141. *New Potential Chemotherapeutic Agents. Part V. Basically-substituted* iso*Alloxazines.*

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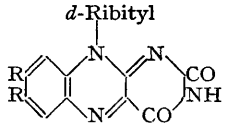
A series of *iso*alloxazines modelled on riboflavin, but with the ribityl side-chain replaced by diethylaminoalkyl groups and methyl, chloro, and methoxyl substituents at the 6-position, has been synthesised from alloxan and the appropriate *o*-phenylenediamines. The products, isolated as *monohydrochlorides*, exhibit in solution the intense fluorescence characteristic of this class of compound.

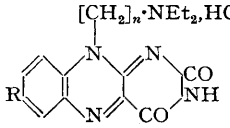
According to Fildes, McIlwain, Woods, and others (for references see Hinshelwood, *Ann. Reports*, 1944, **41**, 25), the inhibitory action of many chemotherapeutic agents on the metabolism of bacteria, and probably of other micro-organisms, *e.g.*, protozoa, is due to their interference with the utilisation of growth factors to which they bear a close structural resemblance. It is believed that by virtue of this similarity of structure antibiotic substances are able to compete with the normal metabolites for their specific enzymes, and considerable evidence now exists to show that it is often the oxidation–reduction enzymes which are thus inhibited.

Among the factors necessary for bacterial growth is riboflavin (I, R = Me), a constituent of several widely occurring enzymes. Two examples have lately been recorded of bacterial inhibition by riboflavin analogues,

i.e., 6 : 7-dichloro-9-*d*-ribityl*iso*alloxazine (I; R = Cl) (Kuhn, Weygand, and Möller, *Ber.*, 1943, **76**, 1044) and 2 : 4-diamino-6 : 7-dimethyl-9-*d*-ribityldihydrophenazine (Woolley, *J. Biol. Chem.*, 1944, **154**, 31), both of which



(I).



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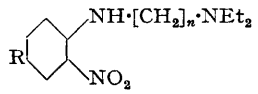

(III.)

are antagonised by riboflavin, and Madinaveitia (*Biochem. J.*, 1944, **38**, xxvii) has reported that the antibacterial activity of certain antimalarial drugs is also reversed by the vitamin.

The experiments now to be described, which were begun in June, 1944, are part of a more comprehensive undertaking to provide a series of synthetical variants of the riboflavin molecule for examination both as potential antimalarials and as general bacteriostatic agents. Since many of the most successful cell inhibitors are basically-substituted, the modification initially contemplated in the riboflavin molecule was the replacement of the 9-ribityl substituent by basic side-chains, for example, the dialkylaminoalkyl groups typical of the current antimalarial drugs, combined with variations in the substituents of the aromatic nucleus. In view of the recent communication of Hall and Turner (*J.*, 1945, 699) describing their attempts to prepare precisely similar derivatives *e.g.*, (II; R = H, $n = 3$), we present this account of our experiments.

The basically-substituted *iso*alloxazines (II) were obtained by the standard method of synthesis, *viz.*, condensation of the *o*-phenylenediamines derived by catalytic reduction of 4-substituted-2-nitroalkylanilines (III) with alloxan, generally in hot 85—90% ethanolic hydrogen chloride. In this way were obtained 9-β-diethylaminoethyl*iso*alloxazine, and the 6-methyl-, 6-chloro- and 6-methoxy- derivatives of both 9-β-diethylaminoethyl- and 9-γ-diethylamino-*n*-propyl-*iso*alloxazine, all of which separated in satisfactory yields from their reaction mixtures as highly crystalline hydrated *monohydrochlorides*. Condensation in boric–acetic acid solution (Kuhn and Weygand, *Ber.*, 1935, **68**, 1282) was tried in one case, but was less successful owing to the difficulty of removing boric acid from the product. The amine hydrochlorides are very freely soluble in water giving yellow to orange-yellow solutions with a brilliant green fluorescence. The *iso*alloxazine bases are also highly water-soluble, but crystalline *picrates* were precipitated on the addition of sodium picrate to aqueous solutions of the hydrochlorides.

The synthesis of the necessary nitroamines (III) was effected either from sodium salts of the *N*-acylated nitroanilines by alkylation with diethylaminoalkyl chlorides followed by acid hydrolysis of the acyl group, or by the condensation of *o*-chloronitrobenzenes with diethylaminoalkylamines. Thus, the action of cold 90% sulphuric acid on 2-nitro-*p*-toluenesulphon-(β-diethylaminoethyl)anilide gave (III; R = H, $n = 2$), an intermediate for the amine (II; R = H, $n = 2$), and a similar process was applied to the synthesis from their *p*-toluenesulphonamides of the compounds (III; R = OMe, $n = 2$) and (III; R = OMe, $n = 3$). The amine (III; R = OMe, $n = 2$) was also obtained by the action of hot hydrochloric acid on its *acetyl* derivative, for which 3-nitro-4-acetamidoanisole was condensed with β-chloroethyldiethylamine in presence of sodamide (cf. Eisleb, *Ber.*, 1941, **74**, 1433). The same alkylation technique was also used for the synthesis of the 4-methyl compound (III; R = Me, $n = 2$) and of its analogue (III; R = Cl, $n = 2$), but these and the remaining nitroamines (III; R = Me, $n = 3$) and (III; R = Cl, $n = 3$) were more conveniently prepared by the second general method, *i.e.*, heating the *o*-chloronitrobenzenes with β-diethylaminoethyl- or γ-diethylamino-*n*-propyl-amine and sodium acetate. The yields of β-diethylaminoethylamine from the catalytic reduction of diethylaminomethyl cyanide have been considerably improved by a slight elaboration of the recorded procedure (cf. Winans and Adkins, *J. Amer. Chem. Soc.*, 1933, **55**, 4167).

From chloro-2 : 4-dinitrobenzene and γ-diethylamino-*n*-propylamine, 2 : 4-*dinitro-γ-diethylamino*-n-*propyl-aniline* has been prepared, but its reduction and condensation with alloxan have failed to give the expected 6-amino*iso*alloxazine. Experiments on the preparation of these amino derivatives are continuing.

The results of biological tests on some of the foregoing *iso*alloxazines will be published in due course.

Experimental.

2-Nitro-β-diethylaminoethylaniline (III; R = H, $n = 2$).—A mixture of β-chloroethyldiethylamine hydrochloride (43 g.) and 2-nitro-*p*-toluenesulphonanilide (58 g.) (Usherwood and Whiteley, *J.*, 1923, **123**, 1084) dissolved in alcohol (300 c.c.) with an alcoholic solution of sodium (11·5 g. in 250 c.c.) was heated under reflux for 36 hours on a steam-bath. The product obtained by evaporation of the filtered liquid was shaken with water (150 c.c.) and concentrated hydrochloric acid (50 c.c.), and, after removal of unchanged 2-nitro-*p*-toluenesulphonanilide, the solution was treated with aqueous sodium hydroxide (50%) and extracted with chloroform. 2-Nitro-*p*-toluenesulphon-(β-diethylaminoethyl)anilide was thus obtained as a pale brown syrup (75 g.) and identified by the *picrate*, which crystallised from ethanol–acetone in lemon-yellow flat prisms, m. p. 152—154° (Found: C, 47·9; H, 4·4; N, 13·2. $C_{19}H_{25}O_4N_3S,C_6H_3O_7N_3$ requires C, 48·4; H, 4·5; N, 13·5%).

Ice-cold sulphuric acid (250 c.c. of 90%) was cautiously added to the above sulphonanilide (74 g.) and the mixture left for 12 hours at room temperature. The resulting solution was then slowly poured into a mixture of ice and excess of aqueous ammonia and the liberated base collected in ether and distilled. The pure *nitroamine* (37 g.) was a bright red oil, b. p. 164—166°/0·05 mm. (Found: C, 61·1; H, 7·8; N, 17·2. $C_{12}H_{19}O_2N_3$ requires C, 60·7; H, 8·0; N, 17·7%), and formed a *picrate* which separated from alcohol–acetone in orange-yellow rods, m. p. 158° (Found: C, 46·4; H, 5·0. $C_{12}H_{19}O_2N_3,C_6H_3O_7N_3$ requires C, 46·4; H, 4·7%).

*9-β-Diethylaminoethyliso*alloxazine *Hydrochloride* (II; R = H, $n = 2$).—The nitroamine (III; R = H, $n = 2$)

(10 g.) was dissolved in ethanol (100 c.c.) and reduced over Raney nickel at room temperature with hydrogen at 50 atm. The resulting triamine was not isolated, but after filtration the almost colourless liquid was immediately acidified with concentrated hydrochloric acid (12·5 c.c., 3 mols.) diluted with a little alcohol, and heated on a steam-bath with a solution of alloxan monohydrate (6·8 g., 1 mol.) in water (7—8 c.c.). Within a few minutes a chrome-yellow crystalline precipitate appeared which was collected after the solution had cooled to room temperature. The product (7·5 g.) readily dissolved in water to a bright yellow solution with a brilliant yellow-green fluorescence, and was most conveniently recrystallised by the addition of a small amount of water to a suspension of the solid in boiling ethanol. 9-*Diethylaminoethyl*iso*alloxazine hydrochloride* separated on cooling as glittering yellow rectangular plates, m. p. 282—283° (decomp.) (Found on a specimen dried at 100° in a high vacuum: C, 55·1; H, 5·8; N, 21·1; Cl, 9·6. $C_{16}H_{19}O_2N_5$,HCl requires C, 54·9; H, 5·8; N, 20·0; Cl, 10·2%). Mixing aqueous solutions of the hydrochloride and of sodium picrate precipitated the *picrate*, which crystallised from ethanol–water in yellow-green prisms, m. p. 226° (decomp.) (Found: C, 49·0; H. 4·2; N, 20·5. $C_{16}H_{19}O_2N_5$,$C_6H_3O_7N_3$ requires C, 48·7; H, 4·1; N, 20·7%).

β-Diethylaminoethylamine (cf. Winans and Adkins, *loc. cit.*).—A mixture of diethylaminomethyl cyanide (20 g.) (Luten, *J. Org. Chem.*, 1939, **3**, 588) and Raney nickel in a high-pressure hydrogenator was cooled to −30°, and a solution of liquid ammonia (20 c.c.) in ether (50 c.c.) added. The apparatus was then filled with hydrogen at 96 atm. and rapidly heated (15—20 minutes) to 140°. When cold, the contents were slowly brought to room pressure and filtered from catalyst, and the product and ether washings fractionated. No high-boiling by-products were formed, and the β-diethylaminoethylamine (12·8 g., 61·5%) of b. p. 144—150° was characterised as the dipicrate, m. p. 211° (decomp.) (Found: C, 37·7; H, 3·9. Calc. for $C_6H_{16}N_2$,$2C_6H_3O_7N_3$: C, 37·7; H, 3·8%).

3-*Nitro*-p-N-(*β-diethylaminoethyl*)*toluidine*, (III; R = Me, *n* = 2).—(i) 4-Chloro-3-nitrotoluene (4·11 g., 1 mol.) and β-diethylaminoethylamine (3·1 g., 1·1 mol.) mixed with anhydrous sodium acetate (5·0 g.) were heated in an oil-bath rising from 100° to 140° during 7 hours. The cooled product was shaken with dilute hydrochloric acid, unchanged chloronitrotoluene extracted with ether, and after addition of sodium hydroxide the liberated 3-*nitro*-p-N-(*β-diethylaminoethyl*)*toluidine* isolated as a deep-red oil (1·8 g.), b. p. 125—135°/0·033 mm. (Found: C, 62·4; H, 8·3; N, 17·6. $C_{13}H_{21}O_2N_3$ requires C, 62·2; H, 8·4; N, 16·7%). The *picrate* formed minute yellow needles from alcohol–benzene, m. p. 176° (Found: C, 47·5; H, 5·0; N, 18·1. $C_{13}H_{21}O_2N_3$,$C_6H_3O_7N_3$ requires C, 47·5; H, 5·0; N, 17·5%).

(ii) To a solution of 3-nitroacet-*p*-toluidide (5·5 g.) and β-chloroethyldiethylamine (4·25 g., 1·1 mol.) in dry toluene (50 c.c.), powdered sodamide (1·1 g., 1·1 mol.) was added during 15 minutes (cf. Eisleb, *loc. cit.*). The mixture, vigorously stirred, was slowly heated to 100° (1 hour), and after 3 hours at 100° was heated to boiling for a further 1 hour. The filtered solution was extracted with hydrochloric acid (5%), and from the aqueous extract, by shaking with sodium hydroxide and ether, the *acetyl* derivative of (III; R = Me, *n* = 2) was obtained as a yellow-red oil (4·1 g., 49·4%), b. p. 170—180°/0·33 mm. (Found: C, 61·2; H, 8·0; N, 14·7. $C_{15}H_{23}O_3N_3$ requires C, 61·4; H, 7·9; N, 14·3%), characterised by its *picrate* which separated from aqueous ethanol in yellow prisms, m. p. 119° (Found: C, 48·1; H, 4·9. $C_{15}H_{23}O_3N_3$,$C_6H_3O_7N_3$ requires C, 48·2; H, 5·0%). Hydrolysis of the acetyl compound (4·1 g.) with concentrated hydrochloric acid (25 c.c.) for 4—5 hours on a steam-bath gave 3-nitro-*p*-*N*-(β-diethylaminoethyl)toluidine, b. p. 150—160°/0·4 mm., which was identified by its picrate, m. p. and mixed m. p. 176°.

6-*Methyl*-9-*β-diethylaminoethyl*iso*alloxazine Hydrochloride*, (II; R = Me, *n* = 2).—The nitroamine (III; R = Me, *n* = 2) (2·66 g.) was hydrogenated in methanol (30 c.c.) over Raney nickel at room temperature and 2 atm. The colourless solution was filtered into concentrated hydrochloric acid (3·0 c.c., 3 mols.), a hot saturated aqueous solution of alloxan hydrate (1·87 g., 1·1 mol.) added, and the mixture gently heated under reflux for 45 minutes. The dark fluorescent solution was evaporated under diminished pressure, and the residue dissolved in boiling ethanol (10 c.c.). Acetone was added, crystallisation initiated by seeding, and next day the product (2·32 g., 62%), m. p. 220°, was collected. Repeated crystallisation from aqueous ethanol gave the *iso*alloxazine *hydrochloride trihydrate* as minute yellow plates, m. p. 259° (decomp.) (Found: C, 48·7; H, 6·6; N, 17·2; Cl, 8·5. $C_{17}H_{21}O_2N_5$,HCl,$3H_2O$ requires C, 48·9; H, 6·7; N, 16·8; Cl, 8·5%. Found after drying in a high vacuum at 120°: C, 55·4; H, 6·1; N, 19·0; Cl, 9·8. $C_{17}H_{21}O_2N_5$,HCl requires C, 56·1; H, 6·1; N, 19·3; Cl, 9·8%). The *picrate*—yellow-orange prisms from aqueous ethanol—had m. p. 225—227° (decomp.) (Found: C, 47·9; H, 4·6; N, 19·3. $C_{17}H_{21}O_2N_5$,$C_6H_3O_7N_3$,H_2O requires C, 48·1; H, 4·5; N, 19·5%).

When the nitroamine (III; R = Me, *n* = 2) (2·37 g.) dissolved in acetic acid (30 c.c.) was hydrogenated at room temperature and 2 atm. over palladised charcoal, and the colourless filtrate added to hot acetic acid (60 c.c.) containing alloxan hydrate (1·9 g.) and boric acid (2 g.), condensation occurred rapidly. After 30 minutes at 30°, the solution was evaporated to dryness under reduced pressure, and the residue triturated at 5° with water (10 c.c.) and concentrated hydrochloric acid (1 c.c., *ca.* 1·05 mol.). The product obtained on filtration from boric acid and evaporation to dryness was dissolved in alcohol, and the filtered solution seeded and left till next day. Recrystallisation of the yellow precipitate (1·07 g., 31·2%), m. p. *ca.* 246° (decomp.), from alcohol–water gave the pure *iso*alloxazine hydrochloride as yellow plates, m. p. 259° (decomp.) (Found after drying at 120° in a vacuum: C, 55·6; H, 6·4; Cl, 9·6%).

3-*Nitro*-p-N-(*γ-diethylamino*-n-*propyl*)*toluidine* (III; R = Me, *n* = 3).—From the product obtained by heating γ-diethylamino-*n*-propylamine (5 g.), 4-chloro-3-nitrotoluene (8 g.), and anhydrous sodium acetate (10 g.) at 100° rising to 140° during 5 hours, unchanged chloronitrotoluene was removed by shaking with dilute hydrochloric acid and ether. From the aqueous solution 3-*nitro*-p-N-(*γ-diethylamino*-n-*propyl*)*toluidine* was precipitated by alkali as a thick orange-red oil, b. p. 145—155°/0·1 mm. (Found: C, 63·3; H, 8·8. $C_{14}H_{23}O_2N_3$ requires C, 63·4; H, 8·7%), characterised by its *picrate* which crystallised as yellow-red plates, m. p. 155·5°, from aqueous ethanol (Found: C, 48·4; H, 5·0; N, 16·4. $C_{14}H_{23}O_2N_3$,$C_6H_3O_7N_3$ requires C, 48·6; H, 5·3; N, 17·0%).

6-*Methyl*-9-*γ-diethylamino*-n-*propyl*iso*alloxazine Hydrochloride* (II; R = Me, *n* = 3).—A methanol solution (40 c.c.) of the amine (III; R = Me, *n* = 3) (4 g.) hydrogenated over Raney nickel at room temperature and 2 atm. was rapidly filtered into concentrated hydrochloric acid (8 c.c.) and mixed with a hot saturated aqueous solution of alloxan hydrate (2·6 g.). After several minutes on a steam-bath, the solution was concentrated under reduced pressure and the *iso*alloxazine *hydrochloride* precipitated with acetone as a dark brown powder (3·07 g., 48·8%), m. p. 270° (decomp.). Repeated crystallisation from aqueous ethanol gave the dihydrate as yellow-red plates, m. p. 286° (decomp.) (Found: C, 52·6; H, 6·7; N, 17·0; Cl, 9·1. $C_{18}H_{23}O_2N_5$,HCl,$2H_2O$ requires C, 52·3; H, 6·8; N, 16·9; Cl, 8·6%. Found on drying in a vacuum at 100°: loss 3·3; C, 54·4; H, 6·7; N, 17·1. $C_{18}H_{23}O_2N_5$,HCl,H_2O requires loss 4·4; C, 54·6; H, 6·6; N, 17·7%. Found on drying in a vacuum at 120°: loss 7·4; C, 56·9; H, 6·6. $C_{18}H_{23}O_2N_5$,HCl requires loss 8·7; C, 57·2; H, 6·4%). The *picrate* separated from aqueous ethanol, in red prisms, m. p. 258—260° (decomp.) (Found: C, 50·5; H, 4·6; N, 19·1. $C_{18}H_{23}O_2N_5$,$C_6H_3O_7N_3$ requires C, 50·5; H, 4·6; N, 19·6%).

4-*Chloro*-2-*nitro*-N-(*β-diethylaminoethyl*)*aniline* (III; R = Cl, *n* = 2).—4-Chloro-2-nitroacetanilide (8·75 g., 1 mol.), β-chloroethyldiethylamine (5·95 g., 1·1 mol.), and sodamide (1·72 g.) were condensed in toluene solution (100 c.c.) as already described for the compound (III; R = Me, *n* = 2). The resulting 4-*chloro*-2-*nitroacet*-N-(*β-diethylaminoethyl*)-*anilide* (8·2 g., 67·5%) was a yellow oil, b. p. 170°/0·35 mm. (Found: C, 53·9; H, 6·3; N, 12·8; Cl, 11·5. $C_{14}H_{20}O_3N_3Cl$ requires C, 53·6; H, 6·4; N, 13·4; Cl, 11·3%), and was characterised as a *picrate* crystallising from aqueous ethanol in yellow prisms, m. p. 144° (Found: C, 44·1; H, 4·2; Cl, 6·9. $C_{14}H_{20}O_3N_3Cl$,$C_6H_3O_7N_3$ requires C, 44·2; H, 4·2; Cl, 6·5%).

(i) The above acetylated amine (7·1 g.) was heated on a steam-bath for 4—5 hours with concentrated hydrochloric acid (25 c.c.), and the deep red solution basified and extracted with ether. Distillation gave 4-*chloro*-2-*nitro*-N-(β-*diethylaminoethyl*)*aniline* (5·45 g., 88·6%), b. p. 158—163°/0·4 mm., which afterwards crystallised in dark red prisms, m. p. 53—54° (Found: C, 53·4; H, 6·6; N, 14·9; Cl, 13·4. $C_{12}H_{18}O_2N_3Cl$ requires C, 53·1; H, 6·6; N, 15·5; Cl, 13·1%). The *picrate* crystallised from aqueous ethanol in minute yellow plates, m. p. 204° (Found: C, 43·2; H, 4·4; N, 16·9; Cl, 7·2. $C_{12}H_{18}O_2N_3Cl,C_6H_3O_7N_3$ requires C, 43·2; H, 4·2; N, 16·8; Cl, 7·1%). The *picrolonate*, orange-yellow plates from aqueous ethanol, had m. p. 205° (decomp.) (Found: C, 49·4; H, 4·7; Cl, 6·6. $C_{12}H_{18}O_2N_3Cl,C_{10}H_8O_5N_4$ requires C, 49·2; H, 5·0; Cl, 6·6%).

(ii) A mixture of 2 : 5-dichloronitrobenzene (8·6 g.), β-diethylaminoethylamine (3·06 g.), and anhydrous sodium acetate (7 g.), heated at 100—135° for 5 hours, on working up gave the amine (III; R = Cl, *n* = 2) (3·63 g., 50·4%), m. p. 52° undepressed on mixing with a specimen prepared by method (i).

6-*Chloro*-9-β-*diethylaminoethyl*iso*alloxazine Hydrochloride* (II; R = Cl, *n* = 2).—The alkylated chloronitroaniline (4·08 g.) was reduced in methanol (40 c.c.) over Raney nickel at room temperature and 2 atm., and the resulting colourless solution filtered and heated on a steam-bath with concentrated hydrochloric acid (4·6 c.c., 3 mols.) and saturated aqueous alloxan (2·66 g.) for 30 minutes. The sticky product obtained on evaporation, dissolved in alcohol (15 c.c.) and precipitated with dry acetone, gave on washing with ethanol a yellow-green solid (2·46 g., 42·5%), m. p. 243° (decomp.). By repeated crystallisation from ethanol–water, the *iso*alloxazine *hydrochloride* was obtained as a *monohydrate* in scintillating yellow-brown needles, m. p. 252—253° (decomp.) (Found: C, 47·9; H, 5·5; N, 16·9; Cl, 18·1. $C_{16}H_{18}O_2N_5Cl,HCl,H_2O$ requires C, 47·7; H, 5·2; N, 17·4; Cl, 17·6%. Found on a specimen vacuum dried at 120°: C, 49·9; H, 4·9; N, 18·0; Cl, 18·9. $C_6H_{18}O_2N_5Cl,HCl$ requires C, 50·0; H, 4·9; N, 18·2; Cl, 18·5%). The *picrate* was obtained as yellow prisms, m. p. 241—243° (decomp.), from ethanol–water (Found: C, 45·7; H, 3·7; Cl, 6·6. $C_{16}H_{18}O_2N_5Cl,C_6H_3O_7N_3$ requires C, 45·8; H, 3·6; Cl, 6·6%).

4-*Chloro*-2-*nitro*-N-(γ-*diethylamino*-n-*propyl*)*aniline* (III, R = Cl; *n* = 3).—γ-Diethylamino-*n*-propylamine (3·9 g.), 2 : 5-dichloronitrobenzene (8·6 g.) and anhydrous sodium acetate (7 g.) were heated from 100° to 140° during 3 hours, and the product was worked up in the usual way. The *amine* (III; R = Cl, *n* = 3) (5·7 g.), a thick orange-red oil, b. p. 164—165°/0·33 mm., crystallised in large prisms, m. p. 38° (Found: C, 54·6; H, 7·0. $C_{13}H_{20}O_2N_3Cl$ requires C, 54·6; H, 7·0%), and was characterised by a *picrate* which separated from ethanol–water in orange-red plates, m. p. 168·5° (Found: C, 44·4; H, 4·9; N, 15·8; Cl, 7·1. $C_{13}H_{20}O_2N_3Cl,C_6H_3O_7N_3$ requires C, 44·4; H, 4·5; N, 16·3; Cl, 6·9%).

6-*Chloro*-9-γ-*diethylamino*-n-*propyl*iso*alloxazine Hydrochloride* (II; R = Cl, *n* = 3).—The colourless solution obtained by hydrogenation of the nitroamine (III; R = Cl, *n* = 3) (4 g.) in methanol (40 c.c.) over Raney nickel was heated with concentrated hydrochloric acid (8 c.c.) and alloxan hydrate (2·35 g.) in hot water (4 c.c.) on a steam-bath for 30 minutes. Evaporation left a dark oil which when crystallised from alcohol gave a brown powder (2·8 g., 49·1%), m. p. 282° (decomp.). Recrystallisation from ethanol–water afforded the *iso*alloxazine *hydrochloride monohydrate* as yellow plates, m. p. 285—286° (decomp.) (Found: C, 49·1; H, 5·4; N, 16·7; Cl, 16·9. $C_{17}H_{20}O_2N_5Cl,HCl,H_2O$ requires C, 49·0; H, 5·5; N, 16·8; Cl, 17·1%. Found after drying in a vacuum at 100°: loss, 2·1; C, 50·0; H, 5·4; N, 17·3; Cl, 18·0. $C_{17}H_{20}O_2N_5Cl,HCl,\frac{1}{2}H_2O$ requires loss 2·2; C, 50·1; H, 5·4; N, 17·2; Cl, 17·4%). The *picrate* crystallised from ethanol–water in light brown prisms, m. p. 263° (decomp.) (Found: C, 46·6; H, 4·1; N, 18·3; Cl, 6·3. $C_{17}H_{20}O_2N_5Cl,C_6H_3O_7N_3$ requires C, 46·7; H, 3·9; N, 19·0; Cl, 6·0%).

3-*Nitro*-4-β-*diethylaminoethylaminoanisole* (III; R = OMe, *n* = 2) (cf. King, Beer, and Waley, this vol., p. 92).—3-Nitro-4-acetamidoanisole (10·5 g.) and β-chloroethyldiethylamine (7·5 g., 1·1 mol.) were condensed in the usual way by means of sodamide (2·2 g., 1·1 mol.) in toluene to give a brown oil (13·5 g.), identified as 3-nitro-4-acet-(β-diethylaminoethyl)-amidoanisole by its *picrate*, which crystallised from ethanol in yellow rhombic tablets, m. p. 156—157° (Found: C, 47·1; H, 4·6. $C_{15}H_{23}O_4N_3,C_6H_3O_7N_3$ requires C, 46·8; H, 4·8%). Hydrolysis of the acetamido compound with boiling concentrated hydrochloric acid for 1 hour, followed by evaporation at 60° and the addition of alkali, gave 3-nitro-4-β-diethylaminoethylaminoanisole, shown by its picrate, m. p. 181°, to be identical with the base already obtained through the *p*-toluenesulphonamide (*loc. cit.*).

6-*Methoxy*-9-β-*diethylaminoethyl*iso*alloxazine Hydrochloride* (II; R = OMe, *n* = 2).—The alkylated nitroanisidine (8·2 g.) dissolved in ethanol (50 c.c.) and acidified with concentrated hydrochloric acid (9 c.c., 3 mol.) was reduced at 2—3 atm. in presence of palladised charcoal. The colourless solution filtered from catalyst, together with alcohol washings (40 c.c.), was added to a solution of alloxan hydrate (4·8 g.) in hot water (5 c.c.) and the mixture heated on a steam-bath for 2—3 minutes. The orange-brown crystalline solid (6·5—7 g.), m. p. 235—238° (decomp.), which separated on cooling was collected and washed with ethanol. It was best crystallised from a little water, though with very considerable loss; dilution with alcohol, in which the *iso*alloxazine is very sparingly soluble, did not produce any rapid further precipitation, and lengthy contact with the solution appeared to result in some decomposition. The recrystallised salt, minute orange needles, m. p. 247° (decomp.), was evidently a hydrated monohydrochloride (Found: Cl, 7·7%); it was characterised as a *picrate* which crystallised from water in feathery red plates, m. p. 182° (decomp., rapid heating) (Found: C, 44·4; H, 4·8; N, 17·7. $C_{17}H_{21}O_3N_5,C_6H_3O_7N_3,3H_2O$ requires C, 44·1; H, 4·8; N, 17·9%. Found after drying at 100°: loss, 3·0; C, 44·9; H, 4·8; N, 18·1. $C_{17}H_{21}O_3N_5,C_6H_3O_7N_3,2H_2O$ requires loss, 3·0; C, 45·4; H, 4·6; N, 18·4%).

3-*Nitro*-4-γ-*diethylamino*-n-*propylaminoanisole* (III; R = OMe, *n* = 3).—A solution of sodium (5·7 g.) in alcohol (400 c.c.) containing 3-nitro-4-*p*-toluenesulphonamidoanisole (36 g.) and γ-chloro-*n*-propyldiethylamine hydrochloride (25 g.) was heated under reflux for 36 hours. After removal of sodium chloride and alcohol, the unreacted sulphonamide was extracted from a solution of the product in ether by aqueous sodium hydroxide, and the dried ethereal solution evaporated. On being kept, the pale orange oil solidified to crystalline 3-nitro-4-*p*-toluenesulphon-(γ-diethylamino-*n*-propyl)amidoanisole, which was freely soluble in the ordinary solvents except light petroleum, and had m. p. 75—76°. The base was identified by its *picrate* which crystallised from ethanol containing acetone in bright yellow needles, m. p. 118° (Found: C, 49·2; H, 5·0; N, 11·8. $C_{21}H_{29}O_5N_3S,C_6H_3O_7N_3$ requires C, 48·8; H, 4·8; N, 12·6%).

The above sulphonamide (45 g.) was mixed at 0° with sulphuric acid (300 g., 90%) and occasionally shaken until dissolved (14 hours). After 24 hours, the solution was added to ice and aqueous ammonia, the red syrupy precipitate being finally basified with sodium hydroxide. Ether extraction isolated the nitroamine as a deep red oil (29·5 g.) characterised by a *picrate*, crystallising from alcohol containing acetone in blood-red fine prisms, m. p. 113° (Found: C, 47·4; H, 5·3. $C_{14}H_{23}O_3N_3,C_6H_3O_7N_3$ requires C, 47·1; H, 5·1%).

6-*Methoxy*-9-γ-*diethylamino*-n-*propyl*iso*alloxazine Hydrochloride*, (II; R = OMe, *n* = 3).—The foregoing nitroamine (8·4 g.) was hydrogenated over palladised charcoal and condensed with alloxan in the presence of hydrochloric acid, as described for the preparation of (II; R = OMe, *n* = 2). The yield of dried product, m. p. 298—299° (decomp.), was 3·1 g., and a further 1·2 g. was precipitated on diluting the reaction solvent with ethanol. When alcohol was added to a solution of the salt in a little water, the hydrated hydrochloride (Found: Cl, 7·3%) slowly separated in clusters of orange needles, m. p. 300° (decomp.), from which was prepared the *picrate*, red tablets from ethanol–water, m. p. 261° (decomp. rapid heating) (Found after drying at 100°: C, 46·0; H, 4·4; N, 17·6. $C_{18}H_{23}O_3N_5,C_6H_3O_7N_3,2H_2O$ requires C, 46·3; H, 4·8; N, 18·0%).

2 : 4-*Dinitro-γ-diethylamino*-n-*propylaniline*.—The mixing of chloro-2 : 4-dinitrobenzene (2 g., 1 mol.) and γ-diethylamino-*n*-propylamine (1·3 g., 1 mol.) was accompanied by considerable evolution of heat, and the light brown resinous product crystallised on dissolving in alcohol. 2 : 4-*Dinitro-γ-diethylamino*-n-*propylaniline hydrochloride* separated in chrome-yellow prisms, m. p. 190—191° (Found : N, 16·5. $C_{13}H_{20}O_4N_4$,HCl requires N, 16·7%). The hydrochloride readily dissolved in water, and on addition of alkali the solid *amine* separated. Recrystallisation from a small volume of methanol gave deep yellow plates, m. p. 87—88°, which turned orange on long exposure to light (Found : C, 52·4; H, 6·7; N, 18·6. $C_{13}H_{20}O_4N_4$ requires C, 52·7; H, 6·7; N, 18·9%). When this was reduced in ethanol and combined with alloxan in presence of hydrochloric acid (3 mol.), a nearly black deliquescent amorphous solid was obtained which gave maroon-coloured solutions devoid of fluorescence, even in ultra-violet light.

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