331. *New Syntheses of Basic Amino-acids and Glycine.*

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When α-amino-dicarboxylic acids were submitted to the Schmidt hydrazoic acid reaction, the carboxyl group remote from the α-amino-group was replaced by an amino-group. Thus, *d*-glutamic, α-aminoadipic and α-aminopimelic acids furnished *d*-αγ-diamino-*n*-butyric acid, *dl*-ornithine and *dl*-lysine respectively, in good yield. A convenient method for the isolation of basic amino-acids as their dipicrates is described.

The synthesis of *dl*-lysine in 60% yield directly from ethyl *cyclo*hexanone-2-carboxylate and two molecules of hydrazoic acid has been realised. *dl*-Ornithine (40% yield) was produced by similar treatment of ethyl *cyclo*pentanone-2-carboxylate.

Malonic acid, recorded in the literature as being inert towards hydrazoic acid, reacted with this reagent under suitable conditions, and glycine was isolated. It is suggested that this reaction provides a new general method for the synthesis of α-amino-acids.

Lysine occurs fairly abundantly in the hydrolysis products of many proteins, but is not readily accessible owing to the tedious and expensive procedure necessary for its isolation. The synthetic methods of Fischer and Weigert (*Ber.*, 1902, **35**, 3772), Sörensen (*Chem. Zentr.*, 1903, ii, 33), v. Braun (*Ber.*, 1909, **42**, 839), and Sugasawa (*J. Pharm. Soc. Japan*, 1927, 1044) do not give satisfactory yields and in general are too complex to be regarded as practical methods for the preparation of the *dl*-amino-acid. The synthesis in six steps from *cyclo*hexanone (Eck and Marvel, *J. Biol. Chem.*, 1934, **106**, 387) was more satisfactory in respect of yield (23%), but entailed laborious preparative work. The existing methods for the production of ornithine (I, $x = 3$) are equally unsatisfactory. Its preparation from arginine is expensive, and poor yields are obtained by the existing synthetic methods, which involve numerous intermediate stages (Fischer, *Ber.*, 1901, **34**, 454; Sörensen, *Chem. Zentr.*, 1903, ii, 35; Fischer and Zemplén, *Ber.*, 1909, **42**, 1022; I. G. Farbenind. A.-G., D.R-P., 1937, 650,999 and 652,550).

The facility with which carboxylic acids are converted into amines by the Schmidt method, employing hydrazoic acid (Schmidt, D.R-P. 500,435, 544,890; E.P. 307,798; F.P. 671,388; v. Braun and co-workers, *Annalen*, 1931, **490**, 125; *Ber.*, 1931, **64**, 2866; 1933, **66**, 684; 1934, **67**, 225; Adamson and Kenner, J., 1934, 842), and the known stability of amino-monocarboxylic acids towards such treatment (Oesterlin, *Z. angew. Chem.*, 1932, **45**, 536) suggested a shorter path to the preparation of the basic amino-acids. It was considered that in the case of amino-dicarboxylic acids, the protective effect of the α-amino-group would be confined to the proximate carboxyl group, and reaction between the second carboxyl group and hydrazoic acid would not be inhibited.

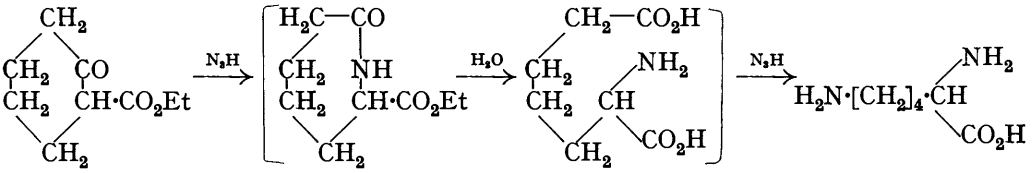
d-Glutamic acid, suspended in sulphuric acid, reacted with hydrazoic acid in chloroform (or with sodium azide) and *d*-αγ-diamino-*n*-butyric acid (I, $x = 2$) was isolated in 42% yield. The advantages of this direct synthesis are apparent when compared with the existing methods, by which yields of less than 20% are obtained after several intermediate reactions (Karrer, Escher, and Widmer, *Helv. Chim. Acta*, 1926, **9**, 301; Akabori and Numano, *Bull. Chem. Soc. Japan*, 1936, **11**, 214). As expected, the process was more efficient in the case of the higher homologues of glutamic acid, α-aminoadipic and α-amino-pimelic acids furnishing *dl*-ornithine and *dl*-lysine in 75% and 74% yields respectively. The properties of the several derivatives prepared from each product corresponded closely to those recorded in the literature.

$$HO_2C\cdot[CH_2]_x\cdot CH(NH_2)\cdot CO_2H \xrightarrow{N_3H} H_2N\cdot[CH_2]_x\cdot CH(NH_2)\cdot CO_2H \quad (I.)$$

In the preliminary experiments, isolation of the products was effected by the phosphotungstic acid method, as used for the separation of lysine from protein hydrolysates (Kossel and Patten, *Z. physiol. Chem.*, 1903, **38**, 39; Vickery and Leavenworth, *J. Biol. Chem.*, 1928, **79**, 377); later, this tedious and expensive stage was eliminated by taking advantage of the ready formation of the amino-acid dipicrates. *d*-αγ-Diamino-*n*-butyric acid in neutral or weakly acidic solution is precipitated as the *dipicrate*, m. p. 180—181°, when

mixed with excess of aqueous picric acid. dl-*Lysine dipicrate*, which is precipitated quantitatively under the same conditions, may be recrystallised without loss, and in comparison with the monopicrate has a definite m. p. (188—190°) and is insoluble in excess of aqueous picric acid. The conversion of the dipicrates into the dihydrochlorides is readily accomplished.

It is known (Schmidt, *Ber.*, 1924, **57**, 704; 1925, **58**, 2413; E.P. 252,460; D.R.-P. 455,585) that cyclic ketones suffer ring enlargement when treated with hydrazoic acid in the presence of a suitable catalyst, and the resulting lactam may be isolated in good yield. A direct synthesis of the basic amino-acid from the appropriate *cyclo*ketone-2-carboxylic acid ester has been effected by combining this ketonic reaction with that of the α-amino-dicarboxylic acids, described above. According to Schmidt (*loc. cit.*), when dry hydrogen chloride is passed into a solution of ethyl *cyclo*hexanone-2-carboxylate and hydrazoic acid in benzene, and the product hydrolysed, α-aminopimelic acid hydrochloride is obtained. Not only this product, but *dl*-lysine was isolated in 25% yield (as dipicrate) when Schmidt's experiment was repeated. Since lysine was absent from the product when concentrated sulphuric acid was used as catalyst, it is evident that the presence of a trace of water is necessary to effect hydrolysis of the lactam, prior to attack by a second molecule of hydrazoic acid.



The yield of *dl*-lysine was increased to 60% by submitting the product, resulting from the initial hydrogen chloride treatment, to further action of hydrazoic acid under the catalytic influence of concentrated sulphuric acid.

dl-Ornithine was obtained in 40% yield when the method was applied to ethyl *cyclo*pentanone-2-carboxylate. The decrease in yield on passing from the *cyclo*hexanone to the *cyclo*pentanone derivative, which may be attributed to a greater resistance to ring enlargement, is also experienced in the analogous reaction between cyclic ketones and diazohydrocarbons (Adamson and Kenner, this vol., p. 183).

Glycine from Malonic Acid.—The protective effect of an amino-group exerted on a carboxyl group in the same molecule may be expected to increase as the two groups are brought together. Thus, adipic acid is converted into tetramethylenediamine by hydrazoic acid to an extent of 83% (Schmidt, *loc. cit.*), but succinic acid yields only 8% of ethylenediamine (Oesterlin, *loc. cit.*). It seemed likely, therefore, that reaction with malonic or substituted malonic acids would cease when only one of the carboxyl groups had been replaced by the amino-group. Contrary to the experience of Oesterlin (*loc. cit.*), who reported that malonic acid was inert towards hydrazoic acid, reaction occurred slowly (more rapidly on raising the temperature) and glycine was identified as a product. It is suggested that this reaction may be extended to derivatives of malonic acid, thus providing a new facile method for the synthesis of α-amino-acids. Investigations on these lines are being actively pursued.

Experimental.

The solutions of hydrazoic acid in chloroform, prepared from sodium azide and sulphuric acid (v. Braun, *loc. cit.*), were partly dried by standing with anhydrous sodium sulphate for a short time.

d-*αγ-Diamino*-n-*butyric Acid.*—(i) Hydrazoic acid (4·8 g.; 1·15 mols.) in chloroform (48 c.c.) was added in portions during 6 hours to *d*-glutamic acid (14·7 g.), concentrated sulphuric acid (25 c.c.), and chloroform (10 c.c.) stirred at 43—46°; approximately 1 mol. of nitrogen was then evolved. Stirring was continued for 4 hours, the product poured on ice, and the aqueous layer diluted (700 c.c.) and treated with 30% aqueous phosphotungstic acid until precipitation was complete. After standing overnight, the white precipitate was washed with 5% sulphuric acid, dissolved in 50% aqueous acetone (500 c.c.), and decomposed by excess of baryta. Barium phos-

photungstate was filtered off, washed with dilute baryta solution, and barium removed from the filtrate and washings by carbon dioxide. The solution was evaporated to a small bulk at 50°, filtered to remove barium carbonate, and concentrated further (15 c.c.). Saturated oxalic acid was added until the solution was weakly acidic, followed by alcohol until there was no further precipitate on stirring. The neutral oxalate (7·7 g.; 41% yield), m. p. 211—215° (decomp.), which crystallised almost quantitatively from aqueous alcohol in clusters of white needles, m. p. 216° (decomp.), had $[\alpha]_D^{18°}$ + 8·3° (*c*, 3·98, anhydrous salt in water) (Found in material dried at 110°/12 mm.: C, 36·8; H, 6·8; N, 16·8. Calc. for $2C_4H_{10}O_2N_2,C_2H_2O_4$: C, 36·8; H, 6·8; N, 17·2%. Found: loss of weight on drying, 14·2. Calc. for $2C_4H_{10}O_2N_2,C_2H_2O_4,3H_2O$: H_2O, 14·2%). Karrer, Escher, and Widmer (*loc. cit.*) record m. p. 205° (decomp.), $[\alpha]_D^{18°}$ + 7·8° (*c*, 1·154) for their product, which was anhydrous when crystallised from aqueous alcohol.

(ii) Sodium azide (4 g.; 1·25 mols.) was added in small portions to *d*-glutamic acid (7·35 g.), concentrated sulphuric acid (25 c.c.), and chloroform (15 c.c.) stirred at 45—50°. When evolution of nitrogen had ceased (3 hours), the product was poured on ice, and the neutral oxalate (3·2 g.; 33% yield) isolated from the aqueous extract as described above.

d-α*γ*-*Diamino*-n-*butyric acid dipicrate*. The oxalate (0·88 g.) in water (10 c.c.) and picric acid (2·4 g.) in water (175 c.c.) were mixed; after 24 hours, the long yellow needles (2·45 g.; 93% yield) were recrystallised from hot water, m. p. 180—181° (Found: C, 33·6; H, 3·0; N, 19·6. $C_4H_{10}O_2N_2,2C_6H_3O_7N_3$ requires C, 33·3; H, 2·8; N, 19·5%).

(iii) The aqueous extract (200 c.c.) obtained from the reaction product by pouring on ice as in expt. (i) was treated with hot saturated baryta until only weakly acidic to Congo-red. After removal of barium sulphate by centrifugation, picric acid (30 g.) in hot water (700 c.c.) was added and 36 hours later the dipicrate, m. p. 178—180°, which had separated (24 g.; 42% yield) was dissolved in hot water (250 c.c.) and concentrated hydrochloric acid (100 c.c.) added. After cooling and removal of picric acid the filtrate was extracted with ether and evaporated to dryness on the steam-bath. The residual crystals of the *dihydrochloride* were ground with absolute ethyl alcohol, filtered off, and dried in a vacuum. Yield, 7·5 g. (39%); m. p. 195—196° (decomp.), $[\alpha]_D^{18°}$ + 14·6° (*c*, 3·67 in water) (Found: N, 14·6; Cl, 37·2. $C_4H_{10}O_2N_2,2HCl$ requires N, 14·7; Cl, 37·2%).

Similar results were obtained when the preparation was conducted on a larger scale (1 g.-mol.).

dl-*Lysine*.—(a) *From α-aminopimelic acid*. (i) Hydrazoic acid (0·58 g.; 1·2 mols.) in chloroform (6 c.c.) was added gradually to a solution of α-aminopimelic acid (Dieckmann, *Ber.*, 1900, **33**, 597; Wolff, *Annalen*, 1890, **260**, 119) (2·0 g.) in sulphuric acid (9 c.c.) and chloroform (5 c.c.) stirred at 42—43°. After 3 hours, when evolution of nitrogen had ceased, the product was poured on ice, and the aqueous layer diluted, until the concentration of sulphuric acid was 5%, and treated with excess of 30% aqueous phosphotungstic acid. The precipitate supplied a solution of the amino-acid carbonate by the usual procedure of solution in 50% aqueous acetone, decomposition by baryta, removal of barium by carbon dioxide, and evaporation (compare Kossel and Patten, *loc. cit.*; Vickery and Leavenworth, *loc. cit.*). The final concentrate, which was free from barium, was divided into two parts. One part was evaporated to a thin syrup, alcoholic picric acid added, and the monopicrate (1·4 g.; 66% yield) collected and recrystallised from hot water. In agreement with previous observations (*e.g.*, Kossel, *Z. physiol. Chem.*, 1899, **26**, 586), the product had no definite m. p., but darkened above 215° and decomposed at *ca.* 235° (Found: C, 38·2; H, 4·5; N, 18·7. Calc. for $C_6H_{14}O_2N_2,C_6H_3O_7N_3$: C, 38·4; H, 4·5; N, 18·7%). The other portion, treated with hydrochloric acid (*ca.* 10%), followed by evaporation, gave the dihydrochloride (0·92 g.; 74% yield), m. p. 180—183°, raised to 187—189° by grinding with ethyl alcohol.

(ii) *dl*-Lysine, isolated as the dihydrochloride (55% yield) by the method outlined above, was obtained when sodium azide was added in small portions to a solution of α-aminopimelic acid in sulphuric acid and chloroform at 45°.

dl-*Lysine dipicrate*. *dl*-Lysine dihydrochloride (1·09 g.) in water (5 c.c.) and picric acid (2·5 g.; 2·2 mols.) in water (150 c.c.) were mixed and after 24 hours the yellow needles (2·88 g.; 96% yield) were recrystallised from a small volume of hot water and dried in a vacuum, m. p. 188—190° (sintering at 169°) (Found: C, 35·8; H, 3·3; N, 18·7. $C_6H_{14}O_2N_2,2C_6H_3O_7N_3$ requires C, 35·8; H, 3·3; N, 18·5%).

(b) *From ethyl* cyclo*hexanone*-2-*carboxylate*. (i) A slow stream of dry hydrogen chloride was passed for 3 hours through a solution of ethyl *cyclo*hexanone-2-carboxylate (10·5 g.) in benzene (108 c.c.) containing hydrazoic acid (3·7 g.; 1·4 mols.), cooled to 3° by stirring in an ice-bath. After 12 hours, concentrated hydrochloric acid (50 c.c.) was added, and the aqueous

layer separated, diluted with water (100 c.c.), and boiled under reflux for 3 hours. The gum obtained by evaporation was dissolved in water and divided into two parts. One part was warmed with copper carbonate, filtered while hot, and kept for several days; the blue crystals which separated, when decomposed by hydrogen sulphide, yielded impure α-aminopimelic acid, m. p. 215° (decomp.) (0·4 g.; 8% yield based on the ester used). The other part was warmed with lead carbonate, filtered, freed from lead by hydrogen sulphide, and evaporated. Addition of alcohol precipitated a white solid (2·4 g.), m. p. 265—275° (decomp.) (Found: N, 11·5%), which was readily soluble in water and gave a white precipitate with phosphotungstic acid. A solution of the substance (0·4 g.), mixed with excess of 1% aqueous picric acid, deposited *dl*-lysine dipicrate (0·6 g.; 18% yield), m. p. 183—186°, raised to 188—190° after recrystallisation from hot water; there was no depression in m. p. on admixture with the dipicrate prepared from *dl*-lysine dihydrochloride as described above.

(ii) Dry hydrogen chloride was led for 3 hours through a solution of ethyl *cyclo*hexanone-2-carboxylate (34 g.) in chloroform (150 c.c.) containing hydrazoic acid (10·1 g.; 1·2 mols.) at 3—5°. After a further 3 hours' stirring, a second portion of hydrazoic acid (1·2 mols.) was added, and the hydrogen chloride treatment repeated for 3 hours. Concentrated hydrochloric acid (100 c.c.) was then added, and the aqueous layer separated, diluted with water (200 c.c.), and boiled under reflux for 2 hours (charcoal added towards the end). Evaporation below 60° left a brown gum (34 g.), which was dissolved in water (100 c.c.) and added to a hot solution of picric acid (39 g.) in water (1·5 l.). After standing overnight in the ice-chest, the dipicrate (30 g.; 25% yield), m. p. 183—187°, was collected and dried in a vacuum. A portion was converted into the dihydrochloride, m. p. 188—190° (Found: N, 12·6; Cl, 32·4. $C_6H_{14}O_2N_2$,2HCl requires N, 12·8; Cl, 32·5%). Eck and Marvel (*loc. cit.*) record m. p. 188—190°; other values in the literature cover the range 183° to 186°.

Benzoylation of the dihydrochloride furnished *NN*-dibenzoyl-*dl*-lysine, m. p. 144—145° (Found: C, 67·9; H, 6·5; N, 7·7. Calc. for $C_{20}H_{22}O_4N_2$: C, 67·8; H, 6·2; N, 7·9%). Fischer and Weigert (*loc. cit.*) record m. p. 145—146°. *dl*-Lysine methyl ester dihydrochloride, prepared by the usual methods, had m. p. 217—218° (compare Fischer and Weigert, *loc. cit.*, m. p. 218°).

(iii) Ethyl *cyclo*hexanone-2-carboxylate (28·4 g.) in chloroform (125 c.c.) containing hydrazoic acid (8 g.; 1·15 mols.) was stirred below 5° in a stream of hydrogen chloride for 3 hours. Concentrated hydrochloric acid (150 c.c.) was added, and the aqueous layer separated, diluted with water (150 c.c.), boiled under reflux for 2 hours, and evaporated under reduced pressure. The residual gum was dissolved in concentrated sulphuric acid (60 c.c.) and chloroform (50 c.c.) at 45°. A solution of hydrazoic acid (8 g.; 1·15 mols.) in chloroform (75 c.c.) was added in 5 c.c. portions during 6 hours. After evolution of nitrogen and carbon dioxide had ceased (8 hours in all), the mixture was stirred overnight at room temperature. The product was poured on ice, the aqueous layer separated, and hot saturated baryta added until the solution was only weakly acidic. Barium sulphate was removed by centrifugation, and picric acid (53 g.) in hot water (1 l.) added to the supernatant liquid (1·2 l.). After standing overnight in the ice-chest, the dipicrate (60·5 g.; 60% yield), m. p. 184—187°, was collected and dried in a vacuum over sulphuric acid. Decomposition by hydrochloric acid yielded the pure dihydrochloride (19·6 g.; 54% yield).

In an earlier experiment on the same scale, a solution of the carbonate was obtained by the phosphotungstate separation and aliquot portions furnished the monopicrate (52% yield) and the dihydrochloride (54% yield).

(iv) Ethyl *cyclo*hexanone-2-carboxylate, dissolved in concentrated sulphuric acid and treated in the usual manner with hydrazoic acid (2·2 mols.) in chloroform, yielded only a trace of *dl*-lysine dipicrate.

dl-*Ornithine.*—(a) *From α-aminoadipic acid.* Hydrazoic acid (0·45 g.; 1·2 mols.) in chloroform (5 c.c.) was gradually added to a stirred solution of α-aminoadipic acid (Dieckmann, *loc. cit.*; Wolff, *loc. cit.*) in sulphuric acid (7 c.c.) at 45°; nitrogen and carbon dioxide were evolved. The product, treated as in the case of *dl*-lysine in (a, i) above, yielded a solution of *dl*-ornithine carbonate, which was neutralised with 10% sulphuric acid and evaporated on the steam-bath.

dl-Ornithine sulphate (1·2 g.; 75% yield) was obtained in white plates, m. p. 223° (decomp.), from hot 60% aqueous alcohol (Found: N, 15·5; S, 8·8. Calc. for $2C_5H_{12}O_2N_2,H_2SO_4$: N, 15·4; S, 8·8%). The m. p.'s cited in the literature range from 213° to 234°.

NN-Dibenzoyl-*dl*-ornithine, m. p. 186—187° (Found: C, 67·1; H, 6·0; N, 8·4. Calc. for $C_{19}H_{20}O_4N_2$: C, 67·1; H, 5·9; N, 8·2%), was isolated from a portion of the product by benzoylation (compare Fischer, *loc. cit.*, m. p. 187—188°).

dl-Ornithine sulphate (1·74 g.) in water (20 c.c.) was added to picric acid (4·6 g.) in water

(300 c.c.). After 36 hours the precipitate of *dl*-ornithine dipicrate was collected and dried in a vacuum. Yield, 4·97 g. (93%); m. p. 195—197° (decomp.), raised to 198—200° (decomp. at 209°) by recrystallisation from hot water (Found: C, 34·8; H, 3·0; N, 19·3. Calc. for $C_5H_{12}O_2N_2,2C_6H_3O_7N_3$: C, 34·6; H, 3·1; N, 19·0%) [Kossel and Weiss, *Z. physiol. Chem.*, 1910, **68**, 163, and Vickery and Cook, *J. Biol. Chem.*, 1931, **94**, 393, record m. p. 195° and 208° (decomp.) respectively].

(b) *From ethyl* cyclo*pentanone-2-carboxylate.* (i) The ester (31·2 g.) was submitted to precisely the same treatment as described for the preparation of *dl*-lysine in expt. (b, iii) above, proportionate quantities of materials being used. The product was poured on ice (200 g.), and the aqueous layer separated. The solution was rendered weakly acidic by addition of hot saturated baryta, barium sulphate removed by centrifugation, and picric acid (60 g.) in hot water (1·5 l.) added to the supernatant liquid. After 36 hours, the dipicrate (47 g.; 40% yield) was separated by filtration and dried in a vacuum.

(ii) In an earlier experiment 23·4 g. of the ester were employed, and isolation effected by the phosphotungstic acid separation (Kiesel, *Z. physiol. Chem.*, 1921, **118**, 254). The resulting carbonate solution was neutralised with 10% sulphuric acid and evaporated to dryness. *dl*-Ornithine sulphate (9·5 g.; 35% yield), m. p. 223°, was obtained from the residual paste by stirring with ethyl alcohol, filtration, and drying in a vacuum.

Glycine.—(i) Hydrazoic acid (2·4 g.; 1·1 mols.) in chloroform (35 c.c.) was added gradually during 2 hours to a solution of malonic acid (5·2 g.) in sulphuric acid (15 c.c.) at 40°. After 8 hours, when approximately 1 mol. of nitrogen had been evolved, the product was poured on ice (100 g.), and the aqueous layer separated and treated with hot 25% baryta solution until neutral to litmus. After removal of barium sulphate by filtration, concentrated hydrochloric acid (10 c.c.) was added, and the liquid evaporated to dryness under reduced pressure. Ethyl alcohol (20 c.c.) was added to the yellow residue, the mixture saturated with dry hydrogen chloride and boiled under reflux for 1 hour, and the liquid filtered through a sintered glass funnel. Glycine ethyl ester hydrochloride (1·7 g.; 25% yield), m. p. 143—144°, separated on cooling, and a further quantity (0·3 g.; 4%) was obtained from the mother-liquor.

3 : 5-Dinitrobenzoylglycine (Saunders, *Biochem. J.*, 1934, **28**, 580), prepared from a portion of the product, had m. p. 178°, not depressed by admixture with an authentic specimen.

(ii) By conducting the reaction at 50°, glycine ethyl ester hydrochloride was isolated as in (i) in a yield of 46%.

The author wishes to thank Sir Robert Robinson, F.R.S., and Dr. James Walker for their interest in the work, and Imperial Chemical Industries, Ltd., for permission to publish the results.

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