*J. Pharm. Pharmac.*, 1973, **25**, 193–200 *Received September 5, 1972*

# The oxidation of 6-hydroxydopamine*

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The oxidation of 6-hydroxydopamine (I) with molecular oxygen or potassium ferricyanide in aqueous solution has been shown to lead mainly to the formation of the uncyclized quinone 2-(β-aminoethyl)-5-hydroxy-*p*-benzoquinone (VII). However this quinone cyclized slowly, presumably to norepinochrome (i.e. indoline-5,6-quinone, XII), since 5,6-dihydroxyindole (II), known to be readily formed by rearrangement of norepinochrome (XII), can be isolated from these solutions. 5,6-Dihydroxyindolines (cf. XIII) can also be isolated (as their acetyl derivatives) from the products obtained by the addition of reducing agents to solutions containing 6-hydroxydopamine oxidation products. No evidence was found to suggest that the possible alternative cyclization of the quinone VII to the *p*-quinonoid aminochrome 6-hydroxyindoline-4,7-quinone (III) occurred to any significant extent.

It has been known for about 10 years now that 6-hydroxydopamine (i.e. 2,4,5-trihydroxyphenylethylamine, I) produces a substantial and relatively persistent depletion of noradrenaline from peripheral sympathetically innervated organs (Porter, Totaro & Stone, 1963; Porter, Totaro & Burcin, 1965; Laverty, Sharman & Vogt, 1965; cf. Heikkila & Cohen, 1971, 1972; De Champlain & Nadeau, 1971; Malmfors & Thoenen, 1971; Sachs & Jonsson, 1972 for other references). At one stage it was suggested that this depletion may have been due to either irreversible damage having occurred at the amine storage sites or by I (and/or one of its metabolites) acting as a false adrenergic nerve transmitter (Porter & others, 1963; Stone, Porter, & others, 1964; Laverty & others, 1965).

It has recently been shown however that the striking and dramatic physiological effects produced by I are due to its ability to selectively destroy peripheral adrenergic nerve terminals without having any apparent effect on other body tissues (Tranzer & Thoenen, 1967; Thoenen & Tranzer, 1968; Thoenen, Tranzer & Häusler, 1970; De Champlain & Nadeau, 1971; cf. Malmfors & Thoenen, 1971). The progressive damage to the noradrenergic reward system that can be attributed to I has been proposed as a possible biochemical aetiology of schizophrenia (Stein & Wise, 1971).

The mechanism by which this chemically induced sympathectomy is produced is not fully understood. Two factors appear to be important in the process however; (*i*) the efficient accumulation of I in the adrenergic nerve endings and (*ii*) the susceptibility of I to oxidation, particularly at "physiological pH" values. It has been suggested by some workers that the effective agent is hydrogen peroxide, formed during the autoxidation of I (Heikkila & Cohen, 1971, 1972). The possibility that quinonoid products that readily form on oxidation of I can take part in a covalent binding process with nucleophilic groups (such as -SH, $-NH_2$ and -OH) present in the bio-

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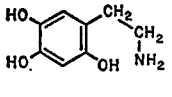
logical macromolecules may be responsible for the irreversible alteration of the biological structures concerned has also been proposed (Thoenen & others, 1970; Saner & Thoenen, 1971).



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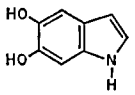
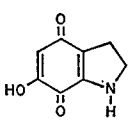


II

The synthesis and oxidation of 6-hydroxydopamine (I) were first described nearly 20 years ago (Harley-Mason, 1953) when it was reported that intensely red solutions were obtained on oxidation of I with potassium ferricyanide in aqueous sodium bicarbonate. When these solutions, which contained oxidized I, were allowed to stand at room temperature in a hydrogen atmosphere for 24 h, 5,6-dihydroxyindole (II) could be isolated in good yield from the ethyl acetate soluble products that formed in solution (Harley-Mason, 1953).

Some years later, however, it was suggested that the red product ($\lambda_{max}$, 475 nm) obtained on autoxidation of I in weakly acid solution was 6-hydroxyindoline-4,7-quinone (III) (Senoh & Witkop, 1959). The product III was reported to give 4,6,7-trihydroxyindoline (IV) on reduction with sodium dithionite, however, it did not apparently undergo the expected internal oxidation-reduction reaction [typical of the related indoline-5,6-quinones, i.e. the *ortho*-quinonoid aminochromes (cf. reviews by Heacock, 1959 and Heacock & Powell, 1972)] with alkali to give 4,6,7-trihydroxyindole (V) (Senoh & Witkop, 1959; Daly, Horner & Witkop, 1961).



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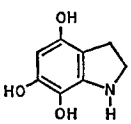

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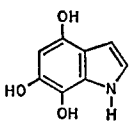

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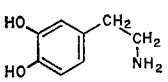
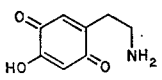


VI

In a recent investigation into the possibility that I might be formed as an intermediate during the melanization of dopamine (i.e. 3,4-dihydroxyphenylethylamine, VI) it was shown that autoxidation of I, in the presence of catalase at pH 8, did give rise to an alkali soluble melanin, but only in very low yield (Swan, 1963; Chapman, Percival & Swan, 1970). These workers also showed that addition of mushroom polyphenol oxidase to solutions of I in air rapidly led to the formation of a deep red solution ($\lambda_{max}$, 214, 272 and 490 nm) which it was believed contained the *p*-quinonoid aminochrome III mentioned above (Chapman & others, 1970).

It has been reported recently that the products obtained on oxidation of I are dependent on the pH of the medium (Saner & Thoenen, 1971). It was suggested that whilst I is very unstable in neutral or alkaline solution, its solutions at pH 3 appear to be stable for several hours. However a red product did form slowly (10–20 h) ($\lambda_{max}$, 480 nm) believed to be the uncyclized quinone [i.e. 2-($\beta$-aminoethyl)-5-hydroxy-*p*-benzoquinone, (VII), or its zwitterion (VIIa)]. This first oxidation product formed and cyclized much more rapidly at higher pH, but Saner & Thoenen (1971) believed that this compound underwent rapid ring-closure to 4,6,7-trihydroxyindoline (IV) which was further oxidized to 6-hydroxyindoline-4,7-quinone (III). These workers suggested that the *para*-quinonoid aminochrome III did in fact, rearrange to 4,6,7-

trihydroxyindole (V) and this compound was capable of undergoing further oxidation to 6-hydroxyindole-4,7-quinone (VIII). The oxidation products of I were observed to react readily with bovine serum albumin and it was suggested that the nucleophilic groups (i.e. -SH, -OH, $-NH_2$, etc.) present in the albumin reacted to form covalently bonded products with the quinones (Thoenen & others, 1970; Saner & Thoenen, 1971).



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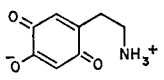

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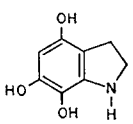

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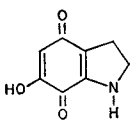

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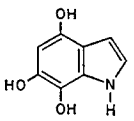

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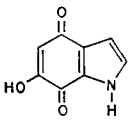


VIII

## RESULTS AND DISCUSSION

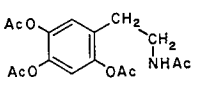
A dilute ($10^{-3}$ M) solution of 6-hydroxydopamine (I) in acetate buffer (pH, 5–6) was oxidized by bubbling oxygen gas through the solution. The absorption maximum at 288 nm in the ultraviolet/visible spectrum of the solution due to 6-hydroxydopamine (I) was slowly replaced by two peaks at 486 and 268 nm. These peaks, which reached their maximum intensity after about 45 min, had extinction coefficients of 1940 and 9510 respectively, based on total conversion of all the amine I originally present. Similar findings had been reported earlier (Senoh & Witkop, 1959), when it was suggested that the compound responsible for the peaks at about 486 and 268 nm was the *para*-quinonoid aminochrome, 6-hydroxyindoline-4,7-quinone (III). In view of the earlier findings of Harley-Mason (1953), involving the isolation of 5,6-dihydroxyindole (II) which suggested that the alternative cyclization to an indoline-5,6-quinone might have occurred, it was decided to attempt to isolate some of the products formed (as suitable derivatives) on oxidation of I under varying conditions.

The oxidation of 6-hydroxydopamine (I), was therefore carried out with molecular oxygen in the manner described above; terminated after 1 h by the addition of an excess of sodium borohydride, which resulted in the immediate discharge of the colour of the solution. The reduction products were acetylated immediately by the addition of sodium bicarbonate and acetic anhydride to the reaction mixture. Thin-layer chromatography (stationary phase: silica gel; solvent: ethyl acetate) of a dichloromethane extract of the reaction mixture after acetylation, revealed the presence of one major product ($R_F$, 0·18) which gave a red colour with 1 N sodium hydroxide and a trace of a second product ($R_F$, 0·46) which gave a brown colour with sodium hydroxide after heating. The major product was obtained as a white crystalline solid, after purification by column chromatography on silica gel. Its physical characteristics were identical to those of $O^2$,$O^4$,$O^5$,*N*-tetraacetyl-6-hydroxydopamine (IX) which had also been prepared by direct acetylation of 6-hydroxydopamine (I) with acetic anhydride in aqueous sodium bicarbonate.

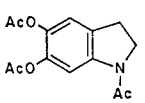
The tetraacetyl derivative of I (i.e. IX) had previously been prepared by Laverty & Sharman (1965) but its physical characteristics were not described by these authors.

When the oxidation reaction was allowed to proceed for 1·5 h before the addition of the sodium borohydride, and the acetylated reaction products worked up as described above, thin-layer chromatography revealed the presence of IX along with an increased amount of the second product ($R_F$, 0·46). A trace of a third product ($R_F$, 0·87), which gave a grey colour with 1 N sodium hydroxide and a violet colour with Ehrlich's reagent, was also observed.

A third experiment was then carried out in which the oxygen gas was again bubbled through the 6-hydroxydopamine (I) solution for 1·5 h, however in this case nitrogen was subsequently bubbled through the red solution for a further 2 h. The three products mentioned above were again detected after working up the reaction mixture, but the relative amounts of the second ($R_F$, 0·46) and especially the third ($R_F$, 0·87) products were considerably higher. Upon column chromatography of the reaction mixture the first two products were isolated as white crystalline solids and the third as a colourless oil. The first product again proved to be identical with 6-hydroxydopamine tetraacetate (IX). The pmr, ultraviolet infrared, and mass spectrometric characteristics of the second product were identical to those reported in the literature for $O^5$,$O^6$,*N*-triacetyl-5,6-dihydroxyindoline [i.e. 5,6-diacetoxy-1-acetylindoline (X) (cf. Piattelli-Oriente, Sciuto & Piattelli, 1970)]. These authors had previously obtained this product by acetylation of the reduced oxidation products of dopamine (VI). The third product, which was isolated in very low yield, exhibited chromatographic, mass spectrometric and u.v. spectral properties identical to those of 5,6-diacetoxyindole (XI) which had been prepared as described in the literature (Heacock, Mahon & Scott, 1961).




IX

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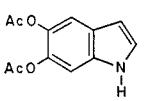



XI

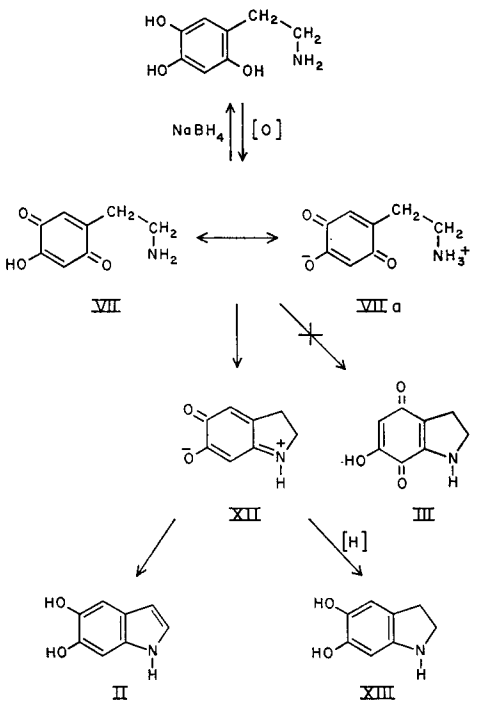
Harley-Mason had previously reported the synthesis of 5,6-dihydroxyindole (II) in relatively high yield by the oxidation of 6-hydroxydopamine (I) with potassium ferricyanide, using a slight excess of the former, followed by storing the reaction mixture under hydrogen for 24 h. Upon repetition of Harley-Mason's procedure, followed by acetylation of the products, 5,6-diacetoxyindole (XI) was obtained after column chromatography in relatively low yield in crystalline form. Thin-layer chromatography of the reaction mixture revealed the presence of several additional products. However, these products were not identified.

Oxidation of 6-hydroxydopamine (I) hydrobromide in the presence of two equivalents of sodium acetate was also carried out in dilute methanolic solution at a concentration of I identical to those used in the reaction in aqueous buffer ($10^{-3}$ M). After bubbling oxygen through the solution for 45 min, the oxidation was terminated by the addition of an excess of sodium borohydride and the products acetylated using the procedure described above. Upon column chromatography on silica gel the products IX, X and XI were isolated as crystalline solids; the yields of the cyclized products X and XI were larger than in the aqueous reaction, despite the fact that the oxidation time was less.

Oxidation of 6-hydroxydopamine (I) by potassium ferricyanide in more concentrated solutions ($1{\cdot}67 \times 10^{-2}$ M) appeared to follow a course similar to the oxidations

which were carried out using molecular oxygen as the oxidizing agent. Reduction of the deep red oxidized solution with sodium borohydride a few seconds after the addition of the ferricyanide, followed by acetylation of the products lead almost exclusively to the formation of the tetraacetate IX. When the borohydride was added after 1/2 h, however, substantial amounts of the cyclic derivatives X and XI were detected by thin-layer chromatography.

As a result of these investigations it can be concluded that 6-hydroxydopamine (I) is oxidized readily, either by oxygen or potassium ferricyanide, to the open-chain quinone VII. This compound undergoes a relatively slow cyclization reaction to give the aminochrome norepinochrome (i.e. XII), which gradually rearranges to 5,6-dihydroxyindole (II) (cf. Harley-Mason, 1953). Alternatively, reduction of XII leads to the formation of 5,6-dihydroxyindoline (XIII). No evidence could be found for cyclization of the open-chain quinone VII to 4,6,7-trihydroxyindoline (IV) as previously postulated (Senoh & Witkop, 1959; Saner & Thoenen, 1971).




During the course of the current investigation a report appeared (Adams, Murrill & others, 1972) in which, it was similarly concluded, on the basis of electrochemical and spectroscopic evidence that the open-chain quinone VII was the major initial product of the oxidation of 6-hydroxydopamine (I) and that the zwitterionic form (i.e. VIIa) of this compound was the main chromophore in solutions of oxidized I at neutral pH values. These workers were concerned mainly with the initial stages of the reaction and they did not deal with any possible subsequent cyclization reactions. The slow rate of cyclization of the open-chain quinone VII (or VIIa) would probably indicate that this compound, rather than any of the cyclized products, has the greatest physiological significance of the 6-hydroxydopamine (I) oxidation products.

PREPARATIVE CHEMISTRY

*$O^2$,$O^4$,$O^5$,N-tetraacetyl-6-hydroxydopamine* [(i.e. 2,4,5-triacetoxy-1-β-*N*-acetylaminoethylbenzene, (IX)] [cf. preparation of *$O^3$*, *$O^4$*,*N*-triacetyladrenaline by Welsh (1952)].

Acetic anhydride (3 ml) was added cautiously in four portions to a solution of 6-hydroxydopamine hydrobromide (200 mg) and sodium bicarbonate (6·5 g) in water (20 ml). Excessive frothing was controlled by the addition of a small amount of ether. After stirring for a further 20 min the aqueous solution was extracted with dichloromethane (6 × 15 ml). The organic extracts were then combined, dried ($Na_2SO_4$), and concentrated in a vacuum to dryness. The residue was dissolved in a few ml of ethyl acetate and light petroleum (60–80°) was added until the solution became markedly cloudy. After standing at room temperature (20°) for several hours a white crystalline solid (218 mg) (m.p. 111–113°) was obtained which after recrystallization from a benzene–hexane mixture gave *$O^2$*,*$O^4$*,*$O^5$*,*N*-tetraacetyl-6-hydroxydopamine (IX) in clusters of small white needles, m.p. 123–126°. $\lambda_{max}$(EtOH) nm (ε) 267 (741), 272 (727); $\nu_{max}$(KBr): 3250, 1780, 1765, 1637, 1569 $cm^{-1}$; δ ($CDCl_3$): 2·08 (3H, s, > *N*-$COCH_3$); 2·25 (6H, s, -*$O^4$*- and -*$O^5$*-$COCH_3$); 2·32 (3H, s, -*$O^2$*-$COCH_3$); 2·73 (2H, m, -$CH_2$-); 3·43 (2H, m, coupled to NH, $J \approx 6$ Hz, > *N*-$CH_2$-); 5·78 (1H, very broad peak, NH); 7·00 (1H, s, probably H-3), 7·07 (1H, s, slightly broadened, probably H-6). Mass spectrum: Found $M^{+}$·337, Calcd for $C_{16}H_{19}NO_7$: 337. Anal. Calcd for $C_{16}H_{19}NO_7$: C, 57·00; H, 5·64; N, 4·15. Found: C, 56·91; H, 5·76; N, 4·24%.

*Oxidation of 6-hydroxydopamine by oxygen in aqueous solution: reduction and acetylation of the products.* Oxygen was bubbled for 1·5 h through a solution of 6-hydroxydopamine hydrobromide (250 mg) in 0·1 M sodium acetate buffer (1000 ml, pH 5·6), after which time nitrogen was bubbled through the aqueous solution for a further 2 h. Sodium borohydride (190 mg, 5 equiv), was added to the rapidly stirred red solution. Sodium bicarbonate (20 g), followed by acetic anhydride (10 ml), were then immediately added to the resulting colourless solution and stirring continued for a further 45 min. The aqueous solution was then extracted with dichloromethane (6 × 500 ml); concentration to dryness of the combined, dried ($Na_2SO_4$) dichloromethane extracts gave a light yellow oil. Thin-layer chromatography on silica gel (Brinkman $F_{254}$ commercially available plates) with ethyl acetate as solvent demonstrated the presence of three major products. These products were separated and isolated by column (2·5 × 25 cm) chromatography on silica gel (Davison, 100–200 mesh). A series of 18 ml fractions were collected with the following solvents being used as eluants: benzene–ethyl acetate (85:15) (1300 ml); benzene–ethyl acetate (50:50), (1800 ml) and ethyl acetate (2700 ml). Fractions 28–41 were combined and the solvent removed in a vacuum, giving a colourless oil which could not be induced to crystallize. The chromatographic, ultraviolet and mass spectral properties of this oil were similar to those of 5,6-diacetoxyindole (XI) which had been prepared by methods described in the literature (Heacock & others, 1961). There were, however, extraneous peaks in the ultraviolet and mass spectra, indicating that the sample of XI, prepared in this manner from 6-hydroxydopamine, was impure.

Fractions 97–115 were combined and concentrated to dryness to give a white crystalline solid (14 mg) which was recrystallized from heptane–benzene to give 5,6-diacetoxy-1-acetylindoline (X) as colourless needles [8 mg, m.p. 211–213°; lit. m.p. 223–225° (Piattelli-Oriente & others, 1970)]. The ultraviolet, infrared, pmr and mass

spectral properties of this compound were identical to those previously reported in the literature.

Fractions 241–280 were combined and concentrated to dryness in a vacuum to give a light yellow crystalline solid which was recrystallized from heptane–benzene to give $O^2$,$O^4$,$O^5$,*N*-tetraacetyl-6-hydroxydopamine (IX) as colourless needles (44 mg, m.p. 120–123°). The identity of this product with samples of IX, which had been prepared by the direct acetylation of 6-hydroxydopamine, was established by the non-depression of m.p. and by a comparison of their infrared, mass spectral and chromatographic properties with those of the synthetic compound.

*Oxidation of 6-hydroxydopamine by oxygen in methanolic solution: reduction and acetylation of the products.* Oxygen was bubbled through a solution of 6-hydroxydopamine hydrobromide (250 mg) and sodium acetate (164 mg, 2 equiv) in methanol (1000 ml) for 45 min. Sodium borohydride (200 mg), followed by conc. HCl (0·5 ml) was then added to the rapidly stirred red solution, resulting in an immediate discharge of the colour of the solution. The solution was then concentrated to dryness in a vacuum and acetic anhydride (7·5 ml) and pyridine (7·5 ml) were added to the residue. After being allowed to stand for 4 h at room temperature (20°) the solution was concentrated to dryness (temperature $\leqslant 50°$). The residue was dissolved in dichloromethane (50 ml) and the organic phase washed with a saturated solution of sodium bicarbonate (3 × 50 ml) and water (3 × 50 ml). The organic phase was then dried ($Na_2SO_4$) and concentrated to dryness in a vacuum. Thin-layer chromatography revealed the presence of three major products, as in the case of the aqueous reaction. After column chromatography on silica gel in the manner described above, 5,6-diacetoxyindole (XI) (1 mg), 5,6-diacetoxy-1-acetylindoline (X) (25 mg) and $O^2$,$O^4$,$O^5$,*N*-tetraacetyl-6-hydroxydopamine (IX) (55 mg) were each isolated as crystalline solids. The identity of these products with samples which had been prepared previously from the aqueous reaction was established by the non-depression of melting points and by the identity of their mass spectral and chromatographic properties.

*Acknowledgements*

The authors wish to thank Mr. D. Embree and Dr. W. D. Jamieson for the mass spectrometric data and Mr. G. McCully for some technical assistance.

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