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STUDIES IN PEROXIDASE ACTION—XVIII*

THE OXIDATION OF 4-CHLORANILINE—FURTHER STUDIES

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Abstract—The main features of the peroxidation of 4-chloroaniline are the production of 2-amino-5-(4-chloroanilino-)benzoquinone-di-4-chloroanil in high yield and the elimination of chloride ion. Two intermediates, (N-(4-chlorophenyl-)*p*-phenylenediamine and 4-chloro-N^2-(4-chlorophenyl-)*o*-phenylenediamine), have been proposed; and it has been shown that while the former may be an intermediate the latter is not: the latter gives rise to 2-amino-5-chlorobenzoquinone-di-4-chloroanil, a compound which we have detected in the 4-chloroaniline oxidation products. This compound is stable to the conditions of oxidation and is not the precursor of the main oxidation product.

The relative yields of the oxidation products have been shown to depend on the initial concentration of 4-chloroaniline.

DANIELS and Saunders[1] recorded the major products of the oxidation of 4-chloroaniline by peroxidase–hydrogen peroxide as: 2-amino-5-(4-chloroanilino-)benzoquinone-di-4-chloroanil (I); tetra-4-chloroazophenine (II); 4,4'-dichloroazobenzene (III) and chloride ion.

It was shown that chloride ion is produced in amount approximately equal to that theoretically required for the formation of I from four molecules of 4-chloroaniline.

In order to elucidate the mechanism of the oxidation it is important to determine at which stage in the production of I elimination of chloride ion takes place. Two possible primary intermediates have been considered; namely N-(4-chlorophenyl-)*p*-phenylenediamine (IV), produced from two molecules of 4-chloroaniline with the elimination of hydrogen chloride; and 4-chloro-N^2-(4-chlorophenyl-)*o*-phenylenediamine (V) produced by oxidative coupling of two molecules of 4-chloroaniline.

We have shown that addition of IV to the initial solution of 4-chloroaniline results in increased yield of I, and diminution of the chloride ion concentration. These results correspond quantitatively to the consumption of IV to produce I by oxidative coupling with two molecules of 4-chloroaniline. The action of peroxidase–hydrogen peroxide on IV alone gives an intractable black solid which is not produced when 4-chloroaniline is present. The addition of IV to the oxidation of 4-chloroaniline does not produce a significant quantity of any compound which is absent from the oxidation products of 4-chloroaniline. It therefore seems probable that IV is an intermediate in the oxidation.

Addition of V to the initial solution of 4-chloroaniline results in the production of 2-amino-5-chlorobenzoquinone-di-4-chloroanil (VI), a compound which we have detected in the oxidation products of 4-chloroaniline. The presence of V increases

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the yield of VI, diminishes that of I and considerably reduces the chloride ion concentration. These results correspond quantitatively to the consumption of V to produce VI by oxidative coupling with a molecule of 4-chloroaniline. The action of peroxidase–hydrogen peroxide on V alone gives an intractable black solid which is not produced when 4-chloroaniline is present. The addition of V to the oxidation of 4-chloroaniline does not produce a significant quantity of any compound which is absent from the oxidation products of 4-chloroaniline. Further experiments have shown that VI is stable to the conditions of the oxidation; it does not undergo Michael addition with 4-chloroaniline at the 5-position followed by chloride ion elimination to give I. It therefore appears that V is not an intermediate in the production of I but is responsible for the production of VI.

There is no evidence that V is produced by an ortho-semidine rearrangement of 4,4′-dichlorohydrazobenzene, the assumed precursor of III in the oxidation. We have shown that under the conditions of the oxidation 4,4′-dichlorohydrazobenzene is converted exclusively to III.

Another feature of the reaction is the dependence of the yields of I and VI on the initial amine concentration. Experiments carried out on solutions of 4-chloroaniline in which the amine concentration has ranged from 1 mM to 40 mM (saturated at pH 4·5) have shown that the percentage by weight of VI in the oxidation product falls while that of I increases as the amine concentration is increased. At 1 mM each is present to the extent of 25%, while at 40 mM the oxidation product contains 0·5% of VI and 85% of I. Attack by 4-chloroaniline on the 4-position of the initially formed intermediate followed by chloride ion elimination to give IV is favoured by increasing amine concentration. A similar effect has recently been observed in the peroxidation of *p*-toluidine.[2]

V is also produced by the action of lead dioxide-acetic acid on 4-chloroaniline. Oxidation under these conditions does not give rise to I, nor to any chloride ion elimination.

EXPERIMENTAL

Materials and equipment. All oxidations were carried out using a purified horse-radish peroxidase (RZ = 0·3), supplied by Seravac Laboratories Ltd., and 20 volume H_2O_2. All chromotography was carried out on alumina. TLC was carried out on alumina 'G' supplied by E. Merck AG, using 25% benzene in light petroleum (b.p. 60–80°) as eluent.

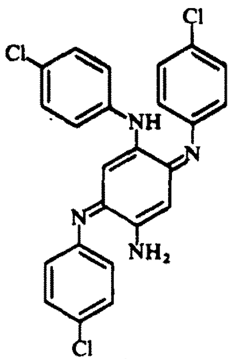
Oxidation of a mixture of 4-chloroaniline and N-(*4-chlorophenyl-*)*p-phenylenediamine* (IV)

Compound IV was prepared as described by I.G. Farbenindustrie AG,[3] m.p. 69° (lit. 71°). (Found: C, 65·5; H, 5·1; N, 13·1. Calc. for $C_{12}H_{11}ClN_2$: C, 65·9; H, 5·1; N, 12·8%.) NMR and mass spectra consistent with the structure of IV. N′-*Benzoyl derivative* m.p. 196°. (Found: C, 71·0; H, 4·9. $C_{19}H_{15}ClN_2O$ requires: C, 70·7; H, 4·7%.)

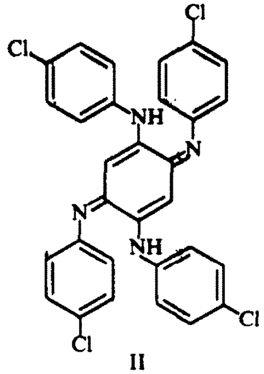
H_2O_2 (1 ml) and peroxidase (1·5 mg) were added to a soln of 4-chloroaniline (0·639 g) and IV (0·109 g) in 1M acetate buffer (50 ml). A red-brown colouration was observed and after 5 min the additions of perioxidase and H_2O_2 were repeated. After ½ hr the red-brown ppt which had formed was filtered off, washed and dried (0·577 g). A parallel experiment containing no IV also gave a red-brown solid (0·531 g). The ppts obtained from the filtrates by treatment with silver nitrate and HNO_3 corresponded to 0·7 and 1·1 mg equivalents of chloride ion respectively. Chromatographic separation of the oxidation products showed that in both cases the product contained 85% by weight of I.

Oxidation of a mixture of 4-chloroaniline and 4-chloro-N^2-(*4-chlorophenyl-*)*o-phenylenediamine* (V)

During 4 hr the following additions were made with stirring to 1M acetate buffer (50 ml, pH 4·5).



I



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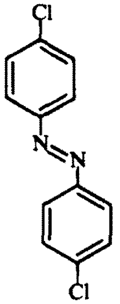
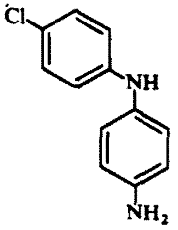
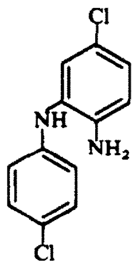


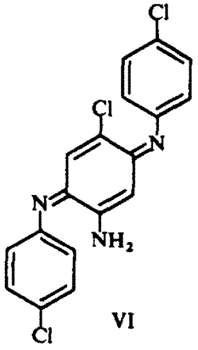
III



IV



V



VI

(a) 4-Chloroaniline (41 mg) and compound V (82 mg, prepared as described by Wilberg;[4] off-white needles, m.p. 90° (lit. 91°)) in dil AcOH (10%, 10 ml).

(b) H_2O_2 (1 ml) every $\frac{1}{2}$ hr.

(c) peroxidase (1 mg) every 2 hr.

A red-purple colour appeared on addition of the enzyme and after $\frac{1}{2}$ hr a red-brown ppt formed. After allowing to stand overnight this was filtered off, washed and dried (117 mg). The pale pink filtrate gave a negative reaction with peroxidase–hydrogen peroxide and contained 8 μg equivts of chloride ion. A parallel experiment in which V was not introduced gave rise to 80 μg equivts of chloride ion in the filtrate. Chromatographic separation of the oxidation products showed that the product of the former experiment contained 40% by wt of VI and 10% of I, and that from the latter experiment 0·5% of VI and 85% of I.

Preparation of compound VI *by the oxidation of* 4-*chloroaniline*

(a) *With peroxidase*-H_2O_2. The oxidation products were obtained according to the method of Daniels and Saunders.[1] The behaviour on TLC was: Compound I, brown, *Rf* 0·3; II, reddish-purple, 0·7; III, yellow, 1·0. The filtered solid from the oxidation gave in addition the following spots: red, *Rf* 0·25; black, 0. The solid (0·1 g) was subjected to preparative TLC and the band at *Rf* 0·25 extracted to give a red solid, (0·5 mg) m.p. and mixed m.p. with authentic sample of 2-*amino*-5-*chlorobenzoquinone-di*-4-*chloroanil*, (VI), 208° (dec). A mixture of the solid with authentic VI could not be separated on TLC.

(b) *With lead dioxide–acetic acid.* 4-Chloroaniline (5 g) was dissolved in ether (120 ml) and glacial AcOH (10 ml). The soln was stirred under reflux and PbO_2 (AR, 25 g) was added in small portions over 15 min. The mixture was heated for 2 hr, and a deep red colour developed. The suspension was filtered to remove lead salts; and the filtrate was shaken with Na_2CO_3aq to remove AcOH and washed with water. The ether layer was dried (Na_2SO_4) and the ether evaporated. A deep brown solid (4·0 g) was obtained.

This solid (1·0 g) was chromatographed using 1:1 Chf: light petroleum (b.p. 60–80°) and the following bands were eluted:

(a) A fast running yellow band; evaporated to a yellow solid (0·35 g) which was recrystallized (n-hexane); m.p. and mixed m.p. with 4,4'-dichloroazobenzene 186° (lit. 188°).

(b) A slower running red band; on partial evaporation and standing deposited red needles of 2-*amino*-5-*chlorobenzoquinone-di*-4-*chloroanil* (0·19 g, 15%) m.p. 210° (dec). (Found: C, 57·3; H, 3·4; N, 11·3. $C_{18}H_{12}Cl_3N_3$ requires: C, 57·4; H, 3·2; N, 11·2%.) Mass spectrum: *m/e* 382, (% of base peak 5); 381,(5); 380,(32); 379,(24); 378,(87); 377,(25); 376,(100); 375,(9); 374,(15); 343,(10); 342,(10); 341,(8); 340,(7); 307,(8); 306,(6); 305,(8). M (Cl = 35): 375. IR spectrum, (Nujol) showed prominent bands at 3420, 3300, 1625, 1585, 1560, 1300, 1240, 1222, 1201, 1165, 1104, 1087, 1007, 887, 880, 847, 805, 697, 689 cm^{-1}. A quantity of slow running tarry material was also obtained.

Analysis of the solid oxidation product on TLC showed that I was not produced, and the aqueous extract from the reaction mixture contained no chloride ion.

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