I. ISOMERIC PHENYLSERINES.[1]

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From several quarters in recent years currency has been given to the suggestion that the protein molecule does not depend entirely on the polypeptide type of anhydride-structure; the diketopiperazine ring is now recognised as a probable unit in the aggregation of groups. Notably Abderhalden has demonstrated in proteins the presence of preformed diketopiperazines, and the production of picrorocellin by an organism so lowly as a lichen gains thereby an added interest.

In representing picrorocellin by a structural formula (Forster and Saville, J., 1922, **121**, 818), the position of the *N*-methyl group remained uncertain, and the most promising method of justifying the constitution

$$C_6H_5\cdot CH(O\cdot CH_3)\cdot CH\cdot N(CH_3)\cdot CO$$
$$\qquad\qquad\qquad\qquad\qquad\qquad\quad |\qquad\quad |$$
$$\qquad\qquad\qquad\qquad\qquad\quad CO—NH—CH\cdot CH(OH)\cdot C_6H_5$$

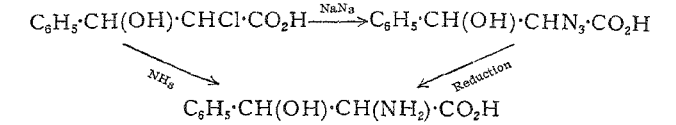
appeared to be synthesis from the appropriate amino-acids. For the above constitution, these would be α-methylamino-β-methoxy-β-phenylpropionic acid, $C_6H_5\cdot CH(O\cdot CH_3)\cdot CH(NH\cdot CH_3)\cdot CO_2H$, and α-amino-β-hydroxy-β-phenylpropionic acid (phenylserine), $C_6H_5\cdot CH(OH)\cdot CH(NH_2)\cdot CO_2H$, but although the latter compound is readily available we have failed to convert it into the corresponding diketopiperazine, of which picrorocellin is the *ON*-dimethyl derivative. Nevertheless, as will be shown later, there is evidence that a diketopiperazine is formed when phenylserine is heated at the temperature of decomposition.

While accumulating the phenylserine required for these experiments, we have encountered an isomeride which appears to have escaped notice, or, if recognised, to have been wrongly described as phenyl*iso*serine. Phenylserine was first prepared by Erlenmeyer, jun. (*Ber.*, 1892, **25**, 3445; Erlenmeyer and Früstück, *Annalen*, 1894, **284**, 36; Erlenmeyer, *ibid.*, 1899, **307**, 84), as the benzylidene derivative arising from glycine condensed with benzaldehyde in aqueous-alcoholic sodium hydroxide; the free amino-acid was observed anhydrous and hydrated, decomposing at the m. p., which was given variously as 196°, 195–196°, and 190° in the former condition and 193–194° or 192–193° in the latter. In the last of the above-quoted papers, Erlenmeyer claims to have recognised in one of his experiments a second form of the acid, decomposing at 187–188°; but the substance was not

[1] Reprinted from the *Journal of the Chemical Society*, 1926, 1943.

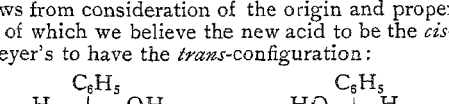
analysed, and further reference to it has not been made by any other investigator. Phenylserine was more recently obtained by Rosemund and Darnsaft (*Ber.*, 1919, **52**, 1734) from glycine ester and benzaldehyde with sodium in ether, and was stated to melt at 192°. Using Erlenmeyer's process, we obtained anhydrous phenylserine with m.p. 200–202° (decomposition).

Owing to initial difficulties in applying this method, we meanwhile prepared phenylserine by reducing α-triazo-β-hydroxy-β-phenylpropionic acid (Forster and Saville, J., 1922, **121**, 2600) with ammonium sulphide. Thus obtained, the amino-acid was quite distinct from Erlenmeyer's, having m. p. 230–232° (decomposition) when anhydrous, and 213° in the hydrated form. Subsequently, the same acid was produced by the action of concentrated ammonia on cinnamic acid chlorohydrin:



Phenylserine.

The production of two distinct individuals finds a simple explanation in the two dissimilar centres of asymmetry possessed by phenylserine. As represented above, this amino-acid may occur in two racemic forms, each comprising an optically active antipodal pair. Allocation of the appropriate configuration to the isomeric phenylserines follows from consideration of the origin and properties of these compounds, of which we believe the new acid to be the *cis*-modification and Erlenmeyer's to have the *trans*-configuration:



cis-Phenylserine, m. p. 230—232°. *trans*-Phenylserine, m. p. 200–202°.

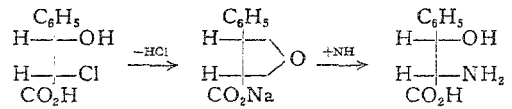
Among the considerations leading to this conclusion is the fact that although addition of chlorine and bromine to cinnamic acid produces externally compensated stereoisomerides (*Ber.*, 1894, **27**, 2039; 1895, **28**, 2235), the action of hypochlorous and hypobromous acids appears to be unidirectional (Read and Andrews, J., 1921, **119**, 1775). At no stage in the sequence, cinnamic acid: chlorohydrin: triazohydrin: phenylserine, is there any experimental evidence of more than one racemic dihydrocinnamic acid derivative being formed.

It is thus natural to expect that the hydroxyl and amino-groups in the phenylserine from this source will be found to have the *cis*-relationship, and this is established by comparing the properties of the two isomerides. Erlenmeyer, for instance, heated his phenylserine with benzoic anhydride and obtained the 'benzoylaminocinnamic acid lactimide' (yellow, m. p. 164°) insoluble in sodium hydroxide and identical with the product of condensing benzaldehyde with hippuric acid:

$$C_6H_5\cdot CHO + H_2C(CO_2H)\cdot NHBz \longrightarrow C_6H_5\cdot CH{:}C\begin{array}{l}\text{——NBz.}\\ \backslash CO/\end{array}$$

We find that the same substance is produced from Erlenmeyer's acid by a much milder method, namely, action of benzoyl chloride suspended in sodium carbonate solution, thus emphasising the surprising facility with which removal of water takes place when favoured by the *cis*-relationship of the hydrogen atom and the hydroxyl group in *trans*-phenylserine. On the other hand *cis*-phenylserine by the same process yields an undehydrated benzoyl derivative (colourless: m. p. 197°) which is freely soluble in sodium carbonate and fails to pass into the lactimide; the latter was not formed even on heating *cis*-phenylserine with benzoic anhydride. Similarly, the *O*-methyl derivative of *cis*-phenylserine, prepared by reducing the corresponding triazo-compound (Forster and Saville, *loc. cit.*), readily yields a benzoyl derivative (colourless, m. p. 208°) which dissolves in sodium carbonate and resists conversion into the lactimide.

Further support to the foregoing representation of the isomeric phenylserines follows from the action of ammonia on sodium phenyloxyacrylate, arising from cinnamic acid chlorohydrin by removal of hydrogen chloride:



Cinnamic chlorohydrin. Phenyloxyacrylate. *cis*-Phenylserine.

Erlenmeyer (*Annalen*, 1892, **271**, 155) erroneously ascribed to the product of this action the constitution of phenyl*iso*serine, $C_6H_5\cdot CH(NH_2)\cdot CH(OH)\cdot CO_2H$, on the ground that its properties differed from those of the phenylserine he obtained from glycine and benzaldehyde. He described it as melting at 220–221°, and announced his intention of confirming the constitution by reducing *iso*nitrosophenylpyruvic acid.

$C_6H_5{\cdot}C(:NOH){\cdot}CO{\cdot}CO_2H \longrightarrow C_6H_5{\cdot}CH(NH_2)\ CH(OH){\cdot}CO_2H.$

As the matter does not appear to have been carried further, however, we can only conclude that phenyl*iso*serine may be erased from the literature, the substance described under that name being incompletely purified *cis*-phenylserine. Erlenmeyer's whole treatment of the subject is most bewildering. In a final paper with Barkow (*Ber.*, 1906, **39**, 793) he states that 'phenyl*iso*serine,' as obtained by the action of cold ammonia on cinnamic acid chlorohydrin, decomposes at 241°, and that sodium phenyloxyacrylate when heated with ammonia yields only the phenyl*iso*serine melting at 220–221°. He leaves the reader to suppose that he regards them as distinct, but at the conclusion of the paper he refers to the less fusible substance as 'phenylserine,' stating that the corresponding active compound has not hitherto been obtained crystalline; he then records a rotation for the copper salt without indicating how the substance was resolved, announcing that detailed communication would follow in another place. We have searched in vain for this communication, although fifteen years elapsed before the author's death.

During the course of these experiments we have made several attempts to prepare α-triazocinnamic acid, $C_6H_5{\cdot}CH{:}CN_3{\cdot}CO_2H$, but without success. Knowing the close attachment of halogen to unsaturated carbon, we did not expect directly to replace chlorine or bromine in α-chloro- or α-bromocinnamic acid by the triazo-group, but until the configuration of *cis*-phenylserine was appreciated it did seem possible to remove the elements of water from α-triazo-β-hydroxy-β-phenylpropionic acid, and persistent failure to accomplish this provides additional evidence in favour of the *cis*-configuration. The result, nevertheless, is most disappointing, because α-triazocinnamic acid, by the ammonium sulphide method of reduction, might conceivably yield α-aminocinnamic acid, belonging to a class of substances which have hitherto eluded all attempts to prepare them.

EXPERIMENTAL.

cis-α-*Amino-β-hydroxy-β-phenylpropionic Acid* (cis-*Phenylserine*).—(*a*) *From* α-*triazo-β-hydroxy-β-phenylpropionic acid.* The triazo-derivative (10 g.) dissolved in dilute ammonia was treated with excess of ammonium sulphide, freshly prepared, when a transient, greenish-black precipitate was formed and almost immediately dissolved. The temperature rose, gas was liberated freely, and the colour diminished. At the conclusion of effervescence, the liquid was evaporated to dryness and the product dissolved in water, filtered from sulphur, again evaporated to dryness, and acidified with dilute acetic acid. Final evaporation left a colourless residue of the amino-acid with ammonium

acetate which was removed by 95 per cent. alcohol, cis-*phenylserine* (7·5g.) remaining as a white powder. It is moderately easily soluble in water and is best purified by precipitation with absolute alcohol from a saturated aqueous solution, crystallising in clustered needles, m. p. 230–232° (decomp. Found: N, 7·8. $C_9H_{11}O_3N$ requires N, 7·7 per cent.). Slow separation from the aqueous alcohol yields the hydrated form, m. p. 213°, and the yellow, resinous material arising from decomposition at the higher temperature is freely soluble in alcohol, but does not yield a diketopiperazine in crystalline form. A concentrated aqueous solution of the amino-acid, when boiled with copper carbonate, gives a sparingly soluble blue salt.

(*b*) *From cinnamic acid chlorohydrin.* The chlorohydrin (5 g.) was shaken with concentrated ammonia until dissolved, and set aside during one week. The residue left on evaporation was dissolved in dilute acetic acid, and the pasty residue from evaporation of this liquid was extracted with 95 per cent. alcohol, which left *cis*-phenylserine (3 g.) undissolved. Purified as above, the product was identical with the foregoing according to the unaltered m. p. of a mixture, and of the mixed benzoyl derivatives (see below).

(*c*) *From sodium phenyloxyacrylate.* The chlorohydrin was treated with excess of alcoholic sodium hydroxide, and the precipitated sodium phenyloxyacrylate separated from sodium chloride by recrystallisation from aqueous alcohol, which deposited lustrous, colourless needles. The salt was shaken with excess of concentrated ammonia and set aside during two weeks, after which the solution was treated as in the foregoing cases. The m. p. (230–232°) was not depressed by admixture with the previous preparations.

The N-*benzoyl* derivative was prepared by shaking with benzoyl chloride (12 g.) a solution of *cis*-phenylserine (3 g.) in water (30 c.c.) containing sodium bicarbonate (15 g.) at intervals during two days and then heating on the water-bath during 30 minutes. The colourless benzoyl derivative precipitated by dilute hydrochloric acid was freed from benzoic acid by repeated extraction with hot petroleum, and was recrystallised from dilute alcohol; m. p. 197° (Found: N, 5·0. $C_{16}H_{15}O_4N$ requires N, 4·9 per cent.). It is soluble in aqueous sodium carbonate, and is thus distinguished from the yellow benzoyl compound arising from *trans*-phenylserine, this being insoluble in sodium hydroxide.

The O-*methyl* derivative was prepared by reducing α-triazo-β-methoxy-β-phenylpropionic acid with ammonium sulphide and proceeding as described in the case of *cis*-phenylserine itself. The product is readily soluble in water, and on rapid precipitation from a concentrated solution by absolute alcohol forms a white powder, m. p. 227–232°

(decomp.). When allowed to evaporate slowly, the solutions deposit slender, colourless prisms which change to rhombic plates during 48 hours; m. p. 215–216° (Found: N, 6·2. $C_{10}H_{13}O_3N,2H_2O$ requires N, 6·1 per cent. After one week in the desiccator: N, 6·8. $C_{10}H_{13}O_3N$, H_2O requires N, 6·6 per cent. Dried at 100°: N, 7·3. $C_{10}H_{13}O_3N$ requires N, 7·2 per cent). The copper salt crystallises from boiling water in bluish-violet prisms.

The *O-methyl-N-benzoyl* derivative of *cis*-phenylserine was prepared by benzoylating the foregoing substance, and crystallises from alcohol in short, thick needles, m. p. 208° (Found: N, 4·8. $C_{17}H_{17}O_4N$ requires N, 4·7 per cent). It dissolves in cold sodium carbonate solution.

The *ethyl ester picrate* separated on mixing the hydrochloride with picric acid (1 mol.), both previously dissolved in hot water, and crystallised from dilute alcohol in yellow needles, m p. 170° (Found: N, 12·9. $C_{17}H_{18}O_{10}N_4$ requires N, 12·8 per cent.).

The *ethyl ester picrate* of the *O*-methyl derivative crystallises from dilute alcohol in yellow needles, m. p. 155° (Found: N, 12·6. $C_{18}H_{20}O_{10}N_4$ requires N, 12·4 per cent.).

The *amide* of *cis*-phenylserine, $C_6H_5{\cdot}CH(OH){\cdot}CH(NH_2){\cdot}CO{\cdot}NH_2$, was prepared by agitating the ethyl ester hydrochloride with concentrated ammonia until completely dissolved; crystals began to separate soon afterwards and were filtered off after 48 hours. It is readily soluble in boiling water, which, on cooling, deposits elongated, rectangular, transparent prisms, m. p. 199–200° (Found: N, 15·7. $C_9H_{12}O_2N_2$ requires N, 15·6 per cent). The amide is soluble in alcohol and is stable towards cold alkali hydroxide, in which it loses ammonia freely on boiling. Fusion is followed by liberation of ammonia and results in a yellow, alcohol-soluble resin; but it was not found possible to isolate a crystalline diketopiperazine from this.

Condensation of Glycine with Benzaldehyde.—For comparison with the new acid, *trans*-phenylserine (Erlenmeyer's) was prepared by condensing glycine with benzaldehyde. By heating these two substances at 130°, Curtius and Lederer (*Ber.*, 1886, **19**, 2462) had obtained benzylamine by an intramolecular change of the initial product, but attempts to effect combination in alcoholic solution have been uniformly unsuccessful. Erlenmeyer found, however, that in presence of sodium hydroxide the condensation proceeds rapidly, yielding

$$C_6H_5{\cdot}CH(OH){\cdot}CH{\cdot}CO_2Na \quad \text{and} \quad C_6H_5{\cdot}CH(OH){\cdot}CH{\cdot}C_6H_5$$
$$\qquad\qquad\quad N{:}CH{\cdot}C_6H_5 \qquad\qquad\qquad\qquad\quad N{:}CH{\cdot}C_6H_5;$$

both compounds lose benzaldehyde when treated with acetic acid, the products being *trans*-phenylserine and diphenylhydroxyethylamine, respectively.

On first attempting to prepare phenylserine by Erlenmeyer's method we repeatedly failed, the product being uniformly the latter of the above two substances. The reason for this was then found to be the fact that if sodium hydroxide is present in a hot, alcoholic suspension of the former substance this passes at varying speeds into the latter. This observation was made also by Erlenmeyer, who gives an explanation (*Annalen*, 1899, **307**, 117) based on (*a*) disruption of the benzylidenephenylserine molecule into benzaldehyde and benzylideneglycine, (*b*) molecular rearrangement of the latter, (*c*) re-condensation of the resulting Schiff's base, $C_6H_5{\cdot}CH_2{\cdot}N{:}CH{\cdot}CO_2Na$, with benzaldehyde and (*d*) removal of sodium glyoxylate. The following alternative explanation, however, has the advantage of simplicity:

$$CHPh(OH){\cdot}CH(CO_2H){\cdot}N{:}CHPh \rightarrow CO_2 + CHPh(OH){\cdot}CH_2{\cdot}N{:}CHPh$$
$$\rightarrow CHPh(OH){\cdot}CHPh{\cdot}N{:}CH_2 \rightarrow CH_2O + CHPh(OH){\cdot}CHPh{\cdot}NH_2.$$

As described by Erlenmeyer, the method makes no provision for this occurrence, and we have modified it accordingly as follows: Glycine (3·7 g.) dissolved in water (20 c.c.) was mixed with alcohol (10 c.c.) and benzaldehyde (10·6 g.); sodium hydroxide (7 g. of 94 per cent.) dissolved in water (20 c.c.) was added, the emulsion being cooled and shaken during five minutes; it then became clear and solid particles began to separate. After about half an hour, the liquid had changed to a paste, which augmented in density during twenty-four hours; the solid was then filtered off, and this product, instead of being extracted with boiling alcohol as recommended by Erlenmeyer, was first freed from sodium hydroxide by repeated treatment with cold alcohol, followed each time by filtration with the aid of the pump. The residue was then allowed to become dry in air, extracted with hot water, and filtered from the benzylidene derivative of diphenylhydroxyethylamine. The sodium salt of benzylidenephenylserine separated from the filtrate, which was turbid, owing to liberation of some benzaldehyde; acetic acid was therefore added, followed by two extractions with ether, and the liquid, thus freed from benzaldehyde, was evaporated to small bulk. Phenylserine (5·5 to 6·0 g.) separated over-night in lustrous, hexagonal laminæ, and was recrystallised either by rapidly cooling a hot, concentrated aqueous solution or by adding absolute alcohol to a cold, concentrated aqueous solution. Thus purified *trans*-phenylserine has m. p. 200–202° (decomp.).

From this material, by the action of hot acetic anhydride, we obtained the acetylaminocinnamic acid lactimide mentioned by Erlenmeyer; it crystallises in pale yellow, silky needles with m. p. 148°. *cis*-Phenylserine, however, when heated with acetic anhydride does not give the lactimide. Furthermore, on benzoylation in sodium carbonate solution, *trans*-phenylserine gave the benzoylaminocinnamic acid lactimide crystallising in pale yellow, silky needles having m. p. 164°, unaffected by admixture with the product of condensing hippuric acid and benzaldehyde in hot acetic anhydride and sodium acetate. In this process of benzoylation there was formed a very small proportion of a colourless substance melting at 160°, when it decomposes to a yellow resin, but the quantity was too small for a decision on the question whether *trans*-phenylserine yields a genuine benzoyl derivative.

Both lactimides are insoluble in cold aqueous sodium hydroxide. A recent paper by Bettzieche (*Z. physiol. Chem.*, 1925, **150**, 177) adds the *p*-toluenesulphonyl derivative and mentions various properties of Erlenmeyer's phenylserine without noticing the difficulty in preparation observed above, although the conversion of the sodium salt of phenylserine into diphenylhydroxyethylamine is confirmed.

Attempts to prepare the Diketopiperazine.—The product of heating *cis*-phenylserine for varying periods over a wide range of temperature was an amber-like resin freely soluble in alcohol. We have uniformly failed to obtain a crystalline substance from it, and also by heating *cis*-phenylserine or its *O*-methyl derivative with anhydrous oxalic acid, hydrogen potassium sulphate and phosphorus trichloride. Heating with glycerol and zinc chloride gave benzaldehyde. Similar experiments with the ester hydrochlorides and picrates were equally unfruitful.

These results are the more disappointing because, although the readiness with which picrorocellin loses water and methyl alcohol diminishes the likelihood of producing the parent diketopiperazine of which picrorocellin is the *ON*-dimethyl derivative, it was reasonable to expect from one or more of the foregoing materials a smooth transformation into 2 : 5-diketo-3 : 6-dibenzylidenepiperazine, of which xanthorocellin is the *N*-methyl derivative and is very sparingly soluble in alcohol.

Even on heating picrorocellin itself, however, when passage to xanthorocellin takes place in two stages (Forster and Saville, *loc. cit.*), it is only the former stage, namely, loss of water, which leads easily to a crystalline product; the conversion of anhydro-

picrorocellin into xanthorocellin by loss of methyl alcohol leads to resinous products from which isolation of xanthorocellin is attended by considerable loss.

We therefore believe that more systematic manipulation of *cis*-phenylserine or, preferably, *trans*-phenylserine in larger quantity would make it possible to isolate the diketopiperazine, because the colour test described by Abderhalden and Komm (*Z. physiol. Chem.*, 1924, **140**, 99) has enabled us to show that it is formed. On heating *cis*-phenylserine at its melting point until decomposition appeared complete (1–2 mins.), covering the residue with a hot solution of 3 : 5-dinitrobenzoic acid in saturated aqueous sodium carbonate, and boiling the mixture (1–2 mins.), we observed the intense red coloration described by Abderhalden and Komm.