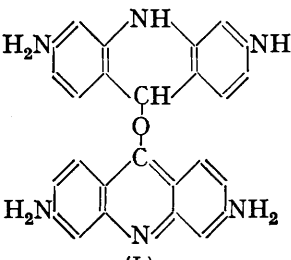
28. *Acridine Syntheses and Reactions. Part I. Synthesis of Proflavine from* m-*Phenylenediamine and its Derivatives.*

By Adrien Albert.

Processes are described which furnish up to 70% of pure proflavine as the result of a single condensation starting from *m*-phenylenediamine (with oxalic or formic acid), from *m*-amino-oxanilic acid, or from mono (or di)-formyl-*m*-phenylenediamine. These processes are of general interest, as they enable a variety of simple benzene derivatives to be rapidly converted into derivatives of acridine in good yield. The mechanism of the initial stages of the reaction is discussed.

Although several members of the acridine series have been made by the action of formaldehyde on primary aromatic amines, it is not possible to obtain proflavine (IV) in this way from *m*-phenylenediamine (Meyer and Gross, *Ber.*, 1899, **32**, 2365). The synthesis most often used is that due to Benda (*Ber.*, 1912, **45**, 1787), who nitrated 4 : 4′-diaminodiphenylmethane and heated the product under pressure with stannous chloride, thus obtaining diaminodihydroacridine, which was finally oxidised to proflavine. The patent literature (D.R.P. 347,819; 1921), however, indicated a method that appeared more economical of time and material: *m*-phenylenediamine (1 mol.), glycerol (2 mols.), zinc chloride (1·33 mols.), and oxalic acid dihydrate (1 mol.) are heated for 2 hours at 190°, and a 60% direct yield of proflavine is claimed.

In an attempt to repeat this work, a tarry mass was produced from which proflavine could be isolated with difficulty. However, modification of the process by heating at 155° for 45 minutes and doubling the amount of glycerol gave 55—65% yields of proflavine (exclusive of some 15% lost in purification) and 10% of the *m*-phenylenediamine remained unaltered. No isomeric diaminoacridines could be isolated, the by-products being traces of tar, resinous material, and a pair of related substances, one of which crystallised from alcohol in cream-coloured needles which, when exposed to the air, changed into the other, an orange substance insoluble in alcohol but dissolved by pyridine (green fluorescence) and hydrolysed by hydriodic acid to 2 : 8-diaminoacridine and 2 : 8-diaminoacridone (Albert and Linnell, J., 1936, 88). The orange substance, characterised as its *tetrabutyryl* derivative, is provisionally assigned the constitution, 2 : 8 : 2′ : 8′-*tetra-amino*-5 : 10-*dihydrodiacridyl* 5 : 5′-*ether* (I).



(I.)

Although more than half the oxalic acid used in the condensation could be recovered unchanged, a reduction in the amount caused a diminution in yield. Presumably, the excess of oxalic acid acts as a source of hydrogen ions, for this excess can be replaced by an equivalent amount of hydrochloric acid without serious reduction in yield. Although unchanged *m*-phenylenediamine was always found after the condensation, the use of more oxalic acid diminished the yield appreciably, as did excess of mineral acid.

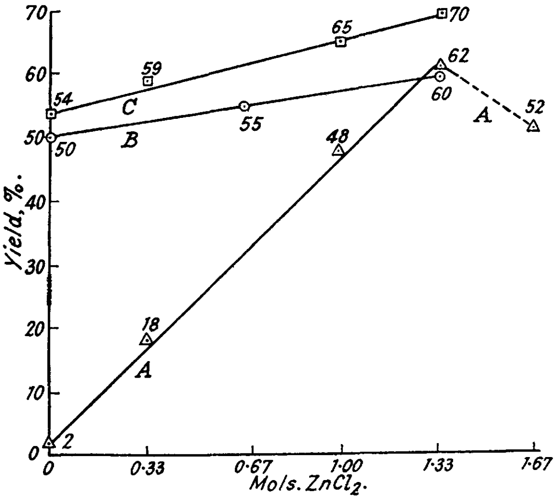
Carbon dioxide began to be liberated at 120° and continued until the condensation was complete, one molecule being liberated for each molecule of proflavine formed.

In the condensation, glycerol may be replaced by those alcohols (sorbitol, glycol, 1 : 2-dihydroxypropane) which, containing two adjacent hydroxyl groups, readily convert oxalic acid into formic acid; alcohols which give hardly any formic acid (1 : 3-dihydroxypropane and phenylethyl alcohol) give no proflavine.

The zinc chloride could be replaced by calcium chloride (30% yield) but not by stannous or aluminium chloride. The yield of proflavine fell in linear proportion when the amount of zinc chloride was decreased (see Fig.). This effect is attributed partly to a fall in hydrogen-ion concentration, since both zinc and calcium chlorides evolve hydrogen chloride freely when triturated with oxalic acid. The zinc salt appears in addition to have a specific catalytic action, since, when it is replaced by hydrochloric acid, the yield rises only to 25%.

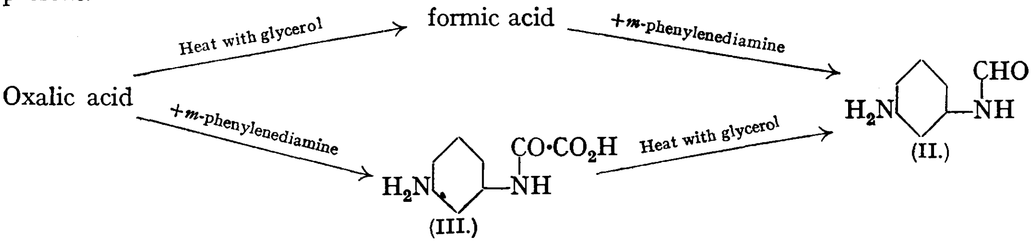
If zinc chloride is omitted from the reaction, the principal product is *m*-amino-oxanilic acid (III), which is not found during the normal proflavine condensation. Although this acid might be considered to be the precursor of proflavine, the highest yield obtainable by heating it with *m*-phenylenediamine and hydrochloric acid is only 23%. On the other hand, m-*aminoformanilide* (II) could be isolated from the main reaction by interrupting it at 130° and, when condensed with *m*-phenylenediamine and hydrochloric acid, gave a 70% yield of proflavine.

m-Amino-oxanilic acid gave *m*-aminoformanilide (5% at 135°) on warming, but only in the presence of glycerol and mineral acid, so that prior hydrolysis cannot be excluded.



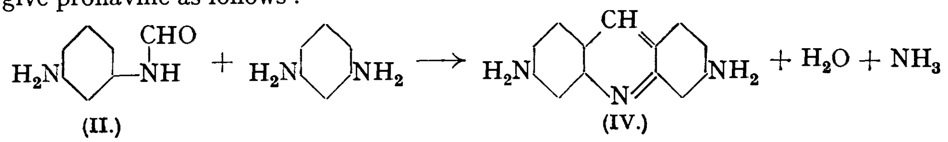
Effect of zinc chloride on the production of proflavine in glycerol.
From (A) m-*phenylenediamine* (2 *mols.*) + *oxalic acid* (2 *mols.*),
(B) m-*phenylenediamine monohydrochloride* (2 *mols.*) + *formic acid* (1 *mol.*),
(C) m-*aminoformanilide hydrochloride* (1 *mol.*) + m-*phenylenediamine monohydrochloride* (1 *mol.*).

The reaction up to this point is summarised in the following scheme and must be considered as following principally the upper route, the lower route being hindered by the zinc chloride present.



Although it might be expected that formic acid could replace oxalic acid in the original reaction, zinc chloride, *m*-phenylenediamine, glycerol, and formic acid (even in excess) gave little proflavine when heated together unless hydrochloric acid also was present. As will be seen from the figure (B), the condensation can be effected in the absence of zinc chloride, thus showing that this salt plays its principal rôle in the first stage of the *original* reaction. In the absence of hydrochloric acid, the condensations with formic acid give moderate yields of the diacridyl ether (I).

The *m*-aminoformanilide produced in the original reaction represents only part of the *m*-phenylenediamine taken and apparently condenses with the remainder of this amine to give proflavine as follows :



This reaction, when effected directly by condensing in equimolecular proportions *m*-aminoformanilide (in 95% yield from *m*-nitroformanilide) with *m*-phenylenediamine dihydrochloride and zinc chloride, gave a particularly high yield (70%) of proflavine. The figure (C) shows that zinc chloride does not greatly influence the course of this reaction, which also takes place in the absence of polyhydric alcohols.

m-Aminoformanilide hydrochloride (2 mols.), heated with glycerol and zinc chloride, gave a 60% yield of proflavine, monoformylproflavine probably being produced first. The latter would be a highly labile substance, *diformylproflavine* being completely hydrolysed on contact for a few minutes with cold dilute alkali solution or warm dilute acid. *s*-Diformyl-*m*-phenylenediamine (1 mol.) and *m*-phenylenediamine dihydrochloride (1 mol.) similarly gave a 47% yield of proflavine.

The exact mechanism of the condensation of *m*-phenylenediamine and *m*-aminoformanilide to proflavine is still under investigation. 3 : 3′-Diamino-*N*-formyldiphenylamine has been identified in the reaction mixture, and this substance gives proflavine on heating with acids. However, further evidence favours 2 : 4 : 2′ : 4′-tetra-aminobenzhydrol as the principal intermediate, and this amine is considered to be the source of diacridyl ethers, which are produced in greatest quantity when the reaction mixture contains an excess of formic acid, Kavache (*Ann. Chim.*, 1918, **10**, 236) having shown that formic acid converts benzhydrol into dibenzhydryl ether.

The various condensations described in the experimental part are equally suitable for other diamines and their derivatives (see also Albert and Large, *Nature*, 1938, **142**, 435; Albert, J., 1939, 920).

EXPERIMENTAL.

General.—All the temperatures recorded are internal. Yields, calculated on the amount of amine taken, refer to purified proflavine and disregard unchanged initial material and proflavine lost in purification.

Condensation using Oxalic Acid.—*m*-Phenylenediamine (*v.i.*, 3 g.) was added at 120° to zinc chloride (5 g.; 1·33 mols.), dissolved in glycerol (10 g.; 4 mols.), in a beaker, and the *m*-phenylenediamine zinc chloride broken up. Oxalic acid dihydrate (3·5 g.; 1 mol.) was added, and the temperature raised during 25 minutes to 155° with gentle stirring (carbon dioxide evolved) and maintained for 45 minutes. The melt was treated with 2·5N-sodium hydroxide (80 ml.), and the precipitate extracted with the minimum quantity of boiling N-hydrochloric acid so that resinous material of low basicity remained undissolved. The filtrate was treated with excess of ammonia to remove zinc, and the precipitate extracted with the minimum quantity of dilute acetic acid. This solution was treated with 20% sulphuric acid (¼ vol.) and left in ice for 4 hours. The red crystals of proflavine sulphate obtained were decomposed by ammonia, and the base dried at 120°. Yield, 1·8 g. (62%) (average of ten experiments). Recrystallisation from 400 parts of boiling water or 55 parts of alcohol produced yellow needles which gave no depression of m. p. with proflavine base synthesised from tetra-aminodiphenylmethane or precipitated from the commercial sulphate (B.D.H.), all the samples melting at 277° [288° (corr.)]. For proflavine, the use of a sealed capillary is considered necessary in order to obtain a sharp m. p. preceded by little sintering.

By stopping the above condensation at various stages, the following amounts of proflavine were found: 140°, 1%; after 5 mins. at 155°, 21%; after 30 mins. at 155°, 53%; 45 mins. at 155°, 61%; 75 mins. at 155°, 48% and much tar. Increasing the amount of oxalic acid by 25% lowered the yields to 32%; decreasing the amount by 25% lowered the yield to 33%, and replacement of the *m*-phenylenediamine by its hydrochloride (5 g.) gave rise to diacridyl ethers (*v.i.*) in 15% yield, no proflavine being formed. However, *m*-phenylenediamine (1·5 g.), *m*-phenylenediamine dihydrochloride (2·5 g.) (equalling in all 3 g. of base), zinc chloride (5 g.), glycerol (10 g.), and oxalic acid dihydrate (1·75 g.; 0·5 mol.) reacted slowly to give a 50% yield of proflavine (65 mins. at 155°).

Condensation using m-*Amino-oxanilic Acid.*—*m*-Amino-oxanilic acid monohydrate (*v.i.*, 2·75 g.; 1 mol.), *m*-phenylenediamine (1·5 g.; 1 mol.), hydrogen chloride (1·0 g., 2 mols., as 3·1 ml. of standardised hydrochloric acid), and zinc chloride (5 g.) in glycerol (10 g.), warmed to 155° during 30 mins. and held there for 45 mins., gave a 23% yield. The use of 1 mol. of hydrogen chloride gave 10%, of 3 mols., 12%, and of 2 mols. in the absence of zinc chloride, 12%, yields.

Condensation using Formic Acid.—*m*-Phenylenediamine (3 g.; 2 mols.), hydrogen chloride (1 g., 2 mols., as 3·1 ml. of concentrated hydrochloric acid), and formic acid (0·72 g. of 90%; 1 mol.) were mixed with a cold solution of zinc chloride (5 g.) in glycerol (10 g.), warmed to 155° during 30 mins., and held there for 45 mins. Yield, 60%. Omission of zinc chloride lowered the yield to 50%, replacing glycerol by phenylethyl alcohol gave an 11% yield, and doubling the quantities of both acids gave diacridyl ethers (*v.i.*) but no proflavine.

Condensation using m-*Aminoformanilide.*—*m*-Phenylenediamine dihydrochloride (2·5 g.; 1 mol.) and *m*-aminoformanilide (1·9 g.; 1 mol.) were added to a cold solution of zinc chloride (5 g.) in glycerol (10 g.), and the mixture warmed to 155° during 25 mins. and held there for 40 mins. Yield, 70%. Less tar was formed in this condensation than in the previous cases. The use of 1 mol. and 3 mols. of hydrogen chloride gave 30% and 35% yields respectively.

m-*Phenylenediamine.*—The material used in the above experiments was freshly distilled and preserved over calcium chloride, which retards its decomposition.

m-*Amino-oxanilic Acid.*—The yield (43%) originally obtained by heating *m*-phenylenediamine (1 mol.) and oxalic acid dihydrate (2 mols.) (Jacobs, *J. Amer. Chem. Soc.*, 1917, **39**, 1451) was increased to 70% when glycerol (5 mols.) was added as a solvent (90 mins. at 140°). The product (+ 1 H_2O), recrystallised from 400 parts of boiling water, had m. p. 237° [245° (corr.)].

m-*Aminoformanilide.*—This was obtained from the oxalic acid condensations (5% at 130°, 10% at 140°) and was freed from *m*-phenylenediamine by rubbing with benzene, in which the latter is more soluble. Further supplies were obtained as follows (compare Albert and Ritchie, *Proc. Roy. Soc. N.S.W.*, 1940, **74**, 77): Raney nickel (10 g.), *m*-nitroformanilide (10 g.), and alcohol (200 ml.) were shaken in hydrogen for 2 hours or until absorption was complete; the filtered liquid was concentrated in a vacuum and allowed to crystallise. Yield, 95%. Further purification, when required, was effected by crystallisation from 3 parts of alcohol. The white needles obtained, m. p. 107°, were soluble in 1 part of boiling alcohol, 150 parts of boiling benzene, *ca.* 50 parts of cold water, more soluble in aqueous ammonia, and very sparingly soluble in ether. The solutions readily became supersaturated. The solid was stable in cold air, but was oxidised in warm air (Found: C, 61·8; H, 5·9; N, 20·9. $C_7H_8ON_2$ requires C, 61·8; H, 5·9; N, 20·6%).

Acyl Derivatives of Proflavine.—2 : 8-*Diformamidoacridine* formed yellow crystals, m. p. 251° (decomp.), sparingly soluble (green fluorescence) in alcohol and most other organic solvents (Found: N, 15·6. $C_{15}H_{11}O_2N_3$ requires N, 15·8%). It was soluble in aqueous sodium hydroxide, proflavine being precipitated through hydrolysis within 3 mins., and was completely hydrolysed in 5 mins. by boiling N-hydrochloric acid. The orange hydrochloride was less soluble in water than that of proflavine.

2 : 8-*Dibutyramidoacridine* formed lemon-yellow crystals (dried at 120°), m. p. 265° [275° (corr.)], not raised by recrystallisation from 7 parts of alcohol, to which it imparted an intense green fluorescence. It was very soluble in boiling water, toluene, chloroform, and acetone (Found: N, 12·8. $C_{21}H_{23}O_2N_3$ requires N, 12·9%). The hydrochloride formed a pale yellow solution in water with intense green fluorescence.

2 : 8 : 2′ : 8′-*Tetra-amino*-5 : 10-*dihydrodiacridyl* 5 : 5′-*ether* (I) was best obtained by refluxing *m*-phenylenediamine dihydrochloride (10 g.), formic acid (10 g.), and glycerol (20 g.), the temperature being raised from 130° to 155° during 90 minutes. The bases formed were precipitated with sodium hydroxide, taken up in cold 0·5% sulphuric acid, chilled, filtered from traces of proflavine sulphate, reprecipitated with sodium hydroxide, and extracted with boiling alcohol. The alcohol-insoluble portion was identical with the substance obtained by exposing the cream-coloured needles, which separated from the alcoholic filtrate, to the air for a day. The former was orange in colour and decomposed at 260° (efferv.) (Found for a sample dried at 25° in a vacuum: C, 65·9; H, 5·7; N, 17·9; loss at 120°, 7·9. $C_{26}H_{22}ON_6,2H_2O$ requires C, 66·4; H, 5·6; N, 17·9; H_2O, 7·7%. The corresponding tetrahydro-compound requires C, 66·1; H, 6·0; N, 17·8%. The completely oxidised tetra-aminodiacridyl ether, $C_{26}H_{20}ON_6,2H_2O$, requires C, 66·6; H, 5·2; N, 18·0%). The last compound is apparently formed on keeping (I) for some weeks; it then becomes insoluble in pyridine. The ether (I) formed dark brown salts, more soluble than those of proflavine, giving no colour with nitrous acid (distinction from proflavine), and coupling with β-naphthol to a port-wine colour. Dr. S. D. Rubbo of the University of Melbourne reports that these salts are protein precipitants and have no antiseptic properties.

2 : 8 : 2′ : 8′-*Tetrabutyramido*-5 : 10-*dihydrodiacridyl* 5 : 5′-*ether*, obtained from (I) (0·26 g.), water (1·5 ml.), butyric acid (0·5 ml.), and butyric anhydride (2 ml.) and recrystallised from acetone–ether, formed buff-coloured crystals, m. p. 250° (not sharp), very sparingly soluble

in water, benzene, and ether and tending to oxidise in the air (Found for material dried in a vacuum: N, 11·75. $C_{42}H_{46}O_5N_6$ requires N, 11·8%).

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