259. *The Chemistry of Adrenochrome and its Derivatives.*

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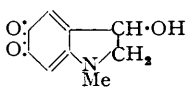
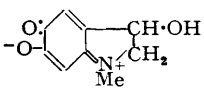
The reduction of adrenochrome (I) has been shown to give rise to two products : 5 : 6-dihydroxy-1-methylindole (II), and an intermediate zwitterion easily converted into 3 : 5 : 6-trihydroxy-1-methylindole (III). The latter product is also obtained by the action of alkali on (I), and on oxidation gives 5 : 6 : 5′ : 6′-tetrahydroxy-1 : 1′-dimethylindigo. From the reduction of 2-bromoadrenochrome, only 2-bromo-5 : 6-dihydroxy-1-methylindole could be isolated.

The oxidation of (II) and the conversion of (I) into "adrenaline black" have been studied, and the structure of the latter is discussed. The preparation of aqueous solutions of (I) by oxidation of adrenaline with buffered persulphate is described.

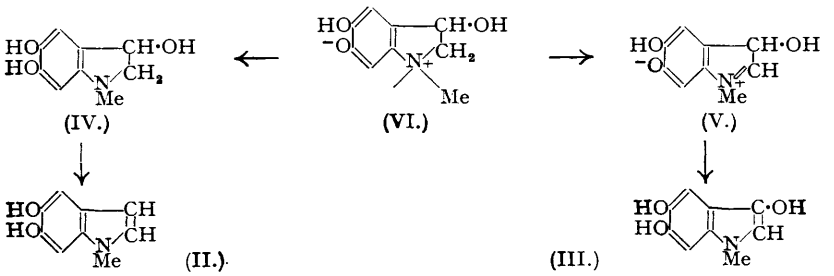
ADRENOCHROME (I) was first isolated by Green and Richter (*Biochem. J.*, 1937, **31**, 596), who obtained it by the oxidation of adrenaline with catechol oxidase in aqueous solution. The preparation was improved by Veer (*Rec. Trav. chim.*, 1942, **61**, 638), who employed silver oxide as oxidising agent, and subsequently by Buchnea (*C.I.O.S. Report* No. 259; a brief account of the method is given in the Experimental section, owing to the inaccessibility of the original). The structure (I) proposed by Green and Richter has been generally accepted, though it has been argued recently (Harley-Mason, *Experientia*, 1948, **4**, 307) that the zwitterionic *p*-quinonoid mesomeric structure (I*A*) makes a large contribution to the resonance hybrid.

(I.)   (I*A*.)

The pharmacological properties of (I) and its derivatives have excited considerable attention in recent years (cf. the review by Bacq, *J. Pharm. Exp. Therap.*, 1949, **95**, [ii], 1), and the present work is aimed at clearing up some of the discrepancies in the pharmacological literature by isolating pure materials from the rather complex transformation products of adrenaline, and submitting them to test independently.

The reduction of adrenochrome was first studied. On hydrogenation of an aqueous solution at room temperature and atmospheric pressure over palladium–charcoal, hydrogen uptake was found to cease when approximately *one* atom of hydrogen per mole of (I) had been absorbed. Ether-extraction of the resulting yellow solution afforded 5 : 6-*dihydroxy*-1-*methylindole* (II), which formed colourless needles, and gave a diacetate identical with that obtained by Bergel and Morrison (*J.*, 1943, 48). Evaporation of a portion of the aqueous solution gave no crystallisable product, but addition of alkali followed by acidification with acetic acid gave a yellow substance $C_9H_{11}O_4N$ which was formulated as 2 : 3 : 5 : 6-tetrahydroxy-2 : 3-dihydro-1-methylindole in a preliminary communication (Harley-Mason, *loc. cit.*). However, it was subsequently found that, on intensive drying, a molecule of water was lost, giving anhydrous 3 : 5 : 6-*trihydroxy*-1-*methylindole* (III); the original material was thus the corresponding *monohydrate* which retains the molecule of water rather firmly. The same results were obtained with sodium dithionite as the reducing agent, though satisfactory yields could only be obtained if the dithionite was fresh (and thus did not contain bisulphite formed by oxidation). Further, an excess of dithionite must be avoided, or the yield of (II) is much reduced, apparently owing to the formation of a bisulphite compound.

The mechanism of the reduction of (I) is thus of particular interest since, instead of the expected leuco-compound (IV), two products are obtained—one a dehydration product of (IV) and the other the zwitterion (V), isomeric with the original quinone. Moreover, these two products are found in approximately equal quantities. This suggests strongly that they are formed by the disproportionation of a common intermediate, the semi-quinone (VI) formed by addition of a hydrogen atom to (I) as represented in the scheme. The ready dehydration of

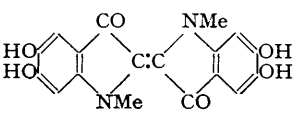


(IV) to (II) is substantiated by the work of Wiesner (*Biochem. Z.*, 1942, **313**, 48) who examined the polarographic reduction of adrenochrome and found that the initial reduction product [which could be re-oxidised to (I)] was rapidly and irreversibly transformed into a product which could not be re-oxidised to (I).

Green and Richter (*loc. cit.*) observed that the reduction of adrenochrome gave an optically active solution; this result has been confirmed and moreover the rotation does not change during 24 hours at room temperature. This indicates clearly that the optical activity cannot be due to (IV) and supports the structure (V) assigned to the zwitterionic reduction product which was not isolated. On treatment with alkali, (V) undergoes irreversible prototropic change to give (III) which is of course optically inactive. The course of this change in very dilute solution was followed by examining the change in the absorption spectrum. It should be noted that Wiesner's polarographic studies (*loc. cit.*) failed to disclose any evidence of semiquinone formation; it is possible that under these conditions direct reduction to (IV) occurs. The only alternative explanation of the previous results, namely that reduction and isomerisation occur simultaneously, seems very unlikely, for it is improbable that these two processes occur at equal rates even when two different reducing agents are employed.

The trihydroxy-compound (III) is bright yellow and is sparingly soluble in water and in organic solvents, giving yellow solutions with a very powerful green fluorescence; dilute solutions soon absorb oxygen from the air, and the fluorescence disappears. Treatment of (III) with acetic anhydride and pyridine gives 3 : 5 : 6-*triacetoxy*-1-*methylindole* which is colourless and non-fluorescent, and can also be obtained by the action of the same reagents on adrenochrome itself, rearrangement as well as acetylation occurring in the latter case. Alkaline hydrolysis of the triacetate in the absence of air gives (III). (III) may also be obtained—and this is the more convenient method of preparation—by the direct action of alkali on an aqueous solution of adrenochrome, followed by acidification with acetic acid [cf. Fischer, *Bull. Soc. chim. Belg.*, 1949, **58**, 205, who clearly obtained the monohydrate of (III)]. It is very probable that (III) is the compound responsible for the green fluorescence observed in determination of adrenaline by Gaddum and Schild's method (*J. Physiol.*, 1934, **80**, 9P) in which the adrenaline is oxidised in alkaline solution: the adrenochrome first produced would be isomerised to (III) by the alkali. A similar suggestion has been put forward by Ehrlen (*Farm. Revy.*, 1948, **47**, 242). Since our work was completed, the isolation of (III) has been reported by Lund (*Acta Pharmacol.*, 1949, **5**, 121), who also records the firm retention of water by the monohydrate.

Acid solutions of (III) absorb oxygen from the air slowly in the cold but more rapidly at the boiling point, giving 5 : 6 : 5′ : 6′-*tetrahydroxy*-1 : 1′-*dimethylindigo* (VII) as a greenish-black precipitate, characterised as its *tetra-acetate*. (VII) closely resembles the unmethylated



(VII.)

compound synthesised previously (Harley-Mason, *J.*, 1948, 1244) in retaining a molecule of water which could not be removed by intensive drying and in giving an intensely permanganate-

coloured solution in aqueous alkali. Its solution in pyridine was however green, in contrast with the blue solution given by the unmethylated compound; this bathochromic effect of *N*-methylation is observed with indigo itself.

The dihydroxyindole (II) is colourless and readily soluble in water and organic solvents, giving, in the latter, solutions which show a rather weak bluish-violet fluorescence in ultra-violet light. Alkaline solutions absorb oxygen from the air very rapidly, giving at first a deep blue colour which rapidly becomes violet and then brown (cf. the oxidation of the unmethylated compound as described by Beer, Clarke, Khorana, and Robertson, *J.*, 1948, 2223). When the solution while still at the blue stage was acidified with acetic acid, a dark precipitate was obtained, partly soluble in alcohol and in ether, giving a deep-blue solution which was stable for several days. When the aqueous filtrate was extracted with ether, the almost colourless ethereal solution exhibited an extremely powerful bluish-violet fluorescence, very much stronger than that exhibited by (II).

The oxidation of (II) by tyrosinase was examined qualitatively. The reaction proceeded fairly rapidly at room temperature and after 2 hours the solution, which had assumed a dirty violet colour, began to deposit a dark precipitate. At this point extraction with ether gave in the ethereal layer the same bluish-violet fluorescence as that noted above. The nature of this fluorescent material, which is probably an early stage in the oxidative polymerisation of (II), is at present under investigation. After oxidation for 24 hours, a copious black precipitate was obtained and an ethereal extract was no longer fluorescent. It is particularly significant that at no period during the oxidation was there any indication of the formation of the indoxyl (III) which, if formed, should have been easily detectable by its intense green fluorescence. This suggests that melanin formation does not involve oxidation at position 3, at any rate as an initial stage.

It has long been known that solutions of adrenaline on oxidation give at first a red colour and then a brown or black precipitate of "adrenaline black" (VIII). Clearly the formation of adrenaline black proceeds *via* adrenochrome, and it has now been found that the transformation from adrenochrome does not require further consumption of oxygen. After storage for 24 hours under nitrogen at room temperature, an aqueous solution of (I) had decomposed completely, giving a precipitate of (VIII) in 75% yield. The yellowish-brown aqueous filtrate on extraction with ether yielded a trace of (II). Addition of alkali to the aqueous filtrate produced a green fluorescence indicating the formation of (III), though the amount formed was too small for isolation. Evidently then, some isomerisation of (I) also occurs. When dried at room temperature, (VIII) gave analytical figures corresponding fairly well to an empirical formula $C_9H_9O_4N$. It forms a black powder, insoluble in most organic solvents with the exception of pyridine, but soluble in aqueous alkalis to give a dark greenish-brown solution. Sodium dithionite reduces an aqueous suspension to an insoluble yellowish-brown leuco-compound, which is readily re-oxidised.

The dihydroxy-compound (II) is a reduction product of (I) and, since the formation of adrenaline black takes place in the absence of oxygen, the process must clearly involve to some extent an oxido-reduction. It will be noted that the empirical formula of (VIII) contains one atom of oxygen more than the original adrenochrome. Moreover the 75% yield of (VIII) obtained indicates clearly that the process cannot involve the simple reaction:

$$\underset{\text{(I.)}}{2C_9H_9O_3N} \longrightarrow \underset{\text{(II.)}}{C_9H_9O_2N} + \underset{\text{(VIII.)}}{C_9H_9O_4N}$$

which would necessitate a maximum yield of 50%; this process is in any case hardly possible since (II) is in fact not a *direct* reduction product of (I), but rather a dehydration product of the unstable leuco-compound (IV).

The most likely explanation is that (VIII) contains tightly bound water [compare (VII), the unmethylated analogue of (VII), and (III)] and that its composition approximates in fact to $C_9H_7O_3N,H_2O$ or perhaps $C_9H_5O_2N,2H_2O$.

Some indication that this may be the case was obtained on micro-analysis of specimens of (VIII) dried at 100° over phosphoric oxide for several days; these specimens certainly had a lower oxygen content, but they were very hygroscopic and consistent results could not be obtained. It would seem that the retention of water occurs in no precise stoicheiometric ratio; this is not surprising in view of the amorphous nature of (VIII) and the fact that it appears to be polymeric.

The anærobic conversion of adrenochrome into adrenaline black was strongly catalysed by mineral acid. In 5% aqueous solution, acidified to *ca.* pH 2 with hydrochloric acid, (I)

decomposed completely to (VIII) in 15 minutes at room temperature, whereas, in the absence of acid, decomposition required 24 hours.

Cohen (*Bull. Soc. chim. Biol.*, 1946, **28**, 104, 107, 354) has suggested that the formation of (VIII) from (I) proceeds *via* the *o*-quinonoid oxidation product of (III), 3-hydroxy-1-methylindole-5 : 6-quinone (described as "oxo-adrenochrome"), which then undergoes chain polymerisation by intermolecular condensation at the 2- and 5-positions with elimination of water. Various attempts were made to oxidise (III) to "oxo-adrenochrome" but with no indication of success, and the only recognisable oxidation product obtained in all cases was the tetrahydroxyindigo (VII), which was formed with great ease and of course differs completely from (VIII). Cohen's structure for (VIII) is thus very improbable, and in any case it should be noted that a mechanism involving condensation of an indoxyl in the 2-position with the carbonyl group of a quinone is *a priori* improbable since both indoxyl and (III) are simply oxidised to the corresponding indigos by *p*-benzoquinone and do not condense with it. The same objections apply to the structure for dopa-melanin proposed by the same author. Furthermore, the melanin structure proposed by Burton (*Chem. and Ind.*, 1948, **313**) contains repeating hydroxyindoxyl units and by analogy with (III) it would be expected that further oxidation to indigoid products would occur extremely readily.

2-Bromoadrenochrome was prepared by a slight modification of the method described by Green and Richter (*loc. cit.*); its formation from (−)-adrenaline generates a new asymmetric centre so that two diastereoisomeric products should be obtained. It is possible that bromoadrenochrome as obtained is such a mixture, but owing to difficulty of recrystallisation separation was not attempted. Reduction of a warm aqueous suspension of the bromo-compound with sodium dithionite gave a considerable amount of insoluble tar; after filtration the yellow filtrate deposited, on cooling, 2-*bromo*-5 : 6-*dihydroxy*-1-*methylindole* as almost colourless needles, characterised by a *diacetate*. No crystalline product could be isolated from the tar.

For the preparation of aqueous solutions of adrenochrome an alternative was sought to the use of silver oxide as oxidising agent. Sodium persulphate can be used advantageously, but the presence of a buffer such as sodium acetate or sodium hydrogen carbonate is necessary. The addition of a trace of a ferrous salt accelerates the reaction considerably. Solutions of adrenochrome prepared in this way can be used conveniently to prepare derivatives such as the oxime and semicarbazone ("Adrenoxyl"), but the yields of (II) and (III) obtained from such solutions are lower than those obtained when silver oxide is employed.

Certain observations in the literature concerning adrenochrome are explicable in the light of the present work. Bergel and Morrison (*loc. cit.*) examined the oxidation of adrenaline by oxygen in the presence of a palladium–charcoal catalyst. The deep-red solution obtained was reduced and acetylation then gave a small amount of material, m. p. 105—110°. This was clearly a mixture of the diacetate, m. p. 104—105°, of (II) and the triacetate, m. p. 112—113°, of (III). Beauvillain and Sarradin (*Bull. Soc. chim. Biol.*, 1948, **30**, 478), on decomposition and reduction of aqueous solutions of (I), observed the presence of two substances, one soluble in ether and exhibiting a blue fluorescence and the other soluble in water exhibiting a greenish-yellow fluorescence; these are evidently (II) and (III) respectively.

A pharmacological investigation of (III) is now being undertaken.

Experimental.

Adrenochrome (I).—(*a*) (After Buchnea, *loc. cit.*) (−)-Adrenaline (18·3 g., 0·1 mol.) was dissolved in dry methanol (500 ml.) containing formic acid (10 ml.). Freshly precipitated silver oxide (73 g., 0·31 mol.) was added and the mixture shaken vigorously for 3 minutes. The temperature rose to 40°. Silver and excess of silver oxide were removed by filtration, and the residue was washed with methanol (100 ml.) at 40°. The deep-red filtrate and washings were cooled to −20° in a carbon dioxide freezing mixture, and after ½ hour the precipitated deep-red crystals were filtered off, washed with a little cold methanol followed by dry ether, and transferred immediately to a vacuum-desiccator which was kept in the dark. The yield was 7·8 g. of anhydrous material (Found : C, 60·7; H, 5·4; N, 7·8. Calc. for $C_9H_9O_3N$: C, 60·3; H, 5·0; N, 7·8%).

(*b*) (−)-Adrenaline (2 g.) was suspended in water (70 ml.), and dilute hydrochloric acid was added dropwise, with shaking, until a clear solution was obtained. Silver oxide (8 g.) was added, the mixture shaken vigorously for 5 minutes and then filtered. The aqueous solution of adrenochrome thus obtained was stable for about 12 hours at 0°.

(*c*) (−)-Adrenaline (1·8 g.) was suspended in water (70 ml.), and acetic acid was added dropwise until a clear solution was obtained. Sodium acetate (3 g.) and sodium persulphate (5 g.) were then added, followed by one drop of 1% ferrous sulphate solution. The mixture was kept at room temperature (20°) for 1·5 hours with frequent shaking, and the clear deep-red solution of adrenochrome thus obtained was used at once.

Reduction of Adrenochrome by Hydrogenation.—Adrenochrome (1 g.) in water (20 ml.) was hydrogenated over a palladium–charcoal catalyst at room temperature and atmospheric pressure. 64 Ml.

(N.T.P.) of hydrogen were absorbed in 20 minutes. A repetition of the experiment gave an uptake of 65·5 ml. [Calc. for 1 atom of hydrogen per mole. of (I), 62·5 ml.]. The yellow solution thus obtained was extracted with peroxide-free ether (3 × 15 ml.), the extract dried (Na_2SO_4), and the ether removed, leaving a yellow gum. Two recrystallisations from light petroleum (b. p. 100—120°) gave colourless needles of 5 : 6-*dihydroxy*-1-*methylindole* (II) (0·3 g.), m. p. 136° (decomp.) (Found : C, 66·2; H, 5·5; N, 8·6. $C_9H_9O_2N$ requires C, 66·3; H, 5·5; N, 8·7%). (II) is very soluble in ether, alcohol, or ethyl acetate, moderately soluble in water or benzene, and sparingly soluble in light petroleum. The crystals darken slowly on exposure to air. A deep red colour is given with the Ehrlich reagent. When a drop of dilute sodium hydroxide was added to an aqueous solution, very rapid oxidation occurred, giving initially a deep purple colour, becoming dirty violet after a few seconds, then brown, and eventually a brownish-black precipitate was deposited. When the solution, while still at the purple stage, was acidified with acetic acid, a dark precipitate was obtained. This was filtered off and the filtrate was extracted with ether. The colourless ethereal extract displayed a very powerful bluish-violet fluorescence. The precipitate was partly soluble in alcohol or ether, giving a deep-blue solution.

Acetylation of (II) (0·2 g.) with acetic anhydride (5 ml.) and pyridine (5 ml.) for 24 hours at room temperature, followed by pouring the mixture into water, and recrystallisation of the precipitate from benzene–light petroleum (b. p. 80—100°), gave 5 : 6-diacetoxy-1-methylindole, colourless prisms, m. p. 104—105° (Found : C, 62·9; H, 5·4. Calc. for $C_{13}H_{13}O_4N$: C, 63·2; H, 5·3%). Bergel and Morrison (*loc. cit.*) give m. p. 100—101°.

The yellow aqueous solution after extraction of (II) was made alkaline with 2N-sodium hydroxide, a deep-yellow solution with a powerful green fluorescence being obtained. This was acidified with acetic acid, and on storage at 0° small yellow prisms of 3 : 5 : 6-*trihydroxymethylindole monohydrate* (0·32 g.), m. p. 228—232°, slowly separated. After recrystallisation from water containing a little sodium dithionite to prevent oxidation, the compound had m. p. 230—232° (Found : C, 55·0; H, 5·8; N, 7·1. $C_9H_9O_3N,H_2O$ requires C, 54·9; H, 5·6; N, 7·1%). When dried at 100° in a high vacuum over phosphoric oxide for 3 days the crystals fell to a yellow powder, which is the anhydrous *indole*, decomposing, without melting, at 285° (Found : C, 60·0; H, 5·3; N, 7·7. $C_9H_9O_3N$ requires C, 60·3; H, 5·0; N, 7·75%). The trihydroxy-compound is readily soluble in sodium hydroxide, sparingly soluble in water, alcohol, acetic acid, or ethyl acetate, and almost insoluble in ether or benzene. The yellow solutions are oxidised in air, becoming brown, and the fluorescence disappears. With Ehrlich's reagent, a deep violet colour is given in the cold.

Acetylation of the trihydroxy-compound (0·2 g.) with acetic anhydride (5 ml.) and pyridine (5 ml.) for 36 hours at room temperature, followed by pouring the mixture into water and recrystallisation of the precipitate from benzene, gave 3 : 5 : 6-*triacetoxy*-1-*methylindole*, colourless prisms, m. p. 112—113° (Found : C, 58·4; H, 5·2. $C_{15}H_{17}O_6N$ requires C, 58·5; H, 5·5%).

The triacetate was also obtained directly from adrenochrome. Adrenochrome (1 g.) was dissolved in pyridine (10 ml.), and acetic anhydride (10 ml.) added. The mixture was kept for 36 hours at room temperature, the colour changing from red to pale brown, and was then poured into water. After 2 hours at 0°, the precipitate was collected and twice recrystallised from benzene. The yield was 0·6 g.

The triacetate (0·5 g.) was hydrolysed by refluxing for 1 hour with 2N-sodium hydroxide (20 ml.) containing a little sodium dithionite, under nitrogen. The deep-yellow solution was cooled and acidified with acetic acid and on storage at 0° deposited the monohydrate of (III) as small yellow prisms.

Preparation of (III) *directly from Adrenochrome.*—Adrenochrome (1 g.) was dissolved in water (30 ml.), and 2N-sodium hydroxide (8 ml.) added with stirring. The colour changed from red to deep yellow, and a powerful green fluorescence appeared. A little sodium dithionite was added to prevent oxidation, and the solution acidified with acetic acid. The trihydroxy-compound rapidly separated and after 2 hours at 0° was collected and recrystallised from water containing sodium dithionite. The yield was 0·65 g. of monohydrate. The same result was obtained with a solution of (I), prepared as described in (*b*) and (*c*) above; in the latter case the yield was smaller.

Reduction of Adrenochrome by Sodium Dithionite.—To a solution of adrenochrome (1 g.) in water (30 ml.) solid sodium dithionite was added with stirring until the initial deep red colour became yellow. The resulting solution was worked up as described above for the hydrogenation, giving (II) (0·32 g.) and (III) (0·28 g.). With solutions of (I) prepared as in (*b*) and (*c*) above similar results were obtained; in the latter case the yield of (II) was very poor. An experiment in which an old sample of dithionite, (containing sodium hydrogen sulphite) was employed gave almost negligible yields, and other experiments in which an excess of dithionite was added gave poor yields of (II).

A solution of adrenochrome (0·1 g.) in water (10 ml.) was reduced with dithionite and examined polarimetrically; at 18° a rotation of −0·53° was observed (10-cm. tube). If half of the adrenochrome is converted into the zwitterion (V) this corresponds to a value $[\alpha]_D^{18}$ −106° for this compound. The rotation was unchanged after 24 hours.

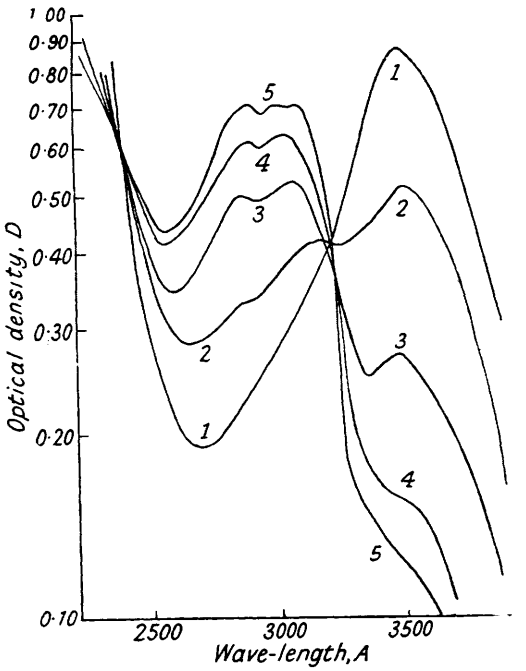
The conversion of the zwitterion (V) into the trihydroxyindole (III) with alkaline catalysis was followed spectrophotometrically. A solution of (I) (1·5 g.) in water (30 ml.) was reduced with dithionite and (II) extracted with ether as above; it was then diluted 5000-fold and the absorption determined (curve 1); $\lambda_{max.} = 3470$; $\varepsilon_{max.} = 7700$. To another sample of the solution a drop of 2N-sodium hydroxide was added and after 5 seconds a drop of acetic acid. The absorption was then determined (curve 2). Curves 3, 4, and 5 were determined exactly similarly after time intervals of 1, 5, and 20 minutes respectively. The last represents complete conversion into (III); $\lambda_{max.} = 2860$ and 3050; $\varepsilon_{max.} = 6700$.

5 : 6 : 5′ : 6′-*Tetrahydroxy*-1 : 1′-*dimethylindigo* (VII).—(*a*) 3 : 5 : 6-Trihydroxy-1-methylindole (0·5 g.) was dissolved in N-sodium hydroxide (5 ml.), and the solution diluted to 100 ml. and then acidified with hydrochloric acid. After the mixture had been kept for 10 days at room temperature exposed to the air with frequent shaking, the black precipitate which had slowly accumulated was collected. It was purified by dissolution in dilute sodium hydroxide under nitrogen, the solution was filtered, and the product was re-precipitated by acetic acid. The *indigo* was collected and dried at 100° in a high vacuum over phosphoric oxide (Found : C, 57·8; H, 4·5; N, 7·4. $C_{18}H_{14}O_6N_2,H_2O$ requires C, 58·1; H, 4·3;

N, 7·5%). It formed a greenish-black powder, soluble in pyridine, and sparingly soluble in acetic acid, giving deep green solutions. It was also soluble in sodium hydroxide, giving an intense permanganate-coloured solution, which rapidly oxidised on exposure to air, becoming brown. Reduction with dithionite gave a yellow vat.

(*b*) 3 : 5 : 6-Trihydroxy-1-methylindole (0·2 g.) was dissolved in acetic acid (50 ml.), and the solution heated on a boiling water-bath for 2 hours with free access to air and frequent shaking. After cooling, the black precipitate was collected and purified as above.

Tetra-acetate of (VI).—(VII) (0·1 g.) was dissolved in pyridine (5 ml.), and acetic anhydride (5 ml.) added. After 3 days at room temperature the mixture was poured into water, and the green precipitate collected. The material proved too insoluble for recrystallisation and was purified by solution in pyridine, filtration, and precipitation by the addition of ethyl acetate. 5 : 6 : 5′ : 6′-*Tetra-acetoxy*-1 : 1′-*dimethylindigo* formed a deep green powder, almost insoluble in all solvents with the exception of pyridine (Found : C, 58·8; H, 5·3; N, 5·2. $C_{26}H_{26}O_{10}N_2$ requires C, 59·3; H, 4·9; N, 5·3%).



Light absorption, at pH 4—4·5, in water of :

(1) *the zwitterion* (V);
(2) *mixture of* (V) *and* (III) *after* 5 *seconds' isomerisation ;*
(3) ,, ,, ,, 1 *minute's* ,,
(4) ,, ,, ,, 5 *minutes'* ,,
(5) 3 : 5 : 6-*Trihydroxy*-1-*methylindole* (III) [20 *minutes' isomerisation of* (V)].

Formation of Adrenaline Black (VIII) *from* (I).—Adrenochrome (2 g.) was dissolved in air-free distilled water (30 ml.), and the solution transferred to a tightly-stoppered flask under nitrogen. After 48 hours at room temperature the deep red colour had become yellowish-brown and a copious precipitate of (VIII) had formed. The precipitate was collected, well washed with air-free water, and dried over phosphoric oxide (yield 1·5 g.) (Found : C, 54·7; H, 4·9; N, 7·0. $C_9H_9O_4N$ requires C, 55·4; H, 4·6; N, 7·2%). Prepared in this way, adrenaline black was a jet-black amorphous powder, almost insoluble in all organic solvents except pyridine in which it gave a greenish-brown solution. It was also soluble in sodium hydroxide, giving a similar coloured solution and was re-precipitated by the addition of acid. An aqueous suspension was slowly reduced by sodium dithionite, giving an insoluble yellowish-brown leuco-compound which was readily re-oxidised on exposure to air.

The aqueous filtrate from the solution of (VIII) was extracted with ether; removal of the ether yielded a very small amount of (II). The aqueous portion was made alkaline and then acidified with acetic acid; the characteristic green fluorescence of (III) was observed, but the amount was too small for isolation.

2-*Bromoadrenochrome.*—(−)-Adrenaline (1·8 g.) was suspended in water (100 ml.) in a stoppered bottle, and acetic acid added until all was dissolved. Sodium acetate (4 g.) was added, followed by bromine (1·5 ml.) which was added dropwise from a burette, with vigorous shaking between each addition. The product separated as deep-red prisms during the addition, which required 15—20 minutes. The mixture was kept at 0° for 1 hour, and the product then filtered off and washed with

ice-cold water. It was at once transferred to a vacuum desiccator, and was stable for about a week at room temperature. The yield was 1·3 g.

2-*Bromo*-5 : 6-*dihydroxy*-1-*methylindole*.—The above compound (0·5 g.) was suspended in water (20 ml.) at 40°, and sodium dithionite was added with very vigorous shaking. After a few minutes the solution became bright yellow and a dark tar separated. The tar was removed by filtration and the filtrate on cooling deposited yellow needles of impure material. After recrystallisation from benzene–light petroleum (b. p. 80—100°), colourless needles of 2-*bromo*-5 : 6-*dihydroxy*-1-*methylindole*, m. p. 121—123° (decomp. : softening at 118°) were obtained (Found : C, 44·9; H, 3·5; Br, 32·8. $C_9H_8O_2NBr$ requires C, 44·6; H, 3·3; Br, 33·0%). The material rapidly darkened on storage.

This product (0·2 g.) was dissolved in pyridine (3 ml.), acetic anhydride (3 ml.) added, and the mixture kept for 24 hours at room temperature and then poured into water. The precipitate was collected and recrystallised from methanol (charcoal), giving colourless prisms of 2-*bromo*-5 : 6-*diacetoxy*-1-*methylindole*, m. p. 164° (Found : C, 47·7; H, 3·8. $C_{13}H_{12}O_4NBr$ requires C, 47·85; H, 3·7%).

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