171. *Syntheses of 9-Substituted Flavins as Antimalarials.*

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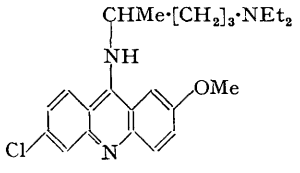
Mepacrine (atebrin) antagonizes the prosthetic group of cytochrome reductase, *viz.*, riboflavin phosphate. With a view of enhancing the action of the drug, compounds (II) and (III) have been synthesized in which the *iso*alloxazine nucleus has been substituted for the acridine nucleus in mepacrine, with retention of the known pharmacodynamic groups of this drug.

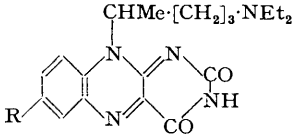
The antimalarial activity of mepacrine depends on inhibition of the respiration of the parasite (Christophers, *Trans. Faraday Soc.*, **1943**, **265**, 333), and seems mainly due to the irreversible combination of the drug with the protein moiety of cytochrome reductase (Haas, *J. Biol. Chem.*, **1944**, **155**, 321). Addition of the prosthetic

group to the enzyme before addition of the drug, however, antagonized the action of mepacrine successfully by suppression of the dissociation of the enzyme. One molecule of riboflavin phosphate " neutralised " about 500 molecules of mepacrine. It appears, therefore, that mepacrine is still rather remote from the structure of the " ideal antagonist " of riboflavin (IV).

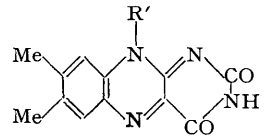
We considered it promising to change the structure of mepacrine (I) by substitution of the *iso*alloxazine for the acridine nucleus, with retention of those parts of mepacrine known for their pharmacodynamic properties.



(I.)



(II; R = H.) (III; R = Cl.)



(IV; R′ = Ribityl.)

While lack of halogen in mepacrine appears dystherapeutic (cf. Williams, "Chemotherapy of Malaria," Lederle Lab. Inc., 1941, p. 218), both condensed benzene rings of the acridine nucleus are by no means indispensable for antimalarial activity (Gilman and Spatz, *J. Amer. Chem. Soc.*, 1944, **66**, 622; Galperin, *Med. Parasit. and Parasit. Dis.*, 1940, **9**, 44; Magidson and Rubtsov, *J. Gen. Chem. Russia*, 1937, **7**, 1896). Although it is impossible to predict *a priori* the biological activity of a new compound, the flavins (II) and (III) seemed a promising approach.

As far as we are aware only one flavin with a basic side chain is known, which has been prepared by Karrer and Naef (*Helv. Chim. Acta*, 1936, **19**, 1029), by condensation of an *N*-(ω-phthalimidoalkyl)phenylenediamine with alloxan. Our flavins were synthesized by condensation of *N*-(ω-diethylaminoalkyl)phenylenediamines with alloxan by the method of Kuhn and Weygand (*Ber.*, 1935, **68**, 1282).

The purification of the flavins was laborious as they could not be induced to crystallize, and numerous attempts to prepare complex salts and derivatives failed to give well-defined products. The isolation of these flavins succeeded only after finding that they gave slightly soluble Reineckates which were easily obtained in the pure state.

While biological tests on the substances described were under progress, a paper describing unsuccessful attempts to prepare flavins substituted at 9 with dialkylaminoalkyl groups came to our attention (Hall and Turner, *J.*, 1945, 699).

Results of biological tests will be published elsewhere.

Experimental.

2-Amino-5-diethylaminopentane was prepared by catalytic reduction of methyl 3-diethylaminopropyl ketone in alcoholic ammonia in presence of Raney nickel. The aminoketone (92 g., 0·59 mol.) was dissolved in 15% alcoholic ammonia (1200 c.c.). Raney nickel (30 g.) was added and the reduction carried out at 40° and at atmospheric pressure. When absorption of hydrogen was complete, the catalyst was filtered off. The alcohol was removed and the product distilled in a vacuum, b. p. 92°/30 mm. Yield, 75 g. (82%).

o-*Nitro*-N-(δ-*diethylamino-α-methylbutyl*)*aniline*.—A solution of *o*-dinitrobenzene (25 g., 0·15 mol.) and 2-amino-5-diethylaminopentane (26 g., 0·16 mol.) in *p*-cymene (400 c.c.) was heated under reflux for 3 hours. After cooling the product was extracted with dilute hydrochloric acid. The acidic liquid was extracted with ether to remove non-basic substances. After cooling strongly excess of 50% aqueous sodium hydroxide was added and the product was extracted by ether. The solvent was removed and the *product* was distilled in a vacuum, b. p. 165°/0·8 mm. A dark red viscous oil was obtained, $n_D^{27°}$ 1·5725. Yield, 25 g. (60%) (Found: C, 64·0; H, 9·3. $C_{15}H_{25}O_2N_3$ requires C, 64·3; H, 9·0%).

N-(δ-*Diethylamino-α-methylbutyl*)-*o*-*phenylenediamine*.—*o*-Nitro-*N*-(δ-diethylamino-α-methylbutyl)aniline (20 g., 0·07 mol.) was dissolved in alcohol (150 c.c.). Palladium catalyst (2% Pd on calcium carbonate, 0·5 g.) was added. The nitro-compound was reduced with slow addition of hydrogen at atmospheric pressure, as the reaction was exothermic. When absorption of hydrogen was complete the catalyst was filtered off, the alcohol was removed, and the product was distilled in a vacuum. The crude product darkened easily, but the pure *product* was quite stable. A colourless oil was obtained, b. p. 144°/0·2 mm., $n_D^{27°}$ 1·5349. Yield, 16 g. (90%) (Found: C, 71·8; H, 11·1. $C_{15}H_{27}N_3$ requires C, 72·3; H, 10·8%).

9-(δ-*Diethylamino-α-methylbutyl*)iso*alloxazine* (II).—*N*-(δ-Diethylamino-α-methylbutyl)-*o*-phenylenediamine (10 g., 0·04 mol.) was dissolved in glacial acetic acid (20 c.c.). Alloxan monohydrate (6·4 g., 0·04 mol.) and boric acid (3 g., 0·05 mol.) in glacial acetic acid (60 c.c.) were heated to boiling, and then cooled to 40°. The solution of the diamine was then added to the solution of the boric acid. A strong green fluorescence immediately appeared. The reaction mixture was kept for two days at 37°. The acetic acid was then distilled off under reduced pressure, and the residue was taken up in water. The solution was steam distilled. The residue was concentrated under reduced pressure to 35 c.c. A part of this solution was neutralised with hydrochloric acid and the flavin was precipitated with a concentrated aqueous solution of Reinecke salt. The yellow precipitate was filtered off and washed with a small amount of water. The *compound* was soluble in acetone. It darkened at 120° and decomposed at about 190°. It was dried in a vacuum over phosphorus pentoxide at 40° to constant weight (Found: C, 38·9; H, 5·2; Cr_2O_3, 11·3. $C_{19}H_{25}O_2N_5[Cr(NH_3)_2(CNS)_4]H,2H_2O$ requires C, 38·9; H, 5·1; Cr_2O_3, 10·7%). Another part of the original solution of the flavin was evaporated to dryness under reduced pressure and extracted with absolute ethanol. The ethanol was removed under reduced pressure and the flavin was taken up in water giving a solution which showed strong green fluorescence. The flavin was adsorbed on frankonite (active charcoal was also effective, while fuller's earth gave poor adsorption). The flavin was eluted with acetic acid–pyridine. Adsorption was very strong and large amounts of eluent were required. The eluent was removed under reduced pressure, and the flavin was taken up in water and precipitated as the Reineckate by the method given above. The same product as from the original solution of the flavin was obtained.

4-*Chloro*-2-*nitro*-N-(δ-*diethylamino-α-methylbutyl*)*aniline*.—2 : 5-Dichloronitrobenzene (19 g., 0·1 mol.) was refluxed

with 2-amino-5-diethylaminopentane (80 g., 0·5 mol.) for 16 hours. Excess of diamine was distilled off under reduced pressure. The residue was taken up in dilute hydrochloric acid. The acidic liquid was extracted with ether. The aqueous layer was cooled, and excess of 50% aqueous sodium hydroxide was added to it. The product was extracted with ether. The solvent was removed and the product was fractionated in a vacuum to give a viscous red *oil*, b. p. 187°/0·5 mm., 177°/0·02 mm., $n_D^{27°}$ 1·5750. Yield, 28 g. (93%) (Found : C, 58·2; H, 7·8. $C_{15}H_{24}O_2N_3Cl$ requires C, 57·4; H, 7·7%).

Attempted catalytic reduction of the above nitro-compound in presence of palladium on calcium carbonate gave a chlorine-free oil which was shown by analysis to be the de-halogenated product N-(δ-*diethylamino-α-methylbutyl*)-o-*phenylenediamine* (Found : C, 73·0; H, 10·7. $C_{15}H_{27}N_3$ requires C, 72·3; H, 10·8%).

4-*Chloro*-N-(δ-*diethylamino-α-methylbutyl*)-1 : 2-*phenylenediamine*.—4-Chloro-2-nitro-*N*-(δ-diethylamino-α-methylbutyl)aniline (14 g., 0·045 mol.) was dissolved in concentrated hydrochloric acid (25 c.c.) at 5°. Tin foil (23 g.) was dissolved in concentrated hydrochloric acid (125 c.c.). This solution was filtered and warmed to 40°. To this the solution of the nitroaniline was added in small portions with stirring. The reaction mixture quickly turned pale yellow. It was heated to 50° for 2 hours. After cooling it was poured into 30% aqueous sodium hydroxide (150 c.c.), the temperature being kept below 30°. A yellow viscous oil floated on top of the alkaline liquid. The product was extracted by ether, the solvent was removed, and the *product* distilled in a vacuum, b. p. 160°/0·05 mm., $n_D^{28°}$ 1·5450. Yield, 11 g. (88%) (Found : C, 64·2; H, 9·3. $C_{15}H_{26}N_3Cl$ requires C, 63·5; H, 9·2%).

6-*Chloro*-9-(δ-*diethylamino-α-methylbutyl*)iso*alloxazine* (III).—This flavin was prepared by the same method as (II). 4-Chloro-*N*-(δ-diethylamino-α-methylbutyl)-1 : 2-phenylenediamine (21·5 g., 0·076 mol.), alloxan monohydrate (12·15 g., 0·076 mol.), boric acid (4·7 g., 0·76 mol.), and glacial acetic acid (115 c.c.) were used. The reaction mixture was worked up as before and a brownish *Reineckate* was obtained. It decomposed slowly between 150° and 200°, the temperature of decomposition depending on the rate of heating (Found : C, 36·7; H, 4·4; Cr_2O_3, 10·9. $C_{19}H_{24}O_2N_5Cl[Cr(NH_3)_2(CNS)_4]H,2H_2O$ requires C, 37·1; H, 4·7; Cr_2O_3, 10·2%).

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