of Brady's reagent. Evaporation of mother liquor under diminished pressure left a residue (238 mg.).

On being purified from diluted acetone, original *O*-dimethylsophorol (57 mg.) was recovered unchanged.

(b) *With palladized charcoal and cinnamic acid.* *O*-Dimethylsophorol (300 mg.) and 30% palladized charcoal[35] (150 mg.) were heated in cinnamic acid (0.7 g.) under stream of carbon dioxide for 2.75 hr. at about 190–200°. The melt having intense odor of β-phenylpropionic acid was dissolved in ether. The residue insoluble in ether, containing catalyst was dissolved in acetone. After the catalyst was filtered off the removal of acetone yielded 45 mg. of residual solid. Recrystallization from diluted acetone gave the dehydro-*O*-dimethylsophorol in pale pink-colored needles m.p. 201–203°, identical with a specimen from selenium dioxide oxidation. From orange-colored ether solution the solvent was distilled off. After washing of residual solid with aqueous-2*N*-caustic alkali and water successively, residue (250 mg.) (m.p. 70–130°) was purified from diluted acetone, giving the original *O*-dimethylsophorol (50 mg.).

The synthesis of DL-*7,2',4'-trimethoxyisoflavanone.* 7,2',4'-Trimethoxyisoflavone (200 mg.), Adams' platinum oxide (20 mg.), and glacial acetic acid (12 cc.) were shaken under hydrogen at 1 atm. for 56 min., 1.3 moles of hydrogen being absorbed. After removal of catalyst and acetic acid (*in vacuo*), DL-7,2',4'-trimethoxyisoflavanone was obtained as colorless needles, m.p. 128–130° (70 mg.) (from acetone-water).

Anal. Calcd. for $C_{18}H_{18}O_5$: C, 68.78; H, 5.77; $3OCH_3$, 29.6. Found: C, 68.20; H, 5.66; OCH_3, 29.0.

Ultraviolet absorptions; (Fig. 8) (alcohol) λ_{max} 229, 272, and 306 mμ (ε, 26800, 20000, and 10420).

The presence of a carbonyl group was confirmed by the infrared absorption spectrum, which showed a strong band at 1677 cm.$^{-1}$ in Nujol mull.

Anhydrosophorol (VIII). Sophorol (500 mg.) was heated under reflux with 4% sulfuric acid (50 ml.) for 2 hr. After the solution was cooled the deposited amorphous solid was collected by filtration, and recrystallized repeatedly from diluted ethanol (alcohol:water 3:2), giving anhydrosophorol (VIII) in colorless needles, m.p. 225–226° (decomp.), which darkened in a few days and gave a faint green ferric reaction in methanol. Original sophorol (200 mg.) was recovered unchanged from the filtrate.

Anal. Calcd. for $C_{16}H_{10}O_5$: C, 68.08; H, 3.57. Found: C, 67.89; H, 3.82.

Anhydrosophorol rapidly decolorizes potassium permanganate in acetone. This compound is easily soluble in acetone, dioxane, and soluble in alcohol, glacial acetic acid, ethyl acetate, while insoluble in water, chloroform, benzene, and light petroleum. It dissolves in hot aqueous 2*N*-sodium hydroxide but not in cold solution. Further, the compound reduces Tollens-reagent, and its warm alkaline solution develops red-orange color with diazobenzenesulfonic acid. The infrared spectrum showed a band at 3420 cm.$^{-1}$ (phenolic hydroxyl).

Durham test[36] *for O-dimethylsophorol.* When the crystals of *O*-dimethylsophorol were treated with a drop of concentrated nitric acid on a porcelain plate a red color developed, which quickly changed to green. The addition of a few drops of ammonia gave a violet color. On the other hand, when acetone solution of the compound was treated by Jones' method[37] a deep violet color was produced.

Acknowledgment. The author expresses his sincere thanks to Professors Toshi Irie, Takeshi Matsumoto, and Tadashi Masamune for their kind interest and encouragement in this work, and to Professor S. Fujise of Tohoku University, Professor A. Robertson and Dr. W. B. Whalley of Liverpool University for their generous gifts of authentic specimens.

The writer also is much indebted to Professor M. Hanzawa of Department of Forestry, Faculty of Agriculture, for his kind supplies of wood material.

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[CONTRIBUTION FROM THE CHEMISTRY DEPARTMENT AND OCEANOGRAPHIC INSTITUTE OF THE FLORIDA STATE UNIVERSITY]

Polycondensation of Thermal Precursors of Aspartic Acid[1]

KAORU HARADA

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Anhydropolyaspartic acid has been synthesized by heating unsubstituted aspartic acid. It is found that the anhydropolyaspartic acid may be prepared by heating monoammonium malate, maleamic acid, and combinations of asparagine and malic acid, maleamic acid and malic acid. Postulated pathways to form anhydropolyaspartic acid are discussed and the resulting polymers are characterized.

A century ago, aspartic acid was prepared by heating ammonium fumarate or ammonium malate.[2] Recently it has been emphasized that thermal homopolymerization accompanies these reactions.[3] On the other hand, heating unsubstituted aspartic acid also yielded a homopolymer which gave aspartic acid upon hydrolysis.[4,5] It is reported in this paper that the infrared absorption spectra of these two aspartic acid homopolymers prepared from

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ammonium fumarate or ammonium malate and from unsubstituted aspartic acid indicate completely identical anhydropolyaspartic acid structure (III, IV).[6] By alkaline treatment these polymers (III, IV) are easily converted to polypeptide structures (I, II).[4,5]

Experiments reported in this paper revealed that there were many ways to synthesize anhydropolyaspartic acid thermally from aspartic acid, fumaric acid, malic acid, maleic acid, and their derivatives and combinations of these compounds. Typical hypothetical routes of polycondensation reactions are indicated in Fig. 1.

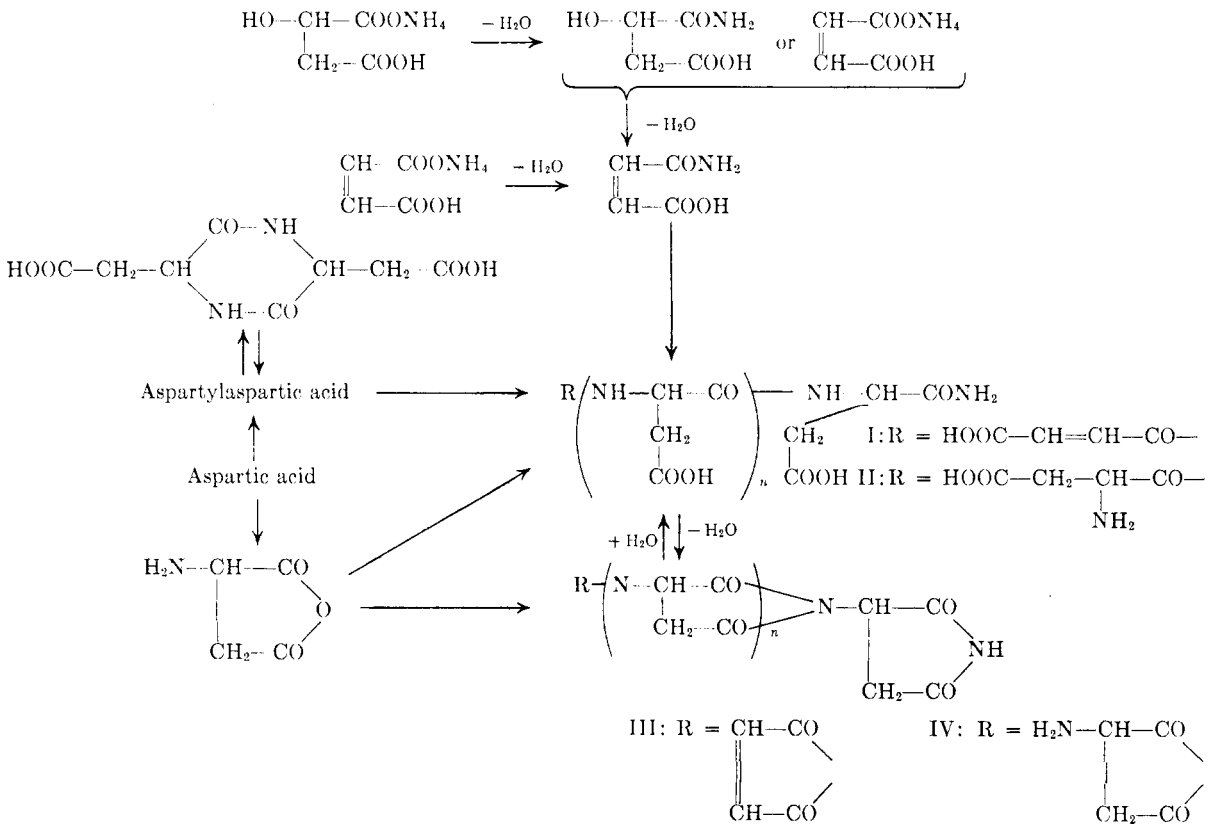

Fig. 1. Flowsheet of postulated reactions

A number of variations of the reactions were attempted in order to verify the proposed pathway illustrated in Fig. 1. The main hypothesis is based on the thermal conversion of ammonium carboxylate to an amide, the addition reaction[7] of the amide to a double bond and a ring closure of the aspartyl group to the imide structure (Fig. 1).

Two kinds of reaction were carried out. In (A), anhydropolyaspartic acid was synthesized from different kinds of derivatives of malic acid, fumaric acid, maleic acid, and also combinations of these compounds. In (B)[8] anhydropolyaspartic acid was prepared from asparagine or monoammonium aspartate and malic acid or fumaric acid. Both reactions resulted in good yields. The products gave positive biuret tests and negative ninhydrin tests. It was found, in general, that higher reaction temperature and longer heating gave larger yields.

EXPERIMENTAL

(A) *Polycondensation of monoammonium dl-malate.* Monoammonium malate was prepared by mixing equimolar quantities of *dl*-malic acid and aqueous ammonia which were then evaporated under reduced pressure. The resulting white crystalline substance was used for this reaction.

Monoammonium malate, 1.50 g. (0.01 mole), was heated in an open test tube in an oil bath under varying conditions. The substance melted easily and began to evolve gas. After cooling, the yellow-brown glassy substance was vigorously rubbed with 15 ml. of water to yield a precipitate. The precipitate was filtered and washed with 15 ml. of water and 10 ml. of ethanol and dried in air. The material was dialyzed for 3.5 days. Yields are reported in Table I.

Polycondensation of maleamic acid. Maleamic acid was prepared by acidification of the ammonium salt of maleamic acid, m.p. 151–152°, which was prepared by ammonolysis of maleic anhydride in dry benzene.[9] Maleamic acid, 1.15 g. (0.01 mole), was heated under nitrogen under varying conditions. The substance melted easily and evolved gas. After cooling, the resulting red-brown glassy substance was

(6) The compound is actually a polymaleimide.

(7) D. S. Breslow, G. E. Hulse, and A. S. Matlack, *J. Am. Chem. Soc.*, **79**, 3760 (1957).

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TABLE I

POLYCONDENSATION OF MONOAMMONIUM *dl*-MALATE

Reaction Condition, °C.[a]	Yield of Polymer: Before dialysis, g.	Yield of Polymer: After dialysis, g.	Yield of Polymer, after dialysis, %[b]
(a) 150	Trace	...	...
160	0.45	0.37	32
170	0.77	0.72	63
180	0.86	0.74	64
190	0.93	0.84	73
200	0.92	0.83	73
Hr.			
(b) 1/4	Trace	...	...
1/2	0.31	0.21	18
1	0.79	0.67	58
2	0.90	0.84	73
4	0.98	0.88	77

[a] In (a), monoammonium *dl*-malate (0.01 mole) was heated for 1.5 hr. In (b), monoammonium *dl*-malate (0.01 mole) was heated at 180°. [b] Yields were based on hydrated polymaleimide,[5] in the following tables.

treated with 15 ml. of water and was rubbed by glass rod to yield a precipitate. The pale red-brown precipitate was filtered, washed with 10 ml. of water and 10 ml. of ethanol, and dried. After pulverizing, the materials were dialyzed for 5 days. Yields are reported in Table II.

TABLE II

POLYCONDENSATION OF MALEAMIC ACID

Reaction Condition, °C[a]	Yield of Polymer: Before dialysis, g.	Yield of Polymer: After dialysis, g.	Yield of Polymer, after Dialysis, %
(a) 150	0.37	0.28	24
160	0.74	0.64	56
170	0.87	0.77	67
180	0.90	0.83	73
190	0.92	0.86	75
200	0.90	0.85	74
Hr.			
(b) 1/2	0.73	0.61	53
1	0.83	0.72	63
2	0.86	0.77	67
3	0.92	0.81	70
5	0.91	0.81	70

[a] In (a) maleamic acid (0.01 mole) was heated for 1 hr. In (b) maleamic acid (0.01 mole) was heated at 170°.

Other reactions studied were: polycondensation of *dl*-monoammonium malate and of maleamic acid, polycondensation of *dl*-malic acid and ammonium maleamate, polycondensation of maleic anhydride and ammonium maleamate, polycondensation of fumaric acid and ammonium maleamate. The procedures and yields were similar in most cases to those in the previous reactions.

(B) *Polycondensation of l-asparagine and dl-malic acid.* *l*-Asparagine monohydrate, 1.50 g. (0.01 mole), and *dl*-malic acid, 1.34 g. (0.01 mole), were ground together in a mortar. The mixture was heated in an open test tube in an oil bath under varying conditions. The mixture melted and began to evolve gas. After cooling the resulting yellow-brown glassy material was rubbed with 15 ml. of water. The resulting light yellow precipitate was filtered and washed with 15 ml. of water and 10 ml. of ethanol and dried. After crushing in a mortar the materials were dialyzed for 5 days. The yields are reported in Table III.

TABLE III

POLYCONDENSATION OF *l*-ASPARAGINE MONOHYDRATE AND *dl*-MALIC ACID

Reaction Condition, °C.[a]	Yield of Polymer: Before dialysis, g.	Yield of Polymer: After dialysis, g.	Yield of Polymer, after dialysis, %
(a) 150	0.53	0.43	19
160	1.65	1.39	60
170	1.87	1.62	70
180	1.88	1.73	75
190	1.93	1.80	78
200	1.97	1.83	79
Hr.			
(b) 1/2	Trace	...	...
1	1.80	1.49	65
2	1.86	1.56	68
4	1.89	1.62	70
6	1.95	1.69	73

[a] In (a), *l*-asparagine monohydrate (0.01 mole) and *dl*-malic acid (0.01 mole) was heated for 2 hr. In (b), the same mixture was heated at 175–180°C.

Polycondensation of monoammonium dl-aspartate and dl-malic acid. Monoammonium *dl*-aspartate was prepared by neutralization of *dl*-aspartic acid with equimolar proportion of aqueous ammonia and evaporation under reduced pressure in a desiccator. The colorless sirup which remained crystallized after 3 days. Crystallization proceeded more rapidly after seeding. The resulting white crystalline substance was used in this reaction.

Monoammonium *dl*-aspartate, 1.50 g. (0.01 mole), was mixed with *dl*-malic acid, 1.34 g. (0.01 mole), and then heated under varying conditions. The material was melted and gas evolution occurred. After cooling, 15 ml. of water was added to yield a precipitate. The slightly colored material was filtered and washed with 15 ml. of water and 10 ml. of ethanol. After crushing in a mortar, the material was dialyzed for 5 days. Yields are recorded in Table IV(a). Other reactions studied were: polycondensation of *l*-asparagine monohydrate and fumaric acid, polycondensation of monoammonium *dl*-aspartate and fumaric [Table IV(b)]. The reaction of procedures are same as above.

Reaction of dl-aspartic acid diketopiperazine diamide and dl-malic acid. *dl*-Aspartic acid diketopiperazine diamide,[6] 1.14 g. (0.005 mole), was ground with *dl*-malic acid, 1.34 g. (0.01 mole) in a mortar. The mixture was heated at 180–183° for 2 hr. Gas evolution occurred. After cooling, the yellow material was rubbed with 15 ml. of water with a glass rod. The mixture was allowed to stand overnight and the precipitate was isolated by centrifugation. This was washed with 10 ml. of water and 10 ml. of ethanol, and dried. A gray-white polymer was obtained after dialysis, 1.68 g.

Conversion of polyimide (III, IV) *to polypeptide* (I, II) *by alkaline treatment.* Polymer (IV) (prepared from *dl*-aspartic acid alone) 0.5 g., was dissolved in 5 ml. of 1.0*N* sodium hydroxide and was heated for 10 min. at 80°. After heating, the solution was cooled rapidly in ice water, acidified with 3.0*N* hydrochloric acid (*p*H 3) and then dialyzed in cellophane tubing for 3 days. No precipitation occurred. The dialyzed solution was dried in a vacuum desiccator under an infrared lamp. A yellow film remained, 0.23 g.

Two grams of polymer(IV) (prepared from *dl*-aspartic acid and orthophosphoric acid) was dissolved in 20 ml. of 1.0*N* sodium hydroxide and was heated for 10 min. at 80°, and the product was treated as described above. A pale yellow gelatin-like substance was obtained, 1.67 g. A

TABLE IV

POLYCONDENSATION OF MONOAMMONIUM *dl*-ASPARTATE AND *dl*-MALIC ACID OR FUMARIC ACID

	Temp., °C.	Yield of Polymer: Before dialysis, g.	Yield of Polymer: After dialysis, g.	Yield of Polymer, after Dialysis, %
(a)[a]	150	0.05	0.02	1.1
	160	0.92	0.77	...
	170	1.56	1.33	57
	180	1.70	1.56	68
	190	1.90	1.74	76
	200	1.87	1.80	78
(b)[b]	150	0.50	0.01	...
	160	0.75	0.15	6
	170	0.90	0.36	16
	180	0.95	0.43	19
	190	1.20	0.98	43
	200	1.70	1.58	69

[a] In (a), monoammonium *dl*-aspartate (0.01 mole) and *dl*-malic acid (0.01 mole) were heated for 1.5 hr. [b] In (b), monoammonium *dl*-aspartate (0.01 mole) and fumaric acid (0.01 mole) were heated for 1.5 hr.

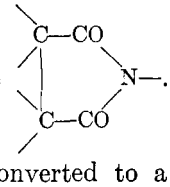
2.0 g. sample of polymer (III) prepared from *l*-asparagine and malic acid was treated with 1.0*N* alkali in the same way as above. A pale yellow-brown substance was obtained, 0.64 g.

Determination of equivalent weight by electrometric titration[10]

The titrations were carried out at room temperature by dissolving in excess 0.102*N* sodium hydroxide and back-titrating with standard 0.476*N* hydrochloric acid using a Beckman model H 2 pH meter. Observed values are as follows: Imide-type polymer (IV), 123; peptide-type polyaspartic acid (II), 128; peptide-type polyaspartic acid sodium salt, 4400.

DISCUSSION

Some of the properties of the imide-type polymers (IV) have already been reported in a previous paper.[5]

Infrared absorption spectra showed that all of the thermal polycondensation products prepared from different materials had the polyimide structure.[5] Typical infrared absorption spectra show bands at 1780 cm.$^{-1}$ and 1701 cm.$^{-1}$ indicating a 5-membered cyclic imide structure  . The polyimide structure can be converted to a polypeptide structure by alkaline treatment.[4,5] Characteristic absorption bands of the resulting peptide-type polyaspartic acid are: 3300 cm.$^{-1}$, 3080 cm.$^{-1}$ (NH stretching); 1710 cm.$^{-1}$ (CO of carboxyl group), 1650 cm.$^{-1}$ (amide I), 1550 cm.$^{-1}$ (amide II). With very weak alkali, *e.g.*, sodium bicarbonate, the imide structure is hydrolyzed slowly; the treatment increases the proportion of peptide structure in the polymer. By refluxing with water, the water-insoluble imide type polymer was converted partially to water-soluble material. The infrared absorption spectra show that this treatment increases the proportion of peptide structure. Completely converted peptide-type polyaspartic acid and DNP derivatives of the polymer are both water soluble and do not precipitate even in strong acid.

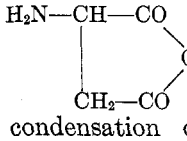
The thermal conversion of ammonium carboxylate to an amide structure is a common reaction. Recently it has been reported that the amide bond of acrylic acid amide reacts with the double bond of other acrylic acid amide molecules to form poly β-alanine in the presence of basic catalysts.[7] Application of the β-alanine formation reaction was studied in this laboratory. It was found that the ammonium acrylate also forms white water-insoluble crystalline poly β-alanine when heated at 160–200° for 1–8 hr. without a basic catalyst. The polymers produce β-alanine upon hydrolysis. Infrared absorption spectra show that the substance is completely polypeptide; 3300 cm.$^{-1}$, 3080 cm.$^{-1}$, NH stretching; 1650 cm.$^{-1}$, amide I; 1550 cm.$^{-1}$, amide II.

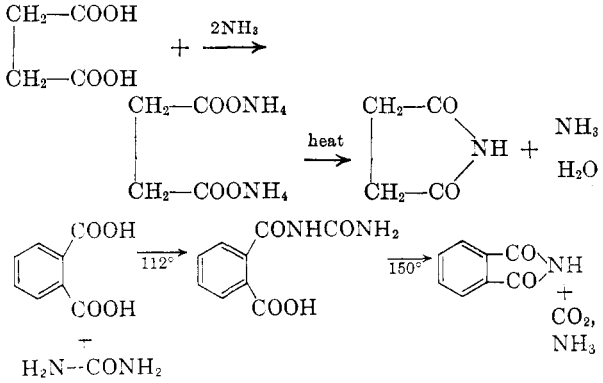
$$n(CH_2{=}CH{-}COONH_4) \downarrow$$
$$CH_2{=}CH{-}CO{-}(NH{-}CH_2{-}CH_2{-}CO)_{n-2}{-}NH{-}CH_2{-}CH_2{-}CONH_2 + nH_2O$$

All reactions for preparing anhydropolyaspartic acid which are presented in this paper are very similar to the above poly β-alanine formation reaction. The amide group or the precursor ammonium carboxylate is the key moiety in forming peptide bonds in that it adds to a double bond of maleic acid or fumaric acid. In the case of malic acid, it should be converted at first to the α-β unsaturated acid upon heating, which could then react with another (unsaturated) dicarboxylic acid amide. After the addition reaction the resulting peptide-type aspartyl residues are converted to imide type structures at high temperature (Fig. 1).

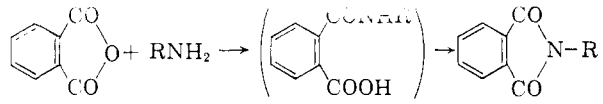
In reaction A, for example, the monoammonium *dl*-malate or the maleamic acid is simultaneously a nitrogen donor and a nitrogen acceptor. In the reaction of ammonium maleamate with malic acid or fumaric acid, the former substance is the nitrogen donor and the latter is the nitrogen acceptor. Although the starting substances are not amino acids, they yield anhydropolyaspartic acid in polycondensation. In reaction B, aspartic acid and monoammonium aspartate are nitrogen donors, whereas malic acid and fumaric acid are nitrogen acceptors. These nitrogen donors are amino acid derivatives and the nitrogen acceptors are not amino acid derivatives and the nitrogen acceptors are not amino acids. Anhydropolyaspartic acid results after copolycondensation in each case.

(10) These samples were prepared by condensation of *dl*-aspartic acid. Peptide-type polyaspartic acid was prepared by alkaline treatment as described in the experimental part. Peptide-type polyaspartic acid sodium salt was prepared by dialysis after alkaline treatment without acidification.

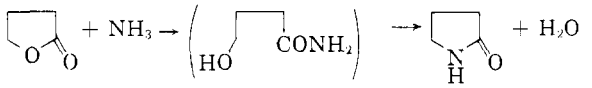
Kovacs and co-workers[4] assumed that aspartic acid anhydride,  , is the intermediate in the condensation of unsubstituted aspartic acid. All of the reactions described in this paper indicate that the aspartic acid anhydride is not a necessary intermediate in the free aspartic acid condensation reaction. The literature provides examples in which an imide is formed without passing through the anhydride step.[11,12] Piutti



found that the reaction of phthalic acid and urea[12] gives *N*-phthalylurea at 112° but at 150° ring closure of the ureide occurs to yield a phthalimide.



Even in the reaction of an anhydride[13] with an amine, the anhydride must pass through the amide step as an intermediate as in the reaction of γ-butyrolactone with ammonia pass the amide step.[14] The amide intermediate in these reactions

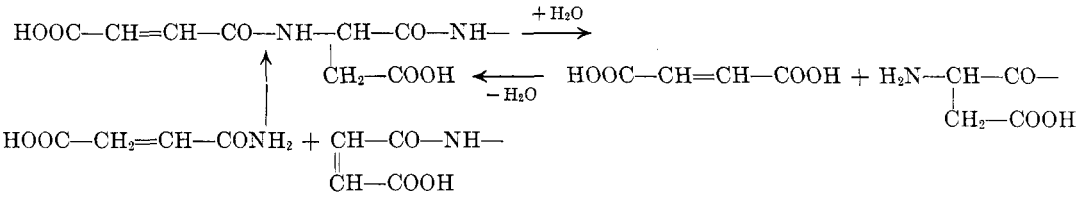


of imide formation corresponds to the peptide bond (I) in the polyimide (III) formation reaction (Fig. 1). Therefore, it is possible to assume that the resulting peptide-type polyaspartic acid (I, II) is converted to the imide-type polymer (III, IV) under these reaction conditions. If aspartic acid anhydride is an intermediate in the formation of the imide type polymer, it must pass through a peptide step rather than function as a direct intermediate.

The reaction of aspartic acid diketopiperazine diamide with malic acid gives almost completely an imide-type polymer. This fact suggests that the diketopiperazine is not stable under these reaction conditions. It is possible to assume that aspartic acid diketopiperazine formed thermally as an intermediate is converted to dipeptide again and that the peptide reacts with another amino acid or peptide to yield polyimide as shown in Fig. 1.

The close agreement of titration values of the imide type (123) and the peptide type (128) polymers shows that the imide-type polymers are completely converted to peptide-type polyaspartic acid by excess alkali during titration. Both titration values agree closely with the calculated value of 115 for peptide-type polyaspartic acid. The titration value of the peptide-type polyaspartic acid sodium salt (4400) shows that almost all of the carboxyl groups of the polymer combine with sodium ions which are held tightly even after 4 days of dialysis.

The molecular weights of some of these polymers were studied by assay with DNFB. The molecular weight of the products prepared by methods A and B indicate values in the range of 15,000–28,000. It is conceivable that some of the polymers do not



have *N*-terminal amino groups (Fig. 1). Therefore, molecular weight determination by the *N*-terminal assay method must be expected to give inaccurate values in such products. However, it is notable that even in the polycondensation of monoammonium malate or maleamic acid fairly high *N*-terminal amino group content was indicated. This suggests that hydrolysis of the peptide bond accompanies the polycondensation reaction.

Since the determination of the ratio of α to β linkages of peptide-type polyaspartic acid prepared by alkaline treatment has been studied by Kovacs and co-workers,[4] it has not been considered here.

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