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STUDIES IN PEROXIDASE ACTION—XX*

THE OXIDATION OF A MIXTURE OF *p*-TOLUIDINE AND 4-CHLOROANILINE

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Abstract—The peroxidase catalysed oxidation of a mixture of *p*-toluidine and 4-chloroaniline gives rise to a large number of products. The major products, aminoanilinobenzoquinone dianils, are all derived from N-*p*-tolylbenzoquinone diimine. Perkin bases derived from 4,4′-dimethyldiphenylamine, azobenzenes and azophenines are also produced. The mechanism of the formation of the products is discussed.

IN VIEW of recent work[1, 2] on the peroxidase catalysed oxidations of *p*-toluidine and of 4-chloroaniline, and the differences in the structures of the compounds obtained and their mechanism of production, it is important to investigate the oxidation of a mixture of these amines. Previous work on the oxidation of amine mixtures by this system has shown[3] that in the case of aniline and *p*-toluidine none of the compounds obtained from the oxidation of the two amines separately is observed, except for slight traces of 4,4′-dimethylazobenzene. Instead, compounds of "intermediate" structure (e.g. the dianil I) are obtained. No other references have been made to such oxidations by the peroxidase system.

The peroxidase catalysed oxidation of a solution containing an equimolar mixture of *p*-toluidine and 4-chloroaniline gave rise to a tarry brown solid. Examination by TLC showed this solid to contain at least fourteen components. Column chromatography (alumina) enabled the components to be separated into the following classes (in order of elution). Each of these was further analysed as indicated.

(a) *Azobenzenes:* constituting 10% of the product by weight. Further chromatography of this fraction showed it to have the following composition: 4,4′-dichloroazobenzene 13·5%, 4-chloro-4′-methylazobenzene 32·5% and 4,4′-dimethylazobenzene 54%.

(b) 4,4′-*Dimethyldiphenylamine:* constituting less than 0·1% of the product by weight.

(c) *Azophenines:* constituting 1% of the product by weight. A deep red crystalline solid was obtained, which had a sharp melting point and the physical properties of an azophenine. Microanalysis showed it to be a mixture of compounds of intermediate composition, containing an average of approximately 1·5 Cl atoms and 2·5 Me groups per molecule. The solid proved unresolvable on TLC.

(d) 4-*p-Toluidino-2,5-toluquinone dianils* (so-called Perkin bases); constituting 15% of the product by weight. Further chromatography showed this fraction to contain 4-

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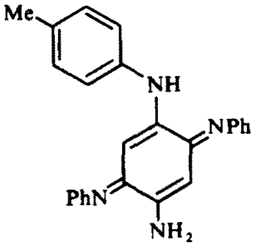
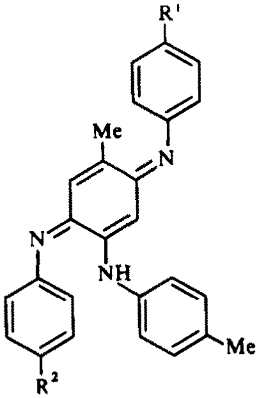
p-toluidino-2,5-toluquinonebis-*p*-tolylimine (II), 4-*p*-toluidino-2,5-toluquinone di-4-chloroanil (III), and the two anils of "intermediate" composition (IV and V).

(e) *Aminoanilinobenzoquinone dianils:* constituting 30% of the product by weight. Further chromatography showed this fraction to contain 2-amino-5-*p*-toluidinobenzoquinone di-4-chloroanil (VI), 2-amino-5-*p*-toluidinobenzoquinonebis-*p*-tolylimine (VII), and the two anils of "intermediate" composition (VIII and IX).

(f) A very slow running tarry material constituting 44% of the product by weight. No compounds could be isolated from this fraction. It appeared to be polymeric and has not been further investigated.



I

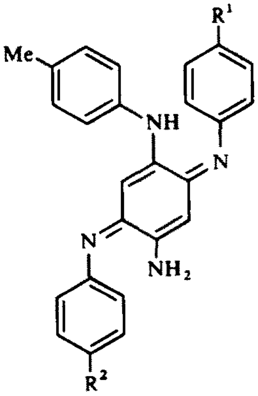


II : $R^1 = R^2 = Me$

III : $R^1 = R^2 = Cl$

IV : $R^1 = Cl$, $R^2 = Me$

V : $R^1 = Me$, $R^2 = Cl$



VI : $R^1 = R^2 = Cl$

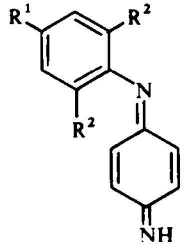
VII : $R^1 = R^2 = Me$

VIII : $R^1 = Cl$, $R^2 = Me$

IX : $R^1 = Me$, $R^2 = Cl$

Chloride ion was produced to the extent required by the weights of aminoanilinobenzoquinone dianils and azophenines formed.

The main feature of the reaction is the high yield of aminoanilinobenzoquinone dianils. These are believed to be formed by oxidative coupling between two molecules of the parent amine and a molecule of N-arylbenzoquinone diimine (X). The diimine itself is formed from nucleophilic attack by a molecule of the parent amine on the 4-position of the enzyme complexed cation XI, followed by elimination of hydrogen chloride. The cation itself is generated by removal of two electrons and a proton by the peroxidase system from a molecule of 4-chloroaniline. In the oxidation of 4-chloroaniline alone the product is 2-amino-5-chloroanilinobenzoquinone di-4-chloroanil (XII),[4] formed via the intermediate N-(4-chlorophenyl)benzoquinone diimine (Xa).

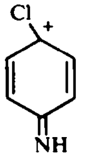


X

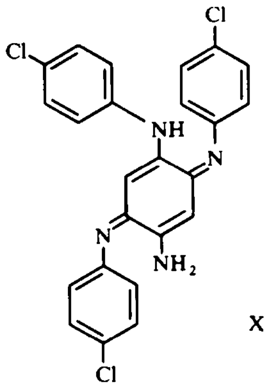
Xa : R^1 = Cl, R^2 = H

Xb : R^1 = Me, R^2 = H

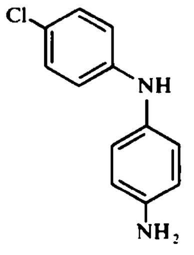
Xc : R^1 = R^2 = Me



XI



XII



XIII

The anils obtained from the oxidation of the amine mixture are all derived from N-*p*-tolylbenzoquinone diimine (Xb). No derivatives of the diimine Xa have been detected, and it may therefore be assumed that Xa is not produced. Further proof of this is afforded by the fact that the addition of N-(4-chlorophenyl)*p*-phenylenediamine (XIII, readily oxidised to the diimine Xa by the peroxidase system) to the oxidation of *p*-toluidine gives rise to an aminoanilinobenzoquinone dianil whose TLC behaviour is different from that of any of those dianils produced in the oxidation of the amine mixture.

One would predict that the diimine Xb would be produced in preference to Xa in the mixed oxidation, since the more nucleophilic amine (*p*-toluidine) would be expected to attack more readily the cation XI formed from 4-chloroaniline.

A similar effect has been observed in the oxidation of mixtures of mesidine and 4-chloroaniline, aminoanilinobenzoquinone dianils derived exclusively from N-mesitylbenzoquinone diimine (Xc) are produced.

The Perkin bases obtained from the oxidation of the *p*-toluidine-4-chloroaniline mixture are all derived from 4,4′-dimethyldiphenylamine. Neither 4,4′-dichlorodiphenylamine nor 4-chloro-4′-methyldiphenylamine has been detected, nor have any derivatives of these amines been observed. Both diphenylamines may be recovered unchanged when introduced into the oxidation. Formation of diphenylamines under these conditions only takes place from amines of comparably high basicity, 4-chloroaniline is not sufficiently basic to perform the reaction.

The yields of azobenzenes in this reaction are of interest. The yield of 4,4′-dimethylazobenzene is enhanced fivefold by the presence of the 4-chloroaniline, and that of 4,4′-dichloroazobenzene diminished fifteenfold by the presence of the *p*-toluidine. It has been proposed that the formation of azobenzenes proceeds via the symmetrical coupling of two ArNH· radicals to form the hydrazobenzene, which is then oxidized to the corresponding azobenzene.

The yield of 4,4′-dichloroazobenzene (15%) from the oxidation of 4-chloroaniline is much higher than that of 4,4′-dimethylazobenzene (1%) from *p*-toluidine, and so the contribution of ArNH· radicals must be greater in the former oxidation. Their increased concentration in the mixed oxidation over that in the oxidation of *p*-toluidine may cause the formation of more *p*-toluidino-radicals by hydrogen exchange between *p*-toluidine and 4-chloroanilino-radicals, thereby increasing the yield of 4,4′-dimethylazobenzene and diminishing that of 4,4′-dichloroazobenzene.

Those of the oxidation products which it has been possible to prepare unambiguously by oxidative coupling experiments have been synthesized, and have been shown to be identical with the corresponding compounds isolated from the oxidation of the amine mixture. The following oxidative couplings have been carried out:

(a) 4,4′-Dimethyldiphenylamine with 4-chloroaniline to prepare 4-*p*-toluidino-2,5-toluquinone di-4-chloroanil (III).

(b) 4,4′-Dimethyldiphenylamine with *p*-toluidine to prepare 4-*p*-toluidino-2,5-toluquinonebis-*p*-tolylimine (II).

(c) N-*p*-Tolyl-*p*-phenylenediamine with 4-chloroaniline to prepare 2-amino-5-*p*-toluidinobenzoquinone di-4-chloroanil (VI).

(d) N-*p*-Tolyl-*p*-phenylenediamine with *p*-toluidine to prepare 2-amino-5-*p*-toluidinobenzoquinonebis-*p*-tolylimine (VII).

EXPERIMENTAL

Materials and equipment The enzyme preparation employed was a purified horseradish peroxidase supplied by Seravac Laboratories Ltd, and had RZ 0·3. The H_2O_2 soln used was approx 20 volume. Column chromatography was carried out on Spence "H" alumina (100-200 mesh), and TLC on plates prepared with Merck alumina "G". The amines used were distilled under reduced press immediately prior to their use.

The oxidation of a mixture of p-toluidine and 4-chloroaniline

p-Toluidine (1·07 g, 10 mmole) and 4-chloroaniline (1·27 g, 10 mmole) were dissolved in acetate buffer (250 ml, 0·5M, pH 4·5). Peroxidase (1 mg) was added every 2 hr and H_2O_2 every $\frac{1}{2}$ hr. A red colour was formed immediately and after 2 hr a reddish brown ppt had appeared. After 24 hr the ppt was filtered off, washed and dried; addition of peroxidase-H_2O_2 to the filtrate gave no coloration. A tarry brown solid was obtained (1·2 g), and the filtrate was retained for chloride ion estimation.

The solid (1·0 g) was chromatographed using benzene in light petroleum (b.p. 60–80°) with a steadily increasing proportion of benzene as eluent. The following bands were eluted.

(a) *Bright yellow band*—on evaporation gave a yellow solid (60 mg). This solid gave the bright yellow colour with conc H_2SO_4 characteristic of an azobenzene. The azobenzenes obtained in this fraction were found to be the *trans*-isomers; the *cis*-isomers were eluted in the slower running red-brown band. The total yield of azobenzenes in the oxidation was estimated by subjection of the crude oxidation product (1 g) to vacuum sublimation with gradient cooling. A yellow sublimate (100 mg) and a less volatile colourless sublimate (1 mg) were obtained.

The yellow sublimate was subjected to column chromatography using n-hexane as solvent; three bands were eluted, which gave the following on evaporation.

(i) Fast running yellow solid (12·5 mg). This was recrystallized (n-hexane) and gave yellow needles, m.p. 186° (Lit.[5] 188°) of 4,4-dichloroazobenzene.

(ii) Yellow solid (30 mg). This was recrystallized (n-hexane) and gave yellow needles, m.p. 155·5° (Lit.[6] 153°) of 4-chloro-4′-methylazobenzene.

(iii) Slow running yellow solid (50 mg). This was recrystallized (n-hexane) and gave yellow needles. m.p. 144° (Lit. 144°) of 4,4′-dimethylazobenzene.

The colourless sublimate (m.p. 76°) was inseparable on TLC from 4,4′-dimethyldiphenylamine (m.p. 79°). No other diphenylamines were observed.

(b) *Red band*—on partial evaporation deep red needles (10 mg) crystallized, which were recrystallized (toluene) m.p. 263°. M.p. of 2,5-di-(4-chloroanilino-)benzoquinonebis-*p*-tolylimine 267°. The IR spectrum of the needles showed marked similarities to, and the R_f on TLC was identical with, those of this compound. (Found: C, 73·5; H, 5·4; Cl, 9·8. $C_{30}H_{20}Cl_2Me_2N_4$ requires: C, 71·5; H, 4·9; Cl, 13·2. $C_{30}H_{20}ClMe_3N_4$ requires: C, 76·6; H, 5·7; Cl, 6·7%). The substance was assumed to be a mixture of azophenines; no further resolution on TLC was possible.

Evaporation of the filtrate after removal of the azophenines gave a red solid (150 mg), which had an IR spectrum similar to that of 4-*p*-toluidino-2,5-toluquinonebis-*p*-tolylimine. On subjection to preparative TLC (15 mg) three bands were observed. These were extracted, evaporated and analysed by mass spectrometry. The results are shown in Table 1.

TABLE 1.

R_f (25% benzene in light petroleum (b.p. 60-80°) as eluent)	Yield of red solid (mg)	m.p.	M.w. from mass spectrum (Cl = 35)
0·65 vw, 0·6 s	8	174·5°	425 (trace at 445)
0·45 s	2	171–173°	425
0·4 s	5	179–80°	405

vw = very weak, s = strong.

The mass spectrum of the fastest running component showed a small peak at *m/e* 445. TLC analysis of this component showed a trace of fast running material which was inseparable from authentic 4-*p*-toluidino-2,5-toluquinone di-4-chloroanil (m.w. (Cl = 35) = 445). The component (R_f 0·4) was identical (TLC, IR, mass) with 4-*p*-toluidino-2,5-toluquinonebis-*p*-tolylimine.

(c) *Red-brown band*—on evaporation gave a red solid (300 mg). This solid had an IR spectrum similar to that of 2-amino-5-(4-chloroanilino-)benzoquinone di-4-chloroanil. On subjection to preparative TLC three bands were observed. These were each extracted, evaporated and analysed by mass spectrometry. The results are shown in Table 2.

TABLE 2.

R_f (25% benzene in light petroleum (b.p. 60–80°) as eluent)	Yield of red solid (mg)	m.p.	M.w. from mass spectrum (Cl = 35)
0·35 s	4	213°	446
0·3 m, 0·27 m	5	213°	426
0·2 w	1	203°	406

w = weak. m = medium. s = strong.

The component (R_f 0·35) was identical (TLC, IR, mass) with 2-amino-5-*p*-toluidinobenzoquinone di-4-chloroanil.

The central band proved on re-examination on TLC to be a mixture of two components, inseparable on the preparative scale; the mass spectrum of the mixture indicated that both components had the same molecular weight.

The component (R_f 0·2) was identical (TLC, IR, mass) with 2-amino-5-*p*-toluidinobenzoquinonebis-*p*-tolylimine.

(d) *Deep brown band*—on evaporation gave a black tar (430 mg). TLC analysis failed to resolve any pure components. The substance has not been further investigated.

Examination of the filtrate. The filtrate obtained after removal of the solid oxidation product was treated with excess standard $AgNO_3$ soln and dil HNO_3. The AgCl was filtered off and washed, the filtrate and washings being subsequently titrated with standard KCNS soln. Wt Cl^- in filtrate = 28·1 mg. Wt Cl^- calculated to be produced when the azophenines and the aminoanilinobenzoquinone dianils obtained are formed from 4-chloroaniline = 29·0 mg.

Preparation of the azobenzenes. Authentic samples of 4,4′-dichloroazobenzene and 4,4′-dimethylazobenzene for comparison purposes were prepared by peroxidase action on 4-chloroaniline and *p*-toluidine respectively as described in the literature.[4, 8] 4-Chloro-4′-methylazobenzene was prepared according to the method of Wieland.[7]

Preparation of 2,5-di-(4-chloroanilino)benzoquinonebis-p-tolylimine. N,N′-Di-*p*-tolyl-*p*-phenylene diamine was prepared from *p*-toluidine and quinol according to the method of Hatschek and Zega,[9] m.p. 182° (Lit. 182°). Benzoquinonebis-*p*-tolylimine was prepared from this substance according to the method of Hughes and Saunders,[10] m.p. 124° (Lit. 123-123·5°).

Benzoquinonebis-*p*-tolylimine (100 mg) was heated for 12 min with glacial AcOH (0·5 ml) and 4-chloroaniline (1 g). A green soln and a red crystalline ppt formed. The ppt was filtered off, washed (EtOH), dried and recrystallized (toluene). Yield of *2,5-di-(4-chloroanilino)benzoquinonebis-p-tolylimine* was 70 mg (37%), m.p. 267°. (Found: C, 71·9; H, 5·2; N, 10·6. $C_{32}H_{26}Cl_2N_4$ requires: C, 71·5; H, 4·9; N, 10·4%); IR spectrum (Nujol) showed prominent bands at 3300, 1600, 1560, 1520 cm^{-1}.

Preparation of 4-p-toluidino-2,5-toluquinone di-4-chloroanil. Finely powdered 4,4′-dimethyldiphenylamine (197 mg) was suspended in stirred acetate buffer (1 1, 0·25M, pH 4·5). Peroxidase (5 mg), H_2O_2 (20 ml) and a soln of 4-chloroaniline (255 mg) in dil AcOH (30 ml, 10%) were added during the course of the reaction. The purple ppt formed was filtered off, washed and dried (200 mg). The solid was chromatographed using cyclohexane as eluent. In addition to the bands containing the usual 4-chloroaniline oxidation products a red fast running band was eluted. On evaporation a red solid, (*4-p-toluidino-2,5-toluquinone di-4-chloroanil*) was obtained which was recrystallized (cyclohexane), yield 80 mg (18%), m.p. 175°. (Found: C, 70·1; H, 5·1; N, 9·7. $C_{26}H_{21}Cl_2N_3$ requires: C, 70·0; H, 4·7; N, 9·4%); IR spectrum (Nujol) showed prominent bands at 3300, 1635, 1590, 1565, 1520 cm^{-1}, and was markedly similar to that of 4-*p*-toluidino-2,5-toluquinonebis-*p*-tolylimine. Mass spectrum: *m/e* 449, (% of base peak 25); 448, (25); 447, (85); 446, (50); 445, (100); 444, (41); 443, (15); 432, (21); 431, (16); 430, (32); 425, (12); 412, (10); 411, (10); 410, (26); 409, (10); 408, (10); 385, (10); 319, (10); 91, (5). M.W. (Cl – 35) = 445.

Preparation of 4-p-toluidino-2,5-toluquinonebis-p-tolylimine. This compound was prepared as described by Holland and Saunders.[1]

Preparation of N-p-tolyl-p-phenylenediamine. This compound was prepared according to the method of I.G. Farbenindustrie AG[11] from *p*-nitrosotoluene and nitrosobenzene. M.p. 115° (Lit. 116–118°).

Preparation of 2-*amino*-5-*p*-*toluidinobenzoquinone di*-4-*chloroanil*. 4-Chloroaniline (700 mg) and N-*p*-tolyl-*p*-phenylenediamine (100 mg) were dissolved in glacial AcOH (2 ml) and the soln diluted to 50 ml with acetate buffer (0·5M, pH 4·5). Peroxidase (2 mg), H_2O_2 (10 ml) and a soln of N-*p*-tolyl-*p*-phenylenediamine (500 mg) in acetate buffer (20 ml, 0·5M) were added during the course of the reaction. After 2 hr a brown ppt formed, which was filtered off, washed and dried (240 mg). The solid was chromatographed using 20% $CHCl_3$ in light petroleum (b.p. 60–80°) as eluent. The bands corresponding to the oxidation products of 4-chloroaniline were obtained, that corresponding in R_f to that of 2-amino-5-(4-chloroanilino-)benzoquinone di-4-chloroanil was collected. This fraction was evaporated and the brown solid, crude 2-*amino*-5-*p*-*toluidinobenzoquinone di*-4-*chloroanil* recrystallized (cyclohexane) as brown needles (50 mg, 4%) m.p. 213°. (Found: C, 67·3; H, 4·5; N, 12·6. $C_{25}H_{20}Cl_2N_4$ requires C, 67·1; H, 4·5; N, 12·5%). IR spectrum (Nujol) showed prominent bands at 3400, 3300, 3230, 1649, 1555 cm^{-1}. Mass spectrum: *m/e* 450, (% of base peak 16); 449, (22); 448, (70); 447, (55); 446, (100); 445, (45); 434, (16); 433, (30); 432, (30); 431, (40); 430, (15); 421, (10); 320, (10); 308, (10); 285, (10); 111, (11); 91, (17); M.W. (Cl = 35) = 446.

Preparation of 2-*amino*-5-*p*-*toluidinobenzoquinonebis*-*p*-*tolylimine*. This preparation was carried out in a similar manner to that of 2-amino-5-*p*-toluidinobenzoquinone di-4-chloroanil except that *p*-toluidine (600 mg) was used in place of 4-chloroaniline. Chromatography gave in addition to the oxidation products of *p*-toluidine a slow running brown band which on elution and evaporation gave crude 2-*amino*-5-*p*-*toluidinobenzoquinonebis*-*p*-*tolylimine*, recrystallized (cyclohexane) as red-brown needles (25 mg, 2%), m.p. 204°. (Found: C, 79·6; H, 6·1; N, 13·6. $C_{27}H_{26}N_4$ requires: C, 79·8; H, 6·4; N, 13·8%); IR spectrum (Nujol) showed prominent bands at 3400, 3280, 3230, 1640, 1610, 1555, 1510 cm^{-1}. Mass spectrum: *m/e* 409, (% of base peak 10); 408, (29); 407, (31); 406, (100); 405, (40); 404, (10); 395, (10); 394, (16); 393, (16); 392, (55); 391, (45); 390, (11); 300, (12); 289, (13); 288, (47); 274, (10); 188, (12); 91, (10). M.W. = 406.

The oxidation of a mixture of mesidine and 4-*chloroaniline*

Mesidine (1·35 g, 10 mmole) and 4-chloroaniline (1·27 g, 10 mmole) were dissolved in acetate buffer (250 ml, 0·5M, pH 4·5). Peroxidase (1 mg purified enzyme) was added every 2 hr and H_2O_2 (1 ml) every ½ hr. A red colour was formed immediately and after 24 hr a brown ppt had appeared. After 72 hr the ppt was filtered off, washed and dried; a brown solid (1·30 g) was obtained. Addition of peroxidase–H_2O_2 to the filtrate gave no further coloration, but a heavy ppt of AgCl was given with $AgNO_3$.

The solid (1·0 g) was chromatographed using benzene in light petroleum (b.p. 60–80°) with a steadily increasing proportion of benzene as eluent. The following bands were eluted.

(a) *Reddish purple band* –on evaporation gave a deep purple solid (40 mg) which after vacuum sublimation gave purple plates, m.p. and mixed m.p. with 2,6-dimethylbenzoquinone-4-(2′,4′,6′-trimethyl-)anil 96°.

(b) *Red-brown band*—on evaporation gave a brown solid (400 mg). Recrystallization (MeOH) gave red-brown needles of 2-*amino*-5-*mesidinobenzoquinone di*-4-*chloroanil* m.p. 141°. (Found: C, 68·3; H, 5·2; C., 14·8; $C_{27}H_{24}Cl_2N_4$ requires: C, 68·2; H, 5·1; Cl, 14·8%); IR spectrum (Nujol) showed prominent bands at 3420, 3270, 1600, 1560 cm^{-1}. Mass spectrum: *m/e* 479, (% of base peak 15); 478, (45); 477, (30); 476, (100); 475, (40); 474, (70); 473, (45); 472, (35); 471, (30); 469, (10); 468, (10); 467, (20); 461, (25); 460, (16); 459, (35); 350, (15); 349, (15); 348, (50); 226, (11); 212, (13); 91, (22). M.W. (Cl = 35) = 474. The mass spectrum of the crude red-brown solid before recrystallization showed traces at *m/e* 482, (25) due to $C_{30}H_{31}ClN_4$ and at 490, (6) due to $C_{33}H_{38}N_4$. UV spectrum (EtOH): λ_{max} 272 mμ. ($\log_{10} \varepsilon$ 4·246); 402, (4·179) λ_{min} 259, (4·218); 321, (4·022); λ_{infl} 352 (4·074).

(c) *Deep brown band*—on evaporation gave a black tar (550 mg). A small quantity of material showing the IR spectrum characteristic of an azophenine was also isolated.

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REFERENCES

[1] V. R. Holland and B. C. Saunders, *Tetrahedron* **22**, 3345 (1966).

[2] V. R. Holland and B. C. Saunders, *Ibid.* **24**, 585 (1968).
[3] G. M. K. Hughes and B. C. Saunders, *J. Chem. Soc.* 3814 (1956).
[4] D. G. H. Daniels and B. C. Saunders, *Ibid.* 822 (1953).
[5] E. Bamberger and O. Baudisch, *Ber. Dtsch. Chem. Ges.* **42**, 3578 (1909).
[6] H. Wieland, *Ibid.* **48**, 1110 (1915).
[7] J. Barsilowsky, *Liebigs. Ann.* **207**, 103 (1881).
[8] B. C. Saunders and P. J. G. Mann, *J. Chem. Soc.* 769 (1940).
[9] A. Hatschek and A. Zega, *J. Prakt. Chem.* **33**, 209 (1886).
[10] I.G. Farbenindustrie AG., B.P. 338. 240 (1930).