VII. THE SYNTHESIS OF *dl*-3:4-DIHYDROXY-PHENYL-*N*-METHYLALANINE.

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(*Received November 26th, 1932.*)

It has recently been shown by Dulière and Raper [1930] that *N*-methyltyrosine, on oxidation with tyrosinase, yields a small amount of a pressor substance. This is of particular interest in view of the close relationship between *N*-methyltyrosine and adrenaline. By oxidation and loss of CO_2 adrenaline might conceivably be produced. The first stage of this oxidation, which is in full accord with what is already known of the action of tyrosinase, involves the introduction of a second hydroxyl group in the *ortho*-position, giving rise to 3 : 4-dihydroxyphenyl-*N*-methylalanine (*N*-methyldopa). It therefore seemed desirable to synthesise this intermediate compound, as the advantages of the use of an *ortho*-dihydric substrate are manifold; notably the protracted time interval which elapses during the first stage of the oxidation is eliminated and the behaviour of the substrate with catechol oxidases and mild oxidising agents is opened to investigation.

The usual methods applied to the synthesis of aromatic amino-acids are not applicable to the synthesis of methyldopa without considerable modification. The amino-nitrogen must be free for methylation, which excludes the use of condensation products of hippuric acid, and both aromatic hydroxyl groups must be protected in order to prevent autoxidation and to avoid the complication of an unnecessary number of methylation products. In the first instance, the hydantoin method, which has been applied to the synthesis of *N*-methyltyrosine by Johnson and Nicolet [1912], was investigated. It was proposed to hydrolyse 1 : 3-dimethylpiperonylhydantoin with baryta to 3 : 4-methylenedioxyphenyl-*N*-methylalanine which would yield methyldopa on treatment with hydriodic acid. The methylation of piperonylhydantoin was attempted with both methyl iodide and methyl sulphate but only very small amounts of the dimethyl-derivative were obtained. Complete methylation of the unsaturated piperonalhydantoin is readily accomplished but in this case reduction of the corresponding dimethyl-derivative cannot be effected with sodium amalgam. Hydrolysis with baryta shatters the molecule at the double bond.

Attention was then directed to the acetylglycine method of synthesis of aromatic amino-acids recently suggested by Dakin [1929]. Piperonal is condensed with acetylglycine in the presence of acetic anhydride and sodium acetate. The resulting azlactone on mild alkaline hydrolysis yields piperonalacetaminoacetic acid, which is readily methylated with methyl sulphate. Treatment of piperonal-*N*-methylacetaminoacetic acid with hydriodic acid in the presence of red phosphorus effects a reduction of the double bond and simultaneous hydrolytic removal of the acetyl- and methylenedioxy-groups with the ultimate production of *N*-methyldopa. In order to minimise oxidation this operation is carried out

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in an inert atmosphere. The substitution for the constant-boiling hydriodic acid of equal volumes of the latter and of acetic anhydride, as employed by Harington and McCartney [1927] in the synthesis of dopa, greatly increases the yield of the final product which is further enhanced by the addition of iodine.

Efforts were made to effect a condensation of piperonal and derivatives of *N*-methylglycine, as such a product would yield *N*-methyldopa directly on treatment with hydriodic acid. The acetyl-, benzoyl- and benzenesulphonyl-derivatives of sarcosine cannot be condensed with piperonal. The respective reagents were heated on the water-bath or boiled on the oil-bath for several hours, but in each case piperonal and the sarcosine derivatives were recovered unaltered. Sarcosine itself behaves in the same manner. The corresponding unmethylated derivatives were therefore investigated, and it was found that acetylglycine and hippuric acid readily condense whereas benzenesulphonylglycine does not. The formation of an azlactone as an initial condensation product appears to be essential, and for ring formation the system —CH_2—NH—CO—R is necessary, *i.e.* the possibility of enolisation to —CH_2—N=C(OH)—R.

Experimental.

Acetylglycine was prepared by the method of Dakin [1929].

Azlactone of piperonalacetaminoacetic acid. An improved yield of this azlactone was obtained by a modification of the method of Dakin [1929]. An intimate mixture of piperonal (12·8 g.), acetylglycine (10 g.), and freshly fused sodium acetate (14 g.) was treated with freshly distilled acetic anhydride (30 cc.) and the whole heated on the water-bath for 7 hours, when the hot reaction mixture was poured into boiling water (500 cc.). On cooling, the crude azlactone was collected and washed with small quantities of alcohol and ether to remove oily impurities; M.P. 170–175°; yield 12·6 g. Crystallisation from benzene or toluene yielded the pure product which separated in bright yellow prisms, M.P. 182°.

Piperonalacetaminoacetic acid. Hydrolysis was effected as described by Dakin [1929]. The crude azlactone (9·6 g.) was dissolved in 3 molecular proportions of warm sodium hydroxide solution (0·5 *N*) and cooled gradually. The mixture was filtered and acidified with dilute hydrochloric acid, and the crude piperonalacetaminoacetic acid was collected and twice recrystallised from dilute acetic acid (10 %); M.P. 119–120°; yield 8 g.

Piperonal-N-methylacetaminoacetic acid. The acid described above (5 g.) in two molecular proportions of sodium hydroxide solution (10 %) was treated with methyl sulphate added in small portions (0·2 cc.) at regular intervals. The solution was vigorously shaken throughout the operation, which was continued until the liquid became acid to litmus, and methylated products began to separate. A third molecular proportion of sodium hydroxide was then added and methylation continued until in all 4 cc. of methyl sulphate had been utilised. Maximum yields were obtained when the methylation had been extended over a period of 3 to 4 hours at room temperature. The oily precipitate obtained on acidification consisted of a mixture of unchanged piperonalacetaminoacetic acid and its *N*-methyl-derivative, together with hydrolytic products resulting from the sodium hydroxide treatment. Separation of the former was effected by the use of chloroform and acetone. The methylated product was found to be soluble in chloroform and but slightly soluble in acetone, whilst the unmethylated acid is soluble in acetone and insoluble in chloroform. The reaction solution was therefore directly transferred from the methylation bottle into a large separating funnel, acidified with dilute hydrochloric acid, and

extracted with chloroform. The residue collected by filtration was returned to the separating funnel and extraction was continued until the crude piperonal-*N*-methylacetaminoacetic acid was completely removed. On removal of the solvent by distillation, the residue was taken up in hot dilute acetone (20 %), boiled for a few moments with a trace of animal charcoal, and filtered. On cooling the pure product separated in well-defined colourless plates, M.P. 196°; yield 2·5 g. A further 0·25 g. was recovered from the acetone filtrate by concentration, chloroform extraction, and crystallisation from acetone.

Piperonal-*N*-methylacetaminoacetic acid is very soluble in warm organic solvents and slightly soluble in cold acetone, ether, and alcohol. It is readily dissolved by cold alkalis and by dilute mineral acids on warming. Crystallisation takes place readily from dilute acetone, alcohol, or acetic acid.

(Found: C, 58·6; H, 4·93; N, 5·42 %. $C_{13}H_{13}O_5N$ requires: C, 59·3; H, 4·94; N, 5·33 %.)

Crystallisation of the residues insoluble in chloroform from dilute acetic acid (10 %) gave unchanged piperonalacetaminoacetic acid (1 g.).

dl-*3 : 4-Dihydroxyphenyl*-N-*methylalanine.* Piperonal-*N*-methylacetaminoacetic acid (5 g.), red phosphorus (5 g.), and resublimed iodine (5 g.) were suspended in a mixture of hydriodic acid (25 cc.; sp. gr. 1·7) and acetic anhydride (25 cc.) contained in a small round-bottomed flask which was fitted by means of a ground-glass joint to the reflux condenser. The system was evacuated and filled with CO_2 by means of a capillary tube led through the condenser. A small stream of gas was then passed continuously and expelled through a water trap to the atmosphere. The mixture was boiled on the sand-bath for 7 hours, cooled under CO_2, and filtered. The phosphorus and iodine residues were washed with 50 % acetic acid (50 cc.) and the combined filtrates added to an excess of lead acetate solution (400 cc. of 20 %). Lead iodide was removed, and washed several times by shaking with water (200 cc.). It was found impossible completely to free the lead iodide from catechol substances as repeated washings always gave an intense green coloration with ferric chloride. The filtrate and wash-waters were made slightly alkaline with dilute ammonia, which brought down the lead salt of *N*-methyldopa as a white flocculent precipitate. The latter was collected, washed, and decomposed in aqueous suspension (250 cc.) with hydrogen sulphide. Lead sulphide was removed, washed, and the combined filtrates were immediately concentrated under diminished pressure, CO_2 being led into the capillary of the Claisen flask. The concentrate (25–50 cc.) was cautiously neutralised to Congo red with a few drops of dilute ammonia (0·1 %) and allowed to crystallise *in vacuo* over sulphuric acid until the mother-liquor attained the bulk of 2–3 cc. The crude product was collected, washed with a few drops of water, and redissolved in the minimum quantity of boiling water containing a drop of sulphurous acid which minimises oxidation. On cooling no crystallisation took place. The solution was therefore neutralised to Congo red and allowed to crystallise *in vacuo*. After two such recrystallisations *N*-methyldopa was obtained in colourless clusters or sheaves of radiating needles which were usually pigmented; M.P. 275°; yield 1·5 g. The use of charcoal as a decolorising agent resulted in the oxidation of appreciable amounts of the product. For analysis the sample was recrystallised four times, M.P. 280°, but still contained traces of a light brown pigment.

(Found: C, 56·8; H, 6·19; N, 6·59 %. $C_{10}H_{13}O_4N$ requires: C, 56·8; H, 6·16; N, 6·63 %.)

The melting-point was not constant but was found to vary considerably with the rate of heating, in which respect the substance behaves in the same manner

as *N*-methyltyrosine. The values recorded above are those taken under like conditions; the rate of heating was controlled by means of a manometer in the gas circuit. *N*-Methyldopa is readily soluble in hot water, sparingly so in cold water, and insoluble in organic solvents. Unlike dopa the methylamino-acid does not crystallise well from water. In neutral aqueous solution with ferric chloride it gives an intense green coloration which passes into reddish-purple on addition of very dilute ammonia. In aqueous solution, autoxidation, with the development of a pink coloration, proceeds more rapidly than with dopa.

The above investigation has been carried out under the supervision of Prof. H. S. Raper, to whom the writer wishes to acknowledge his indebtedness.

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