CCXCV.—*The Isomeric Monohydroxyphenylalanines. Part II. Some Halogen-substitution Products and their Reactions.*

By William Parker Dickinson and Philip Guy Marshall.

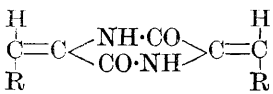
A survey of the literature reveals the fact that the only halogen-substitution products of the monohydroxyphenylalanines which have been studied are monobromo-, dichloro-, dibromo-, and di-iodo-tyrosines. Dibromotyrosine was first obtained by Gorup-Besanez (*Ann. Chem. Pharm.*, 1863, **125**, 281) by the action of bromine vapour on finely powdered tyrosine. This method was later improved upon by Zeynek (*Z. physiol. Chem.*, 1921, **114**, 275), who brominated tyrosine in glacial acetic acid suspension. Zeynek also obtained dichlorotyrosine in a similar manner. Di-iodotyrosine was originally obtained by Wheeler and Jamieson (*Amer. Chem. J.*, 1905, **33**, 368) by iodination in potassium hydroxide solution. Harington (*Biochem. J.*, 1928, **22**, 1434) obtained an improved yield by the use of aqueous ammonia (*d* 0·880) as the solvent.

3-Bromotyrosine was obtained by Johnson and Bengis (*J. Amer. Chem. Soc.*, 1912, **34**, 1061) by the condensation of hydantoin with 3-bromoanisaldehyde, followed by hydrolysis with barium hydroxide and demethylation of the methoxy-acid with hydrobromic acid.

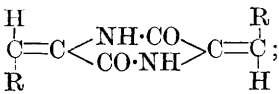
We first attempted the synthesis of the dibromo- and di-iodo-*o*- and -*m*-hydroxyphenylalanines by condensation of the appropriately substituted benzaldehydes with glycine anhydride in the manner previously described by us (J., 1929, 1495). Some difficulty was encountered in the purification of these condensation products and even then the subsequent hydrolysis and reduction with hydriodic acid removed both the bromine and the iodine at the same time, a fact which is hardly surprising in view of the finding of Wheeler and Jamieson (*loc. cit.*) that di-iodotyrosine is decomposed by hydriodic acid. We then had recourse to reduction by zinc dust and acetic acid as used for a similar purpose by Sasaki (*Ber.*, 1921, **54**, 166), who then hydrolysed the reduction product to the amino-acid with barium hydroxide (*ibid.*, p. 2058). Even with this procedure

the iodine was removed almost quantitatively, and the bromine partly when the reduction time was a minimum. Morner (*Z. physiol. Chem.*, 1913, **88**, 137) has reported that dibromotyrosine is debrominated by boiling in aqueous solution with zinc dust.

However, a pure specimen of 2 : 5-*diketo*-3 : 6-*di*-(3′ : 5′-*dibromo*-2′-*acetoxybenzyl*)*piperazine* was obtained. In the purification of the corresponding benzylidenepiperazine two isomerides (of differing m. p. and solubility in glacial acetic acid) were obtained. These probably correspond to *cis*- and *trans*-forms.



cis.



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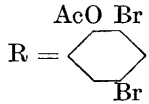

The corresponding diketobenzylidenepiperazine from 3 : 5-dibromo-4-hydroxybenzaldehyde could not be obtained pure and was more susceptible to debromination by zinc dust and acetic acid. This method was finally abandoned in favour of direct bromination or iodination of the hydroxyamino-acids. *Di-iodo*-o- and -m-*hydroxyphenylalanines* were prepared by a modification of Harington's method (*loc. cit.*). Iodination with the theoretical quantity of iodine monochloride failed to give a monoiodo-acid, half the yield of the same di-iodo-acid being obtained. The *o*- and *m*-isomerides were brominated by Zeynek's method (*loc. cit.*); this gave a *monobromo*-derivative which, in the case of the *o*-isomeride, was apt to be contaminated with a little dibromo-compound.

In contradistinction to the *o*- and *m*-hydroxyphenylalanines themselves, their halogen-substitution products are less soluble than those of tyrosine.

Zeynek states that 3 : 5-dibromotyrosine gives a violet colour with ferric chloride. This observation was confirmed and the test was applied to the bromine and iodine substitution products of all three isomerides.

m-Hydroxyphenylalanine was obtained by reduction of 2 : 5-diketo-3 : 6-di-*m*-acetoxybenzylidenepiperazine by zinc dust and acetic acid, followed by hydrolysis with barium hydroxide, but the method is more tedious than that previously described (J., 1929, 1495).

Experimental.

3 : 5-Dibromo-4-hydroxybenzaldehyde, 3 : 5-dibromosalicylaldehyde, and 3 : 5-di-iodosalicylaldehyde were obtained by the methods of Lindemann (*Annalen*, 1923, **431**, 283), Lindemann and Forth (*Annalen*, 1924, **435**, 223), and Henry and Sharp (J., 1922, **121**, 1056), respectively.

2 : 5-*Diketo*-3 : 6-*di*-(3′ : 5′-*dibromo*-2′-*acetoxybenzylidene*)*piperazine*.—A mixture of 3·1 g. of glycine anhydride, 15·5 g. of 3 : 5-dibromosalicylaldehyde, 10 g. of anhydrous sodium acetate, and 25 c.c. of acetic anhydride was heated for 12 hours at about 40°. The cooled mass was extracted thoroughly with hot water, alcohol, and finally acetone. The fawn-coloured product (7·9 g.) was then extracted several times with boiling glacial acetic acid. The residue, after crystallising from nitrobenzene in almost colourless needles, decomposed above 300° (Found : Br, 43·9. $C_{22}H_{14}O_6N_2Br_4$ requires Br, 44·3%). This substance is probably the trans-*isomeride*.

The acetic acid extracts were diluted with water and the precipitate obtained was crystallised twice from glacial acetic acid (charcoal) and then from nitrobenzene, from which it separated in colourless needles, m. p. 287°. This substance, which is probably the cis-*isomeride*, is more than ten times as soluble as the *trans*-isomeride in acetic acid or ethyl acetate, and occurs in smaller quantity (Found : Br, 44·7%).

Reduction. 5 G. of the diketopiperazine, about 2 g. of zinc dust, and 100 c.c. of glacial acetic acid were refluxed gently for 2½ hours with occasional additions of zinc. The liquid was filtered hot, and the filtrate diluted with several volumes of water. The precipitate was collected, washed with water, extracted with small quantities of hot alcohol, and crystallised from glacial acetic acid. After two crystallisations from nitrobenzene, followed by extraction with boiling acetone, 2 : 5-*diketo*-3 : 6-*di*-(3′ : 5′-*dibromo*-2′-*acetoxybenzyl*)*piperazine* was obtained in colourless needles, m. p. 275° (decomp.) (Found : Br, 43·5; N, 3·7, 3·9. $C_{22}H_{18}O_6N_2Br_4$ requires Br, 44·1; N, 3·85%).

2 : 5-*Diketo*-3 : 6-*di*-(3′ : 5′-*dibromo*-4′-*acetoxybenzylidene*)*piperazine*.—This substance was prepared in similar manner to the corresponding *o*-acetoxy-compound. Nitrobenzene, from which it crystallised in pale yellow needles, appeared to produce slight decomposition. The purest product was obtained by dissolving the substance in pyridine, adding an equal volume of benzene, and allowing the piperazine to crystallise, but even then the bromine content was low (Found : Br, 43·1. $C_{22}H_{14}O_6N_2Br_4$ requires Br, 44·3%).

Reduction. No individual product could be obtained by reduction with zinc dust in glacial acetic acid.

Monobromo-m-*hydroxyphenylalanine* was obtained by Zeynek's method (*loc. cit.*) as colourless plates which had a sweet taste; m. p. 260° (decomp.) (Found : Br, 31·2. $C_9H_{10}O_3NBr$ requires Br, 30·8%).

Monobromo-o-*hydroxyphenylalanine*.—A modification of Zeynek's

method was used. The theoretical quantity of bromine was added to a suspension of the hydroxyphenylalanine in glacial acetic acid and, as soon as the solution became colourless, it was diluted with two volumes of water and excess of sodium acetate added. The precipitated *acid*, after thorough extraction with boiling water, had m. p. 256°. It occurs in rectangular plates (Found: Br, 30·6. $C_9H_{10}O_3NBr$ requires Br, 30·8%).

Di-iodo-m-*hydroxyphenylalanine*.—A solution of 1 g. of the hydroxy-acid in 15 c.c. of aqueous ammonia (*d* 0·880) was cooled in ice and 8·8 c.c. of 32% iodine (in potassium iodide) solution were added slowly. After 12 hours, the separated product was washed with and suspended in water, hydrochloric acid added to effect solution, and the *acid* precipitated by addition of sodium acetate. The product had m. p. 230° (decomp.) (Found: I, 59·2. $C_9H_9O_3NI_2$ requires I, 58·7%).

The hydrochloride crystallises from dilute hydrochloric acid (charcoal) in rosettes of slender colourless needles, m. p. 194° (decomp.). It dissociates in water.

Di-iodo-o-*hydroxyphenylalanine* was obtained in similar manner to the *m*-isomeride, the ammonium salt which separated being decomposed with dilute acetic acid. It is insoluble in water and has m. p. 211° (decomp.) (Found: I, 59·5%). A violet colour is obtained with ferric chloride in the cold.

The hydrochloride, m. p. 214° (decomp.), crystallises in needles from dilute hydrochloric acid. It is insoluble in concentrated acid and dissociates in water.

2 : 5-*Diketo*-3 : 6-*di*-m-*acetoxybenzylpiperazine*.—This is prepared in similar manner to the bromine-substituted product. It is dissolved in the smallest amount of hot glacial acetic acid, and water added until a permanent turbidity appears. The product which separates is crystallised several times from absolute alcohol and occurs in colourless needles, m. p. 191—192° (Found: N, 6·6. $C_{22}H_{22}O_6N_2$ requires N, 6·8%).

Hydrolysis. A mixture of 2 g. of the diketopiperazine, 20 g. of barium hydroxide, and 100 c.c. of water was refluxed for 24 hours, 100 c.c. of water were then added, and the liquid was filtered hot and neutralised with 0·1*N*-sulphuric acid (to litmus). After removal of the barium sulphate the liquid was concentrated in a vacuum to about 50 c.c. and 0·1*N*-sulphuric acid was again added until no more barium sulphate was precipitated. After filtration the solution was evaporated in a vacuum almost to dryness, and the separated *m*-hydroxyphenylalanine recrystallised as previously described; m. p. 275° (Found: N, 7·5. Calc. for $C_9H_{11}O_3N$: N, 7·7%).

It gives a pale reddish-violet colour with ferric chloride in the cold as opposed to the deep purple colour given by the *o*-isomeride and the absence of any colour with tyrosine.

All the monohydroxyphenylalanines and their halogen derivatives give an orange-yellow to brown colour on being warmed with ferric chloride.

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