389. *The Synthesis of 7-Amino-alloxazines and -isoalloxazines.*

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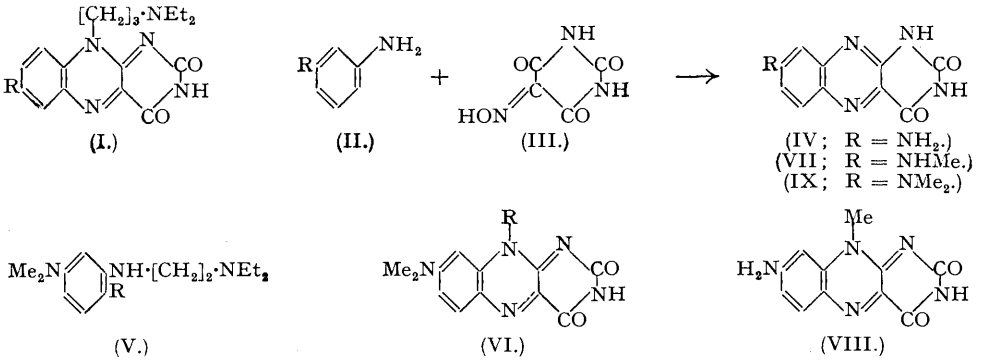
The application of Piloty's synthesis (*Annalen*, 1904, **333**, 44) of 7-aminoalloxazine (IV), from violuric acid and *m*-phenylenediamine, to secondary-tertiary-*m*-diamines has given 7-*dialkylamino*isoalloxazines of type (VI), identical with the condensation products of the appropriately alkylated 1 : 2 : 4-triaminobenzenes and alloxan. Measurements of absorption spectra and chemical examination of the product show that the action of violuric acid on *N*-methyl-*m*-phenylenediamine leads to a mixture of the *alloxazine* (VII) and the iso*alloxazine* (VIII).

In view of their possible microbiological activity, as analogues of riboflavin, various *iso*alloxazines with simple dialkylaminoalkyl groups in the 9-position have recently been synthesised (King and Acheson, *J.*, 1946, 682; Neeman, *ibid.*, p. 811; Adams, Weisel, and Mosher, *J. Amer. Chem. Soc.*, 1946, **68**, 883; Kipnis, Weiner, and Spoerri, *ibid.*, 1947, **69**, 799; Hippchen, *Ber.*, 1947, **80**, 263). In general, these *iso*alloxazines are *benz*-chloro-, -nitro-, -methoxyl- or -methyl derivatives, which have been obtained by condensing in acid solution the corresponding *o*-phenylenediamines with alloxan. *iso*Alloxazines with amino-groups in the benzene nucleus have not so far been described; as already indicated (King and Acheson, *loc. cit.*), 2 : 4-diamino-γ-diethylaminopropylaniline failed, under the usual conditions, to give the expected 6-amino*iso*alloxazine (I; R = NH_2). Certain aminoalloxazines, on the other hand, are already



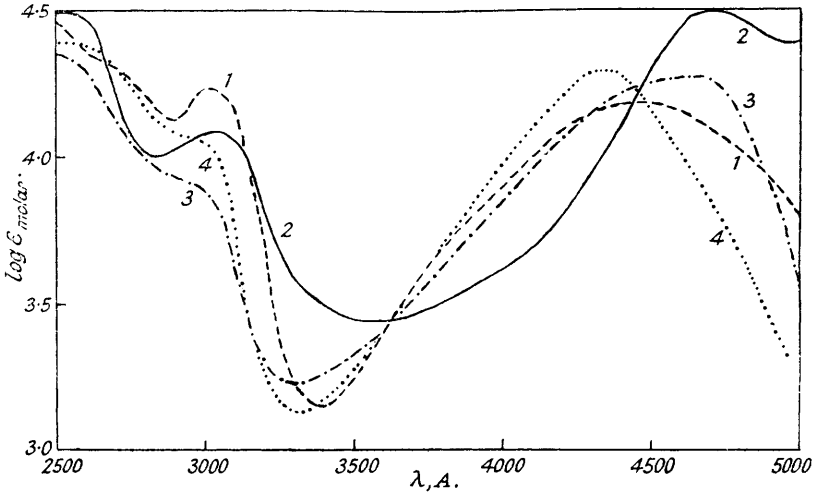
known, having been prepared by Piloty (*Annalen*, 1904, **333**, 44) from *m*-phenylenediamine (II; R = NH_2) and violuric acid (III) or its 1 : 3-dimethyl derivative. The present communication reports the extension of this synthesis to several amino*iso*alloxazines.

Alloxazine formation from violuric acid and a *m*-phenylenediamine depends on the presence in the latter of an activated position *ortho* to the condensing amino-group. From the amine (II; R = NH_2), in which both the 2- and the 6-position are free, either the 5- or the 7-amino-alloxazine may result, but, as recognised by Piloty (*loc. cit.*), the compound most likely to be obtained is the 7-amino-derivative (IV; R = NH_2). Ganapati (*J. Indian Chem. Soc.*, 1938, **15**, 77) attempted to resolve this point by applying the *o*-phenylenediamine–alloxan synthesis to 1 : 2 : 4-triaminobenzene, but the properties of the product (probably a mixture of 6- and 7-aminoalloxazine) and of Piloty's aminoalloxazine, both of which are sparingly soluble red powders with high decomposition points, rendered their comparison inconclusive.

The 7-amino-structure assigned to Piloty's *m*-phenylenediamine–violuric acid condensate now

appears to be confirmed by analogy with the results of the following experiments. 3-*Dimethylamino*-β-*diethylaminoethylaniline* (V; R = H) reacted with violuric acid in hot alcoholic solution giving 7-dimethylamino-9-β-diethylaminoethyl*iso*alloxazine (VI; R = $[CH_2]_2$·NEt_2), which separated as a tetrahydrated *violurate*. Its constitution as the 7-dimethylamino-compound was proved by a synthesis of the base from alloxan and 2-amino-5-dimethylamino-β-diethylaminoethylaniline (V; R = NH_2) in boric–acetic acid solution. The complete identity of both products was established through the above violurate and also by means of the common *picrate*.

Of the materials required for these syntheses, 3-dimethylamino-β-diethylaminoethylaniline (V; R = H) was prepared from 3-*dimethylamino*-p-*toluenesulphonanilide* by alkylation with diethyl-β-chloroethylamine, the resulting oily sulphonamide being hydrolysed by cold sulphuric acid. The triaminobenzene (V; R = NH_2) used in the alloxan synthesis is the reduction product of 2-*nitro*-5-*dimethylamino*-β-*diethylaminoethylaniline* (V; R = NO_2) which was obtained by heating 3 : 4-dinitrodimethylaniline with β-diethylaminoethylamine, the labile 3-nitro-group being displaced (cf. Forster and Coulson, *J.*, 1922, **121**, 1988; Romburgh, *Rec. Trav. chim.*, 1923, **42**, 804). For the preparation of the necessary dinitro-base, direct nitration of 3-nitro-*N*-dimethylaniline (Romburgh, *ibid.*, 1887, **6**, 250; Swann, *J.*, 1920, **3**) was found to be preferable



to the method of Hodgson and Smith (*ibid.*, 1931, 1508), using nitrous acid as nitrating agent, which gives a mixture of products.

The use of a secondary-tertiary amine in the Piloty synthesis admits only of the formation of alloxazines, but from a primary-secondary-*m*-diamine, *e.g.*, *N*-methyl-*m*-phenylenediamine (II; R = NHMe), condensation with violuric acid may give either an alloxazine (VII) or an *iso*alloxazine (VIII). When the experiment was tried, a microcrystalline substance was obtained which was at first believed to be homogeneous. The constitution of this product, whether alloxazine or *iso*alloxazine, was thought to be ascertainable by comparison of its ultra-violet absorption with the spectra of reference compounds of established structure. For this purpose the orange-red microcrystalline 7-*dimethylaminoalloxazine* (IX) was synthesised from *m*-amino-*N*-dimethylaniline and violuric acid, and the corresponding 7-*dimethylamino*-9-*methyl*isoalloxazine (VI; R = Me), a carmine powder, similarly prepared from *m*-methylamino-*N*-dimethylaniline, the latter being obtained from 3-dimethylamino-*p*-toluenesulphonanilide by methylation and hydrolysis.

The absorption spectra of these two reference compounds, the alloxazine (IX) (Curve 1) and the *iso*alloxazine (VI; R = Me) (Curve 2), are shown in the figure: although not in marked contrast, they exhibit an observable difference throughout the whole wave band examined. On plotting the data obtained for the *m*-aminomethylaniline–violuric acid product, the resulting curve (3) was found to lie between those of the two reference compounds, and it became apparent that the material was heterogeneous. Repeated extraction of the red-brown condensate with dilute hydrochloric acid, which was designed to remove the more basic *iso*alloxazine, left an orange solid having a spectrum (Curve 4) approximating closely to that of the alloxazine. In

view of the colour difference between the reference compounds the change of colour to orange on washing with acid is significant.

The absorption curves of the 7-amino-alloxazine and -*iso*alloxazine differ appreciably from those recorded for the 9-diethylaminoalkyl*iso*alloxazines and for riboflavin by Adams, Weisel, and Mosher (*loc. cit.*). In addition to general displacement of the curves towards the lower frequencies, the absorption maxima at *ca.* 3,500 A. exhibited by riboflavin and the compounds of Adams *et al.* have disappeared.

The effect of nitrous acid on the product in question provided further evidence of its heterogeneous character. Diazotisation in sulphuric acid solution precipitated the sparingly soluble 7-N-*nitrosomethylaminoalloxazine*. This was removed, and the filtrate coupled with alkaline β-naphthol, whereupon the presence of the amino*iso*alloxazine was demonstrated by the formation of a deep olive-green *azo-dye*. Diazotisation of 7-aminoalloxazine under the same conditions gave a clear solution which afforded a very similar azo-β-naphthol *compound*. From the relative proportions of these derivatives obtained in the two experiments the percentage of alloxazine to *iso*alloxazine in the mixture is calculated as approximately 40/60. From the action of violuric acid on *m*-amino-β-diethylaminoethylaniline, which was prepared from 3′-*nitro*-p-*toluenesulphon*-(β-*diethylaminoethyl*)*anilide* by removal of the tosyl group and reduction of the resulting *nitro-amine*, a crystalline *violurate* was isolated. This may have contained alloxazine or *iso*alloxazine, but its constitution was not further investigated.

The high proportion of *iso*alloxazine in the mixed product of the diamine–violuric acid condensation is to be expected from the probable course of the reaction, *i.e.*, preliminary addition at the 4- or 6-carbonyl of the pyrimidine followed by ring-closure between the oximino-group and the activated 6-position in the benzene ring. With amino-groups of different basicities, *e.g.*, NH_2 and NHMe, reaction with the more basic centre, *i.e.*, NHMe, would predominate, leading to a higher proportion of *iso*alloxazine. Condensation of the oximino-group with an activated position in the aromatic nucleus as the preliminary step is very improbable, since the intermediate would be analogous to the so-called Kuhling products obtained when the condensation of *o*-diamines with alloxan is carried out in the absence of acid, and these are known to be extremely resistant to cyclisation (Tishler, Wellman, and Ladenburg, *J. Amer. Chem. Soc.*, 1945, **67**, 2165).

Attempts have been made by Ganapati (*loc. cit.*) to extend the Piloty synthesis to other *m*-substituted anilines, but without success, from which he concludes that the *m*-amino-group is specific for this reaction. It is evident, however, that the degree of activation in the aromatic nucleus is the important factor, since from 4-aminoveratrole and violuric acid in acetic acid solution, it has been possible to synthesise, though not in a spectroscopically pure condition, 6 : 7-*dimethoxyalloxazine*. The nature of the product has been demonstrated by an independent synthesis from 4 : 5-diaminoveratrole and alloxan.

In addition to the above 7-amino-compounds, 7-chloro-9-β-diethylaminoethyl- and 7-chloro-9-γ-diethylaminopropyl-*iso*alloxazine have been synthesised by the alloxan method, and characterised by *picrates* and hydrated *hydrochlorides*. The necessary triamines were prepared by the action of 2 : 4-dichloronitrobenzene on the diethylaminoalkylamines, the resulting 2-nitro-5-chloroamines, which were analysed as *hydrochlorides* and *picrates*, being reduced by the catalytic method immediately before condensation.

Experimental.

3-*Dimethylamino*-p-*toluenesulphonanilide*.—*m*-Nitrodimethylaniline (15·3 g.) was reduced in methanol solution (50 c.c.) over Raney nickel, and after filtration from catalyst the resulting diamine was treated with *p*-toluenesulphonyl chloride (18·5 g.). The mixture was heated on a steam-bath for 20 minutes, diluted with water, and basified with ammonia. The precipitated *sulphonamide* crystallised from ethanol in colourless prisms. m. p. 156° (16 g., 68%) (Found : C, 62·0; H, 5·8; S, 10·7. $C_{15}H_{18}O_2N_2S$ requires C, 62·1; H, 6·2; S, 11·0%).

3-*Dimethylamino*-β-*diethylaminoethylaniline* (V; R = H).—Diethyl-β-chloroethylamine (4·2 g.) and 3-dimethylamino-*p*-toluenesulphonanilide (9·0 g.) were added to a solution of sodium (0·72 g.) in ethanol (50 c.c.). After refluxing on a steam-bath for 6 hours, the solvent was evaporated, and the residue treated with water and shaken with chloroform. Evaporation of the chloroform layer gave the alkylated sulphonanilide as an oil which neither solidified nor formed crystalline derivatives. It was therefore hydrolysed with sulphuric acid (100 g. of 94%) at room temperature, the brown solution being diluted with ice-water and basified after 20 hours to give 3-*dimethylamino*-β-*diethylaminoethylaniline* as a pale yellow oil, b. p. 115—117° (bath temp.)/0·03 mm. (4·45 g., 61%) (Found : C, 71·5; H, 10·7. $C_{14}H_{25}N_3$ requires C, 71·5; H, 10·6%). A *monopicrate* separated from aqueous ethanol in very deep red prisms, m. p. 133° (Found : C, 51·4; H, 6·1; N, 18·6. $C_{14}H_{25}N_3,C_6H_3O_7N_3$ requires C, 51·7; H, 6·0; N, 18·1%). The *dipicrate* crystallised from ethanol as a yellow powder, m. p. 143—144° (decomp.) (Found : C, 44·9; H, 4·6. $C_{14}H_{25}N_3,2C_6H_3O_7N_3$ requires C, 45·0; H, 4·5%).

3 : 4-*Dinitro*-N-*dimethylaniline*.—The nitration of 3-nitrodimethylaniline following the directions of Hodgson and Smith (*loc. cit.*) gave only 26% of the desired 3 : 4-dinitro-compound and much 3-nitro-*N*-nitroso-*N*-methylaniline. Direct nitration (cf. Romburgh, *loc. cit.*) was accomplished by adding the finely powdered amine (4 g.) to dilute nitric acid (80 c.c. of 20%). After vigorous shaking for 10 minutes the mixture was diluted with water, and the precipitate collected and dissolved in boiling *n*-propanol. On cooling, 3 : 4-dinitrodimethylaniline (2·07 g., 41%), m. p. 178—179°, separated in yellow needles. Dilution of the mother-liquor with water precipitated 2 : 5-dinitrodimethylaniline, crystallising from ethanol in scarlet prisms (0·7 g., 14%), m. p. 112°.

2-*Nitro*-5-*dimethylamino*-β-*diethylaminoethylaniline* (V, R = NO_2).—3 : 4-Dinitrodimethylaniline (3·84 g., 1 mol.), β-diethylaminoethylamine (3·2 g., 1·5 mols.), and anhydrous sodium acetate (4 g.) were heated in an oil-bath, a vigorous reaction occurring. After 6 hours at 140—160°, the mixture was cooled, and acidified with dilute acid, the aqueous solution then being washed with ether. Addition of alkali gave a product which, when isolated, distilled at 195—205° (bath temp.)/0·1 mm. as a light brown oil (3·9 g., 77%). This consisted of the *nitro-amine* (V; R = NO_2), which crystallised in yellow-brown prisms, m. p. 45—46° (Found : N, 20·3. $C_{14}H_{24}O_2N_4$ requires N, 20·0%). The *picrate* separated from aqueous ethanol in yellow diamond-shaped plates, m. p. 171° (decomp.) (Found : C, 46·8; H, 5·4; N, 18·6. $C_{14}H_{24}O_2N_4,C_6H_3O_7N_3$ requires C, 47·1; H, 5·3; N, 19·3%).

7-*Dimethylamino*-9-β-*diethylaminoethyl*iso*alloxazine* (VI; R = $[CH_2]_2$·NEt_2).—(i) On mixing hot solutions of 3-dimethylamino-β-diethylaminoethylaniline (V; R = H) (2·75 g., 1 mol.) in ethanol (10 c.c.) and of violuric acid (1·84 g., 1 mol.) in water (20 c.c.), a purple colour appeared, rapidly changing to crimson. After 1 hour on a steam-bath the mixture was evaporated under reduced pressure, and the product treated with boiling ethanol (20 c.c.). The residue of *iso*alloxazine violurate (2·35 g., 69% calc. on violuric acid), m. p. 265° (decomp.), crystallised from its deep crimson aqueous solution as red-brown hair-like needles of the *tetrahydrate* (Found : C, 45·4; H, 5·7; N, 22·4. $C_{18}H_{24}O_2N_6,C_4H_3O_4N_3,4H_2O$ requires C, 45·1; H, 6·0; N, 21·5%. Found, after drying at 120° in a vacuum : C, 50·5; H, 5·3; N, 24·3; loss, 10·2. $C_{18}H_{24}O_2N_6,C_4H_3O_4N_3,\frac{1}{2}H_2O$ requires C, 50·6; H, 5·4; N, 25·1; loss, 10·8%. Found, after drying at 150° in a vacuum : C, 51·1; H, 5·5; loss, 11·6. $C_{18}H_{24}O_2N_6,C_4H_3O_4N_3$ requires C, 51·5; H, 5·3; loss, 12·3%). The hydrated *hydrochloride*, which was purified by precipitation with ether from methanolic hydrogen chloride, formed a bright red microcrystalline powder, very soluble in water, m. p. 290° (decomp.) (Found, after drying at 100° in a vacuum : C, 53·0; H, 6·5; N, 19·8; Cl, 8·3. $C_{18}H_{24}O_2N_6,HCl,H_2O$ requires C, 52·6; H, 6·6; N, 20·5; Cl, 8·7%). The *iso*alloxazine *picrate* crystallised from aqueous alcohol in dull red minute needles, m. p. 242° (decomp.) (Found, after drying at 120° in a vacuum : C, 48·9; H, 4·9; N, 21·2. $C_{18}H_{24}O_2N_6,C_6H_3O_7N_3$ requires C, 49·2; H, 4·6; N, 21·5%).

(ii) 2-Nitro-5-dimethylamino-β-diethylaminoethylaniline (V; R = NO_2) (0·9 g., 1 mol.) was reduced in acetic acid (25 c.c.) over palladised charcoal catalyst, and the filtered solution of the amine added to warm acetic acid (30 c.c.) containing alloxan monohydrate (0·6 g., 1·17 mols.) and boric acid (0·9 g.). After 30 minutes at 60°, the deep orange-red solution was evaporated to dryness, and the residue taken up in hot water (15 c.c.). Neutralisation with dilute sodium hydroxide gave the iso*alloxazine* (VI; R = $[CH_2]_2$·NEt_2) (0·47 g., 41%), m. p. *ca.* 278° (decomp.). When purified by acidification of its solution in alkali, the base separated as a monohydrate in hair-like carmine needles, m. p. 298—300° (decomp.) (Found : C, 57·5; H, 6·8; N, 23·0. $C_{18}H_{24}O_2N_6,H_2O$ requires C, 57·8; H, 7·0; N, 22·5%. Found, after drying at 200° in a vacuum : C, 59·3; H, 6·5; loss, 2·4. $C_{18}H_{24}O_2N_6,\frac{1}{2}H_2O$ requires C, 59·2; H, 6·8; loss, 2·4%). The *iso*alloxazine was characterised by the picrate, m. p. 242° (decomp.) (Found : C, 48·6; H, 4·8; N, 21·9%), and violurate, m. p. 264° (decomp.) (Found, after drying at 120° in a vacuum : C, 50·4; H, 5·5; loss, 10·5%), already described.

7-*Dimethylaminoalloxazine* (IX).—*m*-Nitro-*N*-dimethylaniline (1 g.) dissolved in ethanol (20 c.c.) was hydrogenated in presence of Raney nickel, and the filtered liquid added to a solution of violuric acid (0·95 g.) in hot water (10 c.c.). After being heated to boiling for 1 hour, the crimson solution had deposited a dark red solid, which was collected next day (yield 1·03 g., 66·5%) and washed sparingly with water and then ethanol. The *alloxazine* (IX) was purified by dissolving it in aqueous methanol containing sodium hydroxide (1½%) and precipitated by the addition of hydrochloric acid to the boiling deep red solution. The orange-red powder then had m. p. 355—357° (decomp.) (Found, after drying at 120° in a vacuum : C, 54·2, 53·7; H, 4·3, 4·7; N, 26·9, 26·7. $C_{12}H_{11}O_2N_5,\frac{1}{2}H_2O$ requires C, 54·1; H, 4·5; N, 26·3%). The ultra-violet spectrum (Curve 1) was determined in N/20-aqueous sodium hydroxide. From more concentrated alkali the *sodio*-derivative separated in brick-red prisms (Found, after drying at 120° in a vacuum : C, 50·2; H, 4·2. $C_{12}H_{10}O_2N_5Na,\frac{1}{2}H_2O$ requires C, 50·0; H, 3·8%).

m-*Methylamino*-N-*dimethylaniline*.—*m*-Dimethylamino-*p*-toluenesulphonanilide (4 g.) and methyl iodide (2 g.) were added to a solution of sodium (0·32 g.) in ethanol (20 c.c.), and the mixture heated under reflux for 7 hours. The oil obtained on evaporation of the alcohol solidified when treated with dilute acid. Crystallisation from water gave 3-p-*toluenesulphonmethylamido*-N-*dimethylaniline hydriodide* (4·3 g., 72%) in colourless needles, m. p. 163° (decomp.) (Found : C, 44·6; H, 5·0; I, 28·2. $C_{16}H_{20}O_2N_2S,HI$ requires C, 44·4; N, 4·9; I, 29·4%). The free base could not be crystallised, and hydrolysis of the sulphonamide was carried out with the salt (3·85 g.), which was mixed with sulphuric acid (15 c.c. of 98%). A vigorous reaction occurred, and after 24 hours the deep blue solution was diluted with ice-water, filtered from iodine, and basified. The methylaminodimethylaniline, a colourless oil (0·7 g., 52%), was identified by analysis of the *dipicrate*, yellow-brown prisms from alcoholic picric acid, m. p. 124° (decomp.) (Found : C, 41·4; H, 3·6. $C_9H_{14}N_2,2C_6H_3O_7N_3$ requires C, 41·4; H, 3·3%).

7-*Dimethylamino*-9-*methyl*iso*alloxazine* (VI; R = Me).—The *m*-methylaminodimethylaniline (0·45 g.) in ethanol (3 c.c.) was heated with a concentrated aqueous solution of violuric acid (0·47 g.) in a steam-bath for 1¼ hours. The iso*alloxazine* (VI; R = Me) which had precipitated from the deep crimson solution was collected and washed with water and ethanol (yield 0·6 g., 74%). The product was dissolved in boiling 1½% aqueous sodium hydroxde, and on neutralisation with dilute hydrochloric acid gave brick-red needles, m. p. 360° (decomp.) (Found, after drying at 120° in a vacuum : C, 57·8; H, 5·1; N, 25·8. $C_{13}H_{13}O_2N_5$ requires C, 57·6; H, 4·8; N, 25·8%). The ultra-violet spectrum in N/7-aqueous sodium hydroxide is shown as Curve 2.

Condensation of m-*Amino*-N-*methylaniline with Violuric Acid.*—*m*-Nitro-*N*-methylaniline (2 g.) was hydrogenated in methanol (20 c.c.) over Raney nickel, and the resulting solution of the diamine treated with violuric acid (2·1 g.) in boiling water (20 c.c.). After an hour's heating on a steam-bath and addition of water (20 c.c.), the dark red solid which had separated (2·06 g., 93%), m. p. 335—337° (decomp.), was collected and dissolved in 1½% aqueous sodium hydroxide. On acidification of the deep red solution the product separated in a gelatinous form; by repeated solution in alkali and reprecipitation, a red-brown solid, m. p. 340—345° (decomp.), was obtained (Found, after drying at 120° in a vacuum: C, 52·4; H, 4·1; N, 27·2. $C_{11}H_9O_2N_5,\frac{1}{2}H_2O$ requires C, 52·4; H, 4·0; N, 27·8%). The ultra-violet spectrum of the material in N/10-sodium hydroxide is represented by Curve 3.

A specimen of the crude product (0·3 g.) was extracted thrice with hot N-hydrochloric acid (total 120 c.c.), and the residue then purified as above. The orange-red powder had m. p. 352—355° (decomp.), and from its ultra-violet spectrum (Curve 4), which was determined in N/10-alkali, it is believed to consist of 7-*methylaminoalloxazine* (VII) (Found: C, 47·1; H, 4·8; N, 24·4. $C_{11}H_9O_2N_5,2H_2O$ requires C, 47·3; H, 4·7; N, 25·1%. Found, after drying at 120° in a vacuum: C, 52·4; H, 3·9; loss, 9·3. $C_{11}H_9O_2N_5,\frac{1}{2}H_2O$ requires C, 52·4; H, 4·0; loss, 9·7%).

A further specimen of the unpurified product (0·2 g.) was dissolved in warm sulphuric acid (4 c.c. of 70%), and after cooling to 0° was treated with 10% aqueous sodium nitrite (1 c.c.), the salt which precipitated on cooling being disregarded. The mixture was left for a time, then diluted with water (25 c.c.), and the 7-*nitrosomethylaminoalloxazine* (0·08 g.), a yellow powder decomposing >300°, collected and washed with water (Found: C, 46·4; H, 3·3; N, 28·8. $C_{11}H_8O_3N_6,\frac{1}{2}H_2O$ requires C, 46·9; H, 3·2; N, 29·9%. Found, after drying at 120° in a vacuum: C, 49·4; H, 3·3. $C_{11}H_8O_3N_6$ requires C, 48·5; H, 2·9%). The filtrate was added to a solution of β-naphthol (0·12 g.) in aqueous sodium hydroxide (30 c.c. of 15%), and after a short time the deep purple solution was neutralised with acetic acid. The precipitated *azo*-compound (0·15 g.), a deep olive-green powder, m. p. 310° (decomp.), was collected and well washed with water and ethanol (Found: C, 56·1; H, 4·2; N, 17·9. $C_{21}H_{14}O_3N_6,3H_2O$ requires C, 55·7; H, 4·4; N, 18·6%. Found, after drying at 150° in a vacuum: C, 60·1; H, 3·8; loss, 8·0. $C_{21}H_{14}O_3N_6,H_2O$ requires C, 60·6; H, 3·8; loss, 8·0%).

When 7-aminoalloxazine (0·2 g.) was diazotised under exactly the above conditions a clear solution was obtained, which gave with alkaline β-naphthol a deep olive-green *azo*-compound (0·23 g.), m. p. 335—338° (decomp.) after sintering *ca.* 275°, dissolving in alkali to a red solution (Found: C, 57·4; H, 4·0; N, 19·1. $C_{20}H_{12}O_3N_6,2H_2O$ requires C, 57·1; H, 3·8; N, 20·0%. Found, after drying at 120° in a vacuum: C, 61·0; H, 3·3; loss, 7·3. $C_{20}H_{12}O_3N_6,\frac{1}{2}H_2O$ requires C, 61·1; H, 3·3; loss, 6·4%).

3′-*Nitro*-p-*toluenesulphon*-(β-*diethylaminoethyl*)*anilide.*—Finely powdered sodamide (1·6 g.) was added during 25 minutes to a well-stirred solution of diethyl-β-chloroethylamine (5·5 g.) and 3′-nitro-*p*-toluenesulphonanilide (10·5 g.) in toluene (200 c.c.). The mixture was slowly heated (1 hour) to 100°, and after 3 hours at 100° was refluxed for a further hour. The solution was then filtered and extracted 4 times with hot 5% hydrochloric acid (total 400 c.c.). Concentration and cooling of the acid extract gave the alkylated sulphonamide *hydrochloride*, which crystallised in colourless silky needles (12 g., 78%), m. p. 182° (Found: C, 53·1; H, 6·0; N, 9·4. $C_{19}H_{25}O_4N_3S,HCl$ requires C, 53·3; H, 6·1; N, 9·6%). The *picrate* separated from aqueous alcohol in hexagonal yellow tablets, m. p. 156—157° (Found, after drying at 116° in a vacuum: C, 48·0; H, 4·5; S, 5·0. $C_{19}H_{25}O_4N_3S,C_6H_3O_7N_3$ requires C, 48·4; H, 4·5; S, 5·2%). The alkylation was later more conveniently carried out by refluxing a mixture of the components in ethanolic sodium ethoxide for 6 hours, but with some diminution in yield.

3-*Nitro*-β-*diethylaminoethylaniline.*—The foregoing hydrochloride (6·0 g.) was dissolved in sulphuric acid (55 c.c. of 95%), and after standing overnight, the solution was poured into ice-water and basified. The liberated *nitroamine* was collected in ether and on distillation obtained as a yellow-red oil (2·5 g., 75%), b. p. 125—130° (bath temp.)/0·03 mm. (Found: C, 61·1; H, 8·0. $C_{12}H_{19}O_2N_3$ requires C, 60·7; H, 8·0%), characterised by a *picrate*, which crystallised from aqueous alcohol in orange prisms, m. p. 151—152° (Found: C, 46·5: H, 4·8. $C_{12}H_{19}O_2N_3,C_6H_3O_7N_3$ requires C, 46·4; H, 4·7%).

A specimen of the nitroamine (1·5 g.) was catalytically reduced in methanol (30 c.c.), and combined with violuric acid (2 g.) dissolved in water (10 c.c.) by heating on a steam-bath for 50 minutes. On cooling, a red-brown solid (1·6 g.), m. p. 290° (decomp.), was obtained, which dissolved in aqueous ethanol to a yellow solution with green fluorescence. It crystallised from water as a brick-red powder with a green lustre, m. p. 300° (decomp.), and consisted of a *violurate*, possibly of 7-amino-9-β-diethylaminoethyl*iso*alloxazine and of the isomeric alloxazine, but its constitution was not definitely ascertained (Found, after drying at 120° in a vacuum: C, 49·6, 49·3; H, 5·2, 5·2; N, 25·6. $C_{16}H_{20}O_2N_6,C_4H_3O_4N_3$ requires C, 49·5; H, 4·7; N, 26·0%).

6 : 7-*Dimethoxyalloxazine.*—(i) 4-Nitroveratrole (2 g.) was catalytically reduced in dioxan (15 c.c.), and the amine obtained by evaporation to dryness was dissolved in acetic acid (15 c.c.). Violuric acid (1·72 g.) was then added, and the mixture heated under reflux for 10 hours. The dark maroon product (0·34 g.) was collected on cooling, and purified by repeatedly dissolving it in 1% aqueous sodium hydroxide and reprecipitating it with acid. The light yellow-brown 6 : 7-*dimethoxyalloxazine*, m. p. 333—335° (decomp., with darkening >300°), dissolved in alkali to a yellow solution with a brilliant green fluorescence (Found, after drying at 150° in a vacuum: C, 52·5; H, 4·1. $C_{12}H_{10}O_4N_4$ requires C, 52·6; H, 3·6%). The pure alloxazine gave an intense red solution in concentrated sulphuric acid which underwent slight charring on heating.

(ii) 4 : 5-Dinitroveratrole (1·1 g.) in methanol (20 c.c.) was hydrogenated over Raney nickel, and the filtered solution heated with concentrated hydrochloric acid and alloxan monohydrate (0·9 g.) in water (10 c.c.). The yellow precipitate was purified as before, the alkaline solution being kept hot during acidification to minimise gel formation. The resulting alloxazine, m. p. >345° (decomp., with previous darkening), was a *sesquihydrate* (Found: C, 48·2; H, 4·2; N, 18·4. $C_{12}H_{10}O_4N_4,1\frac{1}{2}H_2O$ requires C, 47·8; H, 4·3; N, 18·6%) which lost water slowly at 150° in a vacuum (Found: C, 51·7; H, 4·0%). The intense red solution in sulphuric acid remained clear on heating.

The ultra-violet spectra of the two specimens in N/10-sodium hydroxide show the following characteristics:

Specimen (i).				Specimen (ii).			
λ max.	ε max.	λ max.	ε max.	λ max.	ε max.	λ max.	ε max.
2300	26,900	4050	8700	2200	20,600	4150	10,400
2600	26,900	λ min.	ε min.	2600	26,400	λ min.	ε min.
(3500	6,900) *	3000	2500	3500	7,150	3000	2,090

* The value of ε at 3500 A. : no extinction maximum was apparent in the curve at this wave-length.

5-*Chloro*-2-*nitro*-(β-*diethylaminoethyl*)*aniline*.—2 : 4-Dichloronitrobenzene (11·5 g.), β-diethylaminoethylamine (7 g.), and anhydrous sodium acetate were heated at 100° rising to 150° during 5 hours. Water (35 c.c.) and concentrated hydrochloric acid (18 c.c.) were then added, and the resulting solid collected and washed with ether. 5-*Chloro*-2-*nitro*-(β-*diethylaminoethyl*)*aniline hydrochloride* (11·7 g., 63%) was thus obtained, which on crystallisation from aqueous ethanol formed yellow prisms, m. p. 208—209° (Found : C, 46·9; H, 6·1; Cl, 22·8. $C_{12}H_{18}O_2N_3Cl$,HCl requires C, 46·8; H, 6·2; Cl, 23·1%). A solution in water gave with alkali the *chloronitroamine*, fine yellow needles from ethanol, m. p. 77—78° (Found : C, 53·0; H, 6·8; Cl, 13·0. $C_{12}H_{18}O_2N_3Cl$ requires C, 53·0; H, 6·6; Cl, 13·1%). The *picrate* crystallised from aqueous ethanol in long yellow prisms, m. p. 185—186° (Found : C, 43·6; H, 4·5; Cl, 7·1. $C_{12}H_{18}O_2N_3Cl,C_6H_3O_7N_3$ requires C, 43·2; H, 4·2; Cl, 7·1%).

7-*Chloro*-9-β-*diethylaminoethyl*iso*alloxazine*.—The above nitro-amine (3·1 g., 1 mol.) was catalytically reduced in methanol (40 c.c.), and the colourless solution mixed with concentrated hydrochloric acid (3·5 c.c., 3 mols.) and alloxan monohydrate (2 g., 1·1 mols.) in hot water was heated to boiling for ½ hour. Evaporation to dryness and treatment of the residue with alcohol–acetone gave the iso*alloxazine hydrochloride* as a brown solid (1·8 g., 41%), which after repeated crystallisation from aqueous ethanol formed pale yellow prisms, m. p. 288—289° (decomp.) (Found : C, 49·0; H, 5·1; N, 17·7; Cl, 18·6. $C_{16}H_{18}O_2N_6Cl$,HCl,½H_2O requires C, 48·8; H, 5·1; N, 17·8; Cl, 18·1%). The *picrate* crystallised from ethanol–water in pale yellow needles, m. p. 232° (decomp.) (Found : C, 45·8; H, 3·9; N, 19·0. $C_{16}H_{18}O_2N_6Cl,C_6H_3O_7N_3$ requires C, 45·8; H, 3·6; N, 19·4%).

5-*Chloro*-2-*nitro*-(γ-*diethylaminopropyl*)*aniline*.—A mixture of diethylaminopropylamine (15·6 g.), 2 : 4-dichloronitrobenzene (23 g.), and anhydrous sodium acetate was heated from 100° rising to 150° during 4 hours, and the 5-chloro-2-nitro-(γ-diethylaminopropyl)aniline isolated as *hydrochloride* by the addition of concentrated hydrochloric acid (40 c.c.) and water (80 c.c.). The product (37 g., 96%), m. p. 210°, crystallised from aqueous ethanol in yellow-orange needles, m. p. 224° (Found : C, 48·6; H, 6·5; N, 12·7; Cl, 22·9. $C_{13}H_{20}O_2N_3Cl$,HCl requires C, 48·4; H, 6·5; N, 13·0; Cl, 22·0%). The *amine* was a pale yellow oil, b. p. 155—160°/0·3 mm. (Found : C, 54·6; H, 7·3; N, 14·9. $C_{13}H_{20}O_2N_3Cl$ requires C, 54·6; H, 7·0; N, 14·7%), having a *picrate* which crystallised from aqueous ethanol in yellow prisms, m. p. 169—170° (Found : C, 44·5; H, 4·7; N, 16·2. $C_{13}H_{20}O_2N_3Cl,C_6H_3O_7N_3$ requires C, 44·4; H, 4·5; N, 16·3%).

7-*Chloro*-9-(γ-*diethylaminopropyl*)iso*alloxazine*.—A solution of the amine prepared by catalytic reduction of the foregoing nitro-compound (3·2 g.) in methanol (40 c.c.) gave on being heated to boiling with hydrochloric acid (3·4 c.c.) and alloxan monohydrate (2 g.) in water for ½ hour the iso*alloxazine hydrochloride*. This was isolated by evaporation to dryness and addition of ethanol–acetone as a brown powder (3·1 g., 70%), which on crystallisation from aqueous alcohol gave scintillating microscopic yellow-brown plates, m. p. 291—292° (decomp.) (Found, after drying at 120° in a vacuum : C, 51·1; H, 5·4; N, 17·4; Cl, 17·4. $C_{17}H_{20}O_2N_5Cl$,HCl requires C, 51·2; H, 5·3; N, 17·6; Cl, 17·8%). The *picrate*, minute green-yellow prisms from ethanol–water, had m. p. 258° (decomp.) (rapid heating) (Found : C, 46·9; H, 4·1; Cl, 6·2. $C_{17}H_{20}O_2N_5Cl,C_6H_3O_7N_3$ requires C, 46·7; H, 3·9; Cl, 6·0%).

The 7-chloro*iso*alloxazines in 0·1% aqueous solution were inactive against *B. coli*, but inhibited the growth of diluted (1/1000) *Staph. aureus* broth culture. Similar results were obtained with the *iso*alloxazine (VI; R = $[CH_2]_2$·NEt_2) as hydrochloride, but saturated aqueous solutions of the sparingly soluble compounds (VI; R = Me), (VIII), and (IX) showed no bacteriostatic properties for either organism. We are indebted to Dr. E. P. Abraham, Sir William Dunn School of Pathology, Oxford, for these tests.

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