## 198. *Studies in Peroxidase Action. Part X.* The Oxidation of Phenols.*

By H. Booth and B. C. Saunders.

The oxidation of certain phenols by hydrogen peroxide in the presence of peroxidase has been studied. Mesitol gave 4-hydroxy-3 : 5-dimethylbenzyl alcohol, 4-hydroxy-3 : 5-dimethylbenzaldehyde, and 2 : 6-dimethylbenzoquinone. The reaction involved the stepwise oxidation of the methyl group *para* to the hydroxyl group, since the alcohol (probably the primary oxidation product) was rapidly oxidised by the enzyme system to the aldehyde. The latter was converted, not into the carboxylic acid but into the quinone. Durenol was oxidised mainly to 4 : 4′-dihydroxy-2 : 3 : 5 : 6 : 2′ : 3′ : 5′ : 6′-octamethyldiphenyl. A trace of duroquinone was also isolated.

The much-used guaiacol reaction has been re-examined. Guaiacol was rapidly oxidised to a brown-red solid from which 2 : 2′-dihydroxy-3 : 3′-dimethoxydiphenyl has been isolated. Reductive acetylation of the crude product gave 4 : 4′-diacetoxy-3 : 3′-dimethoxydiphenyl in small yield.

In Parts I—IX, the detailed investigation was recorded of the action of the enzyme peroxidase on various aromatic amines in the presence of hydrogen peroxide. This work has now been extended to include phenols as substrates.

Although it has been known since 1900 that phenols are readily oxidised by the peroxidase system, most investigators have been content to record the colour changes observed; the nature of the products has rarely been examined.[1] In a few cases, however, the oxidation products have been fully characterised. Vanillin yields " dehydrodivanillin " [2] and pyrogallol gives purpurogallin.[3] Catechol is oxidised by the peroxidase system to *o*-benzoquinone, since a mixture of catechol and aniline yields 4 : 5-dianilino-*o*-benzoquinone on oxidation.[4] *p*-Cresol is converted by the enzyme into 2 : 2′-dihydroxy-5 : 5′-dimethyldiphenyl, the analogous terphenyl, and a furan derivative.[5]

We have already shown that oxidation of aniline and of *p*-toluidine by the peroxidase system yields a variety of products because of the free positions in the ring where oxidation

* Part IX, *J.*, 1954, 4630.

[1] Bach and Chodat, (*a*) *Ber.*, 1903, **36**, 600; (*b*) *Arch. Sci. phys. nat.*, 1916, **42**, 56; (*c*) Chodat, *Arch. Génève*, 1907, **24**, 2; Onslow, *Biochem. J.*, 1923, **17**, 2; Szent-Györgyi, *ibid.*, 1928, **22**, 1387; Elliot, *ibid.*, 1932, **26**, 1281; Balls and Hale, *J. Biol. Chem.*, 1934, **107**, 767.

[2] Bourquelot and Marchadier, *Compt. rend.*, 1904, **138**, 1432; Lerat, *Compt. rend. Soc. Biol.*, 1903, **55**, 1325.

[3] Willstätter and Heiss, *Annalen*, 1923, **433**, 17.

[4] Pugh and Raper, *Biochem. J.*, 1927, **21**, 1370.

[5] Westerfield and Lowe, *J. Biol. Chem.*, 1942, **145**, 403.

and condensation can take place.[6] On the other hand, the oxidation of mesidine gave only one compound, *viz.*, 3 : 5-dimethyl-*p*-benzoquinone 1-(2 : 4 : 6-trimethylanil).[7] We thought it likely that similar considerations might apply to the oxidation of subsituted phenols, and therefore investigated the oxidation of mesitol and durenol. There is no reference in the literature to the enzymic oxidation of either phenol, but the oxidation of mesitol by non-enzyme systems is recorded. Using neutralised Caro's acid, Bamberger [8] claimed to have isolated 4-hydroxy-3 : 5-dimethylbenzyl alcohol (I) and 4-hydroxy-2 : 4 : 6-trimethyl-*cyclo*hexa-2 : 5-dienone, but Cosgrove and Waters [9] could not repeat his observations. Ferrous sulphate and hydrogen peroxide oxidise mesitol to a diphenylmethane derivative [10] whereas benzoyl peroxide gives 3 : 5 : 3′ : 5′-tetramethylstilbene-4 : 4′-quinone and 4-benzoyloxy-2 : 4 : 6-trimethyl*cyclo*hexa-2 : 5-dienone.[9] The stilbenequinone is also produced by the action of silver oxide.[11] Fichter and Muller [12] isolated 2 : 6-dimethyl-*p*-benzoquinone and 2-hydroxy-3 : 5-dimethylbenzoic acid by electrochemical oxidation of mesitol. Mesitol has been oxidised by lead tetra-acetate to 2-acetoxy-2 : 4 : 6-trimethyl-*cyclo*hexa-3 : 5-dienone.[13]

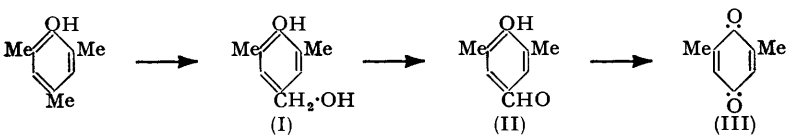
For our reactions, a highly purified specimen of peroxidase was used. An aqueous solution of mesitol, with hydrogen peroxide and peroxidase added intermittently, became yellow and later turbid; later a pale yellow solid separated. The yellow filtrate contained chiefly 4-hydroxy-3 : 5-dimethylbenzaldehyde (II) and 2 : 6-dimethylbenzoquinone (III).

Smith, Opie, Wawzonek, and Prichard [14] prepared this quinone from 3 : 5-xylenol in 74% yield. By their method we obtained a yield of only 65%, and the product was contaminated with a phenol difficult to remove except by selective adsorption on alumina. We found it easier to prepare the pure quinone (required for comparison) by allowing 3 : 5-dimethyl-4-nitrosophenol to react with cuprous oxide and hydrochloric acid in 2-ethoxyethanol.[15]

An authentic sample of 4-hydroxy-3 : 5-dimethylbenzaldehyde was prepared in poor yield by a Reimer–Tiemann reaction and in 50% yield by modification [16] of the Gattermann reaction.

The yellow amorphous solid precipitated during the peroxidase oxidation of mesitol was fractionally sublimed in a high vacuum and gave impure 4-hydroxy-3 : 5-dimethylbenzyl alcohol (I), m. p. 93·5—95·5°. The pure alcohol had m. p. 103·5—105°; the low m. p. of the enzymic product was due to traces of the corresponding aldehyde, since the infrared absorption curve showed a peak where absorption due to this type of carbonyl group normally occurs.

An aqueous solution of the alcohol was very rapidly oxidised to the corresponding aldehyde by the enzyme system. We also noted that 4-hydroxy-3 : 5-dimethylbenzaldehyde was attacked by the system, giving 2 : 6-dimethyl-*p*-benzoquinone. Thus we have

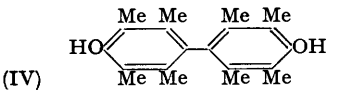



evidence for the enzymic oxidation of mesitol according to the annexed scheme (in particular it should be noted that the carboxylic acid is not formed). Qualitative experiments showed that the quinone was unaffected by the enzyme system and was thus a true end-product of the oxidation.

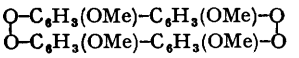
[6] P. J. G. Mann and Saunders, *Proc. Roy. Soc.*, 1935, *B*, **119**, 47; Saunders and P. J. G. Mann, *J.*, 1940, 769.
[7] Chapman and Saunders, *J.*, 1941, 496.
[8] Bamberger, *Ber.*, 1903, **36**, 2030.
[9] Cosgrove and Waters, *J.*, 1951, 388.
[10] *Idem*, *J.*, 1951, 1726.
[11] Goldschmidt and Bernard, *Ber.*, 1923, **56**, 1963.
[12] Fichter and Muller, *Helv. Chim. Acta*, 1935, **18**, 831.
[13] Wessely, Lauterbach-Keil, and Sinwell, *Monatsh.*, 1950, **81**, 1055.
[14] Smith, Opie, Wawzonek, and Prichard, *J. Org. Chem.*, 1939, **4**, 318.
[15] Cf. Sumerford and Dalton, *J. Amer. Chem. Soc.*, 1944, **66**, 1330.
[16] Adams and Levine, *J. Amer. Chem. Soc.*, 1923, **45**, 2373.

Further, mesitol and the intermediate compounds in the oxidation were scarcely attacked by hydrogen peroxide or peroxidase alone. In most of these control experiments, the starting material was recovered in high yield. However, when 4-hydroxy-3 : 5-dimethylbenzaldehyde was treated with hydrogen peroxide alone, a faint yellow colour appeared in the solution after several days. No solid separated and 64—70% of the aldehyde was recovered. It was concluded that hydrogen peroxide alone oxidises the aldehyde so slowly that this reaction may be neglected when the enzyme system is present. Thus all the oxidations described were brought about by peroxidase action.

There is no mention in the literature of the oxidation of durenol, by either inorganic reagents or enzyme systems. Durenol is very slightly soluble in cold water and it was therefore oxidised in fine suspension. Hydrogen peroxide and peroxidase were added intermittently during 5 days. No colour changes were observed and a white precipitate was always present in suspension. The precipitate consisted of unchanged durenol and 4 : 4′-dihydroxyoctamethyldiphenyl (IV). The filtrate yielded traces of crude duroquinone.




(IV)



(V)

Many workers, *e.g.*, Bach and Chodat,[1b] have noted that guaiacol is oxidised by the peroxidase system to a red substance. Ucko and Bansi [17] gave the optimum pH for the reaction as 5—5·2; Chance,[18] however, found that the velocity of reaction of his " complex II " with guaiacol was little affected by pH in the range 3·4—5·3. Bertrand [19] oxidised guaiacol with the enzyme laccase and obtained a red " tetraguaiacoquinone," which he formulated as the peroxide (V). This formula is, of course, improbable because it does not account for the red colour of the compound. Mann [20] used the reaction of peroxidase with guaiacol to investigate the kinetics of peroxidase action.

The action of inorganic oxidising agents on guaiacol has received little attention. Kar [21] reported the oxidation of guaiacol to " tetraguaiacoquinone " by hydrogen peroxide and catalytic amounts of tungstic, molybdic, and vanadic acid sols. Pennington and Ritter [22] showed that one molecule of guaiacol absorbed 3 molecules of periodate, a red colour being produced. By electrochemical oxidation of guaiacol, in sulphuric acid, using a lead anode, Fichter and Dietrich [23] isolated 4 : 4′-dihydroxy-3 : 3′-dimethoxydiphenyl. Potassium nitrosodisulphonate gives methoxy-*p*-benzoquinone.[24]

When a solution of guaiacol in acetate buffer (pH 5·0) was treated with hydrogen peroxide and peroxidase, the product was a brown solid with a green metallic lustre, m. p. 110—160°, soluble in sodium hydroxide and sodium carbonate solutions. With concentrated sulphuric acid it gave a transient green colour and finally a brown solution. The brown solid was easily reduced, *e.g.*, by zinc dust and cold acetic acid, or by phenylhydrazine, to a colourless substance The latter, however, reverted to the coloured form slowly in air and very rapidly in warm alcohol. The colour with alkali and sulphuric acid, and the easy reduction to leuco-compounds, suggested the presence of quinones in the mixture. Most of the solid dissolved in cold benzene, and when the extract was chromatographed on neutral alumina several coloured zones were observed. However, no crystals were obtained by elution from the column.

When the crude mixture was sublimed in a high vacuum, a small quantity of white solid was isolated, which crystallised from *cyclo*hexane in colourless plates, m. p. 141°. Analysis and molecular-weight determinations gave $C_{14}H_{14}O_4$, suggesting a dehydrodiguaiacol. The product was phenolic, and gave a blue colour with 2 : 6-dichloroquinone

[17] Ucko and Bansi, *Z. physiol. Chem.*, 1926, **159**, 235.
[18] Chance, *Arch. Biochem.*, 1949, **24**, 410.
[19] Bertrand, *Bull. Soc. chim. France*, 1904, **31**, 185; *Compt. rend.*, 1903, **137**, 1270; *Ann. Inst. Pasteur*, 1904, **18**, 116.
[20] Mann, *Biochem. J.*, 1931, **25**, 918.
[21] Kar, *J. Indian Chem. Soc.*, 1937, **14**, 291.
[22] Pennington and Ritter, *J. Amer. Chem. Soc.*, 1947, **69**, 187.
[23] Fichter and Dietrich, *Helv. Chim. Acta*, 1924, **7**, 137.
[24] Teuber and Jellinck, *Chem. Ber.*, 1952, **85**, 95.

chloroimide, showing that at least one of the two aromatic rings had an unsubstituted position *para* to a hydroxyl group. The compound evidently had structure (VI) or (VII).


When 2 : 2′-dihydroxy-3 : 3′-dimethoxydiphenyl (VI) was synthesised (see below) it crystallised from *cyclo*hexane in needles, m. p. 142·5—143°, and mixed m. p. with enzymic product 141°. Both compounds gave an orange-red colour with ferric chloride in aqueous alcohol and an orange-red colour with peroxidase and hydrogen peroxide. The infrared absorption spectra were identical. It was concluded that the enzymic product was essentially 2 : 2′-dihydroxy-3 : 3′-dimethoxydiphenyl.

This product (VI) was synthesised from vanillin. With ferric chloride this gave dehydrodivanillin and acetylation gave 2 : 2′-diacetoxy-5 : 5′-diformyl-3 : 3′-dimethoxydiphenyl. Potassium permanganate oxidised the latter to 2 : 2′-diacetoxy-3 : 3′-dimethoxydiphenyl-5 : 5′-dicarboxylic acid, m. p. 277—280° (Elbs and Lerch[25] recorded 140°). The acetyl groups were next removed and the hydroxy-acid was decarboxylated to 2 : 2′-dihydroxy-3 : 3′-dimethoxydiphenyl (VI).

An unsuccessful attempt was made to synthesise the compound (VI) by an Ullmann reaction. 6-Nitroguaiacol was converted into toluene-*p*-sulphonate and then reduced to the amine. Diazotisation and treatment with hydriodic acid gave a poor yield of the toluene-*p*-sulphonate of 6-iodoguaiacol. Preparation of 6-iodoguaiacol from the diazonium salt of 6-aminoguaiacol was unsuccessful owing to the formation of 7-methoxybenz-1 : 2 : 3-oxadiazole, orange crystals, which exploded at 110° and coupled with alkaline β-naphthol to give a red dye.

When the iodoguaiacol toluene-*p*-suphonate was heated with activated copper bronze at 250°, an intractable solid resulted, but a small quantity of the required 3 : 3′-dimethoxy-2 : 2′-di-*p*-toluenesulphonyloxydiphenyl was isolated. Attempts to hydrolyse the ester by two methods were unsuccessful, probably owing to steric hindrance; in this connection it is interesting that heating 2 : 2′-dihydroxy-3 : 3′-dimethoxydiphenyl with toluene-*p*-sulphonyl chloride in hot pyridine for an hour (conditions normally giving good yields) gave only a small yield of the ester.

When the crude oxidation product from guaiacol was heated with acetic anhydride, zinc dust, and sodium acetate, white amorphous solid was obtained. Fractional crystallisation was ineffective, but fractional sublimation gave colourless crystals, m. p. 195·5—197·5°. Analyses indicated that this was probably the diacetate of a dehydrodiguaiacol. The diacetate of 2 : 2′-dihydroxy-3 : 3′-dimethoxydiphenyl was synthesised and had m. p. 133—134°. Therefore the compound isolated was almost certainly 4 : 4′-diacetoxy-3 : 3′-dimethoxydiphenyl. In support of this, the m. p. of our product agrees with that recorded by Fichter and Dietrich[23] for the diacetate of dehydrodiguaiacol obtained by electrochemical oxidation of guaiacol. Fichter and Dietrich suggested that their product was 4 : 4′-dihydroxy-3 : 3′-dimethoxydiphenyl because it did not react with nitrous acid (thus positions *para* to the hydroxyl group were substituted), and with hydriodic acid it gave a tetrahydroxydiphenyl different from the 2 : 3 : 2′ : 3′-tetrahydroxydiphenyl.[26] The isolation of 4 : 4′-diacetoxy-3 : 3′-dimethoxydiphenyl by reductive acetylation of the crude oxidation product shows that the latter contains 3 : 3′-dimethoxydipheno-4 : 4′-quinone. The characteristic red-brown of the crude guaiacol oxidation product is undoubtedly due partly to this quinone. The crude product also probably contains the isomeric 3 : 3′-dimethoxydipheno-2 : 2′-quinone produced by the further oxidation of the dihydroxydiphenyl (VI), but it has not been isolated.

The fact that the oxidation of durenol gives a colourless product is undoubtedly due to the steric effects of the four *o*-methyl groups (cf. IV). Further oxidation of the diphenol (IV) to a coloured diphenoquinone would require the production of a double bond between

[25] Elbs and Lerch, *J. prakt. Chem.*, 1916, **93**, 2.
[26] Barth and Schreder, *Ber.*, 1878, **11**, 1336.

the two rings and this necessitates a shortening of the link. A model indicates that such a shortening, with retention of a planar structure, is not very likely.

## Experimental

*Peroxidase Solution.*—The enzyme used in most experiments was a concentrated solution kindly supplied by Professor Keilin and Dr. Hartree. It was purified by the method of Keilin and Mann.[27] The solution contained 19·4 enzyme units/ml. The preparation did not oxidise catechol in the absence of hydrogen peroxide : therefore tyrosinase and laccase were absent. In oxidation experiments, solutions containing 0·3 or 0·6 enzyme unit/ml. were employed.

*Oxidation of Mesitol.*—Mesitol[28] (2 g.) was dissolved in distilled water (4 l.); the pH was 6·5. Peroxidase solution (10 c.c.; 0·6 unit/c.c.) was added, followed by 20-vol. hydrogen peroxide (2 c.c.). A pale yellow colour developed, followed by turbidity. The solution was stirred continuously and further quantities of enzyme (26 c.c. in all) and hydrogen peroxide (30 c.c. in all) were added at intervals during 24 hr. During oxidation the pH gradually fell to *ca.* 4·5. After addition of enzyme and hydrogen peroxide, the solution was stirred for 24 hr. and set aside for 48 hr., pale yellow solid separating. The mixture was filtered and the precipitate and filtrate were examined.

*Treatment of filtrate.* This was extracted six times with ether. The yellow extracts were dried ($Na_2SO_4$) and the ether distilled off, leaving an orange oil which was steam-distilled.

The non-volatile fraction was extracted with ether and dried ($Na_2SO_4$). Evaporation left a dark red solid which was digested with hot water, and the solution was filtered hot from dark intractable residue. The filtrate, on cooling, deposited white needles of 4-hydroxy-3 : 5-dimethylbenzaldehyde which, recrystallised from aqueous alcohol, had m. p. and mixed m. p. 114·5—115° [Found : C, 71·9; H, 6·8%; *M* (Rast), 130—160. Calc. for $C_9H_{10}O_2$ : C, 71·9; H, 6·7%; *M*, 150]. The 2 : 4-*dinitrophenylhydrazone*, recrystallised from ethanol, m. p. 276—277° (decomp.) (Found : C, 54·4; H, 4·3; N, 17·1. $C_{15}H_{14}O_5N_4$ requires C, 54·5; H, 4·3; N, 17·0%).

The yellow steam-distillate containing orange needles was extracted with ether. Evaporation of the dried extracts gave an orange solid (0·45 g.) which was digested with light petroleum (b. p. 40—60°) at −5° until white and then crystallised from aqueous alcohol as needles (40 mg.) of 4-hydroxy-3 : 5-dimethylbenzaldehyde, m. p. 112—113·5°, mixed m. p. 113—114°. The petroleum extract gave on evaporation a yellow solid (0·4 g.) which crystallised from light petroleum (b. p. 40—60°) as pale yellow needles of pure 2 : 6-dimethyl-*p*-benzoquinone, m. p. and mixed m. p. 71·5—72° (Found : C, 70·4; H, 5·6. Calc. for $C_8H_8O_2$ : C, 70·55; H, 5·9%). The quinone (70 mg.) was reductively acetylated by heating it under reflux with zinc dust (0·2 g.), acetic anhydride (10 c.c.), acetic acid (2 c.c.), and pyridine (0·2 c.c.) for 30 min.; the mixture was filtered hot and poured into water (50 c.c.); on stirring, a white solid separated and crystallised as needles, m. p. 92—93°, from aqueous alcohol (lit.,[29] m. p. 91—92°).

*Treatment of precipitate.* The precipitate (1 g.) was dissolved in acetone (50 c.c.), and the solution filtered, concentrated, and transferred to a sublimation tube. The acetone was removed under a moderate vacuum. Sublimation of the resulting solid at 130—140°/$10^{-4}$ mm. gave, successively, the most volatile fraction, white crystals, m. p. 70—90°, a yellow oil, and a residual solid.

The crystals were contaminated with oil and were resublimed at $10^{-4}$ mm. At 60—80°, the sublimate consisted of white crystals, which were recovered by dissolving them in ether and evaporating the solution [yield 38 mg.; m. p. 94° (softened at 75°)]. Recrystallisation from carbon tetrachloride gave square prisms (11 mg.), m. p. 93·5—95·5° (Found : C, 70·2; H, 7·8. Calc. for $C_9H_{12}O_2$ : C, 71·0; H, 7·95%). The mixed m. p. with pure 4-hydroxy-3 : 5-dimethylbenzyl alcohol (m. p. 103·5—104°) was 96—101°. The infrared absorption spectrum was almost identical with that of an authentic specimen of this alcohol.

The residual solid was resublimed at $5 \times 10^{-5}$ mm. up to 225°, giving successively white crystals of m. p. 75—85°, white crystals of m. p. 85—105°, and yellow oil (140 mg.). The first two fractions were minute and not further examined. The yellow oil solidified to a glass, insoluble in 10% aqueous sodium hydroxide; it gave a precipitate with 2 : 4-dinitrophenylhydrazine; attempts to crystallise it were unsuccessful (Found : C, 77·0; H, 7·5%).

4-*Hydroxy*-3 : 5-*dimethylbenzaldehyde.*—2 : 6-Xylenol (20 g.) was dissolved in dry "AnalaR" benzene (70 c.c.). Zinc cyanide (37 g.) was added, the mixture cooled, and dry hydrogen

[27] Keilin and Mann, *Proc. Roy. Soc.*, 1937, *B*, **122**, 119.
[28] Porter and Thurber, *J. Amer. Chem. Soc.*, 1921, **43**, 1194.
[29] Stern, Robeson, Weisler, and Baxter, *J. Amer. Chem. Soc.*, 1947, **69**, 869.

chloride passed in for 50 min., with stirring. The flask was cooled to 0°, powdered anhydrous aluminium chloride (30 g.) added, and hydrogen chloride again passed in at 50—55° for 4 hr. After cooling, the benzene was decanted from the sticky aldimine hydrochloride. 10% Hydrochloric acid (600 c.c.) was added and the aldehyde liberated by heating the mixture under reflux for an hour. The mixture was steam-distilled to remove benzene and unchanged 2 : 6-xylenol, and then extracted with ether. The extract was shaken with saturated sodium hydrogen sulphite solution; acidification of the sulphite extracts with hot dilute sulphuric acid gave the pure aldehyde (11·5 g., 50% based on unrecovered phenol) which crystallised from aqueous alcohol in needles, m. p. 114—115°.

2 : 6-*Dimethylbenzoquinone.*—3 : 5-Dimethyl-4-nitrosophenol [30] (9 g.) was dissolved in " Methylcellosolve " (90 c.c.) and acetone (10 c.c.). Cuprous oxide (8·4 g.), concentrated hydrochloric acid (28·6 c.c.), and water (36 c.c.) were added and the mixture was heated under reflux for 75 min. The quinone was steam-distilled and extracted from the distillate with ether, which was dried ($Na_2SO_4$) and evaporated, to give the crude quinone (6·67 g., 88%). Crystallisation from light petroleum (b. p. 40—60°) gave yellow needles, m. p. 71·5—72°.

*Peroxidase-catalysed Oxidation of* 4-*Hydroxy*-3 : 5-*dimethylbenzaldehyde.*—4-Hydroxy-3 : 5-dimethylbenzaldehyde (2 g.) was dissolved in distilled water (5 l., pH 6·0). Peroxidase (10 c.c.; 0·6 unit/c.c.) and 20-vol. hydrogen peroxide (2 c.c.) were added, a yellow colour being produced. The solution was stirred for 36 hr. and enzyme (14 c.c.) and hydrogen peroxide (48 c.c.) were added in small portions at intervals. A pale yellow precipitate separated and after a further 60 hr., the solid (0·53 g.) was filtered off. The filtrate was extracted four times with ether and dried ($Na_2SO_4$). Evaporation yielded a yellow oil which was steam-distilled, giving pale yellow needles of 2 : 6-dimethylbenzoquinone (0·6 g.), m. p. 69—70°, a mixed m. p. being 70—71°.

*Peroxidase-catalysed Oxidation of* 4-*Hydroxy*-3 : 5-*dimethylbenzyl Alcohol.*—The alcohol [8] (0·49 g.) in water (150 c.c.) was treated with peroxidase (4 c.c.; 0·6 unit/c.c.) and 20-vol. hydrogen peroxide (2 c.c.). A yellow colour appeared instantly, followed by a turbidity. After 30 min., more hydrogen peroxide (1 c.c.) was added, and at once small needles separated. This solid (0·23 g.) on recrystallisation from aqueous alcohol yielded white needles of 4-hydroxy-3 : 5-dimethylbenzaldehyde, m. p. and mixed m. p. 113—114·5°.

*Peroxidase-catalysed Oxidation of Guaiacol.*—Guaiacol was purified by distillation and the fraction of b. p. 92°/15 mm. collected : this had m. p. 28—28·75°. Sodium acetate (57·6 g. of monohydrate) and 3N-acetic acid (50 c.c.) were dissolved in water and diluted to 2·5 l.

Guaiacol (10 g.) was dissolved in the buffer (750 c.c.; pH 5) and oxidised by gradual addition of 20-vol. hydrogen peroxide (38 c.c.) and peroxidase (10—14 c.c.; 0·3 unit/c.c.) during 3½ days. An initial brown colour changed to red and then a red oil separated. As the oxidation proceeded a dark red solid settled, and was filtered off and dried (9·5 g.).

*Sublimation of oxidation product.* The solid melted and the vacuum was increased slowly to avoid bumping as degassing took place. At 140—160°/8 × $10^{-5}$ mm., a brown crystalline sublimate was obtained. This was resublimed and at 140—160°/2 × $10^{-5}$ mm. a yellow oil sublimed rapidly and crystallised when rubbed with ether. On filtration of the ethereal slurry, a white solid (20 mg.) of m. p. 124—127·5° remained. Recrystallisation from *cyclo*hexane yielded colourless plates of 2 : 2′-*dihydroxy*-3 : 3′-*dimethoxydiphenyl* (15 mg.), m. p. 141°, mixed m. p. 142·5—143° [Found : C, 68·8; H, 6·2%; *M* (Rast), 227—257. $C_{14}H_{14}O_4$ requires C, 68·3; H, 5·8%; *M* 246]. It was soluble in 10% aqueous alkali, being reprecipitated by dilute mineral acid. Concentrated sulphuric acid produced a red-brown colour, which became yellow and finally green. An aqueous solution gave a pale orange-red colour with hydrogen peroxide and peroxidase, or with ferric chloride in the presence of alcohol. With Gibbs's reagent, a blue colour was produced. The infrared absorption spectrum of the compound was identical with that of pure 2 : 2′-dihydroxy-3 : 3′-dimethoxydiphenyl.

*Attempted Preparation of* 6-*Iodoguaiacol.*—Guaiacol was converted into 6-nitroguaiacol [31] and then into 6-aminoguaiacol.[32] The amine (0·71 g.) was dissolved in water (5 c.c.), concentrated sulphuric acid (0·33 c.c.) added, and the solution cooled to 0° and diazotised by sodium nitrite solution (0·36 g. in 5 c.c.). Concentrated sulphuric acid (0·25 c.c.) was added and the mixture poured into potassium iodide (1 g.) in water (1 c.c.) at 0°. The mixture was warmed to 70° for 0·5 hr., some tar being produced. It was cooled and extracted with chloroform, and the extract was washed with sodium thiosulphate solution, then with water, and dried ($MgSO_4$). Evaporation yielded a yellow gum which was extracted with light petroleum (b. p. 60—80°),

[30] Fischer and Cammerloher, *Ber.*, 1901, **34**, 948.
[31] Klemenc, *Monatsh.*, 1912, **33**, 702.
[32] Oxford, *J.*, 1926, 2007.

and on evaporation brown crystals (0·184 g.) were obtained. Two sublimations at 80°/0·05 mm. gave orange prisms of *7-methoxybenz-1 : 2 : 3-oxadiazole*, m. p. 102° (Found: C, 55·6, 55·3; H, 4·4, 4·2. $C_7H_6O_2N_2$ requires C, 56·0; H, 4·0%).

*2-Methoxy-6-nitrophenyl Toluene-p-sulphonate.*—6-Nitroguaiacol (5 g.), heated with toluene-*p*-sulphonyl chloride (10 g.) in pyridine (15 c.c.) at 100° for ½ hr., gave the *toluene-p-sulphonate* (8·9 g., 93%), m. p. 95—95·5° (from methanol) (Found: C, 51·8; H, 4·3; N, 4·4. $C_{14}H_{13}O_6NS$ requires C, 52·0; H, 4·05; N. 4·3%).

*2-Amino-6-methoxyphenyl Toluene-p-sulphonate.*—The foregoing toluenesulphonate (4·8 g.) was treated in hot acetic acid (10 c.c.) with a warm solution of stannous chloride (12 g. of dihydrate) in concentrated hydrochloric acid (20 c.c.), then heated at 100° for 20 min. to complete reduction; the chlorostannate of the base usually crystallised. Excess of 30% sodium hydroxide solution was added, with cooling and stirring. The white residue was filtered off and dried. Crystallisation from methanol gave colourless prisms of *2-amino-6-methoxyphenyl toluene-p-sulphonate* (2·52 g., 57%), m. p. 117·5—118° (Found: C, 57·4; H, 5·2; N, 4·9. $C_{14}H_{15}O_4NS$ requires C, 57·3; H, 5·2; N, 4·8%).

*2-Iodo-6-methoxyphenyl Toluene-p-sulphonate.*—2-Amino-6-methoxyphenyl toluene-*p*-sulphonate (0·733 g.) was dissolved in warm concentrated sulphuric acid (0·2 c.c.), and water (5 c.c.) added; the mixture was cooled to 0° and diazotised with sodium nitrite solution (0·1725 g. in 5 c.c.). A red-brown solid separated. Concentrated sulphuric acid (0·1 c.c.) was added, followed by potassium iodide (0·6 g.) in water (2 c.c.). Gas was evolved and brown solid appeared. After 1 hr. at room temperature, the mixture was warmed to 100° to complete the reaction. The solution, containing tar, was extracted with ether; the extract was washed with sodium thiosulphate solution, water, sodium hydroxide solution, and water. Evaporation gave a brown gum which was extracted with hot light petroleum (b. p. 60—80°). The extract gave on evaporation a gum (200 mg.) which solidified on trituration with methanol. Filtration yielded a yellow solid (93 mg.) which was chromatographed in benzene on alumina, the colour being thus removed. The iodo-compound collected in the first benzene eluates, and on evaporation, crystals (85 mg.; m. p. 68—72°) were obtained. Recrystallisation from light petroleum (b. p. 60—80°) gave the *iodo-ester* as prisms (44 mg.), m. p. 84·5—85° (Found: C, 41·3; H, 3·3. $C_{14}H_{13}O_4SI$ requires C, 41·6; H, 3·2%).

*3 : 3′-Dimethoxy-2 : 2′-ditoluene-p-sulphonyloxydiphenyl.*—Impure iodo-compound (1·71 g.) was heated to 250°, and activated copper bronze [33] (2 g.) was added during 0·5 hr. with stirring. The mixture was then heated at 255° for 20 min., cooled, and extracted with hot methanol (A); the residue was then powdered in a mortar and extracted (Soxhlet) with methanol (B).

Extract A was evaporated, yielding brown amorphous solid (200 mg.), which was extracted with hot light petroleum (b. p. 60—80°) to remove unchanged iodo-compound. The residue, m. p. 140—180°, was adsorbed from benzene on neutral alumina. The column was eluted with benzene and 5 c.c. fractions were collected and evaporated, giving (i) 3·5 mg. the crude diphenyl derivative, m. p. 187—197°; (ii) 24·6 mg. which crystallised from methanol in prisms (14 mg.; m. p. 175—196°); (iii) 29·1 mg. which gave crystals (4 mg.; m. p. 190—200°) from *cyclo*hexane; (iv) material which crystallised from methanol in prisms (7 mg., m. p. 188—202°).

Extract B was evaporated, giving amorphous solid (180 mg.), m. p. 130—190°. This was crystallised twice from aqueous methanol, yielding colourless crystals (30 mg.), m. p. 202—206°. More diphenyl was obtained from the methanolic mother-liquor by chromatography on alumina followed by crystallisation from methanol. The yield of crude 3 : 3′-dimethoxy-2 : 2′-ditoluene-*p*-sulphonyloxydiphenyl was 65 mg. (5%).

The pure crystals from methanol were identical with the solid, m. p. 201—205°, obtained from 2 : 2′-dihydroxy-3 : 3′-dimethoxydiphenyl (prepared by an independent method, see below) and toluene-*p*-sulphonyl chloride.

*2 : 2′-Diacetoxy-5 : 5′-diformyl-3 : 3′-dimethoxydiphenyl.*—" Dehydrodivanillin " [34] (12 g.) was heated under reflux for 30 min. with acetic anhydride (60 c.c.) and anhydrous sodium acetate (3 g.). The solution was poured into ice-water; a brown oil which separated solidified on scratching (15 g., 98%). Crystallisation from methanol gave colourless crystals, m. p. 113·5—115° (Elbs and Lerch [25] gave m. p. 117°).

*2 : 2′-Diacetoxy-3 : 3′-dimethoxydiphenyl-5 : 5′-dicarboxylic Acid.*—The diacetyl-dialdehyde (10 g.) in pure acetone (30 c.c.) was oxidised by potassium permanganate (10 g.) in acetone (300 c.c.). After an hour the precipitate was filtered off and suspended in dilute sulphuric acid. Sodium hydrogen sulphite was added to remove manganese dioxide. The white residue was

[33] Kleiderer and Adams, *J. Amer. Chem. Soc.*, 1933, **55**, 4219.
[34] Cf. Tiemann, *Ber.*, 1885, **18**, 3943.

filtered off and washed with water (yield, 7·9 g., 73%). Crystallisation from ethanol gave colourless needles, m. p. 277—278° (decomp.) (Elbs and Lerch [25] gave m. p. 140°) (Found: C, 57·4; H, 4·6; OMe, 14·7; Ac, 21·9. Calc. for $C_{20}H_{18}O_{10}$: C, 57·4; H, 4·3; OMe, 14·8; Ac, 20·6%).

2 : 2′-*Dihydroxy*-3 : 3′-*dimethoxydiphenyl*-5 : 5′-*dicarboxylic Acid*.—The compound (2·2 g.) was refluxed for 1 hr. with 10% aqueous sodium hydroxide, then cooled and acidified with acetic acid. The white precipitate was filtered off, washed with hot ethanol, and dried (1·6 g., 91%). Crystallisation from glacial acetic acid gave clusters of fine needles, m. p. 306—308° (decomp.) (Elbs and Lerch gave m. p. 295°) (Found: C, 57·3; H, 4·4. Calc. for $C_{16}H_{14}O_8$: C, 57·5; H, 4·2%).

2 : 2′-*Dihydroxy*-3 : 3′-*dimethoxydiphenyl*.—2 : 2′-Dihydroxy-3 : 3′-dimethoxydiphenyl-5 : 5′-dicarboxylic acid (430 mg.) was heated to 300°; decomposition then began with evolution of carbon dioxide and blackening. After 20 min., the mixture was cooled and the solid extracted with warm dilute sodium hydroxide solution, which was acidified and extracted with ether. The latter was washed with sodium hydrogen carbonate solution and water and dried ($Na_2SO_4$). Concentration yielded almost colourless plates of 2 : 2′-*dihydroxy*-3 : 3′-*dimethoxydiphenyl* (136 mg., 43%) which crystallised from *cyclo*hexane as needles, m. p. 142·5—143° (Found: C, 68·6; H, 6·1. $C_{14}H_{14}O_4$ requires C, 68·3; H, 5·7%). The diphenyl gave a pale yellow-brown colour with concentrated sulphuric acid, slowly changing to brown. With aqueous-alcoholic ferric chloride solution it gave an orange-red colour. A red colour appeared in its aqueous solution on addition of peroxidase and hydrogen peroxide.

2 : 2′-*Diacetoxy*-3 : 3′-*dimethoxydiphenyl*.—2 : 2′-Dihydroxy-3 : 3′-dimethoxydiphenyl (88 mg.) was heated under reflux for 30 min. with acetic anhydride (5 c.c.) and anhydrous sodium acetate (0·5 g.). The *diacetate* (77 mg., 65%) separated from aqueous methanol as needles, m. p. 133—134° (Found: C, 65·8; H, 5·6. $C_{18}H_{18}O_6$ requires C, 65·5; H, 5·5%).

3 : 3′-*Dimethoxy*-2 : 2′-*ditoluene*-p-*sulphonyloxydiphenyl*.—The diphenol (50 mg.) was heated at 100° with excess of toluene-*p*-sulphonyl chloride in pyridine for 0·5 hr. The mixture was poured into water, and the oil formed was extracted with ether. The extract was washed with dilute hydrochloric acid, sodium carbonate, and sodium hydroxide solutions, and on evaporation gave a gum which crystallised on addition of methanol. Crystallisation from methanol gave 3 : 3′-dimethoxy-2 : 2′-ditoluene-*p*-sulphonyloxydiphenyl (3 mg.), m. p. 201—205°.

*Reductive Acetylation of the Guaiacol Oxidation Product*.—The product (10·6 g.) was dissolved in acetic anhydride (50 c.c.), giving a deep red solution. Zinc dust (11 g.) and sodium acetate (2 g.) were added, the solution becoming nearly colourless. The reaction was completed under reflux (30 min.). Acetic acid was added and the mixture was boiled and filtered hot, the residue being washed with hot acetic acid. The combined filtrates were poured into cold water; a white solid separated. The yield of mixed acetates, m. p. 75—95°, was 13 g.

The mixture (1·22 g.) was sublimed in a long Pyrex tube. At $5 \times 10^{-5}$ mm., white crystals sublimed at 180—220°, and at 270° a yellow oil distilled (not examined). The solid, m. p. 90—160°, was purified by repeated fractional sublimation and then crystallised from aqueous methanol as needles (11·3 mg.), m. p. 195·5—197·5° (Fichter and Dietrich [23] gave m. p. 198° for 4 : 4′-diacetoxy-3 : 3′-dimethoxydiphenyl) [Found: C, 65·6; H, 5·8; OMe, 19·5; Ac, 29·4%; *M* (Rast), 340. Calc. for $C_{18}H_{18}O_6$: C, 65·5; H, 5·5; OMe, 18·8; Ac, 26·1%; *M*, 330].

*Durenol*.—Duridine [35] (7 g.) was dissolved in warm concentrated sulphuric acid (7 c.c.) and water (20 c.c.). Water (60 c.c.) was then added and the solution cooled to 5° and diazotised by slow addition of sodium nitrite solution (3·5 g. in 15 c.c.). The solution was finally warmed to 100° for 20 min.; a pale yellow precipitate of durenol (6·9 g., 98%) separated and was purified by steam-distillation, followed by 2 crystallisations from light petroleum (b. p. 60—80°). The pure durenol was obtained in silvery plates, m. p. 116—117°.

*Peroxidase-catalysed Oxidation of Durenol*.—Durenol (600 mg.) was dissolved in hot distilled water (2 l.). On cooling rapidly, part of the durenol crystallised in minute needles. Peroxidase (30 c.c.; 0·6 unit/c.c.) and 20-vol. hydrogen peroxide (26 c.c.) were added in small amounts, at intervals, during 5 days. No colour changes were observed. The solution was filtered and the precipitate (308 mg.) and filtrate were examined.

The dry precipitate (m. p. 106—160°; 240 mg.) was sublimed at 70—80°/40—50 mm. Unchanged durenol (100 mg.) was recovered and the residue crystallised from aqueous methanol and then from light petroleum (b. p. 60—80°), giving colourless prisms (41 mg.) of 4 : 4′-*dihydroxy*-2 : 3 : 5 : 6 : 2′ : 3′ : 5′ : 6′-*octamethyldiphenyl*, m. p. 197—198·5° [Found: C, 80·7; H, 8·6%;

[35] Birtles and Hampson, *J.*, 1937, 10.

*M* (Rast), 300. $C_{20}H_{26}O_2$ requires C, 80·5; 8·8%; *M*, 298]. The *diacetate* (30·4 mg.), prepared by use of acetic anhydride and sodium acetate and crystallised from methanol, had m. p. 179—180° (Found: C, 75·4; H, 8·2. $C_{24}H_{30}O_4$ requires C, 75·3; H, 7·9%).

The filtrate was extracted with ether. Evaporation of the dried extracts yielded a gum (274 mg.). Sublimation at 80—90°/40—50 mm. gave yellow crystals (2 mg.; m. p. 105—107°) with a quinone-like smell. This compound was duroquinone, as shown by comparison of the infrared absorption spectrum with that of authentic duroquinone. An *X*-ray diffraction photograph gave the length of one side of the unit cell as 6·9 Å, the same as that of the authentic quinone.

We are grateful to the D.S.I.R. for a maintenance grant (to H. B.).

UNIVERSITY CHEMICAL LABORATORY, CAMBRIDGE. [*Received, March 24th*, 1955.]