466. *Studies in Peroxidase Action. Part VI.** (a) *The Oxidation of* p-*Anisidine : a Further Example of Ready Demethoxylation.* (b) *Some Theoretical Considerations.*

By D. G. H. Daniels and B. C. Saunders.

The oxidation of *p*-anisidine by hydrogen peroxide in the presence of peroxidase at pH 4·5 is described. From the crude solid oxidation product, 2-amino-5-*p*-anisidinobenzoquinone di-*p*-methoxyphenylimine and tetramethoxyazophenine have been obtained crystalline. During the oxidation, the methoxyl group is eliminated as methyl alcohol.

Some suggestions for the mechanism of the peroxidase oxidation of amines are put forward.

In Part IV of this series (Saunders and Watson, *Biochem. J.*, 1950, **46**, 629) we reported that 4-methoxy-2 : 6-dimethylaniline was readily oxidised to 3 : 5-dimethylbenzoquinone 1-(4-methoxy-2 : 6-dimethyl)anil. This involved the elimination of a methoxyl group which appeared as methyl alcohol in the product. We have now examined the peroxidase oxidation of the simpler *p*-anisidine. It might have been expected that the products would have been exactly analogous in constitution to those obtained by the peroxidase oxidation of *p*-toluidine (Saunders and P. J. G. Mann, *J.*, 1940, 769). This was not so, nor was the reaction parallel with the oxidation of 4-methoxy-2 : 6-dimethylaniline, but the information gained with the latter substrate proved a valuable guide in elucidating the structure of the compounds obtained from *p*-anisidine.

There is no reference in the literature to the enzymic oxidation of *p*-anisidine. In fact, very little precise work on its oxidation, even by chemical means, has been recorded. Colorations with ferric chloride, chromic anhydride, and hypochlorous acid in acid solution have been mentioned, but no serious attempt has been made to investigate their nature. Bromine-water at low temperatures gives blue and violet compounds and then *p*-benzoquinone (Wieland, *Ber.*, 1910, **43**, 714). Peroxymonosulphuric acid oxidises *p*-anisidine to *p*-nitrosoanisole contaminated with *p*-nitroanisole (Baeyer and Knorr, *Ber.*, 1902, **35**, 3034).

For our reaction, a highly purified specimen of peroxidase was kindly supplied by Prof. Keilin and Dr. E. P. Hartree. This was diluted and in conjunction with hydrogen peroxide was used to oxidise a 2% solution of *p*-anisidine in dilute acetic acid. A deep violet colour was produced immediately, within two minutes there was a reddish-brown turbidity, and a red-brown solid gradually separated. Further additions of hydrogen peroxide and the enzyme were made intermittently and the reaction was stopped when about 70% of the *p*-anisidine had been converted into insoluble products, as further reaction tended to produce tar.

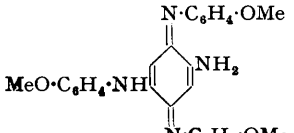
Ether dissolved 95% of the solid oxidation product. By chromatography of a benzene solution of the solid obtained on concentration of the solution, three compounds were isolated : (*a*) 4 : 4′-dimethoxyazobenzene in very small quantity; (*b*) dark red needles, m. p. 236°, hereinafter called compound (II)—this represented 5% of the crude oxidation product; (*c*) lustrous cerise-red leaflets, m. p. 164°, hereinafter called compound (I), and representing *ca.* 80% of the crude oxidation product.

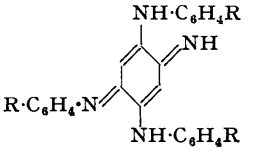
Elementary analysis and molecular-weight determination showed compound (I) to be $C_{27}H_{26}O_3N_4$, and determination of methoxyl groups and quantitative acetylation gave the partial formula $C_{24}H_{15}N_3(OMe)_3{\cdot}NH_2$. It thus appears that it is produced by condensation of four molecules of *p*-anisidine with the elimination of one methoxyl group. Hence, as we have previously obtained quinone anils by peroxidase oxidations, we suggest that compound (I) is 2-amino-5-*p*-anisidinobenzoquinone di-*p*-methoxyphenylimine, which has not previously been described. Quinone anils are often hydrolysed by mineral acids, but this did not occur in a simple manner with compound (I). With boiling dilute sulphuric acid, a complex mixture resulted, and with concentrated mineral acids an intense violet colour was produced, due presumably to the cation derived from (I).

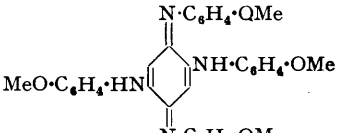
Elementary analysis and molecular-weight determinations showed compound (II) to be $C_{34}H_{32}O_4N_4$ which is derived from five *p*-anisidine residues with the elimination of one methoxyl and one amino-group. Moreover, it contained 4 methoxyl groups per mol. If the structure

* Part V, *J.*, 1950, 3519.

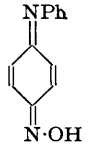
assigned to compound (I) is correct, then the most probable structure for (II) is 2 : 5-di-*p*-anisidinobenzoquinone di-*p*-methoxyphenylimine (or tetra-*p*-methoxyazophenine), *i.e.*, the NH_2 group of (I) is replaced by $NH{\cdot}C_6H_4{\cdot}OMe$. If so, then it should be possible to convert (I) into (II) by reaction with *p*-anisidine. This was found to be the case; the yield of compound (II) was good and ammonia was set free as expected.

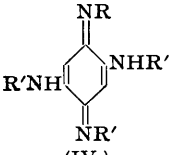

(I.)


(Ia.)


(II.)

Tetra-*p*-methoxyazophenine was first reported by Busch and Bergman (*Z. Farb. Text.*, 1905, **4**, 113), who prepared it by heating 4 : 4′-dimethoxydiazoaminobenzene, $MeO{\cdot}C_6H_4{\cdot}NH{\cdot}N{:}N{\cdot}C_6H_4{\cdot}OMe$, with *p*-anisidine and hydrochloric acid. They gave m. p. 242°. On repeating their work we found that the m. p. could not be raised above 236° even after repeated recrystallisations. However, the product which we obtained was identical with our oxidation product (II). This method of preparation does not demonstrate the structure unequivocally and we therefore sought an alternative and unambiguous synthesis of (II). Fischer and Hepp (*Ber.*, 1888, **21**, 667) heated together *p*-nitrosodiphenylamine [presumably acting in the tautomeric oxime form (III)], *p*-chloroaniline and hydrochloric acid, and obtained the trichloroazophenine (IV; R = Ph, R′ = C_6H_4Cl). On the other hand, using *p*-toluidine in place of *p*-chloroaniline, they obtained tetramethylazophenine (IV; R = R′ = C_6H_4Me) by a reaction in which the anil group must have been replaced by the *p*-toluidino-group (Fischer and Hepp, *Ber.*, 1887, **20**, 2480). By heating together *p*-nitrosodiphenylamine, *p*-anisidine, and hydrochloric acid we effected, in an analogous manner, the synthesis of a substance identical with our compound (II). The structures suggested above for (II) is thereby confirmed, and hence that of (I).


(III.)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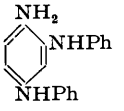
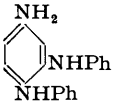

(Va.)


(Vb.)

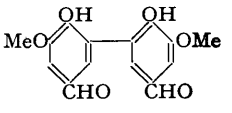
Precise Structure of Compound (I).—Compound (I) can alternatively be written in the imino-form (I*a*; R = OMe). The structure of the corresponding compound (I*a*; R = H) was discussed by Goldschmidt and Wurzschmitt (*Ber.*, 1922, **55**, 3226) who preferred (I*a*) for two reasons: (i) its formation from *p*-quinone imine anil and aniline, (ii) loss of ammonia when hydrolysed by mineral acids. Reaction (i) proceeds presumably by the addition of aniline, oxidation of the resulting aminodianilinobenzene (V*a* and V*b*) to a quinonoid compound, and repetition of the process. The fact that (ii) occurs only in the presence of mineral acid indicates that salt formation precedes hydrolysis. Thus in reactions (i) and (ii) an imino-group would in any case acquire a second hydrogen atom, *i.e.*, (I) and (I*a*) are indistinguishable by the above chemical tests.

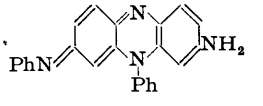
Discussion.—The following is a summary of peroxidase-catalysed reactions in which the products have been isolated and their structures elucidated.

Substrate.	Products.
Vanillin (Bourquelot and Marchadier, *Compt. rend.*, 1904, **138**, 1432)	Divanillin (VI)
Pyrogallol (Willstätter and Heiss, *Annalen*, 1923, **433**, 17)	Purpurogallin (for constitution see Barltrop and Nicholson, *J.*, 1948, 116; Haworth, Moore, and Pauson, *ibid.*, p. 1046)
o-Phenylenediamine [Chodat, Abderhalden's "Handbuch," 1925, (4), **1**, 319]	2 : 3-Diaminophenazine

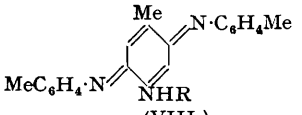
Substrate.	Products.
Catechol plus aniline (Pugh and Raper, *Biochem. J.*, 1927, **21**, 1378)	4 : 5-Dianilino-*o*-benzoquinone
Aniline (P. J. G. Mann and Saunders, *Proc. Roy. Soc.*, 1935, *B*, **119**, 47)	2 : 5-Dianilinobenzoquinone imine anil (I*a*, R = H), *pseudo*-mauvine (VII), indulines-3B and -6B, and aniline-black
p-Toluidine (Saunders and P. J. G. Mann, *J.*, 1940, 769)	4-Amino-2 : 5-toluquinone di-*p*-tolylimine (VIII; R = H), 4-*p*-toluidino-2 : 5-toluquinone di-*p*-tolylimine (VIII; R = C_6H_4Me), 4 : 4′-dimethyldiphenylamine, a small quantity of 4 : 4′-dimethylazobenzene, and traces of 4-amino-2 : 5-toluquinone 2-*p*-tolylimine (IX; R = H) and 4-*p*-toluidino-2 : 5-toluquinone 2-*p*-tolylimine (IX; R = C_6H_4Me)
p-Cresol (Westerfield and Lowe, *J. Biol. Chem.*, 1942, **145**, 463)	2 : 2′-Dihydroxy-5 : 5′-dimethyldiphenyl, the corresponding triphenyl, and a furan derivative
Mesidine (Chapman and Saunders, *J.*, 1941, 496)	3 : 5-Dimethylbenzoquinone 1-(2 : 4 : 6-trimethyl)-anil (X; R = Me)
Dimethylaniline (Naylor and Saunders, *J.*, 1950, 3519)	*NNN′N′*-Tetramethylbenzidine and its oxidation products
4-Methoxy-2 : 6-dimethylaniline (Saunders and Watson, *Biochem. J.*, 1950, **46**, 629)	3 : 5-Dimethylbenzoquinone 1-(4-methoxy-2 6-dimethyl)anil (X; R = OMe)
Mesitol (Booth and Saunders, *Nature*, 1950, **165**, 567)	2 : 6-Dimethylbenzoquinone and 4-hydroxy-3 : 5-dimethylbenzaldehyde

(VI.)

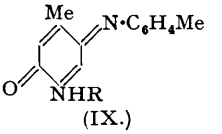




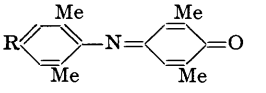
(VII.)



(VIII.)



(IX.)

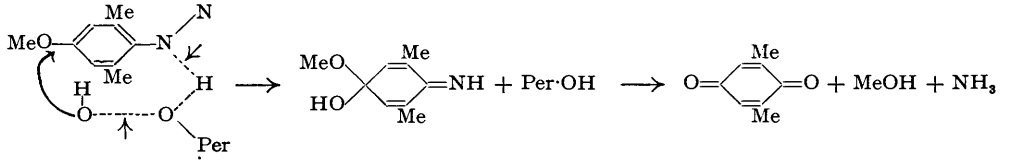


(X.)

Two points should be noted : (i) Primary amines give products mainly of the quinone imine type. A precursor of the general type PhN:C_6H_4:NH (*o*- and *p*-) could account for these products by amine additions or hydrolysis. The possibility of such an intermediate has long been recognised (Goldschmidt and Wurzschmitt, *loc. cit.*). (ii) Robinson (*J.*, 1941, 220) has pointed out the close analogy between the oxidation of amines and phenols and the benzidine transformation, which is essentially a cationoid-type reaction (Dewar, " Electronic Theory of Organic Chemistry," p. 239). The oxidations may involve similar cationoid attack on the free amine or phenol. On the other hand, in instances where peroxidase products can also be obtained by inorganic oxidising agents there are no fixed requirements of pH, suggesting that neutral free radicals may be involved. These oxidations have usually been discussed on this basis in the past.

So far in this series of papers on peroxidase oxidation of amines, we have postulated two distinct radical intermediates : (i) ArNH· (or ArN:) formed by dehydrogenation (Saunders and P. J. G. Mann, *J.*, 1940, 769) and (ii) HO· derived from the hydrogen peroxide (Saunders and Watson, *Biochem. J.*, 1950, **46**, 629). The first kind was suggested by Goldschmidt (*Ber.*, 1920, **53**, 35), who oxidised ethereal solutions of amines by lead dioxide to compounds of the type PhN:C_6H_4:NH and PhN:NPh. Coupling of the radicals in pairs could give rise to both these products, since the odd electron can be localised either on the nitrogen or on the *o*- and *p*-carbon atoms. If this kind of radical-coupling is a step in peroxidase oxidations, the almost complete absence of azo-compounds from the products is difficult to understand. The hypothesis that, under these conditions, the radical attacks an unchanged amine molecule would avoid this difficulty, since at any one moment (because of the low concentration of the catalyst) the primary dehydrogenation product must be surrounded by an excess of unchanged amine molecules. It is not clear whether such a resonance-stabilised radical possesses sufficient energy to do this.

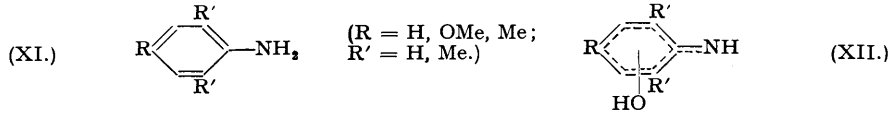
To explain the oxidation of 4-methoxy-2 : 6-dimethylaniline we have recently (Saunders and Watson, *loc. cit.*) suggested the second kind of mechanism. An activated complex containing the substrate and hydrogen peroxide could give rise to a quinone, ammonia, and methanol. It is noteworthy that, in this scheme, we can replace hydrogen peroxide by the secondary peroxidase–hydrogen peroxide complex, Per·OOH (cf. Chance, *Arch. Biochem.*, 1949, **22**, 224). The original enzyme Per·OH is then regenerated as follows :



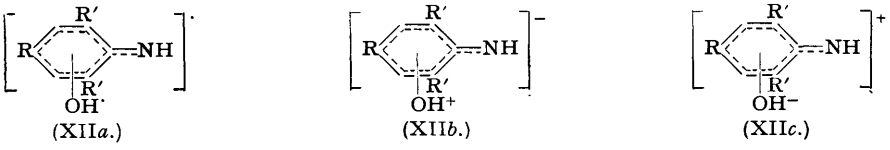
In this scheme it is not necessary to picture the HO radical as being free, *i.e.*, existing in a solution of hydrogen peroxide and peroxidase in the absence of substrate.

Proposed Generalised Scheme for Peroxidase Oxidation.—As it stands, the above scheme does not appear to be applicable to all cases, since it produces a quinone with at least one carbonyl group free, and it has been shown (Suida and Suida, *Annalen*, 1918, **416**, 134) that such quinones do not usually produce anils when they react with amines under mild conditions. The following modifications are suggested.

Let us assume that the oxidation of an aromatic amine (XI) takes place with simultaneous removal of a hydrogen atom and attachment of hydroxyl radical as in Saunders and Watson's

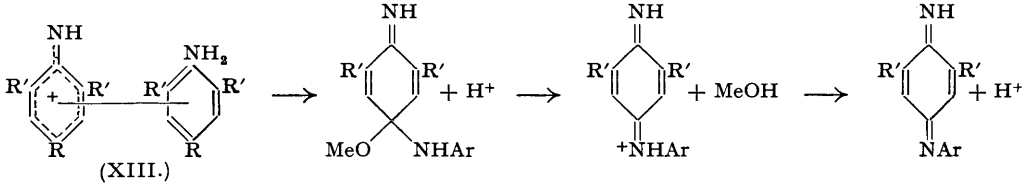


scheme. The resultant loose combination of the two radicals may be represented in the π-complex notation as (XII), of which the canonical forms are (XII, *a*, *b*, *c*). Of these, one would expect (XII*c*) to be favoured, especially when the amine carries substituents such as OMe (−*E*) which stabilise a cation. An anionoid replacement of the hydroxyl ion of this form by a molecule of unchanged amine, giving a new complex (XIII), should lead to a release of energy

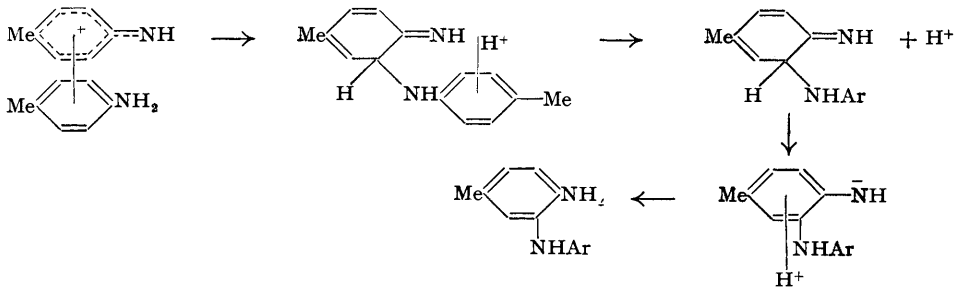


owing to the possibility of increased resonance. The NH^+ group in the cationoid half of this is of powerfully $+E$ type; therefore there is likely to be attack upon the positions *ortho* and *para* to it by anionoid entities, and in particular by the anionoid half of the complex. The mode of rearrangement may vary according to the substituent present.

(i) R = OMe, R′ = Me or H. Here R can form a stable anion, so it undergoes simple replacement (cf. *p*-chloronitrobenzene and *p*-methoxynitrobenzene).



(ii) R = Me or H, R′ = H. Here neither R nor R′ can form a stable anion. Rearrangement leading to an aminodiphenylamine may occur. With *p*-toluidine, one of the *o*-positions will be attacked, as H has less $-I$ effect than Me :



The resultant aminodiphenylamine would undoubtedly have a lower oxidation–reduction potential than the parent amine, and thus would be readily oxidised to the quinoneimine anil assumed to be the precursor of the peroxidase products.

(iii) R = R′ = Me. Here, if the complex (XIII) is formed, the course of its rearrangement is obscure. If the *p*-methyl group is ejected as CH_3^- then methane should be produced : if as CH_3^+ then it might combine with HO^- to form methanol. This point (Chapman and Saunders, *J.*, 1941, 496) is not yet decided.

The complex (XIII) is identical with the one believed to be the essential intermediate in the rearrangement of hydrazobenzene to benzidine (Dewar, " Electronic Theory of Organic Chemistry," p. 237). It should be noted, however, that the benzidine rearrangement is carried out in reducing or indifferent conditions whereas the peroxidase system will favour electron withdrawal from the substrates. The relative potential energies of the orientations of (XIII) leading to benzidine, diphenyline, and semidine, under the conditions of the benzidine rearrangement, are not necessarily those which apply to our conditions. It should be noted that the oxidation–reduction potentials of benzidine and *p*-aminodiphenylamine are $E_0^{25} = 921$ mv. and 751 mv., respectively, indicating that *p*-aminodiphenylamine is more easily oxidised than benzidine. It must be emphasised that we are not suggesting that both these substances are actually produced, but rather that the orientations of (XIII) corresponding to these substances possess potential energies proportional to the above values of E_0^{25} under the prevailing oxidising conditions.

It is noteworthy that where aminodiphenylamine formation is prevented, as in the oxidation of dimethylaniline (Naylor and Saunders, *J.*, 1950, 3519), the oxidation product is a substituted benzidine.

Conclusion.—The theory discussed above depends upon ionic reactions, although free-radical steps are involved. This view may require modification when more experimental and theoretical evidence becomes available. At present we suggest that the essential steps are : (i) Formation of the secondary complex Per·OOH between peroxidase and hydrogen peroxide. (ii) Reaction of this with one amine molecule, leading to its simultaneous dehydrogenation and hydroxyl radical addition, possibly forming a π-complex (XII). (iii) Anionoid replacement of HO^- by a molecule of unchanged amine in favourable circumstances, giving the complex (XIII). (iv) Rearrangement of (XIII) to an *N*-aryl-quinone di-imine either directly or *via* an aminodiphenylamine. (v) Amine addition to the quinone di-imine if this is not sterically hindered, otherwise hydrolysis of the imino-group.

Experimental.

Oxidation of p-*Anisidine.*—The concentrated solution of peroxidase as supplied by Prof. Keilin and Dr. Hartree had an activity of 19·4 units/ml., and a P.N. of 1310. This was diluted with distilled water to an activity of 0·12 unit/ml. before use. Anisidine was distilled under reduced pressure and then recrystallised from benzene as colourless needles, m. p. 57°. The purified amine (10 g.) was dissolved in glacial acetic acid (11·5 ml.) and diluted with water (500 ml.), giving a solution of pH *ca.* 4·5. To this were added hydrogen peroxide (1 ml., 20-vol.) and peroxidase solution (4 ml.). An intense violet colour was produced and within 2 minutes there was a reddish-brown turbidity in the bulk of the solution, but the violet colour persisted for as long as 6 hours in isolated drops on the side of the flask, where the enzyme was in large excess. Hydrogen peroxide was added at the rate of 1 ml. per 45 minutes until in all 40 ml. had been added. Further quantities of enzyme (2 ml.) were added every 3 hours. A red-brown solid gradually separated and this was filtered off at the end of the reaction; its yield was 7 g.

Treatment of the Solid Oxidation Product.—The product (10 g.) was extracted in a Soxhlet apparatus successively with light petroleum (b. p. 40—60°), ether, ethanol, and acetone, the residue from one solvent being extracted by the next. The quantities removed by each solvent were respectively 0·1, 9·5, 0·3, 0·1 g. It was shown chromatographically that there was no significant difference in the composition of the light petroleum and ether extracts, so the former extraction was omitted in subsequent experiments.

Chromatography of the Ethereal Extract.—The ethereal extract was concentrated, and the solid which separated was dissolved in benzene and examined on alumina. The following principal bands were obtained: (*a*) yellow, (*b*) red, (*c*) brownish-red. Higher up the column, subsidiary bands appeared thus: (*d*) brown, (*e*) crimson, (*f*) pale violet, (*g*) brown, (*h*) brown, (*i*) black.

The lowest band (*a*) gave *ca.* 10 mg. (*i.e.*, from 10 g. of crude oxidation product) of a yellow substance of m. p. 145—160°, not obtained pure. However, it gave a red colour with concentrated sulphuric acid identical with that obtained with authentic 4 : 4′-dimethoxyazobenzene, m. p. 164°. Furthermore, a mixture of the yellow substance and 4 : 4′-dimethoxyazobenzene could not be separated into two components by chromatographic methods.

Compound (II).—Evaporation of band (*b*) gave a red solid which recrystallised from toluene as dark red needles, m. p. 235—236° (Found: C, 72·9; H, 5·62; N, 9·8; OMe, 21·0%; *M*, semimicro-ebullioscopic in ethylene dibromide, 590±30. Calc. for $C_{34}H_{32}O_4N_4$: C, 72·9; H, 5·71; N, 10·0; 4OMe, 22·1%; *M*, 560). The compound gave a violet colour, changing to blue on warming, with concentrated sulphuric acid, and could not be acetylated with acetic anhydride. Its mixed m. p. with authentic tetra-*p*-methoxyazophenine, prepared as described below, was 236°. A mixture of (II) and the authentic azophenine in benzene was not resolvable chromatographically on alumina.

Compound (I).—Evaporation of band (*c*) gave 2-*amino*-5-p-*anisidinobenzoquinone di*-p-*methoxyphenylimine*, lustrous cerise-red leaflets which, recrystallised from alcohol, had m. p. 164° (Found: C, 71·5; H, 5·76; N, 12·2; OMe, 20·4%; *M*, cryoscopic in ethylene dibromide, 450±25. $C_{27}H_{26}O_3N_4$ requires C, 71·3; H, 5·73; N, 12·33; 3OMe, 20·5%; *M*, 454). The compound gave an intense violet colour, changing to blue on warming, with concentrated sulphuric, hydrochloric, and nitric acids. It was recovered unchanged after treatment with 70% alcoholic sulphuric acid for 1 hour at room temperature, and tar resulted from boiling with 70% aqueous sulphuric acid. It was decolorised by reduction with zinc and acetic acid, the colour being rapidly restored by shaking with air.

Acetyl derivative. (*a*) The foregoing amino-compound was dissolved in excess of acetic anhydride and heated on a steam-bath for 10 minutes. The product solidified and the *acetyl* compound, recrystallised from acetone as dark red needles, had m. p. 213° (Found: C, 70·5; H, 6·02; N, 11·3. $C_{29}H_{28}O_4N_4$ requires C, 70·2; H, 5·64; N, 11·3%).

(*b*) The amino-compound (0·137 g.) was acetylated with a standardised mixture of acetic anhydride and pyridine, the excess of the anhydride being then titrated with 0·25N-sodium hydroxide solution. The quantity of acetic anhydride used in the acetylation was 0·036 g., and was thus equivalent to 1·1 ± 0·1 amino-groups per mol. The acetyl derivative from this experiment had m. p. 203—205° and was a slightly impure form of the acetyl derivative prepared as above.

Benzoyl derivative. Benzoylation could not be effected by the Schotten–Baumann method. When, however, the amino-compound was triturated with benzoyl chloride for 5 minutes in the cold and the excess of the acid chloride was removed with 10% sodium hydroxide solution, the *benzoyl* derivative was obtained; recrystallised from benzene as red-brown needles, it had m. p. 203—204° (Found: N, 10·6. $C_{34}H_{30}O_4N_4$ requires N, 10·0%).

Examination of the Filtrate from the Solid Oxidation Product.—The entire filtrate (3 l.) from the oxidation of 50 g. of *p*-anisidine was distilled through a Fenske column. A first fraction of 50 ml. (b. p. 98·5—99·5°) was collected. The presence of methanol in this solution was established as follows: (*a*) The solution (5 ml.) was mixed with potassium permanganate solution (2·5 ml.; 2%) and dilute sulphuric acid (5 ml.), and distilled into Schiff's reagent (5 ml.); a magenta coloration showed the production of formaldehyde. Control experiments without the solution, and with dilute methanol itself were carried out. (*b*) The solution (10 ml.) was mixed with sodium hydroxide solution (10 ml.; 10%) in a small mortar. Powdered 3 : 5-dinitrobenzoyl chloride (1 g.) was added, and the mixture ground together for 2 minutes, filtered off, and recrystallised from light petroleum; the m. p. and mixed m. p. with authentic methyl 3 : 5-dinitrobenzoate was 109°. (*c*) Methyl *p*-nitrobenzoate was similarly prepared, the m. p. and mixed m. p. with authentic specimen being 96°.

2 : 5-*Di*-p-*anisidinobenzoquinone Di*-p-*methoxyphenylimine. Tetra*-p-*methoxyazophenine.*—(*a*) 4 : 4′-Dimethoxydiazoaminobenzene (1·3 g.; prepared by Henriques's method, *Ber.*, 1892, **25**, 3064), *p*-anisidine (3 g., 5 mols.), and a few drops of concentrated hydrochloric acid were heated together at 100° for 9½ hours. The product was cooled and washed with water and then ethanol, and a residue (0·12 g.) of a dark red solid was left. This was recrystallised three times from toluene and had m. p. 235—236°, unchanged on admixture with compound (II) derived from the oxidation product.

(*b*) Pure *N*-nitrosodiphenylamine (34·5 g.) was subjected to the Fischer–Hepp transformation by passing dry hydrogen chloride into a suspension in alcohol. The *p*-nitrosodiphenylamine hydrochloride (32 g.) was obtained without first isolating the free base. An intimate mixture of the hydrochloride (3 g.) and *p*-anisidine (6 g.) was heated to 100° for 8 hours. The dark, tarry product was cooled and washed successively with water and ethanol, leaving a dark red crystalline residue (3·25 g., 43%). This azophenine was recrystallised four times from toluene and then had m. p., and mixed m. p. with compound (II) from the oxidation product, 235°. The synthetic azophenine and compound (II) were not separated when the benzene solution was subjected to chromatography on alumina.

Synthesis of Compound (II) *from Compound* (I).—An intimate mixture of compound (I) (0·45 g.), *p*-anisidine, and a few drops of concentrated hydrochloric acid was heated to 100° for 8 hours. After the cooled mass had been washed, a dark red solid was again obtained (0·34 g.; 60%), as described in the foregoing experiment. Recrystallised from toluene, it had m. p. 235°. The aqueous washings were made strongly alkaline with sodium hydroxide solution and, on warming, ammonia was evolved.

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