190. *The Oxidation of Derivatives of* o-*Phenylenediamine. Part III.* Isomeric Phenazine Pigments and* apo*Safranones obtained by Oxidation of Chlorinated 2-Aminodiphenylamine Hydrochlorides.*

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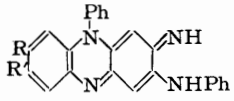
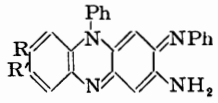
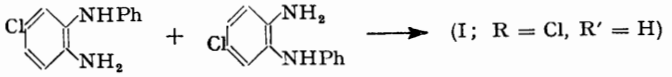
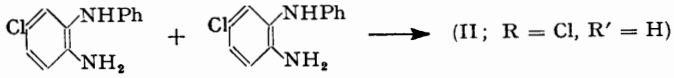
Oxidation of chlorinated 2-aminodiphenylamine hydrochlorides by ferric chloride and by *p*-benzoquinone results in the elimination of chlorine when the latter is present in the 4- or the 5-position, or in both. The products of oxidation are chlorinated anilino*apo*safranines or the corresponding chlorinated isomeric phenazines, or in some cases, a mixture of the two isomers. From a study of the oxidation of five 2-amino-4- or -5-chlorodiphenylamines no generalisation was possible as to the mode of elimination of the chlorine. The *p*-benzoquinone oxidation has been shown to yield also a third type of phenazine, chlorinated *apo*safranones, formed by condensation of the quinone with the diphenylamine derivatives. Some of the dichlorinated phenazines have very high activity in experimental tuberculosis in mice.

IN Part I [1] it was shown that oxidation of 2-aminodiphenylamine hydrochloride by ferric chloride or by *p*-benzoquinone gave a mixture of isomers (I and II; R = R′ = H). Oxidation of 2-amino-4- and -5-chlorodiphenylamine hydrochloride had previously been shown [2,3] to give phenazines to which the structures (I; R = H, R′ = Cl) and (I; R = Cl, R′ = H) respectively had been assigned. In both these oxidations a chlorine atom is eliminated but, as the existence of the parent compound (II; R = R′ = H) had not been established at that time, it was not certain that the phenazines obtained by oxidation of the 2-amino-4- and -5-chlorodiphenylamine hydrochlorides were of type (I) rather than of type (II). Further, since chromatography had not been applied in the earlier work,[2,3] it was not improbable that isomeric pigments of types (I) and (II) had been obtained in each case and that the existence of the second isomer had escaped detection.

(I)   (II)

In the oxidative condensation of 2-amino-5-chlorodiphenylamine hydrochloride the chlorine atom may theoretically be eliminated in two ways, (*a*) or (*b*), as shown :

(*a*) 

(*b*) 

If it could be shown that the oxidation took course (*a*) or (*b*), then a certain method would be available for the preparation of isomers of types (I) and (II). Accordingly, the oxidation of 2-amino-5-chlorodiphenylamine hydrochloride by ferric chloride and by *p*-benzoquinone was re-investigated. With both reagents the only product identified was the compound (II; R = Cl, R′ = H), which was isolated pure in about 20% yield and gave the colour reactions with sulphuric acid and with acetic anhydride expected [1] for the isomer of type (II).

It will be noted that if the chlorine is eliminated in the same way during oxidation of the 4-chloro-derivative, then the product of the reaction must be in this case the isomer

* Part II, preceding paper.

[1] Barry, Belton, O'Sullivan, and Twomey, *J.*, 1956, 888.
[2] Barry and Belton, *Proc. Roy. Irish Acad.*, 1953, **55**, *B*, 149.
[3] Kehrmann and Guggenheim, *Ber.*, 1901, **34**, 1218.

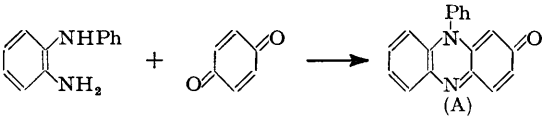
(I; R = H, R′ = Cl). Investigation showed that ferric chloride oxidation of 2-amino-4-chlorodiphenylamine hydrochloride did in fact produce in fair yield only this compound. However, with *p*-benzoquinone there was no evidence of its formation but the isomer (II; R = H, R′ = Cl) was isolated in very poor yield. The latter oxidation was complicated by the fact that the main crystalline compound isolated proved to be a monochloro*apo*safranone (cf. Part I [1]). (These chlorinated *apo*safranones were isolated in almost all cases when chlorinated 2-aminodiphenylamine hydrochlorides were oxidised by *p*-benzoquinone. They are discussed later in this paper.) It was thus clear that the elimination of chlorine during the oxidation could proceed in two ways, and it was therefore expected that oxidation of 2-amino-4 : 5-dichlorodiphenylamine hydrochloride would result in the loss of two chlorine atoms and the production of both isomers (I and II; R = R′ = Cl). However only the latter was formed by oxidation with ferric chloride, and *p*-benzoquinone oxidation yielded only a dichloro*apo*safranone.

Two further compounds containing chlorine in the 4-position were oxidised by both reagents. 2-Amino-4 : 4′-dichlorodiphenylamine hydrochloride gave, with ferric chloride, a mixture of isomers, that of type (II) in greater amount, whereas with *p*-benzoquinone only this isomer was isolated, together with a dichloro*apo*safranone. 2-Amino-4 : 2′ : 4′-trichlorodiphenylamine hydrochloride,[4] gave with ferric chloride the isomer of type (II), while from the *p*-benzoquinone oxidation only a trichloro*apo*safranone could be isolated. On the basis of these results no generalisation seems possible as to the mode of elimination of chlorine during these oxidative condensations.

2-Amino-6-chlorodiphenylamine hydrochloride, which does not lose chlorine on oxidation, gave with ferric chloride only the isomer of type (I), and with *p*-benzoquinone a monochloro*apo*safranone. Finally, 2-amino-4′-chlorodiphenylamine hydrochloride gave a mixture of both types with both reagents, type (I) preponderating when ferric chloride and type (II) when *p*-benzoquinone was used. This result agreed with the behaviour of the parent 2-aminodiphenylamine hydrochloride under similar conditions.

The isomer of type (I) obtained by ferric chloride oxidation of 2-amino-6-chlorodiphenylamine hydrochloride (2-anilino-1 : 6-dichloro-3 : 5-dihydro-3-imino-5-phenylphenazine) gave with concentrated sulphuric acid a red-blue colour in place of the brown-red colour usually obtained with isomers of this class. Any doubt as to its nature was dispelled, however, when in warm acetone in the presence of syrupy phosphoric acid it gave a yellowish-green fluorescent glyoxalinophenazine such as is obtained by condensation of other anilino-*apo*safranines with ketones.[1]

As mentioned in Part I,[1] the filtrate, obtained after removal of the insoluble material resulting from the *p*-benzoquinone oxidation of 2-aminodiphenylamine hydrochloride, contained a number of other products. One such (compound A) was isolated in very small yield as brown plates, m. p. 243—245°, and had the formula, $C_{18}H_{12}ON_2$. In the *p*-benzoquinone oxidations of chlorinated 2-aminodiphenylamine hydrochlorides discussed earlier in this paper, similar materials were sometimes isolated, containing chlorine and,



perhaps for this reason, less soluble than compound (A). None of these compounds was encountered on ferric chloride oxidation. They were insoluble in alkali and probably did not contain a hydroxyl group. They are probably condensation products of the benzoquinone and the diphenylamine derivative, formed as shown, and compound A was shown to be *apo*safranone [5, 6, 7] by comparison with a sample. *apo*Safranone was synthesised by Kehrmann and Bürgin's method.[5]

[4] Barry and Belton, *Proc. Roy. Irish Acad.*, 1955, **57**, *B*, 141.
[5] Kehrmann and Bürgin, *Ber.*, 1896, **29**, 1819.
[6] Jaubert, *Ber.*, 1895, **28**, 275.
[7] Kehrmann, *Ber.*, 1897, **30**, 2623.

Chlorinated *apo*safranones were isolated from the *p*-benzoquinone oxidation of 2-amino-4-chloro-, -6-chloro-, -4 : 4'-dichloro-, -4 : 5-dichloro-, -4'-chloro-, and -4 : 2' : 4'-trichlorodiphenylamine hydrochlorides, but not of 2-amino-5-chlorodiphenylamine hydrochloride.

A multiplicity of coloured materials results from the oxidation of 2-aminodiphenylamine bases. On the whole, with the exception of a small amount of the phenazine (II; R = R' = H), these appear to be different from the products of oxidation of the amine hydrochlorides. They have not, however, been thoroughly investigated.

The effect of the chlorine substituents on the activity of the isomers of types (I) and (II) in experimental tuberculosis in mice is interesting. The original anilino*apo*safranine (I; R = R' = H) produces an increased median survival time (M.S.T.) over the controls in mice (M.S.T., 14 days) infected intravenously with the virulent bovine *Ravenel Rv* strain of *Mycobacterium tuberculosis* (inoculum 0·1 mg.) of about 6 days, while the isomer (II; R = R' = H) gives an increased M.S.T. of 12·5 days. The drugs were fed in the diet for the first 14 days at a dosage level of 100 mg./kg. of mouse for (I; R = R' = H) and 125 mg./kg. for (II; R = R' = H). This higher activity of the type (II) isomer is in agreement with the similar findings reported in Part II [8] for other pairs of isomers. When the two isomers prepared from 2-amino-4'-chlorodiphenylamine were screened in mice, an enormous increase in activity was found: the dichlorinated products fed at about the same dosage levels showed a greatly enhanced protective effect, extending the M.S.T. to about 140 days in each case. This enhancement is brought about by the substitution of two chlorine atoms on the periphery of the molecule and may be due in part to an increased fat-solubility which also serves to anchor the molecule in a particular orientation. Where the chlorine is substituted in the phenazine nucleus as in the compounds (I; R = H, R' = Cl) and (II; R = Cl, R' = H), the compounds appear to have negligible activity. The detailed biological experiments will be reported elsewhere.

Experimental

Oxidation of 2-Amino-4'-chlorodiphenylamine Hydrochloride.—(a) *With ferric chloride.* The amine hydrochloride (5 g.) in 1 : 2 aqueous alcohol (150 c.c.) was stirred for 3 hr. with 10% aqueous ferric chloride (200 c.c.). The precipitate was converted into the base with alcoholic sodium hydroxide and chromatographed in benzene on alumina (Merck). Two main red fractions were obtained: the first (0·25 g.), dark red plates (benzene), m. p. 255—257°, was 2-*amino*-5-*chlorophenyl*-3-p-*chlorophenylimino*-3 : 5-*dihydrophenazine* (Found: C, 67·1; H, 3·6; N, 13·1; Cl, 16·6. $C_{24}H_{16}N_4Cl_2$ requires C, 66·8; H, 3·7; N, 13·0; Cl, 16·5%). The second (3·5 g.) was the (I) type isomer, 2-p-*chloroanilino*-5-p-*chlorophenyl*-3 : 5-*dihydro*-3-*iminophenazine*, a brick red powder (benzene), m. p. 280—283° decomp. (Found: C, 66·6; H, 3·6; N, 13·0; Cl, 16·2%).

(b) *With* p-*benzoquinone.* The amine hydrochloride (10 g.) in water (350 c.c.) and ethanol (80 c.c.) at 80° was stirred for 3 hr. with an aqueous solution of *p*-benzoquinone (7·0 g. in 250 c.c.). The precipitate (6·7 g.) was converted into the base as above and chromatographed as before. 2 G. of the crude base yielded 1·3 g. of the type (II) isomer and 0·3 g. of the type (I) isomer.

10-p-*Chlorophenyl*-2 : 10-*dihydro*-2-*oxophenazine.*—The filtrate from reaction (b) above was made alkaline with sodium hydroxide, and the dark material separating was chromatographed in benzene on alumina, giving, as main product, dark red needles of the apo*safranone*, m. p. 241—242° (benzene), in about 4% yield based on the diphenylamine hydrochloride (Found: C, 70·1; H, 3·8; N, 9·1; Cl, 11·3. $C_{18}H_{11}ON_2Cl$ requires C, 70·5; H, 3·6; N, 9·1; Cl, 11·6%). The parent *apo*safranone isolated similarly from the *p*-benzoquinone oxidation of 2-aminodiphenylamine hydrochloride has already been described (cf. Part I [1]). It was also prepared by the method of Kehrmann and Bürgin.[5] These authors' description is lacking in some experimental detail which we have supplied:

2 : 10-*Dihydro*-2-*oxo*-10-*phenylphenazine* (apo*safranone*).—*N*-2 : 4-Dinitrophenyl-*N'*-phenyl-*o*-phenylenediamine (5 g.) was mixed with benzoic acid (50 g.) and boiled (15 min.). The cooled mass was ground and extracted repeatedly with dilute aqueous ammonia. The residue was washed with water, dried, extracted with benzene, and chromatographed. The main fraction eluted with benzene weighed 1·1 g. and was analytically pure.

[8] Barry, Belton, O'Sullivan, and Twomey, preceding paper.

Oxidation of 2-Amino-5-chlorodiphenylamine Hydrochloride.—(a) *With ferric chloride.* This was carried out as above. The only product isolated (20%) was *2-amino-7-chloro-3 : 5-dihydro-5-phenyl-3-phenyliminophenazine* (II; R = Cl, R′ = H), red needles (from benzene), m. p. 264—265° (Found: C, 72·4; H, 4·3; N, 13·8; Cl, 9·0. $C_{24}H_{17}N_4Cl$ requires C, 72·6; H, 3; N, 14·1; Cl, 8·9%).

(b) *With* p-*benzoquinone.* The only product isolated was again (II; R = Cl, R′ = H) in a yield of 20%.

Oxidation of 2-Amino-4-chlorodiphenylamine Hydrochloride.—(a) *With ferric chloride.* This gave *2-anilino-8-chloro-3 : 5-dihydro-3-imino-5-phenylphenazine* (I; R = H, R′ = Cl) (20% of purified material), dark red needles (from benzene), m. p. 186—187° (Found: C, 72·3; H, 4·3; Cl, 9·2. $C_{24}H_{17}N_4Cl$ requires C, 72·6; H, 4·3; Cl, 8·9%).

(b) *With* p-*benzoquinone.* The oxidation was carried out as usual. Chromatography of the basified material gave one red-purple benzene eluate which on concentration gave reddish-brown crystals, m. p. 303—305° raised to 308—310° on recrystallisation from benzene. The mother-liquor on keeping gave *2-amino-8-chloro-3 : 5-dihydro-5-phenyl-3-phenyliminophenazine* as dark red needles (from benzene), m. p. 243—245°, identified from colour reactions (yield, 2%) (Found: C, 72·5; H, 4·3; N, 14·0; Cl, 8·7%). The other product was the *apo*safranone, *8-chloro-3 : 5-dihydro-3-oxo-5-phenylphenazine* (Found: C, 70·0; H, 3·9; N, 8·9; Cl, 11·4. $C_{18}H_{11}ON_2Cl$ requires C, 70·5; H, 3·6; N, 9·1; Cl, 11·6%).

Oxidation of 2-Amino-6-chlorodiphenylamine Hydrochloride.—(a) *With ferric chloride.* The only product identified was *2-anilino-1 : 6-dichloro-3 : 5-dihydro-3-imino-5-phenylphenazine*, reddish-brown crystals (from benzene), m.p. >320° (Found: C, 67·6; H, 4·0; N, 12·6. $C_{24}H_{16}N_4Cl_2,\frac{1}{4}C_6H_6$ requires C, 67·9; H, 3·9; N, 12·4%).

(b) *With* p-*benzoquinone.* The only product obtained from the column was a chloro*apo*safranone, *1-chloro-8 : 10-dihydro-8-oxo-10-phenylphenazine*, brown-red needles (from benzene), m. p. 267—268° (yield, 60%) (Found: N, 9·4; Cl, 11·6%).

Oxidation of 2-Amino-4 : 5-dichlorodiphenylamine Hydrochloride.—(a) *With ferric chloride.* The only product identified from the column was *2-amino-7 : 8-dichloro-3 : 5-dihydro-5-phenyl-3-phenyliminophenazine* (II; R = R′ = Cl), red needles (from benzene), m. p. 261—263° (yield, 25%) (Found: C, 67·2; H, 3·8; N, 12·5; Cl, 16·1. $C_{24}H_{16}N_4Cl_2$ requires C, 66·8; H, 3·7; N, 13·0; Cl, 16·5%).

(b) *With* p-*benzoquinone.* The only product obtained crystalline was the dichloro*apo*safranone. It was identified by colour reactions but there was not sufficient for analysis.

Oxidation of 2-Amino-4 : 4′-dichlorodiphenylamine Hydrochloride.—(a) *With ferric chloride.* Both isomers were obtained after chromatographic separation. *8-Chloro-5-*p-*chlorophenyl-2-*p-*chlorophenylamino-3 : 5-dihydro-3-iminophenazine* (15%) was obtained as an orange powder (from benzene), m. p. <330° (Found: C, 62·1; H, 3·3; Cl, 23·1. $C_{24}H_{15}N_4Cl_3$ requires C, 61·9; H, 3·2; Cl, 22·9%). *2-Amino-8-chloro-5-*p-*chlorophenyl-3-*p-*chlorophenylimino-3 : 5-dihydrophenazine* was got in 65% yield as dark red needles (from benzene), m. p. 279—282° (Found: C, 62·4; H, 3·6; N, 11·6; Cl, 22·3. $C_{24}H_{15}N_4Cl_3,\frac{1}{4}C_6H_6$ requires C, 63·1; H, 3·4; N, 11·5; Cl, 22·0%).

(b) *With* p-*benzoquinone.* Only the isomer of type (II) and the chlorinated *apo*safranone were identified. The latter, *8-chloro-5-*p-*chlorophenyl-3 : 5-dihydro-3-oxophenazine*, was obtained in poor yield as dark red needles (from benzene), m. p. 237—239° (Found: C, 63·1; H, 3·2; N, 8·1; Cl, 21·1. $C_{18}H_{10}ON_2Cl_2$ requires C, 63·3; H, 2·9; N, 8·2; Cl, 20·8%).

Oxidation of 2-Amino-4 : 2′ : 4′-trichlorodiphenylamine Hydrochloride.—(a) *With ferric chloride.* Only the type (II) isomer, *2-amino-8-chloro-5-(2 : 4-dichlorophenyl)-3-(2 : 4-dichlorophenylimino)-3 : 5-dihydrophenazine*, was isolated; it formed dark red crystals (from benzene), m. p. 236—237° (Found: C, 54·8; H, 2·8; N, 9·5; Cl, 32·1. $C_{24}H_{13}N_4Cl_5,\frac{1}{4}C_6H_6$ requires C, 55·2; H, 2·7; N, 10·0; Cl, 32·0%).

(b) *With* p-*benzoquinone.* Only the chlorinated *apo*safranone, *8-chloro-5-(2 : 4-dichlorophenyl)-3 : 5-dihydro-3-oxophenazine*, (20%) was identified; it formed dark red needles, m. p. 215—217°, from benzene (Found: C, 57·0; H, 2·8; N, 7·6; Cl, 28·2. $C_{18}H_9ON_2Cl_3$ requires C, 57·5; H, 2·4; N, 7·5; Cl, 28·4%).

Grateful acknowledgment is made to J. R. Geigy S.A., Basle, Switzerland, for financial aid.

LABORATORIES OF THE MEDICAL RESEARCH COUNCIL OF IRELAND,
TRINITY COLLEGE, DUBLIN. [*Received, October 7th*, 1955.]