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STUDIES IN PEROXIDASE ACTION—XIX*

THE OXIDATION OF 2,4-,2,6- AND 2,4,6-SUBSTITUTED ANILINES AND AN EXAMPLE OF METHYL GROUP MIGRATION

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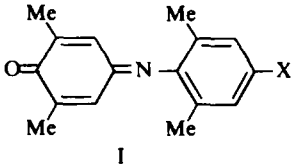
Abstract—2,6-Dimethylaniline, 2,4-dimethylaniline, 4-chloro-2,6-dimethylaniline, 4-bromo-2,6-dimethylaniline and 2,6-dimethyl-4-iodoaniline have been oxidized by the peroxidase system and the products examined in detail. 2,4-Dimethylaniline gives 2,5-dimethyl-*p*-benzoquinonebis-(2,4-dimethyl)anil thus providing the first recorded example of the migration of a methyl group during a peroxidase catalysed oxidation.

THE peroxidase catalysed oxidation of mesidine and of 2,6-dimethyl-4-methoxyaniline[2] yielded a single crystalline product in high yield in each case. With mesidine, the Me group *para* to the NH_2 group was eliminated with great ease as formaldehyde and the MeO group from the methoxyaniline appeared as MeOH. The crystalline products were substituted quinone anils (I, X = Me and OMe respectively). It should be noted by comparison that the unsubstituted aniline itself gives at least five products.



I

It is essential to know whether less highly substituted anilines can still give unique products in high yield and accordingly 2,6-dimethylaniline was oxidized with peroxidase and hydrogen peroxide. A rapid reaction took place and a purple tarry precipitate was obtained. By chromatography ill-defined polymeric substances were obtained together with a bright red crystalline compound, $C_{16}H_{17}NO$, m.p. 76–77°. Acid hydrolysis converted this into 2,6-dimethyl-*p*-benzoquinone and 2,6-dimethylaniline. Reductive acetylation of the red compound gave colourless needles, $C_{18}H_{21}NO_2$, being the O-acetyl derivative of 4-hydroxy-3,5,2′,6′-tetramethyldiphenylamine (II, X = H).

These results suggested that the red needles were 2,6-dimethyl-*p*-benzoquinone-4-(2′,6′-dimethyl)anil (I, X = H). This supposition was confirmed by two independent syntheses: (a) by condensation of 2,6-dimethylaniline and 2,6-dimethyl-*p*-benzoquinone and (b) by reaction between 2,6-dimethylaniline and 2,6-dimethyl-*p*-benzoquinone-4-toluene-*p*-sulphonimide (III).

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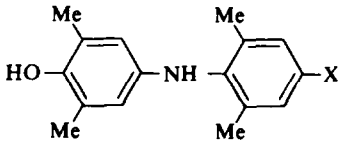
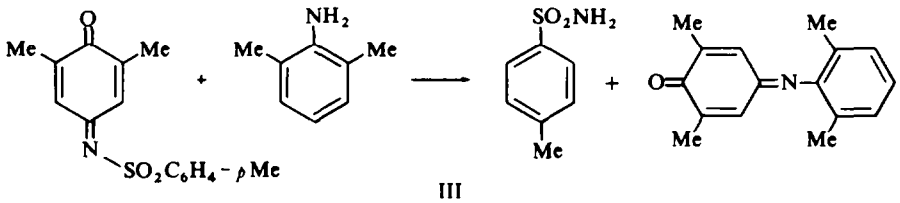


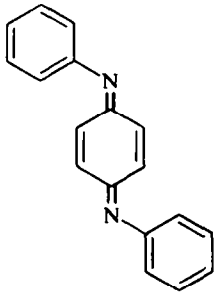
II



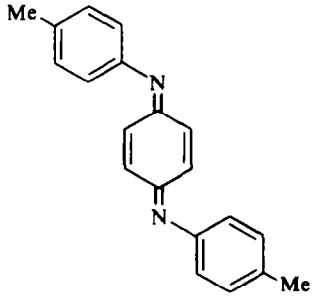
III

The peroxidase oxidation of 2,6-dimethylaniline therefore shows that the expected anil (I, X = H) was obtained, but not in high yield, indicating that additional substitution in the 4-position is necessary to prevent the formation of large quantities of ill-defined high molecular weight material. These results also show that 2,6-dimethylaniline is not a suitable donor for the quantitative determination of peroxidase activity.

The effect of substitution at positions 2 and 4 was investigated by examining the enzymic oxidation of 2,4-dimethylaniline. The reaction was rapid. Chromatography of the product yielded orange-red plates of 2,4,2′,4′-tetramethylazobenzene and a red microcrystalline material (IV), m.p. 153–155°, $C_{24}H_{26}N_2$. Molecular weight determinations agreed with this formula. The IR spectrum showed no NH bands and was similar to the spectra of *p*-benzoquinone dianil (V) and *p*-benzoquinone di-*p*-tolylimine (VI). The UV spectra of IV, V and VI were also very similar. The



V

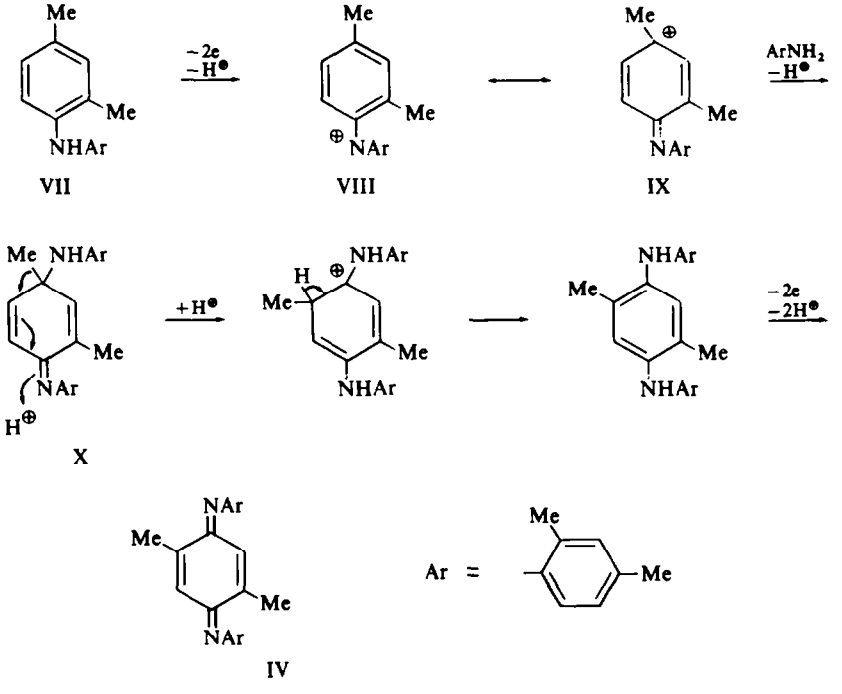


VI

hydrolysis of IV with dilute H_2SO_4 yielded rather surprisingly 2,5-dimethylbenzoquinone and 2,4-dimethylaniline. The compound IV must therefore be 2,5-dimethyl-*p*-benzoquinonebis-(2′,4′-dimethyl)anil. Mass, IR and NMR spectra support this structure.

These results show that migration of a Me group must have taken place during the oxidation of 2,4-dimethylaniline. This is the first recorded case of migration occurring in a peroxidase catalysed oxidation.

The mechanism of formation of 2,5-dimethyl-*p*-benzoquinonebis-(2′,4′-dimethyl) anil is believed to proceed via 2,4,2′,4′-tetramethyldiphenylamine, (VII), produced from two molecules of 2,4-dimethylaniline with the elimination of ammonia in the manner observed in the oxidation of *p*-toluidine.[3] Enzymic oxidation of the diphenylamine gives rise to the diarylnitrenium ion VIII, a mesomer of which (IX) undergoes nucleophilic attack by a molecule of 2,4-dimethylaniline to give the non-aromatic system X. Me group migration to the 5 position of the non-aromatic ring, (the β carbon atom of an αβ unsaturated ketone anil), followed by rearomatization and oxidation gives the observed product IV. Similar Me group migrations have been recorded in the action of acid on arylhydroxylamines,[4] and in the peroxytrifluoroacetic acid oxidation of alkylated phenols.[5]



The enzymic oxidation of 4-chloro-2,6-dimethylaniline was next examined. In unbuffered solution the oxidation was incomplete and accompanied by a fall in pH. When the reaction was carried out in acetate buffer at pH 4·5 a red crystalline solid, m.p. 107·5–108·5°, $C_{16}H_{16}ClNO$ was obtained. Reductive acetylation gave colourless needles, $C_{18}H_{20}ClNO_2$. The red compound was hydrolysed by acid to 2,6-dimethyl-*p*-benzoquinone and 4-chloro-2,6-dimethylaniline. The IR spectrum of the red compound showed absence of NH and OH bands and was similar to that of 2,6-dimethyl-*p*-benzoquinone-4-(2′,4′,6′-trimethyl)anil. Light absorption spectra were also similar. The red oxidation product was therefore 2,6-dimethyl-*p*-benzoquinone-4-(4′-chloro-2,6-dimethyl)anil, (I, X = Cl). This structure was confirmed by two independent syntheses: (a) By the condensation of 2,6-dimethyl-*p*-benzoquinone and

4-chloro-2,6-dimethylaniline. (b) By the reaction between 4-chloro-2,6-dimethylaniline and 2,6-dimethyl-*p*-benzoquinone-4-toluene-*p*-sulphonimide. In each case red needles were obtained identical in every respect with the enzymic oxidation product. Examination of the aqueous filtrate showed the presence of chloride ions and quantitative measurements showed 95% Cl^- assuming I(X = Cl) to be the only organic product. These results show conclusively that elimination in the *para* position has taken place and confirms the fact that 2,4,6-trisubstituted anilines yield, in general, a single product. Furthermore 4-chloro-2,6-dimethylaniline warrants investigation as a suitable donor in the quantitative measurement of peroxidase activity.

4-Bromo-2,6-dimethylaniline similarly gave red crystalline 2,6-dimethyl-*p*-benzoquinone-(4'-bromo-2,'6'-dimethyl)anil (I, X = Br) in good yield by peroxidatic oxidation. Two chemical syntheses, reductive acetylation, and spectra confirmed the structure. Bromide ion was produced in 85% yield.

4-Iodo-2,6-dimethylaniline, not previously described, gave dark red crystals of 2,6-dimethyl-*p*-benzoquinone-4-(4'-iodo-2',6'-dimethyl)anil. The filtrate did not contain I^- but only free iodine. The iododimethylaniline, since it gives free iodine, is a suitable iodine donor in transiodination reactions, under peroxidase conditions, as demonstrated by Saunders and Stark.[6]

EXPERIMENTAL

Oxidation of 2,6-dimethylaniline

2,6-Dimethylaniline (5 g distilled under N_2, b.p. 120°/31 mm) was dissolved in distilled water (1 l.) containing sufficient glacial AcOH to give pH 4·5. No coloration was produced on the addition of H_2O_2 soln (2 ml 20 vol). A peroxidase preparation (2 ml P.N. 50,) from turnips produced an immediate violet coloration and a purple ppt separated. Similar additions of enzyme and H_2O_2 soln were made at 2½ hr intervals for 36 hr. The purpose amorphous oxidation product (3·1 g) was filtered off and dried.

Examination of the precipitate. The oxidation product (3·1 g) was extracted (Soxhlet) with light petroleum (b.p. 40–60°, 2 × 150 ml) until the extract was colourless. Evaporation of the combined extracts gave a black glass (1·88 g). The tarry residue in the thimble could be extracted only with EtOAc and was not further examined.

The black glass, dissolved in light petroleum (b.p. 40–60°, 20 ml) was chromatographed on an alumina column and developed with the same solvent. The following bands appeared:

(i) fast-moving bright red; (ii) much slower-moving diffuse bright blue; (iii) narrow slower-moving purple.

At the top of the column were extremely slow moving bands which were not further investigated.

Eluate from band (i). Evaporation gave bright red needles of 2,6-*dimethyl*-p-*benzoquinone*-4-(2',6'-*dimethyl*)*anil*, (I, X = H) (515 mg) m.p. 76–7°. After sublimation at 0·1 mm m.p. 77°. (Found: C, 80·6; H, 7·15; N, 5·75. $C_{16}H_{17}NO$ requires: C, 80·4; H, 7·35; N, 5·85%).

Hydrolysis. The anil (180 mg) was heated under reflux with dil H_2SO_4 (10 ml, 10%) during 40 min. A pale yellow solid separated in the condenser, and was purified by sublimation (60°/0·1 mm) giving yellow plates, m.p. 72° not depressed on admixture with 2,6-dimethyl-*p*-benzoquinone, m.p. 73°.

The acid soln was poured into 30% NaOH aq soln (20 ml) and benzoyl chloride (0·25 ml) added. After standing overnight the solid was filtered off and recrystallized from aqueous MeOH to give colourless needles, m.p. 167°. (Lit. m.p. N-benzoyl-2,6-dimethylaniline, 165°).

Reductive acetylation of the anil. The compound (100 mg) was reductively acetylated by heating under reflux with Zn dust (500 mg), Ac_2O (7 ml), and pyridine (0·2 ml) during 20 min. The excess of Zn was filtered off and the filtrate poured into ice (ca. 50 g). After standing overnight the light brown solid was filtered off and recrystallized from aqueous AcOH to give colourless needles of O-*acetyl* derivative of 4-*hydroxy*-3,5,2',6'-*tetramethyldiphenylamine* (79 mg) m.p. 128·5–130. (Found: N, 5·2. $C_{18}H_{21}NO_2$ requires: N, 4·95%).

Synthesis of 2,6-dimethyl-p-benzoquinone-4-(2',6'-dimethyl)anil

(i) *Condensation of 2,6-dimethyl-p-benzoquinone and 2,6-dimethylaniline.* To 2,6-dimethyl-*p*-benzo-

quinone (194 mg) dissolved in 50% aqueous Me_2CO (7 ml) was added 2,6-dimethylaniline (160 mg) dissolved in glacial AcOH (0·5 ml). The mixture immediately became dark red and was heated under reflux for 60 hr. The solvent was removed under reduced press and excess of quinone sublimed off. The dark brown residue was dissolved in light petroleum (b.p. 40–60°, 10 ml) and chromatographed on alumina. The eluate from the single, fast-running red band, on evaporation gave bright red needles, (144 mg) m.p. 77° not depressed on admixture with the red compound from the enzymic oxidation.

(ii) *Condensation of 2,6-dimethylaniline and 2,6-dimethyl-p-benzoquinone-4-toluene-p-sulphonimide.* To 2,6-dimethyl-*p*-benzoquinone-4-toluene-*p*-sulphonimide (300 mg) dissolved in dioxan (6 ml) was added 2,6-dimethylaniline (140 mg) dissolved in glacial AcOH (0·5 ml). After heating to 100° for 5 min, the solvent was removed under reduced press to give a dark red glass. This was treated with light petroleum (b.p. 40–60°, 10 ml) and the dark red soln filtered. The white solid, m.p. 137°, was characterized as toluene-*p*-sulphonamide. The filtrate was chromatographed on alumina and the light petroleum eluate of the single red band gave bright red needles (210 mg) m.p. 77° not depressed on admixture with the red enzymic product or the red compound from (i) above.

Band (ii). The bright blue band (ii) was eluted with a mixture of light petroleum (b.p. 40–60°) and benzene (1:1). Evaporation gave black crystals (115 mg) m.p. 123–137°. The solid was dissolved in 40–60 petroleum–benzene mixture (25% benzene) and rechromatographed on alumina. Multiple bands appeared from which 3 fractions of black micro-crystalline material were obtained which melted in the range 130–160°. M (Rast) 640; 820, 900. Further purifications by chromatography and fractional crystallization failed to yield pure material.

Band (iii). This was eluted with benzene. Evaporation of the eluate gave violet micro crystals (300 mg) m.p. 300°, M (Rast) ca. 1000. Further purification by chromatography on alumina showed the material to be a complex mixture of highly coloured compounds and was not further examined.

Oxidation of 2,4-dimethylaniline

2,4-Dimethylaniline was oxidized in a similar manner to that described for 2,6-dimethylaniline. A dark red colouration and finally a dark red ppt appeared. The solid product was filtered off with the aid of Hiflo (4 g) and dried.

Examination of the precipitate. The ppt was extracted (Soxhlet) with light petroleum (b.p. 40–60°, 2 × 150 ml) until the extract and Hiflo were colourless. Evaporation of the combined petroleum extracts gave a dark red glass (1·96 g), this was dissolved in the minimum volume of light petroleum (b.p. 40–60°) and chromatographed on alumina. After development with the same solvent, the following bands appeared:

(i) fast-running pale yellow; (ii) slower-running bright red; (iii) much slower-running brown; (iv) purple, which remained at the top of the column.

The eluates of bands (i) and (ii) were collected separately, bands (iii) and (iv) were removed from the column by eluting with benzene containing 10% EtOH and the combined eluate evaporated and subjected to further chromatography.

Band (i). The eluate on evaporation gave orange-red plates (65 mg) m.p. 121–25°, recrystallisation (EtOH) gave orange-red plates, m.p. 127–29°, (lit., 2,4,2',4'-tetramethylazobenzene: m.p. 129°). (Found: C, 80·7; H, 7·8; N, 11·6. Calc. for $C_{16}H_{16}N_2$: C, 80·6; H, 7·6; N, 11·8%).

Band (ii). Evaporation of the eluate gave dark red needles (160 mg), m.p. 153–5°, recrystallisation (EtOH) gave dark red needles, of *2,5-dimethyl-p-benzoquinonebis-(2',4'-dimethyl)anil* (IV), m.p. 153–5°. (Found: C, 84·0; H, 7·6; N, 8·1. $C_{24}H_{26}N_2$ requires: C, 84·2; H, 7·6; N, 8·2%). *Mass spectrum m/e* 346, (% of base peak 35); 345, (100); 344, (22); 343, (98); 342, (20); 328, (15); 327, (60); 326, (10); 325, (10); 312, (10); 311, (60); 310, (40); 309, (10). *NMR spectrum* (5% soln in CCl_4) showed a singlet (6 Me protons) at 7·94 τ; broad singlet (6 Me protons) at 7·87 τ; singlet (6 Me protons) at 7·67 τ and a complex structure containing 4 broad peaks (8 aromatic protons) at 2·96–3·59 τ. *IR spectrum* (nujol) showed prominent bands at 1590, 1570, 1281, 1160, 1035, 1003, 889, 870, 817, 796, 762 and 717 cm^{-1}. *UV spectrum* (EtOH, 95%): λ_{max} 302 mμ, ($\log_{10} \varepsilon$ 4·387); 465, (3·768); λ_{min} 244 mμ, (3·944); 382, (3·275); $\lambda_{inflection}$ 227 mμ, (4·098).

Hydrolysis. The compound IV (75 mg) was heated under reflux for 3 hr with dil H_2SO_4 (3N, 50 ml) and the soln steam-distilled. The distillatte was extracted (ether) and the extract washed, dried and evaporated. The yellow brown residue was sublimed (90°/15 mm) to orange prisms (9 mg, 30%) m.p. 124°, (lit. m.p. 2,5-dimethylbenzoquinone 125°). (Found: C, 70·4; H, 6·1. Calc. for $C_8H_8O_2$: C, 70·6; H, 5·9%).

The residue after steam-distillation was made alkaline (NaOH) and shaken with benzoyl chloride (100 mg) for ½ hr. The product was filtered off, washed and dried. Vacuum sublimation (150°/15 mm) gave colourless needles m.p. 194° (57 mg, 58%), (lit. m.p. N-benzoyl-2,4-dimethylaniline 192°).

Quinone dianil. Prepared from N,N'-diphenyl-*p*-phenylenediamine. Recrystallized (EtOH) as red needles: m.p. 179° (Lit. 176–180°) UV spectrum (95% EtOH): λ_{max} 304 mμ, ($\log_{10}\varepsilon$ 4·429); 443, (3·900); λ_{min} 238 mμ, (3·832); 360, (3·302). *IR spectrum* (nujol) showed prominent bands at 1581, 1321, 1210, 1170, 1115, 1075, 952, 912, 866, 857, 784, 728 and 697 cm^{-1}.

Quinone di-p-tolylimine. N,N'-Di-*p*-tolyl-*p*-phenylene diamine (2·0 g) in benzene (75 ml) and yellow mercuric oxide (4·0 g) were heated under reflux, with stirring, for 8 hr, cooled, filtered, and the filtrate evaporated. The residue was recrystallised (EtOH) to give red needles (1·7 g), m.p. 123° (lit. 123°). *UV spectrum* (95% EtOH): λ_{max} 223 mμ, ($\log_{10}\varepsilon$ 4·087); 270, (4·210); 310 (4·440); 464–5, (4·065). λ_{min} 218 mμ, (4·076); 240, (3·890); 277, (4·203); 368, (3·320). *IR spectrum* (nujol) showed prominent bands at 1575, 1315, 1212, 1102, 861, 833, 823, 787 and 738 cm^{-1}.

Preparation of 4-chloro-2,6-dimethylaniline

The amine was prepared by the method of Dadswell and Kenner,[7] and was recrystallized from EtOH–ether mixture. 4-Chloro-2,6-dimethylaniline began to sublime at 155°, without melting. (Found: C, 50·1; H, 5·9; N, 7·3. Calc. for $C_8H_{11}Cl_2N$: C, 50·0; H, 5·8; N, 7·3%).

IR spectrum (nujol) showed prominent bands at 3409, 3316, 1632, 1595, 1231, 1031, 887, 857 and 792 cm^{-1}.

A portion of the hydrochloride was treated with dil aqueous NH_3 yielding the free base, m.p. 42°. (Lit. 42°). Paper chromatography of the free base in the amyl alcohol, MeOH, benzene, 2N HCl system showed a single spot R_f 0·7.

Oxidation of 4-chloro-2,6-dimethylaniline

4-Chloro-2,6-dimethylaniline (1·9 g) was added to an NaOAc–AcOH buffer (1 l., 0·5 M, pH 4·5). H_2O_2 (2 ml, 20 vol) was added, no coloration being observed. The addition of peroxidase (2 ml P.N.50) produced immediately a transient purple coloration which soon gave a bright red ppt. Peroxidase and H_2O_2 solns were added at 12 hr intervals until no further coloration was produced; Hiflo (2 g) was then added and the ppt filtered off. The filtrate had pH 4·5.

Examination of the solid oxidation product

The red product absorbed on Hiflo was extracted (Soxhlet) with light petroleum (b.p. 60–80°, 4 × 75 ml), until the Hiflo was colourless. Removal of the solvent from the extracts yielded a red glass (1·15 g); this was dissolved in 40–60° light petroleum, 5% benzene mixture (10 ml), chromatographed on alumina, developed with the same solvent mixture. After development, three coloured bands were observed:

(i) fast running dark-red; (ii) very slow running faint orange; (iii) narrow purple band which remained at the top of the column.

The eluate from band (i) was evaporated to dark red needles (1·04 g) m.p. 105–7°. Recrystallization (aqueous EtOH) gave dark red needles of 2,6-*dimethyl*-p-*benzoquinone*-4-(4'-*chloro*-2',6'-*dimethyl*)anil (900 mg) m.p. 107·5–108·5. (Found: C, 70·6; H, 6·0; N, 5·0. $C_{16}H_{16}ClNO$ requires: C, 70·2; H, 5·9; N, 5·1%). *UV spectrum* (95% EtOH); λ_{max} 275 mμ;, ($\log_{10}\varepsilon$ 4·49); 472–8, (3·03), λ_{min} 241 mμ, (3·65); 370, (2·42). *IR spectrum* (Nujol) showed prominent bands at 1645, 1620, 1620, 1320, 1211, 1161, 1037, 1022, 941, 925, 882, 859, 789, 774 and 715 cm^{-1}.

Hydrolysis of the chloroanil. The compound (200 mg) was heated under reflux for 20 min with H_2SO_4 (3N, 10 ml). Pale yellow crystals separated in the condenser which was dissolved with Me_2CO (5 ml). Removal of Me_2CO under reduced press, followed by recrystallization (light petroleum (b.p. 40–60°)) gave yellow needles m.p. 70°, not depressed on admixture with 2,6-dimethyl-*p*-benzoquinone, m.p. 72°.

The acid soln was made alkaline and extracted with ether (3 × 15 ml); the combined extracts dried (KOH) and evaporated to dryness under reduced press. The dark brown residue was purified by vacuum sublimation, yielding a white crystalline solid, m.p. 41°, not depressed on admixture with 4-chloro-2,6-dimethylaniline, m.p. 41°.

Reductive acetylation of the chloroanil. The compound (90 mg) was heated under reflux for 20 min with Ac_2O (7·5 ml), Zn dust (0·5 g) and pyridine (0·2 ml). The mixture became colourless immediately. The excess of Zn dust was removed by filtration, the filtrate evaporated to small volume, dissolved in EtOH (5 ml) and poured onto ice. After standing overnight, the solid product was filtered off and recrystallized (MeOH), yielding colourless needles of the O-*acetyl* derivative of 4-*hydroxy*-4'-*chloro*-3,5,2',6' *tetramethyl-diphenylamine* (50 mg) m.p. 140–41°. (Found: C, 68·2; H, 6·5; N, 4·6. $C_{18}H_{20}ClNO_2$ requires: C, 68·0; H, 6·4; N, 4·4%).

Condensation of 4-chloro-2,6-dimethylaniline and 2:6-dimethyl-p-benzoquinone

2,6-Dimethyl-*p*-benzoquinone (235 mg) and 4-chloro-2,6-dimethylaniline (213 mg) were dissolved in 50% aqueous Me_2CO (7 ml) containing glacial AcOH (0·2 ml). This soln was treated in a similar manner to that described for the synthesis of 2,6-dimethyl-*p*-benzoquinone-4-(2',6'-dimethyl)anil. The eluate from the fast running dark red band gave, on evaporation, dark red needles (114 mg) m.p. 91–98°. Recrystallization (EtOH) gave dark red needles m.p. 108·5–109°, not depressed on admixture with the red compound from the enzymic oxidation. (Found: N, 5·4. Calc. for $C_{16}H_{16}ClNO$: N, 5·1%). The UV and IR spectra were comparable with those of the enzymic product.

Reaction of 4-chloro-2,6-dimethylaniline with 2,6-dimethyl-p-benzoquinone-4-toluene-p-sulphonimide

2,6-Dimethyl-p-benzoquinone-4-toluene-p-sulphonimide (300 mg) in glacial AcOH (10 ml) was added to 4-chloro-2,6-dimethylaniline (170 mg) dissolved in glacial AcOH (5 ml) at room temp. The mixture immediately turned dark red and was allowed to stand (10 min). The solvent was removed; the resultant red glass, dissolved in light petroleum (b.p. 40–60°; 15 ml) gave a dark red soln containing a crystalline suspension which was removed by filtration, and characterized as toluene-*p*-sulphonamide, m.p. 137°. The filtrate and washings were evaporated to 10 ml and chromatographed on alumina. Development showed two bands: a very fast-running dark red band and a narrow purple band which remained at the top of the column. The eluate from the former yielded, on evaporation, dark red needles (240 mg), m.p. 107°, which on recrystallization (EtOH) had m.p. 108°, not depressed by the red compound from the enzymic oxidation.

Reductive acetylation under conditions described for the enzymic product gave colourless needles m.p. 137–139°, not depressed on admixture with the product of the reductive acetylation of the red enzymic oxidation product.

Bands (ii) *and* (iii). The eluate of band (ii) gave traces of a noncrystallisable glass and was not further investigated. Band (iii) could not be eluted from the column with pure $CHCl_3$ and was not investigated.

Estimation of chloride ion in the filtrate. To the filtrate from oxidation of 4-chloro-2,6-dimethylaniline (508 mg) by pure peroxidase in NaOAc–AcOH buffer (pH 4·5; 1 l, 0·25 M) after evaporation to 100 ml and acidification with conc HNO_3 (10 ml) was added $AgNO_3$ soln (25 ml, 0·1 N). The AgCl was filtered off and the filtrate titrated with 9·4 ml of KCNS soln (0·098 N). Wt of Cl^- = 55 mg; 508 mg of 4-chloro-2,6-dimethylamsine contains 117 mg of Cl.

4-Bromo-2,6-dimethylaniline. The pure amine had m.p. 50–51°; IR spectrum (Nujol) showed prominent bands at 3408, 3315, 1635, 1225, 1030, 860, 855 and 733 cm^{-1}.

N-Benzoyl derivative, m.p. 185–186°. (Found: C, 58·9; H, 4·7; N, 4·7. $C_{15}H_{14}BrNO$ requires: C, 59·15; H, 4·6; N, 4·7%).

Oxidation of the free amine. The amine (3 g) was oxidized with H_2O_2/peroxidase in the usual way. A transient purple colour changed rapidly to orange-red and a red ppt was formed. Hiflo (2 g) was added and the ppt filtered off and extracted with light petroleum (b.p. 40–60°, 3 × 150 ml). Evaporation of the extract yielded red needles (2·2 g), which were dissolved in a 40–60° light petroleum, benzene mixture (10 ml, 10% benzene) chromatographed on alumina, and eluted by the same solvent mixture.

After development, three bands appeared: (i) fast-running, wide, dark red; (ii) much slower-running, very narrow, brown; (iii) faint purple, remained at the top of the column.

The eluate from band (i) yielded dark red needles (1·72 g) m.p. 105–107°. Recrystallization (aqueous MeOH gave dark red needles *2,6-dimethyl-p-benzoquinone-4-(4'-bromo-2',6'-dimethyl)anil* (1·5 g) m.p. 111–112°. (Found: C, 60·1; H, 4·95; N, 4·7: $C_{16}H_{16}BrNO$ requires: C, 60·0; H, 5·05; N, 4·5%). UV spectrum (95% EtOH): λ_{max} 234–6 mμ, ($\log_{10}$ ε 3·928); 274–5, (4·505); 469–470, (3·037); λ_{min} 224–226 mμ, (3·899); 244–245, (3·887); 370, (2·532). *IR spectrum* (Nujol) showed prominent bands at 1645, 1615, 1580, 1322, 1196, 1160, 1035, 1025, 938, 927, 860, 788 and 772 cm^{-1}.

Reductive acetylation of the bromoanil. The compound (190 mg) was heated under reflux with Ac_2O (6 ml), pyridine (0·2 ml) and Zn dust (600 mg). The red colour disappeared almost immediately and after 20 min the mixture was filtered; the filtrate was poured into ice and allowed to stand overnight. The white precipitate was filtered off and recrystallized from aqueous AcOH and gave colourless needles of the *O-acetyl* derivative of *4-hydroxy-4'-bromo-3,5,2',6'-tetramethyldiphenylamine* (180 mg) m.p. 130·5–132°. (Found: C, 59·6; H, 5·4; N, 4·0; Br, 22·1. $C_{18}H_{20}BrNO_2$ requires: C, 59·7; H, 5·5; N, 3·9; Br, 22·1%). *IR spectrum* (Nujol) showed prominent bands at 3397, 1758, 1612, 1215, 1189, 1031, 911, 855, 851 and 792 cm^{-1}.

Hydrolysis of the bromoanil. The compound (130 mg) was heated under reflux with 10% H_2SO_4 soln (15 ml) during 30 min. A pale yellow deposit formed in the condenser, and was removed with Me_2CO

(5 ml). Removal of solvent gave pale yellow crystals, m.p. 65°, purification by vacuum sublimation gave yellow prisms, m.p. 71° not depressed on admixture with 2,6-dimethyl-*p*-benzoquinone, m.p. 72°.

The acid soln, after extraction with ether (2 × 10 ml), was amde alkaline and concentrated, 30% NaOH aq (1 ml) and benzoyl chloride (0·2 ml) were added and the mixture allowed to stand overnight. The ppt was filtered off, washed and recrystallized (aqueous EtOH, with decolorization (charcoal)), to give colourless needles, m.p. 184–186°, not depressed on admixture with N-benzoyl-4-bromo-2,6-dimethylaniline, m.p. 186°.

Condensation of 2,6-*dimethyl*-p-*benzoquinone and* 4-*bromo*-2,6-*dimethylaniline*

2,6-Dimethyl-*p*-benzoquinone (200 mg) and 4-bromo-2,6-dimethylaniline (200 mg) were dissolved in 50% aqueous Me_2CO (7 ml) containing glacial (AcOH) (0·5 ml). This soln was treated in a similar manner to that described for the synthesis of 2,6-dimethyl-*p*-benzoquinone-4-(2',6'-dimethyl)anil. The eluate from the fast running red band gave red needles (110 mg), m.p. 109–110°; recrystallization (EtOH) gave dark red needles m.p. 111–112° not depressed on admixture with the red product from the enzymic oxidation. The UV and IR spectra were comparable with those of the enzymic product.

Reaction of 4-*bromo*-2,6-*dimethylaniline and* 2,6-*dimethyl*-p-*benzoquinone*-4-*toluene*-p-*sulphonimide*

To 2,6-dimethyl-*p*-benzoquinone-4-toluene-*p*-sulphonimide (287 mg) dissolved in dioxan (4 ml) was added 4-bromo-2,6-dimethylaniline (199 mg) and the reaction mixture was heated to 100° for 3 min. The solvent was removed under reduced press and the dark red residue extracted with benzene (15 ml) yielding a dark red soln in which was suspended a solid. This was removed by filtration, washed, and characterized as toluene-*p*-sulphonamide, m.p. 137°. The filtrate was evaporated and the resulting red glass dissolved in light petroleum (20 ml, b.p. 40–60°), and chromatographed on alumina. The eluate from the single fast-running dark red band gave dark red needles (240 mg), m.p. 105–110°, recrystallization (EtOH) raised m.p. to 111–112° not depressed on admixture with the red enzymic oxidation product nor the condensation product of 2,6-dimethyl-*p*-benzoquinone and 4-bromo-2,6-dimethylaniline. IR spectra were also identical. A second band was eluted from the column by a benzene–$CHCl_3$ mixture (10% $CHCl_3$), evaporation gave 22 mg of amorphous material. A third band could not be eluted from the column by 50% benzene $CHCl_3$ mixture and was not further investigated.

Estimation of Br^-. 4-Bromo-2,6-dimethylaniline (770 mg) was suspended in NaOAc–AcOH buffer (0·2M, 750 ml), pH 4·5) and oxidized by pure peroxidase and H_2O_2 soln. When oxidation was complete the filtrate contained 134 mg Br^-. Br introduced into the system = 308 mg.

4-*Iodo*-2,6-*dimethylaniline*. Redistilled ICl (8·15 g) dissolved in glacial AcOH (50 ml) was added slowly to a cooled (below 10°) solution of 2,6-dimethylaniline (8·4 g) in glacial AcOH (50 ml). The colour of the ICl disappeared and white crystals separated. The reaction mixture was set aside for 12 hr at 0°, the amine HCl was filtered off, washed with ether, and dried. The free base was liberated with dil aqueous NH_3, filtered off, dried, and recrystallized from light petroleum (b.p. 40–60°) to give colourless needles of 4-*iodo*-2,6-*dimethylaniline* (6·88 g), m.p. 49·5–50·5°. (Found: C, 38·7; H, 4·0; N, 5·7. $C_8H_{10}INO$ requires: C, 38·9; H, 4·1; N, 5·8%). *IR spectrum* (nujol) showed bands at 3402, 3199, 1628, 1587, 1270, 1225, 1034, 857, 845 and 730 cm^{-1}.

N-*Benzoyl derivative*, m.p. 195–196°. (Found: C, 51·2; H, 3·9; N, 3·9. $C_{15}H_{14}INO$ requires: C, 51·3; H, 4·0; N, 4·0%).

Oxidation of 4-*iodo*-2,6-*dimethylaniline*

4-Iodo-2,6-dimethylaniline was oxidized in NaOAc–AcOH buffer as for the corresponding chloro-compound. When the reaction was complete Hiflo (2 g) was added and the product filtered off and dried. This was extracted (Soxhlet) with light petroleum b.p. 40–60°, (3 × 120 ml) until the extract was colourless. The extracts were evaporated to yield a dark red glass (820 mg). This was dissolved in light petroleum b.p. 40–60° (10 ml) and benzene (25 ml) chromatographed on an alumina column and eluted with the same mixture. After development, three bands were observed:

(i) fast-running red; (ii) very faint, much slower-running, brown; (iii) violet which remained at the top of the column.

The eluate from band (i) gave dark red needles (700 mg) m.p. 124–126°, recrystallization (aqueous EtOH) gave dark red needles of 2,6-*dimethyl*-p-*benzoquinone*-4-(2',6'-*dimethyl*-4'-*iodo*)*anil* (640 mg), m.p. 125·5–126·5°. (Found: C, 52·4; H, 4·6; N, 3·9. $C_{16}H_{16}INO$ requires: C, 52·6; H, 4·4; N, 3·8%); *UV spectrum* (95% EtOH): λ_{max} 240 mμ, ($\log_{10}\varepsilon$ 4·017); 276, (4·447); 474, (3·072); λ_{min} 223 mμ, ($\log_{10}\varepsilon$ 3·943); 246,

(3·977); 374, (2·768); *IR spectrum* (Nujol) showed prominent bands at 1645, 1612, 1575, 1313, 1208, 1159, 912, 850 and 771 cm^{-1}.

Hydrolysis of the iodoanil from band (i). The iodanil (192 mg) was heated under reflux with 10% H_2SO_4 (10 ml) during 30 min. A yellow crystalline deposit appeared in the condenser and was characterized as 2,6-dimethyl-*p*-benzoquinone, m.p. 72°.

The iodoanil (10 mg) was hydrolysed by heating under reflux with H_2SO_4 (3 N, 1 ml); the hydrolysate was extracted (ether, 3 × 5 ml), then made alkaline and again extracted (ether, 3 × 5 ml). This extract was reduced to 5 ml and examined by paper chromatography; the chromatogram showed a single spot R_f, 0·79, identical with authentic 4-iodo-2,6-dimethylaniline.

Attempted reductive acetylation of the iodoanil

(i) The iodoanil (200 mg) was heated under reflux with Ac_2O (10 ml) pyridine (0·2 ml) and Zn dust (0·5 mg) during 30 min. The mixture was filtered, the filtrate evaporated to small volume and the residue dissolved in EtOH (10 ml) was poured onto ice and allowed to stand overnight. The brown solid was filtered off and thrice recrystallized (aqueous AcOH) giving brown micro crystals, m.p. 131–140°. The range could not be narrowed by repeated recrystallization, during which the product became increasingly red in colour.

(ii) The iodoanil (100 mg) dissolved in Ac_2O (20 ml) containing NaOAc (50 mg) was catalytically reduced (H_2/Adams catalyst (10 mg)). A rapid uptake occurred and the red colour was immediately discharged. After 15 hr on contact with the air the red colour of the iodoanil reappeared showing that no acetylation of the reduced compound had occurred.

Synthesis of the iodoanil

Condensation of 2,6-dimethyl-p-benzoquinone and 4-iodo-2,6-dimethylaniline. 2,6-Dimethyl-*p*-benzoquinone (172 mg) and 4-iodo-2,6-dimethylaniline (227 mg), dissolved in 50% aqueous Me_2CO (10 ml) and glacial AcOH (0·2 ml) were heated under reflux during 70 hr. Treatment in a similar manner to that described for the synthesis of 2,6-dimethyl-*p*-benzoquinone-4-(2′,6′-dimethyl)anil gave on evaporation of the eluate dark red needles (87 mg), m.p. 122–126°.Recrystallization (aqueous EtOH) gave dark red needles, m.p. 126–127° not depressed on admixture with the red compound from band I. The UV spectrum was comparable with that of the enzymic product.

Condensation of 2,6-dimethyl-p-benzoquinone-4-toluene-p-sulphonimide and 4-iodo-2,6-dimethylaniline. To 4-iodo-2,6-dimethylaniline (247 mg) dissolved in glacial AcOH was added 2,6-dimethyl-*p*-benzoquinone-4-toluene-*p*-sulphonimide (300 mg): a dark red colouration appeared immediately and the reaction mixture was heated to 100° for 3 min. The solvent was removed under reduced press, and the residual dark red glass treated with light petroleum (b.p. 40–60°) containing 20% benzene (20 ml). Toluene-*p*-sulphonamide was filtered off, the dark red filtrate and washings were chromatographed on alumina and the eluate from the fast-running red band gave, on evaporation, dark red needles (365 mg), m.p. 125–126° not depressed on admixture with the red enzymic oxidation product. The IR spectrum was comparable with that of the enzymic product.

Examination of the filtrate. The filtrate was diminished in volume under reduced press; the residue contained no I^-. The distillate was pale yellow and contained only I_2, it gave a blue coloration with starch. This was rapidly discharged by $Na_2S_2O_3$ (2 ml, 0·1 N).

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