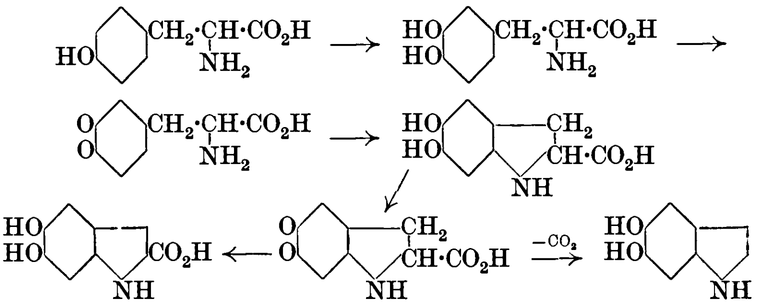
LXII.—*Synthesis of 5 : 6-Dimethoxyindole and its 2-Carboxylic Acid.*

By Albert Edward Oxford and Henry Stanley Raper.

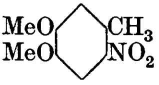
It has been recently shown by Raper (*Biochem. J.*, in the press) that when tyrosine is oxidised by the enzyme tyrosinase, a series of changes occurs which results in the production of 5 : 6-dihydroxyindole and to a lesser extent of 5 : 6-dihydroxyindole-2-carboxylic acid. These changes, which represent a new type of indole synthesis, are as follows :



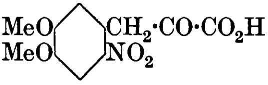
Both 5 : 6-dihydroxyindole and its 2-carboxylic acid are very easily oxidised and can only be isolated as dimethoxy-compounds by methylation in a neutral atmosphere. For purposes of identification, it was necessary to synthesise them, since they had not been previously prepared. Perkin and Rubenstein (J., 1926, 357) have described a synthesis of ethyl 5 : 6-dimethoxyindole-2-carboxylate, but the yields were poor and the ester was not easily converted into the free acid because the latter tended to produce tarry products on heating in the presence of acids.

Attempts to obtain 5 : 6-dimethoxyindole by condensing *as*-dichlorodiethyl ether with 4-aminoveratrole (compare Berlinerblau, *Monatsh.*, 1887, **8**, 180) proved fruitless. The method finally adopted was that used by Blaikie and Perkin (J., 1924, **125**, 296) in the preparation of certain of the monomethoxyindoles.

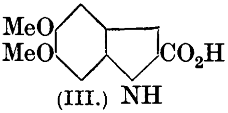
6-Nitrohomoveratrole (I), when condensed with ethyl oxalate in the presence of potassium ethoxide, yields 2-nitro-4 : 5-dimethoxyphenylpyruvic acid (II), from which 5 : 6-dimethoxyindole-2-carboxylic acid (III) is obtained by reduction with ferrous sulphate and ammonia.



(I.)



(II.)



(III.)

The last-named acid loses carbon dioxide at its melting point, yielding 5 : 6-dimethoxyindole.

The series of changes described above which tyrosine undergoes as a result of the action of the enzyme tyrosinase is completed by the formation of a black, insoluble pigment—melanin. This is produced undoubtedly by the oxidation in air of 5 : 6-dihydroxyindole. Similar pigments are produced in nature, both in animals and in plants, and they are known to contain within their molecule some cyclic structure in which nitrogen is present. The production of dihydroxyindole from tyrosine indicates, therefore, that the nitrogen-containing cyclic structure is present before melanin is formed and suggests that tyrosine and not tryptophan may be the chief mother-substance of melanin pigments in nature.

The first stage of the oxidation brought about by the enzyme, *i.e.*, the production of 3 : 4-dihydroxyphenylalanine from tyrosine, is of considerable interest in that it shows a possible method by which certain pyrocatechol derivatives may arise in nature. The 3 : 4-dihydroxy-grouping—often methylated—is of relatively frequent occurrence, *e.g.*, in adrenaline, 3 : 4-dihydroxyphenylalanine, vanillin, eugenol and other naturally occurring products.

EXPERIMENTAL.

2-*Nitro*-4 : 5-*dimethoxyphenylpyruvic Acid* (II).—Potassium (9·9 g.) was pulverised under boiling benzene (250 c.c.). After the liquid had been allowed to cool, and ether (250 c.c., dry and free from alcohol) had been added, a solution of 100% alcohol (14·5 c.c.) in dry ether (15 c.c.) was slowly run in with shaking, after which the mixture was kept for ½ hour. Ethyl oxalate (37·2 g.) was now added followed by a solution of 6-nitrohomoveratrole (50 g.) in dry benzene (300 c.c.). The mixture, protected from atmospheric moisture and carbon dioxide, was kept at 35—40° for 24 hours with occasional shaking.* Water (500 c.c.) was now added to dissolve the cake of the potassium derivative of 2-nitro-4 : 5-dimethoxyphenylpyruvic acid which had separated, and the purple aqueous layer, after separation, was washed with a little benzene and acidified with a slight excess of dilute hydrochloric acid. A copious sandy precipitate of the above acid was immediately formed, which when dried was sufficiently pure for use in the next stage of the synthesis. A further small amount could be isolated by exhaustively extracting the combined benzene–ether layers with 5% caustic soda solution and then acidifying the alkaline extract. The total yield was 40 g. or 60% of the theoretical, and most (16 g.) of the unchanged 6-nitrohomoveratrole was recovered by evaporation of the combined benzene–ether layers.

2-Nitro-4 : 5-dimethoxyphenylpyruvic acid is sparingly soluble in water and readily soluble in the usual organic solvents with the exception of light petroleum, and glacial acetic acid, from which it crystallises in clusters of bright yellow, microscopic needles, m. p. 193—194°, with evolution of gas at 197° (Found : C, 48·85; H, 4·0. $C_{11}H_{11}O_7N$ requires C, 49·1; H, 4·1%). It dissolves in dilute aqueous caustic soda to a deep permanganate-purple solution, the colour being discharged on long standing. Its aqueous-alcoholic solution gives a deep greenish-black colour with ferric chloride stable to acetic acid but destroyed by dilute mineral acids.

The *phenylhydrazone* was obtained by boiling a solution of the acid (0·32 g.) and phenylhydrazine (0·13 g.) in glacial acetic acid (20 c.c.) for 30 minutes. On dilution with much water the phenylhydrazone separated as a sandy precipitate which was crystallised first from benzene and then from 50% acetic acid, from which it separated in minute plates (parallelipipeds) which darken at 165° and melt at 170° with vigorous decomposition (Found : C, 57·3; H, 5·15. $C_{17}H_{17}O_6N_3$ requires C, 56·8; H, 4·8%).

2-*Nitro*-4 : 5-*dimethoxyphenylacetic Acid.*—The pyruvic acid (0·42 g.), dissolved in 2% caustic soda solution (3·5 c.c.) and cooled in ice, was oxidised by the careful addition of hydrogen peroxide (20 volume; 8 c.c. in all) until the colour had been discharged; more 2% caustic soda solution and hydrogen peroxide were then cautiously added until the liquid was permanently decolorised. The pale

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* The mode of effecting the condensation described by Kermack, Perkin, and Robinson (J., 1921, **119**, 1630) which gave good results with *o*-nitro-*p*-tolyl methyl ether, *viz.*, digestion of the *o*-nitrotoluene with an alcoholic solution of sodium ethoxide and ethyl oxalate, is not to be recommended for the above, since one obtains a gummy product, very difficult to purify and not giving satisfactory results if used as such for the preparation of the indolecarboxylic acid.

yellow precipitate obtained on acidification was collected and crystallised from dilute acetic acid, from which it separated in almost colourless needles, m. p. 202—204° with slight decomposition to a red liquid (Found: C, 50·2; H, 4·5. $C_{10}H_{11}O_6N$ requires C, 49·8; H, 4·6%). It is readily soluble in ethyl acetate and glacial acetic acid, and sparingly soluble in benzene, chloroform, and cold water.

5 : 6-*Dimethoxyindole*-2-*carboxylic Acid* (III).—2-Nitro-4 : 5-dimethoxyphenylpyruvic acid (38 g. of crude product) was dissolved in ammonia (213 c.c., *d* 0·880, mixed with 300 c.c. of water) and reduced by the addition of a hot solution of ferrous sulphate (274 g. of hydrated crystals in 300 c.c. of water). The mixture was heated on the steam-bath for 30 minutes with frequent shaking, and finally gently boiled for the same length of time. The liquid was filtered while hot, and the precipitate of ferric oxide washed with hot water containing a little ammonia. The dark brown filtrate was acidified by the addition of a very slight excess of *N*-sulphuric or, preferably, *N*-phosphoric acid, and the precipitated indolecarboxylic acid at once filtered off and dried in a vacuum. The yield is poor, but can be increased somewhat by immediately extracting the mother-liquor with ether. Purification of the acid is best effected by crystallising it first from hot water (charcoal) and then repeatedly from benzene, from which it separates in colourless plates, m. p. 202—203° (decomp.). Well-formed, rhombohedral tablets are deposited if a hot solution of the acid (0·5 g.) in acetone (10 c.c.) and benzene (100 c.c.) is allowed to cool slowly. The acid can also be crystallised from chloroform, in which it is sparingly soluble in the cold (Found: C, 59·9; H, 5·3. $C_{11}H_{11}O_4N$ requires C, 59·7; H, 5·0%). On warming with *p*-dimethylaminobenzaldehyde and a drop of hydrochloric acid a pale purple colour is obtained which becomes redder on the addition of a trace of nitrite.

When a trace of nitric acid is added to a very dilute solution of the acid in glacial acetic acid, a yellow colour is developed, deepening to golden-yellow on warming. The acid itself dissolves in concentrated sulphuric acid to an orange solution, but addition of a trace of nitric acid merely brings about partial decoloration to pale yellow.

Attempts to obtain the ethyl ester (already described by Perkin and Rubenstein, *loc. cit.*) by the ordinary methods were unsuccessful owing to the instability of the acid in presence of mineral acids.

5 : 6-*Dimethoxyindole*.—5 : 6-Dimethoxyindole-2-carboxylic acid, contained in a small Claisen flask with a long side-arm, was heated in an oil-bath at 205—215° for ½ hour. When a quiet melt had been obtained, the flask was transferred to a fusible-metal bath, and its

contents distilled in a vacuum. The indole came over at 198°/8 mm. and solidified at once in the side-arm, which was afterwards severed from the flask and ground up with dilute potassium carbonate solution, and the solid material collected and extracted with boiling 95% alcohol, from which the indole separated, on cooling, in a state of purity, m. p. 154—155° unaltered by further crystallisations. 5 : 6-Dimethoxyindole crystallises from benzene in rods, from 95% alcohol and from light petroleum (in which it is very sparingly soluble) in colourless, glistening leaflets, and from 50% alcohol, by slow deposition, in long, coarse needles (Found : C, 67·6; H, 6·2; N*, 8·2; MeO, 34·0. $C_{10}H_{11}O_2N$ requires C, 67·8; H, 6·2; N, 7·9; MeO, 35·0%).

This indole quickly darkens on keeping and is very sensitive to oxidising agents in the presence of acids. Although insoluble in cold, dilute hydrochloric acid, it slowly develops a brown colour in contact with this acid, and, on warming, dissolves with almost immediate production of the brown colour. Addition of ferric chloride to an aqueous-alcoholic solution of the indole produces a permanent deep green coloration. With *p*-dimethylaminobenzaldehyde and hydrochloric acid the indole gives a rich purple colour and on addition of a trace of nitrite this changes to a deep crimson.

When equivalent quantities of the indole and of picric acid were dissolved in hot acetone or methyl alcohol, a deep red solution was obtained; this, on evaporation, yielded a deep purple, gummy picrate, which could not be obtained crystalline.

1-*Acetyl*-5 : 6-*dimethoxyindole* is formed in poor yield when 5 : 6-dimethoxyindole (0·4 g.) is boiled for 5½ hours with acetic anhydride (10 c.c.) and freshly-fused sodium acetate (1·2 g.). The cooled mixture is diluted with ether and filtered, and the filtrate evaporated in a vacuum, finally at 80°. The red, oily residue is then repeatedly extracted with boiling light petroleum (b. p. 60—80°), from which the acetyl derivative crystallises in minute, yellowish-brown, ill-defined leaflets, m. p. 150—152° (Found : C, 66·4; H, 6·2. $C_{12}H_{13}O_3N$ requires C, 65·8; H, 6·0%). A mixture of the indole and this derivative melted at 144—147°.

Neither 5 : 6-dimethoxyindole nor its 1-acetyl derivative gives the brucine reaction (compare Perkin and Rubenstein, *loc. cit.*, on ethyl 5 : 6-dimethoxyindole-2-carboxylate). In each case addition of a

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* All the compounds described in this communication were analysed for nitrogen by the Pregl–Dumas method, and high results (0·3—1·5%) were consistently obtained. It seems well established that certain anisyl and veratryl derivatives during combustion are liable to give off methane, which gas is not completely oxidised when passed over hot copper oxide (compare Bradley and Robinson, J., 1926, 2361).

little nitric acid to a very dilute solution of the substance in glacial acetic acid produces a yellow tint only, changing to golden-brown on gentle warming. On standing, the colour becomes brownish-red. The indole gives no coloration, and the acetyl derivative a pale green colour, with concentrated sulphuric acid, but in each case addition of a trace of nitric acid at once produces an intense greenish-brown colour, changing quickly to brownish-red and, on standing, to pure red.

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