CXCIII.—*The Isomeric Monohydroxyphenylalanines. Part I. A New Synthesis of the* o- *and* m-*Isomerides and a Comparison of their Properties with those of Tyrosine.*

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THE synthesis of tyrosine was first accomplished by Erlenmeyer (*Annalen*, 1899, **307**, 138; 1883, **219**, 161). Blum (*Arch. exp. Path. Pharm.*, 1908, **59**, 286) prepared the *o*- and *m*-isomerides by Erlenmeyer's second method and showed that they gave a positive Millon's reaction.

These acids were prepared by us with a view to detailed comparison with tyrosine by the method introduced by Sasaki (*Ber.*, 1921, **54**, 163) for tyrosine and phenylalanine and later used by Hirai (*Biochem. Z.*, 1926, **177**, 449).

The glycine anhydride used was prepared most satisfactorily by a modification of Fischer's method (*Ber.*, 1906, **39**, 2893).

The condensation of salicylaldehyde and glycine anhydride in the presence of sodium acetate and acetic anhydride gave a poor yield of 2 : 5-*diketo*-3 : 6-*di*-o-*acetoxybenzylidenepiperazine*. This result was due to the formation of coumarin as a by-product, for the replacement of salicylaldehyde by its methyl ether in the condensation increased the yield of piperazine derivative to 46%.

As Perkin's method of preparing salicylaldehyde methyl ether (*Annalen*, 1868, **145**, 302) proved to be unsatisfactory, methyl sulphate was used under suitable conditions.

Hirai's method (*loc. cit.*) for isolating the free amino-acids was found more satisfactory than that used by Sasaki in the synthesis of tyrosine. Both *o*- and *m*-hydroxyphenylalanines produced a blue colour with Folin's phenol reagent, and these colours (which were more intense) were compared quantitatively with that produced by tyrosine.

EXPERIMENTAL.

Glycine Anhydride.—A solution of 56 g. of glycine ester hydrochloride in 30 c.c. of water was stirred while 33 c.c. of 46% sodium hydroxide solution were added during 1 hour, the temperature being maintained at about −10°. After ½—1 hour's stirring, the whole was kept at room temperature for 3 days; the yellow product was then washed with a small quantity of cold water and recrystallised from 60—70 c.c. of hot water (charcoal), giving colourless needles (8·2 g.; yield, 36%).

2 : 5-*Diketo*-3 : 6-*di*-o-*acetoxybenzylidenepiperazine*.—A mixture of

28 g. of salicylaldehyde, 11 g. of glycine anhydride, 30 g. of anhydrous sodium acetate, and 40 c.c. of acetic anhydride was heated for 9 hours at about 125° and for a further 2 hours at about 135°. The dark brown mass, when cold, was treated twice with hot water and the solution was decanted from the tarry residue, which was extracted with hot alcohol until the filtrate was almost colourless. The yellow-brown powder obtained (6·2 g.; yield, 15·8%), after recrystallisation from 95% acetic acid, melted at 272° (Found by micro-Kjeldahl: N, 6·9. $C_{22}H_{18}O_6N_2$ requires N, 6·9%). The *compound* gives an orange-yellow colour in concentrated sulphuric acid.

Salicylaldehyde Methyl Ether.—A mixture of 50 g. of salicylaldehyde, 200 c.c. of 2*N*-sodium hydroxide, and 30 c.c. of methyl sulphate was heated on the water-bath for 1 hour with occasional shaking. An ethereal extract of the cold product was shaken with dilute sodium hydroxide solution (at least four times, until the aqueous liquor was almost colourless), dried with potassium carbonate, and evaporated. The residue distilled at 242—245° (yield, 27 g.).

2 : 5-*Diketo*-3 : 6-*di*-o-*methoxybenzylidenepiperazine.*—A mixture of 5 g. of glycine anhydride, 12 g. of salicylaldehyde methyl ether, 10 g. of anhydrous sodium acetate, and 20 c.c. of acetic anhydride was heated gradually to 140° and kept at this temperature for 12—15 hours. The cooled brown mass, after being extracted with hot water and repeatedly with boiling alcohol, left a *product* (7·15 g.; yield, 46·6%) which crystallised from glacial acetic acid in yellow needles, m. p. 268°, and gave an orange-yellow colour in concentrated sulphuric acid (Found: N, 8·1, 8·0. $C_{20}H_{18}O_4N_2$ requires N,8·0%).

2 : 5-*Diketo*-3 : 6-*di*-o-*ethoxybenzylidenepiperazine*, prepared by an analogous method, crystallised from toluene–ethyl acetate in yellow needles, m. p. 205—206°, and gave a cherry-red colour in concentrated sulphuric acid (Found by micro-Kjeldahl: N, 7·4. $C_{22}H_{22}O_4N_2$ requires N, 7·4%).

Reduction of 2 : 5-*Diketo*-3 : 6-*di*-o-*methoxybenzylidenepiperazine.*—A mixture of 8 g. of the diketopiperazine, 6 g. of red phosphorus, and 35 c.c. of hydriodic acid (Zeisel) was refluxed for 7 hours and then diluted with 100 c.c. of water. The cooled solution was filtered and evaporated under reduced pressure at 40—45° in a current of carbon dioxide to remove the bulk of the hydriodic acid. The pale yellow solid obtained was dissolved in warm water, and the solution acidified strongly with acetic acid. From this point the procedure adopted was almost identical with that of Hirai (*loc. cit.*). After the evaporation under reduced pressure, alcohol was added to the concentrated solution of the amino-acid, the precipitated acid was

collected and dissolved in the minimum amount of hot water, and the filtered solution treated with 3 volumes of alcohol; the acid then crystallised in colourless plates, m. p. 249—250° (confirming Blum's m. p.) (Found by micro-Kjeldahl: N, 7·8. Calc.: N, 7·7%).

o-Hydroxyphenylalanine may also be prepared by reduction of the corresponding diacetoxy- and diethoxy-benzylidenediketopiperazines.

2 : 5-*Diketo*-3 : 6-*di*-m-*acetoxybenzylidenepiperazine*.—A mixture of 11 g. of glycine anhydride, 24 g. of *m*-hydroxybenzaldehyde, 30 g. of anhydrous sodium acetate, and 45 c.c. of acetic anhydride was heated gradually to 135—140° and maintained at this temperature for 7 hours. The condensation *product*, which was isolated in the same way as that obtained from salicylaldehyde methyl ether, was a light yellow substance (28 g.; yield, 90%) which crystallised from glacial acetic acid in pale yellow plates, m. p. 272° (Found: N, 7·2. $C_{22}H_{18}O_6N_2$ requires N, 6·9%).

Reduction. 10 G. of the diketopiperazine, 5 g. of red phosphorus, and 50 c.c. of hydriodic acid (*d* 1·7) were refluxed for 12 hours and, after cooling, diluted with water. The filtered solution was made strongly acid with acetic acid, and then, by the procedure described in the preceding reduction, 5·5 g. (62%) of *m*-hydroxyphenylalanine were obtained in colourless leaflets, m. p. 275° (Blum gives 279—280°) (Found: N, 7·65. Calc.: N, 7·7%).

Colour Reactions of the Three Hydroxyphenylalanines.—Millon's reaction. Folin and Ciocalteu's modified form of Millon's test (*J. Biol. Chem.*, 1927, **73**, 637) is, as these authors point out, more selective, and acids such as tryptophan develop no colour when so treated. A standard tyrosine solution in 2*N*-sulphuric acid containing 1 mg. per c.c. was used for comparison. To 5 c.c. of this solution, in a 100 c.c. graduated flask, were added 4 c.c. of 15% mercuric sulphate and 12 c.c. of 1·5% mercuric sulphate solutions and 6 c.c. of 7*N*-sulphuric acid. 5 C.c. of similar solutions of the *o*- and *m*-isomerides were treated in the same manner, and all the flasks were heated in a boiling water-bath for 15 minutes. They were then cooled rapidly, 1 c.c. of 2% sodium nitrite solution was added to each, and, after dilution to 100 c.c., the solutions were immediately compared in a colorimeter.

o-Hydroxyphenylalanine gave a much less orange shade and could not be accurately compared with the other two, but the intensity was roughly 35% of that produced by tyrosine. *m*-Hydroxyphenylalanine gave a shade identical with that given by tyrosine, and the intensity was 60% of that of tyrosine.

With Folin's phenol reagent (*J. Biol. Chem.*, 1927, **73**, 644). Equivalent quantities of solutions of tyrosine and its isomerides

were measured into 100 c.c. graduated flasks and treated in exactly the same manner as recommended by Folin and Ciocalteu for the estimation of tryptophan in protein hydrolysates, except that the sodium cyanide was unnecessary owing to the absence of mercury. The flasks were kept for 45 minutes to allow proper development of the blue colour, and comparison was then made in the colorimeter. Quantitative results were obtained as follows, tyrosine being taken as standard: Tyrosine, 100%; *o*-hydroxyphenylalanine, 126·5%; *m*-hydroxyphenylalanine, 123·5%; tryptophan, 84·7% (Folin and Ciocalteu give 84·3%).

The following acids gave no colour with this reagent: cystine, glycine, phenylalanine, alanine. These results seem to suggest that a benzene nucleus together with some substituent other than an " alanine " side-chain is necessary for the development of a colour with Folin's phenol reagent.

We wish to record our thanks to the Chemical Society for a grant which partly defrayed the cost of this investigation.

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[*Received, April 29th*, 1929.]