A CHLOROHYDROXY-TRIPHENYLMETHANE DYE

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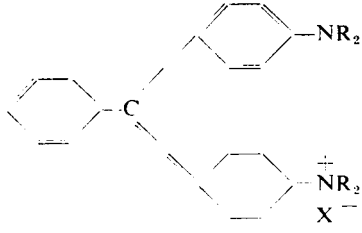
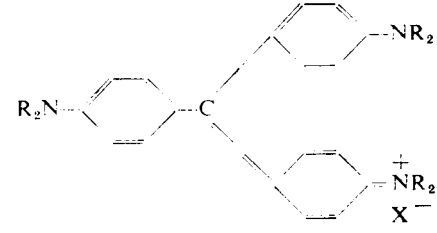
Ehrlich and Bechhold[1] first showed conclusively that the introduction of a halogen atom into the aromatic nucleus produced an increase in the bactericidal activity of phenols. Karpow[2] had already demonstrated that of the three isomeric chlorophenols the para-compound was the most active. These results received further confirmation in later work carried out by Kurodo[3] although Klarmann[4] showed that the activities of the three chlorophenols increased in the order *ortho-*, *para-*, *meta-*. An increase in the molecular weight of both simple phenols and halogenated phenols by the introduction of alkyl, aryl and aryl-alkyl groups into the molecule causes a marked increase in activity. Klarmann, Schternov and Gates[5] tested a series of alkylchlorophenols against 6 different micro-organisms and showed that whereas a peak of activity against *Eberthella typhi* was obtained in those compounds with 5 carbon side chains the peak of activity against *Staphylococcus aureus* and *Streptococcus hæmolyticus* was only attained when the side chain contained 7 or 8 carbon atoms. Furthermore *o*-alkyl-*p*-chlorophenols are more active than *o*-chloro-*p*-alkylphenols. In a corresponding series of benzylhalophenols Klarmann, Gates and Schternov[6] showed that a halogen which is *ortho* to a hydroxyl group increases the phenol coefficient less than when in the *para*-position, though the general level of activity in these diphenylmethane derivatives is lower than that for the corresponding alkylhalophenols. 5-Chloro-2-hydroxydiphenylmethane has approximately half the activity of 2-*n*-amyl-4-chlorophenol toward *Staph. aureus* and *Strep. hæmolyticus*. Contrary to the greater activity against both Gram-negative and Gram-positive organisms of bromophenols over chlorophenols in the simpler series, the monobromo derivatives of 2- and 4- hydroxydiphenylmethanes are less effective against Gram-negative organisms yet more so against Gram-positive types than the corresponding chloro compounds.

Basic dyes of the triphenylmethane series such as malachite green (I, $R=CH_3$, $X=Cl$), brilliant green (I, $R=C_2H_5$, $X=HSO_4$) and crystal violet (II, $R=CH_3$, $X=Cl$) are well established as antibacterial agents, yet little attention has been paid to the possibilities of their chloro- and hydroxy- derivatives as potential bactericides. New solid green 3B,



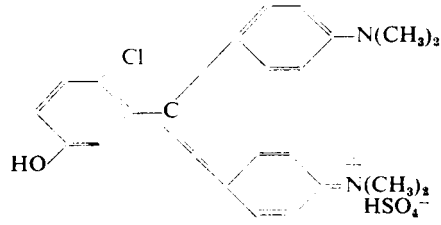
I



II

which is 2-chloro-4′:4″-*bis*dimethylaminotriphenylmethyl chloride was found by Beckwith[7] to be active against typhoid in rabbits. No comparison of the activity of this compound with that of malachite green is recorded. Kligler[8] compared the activity of victoria green, 2:5-dichloro-4′:4″-*bis*dimethylaminotriphenylmethyl chloride with that of malachite green, and showed that the former was slightly more active than the latter. The effect of introducing an hydroxy-group into the molecule of malachite green structure was studied by Fairbrother and Renshaw[9]. 3-Hydroxy-4′:4″-*bis*dimethylaminotriphenylmethyl chloride was found to be too insoluble for testing, while the more soluble patent blue V, monosodium - 5 - hydroxy - 4′:4″-*bis*dimethylaminotriphenylmethyl-2:4-disulphonate possessed no antiseptic properties. Simon and Wood[10] had concluded earlier that all predominantly acidic triphenylmethane dyes are inactive and these findings were confirmed by Fairbrother and Renshaw[9]. But, in view of the presence of two sulphonic acid groups in the molecule of patent blue V, no conclusion can be drawn as to the effect of introducing the hydroxy group into the triphenylmethane molecule.

The compound III, 2-chloro-5-hydroxy-4′:4″-*bis*dimethylaminotriphenylmethyl sulphate, embodies the salient properties of two types of antibacterial substances. One of the aromatic rings introduces the

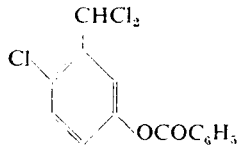


III

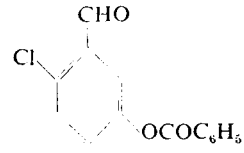
parachlorophenol groupings into the molecule, while an arylalkyl side chain is duplicated in the main triphenylmethane structure. Such a compound might be expected to combine the selective antibacterial action of the triphenylmethane dyes with the more general bacterial toxicity associated with phenolic substances and provide the basis for a new and powerful series of bactericides.

For the synthesis of III, 2-chloro-5-hydroxytoluene was chosen as a convenient starting point on account of its ready availability. The required orientation of chloro- and phenolic groups with respect to the alkyl side chain was thus already established. The oxidation of the alkyl side chain to the aldehyde was accomplished by chlorination to introduce two chlorine atoms, followed by hydrolysis. In the first instance the phenolic group was protected by acetylation and the acetyl derivative chlorinated in bright light at 110°C. to give the theoretical increase in weight for the introduction of two chlorine atoms. On cooling the product solidified and after re-crystallisation was identified as 2:4:6-trichloro-3-acetylcresol, m.pt. 35°C. It seemed possible that side chain chlorination could be achieved with a derivative of higher boiling-point and this expectation was realised with benzoyl-2-chloro-5-hydroxy-

toluene. This compound was chlorinated, in bright light, at an initial temperature of 140°C. which was slowly increased to 180°C. during the course of the reaction. 4-Chloro-3-dichloromethylphenyl benzoate (IV)

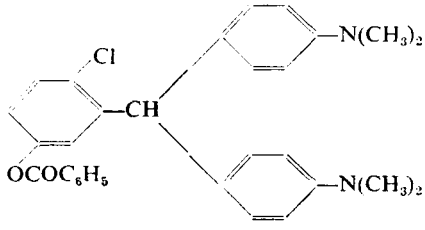


IV

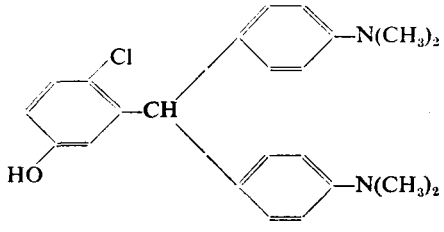


V

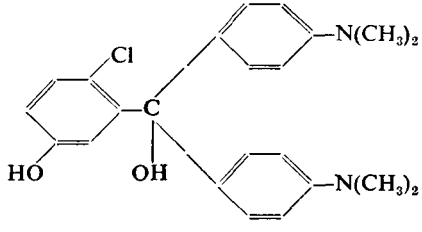
was obtained as a pale yellow, viscous oil after fractional distillation *in vacuo*. This substance was easily converted into the corresponding aldehyde, 2-chloro-5-benzoylhydroxy-benzaldehyde (V) using the method of Hammick[11], boiling with alcoholic silver nitrate solution for 20 minutes. After concentration V crystallises from the filtrate as a white microcrystalline solid which readily forms a 2:4-dinitrophenylhydrazone and a semicarbazone. Its identity was confirmed by titration using hydroxylamine hydrochloride.



VI



VII



VIII

2-Chloro-5-benzoylhydroxy-4′:4″-*bis*dimethylaminotriphenylmethane (VI) was obtained in 70 per cent. yield by condensing the aldehyde (V) with dimethylaniline, using phosphorus oxychloride as the condensing

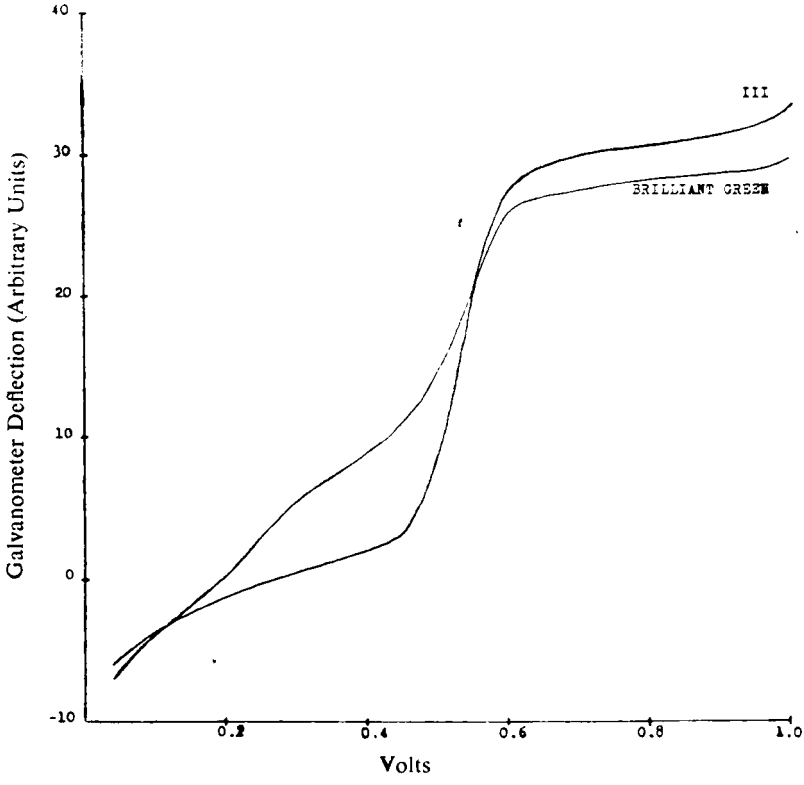
agent and *o*-dichlorobenzene as solvent, according to the conditions of Stryker[12]. The product twice recrystallised from alcohol (95 per cent.) was a white crystalline powder and readily formed a picrate which, on analysis and titration with standard sodium hydroxide solution, was shown to possess the formula $B_1[C_6H_2(NO_2)_3OH]_2$.

Debenzoylation of VI to 2-chloro-5-hydroxy-4′:4″-*bis*dimethylamino-triphenylmethane (VII) was achieved by refluxing with alcoholic solution of potassium hydroxide. The crude leucobase (VII) was separated in 98 per cent. yield in the form of dark green crystals. Purification was effected by chromatographic adsorption from benzene on a column of activated alumina, followed by recrystallisation from alcohol (95 per cent.) to give a white crystalline product in 90 per cent. yield. The picrate was obtained as a yellow crystalline solid and was shown by analysis and by titration against standard solution of sodium hydroxide to have the formula $B_1[C_6H_2(NO_2)_3OH]_2$.

Oxidation of VII to the carbinol base, 2-chloro-5-hydroxy-*bis*dimethylamino-triphenylmethylcarbinol (VIII) was carried out by the method of Minevitch[13] in 70 per cent. acetic acid at 0° to 5°C., using lead peroxide paste. The carbinol base (VIII) was obtained as a dark green scale product in 89 per cent. yield after purification by chromatographic adsorption on activated alumina from chloroform and elution from the column with the same solvent. It has no distinct melting-point and undergoes slow fusion with decomposition when heated for any length of time at temperatures over 100°C. It is insoluble in water, benzene, light petroleum (b.pt. 50° to 60°C.), ether and carbon tetrachloride; soluble in alcohol and very readily soluble in chloroform and acetone. The picrate of VIII was obtained as a dark yellowish green powder and was shown by analysis to possess the formula $B_1[C_6H_2(NO_2)_3OH]_2$.

The conversion of the carbinol base (VIII) to the dyestuff, 2-chloro-5-hydroxy-4′:4″-*bis*dimethylaminotriphenylmethyl sulphate (III) was accomplished by agitating a chloroform solution of VIII with water containing the calculated amount of sulphuric acid, and evaporating to dryness. The identity of the product was established by microanalysis, though direct titration on a semi-micro scale with titanous sulphate solution using the method defined for brilliant green in the British Pharmacopœia failed to give consistent results. Further confirmation of the identity of III was obtained by a study of its polarographic wave in comparison with that of a pure sample of brilliant green. The waves were plotted using a voltamescope on solutions buffered at approximately *p*H2 with hydrochloric acid and 0·1N potassium chloride, the latter substance serving also as the ground electrolyte. Gelatin at a concentration of 0·02 per cent. was used for " maximum " suppression and alcohol to facilitate solution of the dyestuffs. A blank was carried out to demonstrate the absence of interference of the ancillary substances present in the solution. Two typical waves are shown in Figure I. These are followed closely by a large hydrogen wave. The waves of the dyestuff (III) and of brilliant green are shown to be comparable. The half-wave potentials are

0·535V and 0·53V for III and brilliant green respectively, and are measured with respect to the pool mercury anode. The waves are completely cathodic, which may be taken as evidence of completeness of oxidation to the dye form.



The dye III is only sparingly soluble in water, insoluble in ether, benzene and light petroleum, but soluble in acetone, chloroform and alcohol. It is capable of dyeing both silk and wool directly, and cotton in the presence of suitable mordants. The green colours so obtained remain fast on exposure to ultra-violet light for short periods, but are not fast to the action of boiling soap solutions. Preliminary bacteriological tests in aqueous solutions showed that III inhibited the growth of *Streptococcus pyogenes* at a dilution of 1/80,000 and *Staphylococcus aureus* at more than 1/160,000. A 1/1000 solution did not inhibit the growth of *Escherichia coli* and *Pseudomonas pyocyanea.*

Conclusion

The chlorohydroxy-triphenylmethane dye III has been shown to possess a low order of antibacterial activity. The results of Fairbrother and Renshaw indicated that the presence of an acidic group in the molecule of a basic triphenylmethane dye, would decrease the activity. The intro-

duction of the phenolic group into the molecule of the dye III has demonstrated this effect and any enhancement of potency due to the presence of the chloro-group was insufficient to compensate for the reduction in activity due to the phenolic group.

EXPERIMENTAL

3-*Methyl*-4-*chlorophenyl benzoate*.—2-chloro-5-hydroxytoluene (71·2 g.) was dissolved in 5N sodium hydroxide solution (100 ml.). Benzoyl chloride (70·2 g.) was added slowly and the mixture shaken continuously for 10 minutes. A further addition of 5N sodium hydroxide solution (10 ml.) was made and the shaking repeated. The solid product was filtered from the solution, washed first with dilute sodium hydroxide solution, then with water, dried and recrystallised from alcohol (95 per cent.). Yield 110·5 g. (90 per cent.), m.pt. 86°C.

3-*Dichloromethyl*-4-*chlorophenyl benzoate (IV)*.—3-methyl-4-chlorophenyl benzoate (110·5 g.) was heated to 140°C. and a current of dry chlorine was slowly passed into the molten material for 9 hours. During the course of the chlorination the temperature was allowed to rise slowly to 180°C., and the apparatus was exposed to sunlight. The product, a dark brown viscous oil, was distilled *in vacuo*, and the second fraction, b.pt. 203° to 204°C./4 mm. Hg, was collected. Yield 94 g. (67 per cent.). μ. 1·6013. Found: C, 52·90; H, 2·94; Cl, 35·36. $C_{14}H_9O_2Cl_3$ requires C, 53·26; H, 2·88; Cl, 33·76 per cent.

2-*Chloro*-5-*benzoylhydroxybenzaldehyde* (V).—Silver nitrate (56 g.) was dissolved in distilled water (60 ml.), heated to 80°C. and added with continuous stirring to a solution of IV (51 g.) in boiling alcohol (95 per cent.) (250 ml.). The whole was refluxed for 20 minutes with continuous and vigorous stirring and then allowed to cool. Neutralisation was effected by the careful addition of the calculated amount of calcium carbonate and the precipitated silver chloride removed by filtration, washed with alcohol (95 per cent.) and dried. The filtrate was evaporated to dryness, the residue extracted with boiling absolute alcohol and filtered. On cooling V separated as a white crystalline solid, m.pt. 94° to 94·5°C. (Corr.). Yield (i) 34·1 g. A further yield of product was obtained by submitting the dried precipitated, silver chloride to continuous extraction with alcohol (95 per cent.), evaporating off the excess of alcohol and allowing to crystallise. Yield (ii) 1·5 g. Finally the combined mother liquors were concentrated and shaken with a saturated solution of sodium bisulphite. The aldehyde-bisulphite compound separated from the solution as a white solid which was filtered, washed with alcohol and then decomposed with sodium carbonate solution. The purified product was extracted with ether, the solution dried with anhydrous sodium sulphate, and the ether removed by evaporation. Yield (iii) 2·1 g. Total yield 37·7 g. (89·5 per cent.). Found: C, 63·19; H, 3·82; Cl, 14·62 per cent. Eq. wt., 263·1. $C_{14}H_9O_3$ requires C, 64·49; H, 3·48; Cl, 13·61 per cent. Eq. wt., 260·5. The aldehyde (V) forms a

2:4-dinitrophenylhydrazone, which crystallises from absolute alcohol in orange prisms, m.pt. 240° to 241°C. (Corr.). Found: C, 54·55; H, 3·05; N, 12·80; Cl, 8·76 per cent. $C_{20}H_{13}O_6N_4Cl$ requires C, 54·46; H, 2·97; N, 12·71; Cl, 8·05 per cent. The semicarbazone of V is a white microcrystalline solid. It is practically insoluble in alcohol, but may be recrystallised from dry acetone, m.pt. 225° to 226°C. (Corr.). Found: C, 56·31; H, 3·27; N, 12·8; Cl, 11·8 per cent. $C_{15}H_{12}O_3N_5Cl$ requires C, 56·68; H, 3·81; N, 13·22; Cl, 11·16 per cent.

2-Chloro-5-benzoylhydroxy-4′:4″-bisdimethylaminotriphenylmethane (VI)—V (13 g.) was refluxed at 100°C. for 3 hours with dimethylaniline (12·2 g.) and phosphorus oxychloride (7·7 g.), using *o*-dichlorobenzene (23 g.) as solvent. 5N sodium hydroxide solution (30 ml.) and water (30 ml.) were added and the *o*-dichlorobenzene removed by steam distillation. The crude product separated as a dark green sticky mass, which was then extracted with dilute hydrochloric acid (10 per cent.). The extract was filtered, diluted with distilled water (2 l.) and neutralised by the careful addition of strong ammonia solution. A pale green flocculent precipitate was obtained, which was filtered, washed dried and recrystallised from alcohol (95 per cent.), to give a white crystalline solid. Yield 17 g. (70·2 per cent.), m.pt. 150°C. (Corr.). Found: C, 71·74; H, 5·90; N, 5·56; Cl, 7·26 per cent. $C_{30}H_{29}O_2N_2Cl$ requires C, 74·30; H, 6·02; N, 5·77; Cl, 7·31 per cent. Picrate $B_1(C_6H_3O_7N_3)_2$, m.pt. 147·5° to 148°C. (Corr.). Found: C, 52·65; H, 3·59; N, 11·50; Cl, 2·98 per cent. Eq. Wt., 970·1. $C_{42}H_{35}O_{16}N_8Cl$ requires C, 53·47; H, 3·74; N, 11·86; Cl, 3·76 per cent. Eq. Wt., 942·5.

2-Chloro-5-hydroxy-4′:4″-bisdimethylaminotriphenylmethane (VII)—VI (15·7 g.) was refluxed for 4 hours with N/2 alcoholic potassium hydroxide (300 ml.). When cold the solution was just neutralised by the addition of N hydrochloric acid and the alcohol removed by distillation. The solution was made just alkaline by the addition of potassium carbonate, and the solid product which separated was filtered from the solution, washed with water and dried. Yield of crude product 12·13 g. (98 per cent.). This dark green crystalline material was purified by chromatographic analysis, using a 1 per cent. solution in benzene on a column of activated alumina. Development of the chromatogram with a mixture of 10 parts of alcohol (95 per cent.) and 90 parts of benzene caused a separation into four distinct zones, which were coloured (a) dark green, (b) pink, (c) violet, (d) yellow in order from top to bottom of the column. These four fractions were collected separately by continued elution of the column with the developing solvent. Fractions (a), (b) and (c) contained only traces of unidentified impurities. The solvent was removed from fraction (d) under reduced pressure, in a current of hydrogen to minimise oxidation and the pale green solid recrystallised from freshly distilled alcohol (95 per cent.) to give a product which was pure white. Yield 10·91 g., m.pt. 179° to 180° C. (Corr.). Found: C, 72·01; H, 6·37; N, 7·62; Cl, 9·84 per cent. $C_{23}H_{25}ON_2Cl$ requires C, 72·53; H, 6·61; N, 7·35; Cl, 9·31 per cent. Picrate

$B_1(C_6H_3O_7N_3)_2$, m.pt. 187° to 188° C. (Corr.), with initial softening at 185° C. Found: C, 49·83; H, 3·64; N, 13·5; Cl, 3·83 per cent. Eq. wt. 835·1. $C_{35}H_{31}O_{15}N_8Cl$ requires C, 50·07; H, 3·70; N, 13·36; Cl, 4·23 per cent. Eq. wt. 838·7.

2-Chloro-5-hydroxy-4′:4″-bisdimethylaminotriphenylcarbinol (VIII)—VII (5·95 g.) was dissolved in 70 per cent. acetic acid (50 ml.) and the solution cooled to between 0° and 5°C. The theoretical quantity of lead peroxide paste (prepared according to Gattermann[14]) suspended in 70 per cent. acetic acid (40 ml.) was added slowly to the above solution with continuous stirring over a period of about 10 minutes. The reaction was allowed to proceed at 0° to 5°C. for one hour with continuous stirring. Sufficient sodium sulphate solution to precipitate all the lead present was added and stirring continued for 20 minutes. The deep green solution was filtered to remove lead sulphate, made alkaline with sodium bicarbonate, and the precipitated carbinol base extracted with chloroform. The solvent was removed by distillation, and the dark green solid, after drying, continuously extracted with chloroform in a soxhlet apparatus. The chloroform solution was evaporated to 150 ml. and passed through a column of activated alumina. The dark green chromatogram was developed using a mixture of 90 parts of chloroform and 10 parts of alcohol (95 per cent.), when a gradual separation occurred into an upper dark green band and a lower yellow one. The latter was eluted from the column using the same solvent to give a yellow solution, which during the course of evaporation became pale green and finally left a dull green residue (0·15 g.) of unchanged leucobase. The contents of the dark green band on the column were eluted using a mixture of 80 parts of chloroform and 20 parts of alcohol (95 per cent.), and on evaporation of the solvent the product was obtained as a friable, dark, purplish-black solid. Yield 5·39 g. A second fraction (0·10 g.) was obtained by extruding the column, extracting continuously with chloroform for 2½ hours and evaporating the solvent. Total yield 88·6 per cent. Found: C, 68·55; H, 6·42; N, 7·09; Cl, 9·58 per cent. $C_{23}H_{25}O_2N_2Cl$ requires C, 69·56; H, 6·30; N, 7·06; Cl, 8·94 per cent. Picrate $B_1(C_6H_3O_7N_3)_2$. Found: C, 49·65; H, 3·94; N, 13·30; Cl, 4·26 per cent. $C_{35}H_{31}O_{16}N_8Cl$ requires C, 49·12; H, 3·65; N, 13·10; Cl, 4·15 per cent.

2-Chloro-5-hydroxy-4′:4″-bisdimethylaminotriphenylmethyl sulphate (III)—VIII (3 g.) was dissolved in chloroform (30 ml.). The calculated volume of N sulphuric acid solution and water (20 ml.) were added with continuous stirring, and the mixture maintained at 45° to 50°C. for 1 hour. The dye which is almost insoluble in water separates as a sticky mass which is obtained in fine scales on evaporation of the solvents and drying at 100°C. Found: C, 58·92; H, 5·68; N, 5·65; Cl, 7·15; S, 6·79 per cent. $C_{23}H_{25}O_5N_2Cl$ S requires C, 57·9; H, 5·68; N, 5·88; Cl, 7·44; S, 6·71 per cent.

Polarography. Polarographs of the dye (III) and of brilliant green were plotted under the same conditions using a voltamescope, and these are

illustrated in Figure 1. The composition of the two solutions used was as follows:—

Dye (VI), or brilliant green	5×10^{-4} M.
KCl	10^{-1} M.
Gelatin	0·02 per cent.
0·1N Hydrochloric acid	21 ml.
Alcohol (95 per cent.)	50 ml.
Distilled water to	100 ml.

Dissolved oxygen was removed from the solution by passing a stream of hydrogen for 10 minutes, and the polarographs were plotted under the following conditions: temperature 23·5°C., drop time of the mercury cathode 1·4 secs., height of the mercury head 71·5 cm.

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