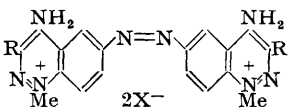
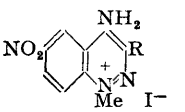
493. *Cinnolines and Other Heterocyclic Types in Relation to the Chemotherapy of Trypanosomiasis. Part IV.* Synthesis of Azocinnoline Derivatives.*

By J. McIntyre and (the late) J. C. E. Simpson.

Azocinnolines containing an azo-substituent at $C_{(6)}$ and other substituents at $C_{(4)}$ have been prepared from appropriate *o*-aminoarylazoacetophenones; the synthesis of these intermediate azo-compounds from arylamines and nitroso-compounds has necessitated a study of the conditions governing the formation of nitroso-compounds by the oxidation of arylamines with Caro's acid. Quaternisation of the diaminoazocinnoline (V; R = NH_2) gives two different dimethochlorides, each of which shows activity against *T. congolense* infections in mice; the monoquaternary salt (VI), on the other hand, is inactive.

The present communication describes experiments directed towards the synthesis of bis-azocinnolinium quaternary salts (A; R = H). The aim of this work was to test the validity of the hypothesis that the trypanocidal compounds produced during the reduction of 6-nitro-4-aminocinnolinium salts (as *B*) are the 6 : 6′-azocinnolinium salts (Keneford, Lourie, Morley, Simpson, Williamson, and Wright, *Nature*, 1948, **161**, 603; *J.*, 1952, 2595).

(*A*)  (*B*) 

The unfavourable physical properties of 4 : 4′-disubstituted 6 : 6′-azoquinolines (Macey and Simpson, preceding paper) suggested that it would be advantageous to introduce the azo-linkage at as late a stage as possible during the synthesis. The possibility of preparing azocinnolines from 6-nitrocinnoline (Morley, *J.*, 1951, 1971) or its 4-substituted derivatives was rejected because 6-nitroquinoline is unsuitable as a starting-point for similar reactions (Macey and Simpson, *loc. cit.*), but the condensation of nitroso- with amino-cinnolines seemed attractive provided that the nitroso-compounds could be made available. The oxidation of 4 : 6-diaminoquinoline with Caro's acid was therefore examined as a model. No identifiable product was formed in neutral solution, and in acid solution the theoretical amount of Caro's acid gave only a small yield of 4-amino-6-nitroquinoline, which became

Table 1. *Oxidation of amines with Caro's acid in neutral and in acid solution.*

	Products formed in acid solution		Products formed in neutral solution		
Amine used	Azoxy	Nitro	Nitroso	Azoxy	Nitro
m-Aminoacetophenone	+(25%)	—	+(50%)	+	—
p-Nitroaniline	+(60%)	—	+(30%)	+(50%)	—
2-Acetyl-4-aminoacetanilide	—	+(20%)	+(80%)	+	—
4-Amino-2-nitroacetanilide	+(50%)	—	+(55%)	+	+
6-Aminophthalide *	+(55%)	—	No product identified		

* Unpublished work.

the main product when the amount of reagent was increased. The formation of nitro- rather than nitroso-compounds by treatment of aromatic amines with Caro's acid is not uncommon (we have ourselves observed it in two instances; see Table 1), and the reaction may be even more difficult to arrest at the nitroso-stage with heterocyclic amines, as 2-, 3-, and 4-aminopyridine all give the corresponding nitro-compounds on oxidation with reagents similar to Caro's acid (Kirpal and Böhm, *Ber.*, 1931, **64**, 767; 1932, **65**, 680; Schickh, Binz, and Schulz, *ibid.*, 1936, **69**, 2593). Experiments (carried out by Dr. J. S. Morley) on the condensation of nitrosobenzene with heterocyclic amines were equally unpromising; in alcohol or acetic acid, 4-aminocinnoline and 4-aminoquinoline failed to react,

* Part III, preceding paper.

as was expected, but 5- and 6-aminoquinoline underwent complex reactions and yielded highly-coloured tars from which no pure substance could be isolated.

In view of these discouraging results, attention was turned to the preparation of 2-amino-5-arylazoacetophenones, which might be expected to yield 6-arylazo-4-hydroxycinnolines by diazotisation and cyclisation. No aminoacetophenone of this type has yet been described, but Elbs and Wogrinz (*Z. Elektrochem.*, 1903, **9**, 429) claimed to have prepared crystalline 3 : 3'-diacetylazobenzene (I; R = R' = H) by electrolytic reduction of *m*-nitroacetophenone; nitration of this might conceivably yield the useful intermediates (I; R = H, R' = NO_2; R = R' = NO_2). However, an attempt (by Dr. J. S. Morley) to reproduce Elbs and Wogrinz's results gave no crystalline product, and the subsequent preparation of (I; R = R' = H) by other routes (see below) showed that the claim of these workers is inaccurate. It was therefore decided to prepare the arylazoacetophenones by condensing appropriately substituted amino- and nitroso-compounds.

Nitroso-compounds have been prepared from arylamines by means of various oxidising agents, of which Caro's acid is the most generally convenient despite the large volumes which its use entails in preparative work. Many examples are known, but no attempt seems to have been made to define the effect of variations in experimental conditions, and in the nature and position of substituents, on the course of the reaction. The arylamine is considered (Sidgwick, " Organic Chemistry of Nitrogen," Oxford Univ. Press, 1942, p. 204) to be converted first into the corresponding hydroxylamino-compound and thence into the nitroso-derivative, which may then either survive or yield an azoxy-compound by condensation with unoxidised hydroxylamino-compound. In our experience (see Table 1) it is essential, in order to obtain a good yield of nitroso-derivative, to work with neutralised Caro's acid, and to add the amine as a solution in dioxan, whereby a well-dispersed suspen-

TABLE 2. *Oxidation of* o-*substituted amines with Caro's acid in acid solution.*

Substituents (NH_2 = 1)	Yield (%) of nitroso-cpd.	Ref.	Substituents (NH_2 = 1)	Yield (%) of nitroso-cpd.	Ref.
2-NO_2	78	1	2-Me-4-NO_2	90	2
2-NO_2-4-Me	75	1	2-Me-5-NO_2	Good	3
2-NO_2-6-Me	60	2			

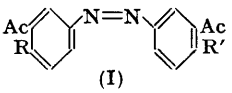
1, Bamberger and Hübner, *Ber.*, 1903, **36**, 3803. 2, Meisenheimer and Hesse, *Ber.*, 1919, **52**, 1161. 3, Chardonnens and Heinrich, *Helv. Chim. Acta*, 1940, **23**, 1399.

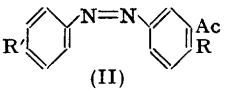
sion is formed which reacts rapidly; in acid solution, on the other hand, little if any nitroso-compound can be isolated, the main product usually being the azoxy-derivative. Examination of the literature confirms this conclusion (the use of dioxan is new), and in addition shows that *o*-substituted amines are a striking exception to this rule in that their oxidation to nitroso-compounds can also be carried out in acid solution (see Table 2); the yields are usually good, and little or no azoxy-compound is formed. These general observations are readily explained by Brand and Mahr's findings (*J. pr. Chem.*, 1931, **131**, 97; 1935, **142**, 153) that condensation of nitroso- with hydroxylamino-compounds (to give azoxy-derivatives) is catalysed both by alkali and by acid (the reaction velocity therefore being presumably minimal in neutral solution), and also that it is hindered by the presence of an *o*-substituent. The conditions which, according to their results, favour the survival of nitroso-compound in the presence of hydroxylamino-compound are thus identical with those which produce optimal yields of nitroso-compound when an amine is treated with Caro's acid.

Interaction between *m*-nitrosoacetophenone and aniline yielded 3-acetylazobenzene (II; R = R' = H), also prepared by the alternative condensation of nitrosobenzene with *m*-aminoacetophenone. Attempts to nitrate (II; R = R' = H) under a variety of conditions were unsuccessful, but the mono-nitro-derivative (II; R = H, R' = NO_2) was prepared by condensing *m*-aminoacetophenone with *p*-nitronitrosobenzene. Efforts to induce reaction between nitrososobenzene and 5-amino-2-nitrosoacetophenone, which would have given the isomer (II; R = NO_2, R' = H), were unsuccessful.

The preparation of 5-arylazoacetophenones containing a protected amino-group at position 2 was then investigated. 5-Amino-2-benzamidoacetophenone and nitrosobenzene

reacted with ease, but the resultant 3-acetyl-4-benzamidoazobenzene (II; R = NHBz, R′ = H) could not be hydrolysed to the free amine. However, interaction of 2-acetamido-5-


(I)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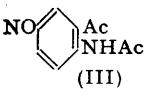
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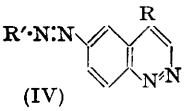
aminoacetophenone (prepared in almost quantitative yield by catalytic reduction of 2-acetamido-5-nitroacetophenone; chemical reduction was less satisfactory) with nitrosobenzene and with *m*-nitrosoacetophenone readily yielded 4-acetamido-3-acetylazobenzene (II; R = NHAc, R′ = H) and 4-acetamido-3 : 3′-diacetylazobenzene (I; R = H, R′ = NHAc) respectively, whence the corresponding amines were easily prepared by acid hydrolysis. The structure of the first of these amines (II; R = NH_2, R′ = H) was proved by deamination to 3-acetylazobenzene (II; R = R′ = H), identical with material prepared as described above. 2-Acetamido-5-nitrosoacetophenone (III) (prepared from the 5-amino-compound) condensed smoothly with 2-acetamido-5-aminoacetophenone, to yield 4 : 4′-diacetamido-3 : 3′-diacetylazobenzene (I; R = R′ = NHAc), but hydrolysis of this to the free amine (I; R = R′ = NH_2) was difficult, probably owing to the extreme insolubility of the compounds concerned; however, it was found that any unhydrolysed material was easily recoverable at the next stage of the synthesis, and could be re-hydrolysed and used again. The constitution of (I; R = R′ = NH_2) was confirmed by deamination to *m* : *m*′-diacetylazobenzene (I; R = R′ = H), also obtained in a less pure condition by interaction of *m*-nitroso- and *m*-amino-acetophenone; the properties of the pure azo-compound are different from those of the material for which this structure was claimed by Elbs and Wogrinz (*loc. cit.*).

The amines (II; R = NH_2, R′ = H), (I; R = H, R′ = NH_2 and R = R′ = NH_2) could not be satisfactorily converted into 4-hydroxycinnolines by diazotisation and cyclisation under the usual conditions (cf. Schofield and Simpson, *J.*, 1945, 520, and later papers). Diazotisation occurred in hydrochloric or sulphuric acid of various strengths, and further re-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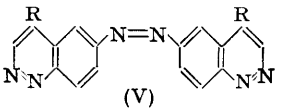
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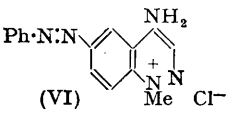
(VI)

action ensued on standing or warming (as shown by the disappearance of the initial coupling reaction with β-naphthol), but the products which separated were amorphous, and remained so even after attempted conversion into acetyl or 4-chloro-derivatives. However, the use of formic acid as diazotisation medium gave satisfactory results, and 4-hydroxy-6-phenylazocinnoline (IV; R = OH, R′ = Ph), 6-*m*-acetylphenylazo-4-hydroxycinnoline (IV; R = OH, R′ = *m*-C_6H_4Ac), and 4 : 4′-dihydroxy-6 : 6′-azocinnoline (V; R = OH) were thus obtained crystalline, the first two of these compounds being further characterised as acetates.

The three 4-hydroxycinnolines were converted smoothly under carefully defined conditions into the corresponding 4-chloro-compounds, from which 4-phenoxy-6-phenylazocinnoline (IV; R = OPh, R′ = Ph) and 4 : 4′-diphenoxy-6 : 6′-azocinnoline (V; R = OPh) were prepared by standard methods and were in turn converted into the amino-compounds (IV; R = NH_2, R′ = Ph) and (V; R = NH_2) by the action of ammonia, phenol, and ammonium chloride (cf. Macey and Simpson, preceding paper).

The transformation of (IV; R = NH_2, R′ = Ph) into 4-amino-6-phenylazocinnoline methochloride (VI) was effected by each of the following methods: (*a*) the base was converted into the methiodide by reaction with methyl iodide in ethanol, and the salt was boiled with an aqueous suspension of silver chloride; (*b*) the base was acetylated, and the product obtained by interaction of the acetyl derivative with methyl toluene-*p*-sulphonate was converted into the methiodide, which was then hydrolysed with dilute hydrochloric acid and finally boiled with silver chloride as in (*a*). The constitution of (VI) follows from the identity of the samples obtained by both routes.

Application of the above methods of quaternisation to (V; R = NH_2) gave unexpected results. When the crude dimethiodide was converted into the dimethochloride, two products were isolated, which we have designated α- and β-4 : 4′-diamino-6 : 6′-azocinnoline dimethochloride. The same two compounds were formed when the base (V; R = NH_2) was acetylated and the product was quaternised with methyl toluene-*p*-sulphonate and then converted by hydrolysis into the diamino-dimethochloride. Analysis of these compounds indicates that they are respectively tri- and hemi-hydrates, but their properties suggest that the differences between them may be more profound than can be accounted for by variations in the degree of hydration. Thus the β-compound is precipitated immediately by the addition of a little hydrochloric acid to its aqueous solution, whereas the α-compound is precipitated only slowly and incompletely under these conditions. Further, the compounds were not interconvertible when crystallised under appropriate conditions, although attempts to differentiate them by paper chromatography (Dr. C. M. Atkinson) were inconclusive.

Biological examination of the compounds, which was carried out by Dr. E. M. Lourie and Dr. J. M. Walker in the Department of Pharmacology, Oxford, has shown that they are beyond doubt distinct entities. For subcutaneous injection into mice infected with *T. congolense*, the maximum tolerated dose (M.T.D.) for the α-compound was between 0·25 and 0·5 mg./20 g., the minimum curative dose (M.C.D.) was between 0·125 and 0·25 mg., and the minimum effective dose (M.E.D.) (causing temporary disappearance of trypanosomes followed by relapse) was 0·031 mg. The β-compound, on the other hand, was much less toxic and much less effective, the corresponding figures being 2—8 (M.T.D.), 2—8 (M.C.D.), and 0·25 mg. (M.E.D.). The mono-quaternary salt (VI) was inactive, and the projected conversion of (IV; R = Cl, R′ = *m*-C_6H_4Ac) into the analogue of (VI) was therefore abandoned.

Experimental

M. p.s are uncorrected.

2-Acetamido-5-aminoacetophenone.—2-Acetamido-5-nitroacetophenone was prepared as previously described (*J.*, 1945, 646; 1947, 237), nitration of acetophenone in 150-g. batches giving 43% of pure *m*-nitroacetophenone and 40% of crude *o*-isomer. Reduction of the acetamido-nitro-ketone was effected in two ways.

(*a*) A mixture of the compound (5·3 g.), acetic acid (52 c.c.), water (50 c.c.), and iron powder (10 g.) was heated on the steam-bath for *ca.* 1 hour. Dilution with water and extraction with ether yielded *2-acetamido-5-aminoacetophenone*, which crystallised from alcohol in small yellow prisms, m. p. 162—164° (2·5 g., 55%) (Found: C, 62·2; H, 6·2; N, 14·7. $C_{10}H_{12}O_2N_2$ requires C, 62·5; H, 6·3; N, 14·6%). On a larger scale this method gave erratic results.

(*b*) The nitro-compound (40 g.) was suspended in ethanol (800 c.c.), mixed with 2% palladium–strontium carbonate (4 g.; prepared according to *Org. Synth.*, **26**, 77), and reduced at 60° with hydrogen maintained at a pressure of 12 atmospheres. Reduction was rapid, and from the filtered solution the pure amino-ketone was isolated in 95% yield.

m-*Nitrosoacetophenone.*—Powdered potassium persulphate ("AnalaR"; 390 g.) and concentrated sulphuric acid (435 c.c.) were stirred together, and, after 1 hour, poured on crushed ice (*ca.* 7 l.) (cf. Meisenheimer and Hesse, *Ber.*, 1919, **52**, 1161). Potassium carbonate (*ca.* 1280 g.) was added until the solution was neutral to litmus, and the solution was then standardised with iodine and thiosulphate. To this neutral Caro's acid solution (≡ 72·9 g. of H_2SO_5) a solution of *m*-aminoacetophenone (35 g.) in the minimum volume of dioxan was quickly added; the mixture was shaken, and after ½ hour the solid which had precipitated was collected and crystallised from ethanol, yielding crude *m*-nitrosoacetophenone, m. p. 76—77° (17·1 g.). This was contaminated with 3 : 3′-diacetylazoxybenzene (see below), of which a small quantity, m. p. 136—138°, was eventually isolated by continued recrystallisation (Found: C, 67·9; H, 5·0. Calc. for $C_{16}H_{14}O_3N_2$: C, 68·1; H, 5·0%). As the nitroso-compound in the filtrates could not be completely purified, and as the crude material was suitable for further use, attempts to obtain it in an analytically pure state were abandoned.

m : m′-*Diacetylazoxybenzene.*—*m*-Aminoacetophenone (1 g.) was added to a Caro's acid solution (not neutralised) (200 c.c.; ≡ 1·94 g. of H_2SO_5); the amine first dissolved, and a solid (0·52 g., 50%), m. p. 133—135°, slowly separated during 2 days. This on recrystallisation from acetic acid and then from ethanol yielded pure m : m′-*diacetylazoxybenzene*, which formed pale

yellow leaflets, m. p. 137—138° (Found: C, 68·2; H, 5·3; N, 9·9. $C_{16}H_{14}O_3N_2$ requires C, 68·1; H, 5·0; N, 9·9%).

p-*Nitronitrosobenzene*.—A solution of *p*-nitroaniline (9 g.) in dioxan (50 c.c.) was added to neutralised Caro's acid (2160 c.c. ≡ 16·5 g. of H_2SO_5), and the mixture was shaken for 20 hours. The solid product was collected (m. p. 103—105°) and steam-distilled, and the steam-volatile material was crystallised from alcohol, giving lemon-yellow needles of *p*-nitronitrosobenzene (2·9 g., 30%), m. p. 117—119° (Bamberger and Hübner, *Ber.*, 1903, **36**, 3803, give m. p. 118·5—119°). Crude *p* : *p*′-dinitroazoxybenzene (5 g.) remained in the distillation flask, and if the distillation was omitted the nitroso-compound could not be completely freed from this material.

2-*Acetamido*-5-*nitrosoacetophenone*.—A slightly warm (supersaturated) solution of 2-acetamido-5-aminoacetophenone (17 g.) in dioxan (250 c.c.) was quickly added to a neutralised Caro's acid solution (3·66 l. ≡ 21·5 g. of H_2SO_5). The mixture was well shaken for 5 minutes, and the solid that separated was collected, washed, dried, and recrystallised from alcohol, from which almost pure 2-*acetamido*-5-*nitrosoacetophenone* (15·9 g., 87%) separated in green needles, m. p. 150—152°. The pure compound had m. p. 152·5—154·5° (Found: C, 57·9; H, 4·7; N, 13·4. $C_{10}H_{10}O_3N_2$ requires C, 58·2; H, 4·9; N, 13·6%).

If the oxidation was conducted with acid Caro's acid solution, only about $\frac{1}{3}$ of the weight of the material employed had separated after 24 hours, and this was identified (m. p. and mixed m. p.) as 2-acetamido-5-nitroacetophenone.

m-*Acetylazobenzene*.—(*a*) A solution of *m*-aminoacetophenone (33·75 g.) and nitrosobenzene (26·75 g.) in acetic acid (250 c.c.) was set aside overnight. The mixture was basified and extracted with ether, and the extract was washed with dilute hydrochloric acid, dried, and evaporated. Crystallisation of the residue from methanol gave m-*acetylazobenzene* (31 g., 56%) as orange plates, m. p. 88—90° (Found: C, 74·6; H, 5·5; N, 12·8. $C_{14}H_{12}ON_2$ requires C, 75·0; H, 5·4; N, 12·5%).

(*b*) Interaction of crude *m*-nitrosoacetophenone (0·75 g.) and aniline (0·47 g.) in acetic acid (5 c.c.) gave the same product, m. p. and mixed m. p. 86—88°.

Nitration of *m*-acetylazobenzene was attempted, with fuming or concentrated nitric acid with or without sulphuric acid, and with potassium nitrate and sulphuric acid, under various conditions, but only unchanged material or intractable products could be isolated.

m-*Acetyl*-p′-*nitroazobenzene*.—Slightly warm solutions of pure *p*-nitronitrosobenzene (1 g.) and *m*-aminoacetophenone (1 g.) in acetic acid (20 and 10 c.c.) were mixed and left overnight. The solid obtained by dilution with water was collected (1·47 g., 83%; m. p. 113—118°) and dissolved in a mixture of benzene and ligroin (b. p. 60—80°) (2 : 1; 60 c.c.). Percolation through a small column of alumina (Merck) and washing with benzene gave almost pure m-*acetyl*-p′-*nitroazobenzene* (m. p. 119—120° after softening at 116°; 1·14 g.), which after crystallisation from ethanol and rejection of the (small) first crops formed orange needles, m. p. 120—122° (Found: C, 61·5; H, 4·1; N, 15·9. $C_{14}H_{11}O_3N_3$ requires C, 62·4; H, 4·1; N, 15·6%).

3-*Acetyl*-4-*benzamidoazobenzene*.—A solution of 5-amino-2-benzamidoacetophenone (5·1 g.; Simpson, Atkinson, Schofield, and Stephenson, *J.*, 1945, 646) in acetic acid (30 c.c.) was treated with nitrosobenzene (2·16 g.). The mixture was warmed slightly, immediately cooled, and set aside. Next day the 3-*acetyl*-4-*benzamidoazobenzene* which had separated was collected (5·58 g., 82%) and crystallised from methanol, from which the pure compound separated in fine yellow needles, m. p. 138—139° (Found: C, 73·1; H, 5·0; N, 12·4. $C_{21}H_{17}O_2N_3$ requires C, 73·4; H, 5·0; N, 12·2%). This compound was unattacked by boiling 2N- or 5N-hydrochloric acid, and boiling aqueous sulphuric acid (20 and 33% v/v) led to decomposition. Some hydrolysis seemingly occurred in presence of alcoholic hydrochloric acid, as ethyl benzoate could be smelt in the reaction mixture, but no useful product could be isolated.

4-*Acetamido*-3-*acetylazobenzene*.—(*a*) Prepared similarly to the above benzamido-analogue from 2-acetamido-5-aminoacetophenone (8·31 g.), nitrosobenzene (4·7 g.), and acetic acid (44 c.c.), 4-*acetamido*-3-*acetylazobenzene* (10·16 g., 84%) crystallised from methanol in orange plates, m. p. 132·5—133° after previous softening (Found: C, 68·4; H, 5·3; N, 15·4. $C_{16}H_{15}O_2N_3$ requires C, 68·3; H, 5·4; N, 14·9%). The yield in this reaction occasionally dropped to 70%, but this could not be traced to any variation in the conditions or in the purity of the reagents.

(*b*) The condensation, carried out in a similar manner, of 2-acetamido-5-nitrosoacetophenone (1·1 mols.) with aniline (1 mol.) gave the azo-compound in almost theoretical yield.

4-*Acetamido*-3 : 3′-*diacetylazobenzene*.—(*a*) Solutions of 2-acetamido-5-aminoacetophenone (19·2 g.) and *m*-nitrosoacetophenone (14·7 g.) in acetic acid (70 c.c. and 45 c.c.) were mixed, and the mildly exothermic reaction was allowed to proceed unchecked. Next day the crude product,

m. p. 144—154° (15·65 g., 49%), was collected and recrystallised from methanol, from which 4-*acetamido*-3 : 3′-*diacetylazobenzene* crystallised in yellow needles, m. p. 157·5—159·5° (Found : C, 66·95; H, 5·3; N, 13·0. $C_{18}H_{17}O_3N_3$ requires C, 66·9; H, 5·3; N, 13·0%).

(*b*) Condensation of *m*-aminoacetophenone (0·68 g.) with 2-acetamido-5-nitrosoacetophenone (1·13 g.) in acetic acid (15 c.c.) gave, after storage overnight, a crude product (1·04 g.) which by fractional crystallisation from methanol gave fairly pure 4-acetamido-3 : 3′-diacetylazobenzene, m. p. 149—151°, and a small quantity of 4 : 4′-*diacetamido*-3 : 3′-*diacetylazoxybenzene*; this crystallised from benzene in yellow prisms, m. p. 227—230° (Found : C, 60·3; H, 5·1. $C_{20}H_{20}O_5N_4$ requires C, 60·6; H, 5·1%).

4 : 4′-*Diacetamido*-3 : 3′-*diacetylazobenzene*.—A hot solution of 2-acetamido-5-nitrosoacetophenone (11·3 g.) in acetic acid (110 c.c.) was cooled to 50° and added, before crystallisation set in, to a solution of 2-acetamido-5-aminoacetophenone (9·6 g.) in acetic acid (50 c.c.). The mixture was shaken for a few minutes until crystallisation of the product commenced, and then left for 2 days. The almost pure 4 : 4′-*diacetamido*-3 : 3′-*diacetylazobenzene* [17·9 g., 94%; m. p. 286—288° (decomp.)] that had separated was recrystallised from acetic acid, from which it formed purple prisms, m. p. 281—283° (decomp.) (Found : C, 62·4; H, 5·65; N, 14·5. $C_{20}H_{20}O_4N_4$ requires C, 63·1; H, 5·3; N, 14·7%).

3-*Acetyl*-4-*aminoazobenzene*.—A mixture of 4-acetamido-3-acetylazobenzene (36·7 g.), alcohol (600 c.c.), and 5N-hydrochloric acid (600 c.c.) was heated under reflux with stirring for 3 hours; the solution was then diluted, basified, and extracted with ether. The extract was dried, concentrated, and mixed with ligroin, which precipitated 3-acetyl-4-aminoazobenzene (27 g., 87%). The pure base crystallised from *cyclo*hexane in orange needles, m. p. 114° after previous softening (Found : C, 69·8; H, 5·2; N, 18·2. $C_{14}H_{13}ON_3$ requires C, 70·2; H, 5·5; N, 17·6%). Deamination was effected by adding, at room temperature, amyl nitrite (0·5 c.c.) to a solution of the base (0·5 g.) in ethanol (50 c.c.) and concentrated sulphuric acid (4 c.c.); after 3 days in an evacuated desiccator no coupling with alkaline β-naphthol was observed, and the addition of water precipitated *m*-acetylazobenzene, m. p. and mixed m. p. 86—89° after crystallisation from methanol.

4-*Amino*-3 : 3′-*diacetylazobenzene*.—Prepared as above from 4-acetamido-3 : 3′-diacetylazobenzene (25·9 g.), alcohol (900 c.c.) and 5N-hydrochloric acid (900 c.c.), crude 4-*amino*-3 : 3′-*diacetylazobenzene*, m. p. 184—185°, separated in theoretical yield on basification of the diluted reaction mixture; it crystallised from alcohol as orange plates, m. p. 189·5—191° (Found : C, 68·2; H, 5·5; N, 14·9. $C_{16}H_{15}O_2N_3$ requires C, 68·3; H, 5·4; N, 14·9%).

3 : 3′-*Diacetyl*-4 : 4′-*diaminoazobenzene*.—Finely-powdered 4 : 4′-diacetamido-3 : 3′-diacetylazobenzene (35 g.), alcohol (500 c.c.), and concentrated hydrochloric acid (500 c.c.) were heated under reflux with stirring for 8 hours. After cooling, the black, finely divided hydrochloride of the diamine was collected and shaken with very dilute aqueous ammonia. The suspension of the yellow base (19·1 g.) was separated roughly by decantation from a small amount (2·4 g.) of unhydrolysed material; it had m. p. 258—261° (decomp.), but could not be recrystallised owing to its insolubility in organic solvents. On a larger scale (60 g.), the proportion of material which escaped hydrolysis was greater, but it was found convenient to use the crude product for conversion into the hydroxycinnoline (see below) as the diacetamido-compound was readily removed after diazotisation of the diamine.

m : m′-*Diacetylazobenzene*.—(*a*) A mixture of 3 : 3′-diacetyl-4 : 4′-diaminoazobenzene (0·3 g.) and hypophosphorous acid (30%; 15 c.c.) was cooled to 0°, and a solution of sodium nitrite (20%; 0·78 c.c.) was slowly added. Nitrogen was evolved; the mixture was kept overnight, and next day the crude product was collected (0·26 g.) and recrystallised from methanol, yielding golden needles of m : m′-*diacetylazobenzene*, m. p. 131—133° (Found : C, 72·1; H, 5·7; N, 10·7. $C_{16}H_{14}O_2N_2$ requires C, 72·1; H, 5·3; N, 10·5%).

(*b*) *m*-Nitrosoacetophenone (0·75 g.) and *m*-aminoacetophenone (0·68 g.) were dissolved in slightly warm acetic acid (5 c.c.), whereupon the solution was immediately cooled and left for 3 hours. The solid (0·6 g.) was collected, and more (0·35 g.) was isolated by ether-extraction of the basified filtrate. Recrystallisation from methanol failed to raise the m. p. above 118—120°, which was however raised by admixture with the sample prepared as in (*a*). The low m. p. was probably due to contamination of the azo-compound by some azoxy-compound present as an impurity in the *m*-nitrosoacetophenone (Found : C, 71·1; H, 5·2; N, 10·6%).

(*c*) When a mixture of *m* : *m*′-diacetylazoxybenzene (0·4 g.) and etched iron (1·2 g.) was heated for 4 hours at 200—240°/15 mm., a sublimate (0·14 g.) was produced which on crystallisation from methanol gave impure *m* : *m*′-diacetylazobenzene, m. p. 117·5—121° [raised by admixture with the sample prepared as in (*a*)].

4-*Hydroxy*-6-*phenylazocinnoline*.—A solution of 3-acetyl-4-aminoazobenzene (6·81 g.) in formic acid (*d* 1·2; 85 c.c.) was treated at 0° to −3° with powdered sodium nitrite ("AnalaR"; 2·27 g.), added slowly with shaking. After 2 days, the reaction was completed by heating the mixture to 80° for a short time, and the solid which had separated (4·25 g., 60%) was collected and crystallised from acetic acid, giving yellow needles, m. p. 298—300°, of 4-*hydroxy*-6-*phenylazocinnoline* (Found: C, 66·3; H, 4·35; N, 20·9. $C_{14}H_{10}ON_4$ requires C, 67·2; H, 4·0; N, 22·4%). The *acetate*, formed when the hydroxycinnoline was refluxed for a few minutes with a large excess of acetic anhydride, separated from acetic acid, ethanol, benzene, or ethyl acetate in crystals, m. p. 179—180° (Found: C, 65·4; H, 4·2; N, 19·5. $C_{16}H_{12}O_2N_4$ requires C, 65·7; H, 4·1; N, 19·2%).

6-m-*Acetylphenylazo*-4-*hydroxycinnoline*.—A solution of 4-amino-3 : 3′-diacetylazobenzene (4 g.) in formic acid (*d* 1·2; 50 c.c.) was cooled in ice and treated with powdered sodium nitrite ("AnalaR"; 1·08 g.), added slowly in portions. After 5 days, the crude 6-m-*acetylphenylazo*-4-*hydroxycinnoline* (m. p. 264—268°; 2·02 g., 50%) which had separated was collected and recrystallised from acetic acid, from which it formed red needles, m. p. 279—280° (decomp.) (Found: C, 64·8; H, 4·15; N, 19·7. $C_{16}H_{12}O_2N_4$ requires C, 65·7; H, 4·1; N, 19·2%). The *acetate*, prepared as for the analogue described above, crystallised from ethyl acetate in yellow needles, m. p. 178—180° (Found: C, 64·6; H, 4·5; N, 16·0. $C_{18}H_{14}O_3N_4$ requires C, 64·7; H, 4·2; N, 16·8%).

4 : 4′-*Dihydroxy*-6 : 6′-*azocinnoline*.—Crude 3 : 3′-diacetyl-4 : 4′-diaminoazobenzene (16 g.) was dissolved in formic acid (*d* 1·2; 300 c.c.), and to the intensely violet solution so obtained was added, at 0° and with stirring, a 20% solution of sodium nitrite ("AnalaR"). After 28 c.c. (87% of the theoretical amount) had been added, diazotisation was complete, as shown by the change in colour to red, and the insoluble material was collected (3·97 g.); this was found to be 4 : 4′-diacetamido-3 : 3′-diacetylazobenzene (see above), and after re-hydrolysis could again be used for diazotisation. The filtered solution, on being kept for 10 days, deposited 4 : 4′-dihydroxy-6 : 6′-azocinnoline (8·54 g.) as dark red needles; it did not melt at 320°, and was completely soluble in dilute sodium hydroxide solution. A further crop (0·37 g.) was obtained by warming the filtrate on the steam-bath; the total yield was 52% based on the crude diamine (69% allowing for recovered diacetamido-compound). The compound was too insoluble in organic solvents to be recrystallised, and could not be obtained crystalline on acidification of a solution in sodium hydroxide. When a suspension of it in a large excess of acetic anhydride was refluxed for ½ hour a product was formed which was insoluble in sodium hydroxide and was probably the diacetyl derivative, but it also did not melt at 300° and was too insoluble to be recrystallised.

4-*Phenoxy*-6-*phenylazocinnoline*.—4-Hydroxy-6-phenylazocinnoline (7 g.), phosphorus pentachloride (7 g.), and phosphorus oxychloride (42 c.c.) were gently warmed together. A clear solution was obtained, which was kept below the b. p. until crystallisation set in, and then warmed for a further ¼ hour. The crude 4-chloro-6-phenylazocinnoline was collected (8·63 g.), well washed with ligroin, and used for the experiments described below (a sample crystallised thrice from ethyl acetate had m. p. 161—162°, clearing at 172°).

(*a*) The crude chloro-compound (6·05 g.) was warmed on the steam-bath with a mixture of phenol (28 g.) and powdered ammonium carbonate ("AnalaR"; 14 g.) until effervescence ceased. The solid which was precipitated by the addition of excess of aqueous sodium hydroxide was taken up in benzene, the solution was filtered (charcoal), and the benzene was removed. Crystallisation of the residue from ethyl acetate gave 4-*phenoxy*-6-*phenylazocinnoline* (4·82 g., 75% based on the hydroxycinnoline) as red needles, m. p. 167—169° (Found: C, 73·3; H, 4·2; N, 16·8. $C_{20}H_{14}ON_4$ requires C, 73·6; H, 4·3; N, 16·8%).

(*b*) Dry ammonia was bubbled through a warm suspension of the crude chloro-compound (0·5 g.) in phenol (10 g.). Complete solution occurred, and at 80° a red crystalline solid began to separate. The temperature was raised to 120° during 20 minutes and kept thereat for 10 minutes, after which the reaction mixture was worked up as in (*a*). The phenoxy-compound (0·16 g.) separated from ethyl acetate and had m. p. 164—167° alone and when mixed with the sample described above.

6-m-*Acetylphenylazo*-4-*chlorocinnoline*.—A mixture of 6-*m*-acetylphenylazo-4-hydroxycinnoline (2·8 g.), phosphorus oxychloride (23 c.c.), and dimethylaniline (0·25 c.c.) was warmed on the steam-bath for 10 minutes (longer heating and/or a higher temperature gave reduced yields) and then poured into dry ether (200 c.c.). The solid product was filtered off, washed with ether, and mixed thoroughly with ice. The mixture was extracted with chloroform, and the extract was washed with very dilute ammonia and water, dried (Na_2SO_4), filtered (charcoal), and evapor-

ated to dryness. Crystallisation of the residue from benzene gave 6-m-*acetylphenylazo*-4-*chlorocinnoline* (2·27 g., 76%) as orange needles, m. p. 194—196° (Found: C, 63·0; H, 3·9; N, 16·8; Cl, 12·0. $C_{16}H_{11}ON_4Cl$ requires C, 61·8; H, 3·6; N, 18·0; Cl, 11·4%).

4 : 4′-*Diphenoxy*-6 : 6′-*azocinnoline*.—The following procedure was the outcome of numerous trials in which the chlorination of the hydroxycinnoline was investigated, with use of a mixture of phosphorus pentachloride and oxychloride, or of phosphorus oxychloride and dimethylaniline, at 50°, 95°, and at the b. p., for various times; the product from each set of conditions was phenoxylated both with phenol and ammonium carbonate at 95°, and with phenol and potassium hydroxide at 90° and at 110°.

A mixture of 4 : 4′-dihydroxy-6 : 6′-azocinnoline (5 g.), phosphorus pentachloride (10 g.), and phosphorus oxychloride (50 c.c.) was heated on the steam-bath for ¼ hour; the temperature was then raised to the b. p. during ¼ hour, and refluxing was continued for a further ¾ hour. After cooling, the crude dichloro-compound (5·3 g.), which did not melt at 300° and could not be recrystallised, was precipitated by the addition of ligroin and collected. It was added to a solution of potassium hydroxide (2·1 g.) in phenol (60 g.); the mixture was heated at 90° for 35 minutes and then poured into iced 5% sodium hydroxide solution (850 c.c.). The resultant suspension was centrifuged, suspended in water, and again centrifuged, and the solid was then dried (5·78 g.) and extracted with much hot benzene. The insoluble matter (2·04 g.) was discarded, and the filtered solution deposited nearly pure 4 : 4′-*diphenoxy*-6 : 6′-*azocinnoline* [m. p. 251—254° (decomp.); 3·53 g., 48% based on the dihydroxycinnoline]; the pure compound on recrystallisation from benzene formed orange-red needles, m. p. 254—258° (decomp.) (Found: C, 71·8; H, 4·5; N, 17·4. $C_{28}H_{18}O_2N_6$ requires C, 71·5; H, 3·9; N, 17·9%).

4-*Amino*-6-*phenylazocinnoline*.—Dry ammonia was passed for ¾ hour into a mixture of 4-phenoxy-6-phenylazocinnoline (2·6 g.), ammonium chloride ("AnalaR"; 0·42 g.), and phenol (22 g.) at 175°. The mixture was cooled, treated with an excess of dilute sodium hydroxide solution, and shaken with ether and benzene. The suspension of organic matter in the ether–benzene was washed with water, and the solid was then collected and dissolved in 50% acetic acid. Basification of the clarified acid solution with ammonia gave 4-*amino*-6-*phenylazocinnoline* (1·7 g., 85%), m. p. 300—301° (decomp.), unchanged by recrystallisation from 2-ethoxyethanol, from which the compound separated in small golden needles (Found: C, 67·0; H, 4·7; N, 26·7. $C_{14}H_{11}N_5$ requires C, 67·4; H, 4·45; N, 28·1%). The *hydrochloride* (Found: Cl, 13·3. $C_{14}H_{12}N_5Cl$ requires Cl, 12·4%) formed golden fibrous needles which decomposed at 275—280° with previous darkening, and was soluble in water but insoluble in very dilute hydrochloric acid.

4-*Acetamido*-6-*phenylazocinnoline*.—Prepared by refluxing the amine (1 g.) with acetic anhydride (20 c.c.) for 5 minutes, 4-*acetamido*-6-*phenylazocinnoline* (0·98 g., 84%) crystallised from 2-ethoxyethanol in dark red leaflets, m. p. 271—275° (decomp.) (Found: C, 65·5; H, 4·5; N, 24·35. $C_{16}H_{13}ON_5$ requires C, 66·0; H, 4·5; N, 24·05%).

4 : 4′-*Diamino*-6 : 6′-*azocinnoline*.—Dry ammonia was passed through a mixture of 4 : 4′-diphenoxy-6 : 6′-azocinnoline (6 g.), powdered ammonium chloride (1·37 g.), and phenol (100 g.), the temperature being gradually raised. At 160° a clear solution was formed, and at 175° a solid began to separate. After 1 hour at this temperature the mixture was cooled and diluted with ether, and the solid was collected. Crystallisation from a mixture of acetic acid (700 c.c.), water (350 c.c.), and 10N-hydrochloric acid (30 c.c.) gave 4 : 4′-*diamino*-6 : 6′-*azocinnoline dihydrochloride* as fine red needles which did not melt at 320° (Found: C, 45·0; H, 4·2; N, 26·65. $C_{16}H_{14}N_8Cl_2,2H_2O$ requires C, 45·15; H, 4·3; N, 26·35%). The salt was insoluble in water, and was decomposed by shaking it for 16 hours with N-sodium hydroxide (200 c.c.) (the completeness of the reaction was proved by titration of the chloride after filtration), yielding 4 : 4′-*diamino*-6 : 6′-*azocinnoline* (3·35 g., 83%) as fine red needles which did not melt at 320° and could not be crystallised owing to insolubility (Found: C, 56·8; H, 4·7; N, 31·6. $C_{16}H_{12}N_8,1·5H_2O$ requires C, 56·0; H, 4·4; N, 32·6%).

4-*Amino*-6-*phenylazocinnoline Methochloride*.—4-Amino-6-phenylazocinnoline methiodide was first prepared by each of the following methods.

(*a*) 4-Amino-6-phenylazocinnoline (0·48 g.), methyl iodide (3·5 c.c.), and ethanol (10 c.c.) were refluxed together for 6 hours, and the alcohol-insoluble methiodide collected; it had m. p. 253—255° (decomp.) (0·65 g., 87%).

(*b*) The amine (0·1 g.), methyl iodide (0·5 c.c.), and phenol (2 g.) were warmed under reflux on the steam-bath for 2 hours; a further 0·3 c.c. of methyl iodide was then added, and heating continued for a further 3 hours. Addition of ether gave the methiodide (0·08 g.), m. p. 251—253° (decomp.) alone and when mixed with the sample from (*a*).

(*c*) 4-Acetamido-6-phenylazocinnoline (0·86 g.) and methyl toluene-*p*-sulphonate (4·3 g.)

were heated together until a clear solution was formed; this occurred at 110°, and after a further 5 minutes at this temperature the melt was cooled and triturated with dry ether. The 4-acetamido-6-phenylazocinnoline methotoluene-*p*-sulphonate was collected (1·35 g., 96%) and crystallised from alcohol–ether, from which it formed reddish needles which decomposed at 188—192°. Treatment of an aqueous solution of this salt (1·91 g. in 300 c.c.) with aqueous potassium iodide precipitated the corresponding methiodide [small red needles from water; m. p. 195—197° (decomp.); 1·46 g., 85%], which (1·35 g.) was boiled under reflux with 0·5N-hydrochloric acid (70 c.c.) for 1¼ hours; the solution was filtered (charcoal), and on cooling deposited the methiodide of the amino-cinnoline (0·71 g., 58%), m. p. 243—246° (decomp.) alone or when mixed with the sample prepared by method (*b*).

The methiodide could not be obtained crystalline by any of the above methods. An aqueous solution of sample (*a*) was boiled under reflux with excess of silver chloride for 1½ hours; the silver halides were removed, and the filtrate concentrated under reduced pressure to small bulk, yielding *4-amino-6-phenylazocinnoline methochloride*, which on crystallisation from aqueous acetone and finally from very dilute hydrochloric acid formed fibrous yellow needles, m. p. 250—255° (decomp.) (Found: C, 59·7; H, 4·6; N, 23·5; Cl, 11·7. $C_{15}H_{14}N_5Cl$ requires C, 60·0; H, 4·7; N, 23·4; Cl, 11·8%). A sample of the methiodide prepared by method (*c*) was similarly treated, and yielded golden needles, m. p. 252—254° (decomp.) alone and in admixture with the above material.

Preparation of α- *and* β-4 : 4′-*Diamino*-6 : 6′-*azocinnoline Dimethochloride*.—(*A*) 4 : 4′-Diamino-6 : 6′-azocinnoline (1·45 g.), phenol (50 g.), and methyl iodide (initially 10 c.c., followed by additions of 5 c.c. and 3 c.c. after successive intervals of 1 hour) were mixed and heated on the steam-bath for 4 hours, a dark red solution being formed from which solid gradually separated. The cold reaction mixture was poured into dry ether and the crude dimethiodide (2·7 g., 98%) collected as an amorphous dark red solid, m. p. 284—288° (decomp.), which could not be crystallised. It was dissolved in approximately 0·05N-hydrochloric acid (1350 c.c.), silver chloride (from 300 c.c. of 0·1N-silver nitrate) was added, and the whole was refluxed for 2 hours. Concentration (reduced pressure) of the filtered solution to 180 c.c. gave the crude dimethochloride [1·46 g., 80%; m. p. 282—288° (decomp.)], which was dissolved in water (150 c.c.) and treated whilst hot with 10N-hydrochloric acid (1 c.c.). The β-compound thus precipitated was removed [0·15 g.; m. p. 312—314° (decomp.) alone and mixed with authentic material (see below)], and from the cold filtrate there separated α-4 : 4′-*diamino*-6 : 6′-*azocinnoline dimethochloride* (1·2 g.) as fine golden-brown needles, m. p. 282—285° (decomp.) (Found: C, 45·9; H, 4·5; N, 24·2; Cl, 15·1. $C_{18}H_{18}N_8Cl_2,3H_2O$ requires C, 45·9; H, 5·1; N, 23·8; Cl, 15·1%).

(*B*) 4 : 4′-Diamino-6 : 6′-azocinnoline (2 g.) was refluxed with acetic anhydride (50 c.c.) for ½ hour. During this time the red crystals of the diamine changed to an ochre-coloured amorphous solid, which was collected after cooling and well washed with dry ether (yield, 2·24 g., 89%); it had m. p. 231—239° (decomp.) after previous darkening and could not be crystallised owing to its insolubility in chlorobenzene, 2-ethoxyethanol, and other usual solvents. An intimate mixture of this diacetyl derivative (2·1 g.) and methyl toluene-*p*-sulphonate (9·6 g.) was heated until the suspension thickened (115—120°). After 10 minutes at 120—125° the mass was cooled and triturated with dry ether and the dimethotoluene-*p*-sulphonate collected as an amorphous solid (4 g., 99%) which could not be crystallised. It was converted into the diamino-dimethochloride by each of the two following methods.

(i) A solution of the salt (1·3 g.) in hot water (50 c.c.) was clarified (charcoal) and heated under reflux for 3 hours with 10N-hydrochloric acid (1 c.c.). The solution after filtration from a trace of insoluble material slowly deposited lustrous golden-brown cubes, m. p. 280—284° (decomp.) (0·52 g.), which consisted of 4 : 4′-diamino-6 : 6′-azocinnoline dimethotoluene-*p*-sulphonate contaminated with some dimethochloride (Found: C, 55·4; H, 4·6; N, 17·8; S, 8·3. Calc. for $C_{32}H_{32}O_6N_8S_2$: C, 55·8; H, 4·7; N, 16·3; S, 9·3. Calc. for $4C_{32}H_{32}O_6N_8S_2 + C_{18}H_{18}N_8Cl_2$: C, 55·3; H, 4·6; N, 17·7; S, 8·1%). A solution of this material (0·45 g.) in hot water (25 c.c.) was treated with 10N-hydrochloric acid (10 c.c.), whereby β-4 : 4′-*diamino*-6 : 6′-*azocinnoline dimethochloride* was quickly precipitated in purplish needles (0·2 g.), m. p. 312—314° (decomp.) (Found: C, 50·7; H, 4·55; N, 25·2; Cl, 16·5. $C_{18}H_{18}N_8Cl_2,\frac{1}{2}H_2O$ requires C, 50·7; H, 4·5; N, 26·3; Cl, 16·6%). The filtrate from the β-compound, when kept, deposited crystals which, after crystallisation from very dilute hydrochloric acid, yielded the α-isomer, m. p. 279—281° (decomp.) alone and when mixed with the sample previously described.

(ii) A clarified (charcoal) solution of the diacetamidoazocinnoline dimethotoluene-*p*-sulphonate (1 g.) in water (50 c.c.) was treated in the cold with a concentrated aqueous solution of sodium iodide (10 c.c.), whereupon the dimethiodide separated in small, dark violet crystals,

m. p. 284—287° (decomp.) (0·7 g., 80%). This salt (0·5 g.) was refluxed in 0·5N-hydrochloric acid (100 c.c.) for 1 hour, and the product which separated on cooling [0·21 g., m. p. 298—302° (decomp.)] was dissolved in 0·1N-hydrochloric acid (50 c.c.) and heated under reflux for 1½ hours with silver chloride (from 30 c.c. of 0·1N-silver nitrate). The filtered solution deposited crystals of almost pure β-compound (0·1 g.), which after recrystallisation from water (crystallisation being induced by a drop of 10N-hydrochloric acid) had m. p. 312—314° (decomp.) alone and when mixed with the specimen described above.

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