189. *The Oxidation of Derivatives of* o-*Phenylenediamine. Part II.* Phenazine Pigments obtained from* N-*Alkyl-*, N-cyclo*Alkyl-*, N-*Alkylphenyl-, and* N-*Alkoxyphenyl-*o-*phenylenediamine Hydrochloride.*

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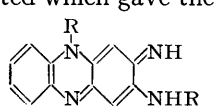
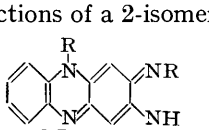
Various *N*-substituted derivatives of *o*-phenylenediamine hydrochloride have been oxidised with ferric chloride and *p*-benzoquinone and the highly coloured phenazine pigments related to 2-anilino-3 : 5-dihydro-3-imino-5-phenylphenazine (anilino*apo*safranine) and its isomer, 2-amino-3 : 5-dihydro-5-phenyl-3-phenyliminophenazine, have been separated. The effect of certain substituent groups on the antituberculosis activity in mice of the isomeric pigments is discussed briefly.

IN Part I [1] it was shown that oxidation of 2-aminodiphenylamine hydrochloride with ferric chloride or *p*-benzoquinone gave, among other products, mixtures of 2-anilino-3 : 5-dihydro-3-imino-5-phenylphenazine (anilino*apo*safranine) and its isomer, 2-amino-3 : 5-dihydro-5-phenyl-3-phenyliminophenazine. For the sake of brevity these are named below the 2-isomer and the 3-isomer respectively. In the present paper the oxidative condensation has been applied to a number of derivatives of *o*-phenylenediamine in the hope of discovering substituted phenazines of higher antituberculosis activity and to collect information which would allow biological activity to be related to chemical structure.

In general the crude oxidation products of the amine hydrochlorides contained both the 2- and the 3-isomer. This was not the case when *N*-alkyl- or *N-cyclo*alkyl-*o*-phenylenediamine hydrochlorides were oxidised. Here the only product isolated was the 3-isomer whether ferric chloride or quinone was employed. When the two isomers were formed, *e.g.*, from *N*-alkylphenyl- or *N*-alkoxyphenyl-*o*-phenylenediamines, as a general rule the 3-isomer preponderated in the mixture from the quinone oxidation and the 2-isomer was present in greater quantity in the ferric chloride oxidation product. However, from the oxidation of *N*-*o*-ethoxyphenyl-*o*-phenylenediamine hydrochloride with either reagent only the 2-isomer was isolated. Oxidation of *N*-*p*-ethoxyphenyl-*o*-phenylenediamine hydrochloride with quinone gave the 3-isomer : in the ferric chloride oxidation, a stable iron complex was isolated which gave the colour reactions of a 2-isomer.



2-Isomer



3-Isomer

* Part I, preceding paper.

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

The *o*-nitroaniline intermediates were made by the usual methods, *i.e.*, by refluxing *o*-chloronitrobenzene with the substituted aniline in nitrobenzene.[2] The *N*-alkyl and *N-cyclo*alkyl derivatives of *o*-nitroaniline were formed by heating the mixture of *o*-chloronitrobenzene and the amine in pyridine,[3] a procedure found to be the most satisfactory for the preparation also of the higheralkoxy phenyl-*o*-nitroanilines. Reduction of the nitrocompounds to the *o*-phenylenediamines was effected in a number of ways—by refluxing them with iron filings in aqueous alcohol in the presence of a small quantity of acetic acid or by warming them with Raney nickel and hydrazine [4] or by using Adams catalyst. It was not possible to crystallise *N-p-iso*butoxyphenyl-*o*-nitroaniline or the corresponding *o*-phenylenediamine, and neither of these compounds has been characterised: a dilute hydrochloric acid solution of the amine was nevertheless oxidised by ferric chloride and benzoquinone and the expected 2- and 3-isomers were isolated analytically pure.

Oxidations were carried out by the method described in Part I,[1] but in aqueous alcohol for amine hydrochlorides of low solubility in water. The precipitated pigment hydrochlorides were converted into the bases and chromatographed in benzene on alumina. The *N*-alkyl- and *N-cyclo*alkyl-phenazines were somewhat unstable and appeared to decompose on the alumina column. Colour reactions [with concentrated sulphuric acid and with acetic anhydride (cf. Part I)] of the products, however, which had been purified by recrystallisation indicated that only the 3-isomer was present in each case. In certain instances analytical data were obtained only on the hydrochloride or picrate. The products obtained by ferric chloride oxidation of *N*-methyl-, *N-n*-heptyl- and *N-cyclo*hexyl-*o*-phenylenediamine have already been prepared and incorrectly described as 2-isomers.[5] These are now listed correctly in the Experimental part.

The pigments were all tested for antituberculosis activity in mice infected intravenously with the *Ravenel* bovine strain of *Mycobacterium tuberculosis* (inoculum 0·1 mg.). The drugs were fed in the diet for 14 days, starting on the day of infection. Some idea of the order of activity may be got from the following instance: 2-amino-5-*p*-ethoxyphenyl-3-*p*-ethoxyphenylimino-3 : 5-dihydrophenazine, fed at a dosage level of 120 mg./kg. for 14 days, produced an increase in median survival time, over the untreated control mice (M.S.T., 14 days), of 90 days. Of the pigments made from *N*-alkyl- and *N-cyclo*alkyl-*o*-phenylenediamines, only the *cyclo*hexyl derivative showed high activity. Pigments derived by oxidation of 2-aminodiphenylamines carrying substituents in the 4'-position all showed enhanced activity and were significantly more active than those derived from the 2-aminodiphenylamines substituted in the 2'-position. On the whole the 3-isomers are more potent antituberculosis agents in mice than the corresponding 2-isomers.

The biological details will be published elsewhere.

Experimental

N-*Substituted* o-*Nitroanilines*.—The following is a typical preparation: 4-*n*-Propoxyaniline (20·6 g.), *o*-chloronitrobenzene (21·0 g.), and pyridine (16 c.c.) were heated in a sealed tube at 200° (20 hr.). The mixture was steam-distilled and the residual oil which solidified was recrystallised from methanol (yield, 11 g.; m. p. 43°) (Found: C, 65·7; H, 6·0; N, 10·5. $C_{15}H_{16}O_3N_2$ requires C, 66·2; H, 5·9; N, 10·3%).

The following new derivatives were characterised: *Allyl-*, red oil, b. p. 174—175°/12 mm. (Found: N, 16·0. $C_9H_{10}O_2N_2$ requires N, 15·8%). n-*Propyl-*, red oil, b. p. 170—172°/12 mm. (Found: N, 14·6. $C_9H_{12}O_2N_2$ requires N, 15·55%). cyclo*Pentyl-*, pale yellow oil, b. p. 161—169°/2 mm. (Found: C, 64·1; H, 6·7; N, 13·3. $C_{11}H_{14}O_2N_2$ requires C, 64·1; H, 6·8; N, 13·6%). o-*Ethoxyphenyl-*, dark red oil, b. p. 202—208°/2 mm. (Found: C, 65·8; H, 5·8; N, 10·6. $C_{14}H_{14}O_3N_2$ requires C, 65·1; H, 5·4; N, 10·9%). p-n-*Propoxyphenyl-*, yellow needles (from methanol), m. p. 43° (Found: C, 65·7; H, 6·0; N, 10·5. $C_{15}H_{16}O_3N_2$ requires C, 66·2; H, 5·9; N, 10·3%). p-iso*Propoxyphenyl-*, orange prisms (from light petroleum), m. p. 79—80° (Found: C, 66·0; H, 6·1; N, 10·4%). *p-iso-* and *p-sec.*-Butoxyphenyl-*o*-nitroaniline were obtained as red oils which decomposed on distillation under reduced pressure.

N-*Substituted* o-*Phenylenediamines*.—The *o*-nitroanilines were reduced in alcoholic solution

[2] Borrodkin, *J. Appl. Chem.* (*U.S.S.R.*), 1950, **21**, 987.
[3] Burger and Fredericksen, *J. Amer. Chem. Soc.*, 1948, **70**, 416.
[4] Malcolm and Furst, *ibid.*, 1953, **75**, 4334.
[5] Barry, Belton, Chambers, Conalty, Kelly, and Twomey, *Proc. Roy. Irish Acad.*, 1953, **55**, *B*, 157.

by Raney nickel [4] or by Adams catalyst. Where it had been found impossible to purify the nitro-compound completely, the latter was best reduced by iron filings and acetic acid. The following are new: Allyl-, an oil which oxidised very rapidly in the air; it was reduced (Adams catalyst) to the *N*-propyl derivative (see below). *n*-Propyl-, an unstable oil, characterised as the 2 : 4-*dinitrophenyl derivative*, red needles (from ethanol), m. p. 109—110° (Found: C, 57·0; H, 5·2; N, 17·8. $C_{15}H_{16}O_4N_4$ requires C, 57·0; H, 5·1; N, 17·7%). The same compound was got from the hydrogenated *N*-allyl derivative (see above). *cyclo*Pentyl-, b. p. 132—138°/2 mm. [2 : 4-*dinitrophenyl derivative*, orange needles (from ethanol), m. p. 127—128° (Found: C, 59·3; H, 5·5; N, 15·8. $C_{17}H_{18}O_4N_4$ requires C, 59·6; H, 5·3; N, 16·3%)]. o-*Ethoxyphenyl-*, plates (from light petroleum), m. p. 79—80° (Found: C, 72·7; H, 7·2; N, 11·7. $C_{14}H_{16}ON_2,\frac{1}{4}H_2O$ requires C, 72·3; H, 7·1; N, 12·0%). This compound colours immediately in air. p-n-*Propoxyphenyl-*, plates (from light petroleum), m. p. 76° (Found: C, 74·5; H, 7·5; N, 11·5. $C_{15}H_{18}ON_2$ requires C, 74·4; H, 7·4; N, 11·5%). p-iso*Propoxyphenyl-*, needles (from aqueous ethanol), m. p. 74—75° (Found: C, 73·9; H, 7·7; N, 11·5%). p-sec.-*Butoxyphenyl-*, needles (from light petroleum), m. p. 57—59° (Found: C, 74·4; H, 7·8; N, 11·2. $C_{16}H_{20}ON_2$ requires C, 75·0; H, 7·8; N, 11·0%. No stable crystalline *p-iso*-butoxyphenyl derivative could be prepared.

Oxidation of N-*Substituted* o-*Phenylenediamine Hydrochlorides. Isolation of the* 2- *and the* 3-*Isomers.*—The *o*-phenylenediamine hydrochlorides were oxidised (*a*) by ferric chloride and (*b*) by *p*-benzoquinone, and the isomeric phenazine bases were separated on alumina (Merck) columns as described in Part I.[1] On elution with benzene the 3-isomer was always the first obtained; in some cases benzene–ether was required to remove the 2-isomer from the column.

The *phenazines* tabulated have been prepared in sufficient quantity for screening in murine tuberculosis.

5-*Aryl*-2-*arylamino*-3 : 5-*dihydro*-3-*iminophenazines* (2-*isomers*) *and* 2-*amino*-5-*aryl*-3-*arylimino*-3 : 5-*dihydrophenazines* (3-*isomers*).

R	Form	Solvent [a]	M. p.	Found (%) C	H	N	Formula	Required (%) C	H	N
				2-*Isomers*						
p-C_6H_4Me	Brown	C_6H_6	282—285°	80·3	5·6	13·9	$C_{26}H_{22}N_4$	80·0	5·6	14·4
o-C_6H_4·OMe	Brick-red	C_6H_6	216—219	75·0	5·4	12·8	$C_{26}H_{22}O_2N_4,\frac{1}{4}C_6H_6$	74·7	5·3	12·7
p-C_6H_4·OMe	Orange	C_6H_6	283—285	74·3	5·7	13·0	$C_{26}H_{22}O_2N_4$	73·9	5·2	13·3
o-C_6H_4·OEt	Dark red plates	C_6H_6	140—142	75·3	6·0	11·8	$C_{28}H_{26}O_2N_4,\frac{1}{4}C_6H_6$	75·4	5·8	11·9
p-C_6H_4·OPr^n	,, needles	C_6H_6	230	75·3	6·5	11·4	$C_{30}H_{30}O_2N_4$	75·3	6·3	11·7
p-C_6H_4·OPr^i	,, ,,	EtOH	186—188	75·1	6·8	11·5	,,	,,	,,	,,
p-C_6H_4·OBu^s	Brick-red needles	MeOH	120—123	75·4	6·6	11·0	$C_{32}H_{34}O_2N_4$	75·9	6·7	11·1
p-C_6H_4·OBu^i	Brown-red needles	EtOH	135—137	76·2	6·9	11·0	,,	,,	,,	,,
				3-*Isomers*						
Me	Red needles	C_6H_6–Lig	176—178	70·3	5·5	23·2	$C_{14}H_{14}N_4$	70·6	5·9	23·5
Allyl [b]	Dark red needles	MeOH	160 [c]	53·3	4·1	18·7	$C_{24}H_{21}O_7N_7,H_2O$	53·6	4·3	18·2
Pr^n [d]	Dark brown	—	—	46·0	5·1	—	[d]	45·4	6·3	—
C_5H_9 [e]	Brown red	Lig	170—171	76·7	7·7	15·7	$C_{22}H_{26}N_4$	76·4	7·5	16·2
n-C_7H_{15}	Dark red needles	—	300	63·4	8·7	—	$C_{26}H_{38}N_4$,2HCl	63·3	8·8	—
p-C_6H_4Me	,, ,,	C_6H_6	243—245	80·5	5·5	14·1	$C_{26}H_{22}N_4$	80·0	5·6	14·4
o-C_6H_4·OMe	Dark red	C_6H_6	227—229	74·8	5·4	12·6	$C_{26}H_{22}O_2N_4,\frac{1}{4}C_6H_6$	74·7	5·3	12·7
p-C_6H_4·OMe [g]	Dark red needles	C_6H_6	228—230	74·7	5·5	12·3	,,	,,	,,	,,
p-C_6H_4·OEt	,, ,,	C_6H_6	225—227	74·7	5·7	12·3	$C_{28}H_{26}O_2N_4$	74·7	5·8	12·4
p-C_6H_4·OPr^n	,, ,,	C_6H_6	194—196	75·2	6·4	11·8	$C_{30}H_{30}O_2N_4$	75·3	6·3	11·7
p-C_6H_4·OPr^i	,, ,,	C_6H_6	215—216	75·0	6·3	11·0	,,	,,	,,	,,
p-C_6H_4·OBu^s	,, ,,	EtOH	183—185	75·6	6·8	11·2	$C_{32}H_{34}O_2N_4$	75·9	6·7	11·1
p-C_6H_4·OBu^i	,, ,,	EtOH	176—178	75·8	6·8	10·9	,,	,,	,,	,,

[a] Lig = ligroin. [b] Picrate. [c] With decomp. [d] Hydrochloride (Found: Cl, 30·5. $C_{18}H_{22}N_4$,4HCl,2H_2O requires Cl, 29·8%); the unstable base gave unsatisfactory analyses. [e] *cyclo*-Pentyl; the *cyclo*hexyl analogue is described in Part I. [f] Hydrochloride (Found: Cl, 15·5. $C_{26}H_{38}N_4$,2HCl requires Cl, 15·6%). [g] Gives a *methosulphate*, grass-green needles (from ethanol), m. p. 300° (decomp.) [Found: C, 61·0; H, 5·1; N, 9·8; S, 6·1. $C_{26}H_{22}O_2N_4,(CH_3)_2SO_4$ requires C, 61·3; H, 5·1; N, 10·2; S, 5·8%], and a *monoacetyl derivative*, dark brown needles (from benzene), m. p. 295° (decomp.) (Found: C, 72·5; H, 5·1; N, 12·0. $C_{28}H_{24}O_3N_4$ requires C, 72·4; H, 5·2; N, 12·1%).

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