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STUDIES IN PEROXIDASE ACTION—XXII*

PEROXIDASE AS A TRANSALKYLATING AGENT

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Abstract—The oxidations of 2,3,4-trimethylaniline, 4-isopropyl-2,6-dimethylaniline, 4-t-butyl-2,6-dimethylaniline and 4-cyclohexyl-2,6-dimethylaniline by peroxidase have been studied in detail. In three instances alkyl group migrations are involved and a mechanism is proposed.

The oxidation of 2,4-dimethylaniline has been studied by Holland *et al.*:[1] one of the products was identified as 2,5-dimethyl-*p*-benzoquinonebis-(2′,4′-dimethyl)anil (1) in which a Me group had migrated from the 4-position to the 5-position on the quinonoid ring. In order to investigate whether this phenomenon is restricted to 2,4-dimethylaniline or is of more general significance, several related aromatic amines have been prepared and oxidised by the peroxidase system. These are 2,3,4-trimethylaniline, 4-isopropyl-2,6-dimethylaniline, 4-t-butyl-2,6-dimethylaniline and 4-cyclohexyl-2,6-dimethylaniline.

Three pathways are open to these amines upon oxidation:

(i) participation of the *ortho*-Me groups,
(ii) migration of the 4-alkyl group or
(iii) elimination of the 4-alkyl group.

ortho-Me group participation has never been observed during the course of a peroxidase oxidation and so pathway (i) can be ruled out with some degree of certainty.

Mesidine is oxidised almost exclusively to 2,6-dimethyl-*p*-benzoquinone-4-(2′,4′,6′-trimethyl)anil[2] (2), a Me group having been eliminated as formaldehyde.[3] The mechanism has been studied in detail by Saunders and Wodak;[4] it has been suggested that loss of a proton from the 4-Me group of mesidine after initial oxidation gives rise to the quinone imine methide (3) to which is added a molecule of water, giving 4-amino-3,5-dimethylbenzyl alcohol (4). Addition of this compound to the peroxidase oxidation of mesidine has been shown to increase the yield of 2. Further oxidation, nucleophilic attack by a second molecule of mesidine, elimination of formaldehyde and hydrolysis gives rise to the final product (Fig 1).

4-t-Butyl-2,6-dimethylaniline, unlike mesidine, has no α-hydrogen at the 4-position, and straightforward hydroxylation at this point is therefore impossible. Thus if a t-Bu group is eliminated, it must be by a hitherto unobserved process. However, in each of the other amines studied, hydroxylation can take place and the alkyl group can be eliminated in the normal way.

The last alternative, migration of the 4-alkyl group could take place in each case, as was observed in the oxidation of 2,4-dimethylaniline.

The oxidations of these amines were carried out under similar conditions to that of mesidine.[1] In each case, the insoluble oxidation products were filtered off at the end of the reaction and the residual solution was examined in order to identify any water soluble fragments from the oxidations.

The oxidation product from 2,3,4-trimethylaniline, a red solid, was chromatographed on silica and two compounds were isolated in low yield. The first, a yellow crystalline solid, was identified by microanalytical and spectral data as 2,3,4,2′,3′,4′-hexamethylazobenzene (5). The compound was synthesised by the method of Chapman and Saunders.[2] The second compound, a red solid was identified as 2,3,5-trimethyl-*p*-benzoquinone-4-(2′,3′,4′-trimethyl)anil (6) by synthetic, spectral, degradative and microanalytical methods. The compound is obviously produced by migration of a Me group in the manner described in Fig 2.

4-Isopropyl-2,6-dimethylaniline was oxidised in a similar manner and a deep red oil was isolated. The oil was a single compound, 2,6-dimethyl-*p*-benzoquinone-4-(4′-isopropyl-2′,6′-dimethyl)anil (7), corresponding to the oxidation product from mesidine. Acetone was identified in the filtrate as its 2,4-dinitrophenylhydrazone. No compounds containing a migrated isopropyl group were found, and no azo compounds were isolated.

Similar conditions were employed for the oxidation of 4-t-butyl-2,6-dimethylaniline, and a deep purple solid was obtained at the end of the reaction. Chromatography showed it to be a mixture of two compounds. The major component was 2,6-dimethyl-*p*-benzoquinone-4-(4′-t-butyl-2′,6′-dimethyl) anil (8), formed in 86% yield by elimination of the

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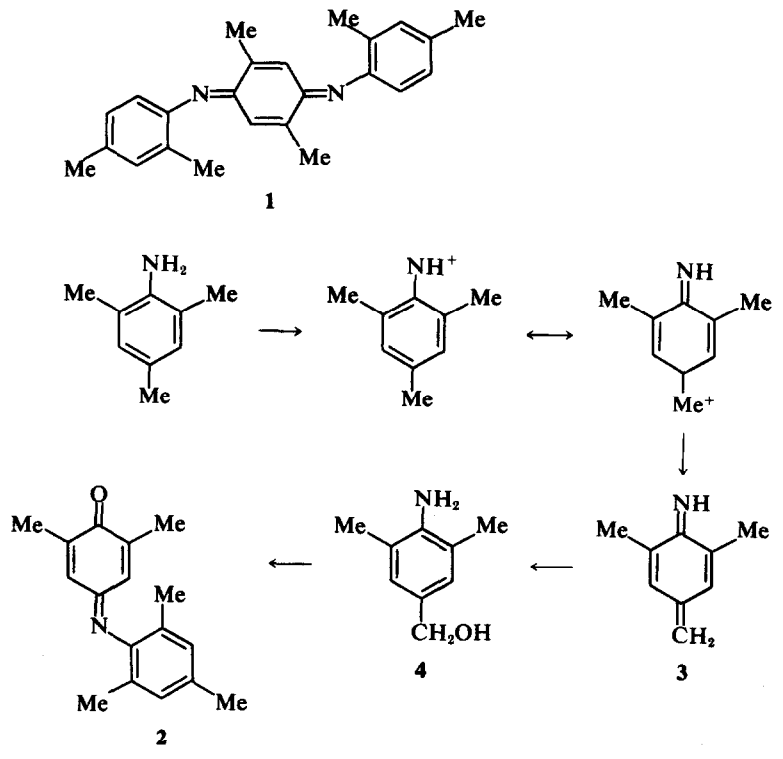


Fig 1.

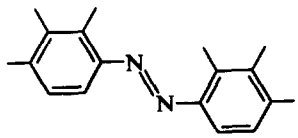
4-t-Bu group. The fate of the eliminated t-Bu group has not yet been ascertained. No carbonyl compounds could be identified in the filtrate. The second component, in only 6% yield, was identified as 3-t-butyl-2,6-dimethyl-*p*-benzoquinone-4-(4′-t-butyl-2′,6′-dimethyl)anil (**9**), formed by migration of the t-Bu group as in Fig 2.

4-Cyclohexyl-2,6-dimethylaniline was oxidised in dilute solution and a red-purple solid was filtered off at the end of reaction. Cyclohexanone was identified in the filtrate as its 2,4-dinitrophenylhydrazone. The solid was chromatographed on silica; three components were separated and identified. The main component, a purple crystalline solid, was obtained in 64% yield and was 3-cyclohexyl-2,6-dimethyl-*p*-benzoquinone-4-(4′-cyclohexyl-2′,6′-dimethyl)anil (**10**), formed by migration of the cyclohexyl group. A smaller quantity (34%) of a red crystalline solid was shown to be 2,6-dimethyl-*p*-benzoquinone-4-(4′-cyclohexyl-2′,6′-dimethyl)anil (**11**). The loss of a cyclohexyl group is required for the production of this compound and this accounts for the cyclohexanone in solution. A small quantity of a third compound (1·5%) was also isolated. This was shown to be 4,4′-dicyclohexyl-2,6,2′,6′-tetramethylazobenzene (**12**) by synthesis from the parent amine by the method of Chapman and Saunders.[2]

The small or zero yields of azo-compounds obtained in these reactions suggest that the dimerisation of 4-alkyl-2,6-dimethylanilino radicals does not take place to any significant extent. This bears out previous work[1,2] on the oxidation of 4-substituted 2,6-dimethylanilines. The main reason for these small yields is that the radical (**13a**) would be expected to be readily oxidised to the cation (**13b**), the intermediate proposed in the mechanisms for peroxidase oxidations (Figs 1 and 2).

A mechanism similar to that of Holland *et al.* for the oxidation of these amines to mono-anils containing a migrated alkyl group can be put forward. Oxidative loss of a proton and an electron yields the radical (**13a**), which on further oxidation, and loss of a proton gives the cation (**13b**). Mesomerism, followed by nucleophilic attack of a second molecule of amine gives the non-aromatic system **14**. Migration of the alkyl group followed by loss of a proton gives the aminodiphenylamine **15**, which undergoes further enzymic oxidation and hydrolysis to the quinone anil **16** (Fig 2).

These experiments *in vitro* show that transalkylations by peroxidase are more widespread than was originally thought, and this may be a major function of the enzyme *in vivo*. The isolation of acetone and cyclohexanone from the oxidations of 4-isopropyl-2,6-dimethylaniline and 4-cyclohexyl-2,6-dimethyl-



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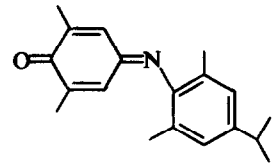

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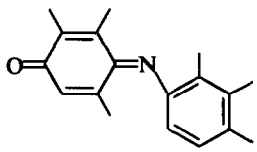

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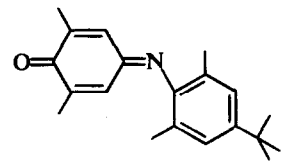

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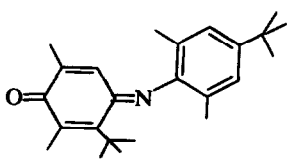

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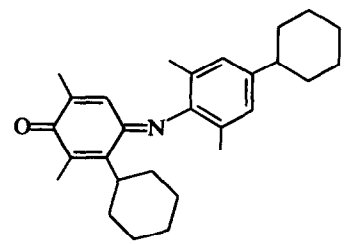

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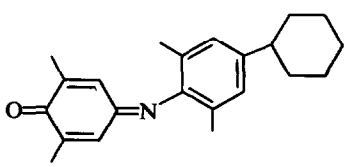

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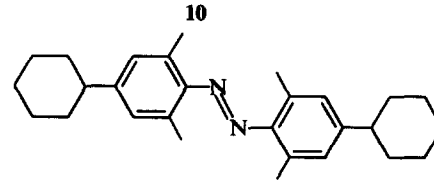

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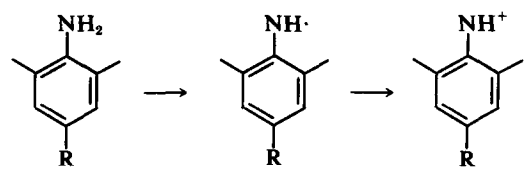
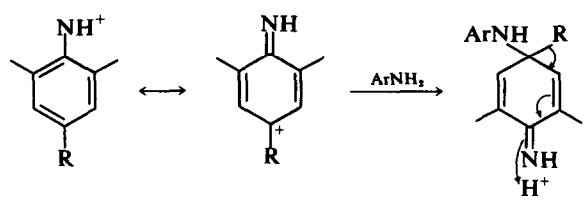


13a 13b



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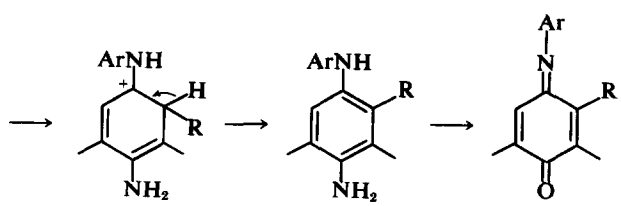

15 16

Fig 2.

anilines respectively further confirms the mechanism of Saunders and Wodak[4] for the elimination of alkyl groups by peroxidase.

EXPERIMENTAL

Materials and equipment. The enzyme preparation was a purified horseradish peroxidase supplied by Seravac Laboratories Ltd., and had RZ O. 3. The H_2O_2 soln was approx 20 volume. Column chromatography was carried out on Spence 'H' alumina (100–200 mesh) or Mallinckrodt 'SilicAR CC-7' (100–200 mesh). No activation was necessary for the latter, but the former was activated by heating to 350°. TLC was carried out on plates prepared with Merck alumina 'G' or Merck silica GF_{254} nach Stahl. The amines used were distilled under reduced pressure or recrystallised immediately before use.

2,3,4-*Trimethylaniline*. 2,3,4-Trimethylnitrobenzene was prepared by nitration of 1,2,3-trimethylbenzene,[5] and was reduced according to the method of Mann and Saunders.[6] It was obtained as a yellow oil, b.p. 128°/16 mm (lit. 136°/20 mm). *NMR spectrum* (5% soln in CCl_4) showed a singlet (3 Me protons) at 8·00τ; broad singlet (6 Me protons) at 7·87τ; perturbed doublet (1 aromatic proton) at 3·70τ ($J = 15$ c/s) and a perturbed doublet (1 aromatic proton) at 3·27τ ($J = 15$ c/s).

The oxidation of 2,3,4-*trimethylaniline*. 2,3,4-Trimethylaniline (1·1 g) was dissolved in glacial AcOH (2 ml) and added to pH 4·8 AcOH/AcONa buffer (500 ml). No coloration was produced on the addition of H_2O_2 soln (2 ml). Peroxidase (2 mg) was added, producing an immediate purple colour in the soln, rapidly depositing a red solid. Similar additions of peroxidase and H_2O_2 were made at 2 hr intervals during 12 hr. The red amorphous oxidation product (1·05 g) was filtered off and dried.

Examination of the precipitate. The red solid, dissolved in light petroleum (b.p. 40–60°) was chromatographed on alumina using the same solvent as eluent. A single yellow band was eluted, the rest of the product remaining on the column. Evaporation of the eluate gave a yellow solid which was crystallised (EtOH) as yellow needles (1·5 mg) of 2,3,4,2',3',4'-*hexamethylazobenzene*, m.p. 185°. (Found: C, 81·35; H, 8·6; N, 10·8. $C_{18}H_{22}N_2$ requires: C, 81·2; H, 8·3; N, 10·5%). *Mass spectrum: m/e* 267, (% of base peak 6); 266, (40); 265, (12); 251, (6); 208, (4); 147, (5); 120, (12); 119, (100); 117, (6); 91, (12); 77, (9) M.W. = 266. *NMR spectrum* (5% in $CDCl_3$) showed a singlet (6 Me protons) at 7·72τ; singlet (6 Me protons) at 7·32τ; singlet (6 Me protons) at 7·32τ; perturbed doublet (2 aromatic protons) at 2·94τ (J = 16 c/s); and perturbed doublet (2 aromatic protons) at 2·56τ (J = 16 c/s). *IR spectrum* (KBr disc) showed prominent bands at 2930, 1595, 1460, 1440, 1375, 1240, 1210, 1170, 1065, 1015, 830, 750, 740 cm^{-1}. *UV spectrum* (EtOH, 95%): λ_{max} 242 mμ ($\log_{10}\epsilon$ 4·103); 345, (4·209); 445, (3·062); λ_{min} 228 mμ, (3·937), 272, (3·450); 408, (2·881).

Further elution of the column with chloroform (10%) in light petroleum (b.p. 40–60°) developed a red band which was eluted and evaporated to give a red solid (21 mg). Recrystallisation (EtOH) gave red needles of 2,3,5-*trimethyl*-p-*benzoquinone*-4-(2',3',4'-*trimethyl*)*anil* m.p. 121°. (Found: C, 80·6; H, 8·0; N, 5·0. $C_{18}H_{21}NO$ requires: C, 80·9; H, 7·9; N, 5·2%). *Mass spectrum: m/e* 270, (% of base peak 13); 269, (12); 268, (11); 267, (80); 266, (11); 253, (20); 252, (89); 238, (26); 224, (15); 169, (24); 134, (11); 92, (15); 92, (100). M.W. = 267. *NMR spectrum* (5% soln in CCl_4) showed doublet (3 Me protons) at 8·08τ (J = 2 c/s); broad singlet (6 Me protons) at 7·92τ; singlet (3 Me protons) at 7·75τ; broad singlet (6 Me protons) at 7·59τ; perturbed doublet (1 aromatic proton) at 3·74τ (J = 14 c/s); doublet (1 aromatic proton) at 3·25τ (J = 2 c/s) and perturbed doublet (1 aromatic proton) at 3·02τ (J = 14 c/s). *IR spectrum* (KBr disc) showed prominent bands at 2940, 1630, 1610, 1485, 1445, 1385, 1350, 1280, 1260, 1210, 1180, 1120, 1040, 1000, 900, 855, 830 cm^{-1}. *UV spectrum* (EtOH): λ_{max} 276 mμ ($\log_{10}\epsilon$ 4·288); 443 (3·244); λ_{min} 240 mμ, (3·650); 389 (2·613).

Hydrolysis of the anil. The anil (50 mg) was heated under reflux with dil H_2SO_4 (1·5M, 20 ml) for 1 hr and the resulting soln was steam-distilled. The yellow distillate was extracted (ether) to give yellow needles m.p. 32° (lit. m.p. 2,3,5-trimethyl-*p*-benzoquinone, 32°). The soln remaining after steam-distillation was made alkaline (10% NaOH) and extracted (ether, 20 ml). The extract was evaporated to 5 ml. TLC showed a single spot, R_f identical with that of 2,3,4-trimethylaniline.

Condensation of 2,3,4-*trimethylaniline with* 2,3,5-*trimethyl*-p-*benzoquinone*. 2,3,5-Trimethyl-*p*-benzoquinone (150 mg) and 2,3,4-trimethylaniline (50 mg) were dissolved in 50% aqueous Me_2CO (7 ml) containing glacial AcOH (0·2 g). The soln became dark red and was heated under reflux for 60 hr. The mixture was made neutral ($NaHCO_3$ soln), and extracted (ether). The extracts were dried (Na_2SO_4) and evaporated to 1 ml. The deep red soln was subjected to a preparative TLC on silica using 10% Me_2CO in light petroleum (b.p. 60–80°) as eluent. The fast-running red band was extracted and recrystallised to give red needles m.p. 120°, not depressed on admixture with the red compound from the enzymic oxidation. The spectra of this compound were identical with those of the enzyme product.

4-*Isopropyl*-2,6-*dimethylaniline*. 5-Isopropyl-1,3-dimethylbenzene (110 g) and Ac_2O (140 ml) were cooled to 5°. A mixture of fuming HNO_3 (50 ml), Ac_2O (50 ml) and glacial AcOH (50 ml) was added with stirring over ½ hr, the temp being maintained at 15–20°. The mixture was subsequently stirred at 25° for 3 hr to complete the nitration. The product was poured onto crushed ice (250 g), the soln was made alkaline (Na_2CO_3) and the supernatant liquid extracted with ether. The extract was washed with water, dried (Na_2SO_4) and evaporated. The residue, a yellow oil (136 g) had b.p. 250°. GLC analysis showed two components, each of which had a similar retention time to that of nitromesitylene. These components proved inseparable by fractional distillation.

This oil (75 g) was reduced by granulated tin and hydrochloric acid. A pale yellow oil (20 g) was obtained which GLC analysis showed to contain two components. The major component, 4-*isopropyl*-2,6-*dimethylaniline* was extracted by preparative GLC (310 mg from 1 g of oil, overall yield 13%) as a colourless liquid. (Found: C, 80·7; H, 10·7; N, 8·7. $C_{11}H_{17}N$ requires: C, 80·9; H, 10·5; N, 8·6%). *IR spectrum* showed prominent bands at 3420, 3350, 2930, 2900, 2850, 1630, 1605, 1525, 1490, 1460, 1440, 1380, 1360, 1330, 1310, 1290, 1260, 1245, 1180, 1140, 1000, 940, 875, 737 cm^{-1}. *NMR spectrum* (5% in CCl_4) showed a doublet (6 Me protons) at 8·80τ (J = 7 c/s); singlet (6 Me protons) at 7·85τ; septuplet (1 methine proton) at 7·24τ; (J = 7 c/s); broad singlet (2 N—H protons) at 6·70τ and singlet (2 aromatic protons) at 3·20τ.

The oxidation of 4-*isopropyl*-2,6-*dimethylaniline*. 4-Isopropyl-2,6-dimethylaniline (150 mg) was oxidised by peroxidase in the usual manner. A deep purple coloration was produced immediately and after 1 hr a deep purple oil

slowly formed on the surface of the soln. The oil was removed after 10 hr; further addition of peroxidase-H_2O_2 produced no coloration in the residual soln. This soln was steam-distilled; 10 ml of distillate were collected. Excess of aqueous 2,4-dinitrophenylhydrazine was added and after 2 min a yellow ppt formed. This was filtered off, washed, dried and recrystallised (EtOH). Yield 10 mg (9%), m.p. and mixed m.p. with acetone 2,4-dinitrophenylhydrazone 125° (lit. 126°).

The oil from the oxidation was chromatographed on alumina, using 15% $CHCl_3$ in light petroleum, b.p. 60–80°, as eluent. One major band appeared, which on elution and evaporation gave a deep purple oil which crystallised on standing. The compound was 2,6-*dimethyl*-p-*benzoquinone*-4-(4′-*isopropyl*-2,6′-*dimethyl*)*anil* m.p. 60° (95 mg, yield 73%) (Found: C, 80·8; H, 8·5; N, 4·8. $C_{19}H_{23}NO$ requires: C, 81·1; H, 8·2; N, 5·0%). *IR spectrum* (nujol) showed prominent bands at 2950, 2910, 2870, 1650, 1620, 1580, 1570, 1480, 1440, 1380, 1325, 1315, 1225, 1215, 1185, 1165, 1135, 1040, 1025, 1005, 945, 915, 873, 788, 727 cm^{-1} and marked similarities to that of 2,6-dimethyl-*p*-benzoquinone-4-(2′,4′,6′-trimethyl)anil. *Mass spectrum: m/e* 283, (% of base peak 9); 282, (14); 281, (50); 268, (8); 267, (22); 266, (100); 252, (10); 238, (6); 224, (5); 222, (4); 208 (4). M.W. = 281. *NMR spectrum* (5% in CCl_4) showed a doublet (6 methyl protons) at 8·71τ (J = 7 c/s); broad singlet (9 methyl protons) at 8·06τ; broad singlet (3 Me protons) at 7·87τ; septuplet (1 methine proton) at 7·12τ (J = 7 c/s); broad singlet (1 aromatic proton) at 3·49τ; singlet (2 aromatic protons) at 3·01τ and broad singlet (1 aromatic proton) at 2·47τ. *UV spectrum* (EtOH): λ_{max} 276 mμ, ($\log_{10}\epsilon$ 4·486); 495, (3·121); λ_{min} 241 mμ, (3·714); 275, (2·592).

No traces of 4,4′-diisopropyl-2,6,2′,6′-tetramethylazobenzene or 3-isopropyl-2,6-dimethyl-*p*-benzoquinone-4-(4′-isopropyl-2′,6′-dimethyl)anil were found in the oxidation product.

Preparation of 4-*t-butyl*-2,6-*dimethylaniline*. 4-t-Butyl-2,6-dimethylnitrobenzene (prepared in a similar manner to 4-isopropyl-2,6-dimethylnitrobenzene) was reduced according to the method of Mann and Saunders.[6] 4-*t-Butyl*-2,6-*dimethylaniline* (79%) was obtained as a pale yellow oil b.p. 135°/15 mm. (Found: C, 81·3; H, 10·8; N, 8·0. $C_{12}H_{19}N$ requires: C, 81·3; H, 10·8; N, 7·9%). *NMR spectrum* (5% soln in CCl_4) showed a singlet (9 methyl protons) at 8·73τ; singlet (6 Me protons) at 7·99τ; broad singlet (2 N—H protons) at 6·85τ and singlet (2 aromatic protons) at 2·97τ. *IR spectrum* showed prominent bands at 3400, 3320, 2920, 2850, 1625, 1605, 1485, 1460, 1440, 1390, 1375, 1360, 1305, 1275, 1220, 1130, 1030, 1010, 1000, 935, 875, 738 cm^{-1}.

Oxidation of 4-*t-butyl*-2,6-*dimethylaniline*. 4-t-Butyl-2,6-dimethylaniline (5 g) was oxidised by peroxidase in the usual way. Within ½ hr a purple coloration was observed and after 70 hr a purple solid had formed and was collected by filtration, washed and dried (4 g). No effervescence was observed during the oxidation.

The solid was chromatographed on alumina using 15% $CHCl_3$ in light petroleum (b.p. 60–80°) as eluent. One major band was eluted and evaporated to give a purple solid. Recrystallisation (MeOH, 80%) gave 2,6-*dimethyl*-p-*benzoquinone*-4-(4′-*t-butyl*-2′,6′-*dimethyl*)*anil* (3·6 g, 86%) as purple plates, m.p. 88°. (Found: C, 81·0; H, 8·2; N, 4·9. $C_{20}H_{25}NO$ requires: C, 81·3; H, 8·5; N, 4·7%) *IR spectrum* (nujol mull) showed prominent bands at 1650, 1620, 1580, 1320, 1305, 1220, 1170, 1120, 1040, 1025, 945, 918, 875, 790, 780, 725 cm^{-1}. *Mass spectrum: m/e* 297, (% of base peak 19); 296, (16); 295, (85); 283, (4); 282, (16); 281, (21); 280, (100); 177, (15); 162, (42); 161, (5); 118, (22); 83, (15); 77, (12). M.W. = 295. *NMR spectrum* (5% in CCl_4) showed singlet (9 Me protons) at 8·64τ; broad singlet (9 Me protons) at 8·04τ; broad singlet (3 Me protons) at 7·87τ; broad singlet (1 aromatic proton) at 3·48τ; singlet (2 aromatic protons) at 2·85τ and broad singlet (1 aromatic proton) at 2·72τ. *UV spectrum* (EtOH): λ_{max} 275 mμ, ($\log_{10}\epsilon$ 4·493); 492, (3·068); λ_{min} 241 mμ, (3·716); 374, (2·551).

A second portion of 4-t-butyl-2,6-dimethylaniline (2 g) was oxidised in the usual way and the purple product isolated. Chromatography on silicic acid gave, in addition to a slow running purple band of the major oxidation product, a faster running deep purple band. This was eluted and evaporated to a deep purple solid. Recrystallisation (light petroleum, b.p. 60–80°) gave purple needles of 3-*t-butyl*-2,6-*dimethyl*-p-*benzoquinone*-4-(4′-*t-butyl*-2′,6′-*dimethyl*)*anil*, m.p. 167° (115 mg, 6%). (Found: C, 82·4, H, 9·3; N, 4·2. $C_{24}H_{33}NO$ requires: C, 82·1; H, 9·4; N, 4·0%). *IR spectrum* (nujol mull) showed prominent bands at 1645, 1580, 1570, 1300, 1260, 1235, 1215, 1180, 1125, 1065, 1040, 1030, 1000, 952, 897, 880, 827, 796, 785, 697 cm^{-1}. *NMR spectrum* (5% in soln in CCl_4) showed singlet (9 Me protons) at 8·70τ; singlet (9 Me protons) at 8·42τ; doublet (3 Me protons) at 8·16τ (J = 3 c/s); singlet (6 Me protons) at 8·08τ; singlet (3 Me protons) at 7·79τ; doublet (1 aromatic proton) at 3·65τ (J = 3 c/s) and singlet (2 aromatic protons) at 3·06τ. *Mass spectrum: m/e* 354, (% of base peak 5); 353, (23); 352, (26); 351, (100); 350, (5); 338, (5); 337, (20); 336, (70); 320, (5); 310, (5); 309, (20); 308, (35); 295, (10); 294, (40); 293, (15); 280, (10); 278, (5); 266, (5); 264, (5); 252, (5); 191, (12); 190, (75) M.W. = 351. *UV spectrum* (EtOH, 95%): λ_{max} 279 mμ, ($\log_{10}\epsilon$ 4·407); 505 mμ, (2·932); λ_{min} 243 mμ, (3·777); 390 mμ, (2·575).

Preparation of 4-*cyclohexyl*-2,6-*dimethylaniline*. 5-Cyclohexyl-1,3-dimethylbenzene was first prepared either by the action of cyclohexyl bromide on 3,5-dimethylphenyl magnesium bromide or by the action of cyclohexene and *m*-xylene in the presence of aluminium chloride. It had b.p. 140°/15 mm. (lit. 138–140°/14 mm.)

NMR spectrum (5% soln in CCl_4) showed two broad peaks (10 aliphatic protons) at 8·0–9·0; broad singlet (6 Me protons and 1 methine proton) at 7·80; singlet (3 aromatic protons) at 3·32. *IR spectrum* showed prominent bands at 3000, 2910, 2830, 1600, 1440, 1370, 1255, 1030, 835, 805, 700 cm^{-1}. *Mass spectrum: m/e* 189, (% of base peak 8); 188, (58); 174, (8); 165, (8); 146, (8); 145, (42); 134, (8); 133, (17); 132, (25); 131, (17); 120, (67); 119, (42); 106, (42); 105, (83); 91, (100); 77, (25). M.W. = 188. *UV spectrum* (EtOH) showed λ_{max} 258 mμ, ($\log_{10}\epsilon$ 2·378); 264, (2·433); 271, (2·373), λ_{min} 228 mμ, (2·104); 259, (2·375); 269, (2·318).

5-Cyclohexyl-1,3-dimethylbenzene (10 g) and Ac_2O (8·9 ml) were cooled to 5°. A mixture of fuming HNO_3 (3·3 ml), glacial AcOH (3·1 ml) and Ac_2O (3·1 ml) was added with stirring over ½ hr, maintaining the temp below 15°. The mixture was allowed to stand at 25° for 1 hr, poured into water, neutralised with Na_2CO_3aq and extracted with ether. The extracts were washed with water and dried (Na_2SO_4). After evaporation, the residue, a yellow oil, was chromatographed on silica using light petroleum (b.p. 40–60°) as eluent. A single yellow band was eluted which on evaporation gave a pale yellow oil (7 g). This was shown (NMR) to be a mixture of 4-cyclohexyl-2,6-dimethylnitrobenzene (70%) and 6-cyclo-

hexyl-2,4-dimethylnitrobenzene (30%). These compounds were not separable by chromatography or by fractional distillation.

This pale yellow oil (6 g) was reduced according to the method of Mann and Saunders.[6] The product, an oil, was chromatographed on silica, using dichloromethane as eluent. Two bands were eluted, the first on evaporation yielded 4-cyclohexyl-2,6-dimethylaniline (2 g, overall yield from the parent hydrocarbon 18%) contaminated with a small quantity of its isomer, 6-cyclohexyl-2,4-dimethylaniline. The second band consisted of pure isomer (1·2 g). The crude 4-cyclohexyl-2,6-dimethylaniline was dissolved in light petroleum (b.p. 30–40°) and cooled. White crystals of *4-cyclohexyl-2,6-dimethylaniline* were obtained (1·5 g, m.p. 40°). (Found: C, 82·7; H, 10·5; N, 6·8. $C_{14}H_{21}N$ requires: C, 82·8; H, 10·3; N, 6·9%). *NMR spectrum* (5% soln in CCl_4) showed 2 broad triplets (10 aliphatic protons) at 8·22τ and 8·68τ; broad singlet (6 Me protons, 1 methine proton) at 7·94τ; singlet (broad, 2 N—H protons) at 6·82τ and singlet (2 aromatic protons) at 3·38τ. *IR spectrum* (KBr disc) showed prominent bands at 3420, 3310, 2900, 2830, 1680, 1480, 1440, 1310, 1240, 1220, 1150, 985, 860, 735 cm^{-1}. *Mass spectrum: m/e* 205, (% of base peak 2); 204, (16); 203, (100); 202, (4); 188, (3); 161, (8); 160, (64); 147, (7); 145, (10); 134, (30). M.W. = 203. *UV spectrum* (ethanol) showed λ_{max} 235 mμ, ($\log_{10}\epsilon$ 3·851); 288, (3·152); λ_{min} 221 mμ, (3·713); 260, (2·517).

Oxidation of 4-cyclohexyl-2,6-dimethylaniline. 4-Cyclohexyl-2,6-dimethylaniline (340 mg) was dissolved in glacial AcOH and added to acetate buffer (500 ml) and oxidised by peroxidase and H_2O_2 in the usual manner. A red colour slowly formed and finally a red solid was filtered off (Hyflo). The filtrate was steam-distilled; 20 ml of distillate were collected. Excess of aqueous acidic 2,4-dinitrophenylhydrazine was added and a yellow ppt separated. This was filtered off, washed with water, dried and recrystallised (EtOH). M.p. and mixed m.p. with cyclohexanone 2,4-dinitrophenylhydrazone 164° (lit. 166°).

The red solid was extracted (Soxhlet) with chloroform. After evaporation, a red solid (307 mg) remained which was subjected to column chromatography on silica, using dichloromethane as eluent. The following bands were eluted.

(a) Bright yellow band—on evaporation gave a yellow solid (5 mg). On spraying the compound with conc H_2SO_4, a deep orange colour developed, characteristic of azobenzenes. The solid was recrystallised (EtOH) and dried to give orange-yellow needles of *4,4′-dicyclohexyl-2,6,2′,6′-tetramethylazobenzene* m.p. 165°. (Found: C, 83·6; H, 9·5; N, 6·8. $C_{28}H_{38}N_2$ requires: C, 83·6; H, 9·5; N, 7·0%). *NMR spectrum* (CCl_4) showed 2 triplets (broad, 20 aliphatic protons) at 8·20τ and 8·64τ; singlet (broad at the base, 12 Me protons, 2 methine protons) at 7·67τ and singlet (4 aromatic protons) at 3·20τ. *IR spectrum* (KBr disc) showed prominent bands at 3000–2900, 1590, 1445, 1370, 1260, 870 cm^{-1}. *Mass spectrum: m/e* 405, (% of base peak 5); 404, (9); 403, (21); 402, (47); 388, (9); 376, (3); 216, (13); 203, (17); 202, (11); 201, (14); 200, (39); 195, (11); 188, (20); 187, (100); 186, (6); 185, (5). M.W. = 402. *UV spectrum* (EtOH): λ_{max} 220 mμ, ($\log_{10}\epsilon$ 4·242); 278, (4·160); λ_{min} 245 mμ, (3·644).

(b) Deep purple band—on evaporation gave a purple solid (196 mg). The compound was recrystallised (EtOH) giving small purple crystals of *3-cyclohexyl-2,6-dimethyl-p-benzoquinone-4-(4′-cyclohexyl-2′,6′-dimethyl)anil* m.p. 188°. (Found: C, 83·4; H, 8·95; N, 3·6. $C_{28}H_{37}NO$ requires: C, 83·4; H, 9·2; N, 3·5%). *NMR spectrum* ($CDCl_3$) showed broad triplets (20 aliphatic protons) at 8·40–9·00τ; doublet (3 Me protons) at 8·16τ (J = 2 c/s); singlet (broad, 6 Me protons, 2 methine protons) at 8·10τ; singlet (3 Me protons) at 7·88τ; quadruplet (1 aromatic proton) at 3·62τ (J = 2 c/s); singlet (2 aromatic protons) at 3·10τ. *IR spectrum* (KBr disc) showed prominent bands at: 2905, 2840, 1635, 1435, 1375, 1315, 1210, 1020, 940, 910, 955 cm^{-1}. *Mass spectrum: m/e* 403, (% of base peak 11); 218 (11); 217, (88); 216, (11); 204, (11); 203, (56); 202, (11); 189, (22); 188, (22); 174, (22); 161, (11); 160, (44); 149, (55); 148, (22); 147, (100); 146, (33); 134, (34); 133, (33); 131, (22); 130, (11); 129, (11). M.W. = 403. *UV spectrum* (EtOH): λ_{max} 220 mμ, ($\log_{10}\epsilon$ 4·242); 278, (4·160); λ_{min} 245 mμ, (3·644).

(c) Slow running red band—on evaporation gave a red solid (104 mg). This compound was recrystallised from ethanol/water mixture. After drying, small red plates of *2,6-dimethyl-p-benzoquinone-4-(4′-cyclohexyl-2′,6′-dimethyl)anil* were produced, m.p. 119°. (Found C, 81·8; H, 8·6; N, 4·1. $C_{22}H_{27}NO$ requires: C, 82·2; H, 8·4; N, 4·1%). *NMR spectrum* ($CDCl_3$) showed 2 triplets (broad, 10 aliphatic protons) at 8·4–9·00τ; singlet (very broad at base, 9 Me protons, 1 methine proton) at 8·08τ; doublet (3 Me protons) at 7·91τ (J = 2 c/s); quadruplet (1 aromatic proton) at 3·58τ (J = 2 c/s); singlet (2 aromatic protons) at 3·12τ; quadruplet (1 aromatic proton) at 2·88τ (J = 2 c/s). *IR spectrum* (KBr disc) showed prominent bands at: 2915, 2860, 1620, 1565, 1435, 1250, 1210, 885, 860 cm^{-1}. *Mass spectrum: m/e* 324 (% of base peak 6); 323, (27); 322, (25); 321, (100); 320, (9); 306, (6); 292, (6); 278, (11); 252, (9); 91, (3). M.W. = 321. *UV spectrum* (EtOH): λ_{max} 275 mμ, ($\log_{10}\epsilon$ 4·510); 490, (3·079); λ_{min} 240 mμ, (3·722); 271, (2·526).

The hydrolysis of 2,6-dimethyl-p-benzoquinone-4-(4′-cyclohexyl-2′,6′-dimethyl)anil. The anil (100 mg) from the peroxidase oxidation of 4-cyclohexyl-2,6-dimethylaniline was added to dilute H_2SO_4 (1·5M, 50 ml) and heated under reflux for 1 hr. A yellow crystalline solid solidified in the condenser. This was removed and dried, m.p. and mixed m.p. with 2,6-dimethyl-*p*-benzoquinone 71° (lit. 72°). The soln remaining in the flask was extracted with ether, made alkaline with 10% NaOHaq and extracted with ether once more. The extracts were dried (Na_2SO_4) and evaporated. Analysis by TLC (silica, dichloromethane) showed a single spot R_f 0·70, identical with that of authentic 4-cyclohexyl-2,6-dimethylaniline.

The preparation of 4,4′-dicyclohexyl-2,6,2′,6′-tetramethylazobenzene. Acetic acid (1·5 ml) and PbO_2 (5 g) were added to 4-cyclohexyl-2,6-dimethylaniline (500 mg) in ether (80 ml) and the mixture was shaken for 4 hr. The solid was filtered off and the ethereal soln was shaken with Na_2CO_3aq, water and dried (Na_2SO_4). Evaporation gave a yellow solid, which, on recrystallisation (EtOH) yielded yellow needles of 4,4′-dicyclohexyl-2,6,2′,6′-tetramethylazobenzene, (412 mg, 82%) m.p. and mixed m.p. with 4,4′-dicyclohexyl-2,6,2′,6′-tetramethylazobenzene derived from the peroxidase oxidation of 4-cyclohexyl-2,6-dimethylaniline 165°.

Preparation of 2,6-dimethyl-p-benzoquinone-4-(4′-cyclohexyl-2′,6′-dimethyl)anil. 4-Cyclohexyl-2,6-dimethylaniline (200 mg) in glacial AcOH (0·5 ml) was added to 2,6-dimethyl-*p*-benzoquinone (135 mg) in 50% aqueous acetone (10 ml) and the mixture was heated under reflux for 12 hr. A deep red colour rapidly developed. The solvents were evaporated under reduced pressure leaving a

dark brown gum. This was dissolved in dichloromethane and subjected to preparative TLC on silica using light petroleum (b.p. 40–60°) and acetone (9 : 1) as eluent. The major component, a deep red band, R_f 0·45, was removed from the plate, extracted into dichloromethane, and filtered. Evaporation of the solvent gave a red solid which was recrystallised (EtOH) to give red crystals of 2,6-dimethyl-*p*-benzoquinone-4-(4′-cyclohexyl-2′,6′-dimethyl) anil, m.p. and mixed m.p. with a sample from the peroxidase oxidation of 4-cyclohexyl-2,6-dimethylaniline 119°. The spectra of the compound derived from peroxidase oxidation were identical with those of the compound obtained in the above preparation.

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