RESEARCH PAPERS

SOME OBSERVATIONS ON THE STRUCTURAL REQUIREMENTS FOR ANTIBIOTIC ACTIVITY IN THE CHLORAMPHENICOL SERIES

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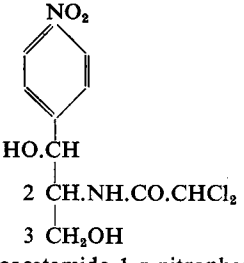
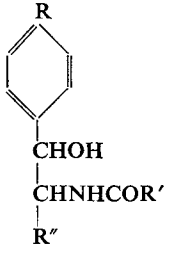
CHLORAMPHENICOL (I) alone, of the major antibotics, possesses a structure which readily lends itself to modification and synthesis[1]. Nevertheless, in spite of this fact, little information exists regarding the specificity of the functional groupings present in the molecule. We have, therefore, sought to modify, in turn, each structural feature, hoping thereby to obtain correlation between chemical constitution and biological activity, and thus pave the way to the preparation of new analogues.



(I) D-(−)-*threo*-2-dichloroacetamido-1-*p*-nitrophenylpropane-1 : 3 diol

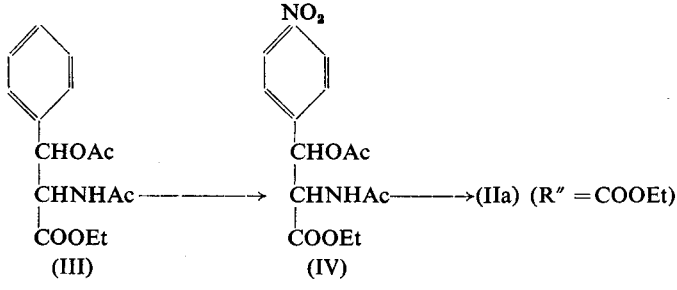


(II) (R = .NO_2; R″ = .CH_2OH)
(IIa) (R = .NO_2; R′ = .$CHCl_2$)
(IIb) (R′ = .$CHCl_2$; R″ = .CH_2OH)
(IId) (R = .NO_2; R″ = H)
(IIe) (R = H; R′ = $CHCl_2$; R″ = CH_2OH)

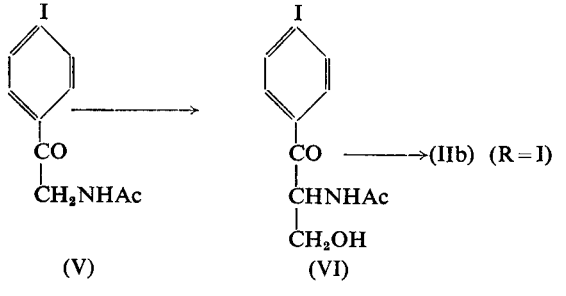
(i) *Modification of the Dichloroacetyl-grouping.* Earlier work by Rebstock, Crooks, Controulis, and Bartz[2] has shown that replacement of the dichloroacetyl-grouping of (I) by acetyl (II; R′ = Me) leads to a seven-fold drop in activity against the test organism *Shigella paradysenteriæ* (Sonnei). The specificity of the dichloroacetyl-grouping, however, has now been systematically examined for the first time by antibacterial study of the corresponding difluoroacetyl (II; R′ = .CHF_2), trifluoracetyl (R′ = .CF_3), trichloroacetyl (R′ = .CCl_3), dichlorofluoroacetyl (R′ = .$CFCl_2$), dibromoacetyl (R′ = .$CHBr_2$), and αα-dichloropropionyl (R′ = .CCl_2Me) derivatives of DL-(I). The latter were prepared in excel-

lent yields by reaction of DL-*threo*-2-amino-1-*p*-nitrophenylpropane-1 : 3-diol with the corresponding halogenated acetic ester at 100°C. DL-*threo*-2-*p*-Acetamidophenylsulphonamido-1-*p*-nitrophenylpropane-1 : 3-diol (IIc), required for comparison, was obtained by employing *p*-acetamidobenzenesulphonyl chloride in the condensation with the DL-chloramphenicol base.

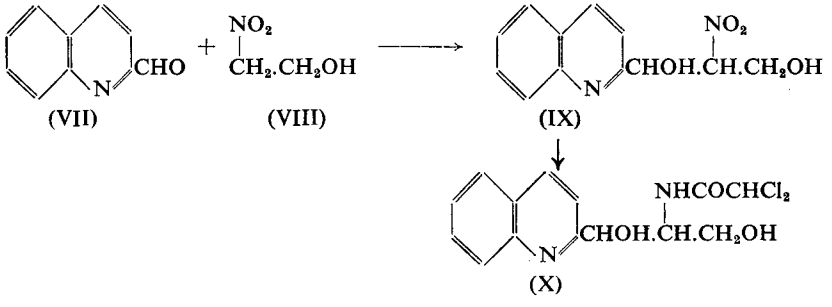
(ii) *Modification of the terminal .CH_2OH grouping.* Two major modifications of the terminal hydroxymethyl grouping were effected. Firstly, DL-2-dichloroacetamido-1-*p*-nitrophenyl ethanol[3] (IId) was prepared by hydrolysis of DL-2-acetamido-1-*p*-nitrophenyl ethanol[4], followed by dichloroacetylation. Secondly, ethyl DL-1-dichloroacetamido-2-hydroxy-1-*p*-nitrophenyl propionate (IIa) (R″ = COOEt) was obtained in good overall yield from DL-phenylserine. The *ON*-diacetyl ethyl ester (III) of the latter compound passed into *ON*-diacetyl *p*-nitrophenylserine ethyl ester (IV) on nitration, the constitution of which followed from its conversion into *p*-nitrobenzoic acid on oxidation. Careful hydrolysis, esterification, and dichloracetylation, finally gave the desired compound (IIa) (R″ = COOEt).



(iii) *Modification of the nitrophenyl-grouping*[3,5]. Replacement of the nitro-grouping by iodine was effected by employing *p*-iodoacetophenone in the chloramphenicol synthesis developed by Long and Troutman[4]. For this purpose *p*-iodo-ω-bromoacetophenone was converted *via* the hexamethylenetetramine salt into *p*-iodo-ω-acetamido acetophenone (V), transformed by reaction with formaldehyde/sodium bicarbonate into α-acetamido-β-hydroxy-*p*-iodopropiophenone (VI). Reduction of the latter compound with aluminium *iso*propoxide, followed by deacetylation and dichloroacetylation, yielded DL-*threo*-2-dichloroacetamido-1-*p*-iodophenylpropane-1 : 3-diol (IIb) (R = I).



The structural and pharmacological similarity between pyridine and nitrobenzene, to which Erlenmeyer, Aeberti, and Sorkin[6] have drawn attention, led us to examine the possibility of replacing the nitrophenyl grouping of (I) by the 4′-pyridyl residue. Difficulties encountered during the preparation of the required intermediates, however, forced us to abandon this project and to turn our attention to the synthesis of the corresponding 2′-quinolyl analogue (X). Its preparation was achieved by reaction of quinoline-2-aldehyde (VII) with nitroethanol (VIII) to give 1-(2′-quinolyl)-2-nitropropane-1 : 3-diol (IX), from which by catalytic hydrogenation and dichloroacetylation, DL-2-dichloroacetamido-1-(2′-quinolyl)-propane-1 : 3-diol (X) was obtained. Further modifications of (I) are described in the experimental section.



(VII) (VIII) (IX) (X)

ANTIBIOTIC ACTIVITY

The foregoing compounds were tested against a variety of organisms, the results being collected in Table I. Antibacterial activity was uniformly of a very low order, and in no case did the antibiotic activity approach that of the parent chloramphenicol.

DISCUSSION

The concept of a spatial correlation between biologically active molecules and the biological substrates upon which they act was first enunciated by Pasteur, and used by him to explain the stereospecificity of enzymes. Its value in the study of drug action is now widely accepted. Both van der Waals forces and hydrogen bonding are assumed to hold the drug molecule in intimate contact with the biological structure at specific receptor sites. Any variations in molecular structure which disturb (a) the spatial relationship, and (b) the "bonding" relationship between the drug and its biological receptor will, therefore, lead to changes in the antibiotic activity of the compound.

The importance of the spatial relationship is well established. Thus L-adrenaline is ten to twenty times as active as the D-isomer in increasing arterial blood pressure. Chloramphenicol forms no exception to this rule. All four stereoisomers of 2-dichloroacetoamido-1-*p*-nitrophenyl-propane-1 : 3-diol are known. Destructive antibiotic properties towards micro-organisms, however, reside in only one[7] of these compounds, the

TABLE I

EFFECTIVE DILUTIONS FOR ANTIBACTERIAL ACTION

COMPOUND	*Micrococcus pyogenes* var. *aureus*	*Escherichia coli*	*Salmonella typhi*	*Salmonella paratyphi* A	*Shigella sonnei*	*Salmonella enteritidis*	*Shigella paradysenteriæ*	*Salmonella stanley*	*Salmonella typhi-murium*	*Bacillus subtilis*	*Mycobacterium tuberculosis*	*Entamœba histolytica*
DL-Chloramphenicol Benzoate	<1000	<1000	<1000	4000	<1000	<1000	<4000	<1000	<1000	<1000	<32,000	
(II) R′=CHF_2	<1000	<1000	<1000	<1000	<1000	<1000	<1000	<1000	<1000	<1000	4000	
(II) R′=CF_3	<1000	<1000	<1000	<1000	<1000	<1000	<1000	<1000	<1000	<1000	<1000	
(II) R′=CCl_3	<500	<500	<500	<500	<500	<500	<500	<500	<500	<500	<2000	<10,000
(II) R′=$CFCl_2$	<2000	<2000	<2000	<2000	<2000	<2000	<2000	<2000	<2000	<2000	<1000	
(II) R′=$CHBr_2$	<1000	<1000	<1000	<1000	<1000	<1000	<1000	<1000	<1000	<1000		
(II) R′=CCl_2Me	<2000	<2000	<2000	<2000	<2000	<2000	<2000	<2000	<2000	<2000	2000	
(IIc)	<1000	<1000	<1000	<1000	<1000	<1000	<1000	<1000	<1000	<1000	<1000	
(IId)	<1000	<1000	<1000	<1000	<1000	<1000	<1000	<1000	<1000	<1000	16,000	
(IIa) R″=COOEt	<1000	<1000	<1000	<1000	<1000	<1000	<1000	<1000	<1000	<1000	4000	
(IIe)	<1000	<1000	<1000	<1000	<1000	<1000	<1000	<1000	<1000	<1000	<2000	
(IIb) R=I	4000	<1000	1000	<1000	1000	<1000	<1000	<1000	4000	8000	16,000	
(X)	200	400	400	400	400	400	400	400	400	400	4000	<10,000
L-Dichloroacetamidotyrosine ethyl ester	<100	<100	<100	<100	<100	<100	<100	<100	<100	<100	8000	<10,000
N-Dichloroacetamido-*p*-nitro-phenylalanine											<1000	
DL-β-Hydroxy-*a*-iodo-β(*p*-nitro-phenyl) propionic ethyl ester	4000	4000	4000	4000	4000	4000	4000	4000	4000	4000	64,000	
DL-2-Acetamido-1(*p*-iodophenyl)-propane-1 : 3-diol	<1000	<1000	<1000	<1000	<1000	<1000	<1000	<1000	<1000	<1000	4000	
2-Dichloroacetamido 1-hydroxy-1 : 2-diphenyl ethane ...	<200	<200	<200	<200	<200	<200	<200	<200	<200	<200	4000	<10,000
Dichloroacetamidodiphenyl methane	500	<500	<500	<500	<500	<500	<500	<500	<500	<500	<20,000	

D-*threo*-isomer, (I), the remaining isologues showing less than 0·5 per cent. chloramphenicol activity in the same microbiological test.

The attachment of the dichloroacetyl in (I) to a receptor site in the biological substrate is made evident by the observation that any modification of this residue leads to loss of antibiotic activity. The strength of the bond between the haloacetyl-grouping and the receptor site, however, is not the only factor involved, as replacement of halogen by the more electronegative fluorine (II) ($R'=CHF_2$) leads to a disappearance of bactericidal properties. As (II) ($R'=\cdot CCl_2Me$) likewise lacks antibiotic effect, the size of the dichloroacetyl cationic head is of critical importance in the chloramphenicol structure.

Dramatic loss of activity is likewise produced by structural variation of the terminal $\cdot CH_2OH$ grouping of (I). Acylated derivatives such as DL-chloramphenicol benzoate (Table I) and chloramphenicol glucuronide[8], as well as compounds in which $\cdot CH_2OH$ is replaced by carbethoxy (IV) or hydrogen[3] (IId), all fail to show significant antibiotic action.

In contrast to the above, variation of the nitro-grouping may be effected within certain limits, providing the substituent is retained in the *p*-position[9,10]. The iodo- (IIb; R=I) (Table) and fluoro-[3] analogues of (I) are without antibiotic action. The chloro- and bromo-derivatives, however, are reported by Buu-Hoi *et al.*[11] to show some *in vitro* activity against *Shigella paradysenteriae,* the latter compound having 1/5 the activity[3] of (I) against *Micrococcus pyogenes* var. *aureus, E. coli,* and *Salmonella paratyphi* B. Nevertheless, such changes produce in all the cases examined an important loss in antibiotic action.

Consideration of these results leads to the conclusion that the chloramphenicol molecule undergoes receptor bonding with fixed receptors in the effector cells through the nitro-grouping, the hydroxyl-groupings, and the dichloroacetyl-residue. The molar volume of these four groupings is also of critical importance, any variation affecting adversely the intimate contact between the drug molecule and the biological structure upon which it acts, thereby preventing development of antibacterial action. Our studies are, admittedly, limited and capable of wide extension. Nevertheless, in so far as the results permit, they point strongly to the conclusion that (I) represents the optimal spatial and structural requirements for antibiotic action in compounds of this type, thereby affording scant encouragement to the preparation of new analogues.

EXPERIMENTAL

M.pts are uncorrected. Microanalyses are by Drs. Weiler and Strauss, Oxford.

Modifications of the Dichloroacetyl-grouping: The following general procedure was employed (cf. Rebstock[1]): DL-*threo*-2-amino-1-*p*-nitrophenylpropane-1:3-diol[2] was heated on the steam bath for 2 to 3 hours with an excess (50 to 100 per cent.) of the appropriate halogenated ester. Excess of the latter was then removed by extraction with light petroleum and the residue purified by crystallisation. The following derivatives

were obtained:—*difluoracetyl,* needles from ethyl acetate, m.pt. 123°C. Found: C, 46·2; H, 5·0; N, 9·5. $C_{11}H_{12}O_5N_2F_2$ requires C, 45·2; H, 4·1; N, 9·6 per cent.; *trifluoroacetyl,* needles from ethyl acetate/light petroleum, m.pt. 100°C. Found: C, 42·8; H, 3·6; N, 8·9. $C_{11}H_{11}O_5N_2F_3$ requires C, 42·9; H, 3·6; N, 9·0 per cent.; *trichloroacetyl,* needles from ethyl acetate, m.pt. 145°C. Found: C, 36·3; H, 3·2; N, 7·3; Cl, 28·8. $C_{11}H_{11}O_5N_2Cl_3$ requires C, 36·9; H, 3·1; N, 7·8; Cl, 29·5 per cent.; *fluorodichloroacetyl,* needles from ethyl acetate, m.pt. 178° C. Found: N, 8·9. $C_{11}H_{11}O_5N_2FCl_2$ requires N, 8·2 per cent.; *dibromoacetyl,* cream-coloured needles from ethyl acetate/light petroleum (b.pt. 40° to 60°C.), m.pt. 156°C. Found: C, 31·7; H, 2·3; N, 6·2. $C_{11}H_{12}O_5N_2Br_2$ requires C, 32·0; H, 2·9; N, 6·8 per cent.; *dichloropropionyl,* plates from water, m.pt. 121°C. Found: N, 8·7; Cl, 21·0. $C_{12}H_{14}O_5N_2Cl_2$ requires N, 8·3; Cl, 21·1 per cent.; p-*acetylaminosulphonyl,* crystals from water, m.pt. 248°C. Found: N, 10·8. $C_{17}H_{19}O_7N_3S$ requires N, 10·3 per cent.

DL-*Chloramphenicol benzoate,* prepared by treating DL-chloramphenicol (1·6 g.) in pyridine solution (2·5 ml.) at 0°C. with benzoyl chloride (700 mg.) and leaving the mixture at room temperature overnight, formed needles from a large volume of water, m.pt. 175°C. Found: C, 50·7; H, 4·0; N, 6·4; Cl, 16·6. $C_{18}H_{16}O_6N_2Cl_2$ requires C, 50·6; H, 3·7; N, 6·5; Cl, 16·6 per cent.

DL-*Dichloroacetamido*-1-p-*nitrophenyl ethanol* (IId), prepared by treating DL-2-amino-1-*p*-nitrophenyl ethanol[4] (5 g.) in methanol (10 ml.) with methyl dichloroacetate (8 ml.) under reflux for 1½ hours, formed needles from aqueous methanol, m.pt. 118°C. Found: C. 41·4; H, 3·8; N, 10·0; Cl, 24·8. $C_{10}H_{10}O_4N_2Cl_2$ requires C, 40·1; H, 3·5; N, 10·0; Cl. 24·2 per cent.

DL-*Ethyl* α-*acetamido*-β-*hydroxy*-β-*phenyl propionate:* DL-Phenyl serine (50 g.), suspended in absolute ethanol (400 ml.), was treated with dry hydrogen chloride at 20°C. for 2½ hours, excess solvent being distilled off under reduced pressure after 48 hours. The residue so obtained in water (150 ml.) and crushed ice (300 g.) was treated with stirring with acetic anhydride (60 g.), followed by hydrated sodium acetate (100 g.) dissolved in water (100 ml.). Stirring was continued for 5 to 6 hours, after which the separated solids were collected and crystallised from ethanol. DL-*Ethyl* α-*acetamido*-β-*hydroxy*-β-*phenyl propionate* (50 g.) formed prismatic needles, m.pt. 175°C. Found: C, 61·8; H, 6·8; N, 5·6. $C_{13}H_{17}O_4N$ requires C, 62·4; H, 6·8; N, 5·6 per cent.

DL-*Ethyl* α-*acetamido*-β-*acetoxy*-β-*phenyl propionate* (III) separated in crystals, m.pt. 167°C. Found: C, 61·0; H, 6·5; N. 4·6. $C_{15}H_{19}O_5N$ require C, 61·4; H, 6·5; N, 4·7 per cent., when the foregoing compound (20 g.) was heated under reflux with acetic anhydride (100 ml.) for 2 hours and the mixture allowed to cool.

Ethyl DL-β-*acetamido*-α-*acetoxy*-α-(p-*nitrophenyl*) *propionate* (IV): The foregoing compound (15 g.) was added in portions with mechanical stirring at −20°C. to concentrated sulphuric acid (25 ml.) and concentrated nitric acid (25 ml.). The temperature of the mixture was allowed to rise to room temperature over 90 minutes, when it was poured on to ice.

The precipitated solids were collected, washed free from acid, and purified form dilute ethanol. Ethyl DL-β-acetamido-α-acetoxy-α-(*p*-nitrophenyl) propionate formed needles, m.pt. 120°C. Found: N, 8·2. $C_{15}H_{18}O_7N_2$ requires N, 8·3 per cent.

When the above nitro-compound (2 g.) was oxidised with sodium dichromate (2 g.) in water (4 ml.) and concentrated sulphuric acid (4 ml.) under reflux for 2 hours, and the mixture poured into water, *p*-nitrobenzoic acid was obtained, m.pt. 241°C., not depressed in admixture with an authentic specimen.

Ethyl DL-β-*amino*-α-*hydroxy*-α-(p-*nitrophenyl*) *propionate:* A stirred mixture of (IV) (10 g.) and 5 per cent. hydrochloric acid was heated on the steam bath for 2½ hours, after which charcoal was added, and the filtered solution evaporated to dryness under reduced pressure. The residue, in absolute ethanol (100 ml.) was treated with a slow steam of hydrogen chloride for 1½ hours at room temperature and the solution left overnight. It was then evaporated to dryness at 30°C., and the residue dissolved in the minimum volume of water and made just alkaline by addition of sodium carbonate solution at 5°C. The precipitated solids were collected and crystallised from ethyl acetate yielding *ethyl* DL-β-*amino*-α-*hydroxy*-α-(p-*nitrophenyl*) *propionate* (5 g.), leaflets, m.pt. 115°C. Found: C. 51·3; H, 5·5; N, 10·6. $C_{11}H_{14}O_5N_2$ requires C, 51·9; H, 5·5; N, 11·0 per cent.

Ethyl DL-β-*dichloroacetamido*-α-*hydroxy*-α-(p-*nitrophenyl*) *propionate* (IIa) (R″ = COOEt): The foregoing amino-ester (2 g.), dissolved in water (25 ml.) and concentrated hydrochloric acid (1 ml.), was cooled with crushed ice (25 g.) and the stirred mixture treated with dichloroacetic anhydride (6 g.) followed by sodium acetate (10 g.) dissolved in water (10 ml.). Stirring was maintained for 4 hours, when the separated solids were collected and crystallised from aqueous ethanol giving *ethyl* DLβ-*dichloroacetamido*-α-*hydroxy*-α-(p-*nitrophenyl*) *propionate* (2 g.), needles, m.pt. 145°C. Found: C, 42·8; H, 3·8; N, 7·0. $C_{13}H_{14}O_6N_2Cl_2$ requires C, 42·8; H, 3·8; N, 7·6 per cent.

ω-*Bromo*-p-*iodoacetophenone hexamethylenetetramine salt:* To a stirred mixture of hexamethylenetetramine (47 g.) in chloroform (350 ml.) was added, in one portion at room temperature, a solution of ω-bromo-*p*-iodoacetophenone (109 g.) in chloroform (350 ml.). Stirring was continued for 4 hours when the product was collected. For purification the material was stirred with ethanol (400 ml.) and then washed on the filter with the same solvent (100 ml.). Repetition of the last two operations gave ω-*bromo*-p-*iodoacetophenone hexamethylenetetramine salt* (140 g.) m.pt. 159°C. Found: C, 36·4; H, 4·0; N, 11·7. $C_{14}H_{18}ON_4BrI$ requires C, 36·2; H, 3·9; N, 12·0 per cent.

ω-*Amino*-p-*iodoacetophenone:* The foregoing hexamine salt (140 g.) was added to a stirred solution of 95 per cent. ethanol (400 ml.) admixed with concentrated hydrochloric acid (200 ml.). After stirring for 16 hours at room temperature the mixture was cooled to 5°C., the crystalline product collected, stirred with water (150 ml.) at 25°C. for 15 minutes,

cooled to 10°C. and filtered. ω-*Amino*-p-*iodoacetophenone hydrochloride* (70 g.) was obtained, m.pt. 275°C. Found: N, 4·0. C_8H_9ONClI requires N, 4·6 per cent.

ω-*Acetamido*-p-*iodoacetophenone* (V): The foregoing product (70 g.) dissolved in water (250 ml.), was cooled with chipped ice (400 g.), and the stirred mixture treated with acetic anhydride (80 g.) added in one portion, followed by sodium acetate (100 g.) dissolved in water (300 ml.). The temperature of the mixture was allowed to rise to 20°C., stirring being continued for a further 4 hours, after which it was strongly acidified with concentrated hydrochloric acid. Crystallisation of the product from ethyl acetate gave ω-*acetamido*-p-*iodoacetophenone* (60 g.), m.pt. 184°C. Found: C, 39·1; H, 3·3; N, 4·3; I, 41·4. $C_{10}H_{10}O_2NI$ requires C, 39·6; H, 3·3; N, 4·6; I, 41·9 per cent.

DL-α-*Acetamido*-β-*hydroxy*-p-*iodopropiophenone* (VI): Sodium bicarbonate (2 g.) was added to a stirred mixture of the foregoing compound (42 g.) in 95 per cent. ethanol (140 ml.) and 36 per cent. formaldehyde solution (26 ml.). After 2 hours at 35°C. the mixture was cooled to 5°C. and the solid product collected, washed with dilute hydrochloric acid and water, and dried *in vacuo,* yielding DL-α-*acetamido*-β-*hydroxy*-p-*iodopropiophenone* (40 g.), m.pt. 159°C. Found: C, 39·8; H, 3·5; N, 4·0; I, 38·0. $C_{11}H_{12}O_3NI$ requires C. 39·6; H, 3·6; N, 4·2; I, 38·1 per cent.

DL-2-*Acetamido* - 1 - p - *iodophenylpropane*-1 : 3-*diol :* α-Acetamido-β-hydroxy-*p*-iodoacetophenone (10 g.) was added to a hot solution of aluminium isopropoxide (12 g.) in isopropyl alcohol (100 ml.) and reduction effected in the usual way over 6 hours. After allowing to cool somewhat, water (25 ml.) was added and the mixture refluxed for 15 minutes and filtered. The residue was re-extracted twice with 100 ml. portions of boiling 80 per cent. isopropyl alcohol. The combined extracts gave DL-2-*acetamido*-1-p-*iodophenylpropane*-1 : 3-*diol* (6 g.), crystals from ethyl acetate (50 ml.) m.pt. 156°C. Found: C. 39·6; H, 3·7; N, 3·8. $C_{11}H_{14}O_3NI$ requires C, 39·6; H, 3·9; N, 4·2 per cent.

DL-2-*Dichloroacetamido*-1-p-*iodophenylpropane*-1 : 3-*diol* (IIb) (R = I): The foregoing compound (6 g.) was heated with 5 per cent. hydrochloric acid for 1 hour at 100°C. and the resulting solution (charcoal) cooled to 5°C. and made alkaline with 20 per cent. sodium hydroxide. The resulting amine was heated with methyl dichloroacetate (10 g.) for 1½ hours on the steam bath giving DL-2-*dichloroacetamido*-1-p-*iodophenylpropane*-1 : 3-*diol,* needles (3 g.) from ethyl acetate, m.pt. 123°C. Found: C, 32·5; H, 3·0; N, 3·3. $C_{11}H_{12}O_3NCl_2I$ requires C, 32·7; H, 3·0; N, 3·5 per cent.

DL-3-(2′-*Quinolyl*)-2-*nitropropane*-1 : 3-*diol* (IX): Quinoline-2-aldehyde (34 g.) (cf. Sharp[12]) dissolved in methanol (250 ml.) was added to a suspension of the sodium salt of β-nitroethanol (27·6 g.) in methanol (250 ml.) at 0°C. The mixture was stirred vigorously for 2 hours at 0° to 5°C., when the solids were collected, washed with ether, and acidified in aqueous solution (1,000 ml.) with glacial acetic acid. The product was collected after 12 hours at 0°C. and purified from ethyl acetate/light

petroleum (b.pt. 80° to 100°C.). *DL-3-(2′-Quinolyl)-2-nitropropane-1:3-diol* (33 g.) formed small buff-coloured prisms, m.pt. 124° to 125°C. Found: C, 57·7; H, 4·6. $C_{12}H_{12}O_4N_2$ requires C, 58·0; H, 4·9 per cent.

DL-2-Dichloroacetamido-1(2′-quinolyl)-propane-1:3-diol (X): The foregoing nitro-alcohol (9·92 g.) was catalytically reduced in methanolic solution (100 ml.) in the presence of palladium charcoal (1 g.). The product was heated with methyl dichloroacetate (7·29 g.) for 1½ hours under nitrogen on a steam bath giving *DL-2-dichloroacetamido-1(2′-quinolyl)-propane-1:3-diol,* needles (5·8 g.) from ethanol, m.pt. 176°C. Found: C, 51·0; H, 3·9; N, 8·9. $C_{14}H_{14}O_3N_2Cl_2$ requires C, 51·1; H, 4·3; N, 8·5 per cent.

*L-Dichloroacetamido-tyrosine ethyl ester, prepared by treating L-*tyrosine ethyl ester (10 g.) in ethanol (20 ml.) with methyl dichloroacetate (10 ml.) under reflux for 2 hours, formed prisms from ethyl acetate, m.pt. 162°C. Found: N, 4·4; Cl, 22·3. $C_{13}H_{15}O_4NCl_2$ requires N, 4·4; Cl, 22·2 per cent.

DL-β-Hydroxy-α-iodo-β-(p-nitrophenyl) propionic acid ethyl ester: Iodine (18 g.) was added in portions to a stirred mixture of *p*-nitrocinnamic acid ethyl ester (15 g.), yellow mercuric oxide (8 g.), water (2 ml.) and ether (saturated with water) (800 ml.). After stirring for 8 hours, the mixture was left overnight at room temperature, filtered, and the filtrate concentrated. *DL-β-Hydroxy-α-iodo-β-(p-nitrophenyl) propionic acid ethyl ester* was obtained, needles (17 g.) from ethanol, m.pt. 134°C. Found: C, 36·8; H, 3·3; N, 3·3; I, 33·8. $C_{11}H_{12}O_5NI$ requires C, 36·2; H, 3·2; N, 3·8; I, 34·8 per cent.

2-Dichloroacetamido-1-hydroxy-1:2-diphenyl ethane, prepared from the corresponding amine and methyl dichloracetate, crystallised from methyl acetate, m.pt. 195° to 196°C. Found: C, 59·0; H, 4·9; N, 4·0. $C_{16}H_{15}O_2NCl_2$ requires C, 5·93; H, 4·7; N, 4·3 per cent.

Dichloroacetamido-diphenyl methane, prepared by heating amino-diphenyl methane (6 g.) with methyl dichloroacetate (9 g.), crystallised (4·2 g.) from ethyl acetate/light petroleum, m.pt. 132°C. Found: C, 61·0; H, 4·4; N, 4·7; Cl, 24·3. $C_{15}H_{13}ONCl_2$ requires C, 61·4; H, 4·1; N, 4·8; Cl, 24·2 per cent.

Antibacterial Tests

The highest dilution at which a compound was found capable of killing the organisms indicated in Table I, with the exception of *Mycobacterium tuberculosis* and *Entamœba histolytica,* was determined as follows:—The compound under test was dissolved in Hedley Wright broth containing 0·3 per cent. of dextrose, and serial dilutions were made into 6″ × ⅝″ test tubes, so that each tube finally contained 5 ml. The tubes were plugged and the contents sterilised by autoclaving at 115°C. for 15 minutes. Each tube was then inoculated with 0·1 ml. of a 24-hour culture of the organism in Hedley Wright broth which had previously been adjusted to an opacity of one-tenth of that of Wellcome Opacity Tube 1. The tubes were incubated at 37°C. for 24 hours and

then subcultured, using a loop of 4 mm. diameter, into 5 ml. quantities of Hedley Wright broth. The subcultures were incubated at 37°C. for 24 hours and then examined for presence or absence of growth.

Tests against Mycobacterium tuberculosis: Serial dilutions in Youman's synthetic medium were made with the compound under test, and 10 ml. amounts of these dilutions were placed in McCartney bottles and sterilised at 115°C. for 15 minutes. Each series of dilutions was made up in triplicate.

The inoculum was prepared as follows: 21-day-old cultures of Human Strain 48 on Lowenstein's malachite green medium were washed off with sterile saline into a tube containing sterile sand and thoroughly ground with a glass pestle. The material was then centrifuged for 10 minutes at 1,000 r.p.m. and the deposit re-ground. The material was then adjusted by dilution with sterile saline to the opacity of Wellcome Opacity Tube 2. Using pipettes delivering 25 drops per ml., one drop of this suspension was then added to each of the McCartney bottles. The bottles were then incubated at 37°C. for 21 days and then examined. If no growth could be observed in a bottle, a microscopical examination was made to determine whether, in fact, *M. tuberculosis* was still present even in small numbers.

Tests against Entamœba histolytica : A 0·1 per cent. solution of the compound under test was made up in distilled water and sterilised by autoclaving at 115°C. for 15 minutes, except where the compound was unstable to heat, when sterilisation was effected by Seitz filtration.

Using aseptic technique a series of tubes was prepared with 14·4 ml. Dobell's liquid medium in the first tube, and 8 ml. Dobell's liquid medium in the succeeding tubes. 1·6 ml. of the sterile 0·1 per cent. solution of the compound under test was added to the first tube, mixed well, and 8 ml. of this solution transferred to the next tube, which thus now contained 16 ml. of solution at half the strength of the first tube. This operation was repeated, giving serial dilutions of the compound starting at 1 : 10,000 and rising to 1 : 640,000. About 0·2 g. of rice starch was now added to each tube in the series, followed by one drop of a well-grown culture of *Escherichia coli,* strain A (Dobell). One drop of a 0·5 per cent. solution of methylene blue was then added to each tube and the tubes incubated at 37°C. for 24 hours.

For preparation of the inoculum, *Entamœba histolytica* (non-encysting strain)* was grown on Dobell's solid medium containing about 0·2 g. of rice starch per tube. The surface of the rice starch in a 3-day-old culture was examined microscopically, and if a large number of amœbæ were seen, about half the rice starch was transferred by means of a Pasteur pipette into a sterile tube. 3 drops of this liquid were then used to inoculate each of the tubes containing dilutions of the compound under test. The tubes were rapidly examined microscopically for amœbæ every day for 3 days. It was also noted whether the tubes remained anaerobic during the test, owing to knowledge of the necessity for maintenance of

* Kindly supplied by Dr. C. Dobell.

strictly anaerobic conditions for the survival of *E. histolytica*. Examinations for survival were made as rapidly as possible owing to the sensitivity of the organism to changes in temperature.

Summary and Conclusions

1. Nineteen analogues of chloramphenicol (I) have been prepared and examined against a variety of organisms.

2. None of these compounds showed significant antibiotic properties.

3. It is concluded that (I) represents the optimal spatial and structural requirements for antibiotic action in compounds of this type.

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References

1. *Cf.* Rebstock, *J. Amer. chem. Soc.*, 1950, **72,** 4800.
2. Rebstock, Crooks, Controulis and Bartz, *ibid.*, 1949, **71,** 2458.
3. *Cf.* Buu-Hoi, Hoan, Jacquignon and Koi, *J. chem. Soc.*, 1950, 2766.
4. Long and Troutman, *J. Amer. chem. Soc.*, 1949, **71,** 2473.
5. *Cf.* Bambas, Troutman and Long, *ibid.*, 1950, **72,** 4445.
6. Erlenmeyer, Aeberti and Sorkin, *Helv. chim. Acta,* 1947, **30,** 2066.
7. Controulis, Rebstock and Crooks, *J. Amer. chem. Soc.*, 1949, **71,** 2463.
8. Glazko, Dill and Rebstock, *J. biol. Chem.*, 1950, **183,** 679.
9. Buu-Hoi and Koi, *C.R. Acad. Sci., Paris,* 1949, **229,** 1343; 1950, **230,** 967.
10. Ruoff and Miller, *J. Amer. chem. Soc.*, 1950, **72,** 1417.
11. Buu-Hoi *et al., C.R. Acad. Sci., Paris,* 1950, **230,** 663.
12. *Cf.* Sharp, *J. Pharm. Pharmacol.*, 1949, **1,** 395.