Cinnolines and Other Heterocyclic Types in Relation to the Chemotherapy of Trypanosomiasis. Part X. The Quaternisation of 4-Aminocinnolines.*

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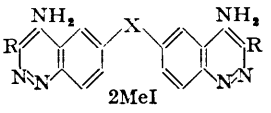
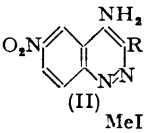
4-Aminocinnolines with methyl iodide in alcohol yield mixtures of two series of quaternary salts in which only the ring nitrogen atoms are involved. The evidence that $N_{(1)}$ is the basic centre in the cinnoline series is reviewed and is supported by degradation studies, but it also has been clearly shown that $N_{(1)}$ is not exclusively the basic centre. Evidence that $N_{(2)}$ may be involved in quaternary salt formation is reported.

Earlier parts of this series have described the synthesis of bis-heterocyclic salts (as I) for test against *T. congolense* in connection with the theory set out in Part I (Keneford, Lourie, Morley, Simpson, Williamson, and Wright, *J.*, 1952, 2595) that the activity of crude solutions of reduced 4-amino-6-nitrocinnoline methiodides (II; R = H or Me) is due to the formation of azo-compounds (as I; X = –N=N–). An alternative interpretation of these results arose from the isolation (Part I, p. 2601) of two active isomeric salts from the quaternisation of 4-amino-6-nitrocinnoline. The possibility that high activity would be associated with a reduction product of only one of these isomers was supported by the appreciably higher activity of one of the isomeric salts isolated from the quaternisation of 4 : 4′-diamino-6 : 6′-azocinnoline (McIntyre and Simpson, *J.*, 1952, 2606). This result emphasised the importance of investigating the nature of the isomerism in the simple cinnolinium salts.

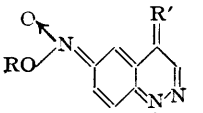


(I)



(II)

MeI



(III): *a*, R = Me, R′ = O.
b, R = H, R′ = NH.

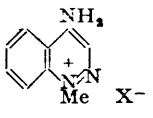
One of the 4-amino-6-nitrocinnolinium salts was readily obtained as massive red needles by recrystallisation from water but the other isomer was separated initially by a tedious hand-separation of the small yellow prisms, seeding of an aqueous solution being ineffective. The variable temperatures at which the isomers melted with decomposition could be standardised (Kofler block) at 222° and 240°, respectively, and the distinct nature of the salts was confirmed by reduction (see below), by spectroscopy (Professor R. A. Morton kindly determined the ultraviolet absorptions), and by paper chromatography. A mixture of *tert.*-butyl alcohol–water–hydrochloric acid was adopted for the chromatography and, in our opinion, this technique provides the best criterion of purity for this class of compound. Large-scale separations, which failed on a cellulose column, were achieved by removal of the bulk of one isomer by recrystallisation from water, conversion of the remainder into methochlorides, and recrystallisation of this mixture from the solvent mixture used for chromatography. This procedure was successful also with the other mixtures of salts which were investigated.

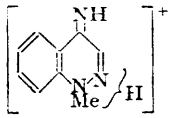
In the 4-amino-6-nitrocinnoline series the task of assigning structures to these compounds was complicated by the possibilities of tautomerism involving the hetero-ring and the 6-nitro-group. Attention was therefore directed to 4-amino- and 4-amino-7-chlorocinnoline which also gave isomeric quaternary salts.

The possibility that one of the salts arose by alkylation of the 4-amino-group was next considered. 4-Methylaminocinnoline, prepared by the action of methylamine on a solution of 4-chlorocinnoline in phenol, formed a hydrochloride which was different from either of the quaternary salts of 4-aminocinnoline. Analogous results were obtained in the 4-amino-6-nitrocinnoline series. Thus the 4-amino-group is not involved in the quaternisation,

* Part IX, *J.*, 1954, 2023.

this behaviour being similar to that in the acridine series where the 5-amino-group has been shown not to add a proton (Craig and Short, *J.*, 1945, 419). Thus only the ring-nitrogen atoms are involved and each of the two isomeric salts can have one only of the following structures, where the proton in (V) may be attached to $N_{(1)}$ or $N_{(2)}$.


(IV)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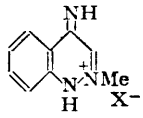

(VI)

Evidence for Quaternisation on $N_{(1)}$.—This may be summarised as follows: (1) The calculations of Longuet-Higgins and Coulson (*J.*, 1949, 971) give the net charges at each nitrogen atom as $N_{(1)} = -0{\cdot}249$ and $N_{(2)} = -0{\cdot}203$. These figures would be conclusive were it not for the uncertainty in the Coulomb integral y_s in the equation $1 - q_r = -\Sigma_s x_{r,s} y_s$ where $1 - q_r$ is the net positive charge at atom r ($N_{(1)}$, $N_{(2)}$) in the cinnoline nucleus (Longuet-Higgins and Coulson, *loc. cit.*) and the fact that the basicity is probably dependent on the change of resonance integral in the C–N bonds on quaternisation. (2) Reactions of the methyl group in 4-methylcinnolines occur more readily than the corresponding reactions of 3-methylcinnoline (Alford and Schofield, *J.*, 1953, 1811) and in general the reactivity of substituents on $C_{(4)}$ is greater than that at other positions. (3) The observation that reduction of 4-phenylcinnolines yields 3-phenylindoles and ammonia (Atkinson and Simpson, *J.*, 1947, 1649), and Barltrop and Taylor's report (*J.*, 1951, 109) that catalytic hydrogenation of heterocyclic quaternary salts did not result in fission of the quaternary alkyl group, suggested a study of the catalytic hydrogenation of the cinnolinium salts. If the reaction had proceeded smoothly and in good yield the use of labelled nitrogen would have shown unambiguously the position of the basic centre. Unfortunately this has not yet been realised but from the reduction of cinnoline methiodide a good yield of ammonia, uncontaminated with methylamine, and a low yield of 1-methylindole were obtained. (4) 4-Acetamido-8-nitrocinnoline did not react with methyl iodide under the usual conditions (see experimental section). This fact will be discussed below.

On the basis of this evidence it is suggested that structure (IV) represents one of the isomeric quaternary salts.

Studies connected with the Ultraviolet Absorption Spectra of the Cinnolinium Salts.—Hearn, Morton, and Simpson (*J.*, 1951, 3325) have discussed the ultraviolet absorption spectrum of cinnoline and have suggested that the weak band at 3900 Å is due to the –N=N– chromophore. Burawoy (*J.*, 1937, 1865) studied the absorption spectrum of azobenzene and showed that this compound absorbed 100 times more strongly in acidic media (where, presumably the chromophore $-N{:}NH^{+}-$ was being measured) than in *cyclo*hexane. It is known (van Allan and Allen, *J. Amer. Chem. Soc.*, 1951, **73**, 5850) that 4-phenylcinnolines do not absorb strongly in this region and it has been shown in this work that 4-methoxy-7-nitrocinnoline is similar in this respect (Fig. 1). Thus intense light absorption in this region of the spectrum is associated with structural changes in the –N=N– group. It was expected, therefore, that differences in structure in this part of the molecule and in particular differences between $-{}^{+}\overset{1}{N}=\overset{2}{N}-$ (IV), $-{}^{+}\overset{1}{N}H-\overset{2}{N}=$ (V), and $-\overset{1}{N}H-\overset{2}{N}^{+}=$ (VI) should be shown in this band. This has proved to be the case. Two types of spectrum have been observed, each corresponding to one of the salts obtained on quaternisation of a 4-aminocinnoline (Fig. 2). An apparent exception was the spectrum of the salt, m. p. 222° (decomp.), derived from 4-amino-6-nitrocinnoline, but this was satisfactorily explained, and the structure of the salt represented as the 1-methiodide of (III*b*), when it was observed that the spectrum of tetrahydro-6-*O*-methyl*aci*nitro-4-oxocinnoline (III*a*) (Schofield and Simpson, *J.*, 1945, 512; Hearn, Morton, and Simpson, *loc. cit.*) was almost identical (Fig. 1). These results have permitted classification of the salts as α and β, those exhibiting a double and a single peak respectively in the 3000—4000 Å band.

Acetylation Experiments and Related Topics.—Comparative experiments on the acetylation of the α- and β-isomers with acetyl chloride substantiated the classification, for only

the former were acetylated, yielding the products obtained on quaternisation of 4-acetamidocinnolines. Only one quaternary salt was obtained from this reaction, as was shown by paper chromatography. Acid hydrolysis of the 4-acetamidocinnolinium salts provided the corresponding α-4-aminocinnoline salts in theoretical yield. Furthermore, the α-salts derived from 6- and 7-nitro-4-aminocinnoline (Part I, *loc. cit.*; Part VIII, *J.*, 1954, 1381) are readily reduced to the diamines, which can be prepared by an alternative route; reduction of β-4-amino-6-nitrocinnolinium salts is more complex and is being studied further. These results indicate that structure (IV) can be assigned to the α-salts and that although the α-isomer from 4-amino-6-nitrocinnoline has the nitronate structure (III*b*), this, too, is quaternised on $N_{(1)}$. Reference was made earlier to the recovery of 4-acetamido-8-nitrocinnoline after prolonged refluxing with ethanolic methyl iodide, in spite of the appreciable solubility of the base in the hot reagent. The importance of the steric effect on the quaternisation reaction is well known (von Braun and Kruber, *Ber.*, 1913, **46**, 3470; Thomas, *J.*, 1913, 594; Meer and Polyani, *Z. phys. Chem.*, 1932, **164**, 1319; Evans,

FIG. 1. FIG. 2. FIG. 3.

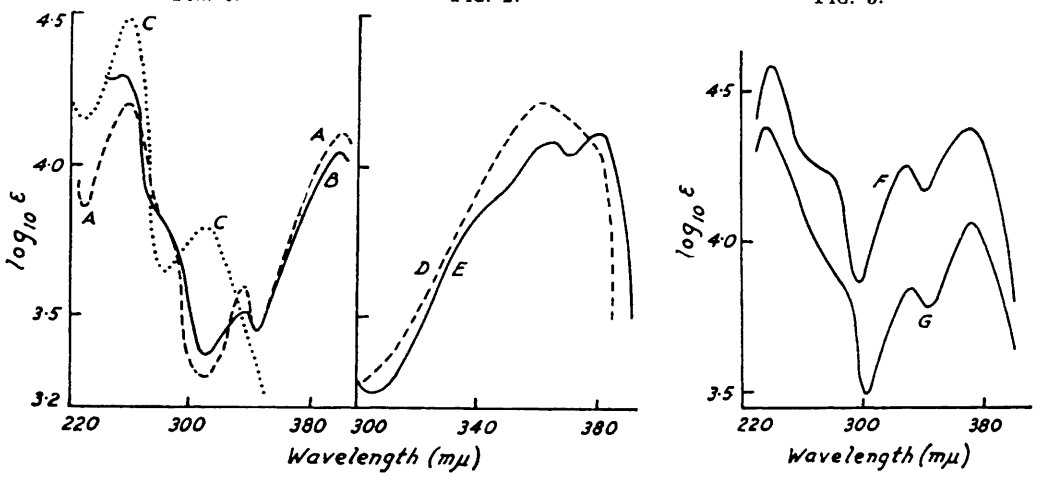


FIG. 1. *Absorption spectra of* (*A*) *tetrahydro-6-*(O-*methyl*acinitro)-4-*oxocinnoline*, (*B*) 4-*amino*-6-*nitrocinnoline methiodide* (α), *m. p.* 222°, *and* (*C*) 4-*methoxy*-7-*nitrocinnoline.*

FIG. 2. *Absorption spectra of* (*D*) *the* β-*methochloride and* (*E*) *the* α-*methiodide of* 4-*amino*-7-*chlorocinnoline.*

FIG. 3. *Absorption spectra of* (*G*) 4-*amino*-6-*nitrocinnoline* β-*methiodide, m. p.* 240°, *and* (*F*) *the dimer, m. p.* 233—234°.

Watson, and Williams, *J.*, 1939, 1348; Brown and Fried, *J. Amer. Chem. Soc.*, 1943, **65**, 1843; Brown and Eldred, *ibid.*, 1949, **71**, 445) and particularly in the analogous 8-nitroquinoline series (Decker, *Ber.*, 1891, **24**, 1984; 1903, **36**, 261; 1905, **38**, 1144); hence this result is regarded as implicit proof, not only that the basic centre of substituted cinnolines is $N_{(1)}$, but also that (IV) represents the structures of the α-isomers.

Degradation of the 4-Aminocinnolinium Salts.—Degradation was studied in three sets of conditions: (*a*) hot water, (*b*) ice-cold N-alkali, and (*c*) refluxing N-alkali until no more basic gases were evolved. Most of the experiments listed under (*a*) and (*c*) were carried out in a micro-Kjeldahl apparatus and the basic gases were analysed by a method based on an observation by François (*J. Pharm. Chim.*, 1907, **25**, 517) that yellow mercuric oxide selectively absorbs ammonia in the presence of methylamine. No basic gas other than ammonia was detected, thus confirming the view that the 4-amino-group was not involved in the quaternisation.

(a) *Treatment with boiling water.* Most of the quaternary salts studied were recovered unchanged when their aqueous solutions were refluxed for 24 hr. However, the β-isomers having a 6- or a 8-nitro-group (the 7-nitro-salt was not isolated) were unstable and yielded

an equimolecular quantity of ammonia and the corresponding 1-methyl-4-cinnolones under these conditions. The nitro-group was the main cause of the ready decompositon, for the parent unquaternised amines were also converted into the corresponding 4-hydroxy-compounds in boiling water.

(b) *Treatment with ice-cold* N-*alkali.* When a slurry of a quaternary salt was treated under these conditions the solid slowly dissolved and an orange precipitate separated. The analysis results for the more stable of these compounds indicated that one molecule of water had been lost; the ultraviolet absorption spectra (cf. Fig. 3) were identical in every respect with those of the parent quaternary salt except that the molecular extinction coefficients observed were exactly twice those of the salt. This was not observed when the spectra of the salts were measured in dilute alkali and it was therefore concluded that the anhydro-bases were dimeric. The dimer from β-4-amino-6-nitro-4-cinnolinium salts rapidly lost ammonia in boiling water and 1-methyl-6-nitro-4-cinnolone was precipitated in quantitative yield. On treatment with dilute acid the dimers were converted into the parent quaternary salts.

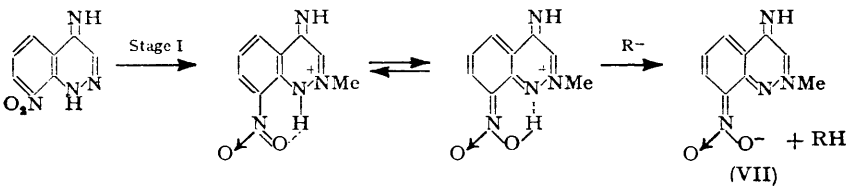
(c) *Treatment with boiling* N-*alkali.* α-4-Amino-6-nitrocinnolinium salts decomposed rapidly with the formation of alkali-soluble tars, in agreement with the representation as salts of (III*b*) and the behaviour of the nitronate (III*a*) under similar conditions (Keneford *et al.*, *J.*, 1950, 1104). The other α-isomers provided one molecular proportion of ammonia and a moderate yield of the 1-methyl-4-cinnolone, the mixtures exhibiting a variety of colour changes (brown, green, and blue).

The quaternary salt obtained from 4-amino-8-nitrocinnoline behaved unusually when treated under the three sets of conditions, for in boiling water ammonia was slowly evolved and 4-hydroxy-8-nitrocinnoline formed. Treatment with cold alkali yielded an extremely unstable dimeric anhydro-base which lost ammonia and formed an intractable tar in warm water; with hot alkali only tars were obtainable.

The reaction of the β-isomers is complex and is still under investigation, although the formation of dimers (above) can be explained more readily by assuming quaternisation to have occurred on $N_{(2)}$.

Evidence for Quaternisation on $N_{(2)}$.—During a study of the effect of alkylating agents in neutral solution on the system NHR–NR′R″ Singh (*J.*, 1913, 604) found that the tertiary nitrogen atom was invariably alkylated. This confirmed in a more general manner Fischer and Tafel's earlier finding (*Annalen*, 1885, **227**, 321) that on quaternisation in neutral solution indazole derivatives were alkylated on $N_{(2)}$. Since the β-4-amino-cinnolinium salts arise from the 4-imino-tautomers the two systems are analogous and this work may be taken as supporting the hypothesis that quaternisation takes place in the 4-aminocinnolines on $N_{(2)}$.

The aqueous decomposition of the quaternary salt derived from 4-amino-8-nitro-cinnoline is slow in comparison with that of the β-6-nitro-analogue and the isolation of 4-hydroxy-8-nitrocinnoline as the only product requires that the proposed mechanism should involve the fission of the C–N bond. The smooth quaternisation of 4-amino-8-nitrocinnoline to give only one product contrasts with the recovery of 4-acetamido-8-nitro-cinnoline in high yield after the same treatment and it is difficult to avoid the conclusion that the reaction proceeds as in stage I below. This is supported by very slow quaternis-



(VII)

ation of 4-amino-3-methyl-8-nitrocinnoline (Part II, *J.*, 1952, 2597), for in this case both ring-nitrogen atoms are sterically hindered.

A similar explanation is advanced for the rupture in 4-amino-8-nitrocinnolinium iodide

of the C–N bond referred to above, for the shift from $N_{(2)}$ to $N_{(1)}$ will be hindered by the 8-nitro-group. The relatively slow degradation of this salt can therefore be explained, for the initial removal of the proton from $N_{(1)}$ will be hindered, and the energy required to rupture the C–N bond will not be gained by its subsequent formation at $N_{(1)}$. The instability of this salt and its anhydro-base can also be understood since on being warmed in alkaline solution the nitronate (VII) would be expected to decompose.

Experimental

M. p.s were determined on the Kofler block, set at the maximum heating rate up to 10° below the m. p. (most important when there was decomposition), and then at 3° per min.

Ultraviolet absorption spectra were determined on a " Unicam " spectrophotometer and $\lambda_{max.}$ values are given below after the corresponding analyses. The compounds were dissolved in alcohol and/or water and determinations carried out in the same 2-mm. silica cells. The instrument was checked at frequent intervals against 4-hydroxy-8-nitrocinnoline, the spectrum of which was determined for us initially by Professor R. A. Morton.

Paper chromatography was carried out with " Whatman No. 1 " paper and a mixture of *tert.*-butyl alcohol (70 c.c.), 6N-hydrochloric acid (1·65 c.c.), and water (to 100 c.c.). The solvent was allowed to flow down the paper from an elevated trough set in an inverted bell-jar, the latter being closed by greased plate glass. After *ca.* 50 hr. the paper was dried in air and examined before an ultraviolet lamp fitted with a " Corning 9863 " filter (Holiday and Johnson, *Nature*, 1949, **163**, 216) which transmits light of 230—400 mμ. The R_F values of quaternary salts are given below after the corresponding analyses. Variation in the concentration of hydrochloric acid (from 0·1N, as specified above, to 1·6N) had little effect on the R_F values. Results with other solvent mixtures were unsatisfactory.

4-Acetamido-6-nitrocinnoline Methiodide.—(*a*) A solution of the cinnoline (2 g.) in ethanol (80 c.c.) was refluxed for 5 hr. with methyl iodide (20 c.c.), and the crystalline precipitate [2·3 g.; m. p. 189—190° (decomp.)] collected. The pure *salt* (m. p. unchanged) crystallised from water in dark red needles (Found: C, 35·1; H, 3·15; N, 15·1; I, 35·3. $C_{11}H_{11}O_3N_4I$ requires C, 35·3; H, 3·0; N, 15·0; I, 33·9%).

(*b*) An intimate mixture of the cinnoline (0·5 g.) and methyl toluene-*p*-sulphonate (0·5 g.) was heated in an oil-bath to 100° (in 8 min.) and then kept at 100—108° for 7 min. The mixture was dissolved in warm water, cooled, and extracted with ether, and the ether-free aqueous layer was treated with saturated potassium iodide solution. The crude methiodide [0·34 g.; m. p. 183° (decomp.)] crystallised from water to give material (0·2 g.) identical (mixed m. p.) with that described in (*a*) (Found: C, 34·8; H, 3·3; N, 14·6; I, 35·5%). The yield by this method is considerably lower if slightly impure material is used.

Hydrolysis of the acetamido-salt (0·75 g.) by refluxing N-hydrochloric acid (24 c.c.) for 1 hr. and treatment of the cold solution with potassium iodide gave *4-amino-6-nitrocinnoline methiodide* (α) (0·6 g.) which crystallised from water as deep red prisms, m. p. 222° (decomp.) (Found: C, 32·6; H, 2·85; N, 16·2; I, 43·85. $C_9H_9O_2N_4I$ requires C, 32·6; H, 2·7; N, 16·9; I, 38·2%), $\lambda_{max.}$ 2550, 3370, 3980 Å (ε 20,000, 3300, and 11,100), R_F 0·71.

Quaternisation of 4-Amino-6-nitrocinnoline.—(*a*) Methyl toluene-*p*-sulphonate and the cinnoline (2 g. of each) were heated together to 105° during 5 min., then kept at 100—105° for 5 min., digested with boiling water (28 c.c.), and filtered hot. The residue [0·4 g.; m. p. 210—215° (decomp.)] was recrystallised (carbon) three times from water, to give golden prismatic needles of the *α-methotoluene-p-sulphonate* (90 mg.), m. p. 254° (decomp.) (Found: C, 50·8; H, 4·35; N, 13·9. $C_{16}H_{16}O_5N_4S$ requires C, 51·05; H, 4·3; N, 14·9%). The cold filtrate yielded crystals which separated from water (32 c.c.; carbon) as a mixture of prismatic needles (0·7 g.), identical (mixed m. p.) with the above, and colourless fluffy needles (0·3 g.), m. p. 263° (slow decomp.); the former crystals alone were collected by gentle warming of the aqueous suspension before filtration. The isomeric *β-salt* (0·2 g.), m. p. 268° (slow decomp.) (depressed on admixture with the α-isomer), separated from water in fine pale yellow needles (Found: C, 50·1; H, 4·0; N, 14·35%). Treatment of aqueous solutions of the α- and the β-methotoluene-*p*-sulphonates with a saturated solution of potassium iodide furnished the corresponding α- and β-iodide, m. p. 222° (decomp.) and 240° (decomp.), respectively.

(*b*) 4-Amino-6-nitrocinnoline (1 part), ethanol (10 parts), and methyl iodide (3 parts) were refluxed together for 3—5 hr. and the crude product was collected after cooling. Recrystallisation from water gave a mixture of thick red needles and golden prismatic needles from which

the former were isolated by cooling the solution very slowly and decanting the supernatant liquid at the first sign of formation of the latter type of crystal. Further recrystallisation of the red needles furnished the pure α-methiodide, m. p. and mixed m. p. 222° (decomp.). Repetition of this procedure with hand-separation of the larger crystals of each type yielded the pure β-*methiodide*, m. p. 240° (decomp.) (Found : C, 32·95; H, 2·9; N, 16·7; I, 38·3%), $\lambda_{max.}$ 2365, 2775, 3310, and 3725 Å (ε 24,000, 8710, 7080, and 11,800 respectively), R_F 0·49. The instability of this salt towards hot water (see below) precludes prolonged fractional crystallisation.

A better method of separation involved isolation of the bulk of the α-methiodide as already described, followed by agitation of the mother-liquors (including the residue obtained by evaporation of the alcoholic reaction filtrate) with freshly precipitated, acid-free silver chloride for several hours at room temperature. The filtrate was evaporated to dryness in a desiccator, and to a solution of the residue (*x* g.) in warm water (14*x* c.c.) was added *tert.*-butyl alcohol (35*x* c.c.) containing 6N-hydrochloric acid (0·8*x* c.c.). The crystalline precipitate was subjected to the same procedure until chromatographically pure. Pure β-4-*amino*-6-*nitrocinnoline methochloride*, m. p. 305° (decomp.), crystallised from water in pale yellow needles and was identical with a sample prepared from the β-methiodide and silver chloride (Found : C, 44·65; H, 3·85; N, 23·5; Cl, 15·3. $C_9H_9O_2N_4Cl$ requires C, 44·9; H, 3·8; N, 23·3; Cl, 14·7%). The α-*methochloride*, m. p. 240° (decomp.), was similarly prepared from the α-methiodide (Found : C, 42·95; H, 4·35; N, 21·4; Cl, 14·1. $C_9H_9O_2N_4Cl,H_2O$ requires C, 41·8; H, 4·3; N, 21·65; Cl, 13·7%).

Quaternisation of 4-*Aminocinnoline.*—This was carried out as described above for the 6-nitro-derivative with the improved method of separation of the isomeric salts. 4-Aminocinnoline (13 g.) gave a crude solid product (20 g.; m. p. 224—240°) which by recrystallisation (3 times) from absolute ethanol furnished yellow prismatic needles of the pure α-*methiodide*, m. p. 258° (9·8 g.) (Found : C, 37·95; H, 3·85; N, 14·55; I, 43·75. $C_9H_{10}N_3I$ requires C, 37·65; H, 3·5; N, 14·6; I, 44·2%), $\lambda_{max.}$ 2150, 3550, and 3700 Å (ε 44,700, 13,200, and 13,800 respectively), R_F 0·72. The derived α-methochloride, m. p. 261°, separated as colourless, silky needles from ethanol. Evaporation of all mother-liquors to dryness, conversion of the mixed salt into chlorides, and treatment (once) with acidified aqueous *tert.*-butyl alcohol as above yielded the pure β-*methochloride*, m. p. 291° (3·8 g.), which formed massive colourless prismatic needles from ethanol (Found : C, 54·8; H, 5·05; N, 20·85; Cl, 17·7. $C_9H_{10}N_3Cl$ requires C, 55·2; H, 5·1; N, 21·4; Cl, 18·1%), $\lambda_{max.}$ 2135 and 3590 Å (ε 3700 and 2400 respectively), R_F 0·68.

Quaternisation of 4-*Amino*-7-*chlorocinnoline.*—When the separation given above was used, the base (3 g.) provided the methiodide (2·3 g.), m. p. 274° (slow decomp.), identical (mixed m. p.) with the compound, m. p. 282—283°, described in Part II (*loc. cit.*) (the difference in m. p. is due to the different rates of heating), $\lambda_{max.}$ 2240, 2800, 2900, 3650, and 3810 Å (ε 38,700, 6920, 6110, 12,200, and 13,200 respectively), R_F 0·78. The mother-liquors yielded, in the usual manner, the pure β-*methochloride*, m. p. 320° (decomp.), which separated from water in colourless prismatic needles (Found : C, 46·9; H, 3·8; N, 18·25; Cl, 31·75. $C_9H_9N_3Cl_2$ requires C, 47·0; H, 3·9; N, 18·3; Cl, 30·8%), $\lambda_{max.}$ 2195, 2410, 2700, 3600 Å (ε 27,200, 17,400, 12,600, and 16,500), R_F 0·64.

4-*Methylamino*-6-*nitrocinnoline Methiodide.*—This was prepared in the usual manner from the base (see later) and methyl iodide in refluxing ethanol. The *salt*, m. p. 222° (decomp.), separated as red needles from water (Found : C, 35·3; H, 3·15; N, 15·8; I, 38·35. $C_{10}H_{11}O_2N_4I$ requires C, 34·7; H, 3·2; N, 16·2; I, 36·7%). Alkaline decomposition gave a theoretical yield of methylamine, identified as picrate.

Acetylation of Cinnolinium Chlorides.—The salt (0·3 g.) was refluxed in "AnalaR" acetyl chloride (15 c.c.) until no further hydrogen chloride was given off. (With the β-salts no appreciable evolution occurred during 5 hr.). The excess of acetyl chloride was removed under reduced pressure and the residual solid was recrystallised from ethanol. Paper chromatograms from solutions of the crude and the pure products were examined and only spots representing the quaternary salts and/or their acetyl derivatives were observed. Acetyl derivatives of the following metho-salts were prepared : 4-*amino*-, m. p. 264° (Found : C, 51·2; H, 5·5; N, 17·2. $C_{11}H_{12}ON_3Cl,H_2O$ requires C, 51·7; H, 5·5; N, 16·4%), and 4-*amino*-7-*chloro*-*cinnoline methochloride*, m. p. 275° (Found : C, 48·2; H, 4·0; N, 14·9. $C_{11}H_{11}ON_3Cl_2$ requires C, 48·5; H, 4·1; N, 15·4%), and 4-amino-7-nitrocinnoline methiodide, m. p. and mixed m. p. 189°.

Quantitative Separation of Ammonia and Methylamine.—The mixture of amines (equiv. to *n* c.c. of 0·1N-acid) was shaken in a light-proof bottle for 2 hr. witb mixed aqueous solutions of sodium carbonate (5 c.c.; 30%) and sodium hydroxide (5 c.c.; 20%) in which was suspended yellow mercuric oxide (*n*/10 g.). The bottle was opened in red light and the contents were filtered through a sintered-glass funnel (porosity 3) into a Buchner flask, connected to the pump through two acid-traps. The filtrate and acid from the traps were transferred to a Kjeldahl

apparatus, made strongly alkaline with sodium hydroxide solution (20%), and distilled in a stream of nitrogen for 1 hr. into two traps in series (each containing *n* c.c. of 0·1N-acid) for estimation in the usual manner. The Kjeldahl apparatus was tested with " AnalaR " ammonium chloride, the ammonia estimated being between 99·5 and 100% of the theoretical value. The following reproducible figures show the accuracy obtainable with " synthetic mixtures " of methylamine (previously purified by shaking with yellow mercuric oxide) and ammonia (from " AnalaR " ammonium chloride). Experiments 2 and 4 represent the limits of accuracy of the method.

Expt. no.	1	2	3	4
Titre of mixture	35·45	40·00	56·00	23·25
Titre of NH_2Me	15·10	0·70	54·9	23·20
Theor. titre of NH_2Me	15·10	0·77	*	23·15

* Commercial " methylamine."

Alkaline Decomposition of Cinnoline Quaternary Salts.—(*a*) The salt was dissolved in water (10—20 parts) and boiled in the micro-Kjeldahl apparatus. The ammonia liberated was estimated as usual and the remaining solution examined on a paper-chromatogram. Most of the salts studied were stable (to 18 hours' refluxing); β-4-amino-6-nitrocinnoline metho-salts gave (after 2½ hr.) 1-methyl-6-nitro-4-cinnolone, m. p. and mixed m. p. 190° (Found: C, 52·55; H, 3·35; N, 21·5. Calc. for $C_9H_7O_3N_3$: C, 52·7; H, 3·4; N, 20·5%). 4-Amino-8-nitrocinnoline methiodide gave 4-hydroxy-8-nitrocinnoline.

(*b*) A solution of the salt (0·5 g.) in warm water (10 c.c.) was rapidly cooled to 5° and the suspension made alkaline by dropwise addition of sodium hydroxide solution. The precipitate was washed with water and dried *in vacuo* over concentrated sulphuric acid. β-4-Amino-6-nitrocinnolinium salts gave the *anhydro-base*, m. p. 230—231°, which separated from ethanol in golden needles (Found: C, 53·5; H, 2·9; N, 28·0. $C_9H_8O_2N_4$ requires C, 52·9; H, 3·95; N, 27·4%). 4-Amino-8-nitrocinnoline methiodide gave an unstable orange solid, m. p. 64° (decomp.).

(*c*) A solution of the salt (0·5 g.) in warm water (10 c.c.) was refluxed with 2N-sodium hydroxide (5 c.c.) for a short time and the precipitate (if any) filtered off after cooling. The β-6-nitro-salts gave the *N*-methyl 4-cinnolones as in (*a*), the corresponding α-salts giving only tars.

4-*Acetamido*-8-*nitrocinnoline*.—The amino-compound (0·4 g.) was refluxed with acetic anhydride (4 c.c.) for 30 min. and the suspension (formed after initial solution) cooled and diluted with ether (100 c.c.). The solid (0·4 g.; m. p. 287—288°), recrystallised from ethyl methyl ketone, gave almost colourless needles of the pure *acetyl derivative*, m. p. 291—292° (Found: C, 52·25; H, 3·65; N, 24·05. $C_{10}H_8O_3N_4$ requires C, 51·7; H, 3·45; N, 24·1%).

4-*Methoxy*-7-*nitrocinnoline*.—A solution of 4-chloro-7-nitrocinnoline (1·5 g.) in benzene (20 c.c.) was refluxed for 1½ hr. with sodium (0·2 g.) in methanol (10 c.c.). Recrystallisation of the precipitate (1·7 g.; m. p. 194—195°) from acetone gave the pure *methoxy-compound*, m. p. 200°, as pale yellow, opaque, flat blades (Found: C, 52·8; H, 3·35; N, 19·4. $C_9H_7O_3N_3$ requires C, 52·7; H, 3·4; N, 20·4%), $\lambda_{max.}$ 2070, 2600, and 3100 (ε 51,700, 38,800, and 6540 respectively).

4-*Methylaminocinnoline*.—4-Hydroxycinnoline (3 g.) was refluxed with phosphorus oxychloride for 30 min. and then the mixture was poured on ice (400 g.) and sodium acetate (25 g.). The chloro-compound was extracted with ether and the washed and dried extract was concentrated to *ca.* 10 c.c. before being added to phenol (30 g.) at 80°. This mixture was heated to 140° and a stream of dry methylamine passed through it for 40 min. before cooling and dilution with ether to precipitate the crude base. Pure 4-*methylaminocinnoline*, m. p. 229° (2·1 g.), was obtained by reprecipitation with sodium hydroxide solution from a solution (carbon) in acetic acid and recrystallisation (colourless needles) from water (Found: C, 66·7; H, 6·1; N, 26·4. $C_9H_9N_3,\frac{1}{4}H_2O$ requires C, 66·1; H, 5·8; N, 25·7%). The *hydrochloride*, m. p. 282°, separated from ethanol as colourless needles (Found: C, 54·1; H, 5·45; N, 21·35; Cl, 18·3. $C_9H_{10}N_3Cl$ requires C, 55·2; H, 5·15; N, 21·5; Cl, 18·1%).

4-*Methylamino*-6-*nitrocinnoline*.—An intimate mixture of 4-chloro-6-nitrocinnoline (5 g.) and phenol (50 g.) was treated with a brisk stream of dry methylamine. After a spontaneous rise in temperature to 90°, orange needles separated from the clear red solution and the passage of methylamine was continued for ½ hr. The cold mixture was diluted with ether and filtered, and the solid (5·7 g.; m. p. 260°) washed free from phenol with ether. The pure *base*, m. p. 345° (decomp.) (4 g.), was obtained by reprecipitation with ammonia from a solution (carbon) in

warm 30% acetic acid (150 c.c.); recrystallisation from *iso*propyl alcohol gave yellow needles (Found: C, 52·8; H, 4·0; N, 24·5. $C_9H_8O_2N_4$ requires C, 52·9; H, 3·95; N, 27·5%).

Reduction of Cinnoline Methiodide.—(*a*) A mixture of the salt (0·5 g.), sodium carbonate (0·1 g.), platinum oxide, and ethanol (50 c.c.) was agitated for 6½ hr. at 100° in a " Baskerville " autoclave with hydrogen (100 atm.). Basic gases (detected on release of the pressure) were passed into acid, and the filtered reaction mixture was distilled into the same acid. Titration with standard alkali indicated a 45% recovery of ammonia; ammonium picrate, m. p. and mixed m. p. 266—269° (decomp.), was isolated from half of the titration liquor whilst a determination for methylamine (as above) showed this to be absent.

(*b*) A solution of the salt (2·4 g.) in ethanol (70 c.c.) was hydrogenated at room temperature and atmospheric pressure in the presence of anhydrous sodium acetate (0·55 g.) and Raney nickel for 24 hr.; the rate of the uptake of hydrogen was negligible (170·5 c.c. used; 1 mol. = 224 c.c.). The mixture was filtered, then evaporated to dryness, and a benzene-soluble oil (" A," 1·4 g.) was separated from the residue of sodium iodide. Distillation of " A " (0·7 g.) gave a green liquid, b. p. 190°/1·5 mm., which immediately became black in air and from it no solid derivative could be prepared.

A solution of " A " (0·7 g.) in ethanol (30 c.c.) was refluxed and treated with small pieces of sodium to maintain a steady evolution of hydrogen during 1 hr. The escaping gases contained only ammonia (70% yield based upon the methiodide). The mixture was evaporated to dryness under nitrgoen, and the residue was dissolved in 1 : 1 benzene–light petroleum (b. p. 60—80°) and chromatographed (alumina; Merck–Brockmann) on a column (18 × 1 cm.). One fraction gave a red crystalline picrate, m. p. 150° (1-methylindole picrate has m. p. 150°).

Attempted Quaternisation of 4-Acetamido-8-nitrocinnoline.—The base (0·14 g.) was refluxed with methyl iodide (1·4 c.c.) in ethanol (5 c.c.) for 3½ hr. and the crystalline precipitate (0·095 g., *i.e.*, 70% recovery) of unchanged material, m. p. and mixed m. p. 290—291°, collected from the cold mixture. An identical experiment gave a 75% recovery of unchanged material and when sufficient ethanol (20 c.c.) to give complete solution was used the product was an intractable red tar.

Reduction of α- and β-4-Amino-6-nitrocinnoline Methiodide.—A solution of the α-salt (0·3 g.) in water (10 c.c.) was treated with one of stannous chloride (0·7 g. of dihydrate) in water (2 c.c.) and concentrated hydrochloric acid (3 c.c.), and the solution heated at 85°. The separation of fine needles which began after 15 min. was complete in 15 min.; the precipitate was collected from the cold mixture and digested with boiling water, then filtered, and the filtrate evaporated to dryness in a desiccator. The resulting solid (0·19 g.; m. p. 298—300°) was identical (mixed m. p.) with 4 : 6-diaminocinnoline methochloride and yielded the pure compound, m. p. 306—310°, on recrystallisation from water.

Formation of Methochlorides from " Anhydro-bases."—The anhydro-base was warmed gently with dilute hydrochloric acid until a clear solution was obtained. This solution was evaporated to dryness in a desiccator, the residue recrystallised from water, and the crystalline product compared with the corresponding authentic quaternary salt by mixed m. p. determination and by paper chromatography.

Aqueous Decomposition of 4-Amino-8-nitrocinnoline and the Derived Methiodide.—An aqueous solution of the compound (*ca.* 0·2 g. in 10 c.c.) was refluxed in a micro-Kjeldahl apparatus from which the acid traps were removed from time to time for titration against standard alkali. The following results show the relative rates of decomposition:

Time (hr.)	1	4	7	10	14	27
NH_3 (%) evolved from salt	—	1·8	—	3·4	—	9·4
" " base	10	25	65	—	80	—

Quaternisation of 4-Amino-8-nitrocinnoline.—After treatment of the base with methyl iodide in ethanol as usual, a sample of the mixture was examined by paper chromatography. Apart from spots which corresponded to the base and 4-hydroxy-8-nitrocinnoline only one spot was obtained and this showed no tendency to " tail " on prolonged running (confirmed in four experiments).

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