452. *The Chemistry of the Melanins. Part I. The Synthesis of 5 : 6-Dihydroxyindole and Related Compounds.*

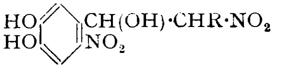
By R. J. S. BEER, KENNETH CLARKE, H. G. KHORANA, and ALEXANDER ROBERTSON.

As part of a comprehensive investigation of the chemistry of the melanins the synthesis of 5 : 6-dihydroxyindole and its monomethyl ethers, and of 5 : 6-dihydroxy-2-methylindole, has been carried out. 5 : 6-Dihydroxyindole and its 2-methyl derivative readily undergo oxidative polymerisation yielding melanins.

FROM a survey of the literature on the constitution and mechanism of formation of the natural and synthetic melanins carried out before the initiation of experimental work now in progress on this subject in these laboratories, it became clear that 5 : 6-*dihydroxyindole* is of considerable interest as the unstable intermediate postulated by Raper (*Biochem. J.*, 1927, **21**, 89) in the tyrosinase-catalysed oxidation of tyrosine to melanin. In this reaction the presence of the indole was inferred from the fact that its dimethyl ether (Oxford and Raper, *J.*, 1927, 417) was isolated after a methylation process. The same dihydroxyindole was considered to be formed by the oxidative action of silver oxide on hydroxytyrosine (Raper, *Biochem. J.*, 1930, **24**, 239) whilst under the same conditions methyl-2-3′ : 4′-dihydroxyphenylethylamine gave 5 : 6-dihydroxy-1-methylindole. Burton (*J.*, 1932, 546) attempted to isolate the latter compound from the oxidation mixture, but was unable to obtain crystalline material, nor was the acetate of his product crystalline (compare Bergel and Morrison, *J.*, 1943, 48).

A study of the oxidation of authentic 5 : 6-dihydroxyindole and of related compounds would be expected to throw light on the formation and structure of melanin, but hitherto workers have been restricted to the use of solutions of unknown concentration, containing considerable amounts of other substances. As a preliminary step in our investigations in this field we have therefore extended the method for the synthesis of hydroxyindoles recently developed in these laboratories (*J.*, 1948, 1605) to the preparation of 5 : 6-dihydroxyindole and of certain related indoles (compare *Nature*, 1948, **161**, 525).


(I.)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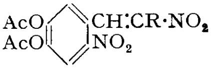
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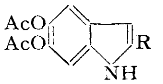
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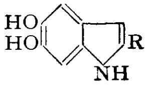
(V.)

According to Harley-Mason (*Nature*, 1947, **159**, 338), nitroprotocatechuic aldehyde (I), the required starting material for the synthesis of (V, R = H), is not satisfactorily prepared by the demethylenation of 2-nitropiperonylidene diacetate with a mixture of acetic anhydride and nitric acid as described by Parijs (*Rec. Trav. chim.*, 1930, **49**, 36). We found, however, that reasonably good yields * of the required nitro-aldehyde (I) could be obtained under certain carefully standardised conditions (see p. 2224). The condensation of the aldehyde (I) and nitromethane was effected by means of alcoholic potash at 0° and, as in our previous work (*loc. cit.*), the resulting alcohol (II, R = H) was not purified but was converted directly into the *diacetoxystyrene* (III, R = H) in very satisfactory yield by treatment with hot acetic anhydride and sodium acetate. Reduction of (III, R = H) with iron filings and acetic acid afforded the *diacetoxyindole* (IV, R = H ; 60%). Since this substance underwent very rapid oxidation in the presence of dilute aqueous alkalis, it seemed likely that the methanolic ammonia deacetylation process used in the synthesis of the much more stable monohydroxyindoles (*loc. cit.*) would fail unless oxygen could be rigorously excluded, and on this account we preferred to attempt the hydrolysis with aqueous-methanolic sodium hydroxide in the presence of a mild reducing agent, sodium hydrosulphite (dithionite). This process furnished a moderate yield of crystalline 5 : 6-dihydroxyindole (V, R = H), which gives a positive Ehrlich and nitroprusside test, forms a red picrate, and shows the expected sensitivity to oxygen in faintly alkaline solution, *e.g.*, in

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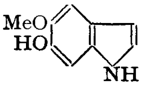
* Probably owing to an error in transcription the yield given in Parijs's communication (*loc. cit.*) exceeds that theoretically possible, *viz.*, 2·50 g. of nitroprotocatechuic aldehyde from 4·0 g. of nitropiperonylidene diacetate, the theoretical yield being 2·46 g.

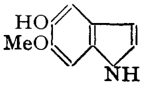
aqueous sodium hydrogen carbonate and to a less extent in neutral aqueous solution (tap water). Solutions of the compound in dilute acetic acid and in non-aqueous solvents are reasonably stable, whilst the solid indole is surprisingly inert. Direct treatment of the alkaline deacetylation mixture with methyl sulphate yielded a crystalline ether, the properties and analysis of which are in agreement with those reported for 5 : 6-dimethoxyindole by Oxford and Raper (*loc. cit.*).

When nitromethane was replaced by nitroethane the same synthetic procedure, with minor modifications, afforded β : 2-*dinitro*-4 : 5-*diacetoxy*-β-*methylstyrene* (III, R = Me) which was converted into 5 : 6-*diacetoxy*-2-*methylindole* (IV, R = Me) and then into 5 : 6-*dihydroxy*-2-*methylindole* (V, R = Me). In this case the initial condensation was less satisfactory and considerable amounts of unchanged nitro-aldehyde were recovered. The dihydroxy-2-methylindole, like 5 : 6-dihydroxyindole, is markedly unstable in faintly alkaline aqueous solution, yielding a dark melanin-like product.

The two monomethyl ethers of 5 : 6-dihydroxyindole, (VI) and (VII), have also been synthesised by the same general method. Thus 6-*acetoxy*-5-*methoxyindole* was obtained from 2-nitro-4-hydroxy-5-methoxybenzaldehyde by way of β : 2-*dinitro*-4-*acetoxy*-5-*methoxystyrene*, and on deacetylation furnished 6-*hydroxy*-5-*methoxyindole* (VI), whilst from 2-nitro-5-hydroxy-4-methoxybenzaldehyde, 5-*hydroxy*-6-*methoxyindole* (VII) was prepared by way of β : 2-*dinitro*-5-*acetoxy*-4-*methoxystyrene* and of 5-*acetoxy*-6-*methoxyindole*.



(VI.)



(VII.)

The deacetylation of the two acetoxy-indoles was effected both by treatment with methanolic ammonia and by the aqueous sodium hydroxide–hydrosulphite method, the latter procedure being definitely superior with 6-hydroxy-5-methoxyindole. Of the two monomethyl ethers, 5-hydroxy-6-methoxyindole is fairly stable in faintly alkaline aqueous solution, whilst the isomeric 6-hydroxy-5-methoxyindole is relatively unstable, but even the latter compound does not undergo oxidative polymerisation with the speed exhibited by 5 : 6-dihydroxyindole or its 2-methyl derivative. Quantitative studies of the latter reaction will be undertaken later, but from our qualitative experiments it appears that the properties of 5 : 6-dihydroxyindole lend support to Raper's view (*loc. cit.*) that this compound is an intermediate in the formation of melanin from tyrosine by enzymatic oxidation.

EXPERIMENTAL.

2-*Nitro*-4 : 5-*dihydroxybenzaldehyde* (2-*Nitroprotocatechuic Aldehyde*) (I).—2-Nitropiperonylidene diacetate (Parijs, *loc. cit.*) (4 g.) was added in small portions during 15 minutes to a mixture of acetic anhydride (20 ml.) and fuming nitric acid (20 ml.; *d* 1·50) which was kept at −12° to −15° and continually agitated. The resulting clear orange-red solution was slowly poured on crushed ice (800 g.) and after the temperature of the mixture had risen to 14° the pale yellow precipitate was collected. When this slightly sticky mass was washed with cold water * (*ca.* 10 ml.), transferred to a round-bottomed flask, and kept for a few minutes, a small quantity of a light orange liquid, containing nitric acid, separated. After this liquor had been decanted the product was heated on the steam-bath with 5% sulphuric acid (15 ml.) for 1 hour, and the resulting hot solution treated with charcoal and filtered. On being kept the cooled filtrate gradually deposited 2-nitro-4 : 5-dihydroxybenzaldehyde as a mass of pale orange, stout rectangular prisms, m. p. 203° (decomp.) (preheated bath). From 4 g. of nitropiperonylidene diacetate the yield of nitroaldehyde varied between 1·25 g. and 1·55 g. and a further quantity of the compound (*ca.* 0·3 g.) was obtained by extraction of the mother-liquor with ether.

β : 2-*Dinitro*-4 : 5-*diacetoxystyrene* (III, R = H).—When potassium hydroxide (1·4 g.), dissolved in water (2 ml.) and 95% alcohol (20 ml.) at 0°, was added dropwise during $\frac{1}{2}$ hour to a manually agitated solution of 2-nitro-4 : 5-dihydroxybenzaldehyde (2 g.) and nitromethane (1·2 g.) in 95% alcohol (20 ml.) maintained at 0° a red precipitate separated. This product became somewhat sticky towards the end of the reaction but on being triturated was converted into a granular powder. After having been kept at 0° for 16 hours the reaction mixture was diluted with ice-water, acidified with dilute hydrochloric acid at 0°, and extracted several times with ether. The combined ethereal extracts were washed three times with aqueous sodium hydrogen sulphite to remove unchanged aldehyde, dried, and evaporated, leaving a product which on occasion partially crystallised. When this material was gently warmed with acetic anhydride (5 ml.) and sodium acetate (1·5 g.) for 15 minutes, and the mixture treated with water (25 ml.), the *dinitrodiacetoxystyrene* (III) (2·6 g.) separated and was collected 1 hour later, and well washed with water. The diacetate, which had m. p. 128—132° (sintering at 124°) and was sufficiently pure to be used in the next stage of the synthesis, was recrystallised from aqueous alcohol, forming slender,

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* Thorough washing at this stage with ice-cold aqueous sodium hydrogen carbonate and then with cold water to remove traces of nitric acid decreased the yield of nitroaldehyde considerably.

elongated, pale orange needles, m. p. 133—134° (sintering at 130°) (Found: C, 46·2; H, 3·4. $C_{12}H_{10}O_8N_2$ requires C, 46·45; H, 3·2%).

5 : 6-*Dihydroxyindole* (V).—When a mixture of the foregoing dinitrodiacetoxystyrene (2 g.), iron powder (10 g.), acetic acid (40 ml.), and absolute alcohol (50 ml.) was gently warmed, a vigorous reaction ensued and this was maintained for 11—12 minutes by occasional heating. The reaction mixture was filtered, the solid washed with small portions of hot alcohol, and the filtrate diluted with water, basified with solid sodium hydrogen carbonate, and extracted five times with ether. Evaporation of the combined dried extracts left a viscous gum which on being kept in a vacuum partially crystallised and was then purified by being extracted several times with hot light petroleum (b. p. 60—80°) containing 20% of benzene., On being kept, the combined extracts deposited 5 : 6-*diacetoxyindole* (0·80 g.), m. p. 130—133° (sintering at 124°), and a further quantity (75—100 mg.) was obtained by concentration of the light petroleum filtrate. Recrystallised from benzene–light petroleum (b. p. 60—80°) (charcoal), this compound (1 g.) formed compact hemispherical clusters of prisms (0·82 g.), m. p. 134—136°, giving strongly positive reactions with the Ehrlich and the nitroprusside reagent (Found: C, 61·5; H, 4·9; N, 6·3. $C_{12}H_{11}O_4N$ requires C, 61·8; H, 4·7; N, 6·0%).

A solution of 5 : 6-diacetoxyindole (0·2 g.) in methanol (5 ml.) at 0° was added to a mixture of sodium hydrosulphite (dithionite) (1 g.) in 10% aqueous sodium hydroxide (5 ml.) kept at 0°, the reaction vessel was closed, and the mixture was vigorously agitated until the purple colouration originally assumed by the solution had disappeared, and then kept at 0° for 15 minutes. After the addition of acetic acid (1 ml.) in water (7 ml.) the resulting pale yellow solution was diluted with water (50 ml.) and extracted several times with ether. The following operations were then carried out in an atmosphere of nitrogen. The combined extracts (*ca.* 50 ml.) were dried over sodium sulphate admixed with a little sodium hydrosulphite, and concentrated to about $\frac{1}{5}$ of the original volume, and the residual liquor was mixed with light petroleum (b. p. 60—80°) (25 ml.) and further concentrated until a small amount of a reddish amorphous material was precipitated; this solid was removed and the liquor was then evaporated until crystalline material began to separate. On being kept, the residue deposited 5 : 6-*dihydroxyindole* (60 mg., 46% of theoretical yield), m. p. 137—138°, which was collected without special precautions and on recrystallisation from benzene–light petroleum (b. p. 60—80°) formed glistening, colourless needles, m. p. 140° (decomp.) (Found: C, 64·7; H, 4·9; N, 9·4. $C_8H_7O_2N$ requires C, 64·4; H, 4·7; N, 9·4%). This compound, which is readily soluble in alcohol, ether, and acetone, appreciably soluble in warm benzene or cold water, and insoluble in light petroleum, gives a deep red colouration with Ehrlich's reagent at room temperature and an intense green ferric reaction in alcohol. On the addition of a drop of aqueous sodium hydroxide an aqueous solution of the indole containing sodium nitroprusside rapidly assumes a dark brown colour which changes to a dirty green on the addition of dilute acetic acid. When stored in a tightly stoppered specimen tube 5 : 6-dihydroxyindole darkens perceptibly in about 14 days, but on exposure to the atmosphere the surface of the crystals becomes dark in the course of 6—8 hours. Solutions of the substance in benzene, ether, or dilute acetic acid are moderately stable, but in tap water a blue colouration appears in the course of a few minutes, whilst the addition of a trace of sodium hydrogen carbonate to an aqueous solution causes rapid oxidation to take place. In the latter case the initially colourless solution becomes violet, and then deep bluish-purple, and eventually a granular black melanin-like precipitate forms.

5 : 6-*Dimethoxyindole*.—5 : 6-Diacetoxyindole (0·2 g.), was deacetylated in the foregoing manner, methyl sulphate (1 g.) was added to the alkaline solution, and the mixture was vigorously agitated in the absence of air for $\frac{1}{2}$ hour, acidified with dilute acetic acid, and extracted with ether. The residue left on evaporation of the dried extracts was digested with boiling light petroleum (b. p. 60—80°), and on being concentrated, the extract deposited 5 : 6-dimethoxyindole in clusters of colourless slender needles, m. p. 142—146°, which on being sublimed in a vacuum at 100—110°/0·05 mm., afforded the pure compound, m. p. 154—155° unchanged on recrystallisation (Found: C, 67·6; H, 6·0. Calc. for $C_{10}H_{11}O_2N$: C, 67·8; H, 6·2%).

β : 2-*Dinitro*-4 : 5-*diacetoxy*-β-*methylstyrene*.—The condensation of 2-nitroprotocatechuic aldehyde (4 g.) with nitroethane (2·8 g.) was effected with potassium hydroxide (3 g.) in 95% alcohol at −10° by the method employed in the case of nitromethane. The mixture was then diluted with an equal volume of water, kept at 0° for 2 days, and thoroughly extracted with ether. Evaporation of the ethereal extracts left the desired product mixed with unchanged aldehyde. This mixture was treated with concentrated aqueous sodium hydrogen sulphite (approx. 50 ml.) for 1 hour, and the required nitro-alcohol isolated by extraction with ether and converted by means of warm acetic anhydride (5 ml.) and sodium acetate (2 g.) into β : 2-*dinitro*-4 : 5-*diacetoxy*-β-*methylstyrene* as in the case of the lower homologue. Triturated with a little cold 95% alcohol, this product was obtained as a colourless powder (1·8 g.) sufficiently pure for conversion into the indole. Crystallised from benzene–light petroleum (b. p. 60—80°), the dinitrostyrene formed fluffy aggregates of colourless slender needles, m. p. 119° (Found: C, 47·8; H, 3·6. $C_{13}H_{12}O_8N_2$ requires C, 48·2; H, 3·7%).

Unchanged aldehyde (1·3 g.) was recovered in the usual manner from the sodium hydrogen sulphite liquors which had been exhausted with ether.

5 : 6-*Dihydroxy*-2-*methylindole* (V, R = Me).—Simultaneous reduction and cyclisation of the foregoing dinitrostyrene (1 g.) by the procedure employed for the lower homologue (III, R = H) gave 5 : 6-*diacetoxy*-2-*methylindole* (IV, R = Me) (0·3 g.), which separated from benzene–light petroleum (b. p. 60—80°) (1 : 2) in compact clusters of colourless, short, stout needles, m. p. 134°, giving with cold Ehrlich reagent an intense red colouration tinged with blue (Found: C, 63·0; H, 5·2; N, 5·9. $C_{13}H_{13}O_4N$ requires C, 63·2; H, 5·3; N, 5·7%).

Deacetylation of the foregoing diacetate (0·2 g.) with aqueous sodium hydroxide containing sodium hydrosulphite according to the method employed for (IV, R = H) gave 5 : 6-*dihydroxy*-2-*methylindole* (0·09 g.) which crystallised from benzene–light petroleum (b. p. 60—80°) in colourless blades, beginning to darken at 180° and becoming black at 200° when heated in a sealed melting-point tube (Found: C, 66·3; H, 5·4; N, 8·3. $C_9H_9O_2N$ requires C, 66·3; H, 5·5; N, 8·6%). This compound, which exhibits an Ehrlich reaction similar to that given by the diacetate, gives an olive-green colouration with alcoholic ferric chloride, rapidly changing to brown. Like the parent 5 : 6-dihydroxyindole (V, R = H) the 2-methyl derivative readily oxidises in aqueous solution containing a little sodium hydrogen carbonate, forming a reddish-brown solution. In organic solvents, *e.g.*, benzene, the substance gradually develops a pink colouration and appears to be somewhat less stable than (V, R = H).

β : 2-*Dinitro*-4-*acetoxy*-5-*methoxystyrene*.—Powdered 6-nitrovanillin (Raiford and Stoesser, *J. Amer. Chem. Soc.*, 1928, **50**, 2559) (0·5 g.), suspended in alcohol (25 ml.), was condensed with nitromethane (0·2 g.) by means of potassium hydroxide (0·3 g.) in the course of 12 hours, and the product, isolated by the general method, was heated with acetic anhydride (2 ml.) and sodium acetate (1·5 g.) until the mixture boiled, and then on the steam-bath for 15 minutes. After the decomposition of the excess anhydride with water the *dinitro-styrene* was isolated, dried, and crystallised from alcohol, forming pale yellow prisms (0·32 g.), m. p. 188° (Found : N, 9·7. $C_{11}H_{10}O_7N_2$ requires N, 9·9%).

6-*Hydroxy*-5-*methoxyindole*.—On reduction and cyclisation by the standard procedure the foregoing dinitro-styrene (0·5 g.) was converted into 6-*acetoxy*-5-*methoxyindole* which was obtained as a brown glass. Extraction of the latter with boiling light petroleum (b. p. 80—100°) gave the pure substance, which separated from the cooled extract in colourless needles (190 mg.), m. p. 135° (Found : N, 7·1. $C_{11}H_{11}O_3N$ requires N, 6·8%). Deacetylation of this derivative (100 mg.) was effected by the methyl alcoholic-ammonia process (*J.*, in the press), giving 6-*hydroxy*-5-*methoxyindole*, which formed flocculent needles (25 mg.), m. p. 111°, from light petroleum (b. p. 80—100°), having an intense reddish-violet ferric reaction in alcohol (Found : C, 66·4 ; H, 5·4 ; N, 8·5. $C_9H_9O_2N$ requires C, 66·3 ; H, 5·5 ; N, 8·6%). It was subsequently found that the sodium hydroxide–sodium hydrosulphite method of deacetylation gave a superior yield of the hydroxyindole (45 mg. from 100 mg. of acetate). A solution of this monomethyl ether in very dilute aqueous sodium hydrogen carbonate develops a purplish tint in about 3 minutes which changes to deep purple in about 10 minutes, whereas in phosphate buffer solution at approximately pH 8 the indole slowly oxidises giving a dark precipitate.

5-*Hydroxy*-6-*methoxyindole*.—From 6-nitro*iso*vanillin (Pschorr, *Ber.*, 1902, **35**, 4396) β : 2-*dinitro*-5-*acetoxy*-4-*methoxystyrene* was prepared by the standard route (yield, 70% of theoretical) and on crystallisation from alcohol formed pale yellow plates, m. p. 162° (Found : N, 9·9. $C_{11}H_{10}O_7N_2$ requires N, 9·9%). This substance was converted into 5-*acetoxy*-6-*methoxyindole* (yield, 63% of theoretical), which separated from light petroleum (b. p. 80—100°) in colourless glistening plates, m. p. 118° (Found : N, 6·8. $C_{11}H_{11}O_3N$ requires N, 6·8%). Deacetylation of this derivative by the methyl-alcoholic ammonia process and by the aqueous sodium hydroxide–sodium hydrosulphite method gave the same yield (63% of theoretical) of 5-*hydroxy*-6-*methoxyindole*, forming colourless needles, m. p. 113°, from light petroleum (b. p. 60—80°), which gave a brown ferric reaction in alcohol (Found : C, 66·3 ; H, 5·5 ; N, 8·3. $C_9H_9O_2N$ requires C, 66·3 ; H, 5·5 ; N, 8·6%).

A solution of this indole in very dilute aqueous hydrogen carbonate assumes a very faint pink tint in the course of 35 minutes, but a phosphate buffer solution at about pH 8 is relatively stable, and does not become coloured within 3 hours.

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UNIVERSITY OF LIVERPOOL.

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