153. *The Chemistry of Adrenochrome. Part II. Some Analogues and Derivatives.*

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

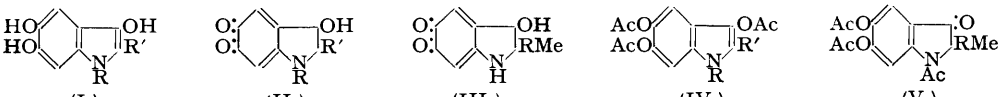
The oxidation of three adrenaline analogues, noradrenaline, α-methylnoradrenaline, and *N-iso*propylnoradrenaline, is described. With potassium iodate these compounds are converted into red crystalline iodoquinones which are isomerised on treatment with acetic anhydride–pyridine and give acetylated 3 : 5 : 6-trihydroxy-2-iodoindoles from which the iodine atom can be removed by reduction. Hydrolysis then yields yellow, intensely fluorescent 3 : 5 : 6-trihydroxyindoles. The iodo-quinones may also be isomerised by zinc acetate. Oxidation with potassium ferricyanide was also examined.

An explanation is advanced of the fact that adrenaline analogues containing a primary amino-group do not give a green fluorescence on autoxidation in alkaline solution.

The oxidation of adrenaline and its analogues in strongly acid solutions is discussed.

THE conversion of adrenaline *via* adrenochrome into 3 : 5 : 6-trihydroxy-1-methylindole (I; R = Me, R′ = H) has been described by one of us (Harley-Mason, *J.*, 1950, 1276; regarded as

Part I of this series), and we have now extended this work by examination of the oxidation of three analogues, namely noradrenaline (" Arterenol "), α-methylnoradrenaline (" Corbasil "), and *N-iso*propylnoradrenaline (" Aleudrine "). Adrenochrome can readily be prepared pure by oxidation of adrenaline with silver oxide in methanol containing formic acid as described in Part I : this process however failed with the three analogues, giving deep-red solutions from which no crystalline product could be obtained. In aqueous solution *N-iso*propylnoradrenaline was readily oxidised by silver oxide to a deep-red solution resembling that of adrenochrome, but noradrenaline and its α-methyl derivative gave dirty reddish-brown solutions from which no product could be isolated. Eventually it was found that the most satisfactory oxidising agent was potassium ferricyanide buffered with sodium hydrogen carbonate in aqueous solution. A solution of the quinone (II; R = Pr^i, R′ = H) so obtained from *N-iso*propylnoradrenaline gave with semicarbazide an orange crystalline semicarbazone and, on treatment with alkali, a deep-yellow solution with a strong green fluorescence. On cautious acidification of this solution with acetic acid a yellow amorphous precipitate was at first obtained, followed by separation of 3 : 5 : 6-trihydroxy-1-*iso*propylindole (I; R = Pr^i, R′ = H) as small yellow prisms.



(I.) (II.) (III.) (IV.) (V.)

On the other hand, the less stable solutions of the quinones (II; R = R′ = H) and (II; R = H, R′ = Me), obtained by similar oxidation of noradrenaline and α-methylnoradrenaline, gave on addition of alkali a yellowish-brown coloration but no fluorescence. A semicarbazone was obtained from (II; R = H, R′ = Me), but not from (II; R = R′ = H).

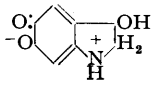
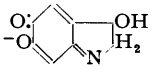
The action of aqueous potassium iodate on adrenaline salts gives 2-iodoadrenochrome (Blaschko and Richter, *J.*, 1937, 601) and we have now found that similar iodo-quinones, namely 2-iodo-1-*iso*propylnoradrenochrome (II; R = Pr^i, R′ = I), 2-iodo-2-methylnoradrenochrome (III; R = I) and 2-iodonoradrenochrome (II; R = H, R′ = I) can be obtained from *N-iso*propyl-, α-methyl-, and noradrenochrome respectively, though in poor yield. Since it was found (Part I, *loc. cit.*) that the action of pyridine and acetic anhydride on adrenochrome resulted in rearrangement as well as acetylation, the action of these reagents on the iodo-quinones was investigated From 2-iodoadrenochrome and from (II; R = Pr^i, R′ = I), 3 : 5 : 6-triacetoxy-2-iodo-1-methylindole (IV; R = Me, R′ = I) and 3 : 5 : 6-triacetoxy-2-iodo-1-*iso*propylindole (IV; R = Pr^i, R′ = I), respectively, were readily obtained in high yield. With (II; R = H, R′ = I) and (III; R = I), *N*-acetylation also occurred, giving 3 : 5 : 6-triacetoxy-1-acetyl-2-iodoindole (IV; R = Ac, R′ = I) and 5 : 6-diacetoxy-1-acetyl-2 : 3-dihydro-2-iodo-3-keto-2-methylindole (V; R = I).

Treatment of the iodo-indoles thus obtained with zinc dust and acetic acid removed the iodine atom. The product (IV; R = Me, R′ = H) from (IV; R = Me, R′ = I) was identical with that obtained directly from adrenochrome. From the alkaline hydrolysis of (IV; R = Ac, R′ = H) and (V; R = H) it was hoped to obtain the dihydroxyindoxyls (I; R = R′ = H) and (I; R = H, R′ = Me); unfortunately both products were very soluble in water and isolation from the small amount of starting material available proved unsuccessful. There can be little doubt, however, that these products were formed, since the yellow solutions displayed the intense green fluorescence characteristic of dihydroxyindoxyls, and, further, the solution obtained from (IV; R = Ac, R′ = H) on acidification and exposure to air slowly deposited a black precipitate whose solution in alkali showed the intense permanganate colour characteristic of tetrahydroxyindigo (Harley-Mason, *J.*, 1948, 1244). The solution obtained from (V; R = H), containing the indoxyl (I; R = H, R′ = Me) in which the 2-position is blocked by a methyl group thus preventing indigo formation, turned dark brown on similar oxidation, but gave no precipitate.

The isomerisation of adrenochrome to 3 : 5 : 6-trihydroxy-1-methylindole by the action of zinc acetate has been described by Fischer, Derouaux, Lambot, and Lecomte (*Bull. Soc. chim. Belg.*, 1950, **59**, 72). We have applied a similar procedure to the iodo-quinones; the action of zinc acetate on 2-iodoadrenochrome and 2-iodo*iso*propylnoradrenochrome followed by sodium dithionite leads directly to the formation, though in rather poor yield, of the dihydroxyindoxyls (I; R = Me, R′ = H) and (I; R = Pr^i, R′ = H), the iodine atom being removed by the reducing agent. 2-Iodonoradrenochrome on similar treatment gave a yellow, strongly fluorescent solution, but the indoxyl could not be isolated. 2-Iodo-2-methylnoradrenochrome gave a yellowish-brown solution which was non-fluorescent, so that evidently the indoxyl was

not formed, probably because isomerisation of (II; R = H, R′ = I) to the iodo-indoxyl would involve the migration of two hydrogen atoms both from the secondary alcohol group in the 3-position, whereas in all the other cases the migration involves one hydrogen atom each from the 2- and the 3-position.

The detection and estimation of small amounts of adrenaline by means of the green fluorescence developed when alkaline solutions are allowed to autoxidise has been described by several authors (Paget, *Bull. Sci. Pharmacol.*, 1930, **37**, 357; Gaddum and Schild, *J. Physiol.*, 1934, **80**, 9P; Ehrlén, *Farm Revy.*, 1948, **37**, 242). That the compound responsible for the fluorescence is the indoxyl (I; R = Me, R′ = H), formed by oxidation of the adrenaline to adrenochrome followed by isomerisation, has been argued earlier (Part I, *loc. cit.*; Ehrlén, *loc. cit.*). Bacq, Fischer, and Lecomte (*Arch. internat. Physiol.*, 1949, **56**, 380) have examined the specificity of this reaction and by a comparative study of adrenaline analogues have shown that a fluorescence is given only by *N*-alkyl analogues. It is evident from our result that even in the absence of a substituent on the nitrogen atom, the corresponding quinone is formed on oxidation, so that failure to give a fluorescence must depend on the subsequent isomerisation following a different course. From noradrenaline the quinone (II; R = R′ = H) is formed on oxidation.

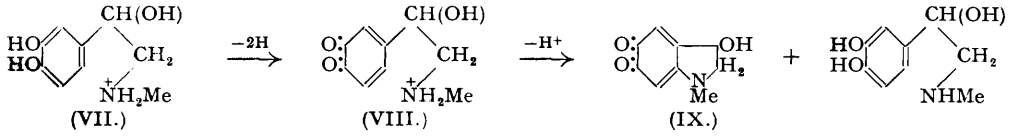
(II*a*.)  $\xrightarrow{-H^+}$  (VI.)

By analogy with adrenochrome, the zwitterionic mesomeric structure (II*a*) must make a large contribution (cf. Harley-Mason, *Experientia*, 1948, **4**, 307), so that the most probable reaction with alkali will be removal of a proton from the nitrogen atom, giving the anion (VI). Since this anion is a dihydrohydroxyindole derivative, it would not be expected to show the fluorescence characteristic of an indoxyl. An isomerisation of this kind is of course not possible in the cases of quinones derived from adrenaline and similar *N*-alkylated compounds, since these contain no hydrogen attached to the nitrogen atom.

In Part I (*loc. cit.*) the preparation of aqueous solutions of adrenochrome by oxidation of adrenaline with buffered persulphate was described. We have now found that oxidation with potassium ferricyanide in the presence of sodium hydrogen carbonate proceeds very much more rapidly, and better yields of products are obtained

The oxidation of adrenaline with lead dioxide at pH 0—1 has been investigated by Rangier (*Compt. rend.*, 1945, **220**, 246) and Ruiz-Gijon (*Nature*, 1950, **166**, 831). Both authors have suggested that under these conditions the orange-yellow solution obtained contains the adrenaline quinone (2-hydroxy-2-3′ : 4′-quinonylethylmethylamine), since reduction leads to the re-formation of adrenaline. We have confirmed these observations and also found that when an alkaline buffer, such as sodium acetate or hydrogen carbonate, is added to the orange-yellow solution (after removal of excess of oxidising agent) the colour at once changes irreversibly to the deep red of adrenochrome. The red colour can then be intensified by the addition of more oxidising agent.

These phenomena may be explained as follows : In strongly acid solution the adrenaline will be present entirely as the cation (VII), which on oxidation will give the quinone (VIII), having the orange-yellow colour usual in *o*-quinones. While the nitrogen atom retains its positive



(VII.) (VIII.) (IX.)

charge, cyclisation does not occur since this involves anionoid attack by the nitrogen on the 6-position of the quinonyl group. When the hydrogen-ion concentration is reduced, the free amine is formed, and cyclisation then occurs to give the deep-red adrenochrome (IX), half of the original quinone being reduced back to adrenaline. Addition of further oxidising agent at this point converts the adrenaline directly to adrenochrome, thus intensifying the colour.

The oxidation of several other (dihydroxyphenyl)ethylamine derivatives, namely noradrenaline, α-methylnoradrenaline, *N-iso*propylnoradrenaline, 2-(3 : 4-dihydroxyphenyl)ethylamine, 2-(3 : 4-dihydroxyphenyl)ethylmethylamine, and 3 : 4-dihydroxyphenylalanine, under the same strongly acidic conditions has also been examined. The same result was obtained in all cases; the orange-yellow quinone was formed and on subsequent addition of an alkaline buffer the deep-red, cyclised quinone was at once obtained.

As the quinones cyclised at once at pH less than about 2, it is unlikely that they are formed as stable entities under biological conditions unless cyclisation is prevented in some other way.

Experimental.

Oxidations with Potassium Ferricyanide.—Adrenaline (1·8 g., 0·01 mol.) was suspended in water (30 c.c.) and acetic acid added dropwise with shaking until all the solid had dissolved. A solution of potassium ferricyanide (13 g., 0·04 mol.) and sodium hydrogen carbonate (4·2 g., 0·05 mol.) in water (70 c.c.) was added with stirring. A brisk effervescence of carbon dioxide occurred, and after 5 minutes, the deep-red adrenochrome solution was treated with 10% sodium hydroxide solution (10 c.c.), giving a deep-yellow solution having a strong, green fluorescence. The solution was then acidified with acetic acid and kept overnight at 0°, a little sodium dithionite being added to prevent oxidation. The 3 : 5 : 6-trihydroxy-1-methylindole monohydrate (1·2 g.), which had separated as small yellow prisms, m. p. 230—232°, was then collected.

Oxidation of *N-iso*propylnoradrenaline sulphate (1·0 g., 0·004 mol.) in the same manner similarly gave a deep-red solution. After addition of alkali, the acidification with acetic acid was performed very slowly; at first a yellow amorphous precipitate separated, and this was filtered off and discarded. Further addition of acetic acid to the filtrate gave 3 : 5 : 6-*trihydroxy*-1-iso*propylindole* (0·3 g.) as very small, yellow nodules, m. p. 214—216° (decomp.) (Found : C, 63·2; H, 6·1; N, 6·8. $C_{11}H_{13}O_3N$ requires C, 63·7; H, 6·3; N, 6·75%). The product was sparingly soluble in water and organic solvents, giving bright yellow solutions showing a strong green fluorescence. Acid solutions rapidly absorbed oxygen from the air giving a black precipitate which dissolved in alkali to an intense permanganate coloured solution, and was thus probably 5 : 6 : 5′ : 6′-tetrahydroxy-*NN′*-di*iso*propylindigo. To a portion of the red solution obtained in the original oxidation, semicarbazide hydrochloride and sodium acetate were added After 24 hours, the N-iso*propylnoradrenochrome semicarbazone*, which had crystallised, was collected and recrystallised from water, giving orange-red plates, m. p. 204° (decomp.) (Found : N, 21·0. $C_{12}H_{16}O_3N_4$ requires N, 21·2%).

Similar oxidation of α-methylnoradrenaline hydrochloride (0·65 g., 0·003 mol.) gave a red solution which on treatment with alkali gave a deep yellow-brown colour, but no fluorescence. Addition of acetic acid gave a light-brown precipitate which could not be purified. Addition of semicarbazide hydrochloride and sodium acetate to the red solution gave 2-*methylnoradrenochrome semicarbazone*, forming orange-yellow needles, m. p. 195—196° (decomp.), from water (Found : N, 23·8. $C_{10}H_{12}O_3N_4$ requires N, 23·7%).

Similar oxidation of noradrenaline hydrochloride (0·6 g., 0·003 mol.) produced a red solution which decomposed rapidly giving a dark precipitate. Addition of alkali gave no fluorescence, and no semicarbazone could be prepared from the original red solution.

Preparation of Iodo-quinones.—A solution of *N-iso*propylnoradrenaline sulphate (1·5 g.) in water (50 c.c.) was treated at 0° with a solution of potassium iodate (1·2 g.) in water (50 c.c.). A brown, rather tarry precipitate soon began to separate; this was filtered off and discarded, and more tarry material was similarly removed until, after 2 hours, crystals began to form. The mixture was kept overnight at 0° and the reddish-brown needles of 2-*iodo*-N-iso*propylnoradrenochrome* (II; R = Pr^i, R′ = I) *hemihydrate* (0·5 g.) were then collected (Found : C, 38·6; H, 4·6. $C_{11}H_{12}O_3NI,0{\cdot}5H_2O$ requires C, 38·6; H, 3·8%).

Noradrenaline hydrochloride (1 g.) was similarly treated with potassium iodate (1 g.). After 2 hours a large amount of black amorphous material was filtered off and the filtrate kept 24 hours at 0°. The product, 2-*iodonoradrenochrome* (II; R = H, R′ = I) (0·25 g.), separated as compact dark-red, almost black, prisms (Found : C, 32·9; H, 2·2; I, 43·0. $C_8H_6O_3NI$ requires C, 33·0; H, 2·1; I, 43·6%).

α-Methylnoradrenaline hydrochloride (1 g.) was similarly treated with potassium iodate (1·1 g.). Some orange, amorphous material was filtered off after 1 hour and the filtrate kept for 6 hours at 0°, after which the crystalline product (0·7 g.) was collected. 2-*Iodo*-2-*methylnoradrenochrome* (III; R = Me, R′ = I) formed deep-red needles (Found : C, 34·8; H, 3·0; N, 4·5. $C_9H_8O_3NI$ requires C, 35·4; H, 2·6; N, 4·6%). When heated, all three iodo-quinones decomposed indefinitely without melting. (II; R = Pr^i, R′ = I) and (II; R = H, R′ = I) decomposed rapidly on storage : (III; R = I) was, however, more stable.

Acetylation of the Iodo-quinones.—The iodo-quinones were dissolved in a mixture of pyridine (5 c.c.) and acetic anhydride (5 c.c.) and the solution kept for 24 hours at room temperature. The mixture was then poured into water, and the separated solid collected and recrystallised from aqueous ethanol (charcoal). From 2-iodo-*N-iso*propylnoradrenochrome (0·4 g.) there was thus obtained 3 : 5 : 6-*triacetoxy*-2-*iodo*-1-iso*propylindole* (IV; R = Pr^i, R′ = I) (0·45 g.), prisms, m. p. 163—164° (Found : C, 44·7; H, 4·1; N, 3·2; I, 26·6. $C_{17}H_{18}O_6NI$ requires C, 44·5; H, 3·9; N, 3·05; I, 27·6%).

2-Iodonoradrenochrome (0·3 g.) gave 3 : 5 : 6-*triacetoxy*-1-*acetyl*-2-*iodoindole* (IV; R = Ac, R′ = I) (0·33 g.), prisms, m. p. 208—209° (Found : C, 42·2; H, 3·0. $C_{16}H_{14}O_7NI$ requires C, 41·8; H, 3·1%).

2-Iodo-2-methylnoradrenochrome (0·4 g.) gave 5 : 6-*diacetoxy*-1-*acetyl*-2 : 3-*dihydro*-2-*iodo*-3-*keto*-2-*methylindole* (V; R = I) (0·3 g.), as pale yellow prisms, m. p. 199—200° (Found : C, 42·1; H, 3·4. $C_{15}H_{14}O_6NI$ requires C, 41·7; H, 3·2%)

2-Iodoadrenochrome (0·5 g.) gave 3 : 5 : 6-*triacetoxy*-2-*iodo*-1-*methylindole* (IV; R = Me, R′ = I) (0·6 g.), prisms, m. p. 150° (Found : C, 42·1; H, 3·5. $C_{15}H_{14}O_6NI$ requires C, 41·7; H, 3·2%).

De-iodination of the Acetylated Iodo-derivatives.—The acetylated iodo-compounds were dissolved in boiling acetic acid (4 c.c.), and zinc dust (2 g.) added slowly over a period of 15 minutes. The solution was then decanted from excess of zinc and poured into water. After some time at 0°, the separated solid was

collected and recrystallised. From 3 : 5 : 6-triacetoxy-1-acetyl-2-iodoindole (0·3 g.) there was obtained 3 : 5 : 6-*triacetoxy*-1-*acetylindole* (IV; R = Ac, R′ = H) (0·15 g.), m. p. 125—127° (needles from aqueous ethanol) (Found: C, 57·2; H, 4·5. $C_{16}H_{15}O_7N$ requires C, 57·6; H, 4·5%). 5 : 6-Diacetoxy-1-acetyl-2 : 3-dihydro-2-iodo-3-keto-2-methylindole (0·3 g.) gave 5 : 6-*diacetoxy*-1-*acetyl*-3-*hydroxy*-2-*methylindole* (V; R = H) (0·13 g.), m. p. 185—187° (prisms from benzene–light petroleum) (Found: C, 59·4; H, 5·1. $C_{15}H_{15}O_6N$ requires C, 59·0; H, 4·9%). 3 : 5 : 6-Triacetoxy-2-iodo-1-methylindole (0·5 g.) gave 3 : 5 : 6-triacetoxy-1-methylindole (IV; R = Me; R′ = H) (0·32 g.), m. p. 113°. The corresponding *iso*propyl analogue gave a gum which crystallised when kept for 3 months; recrystallisation from aqueous ethanol gave 3 : 5 : 6-*triacetoxy*-1-iso*propylindole*, m.p. 87° (Found: C, 61·4; H, 5·7. $C_{17}H_{19}O_6N$ requires C, 61·3; H, 5·7%).

Hydrolysis of the Acetyl Derivatives.—3 : 5 : 6-Triacetoxy-1-acetylindole (0·1 g.) was boiled for 1 hour under nitrogen with 2N-sodium hydroxide (4 c.c.). The solid slowly dissolved giving a yellow solution with an intense green fluorescence. When cool the solution was acidified with acetic acid, but no solid separated even after some while at 0°, and attempts to extract the indoxyl with organic solvents were unsuccessful. After exposure to air for some time, the fluorescence slowly disappeared and a black precipitate separated; this dissolved in aqueous alkali to an intense permanganate-coloured solution.

The triacetyl compound (V; R = H) (0·1 g.) on similar treatment also gave a yellow solution with an intense green fluorescence. Attempts to isolate the indoxyl were similarly unsuccessful.

Isomerisation of Iodoquinones with Zinc Acetate.—2-Iodoadrenochrome (0·5 g.) was stirred with water (10 c.c.), and 20% zinc acetate solution (5 c.c.) added. After 10 minutes' stirring the red colour of the solution had changed to yellow with a green fluorescence. Sodium dithionite (0·2 g.) was then added, the mixture warmed to 60°, and some dark solid which remained undissolved was filtered off. When kept at 0°, the filtrate deposited 3 : 5 : 6-trihydroxy-1-methylindole monohydrate (0·1 g.). Similarly *N-iso*propyl-2-iodonoradrenochrome gave a very small yield of 3 : 5 : 6-trihydroxy-1-*iso*propylindole. 2-Iodonoradrenochrome gave a yellow, fluorescent solution, but no solid product could be isolated. 2-Iodo-2-methylnoradrenochrome gave a brownish-yellow solution which was non-fluorescent.

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