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STUDIES IN PEROXIDASE ACTION—XXI*

THE OXIDATION OF JULOLIDINE

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Abstract—Oxidation of julolidine by the peroxidase system gives initially bis-9,9'-julolidyl. If the oxidation is allowed to continue further a complex mixture of products is obtained, from which bis-6,6'-(8-formyl-1,2,3,4-tetrahydroquinolyl) has been isolated. The C_2 fragment expelled during this reaction appears as acetaldehyde.

IT HAS been observed that the oxidation of N,N,2,6-tetramethylaniline by the peroxidase system proceeds with extreme difficulty.[1] Presumably because of steric strain in the molecule the lone pair orbital on nitrogen is unable to overlap with the aromatic π system. It is known that the dimethylamino group in this molecule is perpendicular to the plane of the ring.[2]

It is therefore important to investigate the action of the peroxidase system on julolidine (I), the structure of which resembles that of N,N,2,6-tetramethylaniline in so far as both systems are tertiary amines and di-*ortho* substituted. Inspection of a model of julolidine shows, however, that in its most stable conformation the lone pair orbital on nitrogen is perpendicular to the plane of the aromatic ring, and overlaps perfectly with the π system of the ring. We would therefore expect on theoretical grounds the peroxidase oxidation of julolidine to take place easily and this has been proved to be the case.

Using one equivalent of hydrogen peroxide, julolidine was converted in the presence of peroxidase to a colourless solid *via* a transient deep red brown intermediate. This solid was shown to be *bis-9,9'-julolidyl* (II), produced by the *p–p'* dimerisation of the julolidyl radical. The formation of this compound is analogous to the production of N,N,N',N'-tetramethylbenzidine by the action of the peroxidase system on N,N-dimethylaniline.[3]

Further addition of hydrogen peroxide to the oxidation solution continued to give a transient deep red brown colouration until three more equivalents had been added. The introduction of further quantities of this reagent had virtually no effect on the colour of the solution, and the reaction was assumed to be complete.

The oxidation of the isolated bis-9,9'-julolidyl behaved similarly when treated with the peroxidase system, and there is no doubt that this compound is the major primary intermediate in the oxidation of julolidine.

The terminal oxidation product was made alkaline (pH 8), whereupon a yellow solid was isolated. TLC analysis showed a complex mixture of products, among which was a fast-running yellow compound. Chromatographic separation gave rise to a

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yellow crystalline solid which was basic and contained an aldehyde function. The compound gave a crystalline semicarbazone. Microanalytical and spectral data showed the compound to be *bis*-6,6′-(8-*formyl*-1,2,3,4-*tetrahydroquinolyl*) (VII). The two-carbon fragment lost on formation of this compound was detected in the oxidation solution as acetaldehyde, trapped by means of its 2,4-dinitrophenylhydrazone.

The mechanism of formation of VII would appear to involve two processes which have been observed before in peroxidase chemistry. Assuming the formation of the dimeric species II is the first step, a subsequent process would be hydroxylation at the α C atom to the nitrogen on the saturated ring, giving rise to the α,α′-dihydroxydiamine III, as formulated in the Scheme below. This process, and the subsequent oxidative cleavage of the C—N bond to give the dialdehyde IV has been observed in the oxidation of N-methylaniline.[4] IV may be regarded as a 2,4,6-trialkylaniline and a similar reaction to that undergone by mesidine under these conditions may be envisaged, in which oxidation gives rise to the quinone imine methide V, followed by addition of water to give the benzyl alcohol derivative VI. The intermediacy of 4-amino-3,5-dimethylbenzyl alcohol has been observed in the oxidation of mesidine.[5] VI is a bis-β-hydroxyaldehyde, and would therefore be expected to undergo the reverse aldol condensation, particularly under the alkaline conditions used in working up the product. Thus the dialdehyde VII and acetaldehyde are generated.

The yield of the dialdehyde VII is less than 2% from julolidine. The remaining slower-running material appears to be highly polymeric and has not been further investigated. There are clearly other courses of reaction open to the proposed intermediates, and it is therefore not surprising that the oxidation product is a complex mixture.

Julolidine has an interesting pharmacological action on the thymus gland. This is being investigated at the moment. It is noteworthy that the thymus contains peroxidase.[6]

EXPERIMENTAL

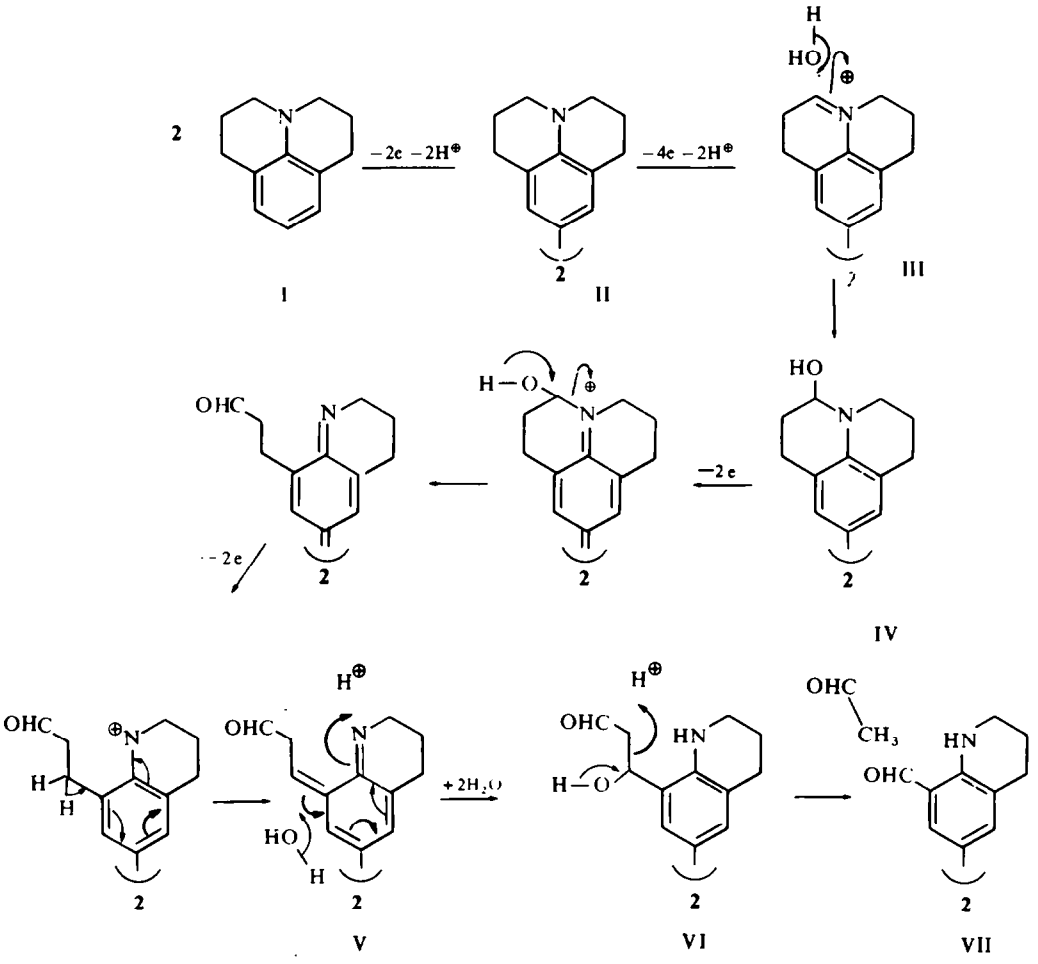
Materials and equipment. All oxidations were carried out using a purified horseradish peroxidase preparation (RZ = 0·3) supplied by Seravac Laboratories Ltd., and 20 volume H_2O_2.

Preparation of julolidine. Julolidine was prepared according to the method described in "Organic Syntheses".[7] Colourless plates m.p. 39°.

Oxidation of julolidine

Isolation of bis-9,9′-julolidyl. Julolidine (13 g) and glacial AcOH (13 ml) were mixed and added in 2 ml aliquots to a soln of peroxidase (20 mg) in acetate buffer (2 l, 0·1 M, pH 4·5). No colouration was observed. For each 2 ml aliquot, H_2O_2 (1·62 ml, 1 molar equiv) was added. The addition took 4 hr. After each addition a deep red brown colouration appeared which slowly faded. The mixture was subsequently made alkaline by careful addition of K_2CO_3 until pH 8 was achieved, (150 g). The pale pinkish brown ppt was filtered off, washed and dried. The solid (4 g) was recrystallised (benzene) as off-white prisms m.p. 209°, yield of *bis*-9,9′-*julolidyl* 3·1 g (24%). (Found: C, 83·5; H, 8·4; N, 7·9. $C_{24}H_{28}N_2$ requires: C, 83·7; H, 8·2; N, 8·1%); IR spectrum (Nujol) showed prominent bands at: 1625, 1500, 1360, 1320, 1245, 1205, 1190, 1160, 1120, 1075, 1055, 890, 880, 865, 855, 740, 735 and 723 cm^{-1}; NMR spectrum (5% soln in $CDCl_3$) showed quintet (J = 6 c/s, 8 methylene protons) at 7·79 τ; triplet (J = 6 c/s, 8 methylene protons) at 7·18 τ; triplet (J = 6 c/s, 8 methylene protons) at 6·85 τ; singlet (4 aromatic protons) at 2·97 τ; UV spectrum (EtOH): λ_{max} 224 mμ ($\log_{10} \varepsilon$ 3·645); 321. (4·444); λ_{min} 263 mμ (3·740).

SCHEME



Isolation of bis-6,6'-(8-formyl-1,2,3,4-tetrahydroquinolyl. The oxidation as described above was repeated using 1 g of julolidine, but in this case 4 molar equivs (6·5 ml) H_2O_2 were added during 24 hr. Further additions caused no significant alteration in the colour of the soln. The soln was then made alkaline (pH 8. K_2CO_3). On standing for a further 12 hr a yellow ppt was deposited, filtered off, washed and dried (700 mg). Further large scale preparations were carried out similarly.

The yellow solid (2 g) was treated with 20% EtOAc in $CHCl_3$, the suspension was filtered and the filtrate chromatographed on silicic acid (Mallinckrodt). The fast-running yellow band was eluted and evaporated to a yellow solid (50 mg). Recrystallisation (benzene) gave yellow prisms of *bis-6,6'-(8-formyl-1,2,3,4-tetrahydroquinolyl)* m.p. 230° (45 mg, 1·7%). (Found: C, 75·0; H, 6·3; N, 8·7. $C_{20}H_{20}N_2O_2$ requires: C, 75·0; H, 6·3; N, 8·7%); IR spectrum (Nujol) showed prominent bands at: 3330, 1675, 1655, 1590, 1530, 1410, 1315, 1275, 1235, 1210, 1195, 1185, 1115, 1090, 1070, 1060, 925, 920, 885, 833, 752, 747, 732 and 723 cm^{-1}. Mass spectrum: *m/e* 321, (% of base peak 25); 320, (100); 292, (5); 291, (5); 263, (5); 235, (5); 160, (30); 132, (10); M.W. $C_{20}H_{20}N_2O_2$ = 320; NMR spectrum (5% soln in $CDCl_3$) showed quintet (J = 6 c/s, 4 methylene protons) at 8·04 τ; triplet (J = 6 c/s, 4 methylene protons) at 7·14 τ; multiplet (broad, 6 protons) at 6·35–6·73 τ; doublet (broad, J = 8 c/s, 4 aromatic protons) at 2·65 τ; and singlet (2 aldehyde protons) at 0·15 τ; UV spectrum (EtOH): λ_{max} 221 mμ ($\log_{10} \varepsilon$ 4·197); 258, (4·711); 304, (4·378); 412, (4·021); λ_{min} 299 mμ (4·185); 282, (3·993); 346, (2·884).

Preparation of bis-6,6'-(8-formyl-1,2,3,4-tetrahydroquinolyl) disemicarbazone. Bis-6,6'-(8-formyl-1,2,3,4-tetrahydroquinolyl) (20 mg) was dissolved in EtOH: benzene (1:1, 5 ml) and a soln of semicarbazide HCl (0·5 g) and NaOAc ($3H_2O$, 0·8 g) in water (3 ml) was added. Heating for 1 hr at 100° gave a yellow crystalline ppt, which was filtered off, washed and dried (20 mg, 74%), m.p. 305° (dec). (Found: C, 60·7; H, 6·4. $C_{22}H_{28}N_8O_2$ requires: C, 60·8; H, 6·0%).

Oxidation of julolidine

Isolation of acetaldehyde. Julolidine (10 g) was oxidised as described above. After removal of the crude bis-6,6'-(8-formyl-1,2,3,4-tetrahydroquinolyl) the filtrate was made acid (dil H_2SO_4) and steam-distilled. 30 ml of distillate were collected in a cooled receiver. Acidic 2,4-dinitrophenylhydrazine reagent was added and the yellow ppt was filtered off, washed and dried. Recrystallisation (EtOH) gave yellow needles (1·0 mg), m.p. and mixed m.p. with authentic acetaldehyde 2,4-dinitrophenylhydrazone 147° (lit. 147°), yield: $7{\cdot}8 \times 10^{-3}$% from julolidine. The low yield is attributed to the high volatility of acetaldehyde and its possible polymerisation before the introduction of the 2,4-dinitrophenylhydrazine reagent.

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