Cinnolines and Other Heterocyclic Types in Relation to the Chemotherapy of Trypanosomiasis. Part IX. Synthesis of Azoquinoxaline Derivatives.*

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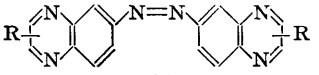
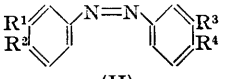
A preparative route to 3 : 3′ : 4 : 4′-tetra-aminoazobenzene is described. Condensation of this compound with butyl glyoxylate gave a mixture of hydroxy-6 : 6′-azoquinoxalines, and thence two pure dichloro-6 : 6′-azoquinoxalines in small yield. Similar condensation of 3 : 4-diaminoazobenzene has given a mixture from which 2(or 3)-hydroxy-6-phenylazoquinoxaline can be isolated directly.

The activity shown by bis(azocinnolinium salts) against *Trypanosoma congolense* (McIntyre and Simpson, *J.*, 1952, 2606, 2615) led us to consider the extension of this work to include ring systems other than cinnolines. The present communication describes attempts to synthesise the corresponding bisazoquinoxaline derivatives (I; R = NH_2) for conversion into the analogous quaternary salts. Of the general methods available for the preparation of aminoquinoxalines (Simpson, "Condensed Pyridazine and Pyrazine Rings," Interscience Publ. Inc., New York, 1953, p. 263) that involving preparation of the hydroxyquinoxaline (I; R = OH) from the di-*o*-diamine (II; $R^1 = R^2 = R^3 = R^4 = NH_2$) was selected.



(I)



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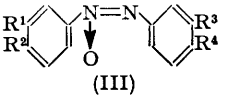

(III)

The key intermediate, 4-amino-2-nitroacetanilide, has been prepared by Chazel (*Ber.*, 1907, **40**, 3177) from *p*-aminoacetanilide by phthaloylation, nitration, and selective hydrolysis of the phthaloyl group. A number of modifications proved necessary for the smooth operation of each of these stages on a much larger scale. We have shown that phthaloylation in an aqueous medium yields roughly equal amounts of *p*-acetamidophenylphthalamic acid and *p*-phthalimidoacetanilide but the latter is exlcusively formed in acetic acid (cf. Vanags and Veinbergs, *Ber.*, 1942, **75**, 1558). Nitration of the phthalimido-compound by Chazel's method was satisfactory on the small scale but in larger preparations unchanged material and a dinitro-compound were isolated: reproducible yields of 2-nitro-4-phthalimidoacetanilide were however obtained by using nitric acid of lower density under controlled conditions. Selective hydrolysis to the amine was effected by Chazel's method. The orientation of the nitro-group which was not established by Chazel follows from deamination of the derived 4-amino-3-nitroazobenzene (see below) to 3-nitroazobenzene (Bamberger and Hübner, *Ber.*, 1903, **36**, 3803).

* Part VIII, *J.*, 1954, 1381.

Oxidation of 4-amino-2-nitroacetanilide with Caro's acid gave results analogous to those with other amines (McIntyre and Simpson, *J.*, 1952, 2606), 4 : 4′-diacetamido-3 : 3′-dinitroazoxybenzene (III; $R^1 = R^3 = NO_2$, $R^2 = R^4 = NHAc$) being the only isolatable product after reaction in acid solution whereas in neutral solution a 56% yield of 2-nitro-4-nitrosoacetanilide was separable from the azoxy-compound. Condensation of 4-amino-2-nitroacetanilide with the derived nitroso-compound gave an almost theoretical yield of the azobenzene derivative (II; $R^1 = R^3 = NO_2$, $R^2 = R^4 = NHAc$), but hydrolysis to the diamine offered some difficulty. The use of alcoholic hydrochloric acid did not give a smooth reaction, and hydrochloric–acetic acid gave a product which was shown to contain partly hydrolysed material (II; $R^1 = R^3 = NO_2$, $R^2 = NHAc$, $R^4 = NH_2$) since reduction yielded an acetamidodiamino-nitroazobenzene as well as the desired tetra-aminoazobenzene. The latter was eventually prepared in high yield by hydrolysis with sulphuric acid to the dinitro-diamine and reduction with sodium hydrogen sulphide.

The mixed hydroxyquinoxalines (I; R = OH) were obtained from 3 : 3′ : 4 : 4′-tetra-aminoazobenzene by reaction with *n*-butyl glyoxylate. Although this reagent has been widely applied by Wolf and his co-workers (*J. Amer. Chem. Soc.*, 1949, **71**, 6) to the synthesis of hydroxyquinoxalines, no convenient method of preparing it is described. We could not reproduce the high yields claimed by Wolf (personal communication) using lead tetra-acetate on *n*-butyl tartrate, but oxidation with sodium periodate provided a good preparative route. The condensation product possessed no definite melting point but the general properties agreed with the representation as (I; R = OH). Phosphoryl chloride and phosphorus pentachloride converted this crude material into a mixture giving, in low yield, two crystalline dichloro-6 : 6′-azoquinoxalines (α and β) by chromatography. Although this result could be reproduced on a small scale, attempts to increase it gave only partly pure crystalline fractions. Attempts to achieve separation of the crude mixture by treatment with phosphorus halides and subsequent phenoxylation gave only a small amount of the α-dichloro-derivative, a result in agreement with later experience (Atkinson, Brown, and Simpson, unpublished work) of the reactivity of simpler quinoxalines.

The possibility of using the azoxy-compounds (as III) as a source of the azo-types was was investigated. The compound (III; $R^1 = R^3 = NO_2$, $R^2 = R^4 = NHAc$) was smoothly hydrolysed to the diamine but an attempt to reduce the latter with sodium hydrogen sulphide gave only 3 : 4 : 3′ : 4′-tetra-aminoazoxybenzene.

Some phenylazo-derivatives were also prepared for use as model compounds; reaction between 2-nitro-4-nitrosoacetanilide and aniline, or 4-amino-2-nitroacetanilide and nitrosobenzene, gave 4-acetamido-3-nitroazobenzene (II; $R^1 = NO_2$, $R^2 = NHAc$, $R^3 = R^4 = H$), readily hydrolysed to the nitro-amine and thence by reduction to 3 : 4-diaminoazobenzene. Condensation of the latter with butyl glyoxylate gave a mixture of 2(and 3)-hydroxy-6-phenylazoquinoxalines, one of which was obtained pure directly by recrystallisation.

Experimental

p-*Aminoacetanilide*.—*p*-Nitroacetanilide (60 g.) in ethanol (600 c.c.) was hydrogenated during 90 min. at 60—70°/40 atm. with 2% palladium–strontium carbonate (4·85 g.). The catalyst was filtered off and the filtrate was treated with charcoal and concentrated to *ca.* 400 c.c., to provide *p*-aminoacetanilide (42 g.), m. p. 163·5—166°; a second-crop (7·9 g.), m. p. 166—167°, was obtained on further evaporation.

p-*Phthalimidoacetanilide*.—*p*-Aminoacetanilide (170 g.) in hot acetic acid (1 l.) was added to a hot stirred solution of phthalic anhydride (170 g.) in acetic acid (1·2 l.). A crystalline solid began to separate almost immediately, and, after 5 hours' refluxing, filtration of the cold reaction mixture gave pure *p*-phthalimidoacetanilide (288 g., 91%), m. p. 287—288°, as colourless needles.

Nitration of p-*Phthalimidoacetanilide*.—(*a*) The compound (60 g.) was added to cooled (ice–salt) nitric acid (240 c.c.; *d* 1·50) at −6° (±1°). After 1 hr. at −6° the mixture was poured on crushed ice (3 l.); the precipitate was collected, washed until acid-free, partially dried (porous plate), and digested for 2 hr. with boiling 95% ethanol (3 l.); the alcohol-soluble fraction (15 g.; m. p. 203—223°), together with an intractable oil formed on removal of solvent, were rejected. The insoluble fraction (24·5 g.), m. p. 240—244°, gave on recrystallisation from

acetic acid pale yellow tabular needles (17·6 g.), m. p. 251—253°, of a *dinitro*-derivative (Found : C, 52·0; H, 3·3; N, 15·3. $C_{16}H_{10}O_7N_4$ requires C, 51·9; H, 2·7; N, 15·1%).

This derivative (16 g.) was kept overnight with aqueous ammonia (400 c.c.; *d* 0·880), and the suspension refluxed for 6 hr. and filtered cold. *4-Amino-x : y-dinitroacetanilide* (10 g.), m. p. 252—254°, crystallised from water in large golden tabular needles (Found : C, 40·3; H, 3·6; N, 23·7. $C_8H_8O_5N_4$ requires C, 40·0; H, 3·4; N, 23·3%).

(*b*) The compound (120 g.) was added during 70 min. to stirred nitric acid (800 c.c.; *d* 1·48—1·49) at −12° to −13°, the mixture held at this temperature for 1 hr., and the clear yellow solution poured on crushed ice (4 l.). The bright yellow solid was washed well and dried over sodium hydroxide *in vacuo*, and the crude solid (135 g.; m. p. 248—250°) was recrystallised from acetic acid (4·5 l.) to provide pure 2-nitro-4-phthalimidoacetanilide (118 g.), m. p. 249—251°.

4-Amino-2-nitroacetanilide.—The precursor (61 g.; m. p. 249—251°) and aqueous ammonia (1·25 l.; *d* 0·880) were slowly (during 6 hr.) heated with stirring to the b. p. and then refluxed for 2 hr. The dark red solution was treated with carbon and cooled to provide either golden needles or dark red tabular needles, m. p. 163—166°, of the amino-compound.

2-Nitro-4-nitrosoacetanilide.—A slightly warm (supersaturated) solution of 4-amino-2-nitroacetanilide (6 g.) in dioxan (40 c.c.) was quickly added to a neutralised Caro's acid solution (900 c.c., containing 80 g. of H_2SO_5), and the mixture was shaken for 10 min. The flocculent ochre precipitate was collected and combined with a small second crop which separated after 3 hr. This crude, washed material was digested successively with ethanol–water (3 : 2; 150 c.c., " A ") and hot water and finally recrystallised from acetic acid. *4 : 4′-Diacetamido-3 : 3′-dinitroazoxybenzene* (0·69 g.) formed bright yellow fibrous needles, m. p. 241—242·5° unchanged by recrystallisation from benzene–light petroleum (b. p. 60—80°) (Found : C, 47·85; H, 3·3; N, 21·1. $C_{16}H_{14}O_7N_6$ requires C, 47·75; H, 3·5; N, 20·9%). The same compound (2·5 g.; 50%), m. p. and mixed m. p. 238—240° (needles from acetic acid), was formed by adding a hot aqueous solution of 4-amino-2-nitroacetanilide (5 g. in 350 c.c.) to Caro's acid solution (650 c.c.; 67 g. of H_2SO_5) containing ice and setting the whole aside for 16 hr.

The mixture of green and yellow needles (4·38 g.), m. p. 102—105°, which separated on cooling the aqueous-alcoholic mother-liquor " A " was recrystallised from 50% aqueous alcohol (80 c.c.), to yield pure *2-nitro-4-nitrosoacetanilide* (3·25 g.), m. p. 102·5—105° (green tabular needles from ethanol) (Found : C, 45·8; H, 3·5; N, 20·0. $C_8H_7O_4N_3$ requires C, 45·0; H, 3·4; N, 20·1%).

4 : 4′-Diacetamido-3 : 3′-dinitroazobenzene.—Slightly warm (supersaturated) solutions of 2-nitro-4-nitrosoacetanilide (3·3 g.) and 4-amino-2-nitroacetanilide (3·3 g.) in acetic acid (25 c.c. each) were mixed and set aside overnight. The precipitate (6 g.), m. p. 274—281°, was collected and recrystallised from acetic acid (2 l.); the *azo*-compound (4·5 g.), m. p. 284—285°, separated as orange needles (Found : C, 50·0; H, 3·7; N, 21·8. $C_{16}H_{14}O_6N_6$ requires C, 49·7; H, 3·8; N, 21·8%).

4 : 4′-Diamino-3 : 3′-dinitroazobenzene.—4 : 4′-Diacetamido-3 : 3′-dinitroazobenzene (6 g.) was dissolved in concentrated sulphuric acid (110 c.c.) by gentle warming, and water (187·5 c.c.) added with stirring and cooling. The suspension was heated at 100° for ½ hr. and then cooled and the dark blue sulphate was collected and treated with very dilute ammonia solution, to give the crude orange *4 : 4′-diamino-3 : 3′-dinitroazobenzene* (4·45 g.), m. p. 338—339°. Purification could be effected by recrystallisation from phenol–ethanol, but was unnecessary for the subsequent reduction. The recrystallised material consisted of red plates or orange needles, m. p. 340—341° (Found : C, 47·9; H, 4·0; N, 27·6. $C_{12}H_{10}O_4N_6$ requires C, 47·7; H, 3·3; N, 27·8%).

When the hydrolysis was carried out by a refluxing mixture of concentrated hydrochloric acid and acetic acid (25 c.c. of each per g.), and the free base recrystallised from phenol–ethanol an apparently homogeneous product (" B "), m. p. >330°, was obtained.

3 : 3′ : 4 : 4′-Tetra-aminoazobenzene.—4 : 4′-Diamino-3 : 3′-dinitroazobenzene (3 g., crude) was heated for 1 hr. with ethanol (30 c.c.) and 26% (w/v) sodium hydrogen sulphide (27 c.c.; freshly prepared), and the crystalline purple solid (2·14 g., 89%), m. p. 252—256° (decomp.), was removed whilst still hot. The pure *tetra-amine*, m. p. 252—256° (decomp.), crystallised as violet needles from aqueous ethanol (Found : C, 59·7; H, 6·0; N, 34·7. $C_{12}H_{14}N_6$ requires C, 59·5; H, 5·8; N, 34·7%).

Similar reduction of the hydrolysis product " B " (above) gave, by fractional recrystallisation from aqueous ethanol, violet needles of 3 : 3′ : 4 : 4′-tetra-aminoazobenzene, m. p. 250° (decomp.) (Found : C, 59·1; H, 6·1%), and dark red, silky needles of *4-acetamido-*3(or 3′) : *4′-diamino-*

3′(or 3)-*nitroazobenzene*, m. p. 245—246° (decomp.) (Found: C, 53·7; H, 4·5; N, 26·2. $C_{14}H_{14}O_3N_6$ requires C, 53·5; H, 4·5; N, 26·7%).

Dihydroxy-6 : 6′-*azoquinoxaline*.—A hot solution of 3 : 3′ : 4 : 4′-tetra-aminoazobenzene (1 g.) in ethanol (170 c.c.) and water (90 c.c.) was refluxed with one of *n*-butyl glyoxylate (1·1 g.) in ethanol (5 c.c.) for 6 hr., then cooled, and the brown solid (1·05 g.), m. p. indefinite, was collected. This material was almost insoluble in organic solvents but readily soluble in dilute aqueous sodium hydroxide from which it was reprecipitated in gelatinous form by dilute acids.

Dichloro-6 : 6′-*azoquinoxaline*.—Dihydroxy-6 : 6′-azoquinoxaline (1 g.), phosphoryl chloride (10 c.c.), and phosphorus pentachloride (2 g.) were refluxed for 50 min., the mixture was poured on crushed ice (*ca.* 300 c.c.), and the maroon solid (1·1 g.), m. p. 250°, was collected. This was extracted with hot benzene (3 × 80 c.c.), and the red extract evaporated to dryness; this solid (0·77 g.) was dissolved in cold benzene (300 c.c.) and poured down a column of Type "H" alumina (100 g.). Elution was continued with pure dry benzene; the first three (100 c.c.) fractions contained (α)-*dichloro*-6 : 6′-*azoquinoxaline*, m. p. 276—277°, which separated from benzene in long red needles (Found: C, 54·1; H, 2·25; N, 24·25; Cl, 20·8. $C_{16}H_8N_6Cl_2$ requires C, 54·1; H, 2·3; N, 23·7; Cl, 20·0%). This was followed by two fractions which consisted of material of m. p. *ca.* 200° and then by (β)-*dichloro*-6 : 6′-*azoquinoxaline*, m. p. 270—272°, which crystallised from benzene in short, orange-red needles (Found: C, 53·75; H, 2·5; N, 23·55; Cl, 20·65%). The mixed m. p. between the α- and β-isomers was 232—245°. (*Note*: It is suspected that the success of this separation is critically dependent on the activity of the alumina used.)

Further elution with benzene gave a negligible amount of material and the solid eluted by chloroform did not crystallise. The combined α- and β-fractions (formed in approximately equal amount) accounted for only 30% of the benzene-soluble chloro-compound.

Diphenoxy-6 : 6′-*azoquinoxaline*.—Dichloro-6 : 6′-azoquinoxaline (0·1 g. crude) was heated with a solution of potassium hydroxide (0·05 g.) in phenol (0·5 g.) for ¾ hr. on a steam-bath, and the mixture was cooled somewhat and poured into 2N-sodium hydroxide (15 c.c.). The orange solid (0·17 g.) had m. p. 205—220° and on its being heated with phenol–ethanol a small fraction (0·025 g.), m. p. 275—285°, remained undissolved. Further solid (0·025 g.), m. p. 230—240°, separated on cooling. Addition of more ethanol precipitated a third fraction (0·03 g.), m. p. 180—210°. None of these fractions was crystalline.

In another experiment the crude product was chromatographed on Type "H" alumina. The only crystalline material obtained was a little of the α-dichloro-compound.

4-*Acetamido*-3-*nitroazobenzene*.—(*a*) Solutions in acetic acid of 2-nitro-4-nitrosoacetanilide (0·18 g. in 2 c.c.) and aniline (0·1 c.c. in 1 c.c.) were mixed and set aside overnight. Addition of water precipitated 4-*acetamido*-3-*nitroazobenzene* (0·23 g.) which crystallised from aqueous methanol in golden-yellow needles, m. p. 143—145° (Found: C, 58·9; H, 4·1; N, 19·7. $C_{14}H_{12}O_3N_4$ requires C, 59·1; H, 4·3; N, 19·7%).

(*b*) Warm solutions in acetic acid of 4-amino-2-nitroacetanilide (19 g. in 125 c.c.) and nitrosobenzene (15 g. in 125 c.c.) were mixed and set aside overnight. Addition of water precipitated the same compound (25 g.) as in (*a*).

4-*Amino*-3-*nitroazobenzene*.—The foregoing acetamido-compound (24·9 g.) was refluxed with ethanol (500 c.c.), and concentrated hydrochloric acid (250 c.c.) was added. The crystals which separated quickly from a clear solution were filtered off when cold, combined with a second crop obtained by dilution, and recrystallised from acetic acid (1·1 l., 73%), to provide orange-red needles (19·8 g.), m. p. 171—173° or 172—174° (from aqueous ethanol), of 4-*amino*-3-*nitroazobenzene* (Found: C, 59·8; H, 3·9; N, 23·1. $C_{12}H_{10}O_2N_4$ requires C, 59·5; H, 4·2; N, 23·1%).

3 : 4-*Diaminoazobenzene*.—The nitroamine (5 g.), ethanol (100 c.c.), and a 19% solution of sodium hydrogen sulphide (20 c.c.) were heated at 50° for 30 min. and the dark red solution was diluted with water (120 c.c.). Orange needles (3·68 g.; m. p. 125—136°) were collected from the cold mixture, and a second crop (0·60 g., m. p. 135—138°) was obtained by addition of water (250 c.c.) to the filtrate. The first crop yielded on recrystallisation (carbon) from a mixture of ethanol (50 c.c.) and water (35 c.c.) orange-red needles (0·66 g.) of the starting material, m. p. and mixed m. p. 172—173°. The second crop was recrystallised (carbon) from the aqueous-alcoholic mother-liquor with added water (75 c.c.) and ethanol (20 c.c.), to provide orange-yellow needles of 3 : 4-*diaminoazobenzene* (3·28 g.), m. p. 135—138° raised to 137·5—139·5° on recrystallisation from benzene–light petroleum (b. p. 60—80°) Found: C, 67·4; H, 5·7; N, 26·7. $C_{12}H_{12}N_4$ requires C, 67·9; H, 5·7; N, 26·4%).

4 : 4′-*Diamino*-3 : 3′-*dinitroazoxybenzene*.—The diacetamido-compound (0·3 g.) was refluxed

for 90 min. with acetic acid (10 c.c.) and concentrated hydrochloric acid (10 c.c.). The bulky orange precipitate [0·24 g.; m. p. 320—325° [decomp.)] was collected cold and recrystallisation from phenol-ethanol gave the *diamino*-derivative, m. p. 328—330° (decomp.), as orange-red tabular needles (Found: C, 45·9; H, 3·4; N, 26·9. $C_{12}H_{10}O_5N_6$ requires C, 45·3; H, 3·2; N, 26·4%).

3 : 3′ : 4 : 4′-*Tetra-aminoazoxybenzene.*—4 : 4′-Diamino-3 : 3′-dinitroazoxybenzene (0·16 g.), butanol (10 c.c.), ethanol (10 c.c.), and a 19% solution of sodium hydrogen sulphide (3·5 c.c.) were heated together on a steam-bath for 1 hr. and the mixture then cooled, carefully acidified with concentrated hydrochloric acid, and clarified. More hydrochloric acid was added to the filtrate, and the amorphous black hydrochloride was collected, dissolved in water, and basified. The *tetra-amino*-compound (0·05 g.) crystallised from water as golden tabular needles, m. p. 231—233° (decomp.) (Found: C, 56·4; H, 5·6; N, 33·2. $C_{12}H_{14}ON_6$ requires C, 55·8; H, 5·5; N, 32·6%).

3-*Nitroazobenzene.*—(*a*) Warm solutions of *m*-nitroaniline (1 g.) and nitrosobenzene (1·3 g.) in acetic acid (5 c.c. each) were mixed and set aside overnight. The mixture was diluted and the product (1·37 g.), m. p. 94°, recrystallised (charcoal) from aqueous ethanol to provide 3-nitroazobenzene, m. p. 95—97° (Bamberger and Hübner, *loc. cit.*, give m. p. 95—96°).

(*b*) 4-Amino-3-nitroazobenzene (0·5 g.) was diazotised at 0° in a mixture of concentrated sulphuric acid (15 c.c.) and water (15 c.c.) with a 20% solution of sodium nitrite (1·2 c.c.). Hypophosphorous acid (40 c.c. of a 30% solution) was added at 0° and the mixture set aside overnight. The precipitate was recrystallised successively from aqueous ethanol and aqueous acetic acid, and the product (0·32 g.), m. p. 86—94°, chromatographed in benzene–light petroleum (b. p. 60—80°) (2 : 1), to provide golden needles (0·25 g.) of 3-nitroazobenzene, m. p. and mixed m. p. with (*a*) 96—98°.

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