Synthesis of a,α'-Diaminodicarboxylic Acids

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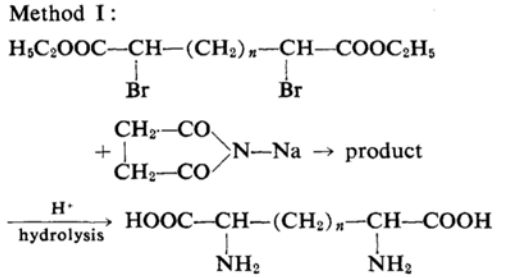
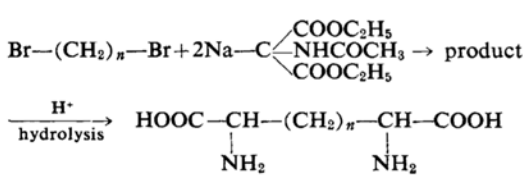
A systematic study of the synthesis of a series of α,α'-diaminodicarboxylic acids, containing a number of biologically and biochemically important amino acids, was made, mainly by amination of the corresponding α,α-dibromo-compounds using succinimide as an aminating reagent (Method I) and by condensation of diethyl acetamidomalonate with α,α'-dibromo-paraffin (Method II).

Among the compounds of this type, of particular interest is α,α'-diaminopimelic acid because of its presence in bacterial products[1,2], and because of its role as a precursor in the biosynthesis of lysine[3,4]. α,α'-Diaminosuccinic acid, with a chemical structure analogous to aspartic acid, asparagine, C_4-dicarboxylic acid and other biologically important substances, has recently been isolated from a natural source[5]. The other amino acids in this series have not yet been found in nature. However, α,α'-diaminoglutaric acid is regarded as a precursor in the biosynthesis of α,γ-diaminobutyric acid.[6] Therefore the availability of these amino acids for biological investigations is desirable.

Method I:



Method II:



Succinimide was first used successfully as an aminating reagent by Akabori, Izumi and Fujiwara for the synthesis of α,α'-diaminopimelic acid (D. A. Pim.)[7]. In the present work this method was tried for the preparation of α,α'-diaminosuccinic (D. A. Suc.), glutaric (D. A. Glu.) and adipic acid (D. A. Adi.). However it was only successful for D. A. Adi. The diethyl acetamidomalonate method was tested in the preparation of D. A. Adi., D. A. Pim., α,α'-diaminosuberic (D. A. Seb.), azelaic (D. A. Aze.), and sebacic acid (D. A. Seb.). It was satisfactory for the synthesis of the three last named. Table I shows the results of the above synthesis.

D. A. Suc. has been prepared by reduction of sodium dihydroxytartarate osazone with sodium amalgam.[8-12] Wenner[13,14] and Mckennis et al.[15] prepared it in high yield by hydrolysis of the corresponding bisbenzylamino compound in the presence of Pd-C and hydrogen at high or atmospheric pressure. In the present work a 44 per cent yield of D. A. Suc. was readily obtained by amination of *meso*-α,α'-dibromosuccinate, obtained easily by bromination of diethyl fumarate in sunlight, using succinimide as an aminating reagent.

D. A. Glu. has been prepared by Carter et al.[16] by hydrogenolysis of diethyl pyrazoline-3, 5-dicarboxylate at a high pressure. Hellmann et at.[17] prepared it by reacting diethyl α-acetamido - α - dimethylaminomethylmalonateiodomethylate with sodium salt of acetamidomalonate, and by hydrolysis of α,α'-diphosphiminoglutaric acid. The final product could not be obtained by amination of diethyl α,α'-dibromoglutarate with succinimide. Therefore the method of Carter et al. was reinvestigated. A modification of their procedures

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Table I

Compounds to be synthesized	Method I: Starting material	Method I: Yield of product	Method II: Starting material	Method II: Yield of product
D. A. Suc.	*meso*-Diethyl α, α'-dibromosuccinic acid	44%	—	—
D. A. Glu.	Diethyl α, α-dibromoglutaric acid	Trace	—	—
D. A. Adi.	Diethyl α, α'-dibromoadipic acid	73%	Ethylene dibromide	Trace
D. A. Pim.	Diethyl α, α'-dibromopimelic acid	81%[7]	Trimethylene dibromide	Trace
D. A. Sub.	—	—	Tetramethylene dibromide	62%
D. A. Aze.	—	—	Pentamethylene dibromide	66%
D. A. Seb.	—	—	Hexamethylene dibromide	79%

was employed and D. A. Glu. was synthesized in a yield of 33 per cent from glycine ethyl ester hydrochloride without separation of the individual intermediates. The method was as follows: to an aqueous solution of glycine ethyl ester hydrochloride was added an aqueous solution of sodium nitrite. An ethereal solution of methyl acrylate was added without separation of ethyl diazoacetate. Since the product could not be crystallized, the substance was reduced in ethereal solution at a high pressure of hydrogen in the presence of Raney's nickel. It was then hydrolyzed in hydrochloric acid.

Simmonds[18] reported the synthesis of D. A. Adi. and D. A. Pim. by the reaction of phthalimide with the corresponding α, α'-dibromo-compounds. Using succinimide instead of phthalimide, these two α, α'-dibromo-compounds were converted to D. A. Adi. and D. A. Pim. with comparatively good yields of 73 and 81 per cent, respectively. However, the use of diethyl acetamidomalonate resulted in very poor yields.

D. A. Sub., D. A. Aze., and D. A. Seb. were prepared by Neuberg[19] and Abderhalden et al.[20] from the corresponding α, α'-dibromo-compounds and ammonia. Simmonds[19] also reported the preparation of D. A. Seb. by a similar method and of D. A. Seb. and D. A. Aze. in 44 and 39 per cent yields, using acetamidomalonate. In the present work the use of acetamidomalonate resulted in yields of 62, 66, and 79 per cent for D. A. Sub., D. A. Aze. and D. A. Seb., respectively. This method seems the most suitable for these three amino acids, since the starting materials are readily available and the required operations are simple.

It is considered that the low yields of D. A. Suc. and D. A. Glu. in the succinimide method, and of D. A. Adi. and D. A. Pim. in the acetamidomalonate method are probably caused by an inhibition of the reaction by steric hindrance.

Table II. The chromatographic behavior of synthetic diaminodicarboxylic acids

	R_f-value: Fee-form	R_f-value: Hydrochloride
D. A. Suc.	0.0312	0.0402
D. A. Glu.	0.0406	0.0522
D. A. Adi.	0.0455	0.0600
D. A. Pim.	0.0570	0.0715
D. A. Sub.	0.0691	0.0878
D. A. Aze.	0.109	0.127
D. A. Seb.	0.157	0.186

The above seven amino acids and their hydrochlorides prepared as described above have R_f-values shown in Table II on paper chromatography using acetic acid-butanol-water (1 : 4 : 1) as solvent.

Experimental

D. A. Suc. — To a solution of 5.5 g. (0.24 mol.) of sodium in 100 ml. of absolute ethanol was added 30 g. (0.30 mol.) of succinimide. This was heated on a water bath to dissolve the succinimide. To this mixture were added 0.5 g. of potassium iodide and 33 g. (0.10 mol.) of *meso*-α, α'-dibromosuccinic acid. The mixture was refluxed on an oil bath for 1 hr. It was cooled and filtered. The filtrate was concentrated in vacuo and the residue was hydrolyzed by refluxing in 100 ml. of concentrated hydrochloric acid and 50 ml. of water for 4 hr. The precipitated succinic acid was removed by filtration and the supernatant was concentrated under reduced pressure. The residual material was dissolved in 70 ml. of methanol and filtered to remove sodium chloride. On neutralization with aniline, D. A. Suc. crystallized immediately as paste or block. This crude solid was washed with 90 per cent methanol. It was dissolved in 100 ml. of warm 20 per cent hydrochloric acid and decolorized. The solution was adjusted to pH 6 with aqueous ammonia and 100 ml. of methanol was added to complete crystallization. White crystalline D. A. Suc. decomposing at 305~310°C was obtained in a yield of 6.0 g. (44 per cent).

Found: C, 32.10; H, 5.62; N, 18.54. Calcd. for $C_4H_8O_4N_2$: C, 32.43; H, 5.44; N, 18.91%.

D. A. Glu.—A solution of 12 g. (0.15 mol.) of nitrite and 0.5 g. of sodium acetate in 15 ml. of

18) D. H. Simmonds, *Biochem. J.*, **58**, 520 (1954).
19) C. Neuberg, *Z. phys. Chem.*, **45**, 99 (1905).
20) E. Abderhalden and W. Zeisset; *Fermentforschung*, **9**, 336 (1928).

water was cooled to 0°C and a cold solution containing 14 g. (0.1 mol.) of glycine ethyl ester hydrochloride was added with stirring, external cooling being used to keep the temperature at 0~2°C. After 30 min. 13 g. (0.15 mol.) of methyl acrylate in 25 ml. of ether was added, and rapid stirring was continued for 1 hr. Then 4 ml. of acetic acid was added to the mixture dropwise, and stirring was continued for 1 hr. The ethereal layer was separated. The aqueous layer was extracted with 70 ml. of ether, and the combined ethereal solutions were dried over sodium sulfate. The ether was slowly removed by distillation until the total volume became 40 ml. This solution was then reduced at 80 kg./cm² by hydrogen in the presence of Raney's nickel for 3 hr. at 60°C and for a further 3 hr. at 120°C. After removal of the catalyst, the filtrate was concentrated under reduced pressure. The residue was hydrolyzed for 3 hr. in 50 ml of boiling concentrated hydrochloric acid. The hydrolysate was decolorized with charcoal and concentrated in vacuo. The residual material was dissolved in 50 ml. of 50 per cent methanol and then 20 ml. of aniline was added with vigorous shaking. D. A. Glu. was crystallized. After storing in a refrigerator, the product was filtered and washed with 10 ml. of water, and then twice with 10 ml. of methanol. The dried product weighed 5.3 g. or 33 per cent of the theoretical yield from glycine ethyl ester hydrochloride. It decomposed at 300~310°C without melting.

Found: C, 36.68 H, 6.40; N, 16.54. Calcd. for $C_5H_{10}O_4N_2$: C, 37.03; H, 6.22; N, 17.28%.

D. A. Adi. — This was prepared in a manner similar to that of D. A. Suc. using 5.5 g. (0.24 mol.) of sodium, 30 g. (0.3 g. mol.) of succinimide, and 36 g. (0.1 mol.) of diethyl α, α'-dibromoadipate. The white crystalline product, reprecipitated from hydrochloric acid and aqueous ammonia, weighed 13.6 g. (73 per cent) and decomposed at 275~277°C without melting.

Found: C, 38.99; H, 7.02; N, 15.49. Calcd. for $C_6H_{12}O_4N_2$: C, 40.90; H, 6.87; N, 15.90%.

D. A. Pim.—The preparation of this compound was reported in a previous paper.[1] The yield was 81 per cent, and the decomposition point was 307~310°.

D. A. Sub.—To 800 ml. of absolute alcohol containing 19 g. (0.825 mol.) of sodium was added 184 g. (0.85 mol.) of diethyl acetamidomalonate. The mixture was dissolved by heating on a water bath. Then 70 g. (0.324 mol.) of tetramethylenedibromide was added and the mixture was refluxed for 10 hr. The hot reaction mixture was filtered, the residue being washed with hot alcohol. The filtrate and the washings were combined and evaporated in vacuo. The residue was hydrolyzed in 1000 ml. of boiling hydrochloric acid for 5 hr. The mixture was concentrated in vacuo and the dry residue was taken up in 100 ml. of water, and again concentrated to dryness. This operation was repeated once more. Then the dry residue was dissolved in 300 ml. of methanol. A mixture of 80 ml. of pyridine and 300 ml. of methanol was added with shaking and the resulting mixture was let stand overnight to precipitate the crude D. A. Sub. This precipitate was washed with methanol and ether and dried. The yield was 53.5 g. This crude compound was suspended in 1000 ml. of water and dissolved by the addition of 10 per cent sodium hydroxide. An aqueous solution containing 100 g. of copper sulphate was added and the mixture was adjusted to pH 5.4 with 10 per cent sodium hydroxide. The precipitate was washed thoroughly with water and was dissolved in 500 ml. of 3 N hydrochloric acid. It was then treated with hydrogen sulfide to remove the copper. The black precipitate was filtered off, and the filtrate was concentrated in vacuo. The residue was taken up in 400 ml. of methanol. A mixture of 80 ml. of pyridine and 200 ml. of methanol was added with shaking and the resulting mixture allowed to stand. After washing with methanol and ether and drying, 41 g. (62 per cent yield) of white crystalline D. A. Sub. were obtained. This did not decompose below 360°C. Paper chromatography showed a single spot with the R_f-value given in Table II.

Found: C, 46.89; H, 7.86; N, 13.79. Calcd. for C, 47.05; H, 7.90; N, 13.72%.

D. A. Aze.—This was prepared as above by condensation of 160 g. (0.74 mol.) of diethyl acetamidomalonate with 65 g. (0.283 mol.) of pentamethylenedibromide using 16.8 g. (0.73 mol.) of sodium in 700 ml. of absolute alcohol. The crude product (55.5 g.) was dissolved in 2000 ml. of hot water and 80 g. of basic copper carbonate monohydrate was added. The mixture was heated at boiling point for some time. The precipitate was collected on a suction funnel and thoroughly washed with water. It was dissolved in diluted hydrochloric acid. The solution was saturated with hydrogen sulfide to precipitate the copper sulfide completely. The precipitate was removed by filtration and the filtrate was concentrated in vacuo. The residue was dissolved in 300 ml. of methanol. Neutralization with a mixture of 80 ml. of pyridine and 450 ml. of methanol gave a white crystalline product. This was collected on a suction filter, washed with water, methanol and ether, and dried. 40.5 g. (66 per cent yield) of D. A. Aze. was obtained. This decomposed above 320°C without melting. Paper chromatography of this compound gave a single spot with the R_f-value given in Table II.

Found: C, 49.74; H, 8.31; N, 12.81. Calcd. for $C_9H_{18}O_4N_2$: C, 49.53; H, 8.31; N, 12.84%.

D. A. Seb.—This compound was prepared in the same way as D. A. Sub. from 23 g. (0.106 mol.) of diethyl acetamidomalonate, 10 g. (0.041 mol.) of hexamethylene dibromide, and 2.3 g. (0.10 mol.) of sodium. The yield was 7.5 g. (79 per cent). It did not decompose below 360°C and gave a single spot on paper chromatography with the R_f-value given in Table II.

Found: C, 51.73; H, 8.70; N, 11.96. Calcd. for $C_{10}H_{20}O_4N_2$: C, 51.70; H, 8.68; N, 12.06%.

Summary

A systematic study was made on the synthesis of α, α'-diaminodicarboxylic acids mainly by amination of the corresponding α, α'-dibromodicarboxylic acids using succinimide as an

aminating reagent and by condensation of diethyl acetamidomalonate with α, ω-dibromoparaffin.

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