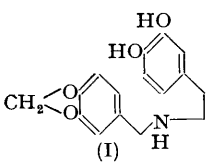
## 104. *The Oxidation of Some 3 : 4-Dihydroxyphenethylamines.*

By E. J. Forbes.

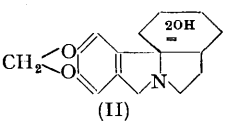
3 : 4-Dihydroxy-*N*-(3 : 4-methylenedioxybenzyl)phenethylamine (I) and its *N*-3 : 4-dihydroxybenzyl analogue (IV) have been oxidised to indoles by gaseous oxygen in the presence of the enzyme polyphenol oxidase and by other agents. The structures of the products, isolated as their methyl ethers, have been proved by independent syntheses.

Although the oxidative coupling of aromatic nuclei has been achieved in other fields, it has not as yet been accomplished in the alkaloid series. Such a reaction has been postulated by Robinson [1] as a step in the biogenesis of the alkaloids of the morphine and aporphine groups. In an attempt to realise this process in the laboratory, Robinson and Sugasawa [2] and Schöpf and Thierfeld [3] oxidised laudanosoline under a variety of conditions. The product, however, was a tetrahydrodibenzopyrrocoline and not norglaucine.

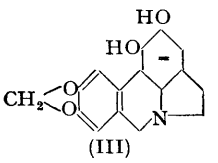
In a biogenetic scheme for lycorine, the oxidation of the amine (I) [4] or its biogenetic equivalent [5] was envisaged as an intermediate step. This could lead either to a blocked hydroaromatic structure (II) [5] or to a reduced phenanthridine (III).[6] Either course involves the formation of an indole ring and the oxidative coupling of two aromatic nuclei. In the oxidation of laudanosoline the prior formation of the indole ring in laboratory experiments stereochemically precluded coupling of the aromatic nuclei, but with amine (I) this would not be so.



(I)



(II)



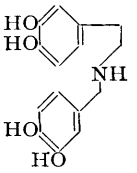
(III)

Harley-Mason [4] oxidised the closely related amine (IV) with potassium ferricyanide in the presence of sodium hydrogen carbonate. The product was undoubtedly the indole (V; R = H), though this was not in fact proved. In the present work this point has been settled, and further efforts have been made to oxidise the amine (IV) beyond the indole stage. The analogous amine (I) has also been submitted to oxidative procedures, but as with the amine (IV) aromatic coupling was not achieved.

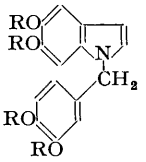
Raper [7] oxidised a series of 3 : 4-dihydroxyphenethylamines to 5 : 6-dihydroxyindoles with gaseous oxygen in the presence of the enzyme tyrosinase, obtained from meal worms.

[1] Robinson, *J.*, 1931, 3163; *J. Roy. Soc. Arts*, 1948, **96**, 807.
[2] Robinson and Sugasawa, *J.*, 1932, 789.
[3] Schöpf and Thierfeld, *Annalen*, 1932, **497**, 22.
[4] Harley-Mason, *J.*, 1953, 200.
[5] Forbes, Harley-Mason, and Robinson, *Chem. and Ind.*, 1953, 946.
[6] Kondo and Uyeo, *Ber.*, 1937, **70**, 1087; Kelly, Taylor, and Wiesner, *J.*, 1953, 2094.
[7] Raper, *Biochem. J.*, 1927, **21**, 89.

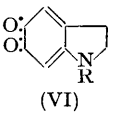
This reaction involves the formation of an intermediate *o*-quinone (VI), which then undergoes autoreduction to the dihydroxyindole in the presence of aqueous sulphur dioxide. It has now been found that a preparation of polyphenol oxidase, readily obtained from mushrooms, gives excellent results. Preliminary experiments, designed to test the effectiveness and specificity of the enzyme, were made on 3 : 4-dihydroxyphenethylamine according to Blaschko and Schlossmann's method.[8] Solutions of the amine hydrochloride



(IV)



(V)



(VI)

in a phosphate buffer were incubated in oxygen with the enzyme preparation, and the uptake of oxygen was measured manometrically. After a rapid uptake of two atoms of oxygen, as required for the formation of the *o*-quinone (VI; R = H), there was practically no further absorption. With the amine (I) there was again a rapid initial uptake of two atoms of oxygen, as expected, but with the amine (IV) a rapid uptake of four atoms was recorded. This latter figure must include two atoms for the formation of the *o*-quinone (VI), one atom for the catechol group which does not participate in ring-closure, and, most disappointingly as the sequel shows, the fourth atom must have been used in dehydrogenating the *o*-quinone nucleus (VI). From these experiments the time required for complete oxidation and also the concentrations of solutions which could be employed without the *o*-quinone's being precipitated were determined. For preparative purposes, a suspension of the enzyme preparation in a dilute solution of the amine hydrochloride in a phosphate buffer of pH 7 was treated with a rapid stream of oxygen. A deep-red colour, due to *o*-quinone formation, rapidly developed. After an appropriate period, as determined by the manometric experiments, the oxygen stream was interrupted. Acetic acid was added to inactivate the enzyme which was then removed. Following the addition of aqueous sulphur dioxide, the solution was put aside. Overnight the *o*-quinone (VI) underwent autoreduction to the 5 : 6-dihydroxyindole, which was methylated before isolation. The products from the amines (I) and (IV) were the indoles (VIII; R = H) and (V; R = Me), no aromatic coupling having taken place. Potassium iodate gave similar results. It is of interest that in Raper's experiments and in the present series the oxidation proceeded only so far as the *o*-quinone (VI), whereas in the oxidation of laudanosoline with chloranil in alcohol in the presence of acetate [2] the quinone was formed only as an intermediate, its colour being discharged quite rapidly without further treatment to give the product, albeit in this case a dihydroindole.

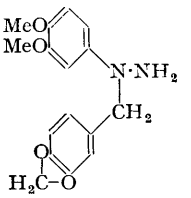
The syntheses of the methyl ethers isolated from the above experiments followed the same path, which will be described only for 5 : 6-dimethoxy-1-(3 : 4-methylenedioxybenzyl)-indole (VIII; R = H). 3 : 4-Dimethoxyaniline condensed readily with piperonaldehyde in refluxing alcohol to give the Schiff's base, 3 : 4-dimethoxy-*N*-(3 : 4-methylenedioxybenzylidene)aniline. This compound was so readily hydrogenated that considerably more than the mol. of hydrogen required for the azomethine linkage was absorbed when either Adams catalyst or palladium on strontium carbonate was used. With Raney nickel, the reduction stopped abruptly after one mol. of hydrogen had been absorbed. The resulting 3 : 4-dimethoxy-*N*-(3 : 4-methylenedioxybenzyl)aniline showed little tendency to darken in the air. It gave a transient red colour, changing to bluish-green, with ferric chloride, probably as the result of demethylation of the group *para* to the nitrogen atom followed by quinone formation (cf. the synthesis of asaronic acid [9]).

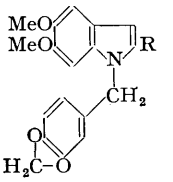
3 : 4-Dimethoxy-(*N*-3 : 4-methylenedioxybenzyl)aniline was converted into the nitrosamine, which was reduced by zinc dust and acetic acid to the hydrazine (VII). This

[8] Blaschko and Schlossmann, *J. Physiol.*, 1940, **98**, I, 130.
[9] Luff, Perkin, and Robinson, *J.*, 1910, 1131.

reduction caused much difficulty, and the yields (based on subsequent products) were low. Catalytic reduction yielded only by-products (cf. Paal and Yao [10]). In practice the hydrazine was not isolated, but was straightway condensed with pyruvic acid and the resultant hydrazone was cyclised with sulphuric acid to 5 : 6-dimethoxy-1-(3 : 4-methylenedioxybenzyl)indole-2-carboxylic acid (VIII; R = $CO_2H$). Overall, the yield of indolecarboxylic acid from the nitrosamine varied from 18 to 27%. When *cyclo*hexanone was used instead of pyruvic acid, the yield of the carbazole was rather better (40%), owing to the greater facility with which the hydrazone underwent cyclisation.

(VII)




(VIII)

The indolecarboxylic acid (VIII; R = $CO_2H$) when warmed with Ehrlich's reagent gave a magenta colour, which disappeared on cooling. When heated to 2—3° above its melting point for a few minutes, the acid was decarboxylated smoothly to give a high yield of 5 : 6-dimethoxy-1-(3 : 4-methylenedioxybenzyl)indole (VIII; R = H), identical with the product of oxidation of the secondary amine (I).

With veratraldehyde in place of piperonaldehyde the above series of reactions gave 5 : 6-dimethoxy-1-(3 : 4-dimethoxybenzyl)indole (V; R = Me), identical with the product of the oxidation of the secondary amine (IV) with oxygen, potassium iodate, or potassium ferricyanide (cf. Harley-Mason [4]).

## Experimental

*Oxidation of* 3 : 4-*Dihydroxy*-N-(3 : 4-*methylenedioxybenzyl*)*phenethylamine with Gaseous Oxygen.*—A preparation of the enzyme, polyphenol oxidase, was made as follows. Fresh mushrooms (195 g.) were passed through a meat-mincer into ice-cold acetone (1·5 l.). The solid was collected, washed twice with ice-cold acetone, and pressed between filter papers. After drying overnight in the air, the solid (7·5 g.) was ground to a fine powder and used without further treatment. (*a*) Preliminary experiments were carried out with 3 : 4-dihydroxyphenethylamine hydrochloride according to Blaschko and Schlossmann's method.[8] Solutions of the hydrochloride were incubated with the enzyme preparation and the uptake of oxygen was measured manometrically. The main bulbs of the manometric flasks contained suspensions of the enzyme powder (the strongest being 100 mg. of powder in 100 c.c. of solution) in a phosphate buffer of pH 7·4 (2·7 c.c.), whilst in the side bulb was placed a solution (0·02M; 0·3 c.c.) of 3 : 4-dihydroxyphenethylamine hydrochloride. The manometers were filled with oxygen, and the flasks were then incubated at 17°. When the contents of the two bulbs were mixed, absorption of oxygen commenced immediately and the solutions became red, owing to *o*-quinone formation. With the most concentrated enzyme suspension, gas absorption was complete (2 atoms) within 6 min.; the more dilute preparations required longer. A similar series of experiments was then carried out with 3 : 4-dihydroxy-*N*-(3 : 4-methylenedioxybenzyl)phenethylamine hydrochloride, in which the concentration of the enzyme suspension remained constant whilst the strength of the hydrochloride was varied from 0·02M to 0·004M. The *o*-quinone was not precipitated at a concentration of 0·004M, and oxygen uptake was complete (2 atoms) within 10 min.

(*b*) A rapid stream of oxygen was bubbled through a suspension of the enzyme preparation (3·5 g.) in a solution of the hydrochloride (0·5 g.) in water (500 c.c.) and a 0·03M-phosphate buffer (50 c.c.; pH 7·4); the flask was shaken meanwhile. The liquid immediately became red, the colour gradually deepening. After 10 min., the oxygen stream was stopped, 1% acetic acid (10 c.c.) was added to the mixture, and the liquid was filtered. Saturated aqueous sulphur dioxide (10 c.c.) was added to the deep-red filtrate, which was then left overnight. After filtration, the straw-coloured liquid was evaporated under reduced pressure to about 20 c.c. The product was then methylated under hydrogen with 20% sodium hydroxide (6·5 c.c.) and

[10] Paal and Yao, *Ber.*, 1930, **63**, 57.

dimethyl sulphate (3 c.c.). When the initial reaction was over, the mixture was heated (steam-bath) for 1 hr. After cooling, the mixture was extracted with a large volume of ether, and the ethereal extract was washed quickly with 2% sulphuric acid. The solution was dried ($K_2CO_3$) and evaporated to dryness, leaving buff crystals (0·2 g.). Recrystallisation from methanol afforded 5 : 6-dimethoxy-1-(3 : 4-methylenedioxybenzyl)indole in needles, m. p. 93° undepressed on admixture with an authentic specimen.

*Oxidation of* N-(3 : 4-*Dihydroxybenzyl*)-3 : 4-*dihydroxyphenethylamine.*—(a) *Gaseous oxygen.* Preliminary and preparative experiments were carried out in the manner described above. Oxygen uptake (4 atoms) was complete in 4 min. From *N*-3 : 4-dihydroxybenzyl-3 : 4-dihydroxyphenethylamine hydrochloride (0·5 g.), 1-(3 : 4-*dimethoxybenzyl*)-5 : 6-*dimethoxyindole* (0·25 g.) was obtained, which after two recrystallisations from methanol (charcoal) afforded prisms, m. p. 121—122° (Found : C, 69·4; H, 6·6; N, 4·1. $C_{19}H_{21}O_4N$ requires C, 69·7; H, 6·4; N, 4·3%). The indole gives a magenta colour with Ehrlich's reagent in the cold and slowly darkens when kept.

(b) *Potassium iodate.* A solution of potassium iodate (0·35 g.) in water (30 c.c.) was added to one of the hydrochloride (0·5 g.) in water (500 c.c.). After 30 min., the red solution was treated with aqueous sulphur dioxide (10 c.c.) and worked up, as previously described, to give 1-(3 : 4-dimethoxybenzyl)-5 : 6-dimethoxyindole (50 mg.), m. p. and mixed m. p. 121—122°.

(c) *Potassium ferricyanide.* The oxidation was carried out according to Harley-Mason's method.[4] The ethyl acetate extract of the tetrahydroxyindole was evaporated and the residue methylated with sodium hydroxide and dimethyl sulphate under hydrogen. Sublimation of the crude product at 120°/0·5 mm. gave 1-(3 : 4-dimethoxybenzyl)-5 : 6-dimethoxyindole, m. p. and mixed m. p. 120—121°.

3 : 4-*Dimethoxyaniline.*—4-Nitroveratrole was obtained by nitrating veratrole with concentrated nitric acid in acetic acid. Hydrogenation of the nitro-compound in alcohol, a Raney nickel catalyst being used at 80° and 75 atm. pressure for 15 min., gave 3 : 4-dimethoxyaniline as a colourless solid, b. p. 160°/14 mm., in almost quantitative yield.

3 : 4-*Dimethoxy*-N-(3 : 4-*methylenedioxybenzylidene*)*aniline.*—A solution of 3 : 4-dimethoxyaniline (43 g.) and piperonaldehyde (45·5 g.) in ethanol (200 c.c.) was heated under reflux for 30 min. On cooling, the solution deposited 3 : 4-*dimethoxy*-N-(3 : 4-*methylenedioxybenzylidene*)-*aniline* (75 g., 94%) which crystallised from ethanol in needles or plates, m. p. 109° (Found : C, 67·1; H, 5·2. $C_{16}H_{15}O_4N$ requires C, 67·4; H, 5·3%).

3 : 4-*Dimethoxy*-N-(3 : 4-*methylenedioxybenzyl*)*aniline.*—A solution of 3 : 4-dimethoxy-*N*-(3 : 4-methylenedioxybenzylidene)aniline (5 g.) in dioxan (30 c.c.) was hydrogenated at atmospheric pressure in presence of several grams of Raney nickel. When hydrogen uptake (420 c.c.) was complete (45 min.), the catalyst was removed, and most of the solvent was removed under reduced pressure. Addition of water to the residue gave 3 : 4-*dimethoxy*-N-(3 : 4-*methylenedioxybenzyl*)*aniline* (4·8 g.). Recrystallisation from ethanol afforded prisms, m. p. 96° (Found : C, 66·9; H, 6·0; N, 4·6. $C_{16}H_{17}O_4N$ requires C, 66·9; H, 5·9; N, 4·9%). The amine gives a transient red colour, changing to bluish-green, when treated in alcohol with a drop of dilute aqueous ferric chloride.

The *hydrochloride* crystallised from dilute hydrochloric acid or ethanol in plates, m. p. 196° (Found : C, 59·4; H, 5·8; Cl, 10·8. $C_{16}H_{17}O_4N$,HCl requires C, 59·4; H, 5·6; Cl, 11·0%).

3 : 4-*Dimethoxy*-N-(3 : 4-*methylenedioxybenzyl*)-N-*nitrosoaniline.*—A solution of sodium nitrite (3·5 g.) in water (20 c.c.) was added to a solution of 3 : 4-dimethoxy-*N*-(3 : 4-methylenedioxybenzyl)aniline (10 g.) in glacial acetic acid (200 c.c.) until excess of nitrous acid was present, as indicated by a sudden change in colour of the solution from dark green to orange red. Addition of water (2 vol.) precipitated 3 : 4-*dimethoxy*-N-(3 : 4-*methylenedioxybenzyl*)-N-*nitrosoaniline*, pale-yellow slender needles (10 g.) (from ethanol), m. p. 119° (Found : C, 60·7; H, 5·3; N, 8·9. $C_{16}H_{16}O_5N_2$ requires C, 60·8; H, 5·1; N, 8·9%).

5 : 6-*Dimethoxy*-1-(3 : 4-*methylenedioxybenzyl*)*indole*-2-*carboxylic Acid.*—A suspension of 3 : 4-dimethoxy-*N*-(3 : 4-methylenedioxybenzyl)-*N*-nitrosoaniline (2 g.) in acetic acid (4 c.c.)–dioxan (6 c.c.) was added at <10° to a vigorously stirred mixture of zinc dust (4 g.) in water (2 c.c.), cooled in ice (*ca.* 15 min.). Pyruvic acid (0·5 c.c.) and, after 1 min., 20% sulphuric acid (8 c.c.) were added to the filtrate. The resultant golden-yellow solution was heated at 50° for 5 min. On cooling, the solution deposited 5 : 6-*dimethoxy*-1-(3 : 4-*methylenedioxybenzyl*)*indole*-2-*carboxylic acid* (0·4—0·6 g.), which was purified through its sodium salt (charcoal). Two recrystallisations from methanol then afforded the acid in fine needles, m. p. 189° (Found : C, 64·0; H, 5·0; N, 4·0. $C_{19}H_{17}O_6N$ requires C, 64·2; H, 4·8; N, 4·0%). When warmed with Ehrlich's reagent the acid develops a magenta colour, which disappears on cooling.

The *methyl ester*, prepared from the acid in dioxan and ethereal diazomethane, crystallised from methanol in felted needles, m. p. 136° (Found : C, 65·2; H, 5·3. $C_{20}H_{19}O_6N$ requires C, 65·0; H, 5·2%).

5 : 6-*Dimethoxy*-1-(3 : 4-*methylenedioxybenzyl*)*indole*.—When 5 : 6-dimethoxy-1-(3 : 4-methylenedioxybenzyl)indole-2-carboxylic acid (0·5 g.) was heated 2—3° above its m. p., carbon dioxide was evolved. After 5 min., gas evolution had ceased, and the residue was dissolved in hot methanol. On cooling, the solution deposited a solid (0·3 g.), which on crystallisation from a small quantity of methanol afforded 5 : 6-*dimethoxy*-1-(3 : 4-*methylenedioxybenzyl*)*indole* in small needles, m. p. 93° (Found : C, 69·3; H, 5·6. $C_{18}H_{17}O_4N$ requires C, 69·5; H, 5·5%). The indole slowly darkens, and gives a magenta colour with Ehrlich's reagent in the cold.

N-(3 : 4-*Dimethoxybenzylidene*)-3 : 4-*dimethoxyaniline*.—A solution of 3 : 4-dimethoxyaniline (8·4 g.) and veratraldehyde (9·1 g.) in ethanol (25 c.c.) was heated under reflux for 30 min. The *Schiff's base*, which separated on cooling, crystallised from ethanol in small pale-yellow needles, m. p. 136° (Found : C, 67·6; H, 6·3. $C_{17}H_{19}O_4N$ requires C, 67·8; H, 6·3%).

N-(3 : 4-*Dimethoxybenzyl*)-3 : 4-*dimethoxyaniline*.—A solution of the Schiff's base (7·5 g.) in dioxan (25 c.c.) was shaken with Raney nickel under hydrogen (45 min.). After filtration, the solution was evaporated under reduced pressure. Addition of water to the residue and crystallisation (7 g.) from methanol gave needles, m. p. 93°. The amine soon turns yellow.

The *hydrochloride* formed feathery needles, m. p. 208°, from methanol (Found : C, 60·2; H, 6·9. $C_{17}H_{21}O_4N$,HCl requires C, 60·2; H, 6·8%). The *nitrosamine* was prepared in acetic acid, and formed pale-yellow irregular platelets (from methanol), m. p. 93° depressed on admixture with the original amine (Found : C, 61·4; H, 6·1; N, 8·1. $C_{17}H_{20}O_5N_2$ requires C, 61·5; H, 6·0; N, 8·4%).

1-(3 : 4-*Dimethoxybenzyl*)-5 : 6-*dimethoxyindole*.—*N*-(3 : 4-Dimethoxybenzyl)-3 : 4-dimethoxy-*N*-nitrosoaniline was reduced with zinc dust and acetic acid in the manner described for the *N*-(3 : 4-methylenedioxybenzyl) analogue. The hydrazine was treated with pyruvic acid, and the resultant hydrazone cyclised to the indolecarboxylic acid with dilute sulphuric acid, as described previously. The yield of the indole-2-carboxylic acid was very small. When heated at 180°/0·1 mm., it gave a sublimate of 1-(3 : 4-dimethoxybenzyl)-5 : 6-dimethoxyindole, m. p. 122° undepressed on admixture with the specimens obtained from the oxidation experiments.

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DYSON PERRINS LABORATORY, OXFORD. [*Received, September 1st*, 1955.]