309. *The Synthesis of α-Amino-γ-(p-hydroxyphenyl)butyric Acid, a Homologue of Tyrosine.*

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Because of the known intermediate formation of indole derivatives in the *in vitro* production of melanin from tyrosine under the influence of tyrosinase, it became of interest to study the action of this enzyme on the next higher homologue of tyrosine. By this means it seemed possible that data relating to the analogous formation of quinoline compounds by enzyme action might be obtained.

α-Amino-γ-(*p*-hydroxyphenyl)butyric acid has been synthesised by two different routes, and some related intermediate compounds are described.

The elucidation of the series of chemical reactions involved in the formation of melanin from tyrosine under the influence of the enzyme tyrosinase has been the subject of several investigations (see Raper *et al.*, *Physiol. Rev.*, 1928, **8**, 245; Evans and Raper, *Biochem. J.*, 1937, **31**, 2162; Clemo and Weiss, *J.*, 1945, 702). In the presence of tyrosinase, a number of compounds structurally related to tyrosine, and possessing great physiological interest (*e.g.*, adrenaline, epinine, tyramine, etc.) are oxidised and give rise, where the side-chain configuration allows, to indole compounds, the formation of which has been established in most cases (Dulière and Raper, *Biochem. J.*, 1930, **24**, 259). It is generally assumed that the *o*-quinone of 3 : 4-dihydroxyphenylalanine undergoes ring closure spontaneously, without mediation of the enzyme. With esters of tyrosine, however, and compounds where the amino-group is fully alkylated, including quaternary ammonium salts, ring closure does not take place after quinone formation, although additional oxygen is utilised, the subsequent course of the reaction being unknown. It is clear from the work of Bloch and Schaaf (*Biochem. Z.*, 1926, **162**, 181) and of Heard and Raper (*Biochem. J.*, 1933, **27**, 36) that under changed conditions of pH and temperature, side reactions occur to a greater or less extent, a fact which the latter authors deem important in relation to the biochemical origin of adrenaline from tyrosine or some similar precursor.

α-Amino-γ-(p-hydroxyphenyl)butyric acid has been synthesised in order to study the action of tyrosinase on it, with particular reference to the fate of the side chain. If the reaction follows an analogous course to the tyrosine–tyrosinase system, the eventual formation of quinoline derivatives would be expected.

Attempts to effect the condensation of *p*-methoxyphenylacetaldehyde with hippuric acid, acetylglycine, or hydantoin failed to yield the anticipated derivatives of α-amino-γ-(*p*-methoxyphenyl)butyric acid, in spite of a large variety of experimental conditions.

Two alternative methods for the synthesis of α-amino-γ-(*p*-hydroxyphenyl)butyric acid were employed : (*a*) β-(*p*-Methoxyphenyl)propionitrile, prepared from anisaldehyde, was reduced by stannous chloride under the usual conditions of the Stephen reaction to yield *p*-methoxyphenylpropaldehyde, which, when condensed with ammonium cyanide, followed by hydrolysis of the resulting aminocyanohydrin, afforded *α-amino-γ-(p-methoxyphenyl)butyric acid*. The latter compound was demethylated by treatment with hydriodic acid and red phosphorus in the usual manner to yield the desired amino-acid.

(*b*) 2-(*p*-Aminophenyl)ethyl bromide, obtained by reducing the corresponding nitro-compound, was converted into 2-(*p*-hydroxyphenyl)ethyl bromide by diazotisation of the amino-group followed by hydrolysis of the diazonium compound in the usual manner. The hydroxy-compound was condensed with the sodio-derivative of benzamidomalonic ester in anhydrous ethanol (cf. Redemann and Dunn, *J. Biol. Chem.*, 1939, **130**, 341), and hydrolysis of the condensation product with aqueous hydrobromic acid gave the desired α-amino-γ-(*p*-hydroxyphenyl)butyric acid. The results of the investigation on the action of tyrosinase on this amino-acid will be reported later.

Experimental.

(All m. ps. are uncorrected.)

p-*Methoxyphenylpropionitrile* (cf. Goldschmidt and Fraenkel, *Monatsh.*, 1914, **35**, 283).—*p*-Methoxyphenylpropionamide (6 g.), obtained by the method of Barger and Walpole (*J.*, 1909, **95**, 1724), dissolved in dry xylene, was boiled vigorously whilst phosphoric oxide (12 g.) was added in small portions with shaking. The mixture was boiled under reflux for 15 minutes, the reaction liquid filtered, and the xylene removed by distillation under reduced pressure on the water-bath. The residue was distilled under reduced pressure, and the fraction, b. p. 160—165/13 mm. (3 g., yield 50%), was collected. It was a colourless oil possessing a characteristic smell.

p-*Methoxyphenylpropaldehyde*.—Finely powdered anhydrous stannous chloride (10 g., 1·5 mols.) was suspended in dry ether (25 c.c.), cooled in ice and saturated with dry hydrogen chloride, until a homogeneous solution was obtained. *p*-Methoxyphenylpropionitrile (5 g., 1 mol.) was then added, the reaction mixture shaken vigorously, and the stoppered reaction vessel set aside at room temperature overnight. The white, crystalline aldimino-stannichloride which separated was collected on a dry filter, and washed with dry ether. It was hydrolysed to the aldehyde by suspension in warm water made definitely alkaline with aqueous sodium carbonate, followed by extraction with ether. The ethereal solution was dried (Na_2SO_4), and the solvent removed. The oily residue was distilled under reduced pressure, giving *p*-methoxyphenylpropaldehyde, b. p. 130—135°/15 mm. (4·8 g., 90%). On condensation with semicarbazide hydrochloride in the usual way, it gave a *semicarbazone*, which on crystallisation from benzene afforded white needles, m. p. 140—142° (Found : C, 59·2; H, 7·0; N, 18·7. $C_{11}H_{15}O_2N_3$ requires C, 59·1; H, 6·8; N, 19·0%). This aldehyde could also be obtained, in poor yield, by distillation under reduced pressure of an intimate mixture of the barium salt of *p*-methoxyphenylpropionic acid with barium formate and silver sand.

α-Amino-γ-(p-methoxyphenyl)butyric Acid.—A modification of Strecker's synthesis due to Lapworth and Cocker (*J.*, 1931, 1371) was employed. *p*-Methoxyphenylpropaldehyde (4·5 g. 1 mol.) was added gradually, with stirring, to a well-cooled solution of ammonia (0·9 g., *d* 0·88, 2 mols.) and hydrogen cyanide (1·4 g., 2 mols.). The reaction mixture was shaken mechanically at room temperature for several hours, and left for 2 days to attain equilibrium. To the mixture, which had now darkened appreciably, was slowly added excess of sulphuric acid (40% w/v; 5—7 mols.). Hydrolysis was effected by heating for 3 hours at 125° until a test portion of the solution gave no reaction for ferrocyanide with excess of sodium hydroxide and ferrous hydroxide. The solution was diluted with several times its volume of water, and powdered barium carbonate (A.R.) was gradually added until the mixture was definitely alkaline to litmus. Steam was passed through the boiling solution until all the ammonia had been driven off. The bulky precipitate of barium sulphate was filtered off, and well washed with boiling, very dilute aqueous sulphuric acid. The combined filtrates were treated with powdered lead carbonate until effervescence ceased, and the solution no longer showed an acid reaction to Congo-red, the mixture filtered, and the residue of lead sulphate washed with water. The combined filtrate and washings were saturated with hydrogen sulphide, the lead sulphide removed, and the filtrate concentrated to a small bulk (50 c.c.). The concentrate was decolorised (norite) and filtered, and on further concentration and cooling, a white crystalline solid separated. After recrystallisation from aqueous alcohol the *amino*-acid formed needles (2 g., 35%), m. p. 245—246° (Found, C, 63·1; H, 6·9; N, 6·75. $C_{11}H_{15}O_3N$ requires C, 63·2; H, 7·1; N, 6·7%).

α-Amino-γ-(p-hydroxyphenyl)butyric Acid.—The above methoxy-acid was demethylated by the method of Harington and McCartney (*Biochem. J.*, 1927, **21**, 852), the acid (5 g.), red phosphorus (5 g.), hydriodic acid (25 c.c., *d* 1·7), and acetic anhydride (25 c.c.) being boiled together under reflux for 1·5 hours. The reaction mixture was filtered through asbestos-wool, the phosphorus well washed with glacial acetic acid, and the filtrate evaporated to dryness in a vacuum. Water was then added, and the evaporation repeated. The residue was taken up in a little water, and sufficient lead carbonate added just to neutralise the trace of acid present and to remove the iodine. The precipitate was filtered off and washed thoroughly with distilled water, the filtrate saturated with hydrogen sulphide, the lead sulphide removed, and the clear filtrate decolorised (norite) and concentrated to small bulk. On cooling, crystals soon developed, which were collected and recrystallised twice from boiling water, whence a white micro-crystalline powder was obtained (1·5 g.) m. p. 265° (Found, C, 61·5; H, 6·7; N, 7·2. $C_{10}H_{13}O_3N$ requires, C, 61·5; H, 6·66; N, 7·18%). The *amino*-acid on treatment with a solution of diazotised *o*-chloroaniline gave a red azo-compound. Nitrogen was evolved on treatment with cold nitrous acid.

p-(2-*Bromoethyl*)*aniline Hydrochloride*.—2-(*p*-Nitrophenyl)ethyl bromide (10 g.), prepared from 2-phenylethyl alcohol by the method of Foreman and McElvain (*J. Amer. Chem. Soc.*, 1940, **62**, 1436), was added in 1 g. portions to a solution of stannous chloride (40 g.) in concentrated hydrochloric acid (100 c.c.) which was heated on the steam-bath, the liquid being shaken vigorously after each addition; the solid nitro-compound melted to an oil, which gradually reacted, and dissolved on shaking, a further portion being then added. When the addition was completed, the mixture was heated on the steam-bath for a further 45 minutes. The reaction mixture was then freed from a little oil by extraction with hot benzene, and the aqueous layer was separated, and cooled in a freezing mixture. An excess of aqueous sodium hydroxide was added, with careful cooling until all the tin, which was at first precipitated as hydroxide, had dissolved. The alkaline solution was thrice extracted with ether, the combined extract washed with water, and the amine was removed from the ether by shaking with 3·5N-hydrochloric acid (22 c.c.). After separation of the aqueous layer, *p*-(2-bromoethyl)aniline hydrochloride (7·75 g.) crystallised out; it recrystallised from water in nearly colourless needles, m. p. 211° (decomp.).

p-(2-*Bromoethyl*)*phenol*.—The foregoing hydrochloride (10 g.) was dissolved in water (100 c.c.) and 30% sulphuric acid (20 c.c.), the mixture warmed to 55°, and a solution of sodium nitrite (3·5 g.) in water (20 c.c.) was added slowly, with mechanical stirring. A dark reddish-brown oil possessing a sharp sickly smell separated, and the mixture was heated on the steam-bath for a further 15 minutes, then cooled;

the oil was extracted with ether, the extract dried (Na_2SO_4), the solvent removed, and the residual oil distilled, yielding *p*-(2-bromethyl)phenol (2·3 g.) as a viscous yellow oil, b. p. 150—155°/12 mm.

α-Amino-γ-(p-hydroxyphenyl)butyric Acid.—Benzamidomalonic ester (2·8 g.), prepared as described by Redemann and Dunn (*J. Biol. Chem.*, 1939, **130**, 341) but with Painter's modification (*J. Amer. Chem. Soc.*, 1940, **62**, 232), was added to a hot solution of sodium (0·25 g.) in absolute alcohol (50 c.c.), and when the solid sodio-derivatives had separated *p*-(2-bromoethyl)phenol (2·3 g.) was added, and the mixture heated under reflux for 3 hours. The bulk of the alcohol was removed by distillation, and the residue diluted with water (100 c.c.) and extracted with ether. The extract was dried (Na_2SO_4), and the solvent removed, leaving ethyl 2-(*p*-hydroxyphenyl)ethylbenzamidomalonate as a brown oil (3 g.). This was hydrolysed by refluxing with 48% aqueous hydrobromic acid (20 c.c.) for 10 hours. The mixture was diluted with boiling water (40 c.c.), filtered, and left in the ice-chest overnight. Benzoic acid separated, and was filtered off, the filtrate being evaporated to dryness in a vacuum. The residual solid was extracted with water (15 c.c.), the extract boiled (norite), filtered, and the filtrate carefully neutralised with aqueous ammonia and kept in the ice chest for 24 hours. α-Amino-γ-(*p*-hydroxyphenyl)butyric acid (0·17 g.), m. p. 265° (decomp.), separated as a microcrystalline powder; recrystallised from 50% aqueous alcohol, it had m. p. 265° (decomp.) undepressed on admixture with the amino-acid synthesised by the previous route (Found : C, 60·3; H, 6·52; N, 7·2%).

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