305. *Aminophenoxazines as Possible Antitubercular Agents. Part I. A New Method for the Synthesis of 3-Aminophenoxazine.*

By B. Boothroyd and Edward R. Clark.

Desulphonation of 3-nitrophenoxazine-1-sulphonic acid (IV; R = SO_3H) and decarboxylation of 3-nitrophenoxazine-1-carboxylic acid (IV; R = CO_2H) have been unsuccessfully attempted as possible routes to 3-aminophenoxazine (I; R = H). Partial reduction of 1 : 3-dinitrophenoxazine (IV; R = NO_2) with sodium hydrogen sulphide yields a complex phenolic substance.

10-Benzyl-3-nitrophenoxazine (V; R = CH_2Ph, R′ = H) and its 8-chloro-analogue (R′ = Cl) have been synthesized and reduced catalytically. The former yields the corresponding amine and finally 3-aminophenoxazine. Partial removal of the chlorine atom accompanies the debenzylation of the 8-chloro-analogue.

The report by Clapp, Clark, English, Fellows, Grotz, and Shepherd (*J. Amer. Chem. Soc.*, 1952, **74**, 1989) on the possible use of phenoxazine derivatives as antitubercular agents prompts the authors to record similar investigations conducted during the last 3 years.

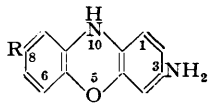
The earliest record of the antitubercular activity of phenoxazine derivatives is de Witt's finding (*J. Inf. Dis.*, 1913, **13**, 378) that New Methylene Blue G.G. stained the tubercle bacillus *in vitro* and penetrated the tubercle *in vivo*. de Witt did not, however, follow this up and phenoxazines, as antitubercular agents, were apparently neglected until the much more recent publications by Okomoto (*Japan. Med. J.*, 1948, **1**, 422; *Chem. Abs.*, 1951, **45**, 5757), Crossley *et al.* (*J. Amer. Chem. Soc.*, 1952, **74**, 573, 578, 584) and Clapp *et al.* (*loc. cit.*).

A number of analogous ring structures and other substances bearing a superficial resemblance to phenoxazine have however been investigated. Thus Freedlander (*Proc. Soc. Exp. Biol. N.Y.*, 1944, **57**, 106) suggested that the antitubercular activity of phenothiazine was due to its oxidation to the 3-quinone. The presence of the quinonoid structure could also be the basis of activity in certain phenazines, *e.g.*, anilinoaposafranine (Barry, Belton, Conalty, and Twomey, *Nature*, 1948, **162**, 622), induline (Kudryavtsev, *Compt. rend. Acad. Sci. U.R.S.S.*, 1941, **33**, 292; *Chem. Abs.*, 1944, **38**, 5878), and 2 : 3-diaminophenazine (Erlenmeyer, Noll, and Sorkin, *Helv. Chim. Acta*, 1949, **32**, 605).

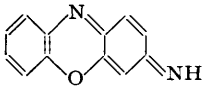
The occurrence of antitubercular activity in these compounds and in various amino-substituted diphenyl ethers (Barry, O'Rourke, and Twomey, *Nature*, 1947, **160**, 800) encouraged the belief that simple aminophenoxazines, capable of oxidation to *o*- or *p*-quinones, would also be active. Further, if the ability to penetrate the tubercle, *in vivo*,

was inherent in the phenoxazine structure then the envisaged substances would be able to concentrate at the site of infection in the animal.

We have therefore attempted to synthesize 3-amino- (I; R = H) and 3-amino-8-chlorophenoxazine (I; R = Cl) for biological testing. [Barry, O'Rourke, and Twomey (*loc. cit.*) found that substitution by chlorine greatly increased the antitubercular activity of 4-aminodiphenyl ethers.]



(I)



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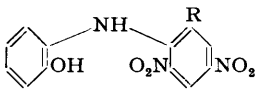


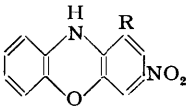
(III)

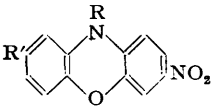
The chloroplatinate of phenoxazim (II) has been prepared (Kehrmann and Grely, *Ber.*, 1909, **42**, 348) from 3 : 7-dinitrophenoxazine, obtained by the direct nitration of phenoxazine. Neither the base nor its salts were apparently obtained pure. This method is not suitable for the synthesis of 3-amino-8-chlorophenoxazine.

The production of 3-nitrophenoxazine (IV; R = H) by heating 2'-hydroxy-2 : 4-dinitrodiphenylamine (III; R = H) with anhydrous sodium acetate in glycerol has been claimed by Kehrmann and Ramm (*Ber.*, 1920, **53**, 2265), but refuted by Brady and Wallace (*J.*, 1930, 1218). The dark brown colour and m. p. (>300°) ascribed to 3-nitrophenoxazine by Kehrmann and Ramm are not consistent with the known properties of well-authenticated nitrophenoxazines : other nitrophenoxazines are well-defined red or violet crystalline compounds with sharp melting points around 200° [*e.g.*, (IV; R = Me) forms red needles, m. p. 212—213° (decomp.) (Boothroyd and Clark, following paper)].

Substitution in the 6-position in the diphenylamine is known to facilitate ring closure; the possibility of removing this group, after ring closure, was therefore investigated. The first synthesis attempted was the desulphonation of 3-nitrophenoxazine-1-sulphonic acid (IV; R = SO_3H). However, hydrochloric or sulphuric acid gave unidentified decomposition products. Acid hydrolysis of 3-aminophenoxazine-1-sulphonic acid, prepared by reduction with tin and hydrochloric acid, gave no better result, though the somewhat similar 4 : 4'-diaminodiphenylamine-2-sulphonic acid has been successfully desulphonated (U.S.P. 2,022,889). Decarboxylation of the corresponding carboxylic acid (IV; R = CO_2H), by heating it with (*a*) glycerol at 250°, (*b*) soda-lime at 265°, or (*c*) quinoline and copper powder, was similarly unsuccessful. [2-Chloro-3 : 5-dinitrobenzoic acid, required for the synthesis of (IV; R = CO_2H), was readily obtained from 3 : 5-dinitrosalicylic acid, phosphorus oxychloride, and diethylaniline; this is a more convenient method than the nitration of *o*-chlorobenzoic acid.] Preferential reduction of the 1-nitro-group of the readily accessible (IV; R = NO_2) (Turpin, *J.*, 1891, **59**, 714), followed by diazotization and reductive decomposition, should give the desired 3-nitrophenoxazine. Treatment of (IV; R = NO_2) with sodium hydrogen sulphide solution yielded a green crystalline product (probably phenolic), having a bright metallic lustre. Digestion with dilute sodium hydroxide solution followed by acidification yielded a greenish-brown product, m. p. 201—203° (decomp.), of empirical formula $C_{26}H_{24}O_9N_6$. Two acidic groupings in a molecule of molecular weight *ca.* 290 were shown by potentiometric titration; the absence of amino-groups was indicated by inability to form a diazo-compound. Further work will be necessary to elucidate the structure of this product.

(IV)





(V)

Ring closure of diphenylamines (as III; R = H) is facilitated by substitution of the amino-hydrogen atom; thus, Roberts and Clark (*J.*, 1935, 1312) converted 2'-hydroxy-*N*-methyl-2 : 4-dinitrodiphenylamine into 10-methyl-3-nitrophenoxazine (V; R = Me, R' = H).

10-Benzyl-3-nitrophenoxazine (V; R = CH_2Ph, R' = H) has now been similarly

prepared. Its catalytic reduction yielded 3-aminophenoxazine (I; R = H), reduction of the nitro-group being followed by fission of the *N*-benzyl linkage. In practice, *N*-benzyl-2'-hydroxy-2 : 4-dinitrodiphenylamine, prepared by the condensation of 1-chloro-2 : 4-dinitrobenzene and *o*-benzylaminophenol, was converted into the corresponding phenoxazine without being isolated. The *o*-benzylaminophenol was obtained in excellent yield by the reduction of the corresponding Schiff's base with lithium aluminium hydride; this was in marked contrast to the poor yields obtained by using magnesium and methyl alcohol (18—24% of theory) or by catalytic hydrogenation with Raney nickel as catalyst (20—25% of theory). *N*-Benzyl-5-chloro-2-hydroxyaniline, used to prepare 10-benzyl-8-chloro-3-nitrophenoxazine (V; R = CH_2Ph, R' = Cl), was obtained similarly.

Reduction of 10-benzyl-3-nitrophenoxazine by using platinum oxide, acetic acid, and hydrogen at 5 atmospheres gave an almost theoretical yield of the 10-benzyl-3-amino-compound. Similarly, palladous oxide in alcohol with hydrogen at atmospheric temperature and pressure, did not promote debenzylation, though the colourless solution obtained rapidly became red in air, owing presumably to oxidation of a small amount of 3-aminophenoxazine to phenoxazim. In acetic acid debenzylation could be induced by raising the temperature to 90°, after the reduction of the nitro-group. Even then, however, only 27% of phenoxazim hydrochloride was isolated, while 48% of 3-amino-10-benzylphenoxazine was obtained. Recrystallization of the phenoxazim hydrochloride was not possible; it was identified as the picrate.

3-Aminophenoxazine (I; R = H) was obtained by using palladium chloride–charcoal in *n*-butyl alcohol. Again the nitro-group was reduced at room temperature, and the solution then heated to 90° to induce removal of the benzyl group. Solutions of the free base in dilute hydrochloric acid rapidly darkened in air owing to oxidation to phenoxazim.

The use of acetic acid in place of *n*-butyl alcohol gave good yields of the monoacetyl derivative of 3-aminophenoxazine, identical with that obtained by acetylation of the free base. The acetyl derivative was insoluble in both hot and cold dilute hydrochloric acid, but in air hydrolysis and oxidation gave a solution of phenoxazim hydrochloride.

Hydrogenation of 10-benzyl-8-chloro-3-nitrophenoxazine in acetic acid solution with palladium chloride—charcoal yielded a mixture, and some dechlorination accompanied debenzylation. The mixture was readily oxidized by air, and the constituents could not be separated by fractional crystallization of the amines or of the picrates formed from the oxidized mixture.

In a preliminary examination, *in vitro*, of 3-aminophenoxazine hydrochloride against *M. tuberculosis* (H37Rv), inhibition of growth was observed down to a dilution of 1 in 1×10^7. Experiments *in vivo* are in progress and details will be published elsewhere.

Experimental

Some analyses are by Mrs. Richards.

Effect of Acid on 3-Nitrophenoxazine-1-sulphonic Acid.—When potassium 3-nitrophenoxazine-1-sulphonate (Ullmann and Herre, *Annalen*, 1909, **366**, 112) was heated with 8N-hydrochloric acid for 5 hr. or with 50% sulphuric acid for 7 hr., much decomposition occurred; in the former experiment a small amount of the S-*benzylthiuronium* salt was obtained, identical with an authentic specimen, m. p. 213—214° (Found: C, 50·75; H, 3·7; N, 11·3; S, 13·45. $C_{20}H_{18}O_6N_4S_2$ requires C, 50·6; H, 3·8; N, 11·8; S, 13·5%).

3-Aminophenoxazine-1-sulphonic Acid.—Granulated tin (3 g.) was slowly added to a stirred refluxing suspension of potassium 3-nitrophenoxazine-1-sulphonate (5 g.) in *ca.* 7N-hydrochloric acid (200 c.c.), and stirring was continued until the mixture was colourless. The insoluble product was recrystallized from water (800 c.c.) containing a small amount of sodium hydrosulphite (dithionite), giving grey needles (2·9 g.), which became red in air (Found: C, 51·5; H, 3·2. Calc. for $C_{12}H_{10}O_4N_2S$: C, 51·7; H, 3·6%). With hot hydrochloric acid only a black solid, insoluble in acid and alkali, was obtained.

2-Chloro-3 : 5-dinitrobenzoic Acid.—Diethylaniline (50 c.c.) was slowly added to a solution of 3 : 5-dinitrosalicylic acid (25 g.) in phosphorus oxychloride (200 c.c.), with cooling. The solution, protected from atmospheric moisture, was heated under reflux for 2 hr. After cooling, the mixture was poured on crushed ice (2 kg.), and the crude product dried and extracted with

hot 50% aqueous ethanol (100 c.c.), leaving a small insoluble residue. The extract, on cooling, first deposited an oil and then a crystalline precipitate, which, on recrystallization from 50% aqueous ethanol, yielded 2-chloro-3 : 5-dinitrobenzoic acid (19·8 g.), m. p. 198—199° (Purgotti and Contardi, *Gazzetta*, 1901, **31**, 527, record m. p. 199°).

3-Nitrophenoxazine-1-carboxylic Acid.—Prepared according to Ullmann's method (*Annalen*, 1909, **366**, 90), this formed red needles, m. p. 292—292·5° (decomp.) (Found : C, 57·2; H, 2·8; N, 9·85. Calc. for $C_{13}H_8O_5N_2$: C, 57·35; H, 2·9; N, 10·3%).

Reduction of 1 : 3-*Dinitrophenoxazine by using Sodium Hydrogen Sulphide.*—Methyl alcohol (225 c.c.) was added to a solution of hydrated sodium sulphide (21 g.) and sodium hydrogen carbonate (21 g.) in water (150 c.c.), and after 15 min. the precipitate was filtered off. This solution of sodium hydrogen sulphide was added during 1½ hr. to a stirred, refluxing mixture of 1 : 3-dinitrophenoxazine (8·4 g.) (Turpin, *loc. cit.*) and methyl alcohol (200 c.c.). The dark brown solution was heated under reflux for a further 15 min. and then left overnight in the ice-box. The mass of green needles (6·2 g.) was washed with 50% aqueous methyl alcohol. Digestion of this green product (0·5 g.) for 1 hr. with hot *ca.* 2N-sodium hydroxide (10 c.c.), neutralization (litmus) of the solution with dilute hydrochloric acid, and crystallization of the *product* (0·45 g.) from 50% aqueous ethanol yielded greenish-brown needles, m. p. 201—203° (decomp.) (Found : C, 55·3; H, 4·3; N, 15·0. $C_{26}H_{24}O_9N_6$ requires C, 55·3; H, 4·25; N, 14·9%).

o-*Benzylaminophenol.*—(*a*) Magnesium turnings were added to a solution of *o*-benzylideneaminophenol (15 g.) (Pictet and Ankersmit, *Annalen*, 1891, **266**, 140) in methyl alcohol. The stirred mixture was warmed gently on a steam-bath for about an hr. in all, the reaction being controlled by use of a cold-water bath when necessary. The excess of methyl alcohol was distilled off and the residue decomposed with ice–water (100 c.c.) and acetic acid (60 c.c.). The black sticky product was dissolved in benzene, the aqueous liquor extracted with benzene (3 × 75 c.c.), and the combined benzene solutions were dried (Na_2SO_4).

Three methods were used for working up the resulting benzene solution : (i) Distillation yielded *o-benzylaminophenol*, b. p. 135—155°/0·1 mm., which crystallized from *cyclo*hexane or ligroin (b. p. 60—80°) in pale brown plates (4·2 g.), m. p. 88—89° (Found : C, 78·45; H, 6·3; N, 7·0. $C_{13}H_{13}ON$ requires C, 78·4; H, 6·5; N, 7·0%). (ii) The benzene solution was extracted with dilute hydrochloric acid (3 × 75 c.c.), the extract neutralized with sodium hydrogen carbonate, and the precipitate dried and extracted with ligroin (b. p. 60—80°). Concentration of the ligroin solution gave the required *o*-benzylaminophenol (2·7 g.), m. p. 88—89°. (iii) The precipitate obtained in (ii) was distilled (b. p. 140—155°/0·1 mm.), and the distillate recrystallized from *cyclo*hexane, yielding *o*-benzylaminophenol, m. p. 88—89°.

(*b*) A mixture of *o*-aminophenol (11 g.) and benzaldehyde (12·7 g.) in ethanol (100 c.c.) was reduced at 70° by using Raney nickel (1·2 g.) as catalyst and an initial hydrogen pressure of 70 atm. The uptake of hydrogen was only *ca.* 75% of that calculated. The catalyst was filtered off, the alcohol evaporated, the residue distilled, and the *o*-benzylaminophenol (5·1 g.) recrystallized from ligroin (b. p. 60—80°).

(*c*) Reduction of *o*-benzylideneaminophenol (6·5 g.) under the conditions described in (*b*) yielded only 1·4 g. of the required product.

(*d*) *o*-Benzylideneaminophenol (6·9 g.), in dry ether (125 c.c.), was during 20 min. added to a stirred suspension of lithium aluminium hydride (0·9 g.) in dry ether (75 c.c.) so that gentle refluxing took place. The mixture was then heated on a steam-bath for 1 hr., water added to destroy the excess of lithium aluminium hydride, and the mixture acidified with dilute sulphuric acid. The ethereal layer was extracted further with dilute sulphuric acid, the combined acid extracts were neutralized (sodium hydrogen carbonate), and the precipitate was dried. Extraction (Soxhlet) of this solid with ether, evaporation of the resulting solution, and recrystallization from *cyclo*hexane yielded *o*-benzylaminophenol (6·3 g.), m. p. 89—90° (mixed m. p. with an authentic specimen 88—89°).

N-*Benzylidene-5-chloro-2-hydroxyaniline.*—5-Chloro-2-hydroxyaniline (14·8 g.) (Mottier, *Arch. Sci. Phy. Nat.*, 1934, **16**, 301; *Chem. Abs.*, 1935, **29**, 3322) was mixed with freshly distilled benzaldehyde (10·1 c.c.); an immediate reaction yielded a fawn-coloured solid. Recrystallization from 60% aqueous alcohol yielded long rectangular orange-brown plates of N-*benzylidene-5-chloro-2-hydroxyaniline* (20·5 g.), m. p. 93·5—94° (Found : C, 66·9; H, 4·0; N, 6·1; Cl, 15·4. $C_{13}H_{10}ONCl$ requires C, 67·4; H, 4·3; N, 6·0; Cl, 15·3%).

N-*Benzyl-5-chloro-2-hydroxyaniline.*—*N*-Benzylidene-5-chloro-2-hydroxyaniline (5·7 g.) was reduced by using lithium aluminium hydride (0·58 g.) as described above. Just sufficient dilute sulphuric acid was added to dissolve the thick yellow precipitate which formed on the addition

of water to the reaction mixture. The yellow ethereal layer was separated and evaporated to dryness. Recrystallization of the residue from *cyclo*hexane yielded plates of *N-benzyl-5-chloro-2-hydroxyaniline* (4·9 g.), m. p. 119—120° (Found: C, 67·2; H, 5·1; N, 6·0; Cl, 14·85. $C_{13}H_{12}ONCl$ requires C, 66·8; H, 5·1; N, 6·0; Cl, 15·2%).

10-Benzyl-3-nitrophenoxazine.—A solution of sodium hydroxide (4 g.) in 50% aqueous alcohol (60 c.c.) was slowly added to a stirred, refluxing solution of *o*-benzylaminophenol (8·5 g.) and 1-chloro-2 : 4-dinitrobenzene (9 g.) in alcohol (85 c.c.). The mixture was heated for a further 1 hr. and then left in the ice-box overnight. The bright red crystals were filtered off, and washed with dilute sodium hydroxide solution and with alcohol. Recrystallization from acetone gave red needles of *10-benzyl-3-nitrophenoxazine* (11·3 g.), m. p. 175—175·5° (Found: C, 71·9; H, 4·2; N, 8·6. $C_{19}H_{14}O_3N_2$ requires C, 71·7; H, 4·4; N, 8·8%).

10-Benzyl-8-chloro-3-nitrophenoxazine.—Condensation of *N*-benzyl-4-chloro-2-aminophenol (2·5 g.) and 1-chloro-2 : 4-dinitrobenzene (2·5 g.), by using sodium hydroxide (1·2 g.) as described above, gave *10-benzyl-8-chloro-3-nitrophenoxazine* (2·6 g.), orange-red needles (from benzene), m. p. 226·5—227° (Found: C, 64·9; H, 3·8; N, 8·3; Cl, 10·3. $C_{19}H_{13}O_3N_2Cl$ requires C, 64·7; H, 3·7; N, 8·0; Cl, 10·1%).

Reduction of 10-Benzyl-3-nitrophenoxazine.—(*a*) 10-Benzyl-3-nitrophenoxazine (1·6 g.) in acetic acid (50 c.c.) was reduced, by using platinum oxide (0·1 g.) and hydrogen at atmospheric pressure. Absorption ceased when sufficient hydrogen to reduce the nitro-group had been taken up. After filtration under nitrogen, the acetic acid solution was made alkaline with 40% aqueous sodium hydroxide. Recrystallization of the precipitate from 1 : 1-glacial acetic acid–2% sodium dithionite solution yielded greenish-grey diamond-shaped plates of *3-amino-10-benzylphenoxazine monohydrate*, m. p. 188—189° (1·5 g.) (Found: C, 74·6; H, 5·9; N, 9·5. $C_{19}H_{16}ON_2,H_2O$ requires C, 74·5; H, 5·9; N, 9·15%). Repetition of the experiment at 90° gave a similar result.

(*b*) 10-Benzyl-3-nitrophenoxazine (2·5 g.) in alcohol (75 c.c.) was reduced, at atmospheric pressure and temperature, by using palladous oxide as catalyst. Uptake of hydrogen ceased when 700 c.c. of the required 830 c.c. had been absorbed. The filtered solution was concentrated to 25 c.c. under nitrogen; addition of water (75 c.c.) precipitated a tar which was taken into benzene. This solution was dried (Na_2SO_4) and the amine hydrochlorides precipitated by dry hydrogen chloride. Extraction with water then yielded a red solution of phenoxazim hydrochloride, a very small amount of which could be isolated by neutralization, extraction with benzene, and precipitation with hydrogen chloride. The water-insoluble hydrochloride, on trituration with benzene, yielded a blue powder (1·7 g.), m. p. 213—215° (decomp.).

Treatment with sodium carbonate solution, extraction with benzene, evaporation of the green benzene solution, and recrystallization of the residue from 50% aqueous acetic acid containing a little sodium dithionite, yielded small green-grey crystals, m. p. 188—189°; m. p. of mixture with 3-amino-10-benzylphenoxazine monohydrate, 188—189°.

(*c*) 10-Benzyl-3-nitrophenoxazine (5·6 g.) was reduced in acetic acid (50 c.c.) by using palladous oxide (1 g.). 1790 c.c. of hydrogen were absorbed initially and then, on heating to 90°, a further 220 c.c. (calc. vol. required, 2215 c.c.). Air was drawn through the filtered solution to oxidize the amines and, after addition of an equal volume of water, the acid was neutralized with ammonia (*d* 0·88). Extraction with benzene yielded a greenish-brown solution which was dried and then concentrated to 150 c.c. under reduced pressure. Passage of dry hydrogen chloride precipitated a mixture of amine hydrochlorides, extraction of which, with dilute hydrochloric acid, gave a red solution and left crude 3-amino-10-benzylphenoxazine hydrochloride (2·8 g.).

The red acid solution was made alkaline and extracted with benzene, the benzene solution dried (Na_2SO_4), and the hydrochloride precipitated as before yielding phenoxazim hydrochloride (1·1 g.). Attempts to prepare the phenylurea and the acetyl derivative were unsucessful. It was characterized as *phenoxazim picrate*, which, after recrystallization from *ca.* 0·75% aqueous picric acid solution, had m. p. 186—187° (decomp.) (Found: C, 50·8; H, 2·7; N, 16·3. $C_{18}H_{11}O_8N_5$ requires C, 50·8; H, 2·6; N, 16·5%).

Carrying out the entire reduction at 90° gave decomposition products and smaller amounts of the two amines.

(*d*) 10-Benzyl-3-nitrophenoxazine (2·2 g.) was reduced in *n*-butyl alcohol (50 c.c.) with 10% palladium chloride–charcoal (1 g.) as catalyst. The volume of hydrogen for the reduction of the nitro-group was smoothly absorbed and, at 90°, rapid debenzylation occurred. The cooled solution was filtered under nitrogen, concentrated under reduced pressure (nitrogen) to *ca.* 5 c.c., and then left in the ice-chest. The solid product was recrystallized from a 1 : 3-alcohol–2%

aqueous dithionite solution, yielding pale fawn-coloured plates of 3-*aminophenoxazine* (0·75 g.), m. p. 172—173° (decomp.) (Found: C, 72·9; H, 4·8; N, 14·35. $C_{12}H_{10}ON_2$ requires C, 72·7; H, 5·05; N, 14·1%). The monoacetyl derivative had m. p. and mixed m. p. 171·5—172·5° (decomp.).

(*e*) Replacement of the *n*-butyl alcohol in (*d*) by acetic acid resulted in the production of 3-*acetamidophenoxazine* (1·1 g.), m. p. 171·5—172·5° (decomp.) (Found: C, 70·2; H, 5·25; N, 11·7. $C_{14}H_{12}O_2N_2$ requires C, 70·0; H, 5·0; N, 11·7%).

THE UNIVERSITY, LEEDS, 2. [*Received, December 2nd*, 1952.]