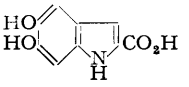
152. *Melanin and Its Precursors. Part II. Model Experiments on the Reactions between Quinones and Indoles, and Consideration of a Possible Structure for the Melanin Polymer.*

By JOHN D. BU'LOCK and JOHN HARLEY-MASON.

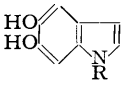
A study has been made of the reactions between indoles and quinones as models of the possible ways in which the melanin intermediate indole-5 : 6-quinone might undergo self-condensation. With certain exceptions, which are discussed, indoles unsubstituted in the 3-position condense with *o*- and *p*-benzoquinone and with 1 : 2- and 1 : 4-naphthaquinone to give intensely coloured indolylquinones. In certain cases these can react at the 2-position, if free, with a further molecule of quinone. With 3-methyl- and 2 : 3-dimethyl-indole, *p*-benzoquinone gives colourless products. The mechanism of these reactions is discussed.

From consideration of the model experiments and the behaviour of known methyl-substituted 5 : 6-dihydroxyindoles on oxidation, a polymeric structure formed by self-condensation of indole-5 : 6-quinone at the 3- and the 7-position, with cross-links involving reaction at the 2-position, is advanced for melanin.

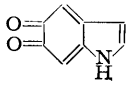
INVESTIGATIONS by Raper *et al.* (Raper, *Biochem. J.*, 1927, **21**, 89; Dulière and Raper, *ibid.*, 1930, **24**, 239) have shown that the enzymic oxidation of tyrosine leads *via* 3 : 4-dihydroxyphenylalanine and a red quinonoid oxidation product derived from it to 5 : 6-dihydroxyindole-2-carboxylic acid (I), 5 : 6-dihydroxyindole (II), and then to melanin. The two dihydroxyindole



(I.)



(II; R = H.)
(III; R = Me.)

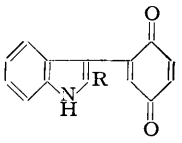


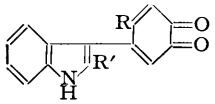
(IV.)

compounds have recently been synthesised (Beer, Clarke, Khorana, and Robertson, *J.*, 1948, 2223), and a comparison of their properties indicates that (II) rather than (I) is the actual precursor of melanin, a conclusion supported by the work of H. S. Mason (*J. Biol. Chem.*, 1948, **172**, 83).

H. S. Mason has also suggested that the formation of indole-5 : 6-quinone (IV) may be the first stage in the oxidative polymerisation of (II) to melanin. In order to throw some light on the possible ways in which (IV), regarded as a bifunctional compound, being both an indole and a quinone, might polymerise, we have studied the interaction of some simple indoles and quinones as models. Apparently, the only comparable reaction on

(V; R = Me.)
(VI; R = H.)





(VII; R = R′ = H.)
(VIII; R = Me, R′ = H.)
(IX; R = H, R′ = Me.)

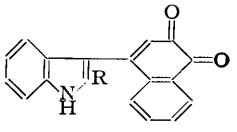
record is that between *p*-benzoquinone and 2-methylindole to give an intensely violet product, 2′-methyl-3′-indolyl-1 : 4-benzoquinone (V), obtained by boiling 2-methylindole (1 mol.) with *p*-benzoquinone (2 mols.) in ethanol (Mohlau and Redlich, *Ber.*, 1911, **44**, 3605). We confirmed this observation and found moreover that the condensation was strongly catalysed by acid, even by a little acetic acid added to the ethanol. When boiling acetic acid alone was

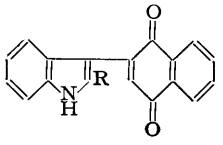
used as solvent, the reaction proceeded a stage further and a mixture of (V) and a very sparingly soluble product formed by the condensation of (V) with a second molecule of 2-methylindole, *viz.*, 2 : 5-di-(2-methyl-3-indolyl)-1 : 4-benzoquinone was obtained. Mohlau and Redlich were unable to isolate any pure product from the reaction of *p*-benzoquinone and indole ; we found, however, that the expected analogue of (V), *viz.*, 3′-indolyl-1 : 4-benzoquinone (VI), can be obtained by reaction in ethanol in the presence of hydrochloric acid. The yield was, however, poor and other ill-defined by-products were also formed. Reaction in boiling acetic acid led to the formation of a sparingly soluble red compound by condensation of two molecules of quinone with one of indole. This compound proved difficult to purify, but was regarded as 2 : 3-di-*p*-benzoquinonylindole by analogy with the corresponding naphthaquinone compound (XIV). 2 : 5- and 2 : 6-Dimethyl-1 : 4-benzoquinone failed to react with indole.

Reactions involving indoles and *o*-quinones were next examined. From indole and *o*-benzoquinone, 4-3′-indolyl-1 : 2-benzoquinone (VII) was readily obtained by reaction in aqueous ethanol at room temperature, and similarly 4-3′-indolyl-5-methyl-1 : 2-benzoquinone (VIII) from indole and 4-methyl-1 : 2-benzoquinone and 4-(2-methyl-3-indolyl)-1 : 2-benzoquinone (IX) from 2-methylindole and *o*-benzoquinone. All three compounds formed deep-violet prisms with a bronze reflex and gave deep-blue or blue-violet solutions in organic solvents. From 2-methylindole and 4-methyl-1 : 2-benzoquinone no product could be obtained, probably owing to steric hindrance (see below). It is noteworthy that the two *o*-quinones are more reactive towards indoles than is the *p*-quinone and that the reaction is complete at room temperature in 5 minutes. No product could be obtained from the attempted reaction of 4 : 5-dimethyl-1 : 2-benzoquinone with indole or 2-methylindole. This quinone was first prepared by Diepolder (*Ber.*, 1909, **42**, 2921) by oxidation of 5-amino-*o*-4-xylenol with aqueous acid dichromate : having encountered some difficulty with this reaction, we sought an alternative. *o*-4-Xylenol was oxidised with alkaline persulphate to give a low yield of 4 : 5-dimethylcatechol (cf. oxidation of *p*-cresol ; G.P. 81,289) and this on oxidation with silver oxide in ether gave an excellent yield of the quinone.

Reactions between indoles and naphthaquinones were then investigated. Herter (*J. Biol. Chem.*, 1904, **1**, 247) observed that indole reacted with solutions of sodium 1 : 2-naphthaquinone-4-sulphonate giving a deep-violet colour, but did not isolate any pure product. We have re-investigated this reaction and have isolated 4-3′-indolyl-1 : 2-naphthaquinone (X) and, from 2-methylindole, 4-(2-methyl-3-indolyl)-1 : 2-naphthaquinone (XI). The yield of the latter was much lower, probably owing to steric hindrance (see below). The same products

(X ; R = H.)
(XI ; R = Me.)





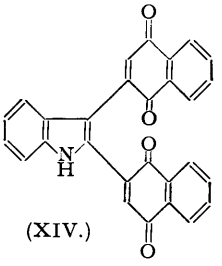
(XII ; R = Me.)
(XIII ; R = H.)

were obtained when 1 : 2-naphthaquinone was employed instead of the 4-sulphonate. 2-Methylindole and 1 : 4-naphthaquinone reacted readily in ethanol containing acetic acid to give 2-(2-methyl-3-indolyl)-1 : 4-naphthaquinone (XII) which formed almost black prisms giving deep-violet solutions in organic solvents, whereas indole and 1 : 4-naphthaquinone in acetic acid gave a mixture of two products, the more soluble giving intensely violet solutions and closely resembling (XII), and the other, very sparingly soluble, forming brilliant red needles which separated from the boiling solvent as the reaction proceeded. Analysis indicated that the first product contained one indole and one naphthaquinone residue and was thus 2-3′-indolyl-1 : 4-naphthaquinone (XIII) analogous to (XII), whereas the second product contained two naphthaquinone residues and was therefore formulated as 2 : 3-di-(1 : 4-naphthaquinon-2-yl)indole (XIV). This formulation is supported by the fact that (XIV) can be obtained by reaction of (XIII) with a further molecule of 1 : 4-naphthaquinone, and that (XII), in which the 2-position is blocked, does not react further with the naphthaquinone. Since (XIII) is presumably first formed, it was hoped that, by conducting the reaction under milder conditions, formation of (XIV) might be avoided, but under all conditions examined the mixture was invariably obtained. 2-Methyl-1 : 4-naphthaquinone failed to react with indole under the above conditions.

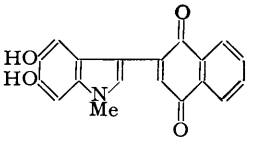
To test the effect on the reaction with quinones of substituents in the indole *Bz*-nucleus, the reaction between 2-methyl-5 : 6-methylenedioxyindole (Burton and Duffield, *J.*, 1950, 78) and *p*-benzoquinone was examined. The presence of the methylenedioxy-group did not appear

to affect materially the course of the reaction, and the expected 2′-methyl-5′ : 6′-methylenedioxy-3′-indolyl-1 : 4-benzoquinone was obtained, with properties very similar to those of (V). However, neither 5 : 6-methylenedioxyindole-2-carboxylic acid nor its ethyl ester could be induced to react with quinones at all, and it appears that the reactivity of the 3-position is completely abolished by the adjacent carboxyl group.

5 : 6-Dihydroxy-1-methylindole (III) (Harley-Mason, *J.*, 1950, 1276) was found to be oxidised to melanin-like products by all the quinones examined, except 1 : 4-naphthaquinone, with which it condensed readily in cold aqueous acetic acid solution to give 2-(5 : 6-dihydroxy-1-methyl-3-indolyl)-1 : 4-naphthaquinone (XV), which separated as deep violet needles. In



(XIV.)



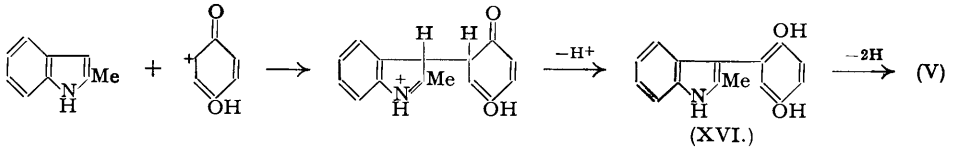
(XV.)

this case it is evident that the hydroxyl groups in the indole nucleus accelerate the reaction considerably, since 1 : 4-naphthaquinone and indole itself react much more slowly under the same conditions. With an excess of the naphthaquinone in boiling acetic acid solution, the product was 5 : 6-dihydroxy-1-methyl-2 : 3-di-(1 : 4-naphthaquinon-2-yl)indole, analogous to (XIV).

All the foregoing experiments describe reactions between quinones and indoles unsubstituted in the 3-position, and the products, except those of type (XIV), all have very similar properties, giving intensely coloured (blue or violet) solutions in ethanol, changing to green or bluish-green on the addition of alkali. To demonstrate that products of this type can only be obtained by reaction at the 3-position, an acetic acid solution of equimolecular amounts of 3-methylindole (scatole) and *p*-benzoquinone was kept at room temperature; the solution slowly became violet-red and a crystalline substance separated, of empirical formula $C_{12}H_{10}ON$; this, if doubled, indicates reaction of 2 mols. of scatole and 1 mol. of *p*-benzoquinone with the loss of 2 hydrogen atoms. A similar product was obtained (though in lower yield) from 2 : 3-dimethylindole and *p*-benzoquinone. Both products were colourless, had high melting points (250—252° and 345—348°, respectively), and were very sparingly soluble in all solvents. They were completely unaffected by aqueous alkali, indicating the probable absence of phenolic hydroxyl groups, but afforded diacetyl derivatives with boiling acetic anhydride. In these the acetyl groups were thus presumably attached to nitrogen, indicating that the molecule probably contains two dihydroindole rings, since indole is not acetylated under these conditions. These compounds were not further studied, and on the above evidence they cannot be assigned a structure, but it is quite clear that they are entirely different from the products obtained from benzoquinone and indoles unsubstituted in the 3-position.

Discussion.

(1) *Mechanism of the Reaction between Indoles and Quinones.*—Mohlau and Redlich (*loc. cit.*) interpret the reaction between 2-methylindole and *p*-benzoquinone as proceeding in two stages : (*a*) addition of the indole to the quinone with the transfer of two hydrogen atoms to give the indolylquinol (XVI); (*b*) oxidation of (XVI) to the corresponding quinone (V) by a second molecule of benzoquinone :

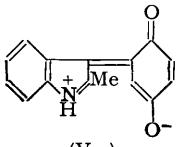


(XVI.)

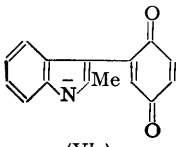
The fact that the reaction is strongly catalysed by acids indicates that the conjugate acid of the quinone is probably the reactive entity involved, so the reaction may be represented as in the scheme. Electron density in the indole nucleus is maximal in the 3-position (Longuet-

Higgins and Coulson, *Trans. Faraday Soc.*, 1947, **43**, 87), so reaction at this point with a strongly cationoid reagent is to be expected. A similar scheme would apply to the reactions of indole and 2-methylindole with *o*-quinones.

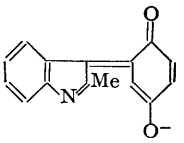
The 3-indolylquinones obtained in this way all have similar properties : They are lustrous crystalline solids, varying in colour from blue-violet to almost black. They are almost insoluble in water but soluble in organic solvents, giving intensely blue-violet solutions. The absorption spectra (Figs. 1, 2, and 3) indicate clearly that they may be regarded as vinylogues of amino-quinones, the indole-nitrogen atom being conjugated with the quinone nucleus. This conjugation is responsible for the principal absorption maximum in the 5200—6000-A. region shown by all the compounds; it results also in a lowering of the oxidation potential of the reduced form (*e.g.*, XVI) so that oxidation to the indolyl-quinone by a second molecule of the original quinone is facilitated. The conjugation implies a considerable contribution from zwitterionic mesomeric structures, such as (V*a*), requiring coplanarity of the indole and quinone nuclei. Inspection of a scale drawing of 4-2′-methyl-3′-indolyl-1 : 2-naphthaquinone shows that there will be considerable steric hindrance of such coplanarity. This is confirmed not only by the slow rate of formation and poor yield of this compound, but also by the greatly reduced intensities of its absorption bonds at 4000 and 5600 A. compared with those at 4000 and 5200 A. in the unhindered compound (X) lacking the methyl group (Fig. 3). The bands at lower wave-lengths correspond to more localised electron transitions and are not affected in this way by the coplanarity criterion. This is a particular case confirming the generalisation that the colour of these compounds is due to the type of conjugation here considered.



(V*a*.)



(V*b*.)



(V*c*.)

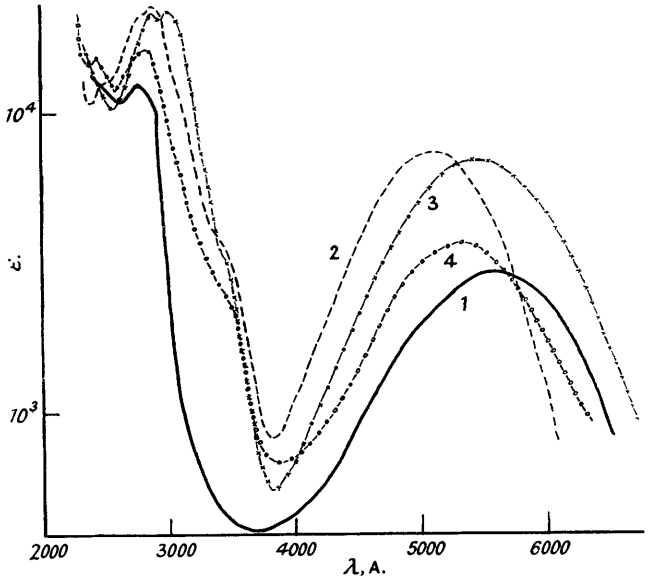
Further evidence in favour of this view is provided by the behaviour of these compounds in alkaline solution. They behave as weak acids and form ions with an intense blue or green colour. Their absorption spectra (Fig. 4) (in some cases only approximate because of the rather rapid decomposition of the solutions) all show intense long-wave-length (*ca.* 6500 A.) absorption which is ascribed to resonance between structures such as (V*b*) and (V*c*). Since the low-energy transition corresponding to the 5100-A. bond of the neutral molecule (V $\longleftrightarrow$ V*a*) involves charge separation whilst that in the anion (V*b* $\longleftrightarrow$ V*c*) does not, the latter transition is even more favoured and the absorption of its anion is of higher intensity. The same steric factors should be operative in the anion of (XI) as in the neutral molecule, and in fact comparison of the absorption spectra (Fig. 4) of the anions of (XI) and (X) shows that the effect is even more marked here than in the neutral molecules.

Inspection of a scale drawing of 5-methyl-4-(2-methyl-3-indolyl)-1 : 2-benzoquinone shows that the 5-methyl group overlaps the indole 4-position and the 2-methyl group, preventing coplanarity. In this case the steric effect is so large that all attempts to obtain this compound by reaction of 4-methyl-1 : 2-benzoquinone and 2-methylindole were unsuccessful, whereas the two compounds (VIII) and (IX), lacking either of the methyl groups, were readily obtained.

The failure of 2 : 5- and 2 : 6-dimethyl-1 : 4-benzoquinones, 4 : 5-dimethyl-1 : 2-benzo-quinone, and 2-methyl-1 : 4-naphthaquinone to react with indoles is also probably due to steric hindrance : Madinaveitia (*Anal. Fis. Quím.*, 1933, **31**, 750) has commented on the lower reactivity of 2-methyl-1 : 4-naphthaquinone than of 1 : 4-naphthaquinone towards nucleophilic reagents. On the other hand, the failure of 5 : 6-methylenedioxyindole-2-carboxylic acid to react with quinones is probably caused by the electromeric effect of the carboxyl group with-drawing electrons from the 3-position.

The following general conclusions may be drawn from the reactions between indoles and quinones studied : (1) Indoles unsubstituted in the 3-position react readily at this position with *o*- and *p*-quinones, undergoing oxidative condensation to give highly coloured products in which the indole and the quinone rings are conjugated and coplanar or nearly coplanar. (2) If the indole 2-position is free, reactions with a second molecule of quinone may occur at this position. (3) The reaction is catalysed by acids, the uncatalysed reaction being much slower. *o*-Quinones are more reactive than *p*-quinones. (4) Substituents in other positions (apart from carboxyl in the 2-position, which prevents reaction altogether) do not directly

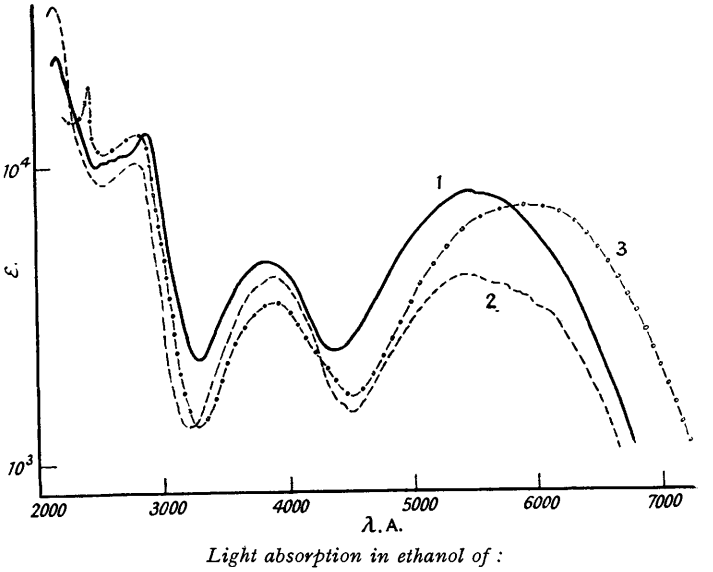
FIG. 1.



Light absorption in ethanol of :

1. 2′-*Methyl*-3′-*indolyl*-1 : 4-*benzoquinone* (V).
2. 2-3′-*Indolyl*-1 : 4-*naphthaquinone* (XIII).
3. 2-(5 : 6-*Dihydroxy*-1-*methyl*-3-*indolyl*)*naphtha*-1 : 4-*quinone* (XV).
4. 2-(2-*Methyl*-3-*indolyl*)-1 : 4-*naphthaquinone* (XII).

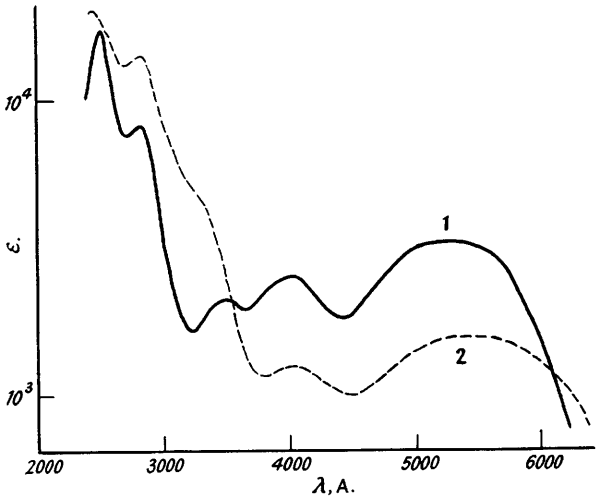
FIG. 2.



Light absorption in ethanol of :

1. 4-3′-*Indolyl*-1 : 2-*benzoquinone* (VII).
2. 5-3′-*Indolyl*-4-*methyl*-1 : 2-*benzoquinone* (VIII).
3. 4-(2-*Methyl*-3-*indolyl*)-1 : 2-*benzoquinone* (IX).

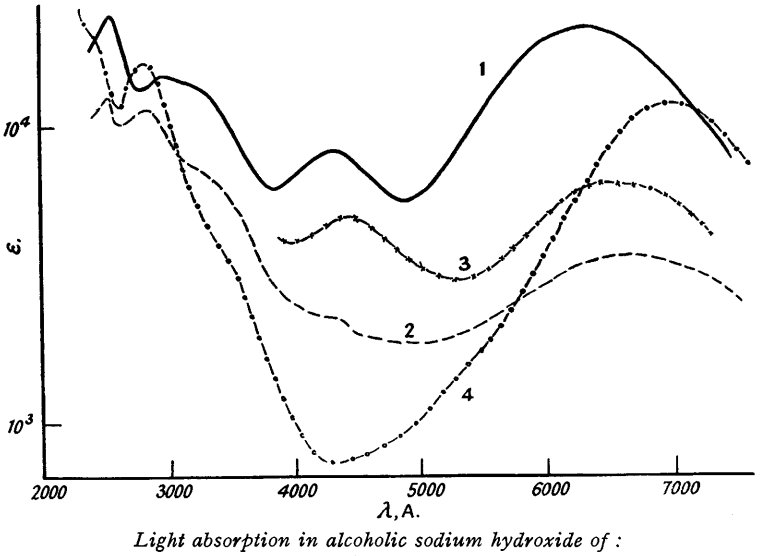
FIG. 3.



Light absorption in ethanol of :

1. 4-3′-*Indolyl*-1 : 2-*naphthaquinone* (X).
2. 4-(2-*Methyl*-3-*indolyl*)-1 : 2-*naphthaquinone* (XI).

FIG. 4.



Light absorption in alcoholic sodium hydroxide of :

1. 4-3′-*Indolyl*-1 : 2-*naphthaquinone* (X).
2. 4-(2-*Methyl*-3-*indolyl*)-1 : 2-*naphthaquinone* (XI).
3. 4-3′-*Indolyl*-1 : 2-*benzoquinone* (VII).
4. 2-(2-*Methyl*-3-*indolyl*)-1 : 4-*naphthaquinone* (XII).

affect the course of the reaction provided that steric effects are not involved : in the one case of a 5 : 6-dihydroxyindole studied, the hydroxyl groups are found to facilitate reaction. (5) Steric effects are particularly important : in cases where overlapping substituents would cause large deviations from coplanarity of the product, the reaction proceeds with difficulty or not at all. (6) When the indole 3-position is blocked, reaction follows a different course, and colourless products are formed.

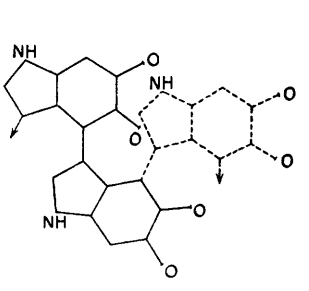
(2) *The Structure of Melanin.*—Three monomethyl-substituted 5 : 6-dihydroxyindoles have recently been synthesised, and their oxidation studied. 5 : 6-Dihydroxy-1- and -2-methylindole give " methyl-melanins " (Burton, *Chem. and Ind.*, 1947, 283) whereas the 3-methyl compound

(Beer, McGrath, Robertson, and Woodier, *J.*, 1949, 2061) does not, thus suggesting that the 3-position of 5 : 6-dihydroxyindole is directly concerned in the oxidative polymerisation to melanin. Experiments on the oxidation of (III) (Harley-Mason, *loc. cit.*) have shown that hydroxylation at position 3 is unlikely to be involved, since the product which would thus be obtained, 3 : 5 : 6-trihydroxy-1-methylindole, was synthesised by another route and found to give 5 : 6 : 5′ : 6′-tetrahydroxy-1 : 1′-dimethylindigo on further oxidation, and this is quite unlike melanin (cf. Harley-Mason, *J.*, 1948, 1244).

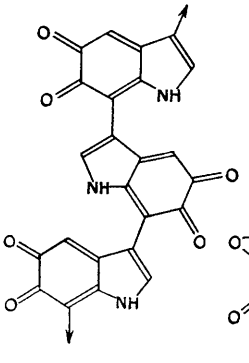
The most likely alternative is that an oxidative condensation occurs at the 3-position. Indirect evidence that this condensation may be similar to the indole–quinone reactions which we have studied, is provided by the fact that 5 : 6-dihydroxyindole-2-carboxylic acid, in contradistinction to the 2-methyl compound, does not give melanin on autoxidation (Beer *et al.*, *loc. cit.*). Further, Clemo and Duxbury (*J.*, 1950, 1795) have shown that enzymic oxidation of tyrosine ethyl ester gives a brown pigment in which the carboxyethyl residue is retained and which differs in many respects from tyrosine-melanin. These results could be accounted for by deactivation of the 3-position by the adjacent carboxyl group [cf. conclusion (4), above].

A polymerisation of indole-5 : 6-quinone (IV) could take place by repeated oxidative condensation involving the 3-position of one molecule and the 4- or 7-position of another, the condensation being analogous to the indole–quinone reactions described above. Scale diagrams (XVII) and (XVIII) show the two structures which would thus be obtained, three of the repeating units being shown in each case.

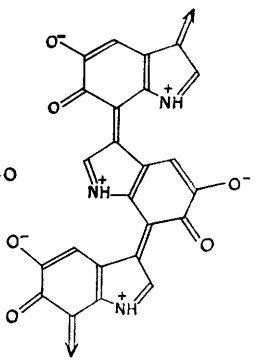
It will be seen that (XVII), the structure derived by condensation in the 4-position, cannot possibly be coplanar and therefore, by analogy with the model experiments above [cf. conclusion (5)], it is unlikely that a product of this structure could be formed at all



(XVII.)



(XVIII.)



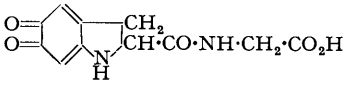
(XVIII*a*.)

easily. No such difficulty arises with (XVIII), and moreover the quinone rings are conjugated with the indole-nitrogen atoms of the neighbouring units (as in the canonical structure XVIII*a*). Extensive conjugation of this kind throughout long chains of varying length would account for the intense light-absorption over a wide range of wave-lengths which melanin displays. The extreme insolubility of melanin suggests that, although polymer chains such as (XVIII) may form " backbone units," some sort of cross-linking is probably present. This could be provided by condensation at the free 2-position with the 4- or the 7-position of another molecule, a process analogous to the formation of indolyl-2 : 3-diquinones described above. Reaction at the 2-position would occur less readily than at the 3-position, but the occurrence of a few such links would be sufficient to build up a highly irregular three-dimensional polymer.

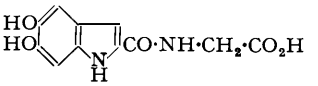
The repeating unit in (XVIII) has the empirical formula $C_8H_3O_2N$: derivation of such a structure by oxidation of tyrosine would require consumption of 5 atoms of oxygen per molecule, in good agreement with Dulière and Raper's results (*loc. cit.*). Analytical figures for tyrosine-melanin lead to empirical formulæ $C_8H_5O_3N$ (*idem*) and $C_{16}H_{10}O_5N_2$ (Burton, *loc. cit.*). The first differs from $C_8H_3O_2N$ by $1H_2O$ and the second from $2C_8H_3O_2N$ by $1H_2O$ and 2 hydrogen atoms. Two other condensed 5 : 6-dihydroxyindole derivatives (5 : 6 : 5′ : 6′-tetrahydroxy-indigo and its *NN*′-dimethyl derivative; Harley-Mason, *loc. cit.*) have been shown to retain water extremely firmly, and it is suggested that this same firm retention occurs in melanin—such retention by a cross-linked amorphous polymer is *a priori* not unlikely.

Bawn and White (quoted by Beer *et al.*, *loc. cit.*) have found that the autoxidation of 5 : 6-dihydroxyindole shows the characteristics of an oxidative chain reaction and is susceptible to trace-metal catalysis. If the polymerisation proceeds by a radical-chain mechanism, the initiating radical is most probably not (IV) but the corresponding semiquinone formed by loss of one hydrogen atom from (II). The model indole–quinone reactions we have described are almost certainly ionic in mechanism and it is possible that a radical-mechanism polymerisation may proceed differently. None the less, we regard a cross-linked polymer based on (XVIII) as the most satisfactory structure for melanin on present evidence. A test of its validity would be provided by the investigation of the oxidation of a 5 : 6-dihydroxyindole substituted in the 7-position, which should not give a melanin, and we are engaged upon the synthesis of such a compound.

Melanins isolated from natural sources are usually conjugated with protein, and in an attempt to throw some light on the nature of this conjugation we have examined the oxidation with mushroom tyrosinase of the dipeptide tyrosylglycine. This oxidation led to a bright red solution having an absorption spectrum almost identical with that of the red quinone obtained by similar oxidation of tyrosine itself. Continued oxidation led to a brown solution, but no precipitate of melanin was obtained. When the red solution was kept under nitrogen it was slowly decolorised and an ethereal extract then exhibited a bluish-violet fluorescence in ultraviolet light and gave a green colour with ferric chloride. Evidently the oxidation of the dipeptide proceeds initially in the same manner as that of tyrosine, through the red quinone



(XIX.)



(XX.)

(XIX) to the dihydroxyindole compound (XX), and it would be expected that a tyrosyl end-group of a protein would behave similarly. Similar behaviour of a tyrosyl residue in the middle of a peptide chain is, however, very improbable, since it has been shown that *N*-formyl- and *N*-acetyl-tyrosine are not oxidised by tyrosinase (Lerner, Fitzpatrick, and Summerson, *Fed. Proc.*, 1949, **8**, 218), so the presence of an unacylated amino-group appears to be essential. In (XX) the 3-position is deactivated so that oxidative polymerisation to give a melanin does not occur, but the 7-position remains free for oxidative coupling with dihydroxyindole derived from unbound tyrosine. In this way a polymer could be build up having an end group such as (XX) in which the glycine residue was replaced by a protein chain.

Experimental.

Reaction of p-*Benzoquinone with Excess of 2-Methylindole.*—A solution of 2-methylindole (1·5 g.) and *p*-benzoquinone (1 g.) in acetic acid (25 c.c.) was heated under reflux for 5 hours. After cooling, the product was filtered off and recrystallized from nitrobenzene. 2 : 5-*Di*-(2-*methyl*-3-*indolyl*)-1 : 4-*benzoquinone* formed small brownish-violet plates, m. p. 294—296°, and was very sparingly soluble in all the usual solvents (Found : C, 78·4; H, 5·1; N, 7·7. $C_{24}H_{18}O_2N_2$ requires C, 78·7; H, 4·9; N, 7·6%). Light absorption in acetone : max. 4980 A.; $\varepsilon = 6620$.

Reaction of p-*Benzoquinone with Indole.*—(*a*) To a solution of indole (1 g.) and *p*-benzoquinone (2 g.) in ethanol (100 c.c.) 5 drops of concentrated hydrochloric acid were added with stirring. After 10 minutes at room temperature, the deep-violet solution was diluted with water (100 c.c.). A precipitate separated, consisting of deep violet needles admixed with greenish-yellow amorphous material. This was collected by filtration and extracted with cold aqueous 2% sodium hydroxide, which dissolved the amorphous material. The residue was recrystallized by warming it with ethanol to 50°, filtering, and cooling the filtrate to −30°. 3′-*Indolyl*-1 : 4-*benzoquinone* formed deep violet-black needles which decomposed without melting at *ca.* 140°, and gave reddish-violet solutions in ethanol, acetic acid, and benzene which decomposed very rapidly on heating (Found : C, 74·9; H, 3·7. $C_{14}H_9O_2N$ requires C, 75·2; H, 4·0%).

(*b*) A solution of indole (1·2 g.) and *p*-benzoquinone (3·5 g.) in acetic acid (50 c.c.) was heated under reflux. The solution rapidly became violet and then red, and some reddish solid soon began to separate. After 2 hours' boiling, the material was filtered off hot and twice recrystallised from nitrobenzene. It formed a dull red powder which remained unmelted below 360°, was almost insoluble in all the usual solvents, and was substantially 2 : 3-*di*-p-*benzoquinonylindole* (Found : C, 74·0; H, 3·4; N, 4·6. $C_{20}H_{11}O_4N$ requires C, 73·0; H, 3·35; N, 4·3%).

Reaction of o-Benzoquinone with Indole and 2-Methylindole.—Catechol (2·5 g.) was dissolved in dry ether (70 c.c.), and the solution shaken for 20 minutes with silver oxide (10 g.) and anhydrous sodium sulphate (10 g.). The solids were filtered off, and the filtrate cooled to −40°. After 10 minutes, the crystals of *o*-benzoquinone were filtered off and dissolved at once in aqueous ethanol (20 c.c.; 1 : 1). A solution of indole (0·2 g.) in aqueous ethanol (10 c.c.; 1 : 1) was added immediately. The mixture at once became deep violet and the product rapidly separated. After 3 minutes it was collected and

purified by dissolution in acetone at room temperature, filtering, and cooling the filtrate to −40°. 4-3′-*Indolyl*-1 : 2-*benzoquinone* (Found : C, 74·3; H, 4·0; N, 6·3. $C_{14}H_9O_2N$ requires C, 75·2; H, 4·0; N, 6·3%) formed small violet needles with a bronze reflex, m. p. 160° (decomp.). Light absorption in ethanol : Max., 2170, 2880, 3780, and 5390—5430 A.; ε = 24,200, 13,500, 4870, and 8140, respectively. The deep-violet solutions in ethanol and acetone decomposed very rapidly when warmed. Alcoholic sodium hydroxide gave a transient intense greenish-blue colour. Reduction with sodium dithionite afforded a colourless solution with a strong violet fluorescence.

Repetition of the above procedure using 2-methylindole in place of indole gave 4-(2-*methyl*-3-*indolyl*)-1 : 2-*benzoquinone*, m. p. 171° (decomp.) (Found : C, 76·3; H, 4·9. $C_{15}H_{11}O_2N$ requires C, 75·9; H, 4·7%). Light absorption in ethanol : Max. 2450, 2820, 3940, and 5950 A.; ε = 19,000, 12,700, 3500, and 7400, respectively. This compound was much more stable in solution than its lower homologue, and was recrystallised from boiling ethanol.

Reaction of 4-*Methyl*-1 : 2-*benzoquinone with Indole.*—(*a*) 4-Methyl-1 : 2-benzoquinone was prepared as described above, 4-methylcatechol being used in place of catechol. The quinone (0·6 g.) in aqueous ethanol (30 c.c.; 1 : 1) was added to a solution of indole (0·3 g.) in ethanol (10 c.c.). The product separated rapidly from the violet solution and was collected and recrystallised from ethanol. 4-3′-*Indolyl*-5-*methyl*-1 : 2-*benzoquinone* formed violet prisms, m. p. 158° (Found : C, 75·4; H, 4·6; N, 5·8. $C_{15}H_{11}O_2N$ requires C, 75·9; H, 4·7; N, 5·9%). Light absorption in ethanol : Max., 2180, 2810, 3880, and 5460—5510 A.; ε = 36,400, 10,530, 4500, and 4520, respectively.

(*b*) To a solution of 4-methylcatechol (1·2 g.) in water (100 c.c.), a solution of indole (1·1 g.) in ethanol (50 c.c.) was added, and to this a solution of potassium ferricyanide (13·2 g.) and sodium hydrogen carbonate (3·4 g.) in water (80 c.c.) was added slowly with stirring. The product, which separated rapidly as deep violet needles, was collected and recrystallised as above.

Reaction of Sodium 1 : 2-*Naphthaquinone*-4-*sulphonate with Indole and* 2-*Methylindole.*—The sulphonate (recrystallised; 0·5 g.) was dissolved in boiling water (30 c.c.) and added to a solution of indole (0·25 g.) in water containing a little ethanol at 100°. The product rapidly separated in crystalline form, and was collected and recrystallised from nitrobenzene. 4-3′-*Indolylnaphtha*-1 : 2-*quinone* formed purple needles, m. p. 272—275° (decomp.) (Found : C, 79·5; H, 4·7; N, 4·9. $C_{18}H_{11}O_2N$ requires C, 79·2; H, 4·1; N, 5·1%). Light absorption in ethanol : Max., 2520, 2800, 3450, 4000, and 5150 A.; ε = 17,000, 8100, 2050, 2500, and 3200, respectively. It was sparingly soluble in acetone, ethanol, and acetic acid, giving reddish-purple solutions.

When the above procedure was repeated but with 2-methylindole in place of indole, the reaction was much slower and the product separated during several hours as an amorphous purple powder. This was purified by chromatography from a chloroform solution on alumina. After elution and removal of the solvent, 4-2′-*methyl*-3′-*indolyl*-1 : 2-*naphthaquinone*, purple needles, m. p. 200—205° (decomp.), was recrystallised from nitrobenzene (Found : C, 79·2; H, 4·7; N, 5·1. $C_{19}H_{13}O_2N$ requires C, 79·4; H, 4·5; N, 4·9%). Light absorption in ethanol : Max., 2500, 2800, 4000, and 5600 A.; ε = 18,000, 14,000, 1200, and 1600, respectively.

Reaction of 1 : 4-*Naphthaquinone with Indole and* 2-*Methylindole.*—Indole (0·4 g.) and 1 : 4-naphthaquinone (0·6 g.) were dissolved in acetic acid (20 c.c.) and left at room temperature. After 7 days, the red needles which had separated were collected and recrystallised from nitrobenzene. 2 : 3-*Di*-(1 : 4-*naphthaquinon*-2-*yl*)*indole* (XIV) formed brilliant scarlet needles, m. p. 356—360° (decomp.), very sparingly soluble in all the usual solvents, giving orange solutions (Found : C, 78·6; H, 3·6; N, 3·3. $C_{28}H_{15}O_4N$ requires C, 78·3; H, 3·5; N, 3·3%). When boiled with sodium hydroxide solution, the compound was slowly decomposed with the liberation of indole. Reduction with sodium dithionite gave a solution with a marked yellow fluorescence. Light absorption in acetone : Max., 3270, 3820, and 4800 A.; ε = 8800, 9000, and 4100, respectively.

The reddish-violet acetic acid mother-liquor was diluted with water (10 c.c.) and kept overnight, and the precipitate collected and twice recrystallised from ethanol. 2-3′-*Indolyl*-1 : 4-*naphthaquinone* formed glittering violet plates with a metallic lustre, m. p. 176° (Found : C, 78·8; H, 4·1; N, 5·3. $C_{18}H_{11}O_2N$ requires C, 79·2; H, 4·1; N, 5·1%). Its solutions in ethanol, acetone, and acetic acid were violet-red. Light absorption in ethanol : Max., 2175, 2830, and 5040—5090 A.; ε = 27,600, 21,550, and 7350, respectively. The diquinone (XIV) was obtained in higher yield as the only product when indole (0·6 g.) and 1 : 4-naphthaquinone (2 g.) in acetic acid (30 c.c.) were refluxed for 2 hours. After cooling, the product, most of which had separated during the heating, was collected and purified as above.

2-Methylindole (0·65 g.) and 1 : 4-naphthaquinone (1·6 g.) were refluxed for 5 hours in ethanol (30 c.c.) containing acetic acid (5 c.c.). 2-2′-*Methyl*-3′-*indolyl*-1 : 4-*naphthaquinone* separated on cooling of the violet solution, and after collection and recrystallisation from nitrobenzene formed very deep violet prisms, m. p. 176—178° (Found : C, 79·1; H, 5·5; N, 4·9. $C_{19}H_{13}O_2N$ requires C, 79·4; H, 5·5; N, 4·9%). Light absorption in ethanol : Max., 2450, 2800, and 5350 A.; ε = 15,100, 16,500, and 3710, respectively.

Reaction of 1 : 4-*Naphthaquinone with* 5 : 6-*Dihydroxy*-1-*methylindole.*—(*a*) The indole (0·2 g.) was dissolved in 20% acetic acid (10 c.c.), and a solution of the quinone (0·3 g.) in acetic acid (5 c.c.) was added. The mixture became violet, and the product soon began to separate. After 24 hours it was collected and recrystallised from ethanol, forming deep violet needles, m. p. 290—293° (decomp.) (Found : C, 71·6; H, 4·1. $C_{19}H_{13}O_4N$ requires C, 71·2; H, 4·1%). 2-(5 : 6-*Dihydroxy*-1-*methyl*-3-*indolyl*)-1 : 4-*naphthaquinone* was soluble in the common organic solvents, giving blue-violet solutions; in aqueous sodium hydroxide it gave a transient blue colour. Light absorption in ethanol : Max., 2870, 3020, and 5450 A.; ε = 21,500, 22,060, and 7050, respectively.

(*b*) 5 : 6-Dihydroxy-1-methylindole (0·1 g.) and 1 : 4-naphthaquinone (0·3 g.) were refluxed in acetic acid (8 c.c.) for 2 hours. The product separated during the heating and was filtered off hot from

the violet solution. After recrystallisation from nitrobenzene, 5 : 6-*dihydroxy*-1-*methyl*-2 : 3-*di*-(1 : 4-*naphthaquinon*-2-*yl*)*indole* (Found : C, 70·4; H, 3·5; N, 4·5. $C_{29}H_{17}O_6N,C_6H_5O_2N$ requires C, 70·2; H, 3·7; N, 4·7%) formed brownish-red needles (containing one molecule of solvent) which decomposed without melting at 345° and were very sparingly soluble in organic solvents to give an orange colour.

Reaction of p-*Benzoquinone with* 2-*Methyl*-5 : 6-*methylenedioxyindole*.—The indole (0·35 g.) and *p*-benzoquinone (0·43 g.) in ethanol (10 c.c.) containing acetic acid (1 c.c.) were boiled under reflux for 2 hours and the product, which crystallised overnight, was collected and recrystallised from benzene. 2-(2-*Methyl*-5 : 6-*methylenedioxy*-3-*indolyl*)-1 : 4-*benzoquinone* formed deep violet prisms, m. p. 191° (decomp.), and gave violet solutions in the common organic solvents (Found : N, 4·8. $C_{16}H_{11}O_4N$ requires N, 5·0%).

Reaction of p-*Benzoquinone with Scatole and* 2 : 3-*Dimethylindole*.—A solution of scatole (1·3 g.) and *p*-benzoquinone (1·1 g.) in acetic acid (30 c.c.) rapidly became reddish-violet. After 24 hours, the precipitate, which was a mixture of greyish prisms (the required product) and dark greenish-black needles (quinhydrone), was collected, washed with ethanol, and extracted with boiling water to remove the quinhydrone. The residue was twice recrystallised from nitrobenzene, and the *substance* formed colourless plates, m. p. 250—252°, very sparingly soluble in all organic solvents and unaffected by aqueous alkali (Found : C, 78·1; H, 5·5; N, 7·4. $C_{24}H_{20}O_2N_2$ requires C, 78·3; H, 5·4; N, 7·4%). When this was refluxed with acetic acid–acetic anhydride (1 : 1) the *diacetyl* derivative, colourless plates (from nitrobenzene), m. p. 360°, was obtained (Found : C, 74·2; H, 5·3; N, 6·2. $C_{28}H_{24}O_4N_2$ requires C, 74·3; H, 5·3; N, 6·2%). Similar treatment of 2 : 3-dimethylindole (4·2 g.) and *p*-benzoquinone (3 g.) in acetic acid (100 c.c.) gave a *substance* which, after recrystallisation (nitrobenzene), formed colourless plates, m. p. 345—348° (Found : C, 78·6; H, 5·9; N, 6·9. $C_{26}H_{24}O_2N_2$ requires C, 78·8; H, 6·1; N, 7·1%); its *diacetyl* derivative, prepared as above, separated from nitrobenzene as colourless plates, m. p. 352—354° (Found : C, 74·6; H, 5·7; N, 5·8. $C_{30}H_{28}O_4N_2$ requires C, 75·0; H, 5·8; N, 5·8%).

4 : 5-*Dimethylcatechol*.—*o*-4-Xylenol (15 g.) was dissolved in a solution of sodium hydroxide (25 g.) in water (1 l.). Potassium persulphate (30 g.) was added, and the mixture stirred at 35° until all was dissolved. After 4 days, the dirty brown solution was saturated with carbon dioxide and then extracted with ether (3 × 100 c.c.) to remove unchanged xylenol. The aqueous solution was acidified with hydrochloric acid, boiled for 0·5 hour, kept overnight, then decanted from tar and extracted with ether (3 × 150 c.c.). The ether was removed, and the residue extracted with water (50 c.c.) at 50°, filtered through charcoal, and evaporated to dryness. The residual gum was sublimed in a vacuum, giving colourless needles of 4 : 5-dimethylcatechol (1·8 g.), m. p. 80—82°.

4 : 5-*Dimethyl*-1 : 2-*benzoquinone*.—4 : 5-Dimethylcatechol (1 g.) was dissolved in dry ether (100 c.c.) and shaken for 20 minutes with silver oxide (4 g.) and anhydrous sodium sulphate (5 g.). The solids were removed by filtration, and the orange filtrate was concentrated to 10 c.c. When the concentrate was kept at 0°, the quinone separated as long orange needles, m. p. 101—102°. It was very much more stable than *o*-benzoquinone and could be kept for 2 months in the dark with little decomposition.

5 : 6-*Methylenedioxyindole*-2-*carboxylic Acid*.—This was prepared precisely as described by Lions and Spruson, *J. Proc. Roy. Soc. N.S.W.*, 1932, **66**, 171, for the 5 : 6-dimethoxy-analogue. *Ethyl* 5 : 6-*methylenedioxyindole*-2-*carboxylate* separated from ethanol as small yellowish prisms, m. p. 180° (Found : C, 70·0; H, 4·9. $C_{12}H_{11}O_4N$ requires C, 61·9; H, 4·7%), and the *acid* formed colourless plates, m. p. 250—251° (efferv.), from benzene (Found : C, 58·6; H, 3·5. $C_{10}H_7O_4N$ requires C, 58·5; H, 3·4%).

The authors thank Dr. R. Pitt-Rivers for a gift of tyrosylglycine.

UNIVERSITY CHEMICAL LABORATORY, CAMBRIDGE. [*Received, October 12th*, 1950.]