the experimental conditions described later, III yielded with phosphorus pentachloride the N-carboxy anhydride of aspartic acid β-benzyl ester (V). The acid chloride (IV), the intermediate formation of which was to be expected, was, as in many other cases, also here not isolated. The results of elementary analysis are consistent with formula V, but do not exclude the isomeric formula (VIII), the N-carbobenzyloxyaspartic acid anhydride (7, 8).

The decision in favor of V can be made on consideration of the general behavior and the properties of the substance in question. It shows the general features of an N-carboxy amino acid anhydride, a so-called "Leuchs Anhydride": on treatment with water, acids, or on heating, it decomposes with the evolution of carbon dioxide. It gives a positive ninhydrin reaction and treatment with dilute acids under mild conditions leads to a product having a considerable portion of its nitrogen in the form of free amino nitrogen. Under such mild conditions the carbobenzyloxy group attached to nitrogen which formula VIII postulates is not split off at all or to a negligible extent only.

As mentioned above the substance develops carbon dioxide on heating. The loss in weight which it suffers is in agreement with the value calculated on the assumption that one molecule of carbon dioxide is evolved from each molecule of the anhydride (V). On heating the substance in question a polymer is formed whose elementary composition conforms to that to be assumed if it is derived from a monomer having the constitution V. In agreement with formula V are also the properties of the polymer obtained from it, *e.g.* its acidic character and its positive biuret reaction. Finally, the substance has a different melting point (111°) from that given for VIII in the literature (8) (124°) and an admixture of it with a sample of N-carbobenzyloxyaspartic acid anhydride (VIII) (8), gives a definite depression.

On the basis of all these facts formula VIII can safely be excluded and formula V accepted.

As in our previous work, the polymerization was carried out by heating the pure, vacuum-dried N-carboxy anhydride of aspartic acid β-benzyl ester (V) in a high vacuum under the experimental conditions described later. The polyaspartic acid β-benzyl ester (VI) thus obtained gives a positive biuret reaction. Determinations of the free amino nitrogen end group were carried out in two different preparations of this polymer to obtain an indication of the average degree of polymerization. As will be shown in the experimental part, the determinations in each polymeric preparation were reproducible within 10% of the value and the average degree of polymerization was about 100, corresponding to an average molecular weight of about 20,000.

Removal of the benzyl groups from the polyester (VI) was satisfactorily effected by reduction with phosphonium iodide in glacial acetic acid [*cf.*, (4, 6b)]. The free polyaspartic acid (VII) was obtained as a white powder with a positive biuret reaction, slightly soluble in hot water, readily soluble in dilute alkali.

Molecular weight estimations of the polyaspartic acid were again based on amino nitrogen determinations using the Van Slyke manometric apparatus. Different preparations of polyaspartic acid showed chain lengths from about 35 to about 80 (*cf.*, Table I).

In the course of our experiments we had the opportunity to study the influence of normal alkali at room temperature and at 40° on the polyaspartic acid β-benzyl ester. It appeared that the ester groups are hydrolyzed by the alkali to a considerable extent, whilst the peptide linkages are not affected.

The starting material for the experiments reported here was L-aspartic acid. The various stages of the synthesis were not followed by polarimetric measurements. It was found, however, that the polyaspartic acid obtained through hydrogenolysis with phosphonium iodide does show optical activity. Thus it can be said that under the conditions set out in the experimental part, racemization, if any, is not complete.

EXPERIMENTAL[1]

Dibenzyl N-carbobenzyloxy-L-aspartate. N-Carbobenzyloxy-L-aspartic acid (7) (7.3 g.) was dissolved in 2 *N* aqueous sodium hydroxide (30 ml.). The solution was carefully neutralized to Phenolphthalein and 2 *N* silver nitrate (21 ml.) was added with vigorous stirring to break up the lumps. After standing for 30 min. in a dark place, the silver salt was collected, washed with ice-water, ethanol, and ether, and dried at 60°; yield, almost theoretical.

Anal. Calc'd for $C_{12}H_{11}Ag_2NO_6$: Ag, 44.86; Found: Ag, 44.6.

To this silver salt (11.3 g.) a solution of benzyl iodide (12.5 g.) in anhydrous ether (25 ml.) was added in small portions so as to control the reaction from becoming too vigorous. After the reaction mixture had cooled to room temperature, it was shaken from time to time during two hours. The ether was then evaporated, and the residue heated to 90° for 30 minutes. It was then thoroughly extracted with ether and the filtered solution concentrated to a syrup (hood); the syrup was then triturated with petroleum ether to remove most of the excess of benzyl iodide. The residue was dissolved in ether (5 ml.) and precipitated with petroleum ether (200 ml.). The light yellow oil thus obtained was dissolved in ether (10 ml.) and saturated aqueous sodium bicarbonate (10 ml.) was added whereupon part of the diester crystallized in the ether layer. The ethereal solution and crystals were washed once with water and petroleum ether (200 ml.) was added to complete crystallization. The diester was collected by suction and washed with petroleum ether and dried *in vacuo*. Yield, 5 g. (49%); m.p. 64° (sintering at 56°).

Anal. Calc'd for $C_{26}H_{25}NO_6$: C, 69.8; H, 5.6; N, 3.1.
Found: C, 70.4; H, 5.6; N, 3.1.

N-Carbobenzyloxy-L-aspartic acid β-benzyl ester. Procedure (a): Dibenzyl N-carbobenzyloxy-L-aspartate (5 g.) was added to a solution of potassium hydroxide (0.62 g.) in benzyl alcohol (9 ml.). The ester dissolved. After keeping for 30 minutes at room temperature the mixture was heated for 10 minutes on a water-bath. On addition of 1 liter of anhydrous ether the potassium salt of the monoester was precipitated as a white soft mass. The potassium salt solidified over phosphorus pentoxide to an extremely hygroscopic

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[1] All melting points are uncorrected.

brittle solid; very soluble in water, soluble in ethanol, and insoluble in ether. Yield 2.8 g. (64%).

Anal. Calc'd for $C_{19}H_{18}KNO_6$: K, 9.9. Found: K, 10.1.

The salt was dissolved in water (30 ml.) and the solution acidified to Congo Red with 2 *N* hydrochloric acid. The acid was precipitated in soft white flakes which soon became crystalline. The acid was filtered, washed with water, and dried over phosphorus pentoxide and potassium hydroxide; m.p. 96–101°. After solution in aqueous sodium bicarbonate and precipitation with hydrochloric acid the melting point was 101–103° with slight sintering at 99°. The melting point was not changed by precipitation of the acid with petroleum ether from an ethereal solution. Yield: 2 g. (78% on the potassium salt, over-all yield calculated on the dibenzyl ester, 50%).

Anal. Calc'd for $C_{19}H_{19}NO_6$: C, 63.8; H, 5.3; N, 3.9; Neut. equiv., 357.
Found: C, 63.3; H, 5.3; N, 4.0; Neut. equiv., 365.

Procedure (b): Dibenzyl N-carbobenzyloxy-L-aspartate (2.0 g.) was added to a solution of potassium hydroxide (0.25 g.) in benzyl alcohol (10 ml.). After standing overnight the benzyl alcohol was distilled off at 90°/0.01 mm. The residue was washed repeatedly with anhydrous ether and dissolved in 10 ml. of water. The aqueous solution was extracted twice with ether and the aqueous layer acidified with 2 *N* hydrochloric acid to Congo Red. The precipitated acid was filtered, washed with water, and dried *in vacuo* over sulfuric acid; m.p. 103°, yield 1.0 g. (65%).

N-Carboxy anhydride of aspartic acid β-benzyl ester. N-Carbobenzyloxy aspartic acid β-benzyl ester (2.8 g.) was dissolved in anhydrous ether (30 ml.) and cooled in an ice-bath. Phosphorus pentachloride (1.70 g.) was added in one portion and the mixture shaken for 15 minutes with ice-cooling and a further 30 minutes at room temperature. After filtration the ether was evaporated *in vacuo* and petroleum ether was added to the residue. The oil obtained was washed with a fresh portion of petroleum ether; it solidified on rubbing, m.p. 105° (decomp.). The anhydride was sparingly soluble in ether. By precipitation with petroleum ether from its ethereal solution material of m.p. 112° was obtained. Yield, 1.6 g. (82%). The melting point did not change on standing for two months over phosphorus pentoxide.

Anal. Calc'd for $C_{12}H_{11}NO_5$: C, 57.8; H, 4.4; N, 5.6.
Found: C, 57.6; H, 4.5; N, 5.6.

Loss of weight on heating to 130° for 4 hours, 17.5%.

Calc'd for one mole of CO_2, 17.7%.

Polyaspartic acid β-benzyl ester. Dry and pure N-carboxy anhydride of aspartic acid β-benzyl ester was introduced into a high vacuum apparatus equipped with a liquid air trap. (The reaction vessel should have a volume of at least 100 ml. for 10 g. of the anhydride). The monomer was heated to 60° at 1 μ for 3 hours. The temperature was then raised. At 80° the pressure was about 15 μ, and could not be lowered by continuous pumping. At 110° the monomer melted and evolution of carbon dioxide became vigorous. The pressure rose to 0.7 mm. and fell off gradually during 4 hours. During the last two hours the temperature was kept at 125°. On cooling the polyester was obtained as a transparent brittle mass, which could be ground to a white powder. It was found that repeated recrystallization of the N-carboxy anhydride of aspartic acid β-benzyl ester did not increase the size of the polymer obtained from it.

Polyaspartic acid β-benzyl ester is soluble in hot glacial acetic acid and hot dimethylformamide; less soluble in hot methanol and acetone; and insoluble in water and ether.

Anal. Calc'd for $(C_{11}H_{11}NO_3)_n \cdot H_2O$, $n = 100$: C, 64.3; H, 5.4; N, 6.8.
Found: C, 64.4; H, 5.4; N, 6.8.

The polyester is soluble in 30% sodium hydroxide and gives a positive biuret reaction.

Molecular weight estimations: α-amino nitrogen determinations were carried out in a Van Slyke manometric apparatus on the polyester dissolved at 50° in glacial acetic acid. On introducing these solutions into the reaction chamber of the apparatus a precipitate formed. Nevertheless the values for a given preparation of the polymer could be reproduced within 10% in independent determinations. Moreover, if weighed amounts of the polyester were kept in 1 *N* aqueous sodium hydroxide at room temperature for 45 minutes, clear solutions were obtained which were used for amino nitrogen determinations. These solutions, on introduction into the reaction chamber of the Van Slyke apparatus, did not form any precipitates; the values obtained with them were in agreement with those obtained with the original ester preparations. It appears therefore that the precipitates formed on using the benzyl ester do not interfere to a considerable degree with the amino nitrogen determination.

Identity (within experimental error) of the values before and after treatment of the polyester with normal aqueous alkali indicate that under such conditions only ester hydrolysis occurs and that the peptide linkages are not affected. This finding is in agreement with the results of Hanby, Waley, and Watson (6a, 6b) who hydrolyzed the methyl ester of polyglutamic acid by treatment with dilute alkali. Although no attempt was made by us to determine quantitatively the extent of ester hydrolysis, it appears that saponification occurred to a considerable extent as the product obtained is water-soluble and a characteristic copper salt was obtained on adding aqueous copper sulfate to its solution.

Polyaspartic acid. Polyaspartic acid β-benzyl ester (4 g.) was dissolved with heating in glacial acetic acid (30 ml.) and a stream of dry hydrogen was passed through the solution which was kept at 55°. Phosphonium iodide (16 g.) was added in four portions at one-hour intervals. The mixture was agitated with a magnetic stirrer. The stream of hydrogen was continued for three hours after the last addition, the temperature being kept between 50° and 60°. The polyaspartic acid separated as a white powder. On addition of ether (100 ml.) a further small quantity of material was precipitated. The solid was washed several times with ether by decantation and then collected and dried by suction. The polyacid was obtained as a white powder, which, however, turned yellow on standing. On boiling in water for a few minutes a material was obtained which remains colorless even when exposed to light and air. Biuret reaction, positive; ninhydrin, negative. It was slightly soluble in hot water, soluble in dilute alkali.

TABLE I

AVERAGE MOLECULAR WEIGHT AND AVERAGE DEGREE OF POLYMERIZATION CALCULATED FROM AMINO NITROGEN FOUND IN VARIOUS POLYMER PREPARATIONS

PREPARATIONS OF	AMINO NITROGEN Found, %	AVERAGE MOL. WEIGHT (about)	AVERAGE DEGREE OF POLYMERIZATION (about)
Polyaspartic acid β-benzyl ester	0.070	20,000	98
Polyaspartic acid	.075	18,600	91
	.20	7,000	61
	.17	8,200	72
	.35	4,000	35
	.15	9,300	81

Anal. Calc'd for $(C_4H_5NO_3)n \cdot H_2O$, n = 80: C, 41.6; H, 4.3; N, 12.2; Neut. equiv., 115.
Found: C, 40.8; H, 4.3; N, 11.9; Neut. equiv., 125.

$[\alpha]_D^{20}$ +6.2° in the presence of one equivalent sodium hydroxide; (*c*, 3%).

Polyaspartic acid is hydrolyzed by 6 *N* hydrochloric acid and yields aspartic acid quantitatively.

For all the experiments presented in this paper L-aspartic acid (Hoffman-La Roche, Basle) was used.

Anal. Calc'd for $C_4H_7NO_4$: N, 10.5. Found: 10.4.

$[\alpha]_D$ in 6 *N* HCl, +24.5°.

No attempt was made to follow in each phase of the synthesis of the polymer the optical activity, or to check the optical purity of intermediates.

Molecular weight estimations: α-amino nitrogen determinations were carried out on a number of different preparations as in the case of the polyaspartic acid β-benzylester. No precipitates were formed during the analysis and the reproducibility of the values for each polymer was about 3%. The results are summarized in Table I.

In the course of the present investigation the following two additional compounds were prepared:

N-Carbobenzyloxyisoasparagine benzyl ester. N-Carbobenzyloxyisoasparagine (7) was treated with aqueous silver nitrate. The silver salt obtained (0.8 g.) was suspended in anhydrous ether (11 ml.) and benzyl iodide (0.5 g.) was added. The reaction mixture was filtered after 3 hours and the ethereal solution washed with aqueous sodium bicarbonate and water, and dried over sodium sulfate. On addition of petroleum ether an oil was precipitated which crystallized on trituration with petroleum ether. It was recrystallized from ethyl acetate-petroleum ether, m.p. 84°.

Anal. Calc'd for $C_{19}H_{20}N_2O_5$: C, 64.0; H, 5.6; N, 7.9.
Found: C, 63.6; H, 5.6; N, 7.9.

N-Carbobenzyloxy-L-aspartic acid hydrazide-hydrazonium salt.

N-Carbobenzyloxy-L-aspartic acid anhydride (7, 8) was treated with an excess of hydrazine hydrate at 0°. After 10 minutes all the anhydride had dissolved. The solution was evaporated to dryness *in vacuo* over sulfuric acid. The resulting oil was crystallized from absolute ethanol on addition of anhydrous ether. The hydrazonium salt was obtained as a white hygroscopic powder very soluble in water, soluble in ethanol.

Anal. Calc'd for $C_{12}H_{19}N_5O_5$: C, 46.0; H, 6.1; N, 22.4.
Found: C, 45.5; H, 6.2; N, 22.1.

The free N-carbobenzyloxy hydrazide acid was not precipitated from the aqueous solution of this salt on adding hydrochloric acid. Similar observations were made in the case of the preparation of the corresponding glutamic acid derivative (10). This glutamic acid derivative has also been described in a recent paper (11) and turned out to be a mixture of the α- and the γ-hydrazides.

SUMMARY

Dibenzyl N-carbobenzyloxy-L-aspartate prepared from the disilver salt of N-carbobenzyloxy-L-aspartic acid and benzyl iodide yielded on partial hydrolysis N-carbobenzyloxy-L-aspartic acid β-benzyl ester. This with phosphorus pentachloride gave the N-carboxy anhydride of aspartic acid β-benzyl ester which on polymerization in a high vacuum yielded polyaspartic acid β-benzyl ester. On reduction with phosphonium iodide the latter gave polyaspartic acid. Chain length values ranging between 35 and 80 units were found.

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