[CONTRIBUTION FROM THE DEPARTMENT OF ORGANIC CHEMISTRY, THE HEBREW UNIVERSITY]

Synthesis of Polyaminomalonic Acid

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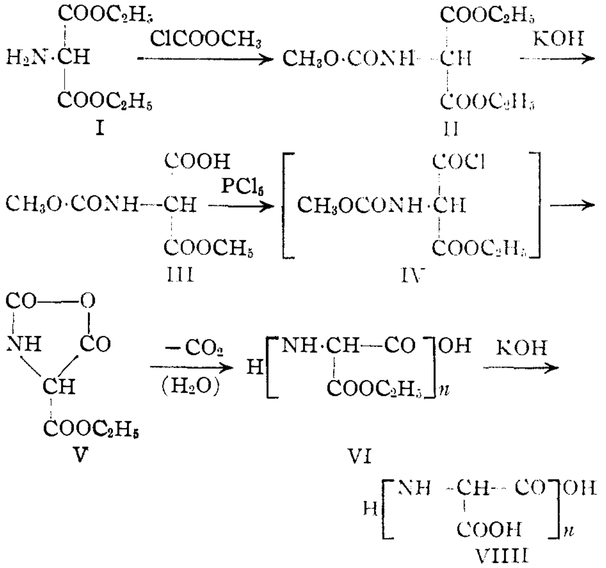
Diethyl aminomalonate gives N-carbomethoxy diethyl aminomalonate with methyl chloroformate. By partial hydrolysis N-carbomethoxy ethyl hydrogen aminomalonate is obtained. The action of phosphorus pentachloride on the latter yields the N-carboxy anhydride of ethyl hydrogen aminomalonate. Polymerization of this anhydride either by heat *in vacuo* or in pyridine solution yields polyethylaminomalonate acid and after hydrolysis polyaminomalonic acid.

Two poly-α-aminodicarboxylic acids have so far been synthesized: polyaspartic acid[1] and polyglutamic acid.[2] As a continuation of this work it seemed of interest to synthesize polyaminomalonic acid[3] which constitutes the first member of the homologous series of polydicarboxylic amino acids.

Polyaminomalonic acid was prepared from diethyl aminomalonate[4] (I) according to the accompanying scheme. N-Carbomethoxy diethyl aminomalonate (II) was obtained in analogy to the corresponding N-benzoyl derivative[5] by the action of methyl chloroformate on diethyl aminomalonate (I) in aqueous pyridine. By partial hydrolysis of the carbomethoxy derivative II with ethanolic potassium hydroxide, N-carbomethoxy ethyl hydrogen aminomalonate (III) was obtained. Action of phosphorus pentachloride on the semi-ester III suspended in petroleum ether yielded, presumably through the chloride IV which was not isolated, the N-carboxy anhydride of ethyl hydrogen aminomalonate V, which when freshly prepared, yielded the polyethylaminomalonate acid VI on polymerization by heating *in vacuo* or by keeping in dried pyridine. The latter is insoluble in water, soluble in alkali and gives positive biuret and ninhydrin reactions. Hydrolysis with cold aqueous normal potassium hydroxide gave the potassium salt VII from which the free polyaminomalonic acid VIII was obtained.



If the polymerization is carried out *in vacuo* at 20–25 mm. and at 90–125°, or in pyridine at room temperature, the resulting polymer is soluble in hot acetic acid. On heating V, however, in high vacuum (10^{-2}–10^{-3} mm.) the polymerization takes place at lower temperature (50–85°) and the resulting product is not soluble in hot glacial acetic acid, but forms gels in it even at very low concentrations.

Polyaminomalonic acid is readily soluble in water. It is precipitated as a voluminous mass from its aqueous solution by addition of ethanol and ether. On drying *in vacuo* this precipitate yields a brittle, shining hygroscopic film, giving positive biuret and ninhydrin reactions.

Van Slyke α-amino nitrogen determinations in various polyaminomalonic acid preparations, obtained by polymerization at about 20 mm. indicated an average degree of polymerization of about 70–85, corresponding to average molecular weight between 7000 and 8500.

The ethyl polyaminomalonate is hydrolyzed to glycine practically quantitatively by boiling it with 7 *N* hydrochloric acid for several hours.

Experimental[6]

N-Carbomethoxy Diethyl Aminomalonate (II).—To an ice-cold mixture of diethyl aminomalonate[4,6] (175 g., 1 mole), pyridine (158 g., 2 moles), and water (800 ml.), methyl chloroformate (104 g., 1.06 moles) was added slowly with mechanical stirring which was continued for an additional 30 minutes. The lower oily layer was separated, and water and pyridine were removed *in vacuo* on a water-bath. The desired substance distilled at 138–141° (6.5 mm.); m.p. 46°, yield 118 g. (50%).

Anal. Calcd. for $C_9H_{15}NO_6$: C, 46.4; H, 6.4; N, 6.0. Found: C, 46.3; H, 6.3; N, 6.1.

N-Carbomethoxy Ethyl Hydrogen Aminomalonate (III).—Pure diester II (10 g., 0.043 mole) was dissolved in absolute ethanol (35 ml.), and mixed with a filtered solution of potassium hydroxide (2.88 g., 0.0513 mole) in absolute ethanol (35 ml.). After standing for two days at room temperature the solution was filtered and the ethanol removed *in vacuo* at 30–40°. The oily residue crystallized generally on standing overnight over sulfuric acid. The crystals (or the still oily product) were dissolved in water (30 ml.), and extracted three times with portions of ether (10 ml. each) to remove the unsaponified matter. The aqueous layer was filtered, acidified with concentrated hydrochloric acid to congo red and extracted three times with portions of ether (15 ml. each). After drying with anhydrous sodium sulfate the ether was evaporated; the oily residue crystallized after two days standing over sulfuric acid, and then over phosphorus pentoxide. It was recrystallized from benzene–petroleum ether. Yield of the pure substance was 6.5 g. (75%), m.p. 73–74°.

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[1] M. Frankel and A. Berger, *Nature*, **163**, 213 (1949); *J. Org. Chem.*, **16**, 1513 (1951); *cf.* A. Berger and E. Katchalski, THIS JOURNAL, **73**, 4084 (1951).

[2] W. E. Hanby, S. G. Waley and J. Watson, *Nature*, **161**, 132 (1948); *J. Chem. Soc.*, 3239 (1950).

[3] *Cf.* M. Frankel, M. Harnik and Y. Levin, *Experientia*, **8**, 98 (1952).

[4] H. R. Snyder and C. W. Smith, THIS JOURNAL, **66**, 350 (1944).

[5] C. E. Redemann and M. S. Dunn, *J. Biol. Chem.*, **130**, 341 (1939).

[6] Elementary analyses by Drs. Weiler and Strauss, Oxford, England; m.p. determinations with Fisher–Johns apparatus.

Anal. Calcd. for $C_7H_{11}NO_6$: C, 41.0; H, 5.4; N, 6.8; Found: C, 41.2; H, 5.4; N, 6.6.

The substance is soluble in water, ether and ethanol.

N-Carboxy Anhydride of Ethyl Hydrogen Aminomalonate (V).—Finely ground phosphorus pentachloride (1.02 g., 0.0049 mole) was added to ethyl hydrogen aminomalonate (III) (1 g., 0.0049 mole) covered with dry petroleum ether (10 ml.). After standing a few hours at room temperature the solid mixture liquefied with strong evolution of hydrogen chloride. The lower oily layer was separated and shaken 3 times with portions of dry petroleum ether (10 ml.). It was then kept at about 70° at 20–30 mm. pressure for two hours (protection with a calcium chloride tube), until the evolution of gas ceased. The clear, colorless oil was purified twice by dissolving in absolute ether and precipitating with dry petroleum ether. This unstable oil evolved a gas even when stored above phosphorus pentoxide. The yield was 0.72 g. (83%). Efforts to obtain it in crystalline form were unsuccessful. It is soluble in ether; water decomposes it immediately, yielding a solution giving a positive ninhydrin reaction.

Polyethylaminomalonate Acid (VI).—The polymerization of the anhydride V was performed in a large vessel because of the sudden foaming in the last stage of the reaction. The freshly prepared anhydride (5 g.) was placed in a 500-ml. round-bottomed flask protected with a calcium chloride tube. The flask was kept at 18–20 mm. in a bath, the temperature of which was raised gradually during 3 hours from 90 to 120°. The substance solidified, with strong evolution of gas. The polymer formed, was a brittle, transparent film of slightly yellow color; after powdering, the colored impurity was removed by washing with ether. The polymer was insoluble in water and the usual organic solvents, soluble in hot acetic acid and in aqueous alkali. It was further purified by dissolving in a large quantity of hot acetic acid, filtering, and precipitating with water and centrifugation. Biuret reaction was positive; aqueous ninhydrin colors the particles violet.

Anal. Calcd. for $(C_5H_7NO_3)_n{\cdot}H_2O$: C, 46.5; H, 5.4; N, 10.9. Found: ($n = \infty$) C, 46.2; H, 5.6; N, 11.0.

On carrying out the polymerization of (V) in a high vacuum system (pressure 10^{-2} to 10^{-3} mm.) a slight polymerization seems to start even at room temperature; at 50° it became very pronounced and comes to an end at 90° (after about 1.5 hours from the beginning of the heating). Polymerization products thus obtained are not soluble even in hot glacial acetic acid (about 4 g. of polymer yielded in 2 l. of boiling glacial acetic acid only a gel which on cooling entirely solidified). The polymer is soluble in 5 *N* alkali in the cold.

Polymerization in Pyridine.—Freshly prepared and purified N-carboxy anhydride of ethyl hydrogen malonate (V) (4.3 g.) was dissolved in pure and dry pyridine (10 ml.) and kept at room temperature during 10 days. During the last days the formation of a suspension or of a gel could be observed. Dry ether was added to the reaction mixture, causing precipitation of the polyethylmalonate acid which was centrifuged, washed with ether, dissolved in warm glacial acetic acid (50–60°) and then precipitated by water. The precipitate was dried *in vacuo* over sodium hydroxide first at room temperature and finally at 100°. The yield was about 11%.

On precipitating the mother liquid of the above preparation by ether, additional amounts of polymer were obtained, increasing the total yield to about 25%. Ninhydrin and biuret reactions were positive as above.

Anal. Calcd. for $(C_5H_7NO_3)_n{\cdot}H_2O$ ($n = \infty$): C, 46.5; H, 5.4; N, 10.9. Found: C, 45.9; H, 5.3; N, 10.8.

Complete Hydrolysis of the Polyethylaminomalonate Acid.—To the polymeric ester VI (0.043 g.) 7 *N* hydrochloric acid (8 ml.) was added and the mixture heated under reflux. Complete solution resulted after 3–4 hours; the heating was continued for about 25 hours; the solution was neutralized by concentrated potassium hydroxide, filtered from potassium chloride which was then several times washed with water in order to extract the amino acid. In an aliquot part of the final solution the total nitrogen and the amino nitrogen were determined. They were found to be identical (amino nitrogen = total nitrogen). Calcd. for a 100% hydrolysis to glycine: 10.8% (in the relations of the hydrolysis experiments); found: 10.3%. Hydrolysis was practically complete (about 96%) yielding glycine.

Polyaminomalonic Acid (VIII). A. Silver Salt of Polyaminomalonic Acid.—The polyester VI (1 g., 0.00774 mole) was dissolved in 1 *N* potassium hydroxide solution in water (14.5 ml.). After 2 days standing at room temperature the dark solution was filtered and neutralized with 7% nitric acid. 2 *N* silver nitrate (5 ml.) was added drop by drop. A voluminous, slightly creamy precipitate appeared. It was washed free of potassium ions by repeated centrifugations with water, and dried over sulfuric acid *in vacuo*. Yield of pure material was 0.8 g. (43%).

Anal. Calcd. for $(C_3H_2NO_3Ag)_nOHAg$ ($n = \infty$): Ag, 51.9. Found: Ag, 51.5.

B. **Polyaminomalonic Acid from the Silver Salt.**—To the freshly prepared silver salt (0.55 g.) 7% nitric acid was added until complete solution was attained (on storage the silver salt becomes insoluble in dilute nitric acid). Five per cent. hydrochloric acid was then added in excess to the filtered solution. Next day the precipitated silver chloride was filtered and the filtrate concentrated, over sulfuric acid and potassium hydroxide, *in vacuo* to a volume of 2–3 ml. Addition of a mixture of ethanol and ether precipitated a white, gelatinous mass. The polyaminomalonic acid was purified by repeated centrifugations with ether, dissolution in little water and reprecipitation with ethanol–ether. After centrifuging and drying over phosphorus pentoxide the polymer was obtained as a yellowish film, very soluble in water, giving positive biuret and ninhydrin reactions; the yield was 0.2 g. (75%).

Anal. Calcd. for $(C_3H_3NO_3)_n{\cdot}H_2O$ ($n = \infty$): C, 35.6; H, 2.97; N, 13.9. Found: C, 35.7; H, 3.3; N, 14.3.

Van Slyke manometric analyses for amino nitrogen in various preparations: (1) 0.165% corresponding to an average degree of polymerization: 85 (average molecular weight 8500). (2) 0.202% corresponding to an average degree of polymerization: 69 (average molecular weight 6900). (3) 0.176% corresponding to an average degree of polymerization: 79 (average molecular weight 7900).

Polyaminomalonic acid was also obtained from the ester VI through hydrolysis with N-potassium hydroxide carried out as above, acidification with 3% hydrochloric acid, dialysis and evaporation to dryness *in vacuo* over sulfuric acid. It constituted a brittle brownish film.

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