757. *Studies in Peroxidase Action. Part VII.* The Oxidation of* p-*Toluidine by Hydrogen Peroxide in the Presence of Ferrous Sulphate.*

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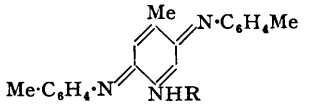
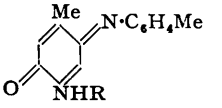
The action of hydrogen peroxide in the presence of the enzyme peroxidase at pH 4·5 on *p*-toluidine has already been investigated. It is now shown that, if the reaction is catalysed by ferrous ions, instead of by peroxidase, a considerable amount of amorphous material is produced together with small quantities of 2 : 7-dimethyl-3-*p*-toluidinophenazine and 4 : 4′-dimethylazobenzene. Traces of di-*p*-tolylamine, 4-*p*-toluidino-2 : 5-toluquinone di-*p*-tolylimine, and 4-*p*-toluidino-2 : 5-toluquinone 2-*p*-tolylimine have also been isolated. The ferrous-catalysed oxidation is therefore not identical with that catalysed by the enzyme.

In Part II (Saunders and P. J. G. Mann, *J.*, 1940, 769) it was shown that the oxidation of *p*-toluidine by hydrogen peroxide, catalysed by the enzyme peroxidase, produced mainly 4-amino-2 : 5-toluquinone di-*p*-tolylimine (I; R = H), 4-*p*-toluidino-2 : 5-toluquinone di-*p*-tolylimine (I; R = *p*-C_6H_4Me), and di-*p*-tolylamine. Traces of 4 : 4′-dimethylazobenzene, 4-amino-2 : 5-toluquinone 2-*p*-tolylimine (II; R = H), and 4-*p*-toluidino-2 : 5-toluquinone 2-*p*-tolylimine (II; R = *p*-C_6H_4Me) were also produced. These compounds accounted for nearly the whole of the solid oxidation product.

(I) 

 (II)

In contrast to this result, we have now shown that if ferrous sulphate is substituted for peroxidase, the rapid reaction which ensues leads mainly to amorphous material. From the mixture we have isolated only traces of 4-*p*-toluidino-2 : 5-toluquinone di-*p*-tolylimine (I; R = *p*-C_6H_4Me), 4-*p*-toluidino-2 : 5-toluquinone 2-*p*-tolylimine (II; R = *p*-C_6H_4Me), di-*p*-tolylamine, and 4 : 4′-dimethylazobenzene. Moreover, there was produced in larger amount a compound which crystallised in orange prisms, m. p. 206—207°, not obtained in the peroxidase oxidation. It showed a strong yellow-green fluorescence in dilute benzene solution, and gave a characteristic green colour with concentrated sulphuric acid, changing to magenta on dilution.

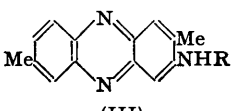
This compound, a base, had the empirical formula $C_{21}H_{19}N_3$. Two series of salts were formed : green, stable only in the presence of excess of concentrated acid, and reddish-violet. These were evidently di- and mono-acid salts respectively. The chloroplatinate, picrate, and picrolonate, all violet, were prepared. It was reversibly reduced to a leuco-compound.

These facts suggested that it might be 2 : 7-dimethyl-3-*p*-toluidinophenazine (III; R = *p*-C_6H_4Me) derived from the union of three *p*-toluidine residues. This supposition was confirmed by synthesis by two independent routes.

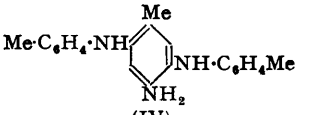
In the first, oxidation of 2 : 5-di-*p*-toluidino-*p*-toluidine (IV) by means of lead dioxide gave (III, R = C_6H_4Me) directly. The reaction giving (I; R = H) and not involving ring-closure predominated, however.

* Part VI, *J.*, 1951, 2112.

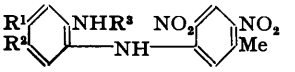
In the second, 3-amino-2 : 7-dimethylphenazine (III; R = H) was prepared by the stannous chloride reduction of 2-amino-4 : 5′-dimethyl-2′ : 4′-dinitrodiphenylamine (V; R^1 = Me, R^2 = R^3 = H) (cf. Ullmann, *Annalen*, 1909, **366**, 91). This compound resulted from the condensation of 3 : 4-diaminotoluene and 5-bromo-2 : 4-dinitrotoluene. Its structure was



(III)



(IV)



(V)

established by the formation of an acetyl derivative, which was identical with the compound (V; R^1 = Me, R^2 = H, R^3 = Ac) from the condensation of 3-acetamido-4-aminotoluene with 5-bromo-2 : 4-dinitrotoluene, and depressed the m. p. of the compound (V; R^1 = H, R^2 = Me, R^3 = Ac) from the condensation of 4-acetamido-3-aminotoluene with 5-bromo-2 : 4-dinitrotoluene. 3-Amino-2 : 7-dimethylphenazine decomposed very rapidly in air. It was characterised by its light absorption and by its acetyl derivative, m. p. 258—260°. 2 : 7-Dimethyl-3-*p*-toluidinophenazine, identical with the oxidation product, was formed by heating 3-amino-2 : 7-dimethylphenazine with *p*-toluidine and hydrochloric acid in a sealed tube filled with nitrogen.

Experimental.

Oxidation of p-*Toluidine.*—*p*-Toluidine (20 g.) was dissolved in acetic acid (23 g.) and diluted with water (1 l.). To this solution was added hydrogen peroxide (2 c.c.; 20-vol.), no colour being produced. Ferrous sulphate solution (*ca.* 1 c.c.; 5% of hydrate) was added, and a magenta colour was produced immediately. Hydrogen peroxide (200 c.c.) and ferrous sulphate solution (*ca.* 12 c.c.) were added over a period of 50 hours. A brown precipitate gradually separated and was filtered off at the conclusion of the reaction, and dried (yield, 8—10 g.). If hydrogen peroxide was added too quickly a strong odour of *iso*cyanide was noticed.

Treatment of the Crude Oxidation Product.—The dry crude product (25 g.) was extracted in a Soxhlet apparatus with *cyclo*hexane. The extract was evaporated and the solid residue (4·1 g.) was dissolved in chloroform. Chromatography on alumina gave a preliminary purification. The eluate was evaporated to dryness, dissolved in benzene, and re-chromatographed, the bands being collected as a "liquid chromatogram." They appeared in the following order :

(i) 4 : 4′-*Dimethylazobenzene.* On concentration of the eluate, deep yellow crystals were obtained which, when recrystallised from light petroleum (b. p. 60—80°), had m. p. 143—144° and gave a yellow colour with concentrated sulphuric acid in the cold. This is the behaviour of 4 : 4′-dimethylazobenzene, m. p. 145°.

(ii) 4-p-*Toluidino*-2 : 5-*toluquinone di*-p-*tolylimine.* The eluate gave, on evaporation, a small amount of an oil which crystallised on cooling. This consisted of a mixture of the toluquinone derivative and di-*p*-tolylamine. When recrystallised from benzene the ditolylamine remained in the mother-liquor and 4-*p*-toluidino-2 : 5-toluquinone di-*p*-tolylimine separated as red needles, m. p. 178—179°, on cooling. With concentrated sulphuric acid the toluquinone gave a characteristic violet colour, rapidly changing to green.

(iii) *Di*-p-*tolylamine.* Evaporation of the eluate from band (iii) gave a very small quantity of di-*p*-tolylamine contaminated with traces of 4 : 4′-dimethylazobenzene. Purification consisted of vacuum-sublimation, followed by reduction of 4 : 4′-dimethylazobenzene to *p*-toluidine by treatment with tin and dilute hydrochloric acid in which the ditolylamine is sparingly soluble. Extraction with light petroleum yielded the compound as needles, m. p. 78—79°.

(iv) 2 : 7-*Dimethyl*-3-p-*toluidinophenazine.* The eluate of this band was concentrated; orange prisms of 2 : 7-*dimethyl*-3-p-*toluidinophenazine* separated on cooling. A further quantity was obtained from the residue in the mother-liquor by sublimation at 170—180°/2 × 10^{-4} mm. After recrystallisation from benzene it had m. p. 206—207° (yield, 0·75 g.) (Found : C, 80·7; H, 6·2; N, 13·7. $C_{21}H_{19}N_3$ requires C, 80·5; H, 6·1; N, 13·4%). Dilute solutions in benzene showed a strong yellow-green fluorescence in ultra-violet light. Crystallisation from aqueous alcohol gave red needles, m. p. 206°, which did not depress the m. p. of the orange variety crystallised from benzene. The red variety also resulted from sublimation in a high vacuum, and was immediately changed to orange when moistened with benzene. Concentrated mineral acids gave green solutions which changed to reddish-violet on dilution. Light absorption in light petroleum : Max., 2450, 2820, 3720, and 4340 Å; ε = 37,000, 39,200, 8860, and 12,300, respectively. Light absorption in concentrated sulphuric acid : Max., 2440, 2990, 4120, 4820, and 6550 Å; ε = 59,400, 38,400, 7600, 14,500, and 11,400, respectively. Light absorption in 10% sulphuric acid : Max., 2380, 3020, 4200, and 5350 Å; ε = 46,200, 33,900, 14,500, and 20,900, respectively.

The following derivatives were readily prepared : *Chloroplatinate :* violet needles from methanol, no m. p. [Found : C, 48·3; H, 4·1; N, 7·7; Pt, 17·75. $(C_{21}H_{19}N_3)_2,H_2PtCl_6,2CH_3·OH$ requires C, 48·0; H, 4·4; N, 7·6; Pt, 17·75%]. *Picrate :* reddish-violet needles from ethanol, no m. p., decomp. *ca.* 225° (Found : C, 60·0; H, 4·3; N, 15·4. $C_{21}H_{19}N_3,C_6H_3O_7N_3$ requires C, 59·8; H, 4·1; N, 15·5%). *Picrolonate :* reddish-violet leaflets from ethanol, no m. p., decomp. *ca.* 215° (Found : C, 64·5; H, 4·8; N, 17·1. $C_{21}H_{19}N_3,C_{10}H_8O_5N_4$ requires C, 64·5; H, 4·7; N, 17·0%).

4-p-*Toluidino*-2 : 5-*toluquinone* 2-p-*Tolylimine*.—The original extract was shaken with aqueous hydrochloric acid (6N.), and a brownish-black precipitate was obtained (from this the phenazine could be isolated). The liquid was filtered, and the aqueous layer made alkaline and extracted with ether. A very small quantity of deep brown-red crystals, m. p. 177°, was obtained on concentrating the ether layer. These crystals gave a transient green colour with concentrated sulphuric acid, and on dilution gave a yellow solution. This is the behaviour of 4-*p*-toluidino-2 : 5-toluquinone 2-*p*-tolylimine (cf. Bornstein, *Ber.*, 1901, **34**, 1279, who gave m. p. 177°).

Oxidation of 2 : 5-*Di*-p-*toluidino*-p-*toluidine*.—This base was prepared from 4-amino-2 : 5-toluquinone di-*p*-tolylimine by reduction with zinc dust and glacial acetic acid (Green, *Ber.*, 1893, **26**, 2774). Excess of zinc was filtered off, and the filtrate diluted with hot water and allowed to cool slowly; almost colourless crystals, m. p. 164°, were obtained.

The compound (1·75 g.), mixed intimately with lead dioxide (7 g.), was heated slowly in a high vacuum. Sublimation commenced at 240°/2 × 10^{-3} mm. The orange-yellow sublimate was dissolved in light petroleum and chromatographed on alumina. Three bands were obtained which were eluted with ethanol: (i) This contained 4-amino-2 : 5-toluquinone di-*p*-tolylimine formed by re-oxidation of the leuco-base; its identity was confirmed by its characteristic blue coloration with concentrated sulphuric acid. (ii) On evaporation of the ethanol a small amount of a pink-red solid was left, which on recrystallisation from light petroleum (b. p. 60—80°) had m. p. 204°; mixed m. p. with 2 : 7-dimethyl-3-*p*-toluidinophenazine from hydrogen peroxide reaction, 204°. It gave the characteristic green colour with concentrated sulphuric acid, becoming pink on dilution. (iii) This band contained a trace of solid which gave a reddish-brown colour with concentrated sulphuric acid : it was not further investigated.

2-*Amino*-4 : 5′-*dimethyl*-2′ : 4′-*dinitrodiphenylamine*.—3 : 4-Diaminotoluene (4 g.), prepared by catalytic reduction of 3-nitro-*p*-toluidine, was dissolved in alcohol and heated for 1½ hours under reflux with 5-bromo-2 : 4-dinitrotoluene (5·2 g.) and anhydrous sodium acetate (2 g.). The *compound* crystallised from the hot solution in orange needles. It was filtered off, washed with water, and recrystallised from ethanol: m. p. 190—192° (decomp.) (5·3 g., 88%) (Found: C, 55·8; H, 4·7; N, 19·0. $C_{14}H_{14}O_4N_4$ requires C, 55·6; H, 4·6; N, 18·6%). The acetyl derivative (yellow needles) had m. p. 250—253°.

2-*Acetamido*-5 : 5′-*dimethyl*-2′ : 4′-*dinitrodiphenylamine*.—4-Acetamido-3-nitrotoluene was reduced catalytically to 4-acetamido-*m*-toluidine, m. p. 130°. It reacted as above with 5-bromo-2 : 4-dinitrotoluene. The *derivative* recrystallised from ethanol as yellow needles, m. p. 259—265° (decomp.) (Found: N, 16·4. $C_{16}H_{16}O_5N_4$ requires N, 16·3%); mixed m. p. with the acetyl derivative of 2-amino-4 : 5′-dimethyl-2′ : 4′-dinitrodiphenylamine (below), 227—232°.

2-*Acetamido*-4 : 5′-*dimethyl*-2′ : 4′-*dinitrodiphenylamine*.—This was prepared similarly, from 5-bromo-2 : 4-dinitrotoluene and 3-acetamido-*p*-aminotoluidine. The latter was obtained by catalytic reduction of 3-acetamido-4-nitrotoluene, formed as the minor product of the nitration of aceto-*m*-toluidide. 2-*Acetamido*-4 : 5′-*dimethyl*-2′ : 4′-*dinitrodiphenylamine* crystallised in yellow needles, m. p. 253—256° (decomp.); mixed m. p. with the acetyl derivative of 2-amino-4 : 5′-dimethyl-2′ : 4′-dinitrodiphenylamine, 253—255° (decomp.) (Found: N, 16·4. $C_{16}H_{16}O_5N_4$ requires N, 16·3%).

Reduction of 2-*Amino*-4 : 5′-*dimethyl*-2′ : 4′-*dinitrodiphenylamine*.—The compound (2 g.), stannous chloride (*ca.* 10 g.), and concentrated hydrochloric acid (8 c.c.) were heated for 5 hours in a sealed tube at 120°. During the reaction, the mixture became dark red. After some while at room temperature, it was diluted three-fold and made alkaline with sodium hydroxide, and the red precipitate filtered off, washed, and dried. It was extracted with ether in an atmosphere of nitrogen. The red extract was chromatographed on alumina. A prominent orange band was eluted as an orange-coloured solution exhibiting a strong green fluorescence. On evaporation of the solvent, a semi-crystalline mass, probably containing 3-amino-2 : 7-dimethylphenazine, was left (yield, crude, 0·2 g., 14%). It recrystallised from benzene–light petroleum (b. p. 60—80°) as orange needles, m. p. 248—249° (decomp.), which rapidly darkened in air. Light absorption of 3-*amino*-2 : 7-*dimethylphenazine* in light petroleum: Max., 2220, 2630, 3690, and 4120 Å; ε = 32,300, 71,300, 9170, and 8070, respectively. 2-Aminophenazine has light absorption in ether: Max., 2650, 3650, and 4880 Å (Kehrmann, Havas, and Grandmougin, *Ber.*, 1914, **47**, 1886). With concentrated sulphuric acid, 3-amino-2 : 7-dimethylphenazine gave a brown colour, which became successively green and magenta on dilution. The *acetyl* derivative recrystallised from methanol as pale yellow needles, m. p. 258—260° (Found: C, 71·6; H, 5·7; N, 16·2. $C_{16}H_{15}ON_3$ requires C, 72·4; H, 5·7; N, 15·9%).

Reaction with p-*toluidine*. The aminophenazine (100 mg.) was mixed with *p*-toluidine hydrochloride (64 mg., 1 mol.) and *p*-toluidine (240 mg., 5 mols.), and heated in a sealed tube filled with nitrogen for 2 hours at 150—160°. After cooling, the residue was washed with methanol and basified, and the brown precipitate filtered, dried, and extracted with ether. After removal of the solvent the excess of *p*-toluidine in the residue was removed by sublimation (100°/15 mm.), and the residue dissolved in benzene and chromatographed. Orange crystals were obtained in small yield, and on recrystallisation from benzene–light petroleum had m. p. 204—205°; mixed m. p. with 2 : 7-dimethyl-3-toluidinophenazine from the hydrogen peroxide reaction, 204—205° (Found: N, 13·7. Calc. for $C_{21}H_{19}N_3$: N, 13·4%).

We are grateful to the Department of Scientific and Industrial Research for maintenance grants to two of us (D. G. H. D. and F. T. N.). Our thanks are offered to Mr. C. L. de Silva for help with some of the experimental work.

UNIVERSITY CHEMICAL LABORATORY, CAMBRIDGE. [*Received, August 15th*, 1951.]