7. *The Synthesis of Amino-acids from Substituted Cyanoacetic Esters.*

By Paul E. Gagnon, Roger Gaudry, and F. E. King.

The synthesis of amino-acids from substituted cyanoacetic esters by the Curtius reaction (Darapsky, *J. pr. Chem.*, 1936, **146**, 250) has been successfully applied to the preparation of valine, phenylalanine, and tyrosine.

$$\underset{\text{(I.)}}{R\cdot CH(CN)\cdot CO_2Et} \longrightarrow \underset{\text{(II.)}}{R\cdot CH(CN)\cdot NH\cdot CO_2Et} \longrightarrow \underset{\text{(III.)}}{R\cdot CH(CO_2H)\cdot NH_2}$$

The failure of experiments to extend the method, for example, to the synthesis of the diamino-acids ornithine and lysine from ethyl α-cyanoadipate and ethyl α-cyanopimelate, indicates that it is not of wide application.

It has been shown that amino-acids may be obtained by applying the Curtius reaction to the monohydrazides of the dicarboxylic acids (Curtius, *J. pr. Chem.*, 1930, **125**, 211), but the preparation of these acid hydrazides is laborious and constitutes a disadvantage of the method. For the synthesis of α-amino-acids, Darapsky (*ibid.*, 1936, **146**, 250) has employed the more accessible alkylcyanoacetic esters (I), which readily afford monohydrazides. These undergo degradation in the usual manner, hydrolysis at the urethane stage (II) also affecting the cyano-group and thus giving the amino-acid (III).

Although apparently of general application, the synthesis has been used to prepare only three amino-acids, namely, leucine, α-amino-*n*-valeric acid, and α-amino-δ-methylhexoic acid (Darapsky, *loc. cit.*). The following experiments were undertaken in order to extend it to amino-acids of other types.

Experience of the reaction was gained by the synthesis of valine (III, R = $CHMe_2$). For this, α-cyano-β-methylbutyrhydrazide was converted by nitrous acid into the azide and thence into the ethyl urethane by heating with alcohol. Hydrolysis with boiling hydrochloric acid afforded *dl*-valine, characterised by the *phenylcarbamyl* derivative, $Ph\cdot NH\cdot CO\cdot NH\cdot CH(CO_2H)\cdot CHMe_2$, which was obtained by reaction with phenyl *iso*cyanate and alkali.

By the same sequence of reactions phenylalanine (III, R = CH_2Ph) was conveniently obtained from *α-cyano-β-phenylpropionhydrazide*, and the amino-acid was analysed in the form of its *phenylcarbamyl* derivative. Similarly, the *hydrazide* of α-cyano-β-anisylpropionic acid gave *dl*-tyrosine when the cyanourethane was hydrolysed by mixed concentrated hydrochloric and acetic acids; with diluted acid, *O*-methyltyrosine was the product.

For attempting the synthesis of ornithine, γ-phenoxypropyl bromide was condensed with ethyl cyanoacetate, and the *hydrazide* of the resulting α-cyano-δ-phenoxyvaleric acid degraded by the standard procedure.

The final hydrolysis with hydrochloric acid gave *α-amino-δ-phenoxyvaleric acid* (III, R = $PhO{\cdot}CH_2{\cdot}CH_2{\cdot}CH_2$) : more drastic reagents, *e.g.*, hydrobromic acid, destroyed the cyanourethane.

The possibility that cyanodicarboxylic acids might yield diamino-acids by the Curtius reaction was investigated with the *dihydrazides* prepared from ethyl cyanoadipate and from its homologue ethyl cyanopimelate. From both, diurethanes appeared to be formed in the usual way, but these were destroyed by the hydrolytic agent.

Ethyl αβ-dicyanopropionate, the appropriate ester for the synthesis of aspartic acid, gave only resinous products with hydrazine, and it therefore seems unlikely that the Darapsky method can afford amino-dicarboxylic acids.

Experimental.

dl-*Valine*.—Hydrazine hydrate (24 g. of 100%) reacted exothermally with ethyl *iso*propylcyanoacetate (Fischer, *Ber.*, 1909, **42**, 2983) (92 g.) to give a pale yellow syrup. This was set aside in an evacuated desiccator to remove alcohol, but could not be crystallised. A solution of the crude hydrazide (46 g.) in hydrochloric acid (400 c.c. of 25%) was cooled to 0° and stirred under a layer of ether (200 c.c.) while sodium nitrite (34 g.), dissolved in water (50 c.c.), was added. The dried ethereal layer was then mixed with absolute alcohol (300 c.c.) and heated on a water-bath under a short fractionating column until the ether had distilled. After boiling for 1 hour to complete the evolution of nitrogen, alcohol was removed under reduced pressure, and the syrupy product heated under reflux with hydrochloric acid (300 c.c. of 20%) in an oil-bath for 48 hours. The straw-coloured solution was then evaporated under diminished pressure, and the residue taken up in water and neutralised with ammonia. The addition of an equal volume of alcohol precipitated *dl*-valine, which was collected on the next day and recrystallised from water (22—23 g.; 60% yield). A crystalline derivative was prepared by dissolving the amino-acid in an equivalent of aqueous sodium hydroxide and shaking with the calculated amount of phenyl *iso*cyanate. The filtered solution was acidified, and this precipitated dl-α-N-*phenylcarbamido*isovaleric acid, which formed colourless thin leaflets, m. p. 149°, after crystallisation from aqueous alcohol (Found : N, 12·0. $C_{12}H_{16}O_3N_2$ requires N, 11·9%).

Ethyl α-Cyano-β-phenylpropionate (cf. Hessler, *Amer. Chem. J.* 1899, **22**, 169 : Walker, J., 1924, **125**, 1622).—Ethyl cyanoacetate (65 g. ; 2 mols.) and benzyl bromide (50 g.) were dissolved in a solution of sodium (6·7 g.) in absolute alcohol (125 c.c.), which was refluxed on a steam-bath for 2 hours. After removal of the alcohol the product was shaken with water and ether, and the dried ethereal extract evaporated and fractionated. The portion, b. p. 160—188°/15 mm., was redistilled, and the α-cyano-β-phenylpropionic ester, b. p. 165—173°/15 mm. (26 g. ; 44% yield), mixed with hydrazine hydrate (6·5 g. of 100%). After some minutes a vigorous reaction set in, and when cool the product solidified. Recrystallisation from alcohol gave the *hydrazide* (21 g.) in shining plates, m. p. 123—124° (Found : C, 63·7; H, 5·8. $C_{10}H_{11}ON_3$ requires C, 63·5; H, 5·8%). From the ester fractionation, ethyl α-cyano-ββ-dibenzylacetate (10 g.), b. p. 190—200°/15 mm., was isolated; it was identified by means of the *hydrazide*, m. p. 235—237°, which crystallised from alcohol in microscopic prisms (Found : C, 72·9; H, 6·0. $C_{17}H_{17}ON_3$ requires C, 73·1; H, 6·1%).

dl-*Phenylalanine*.—α-Cyano-β-phenylpropionhydrazide (10 g.), in ice-cold water (50 c.c.) and concentrated hydrochloric acid (75 c.c.) covered by ether (100 c.c.), was converted into the azide by addition of sodium nitrite (8 g.) in a little water. The dried ethereal solution of the product was added to alcohol (100 c.c.), which was heated to expel the ether, then refluxed for an hour, and finally evaporated. The residue (11 g.) was hydrolysed by refluxing (oil-bath) for 48 hours with hydrochloric acid (200 c.c. of 20%), and the material obtained by evaporation of the filtered liquid (charcoal) under reduced pressure was dissolved in water (10 c.c.). The solution was brought to the isoelectric point (pH = 5·9) with aqueous ammonia, and after an equal volume of alcohol had been added, phenylalanine (4 g., 50% yield) separated, m. p. 265° when crystallised from water. The *phenylcarbamyl* derivative, prepared from an alkaline solution of the amino-acid and phenyl *iso*cyanate, separated from aqueous alcohol as a white powder, m. p. 168—170° (Found : C, 67·9; H, 5·6. $C_{16}H_{16}O_3N_2$ requires C, 67·6; H, 5·6%).

Ethyl α-Cyano-β-anisylpropionate.—Ethyl cyanoacetate (80 g. ; 2 mols.) and anisyl chloride (55 g.) were dissolved in a solution of sodium (8·1 g.) in alcohol (150 c.c.) heated for 3 hours under reflux on a steam-bath, and the oil obtained by pouring into water was collected in ether and distilled. The fraction (39 g. ; 48% yield), b. p. 165—170°/0·2 mm., was treated with hydrazine hydrate (1 mol. of 100%), and after leaving overnight the solid *hydrazide* (36 g.) was obtained, m. p. 122—123° when crystallised from alcohol (Found : N, 19·5. $C_{11}H_{13}O_2N_3$ requires N, 19·2%).

dl-*Tyrosine*.—α-Cyano-β-anisylpropionhydrazide (36 g.) reacted with nitrous acid under the conditions given for the preparation of phenylalanine, except that the formation of the hydrazide was slower. When the viscous urethane obtained by boiling with alcohol was refluxed for 40 hours with 20% hydrochloric acid and the solution neutralised, the sparingly soluble *dl-O*-methyltyrosine (yield, 30%) was obtained (negative Millon test).

Refluxing the urethane (36 g.) with concentrated hydrochloric acid (300 c.c.) and glacial acetic acid (300 c.c.) gave a dark solution, which was then evaporated. The residue was shaken with water, and the insoluble matter removed, addition of ammonia to the charcoaled solution precipitating *dl*-tyrosine in characteristic woolly needles at pH 5·7 (Found after five crystallisations and drying under reduced pressure over phosphoric oxide : C, 59·5; H, 6·6. Calc. for $C_9H_{11}O_3N$: C, 59·6; H, 6·1%) (yield, 3·3 g. ; 11%).

Ethyl α-Cyano-δ-phenoxyvalerate.—Ethyl cyanoacetate (57 g.) and γ-phenoxypropyl bromide (108 g.) were heated in a solution of sodium (11·5 g.) in alcohol (300 c.c.) under reflux on a steam-bath for 1 hour. The crude cyanophenoxyvalerate (50 g. ; 40%), b. p. 175—190°/0·7 mm., was isolated by fractionating the oily product obtained on pouring into water. A mixture with hydrazine hydrate (1 mol. of 100%) solidified on cooling, and by crystallisation from alcohol the *hydrazide* of α-cyano-δ-phenoxyvaleric acid formed colourless minute needles, m. p. 85°, turning pink in air (Found : C, 61·7; H, 6·4. $C_{12}H_{15}O_2N_3$ requires C, 61·8; H, 6·4%).

α-Amino-δ-phenoxyvaleric Acid.—Conversion of the hydrazide of α-cyano-δ-phenoxyvaleric acid into the syrupy urethane followed the general procedure. The product (20 g.) was refluxed with hydrochloric acid (200 c.c. of 20%), and the filtered solution (charcoal) evaporated. The residue was dissolved in water (10 c.c.) and neutralised with ammonia, whereupon dl-*α-amino-δ-phenoxyvaleric acid* (7 g. ; 40% yield) was precipitated. It was recrystallised from boiling water, and separated as a white powder, m. p. 265—267° (decomp.) (Found : C, 63·2; H, 7·1. $C_{11}H_{15}O_3N$ requires C, 63·1; H, 7·2%). The action of phenyl *iso*cyanate on a concentrated alkaline solution of the acid gave a *phenylureide*, which separated from aqueous alcohol as a micro-crystalline powder, m. p. 158° (Found : N, 8·5. $C_{18}H_{20}O_4N_2$ requires N, 8·5%).

Ethyl α-Cyanoadipate.—The condensation of ethyl γ-bromobutyrate (40 g.) and ethyl cyanoacetate (25 g.) in a solution of sodium (4·8 g.) in alcohol (100 c.c.) refluxing on a steam-bath for 2 hours gave, on pouring into water, an oil, from which the fraction (19 g. ; 40% yield), b. p. 178—186°/15 mm., was isolated. It formed with hydrazine hydrate a syrup which eventually solidified and was crystallised, though with appreciable loss, from alcohol. The pure *cyanoadipodi-*

hydrazide, m. p. 128°, was very soluble in water (Found : N, 34·9. $C_7H_{13}O_2N_5$ requires N, 35·2%). It was submitted to the sequence of operations necessary to produce the corresponding diurethane, but when the viscous product was heated (oil-bath) under reflux for 48 hours with hydrochloric acid (20%), only amorphous material was obtained from which no crystalline derivatives (dibenzoate, dipicrate) could be prepared.

Ethyl α-Cyanopimelate.—A mixture of ethyl δ-bromovalerate (35 g.) with ethyl cyanoacetate (37 g.; 2 mols.), heated in a solution of sodium (3·9 g.) in alcohol (100 c.c.) at 100° for 3 hours, gave, after the appropriate treatment, a colourless oil (21 g.; 30% yield), b.p. 183—197°/12 mm., from which was prepared *cyanopimelodihydrazide*. It separated slowly from a fairly large volume of alcohol as a white powder, m. p. 115—116° (Found : N, 33·0. $C_9H_{15}O_2N_5$ requires N, 32·9%). As with its lower homologue, degradation *via* azide and urethane yielded only resinous material.

Ethyl αβ-dicyanopropionate (Thorpe, J., 1906, **89**, 1461) reacted violently with hydrazine hydrate (50%), giving a brown resin, soluble in dilute mineral acid but insoluble in organic solvents. A similar product slowly formed when the reaction was conducted at 0° in alcoholic solution, which was afterwards evaporated in an evacuated desiccator. The ester and aqueous hydrazine (6%) were therefore cautiously mixed and cooled, and the solution then acidified and treated with nitrite under ether in the usual way. A dark flaky solid separated insoluble in ether and not showing any properties of an azide.

The authors thank the Rhodes Trustees for a Scholarship awarded to one of them (R. G.).

LAVAL UNIVERSITY, QUEBEC.
DYSON PERRINS LABORATORY, OXFORD.

[*Received, August 25th*, 1943.]