SOME OBSERVATIONS ON THE STRUCTURAL REQUIREMENTS FOR ANTIBIOTIC ACTIVITY IN THE CHLORAMPHENICOL SERIES. PART II*

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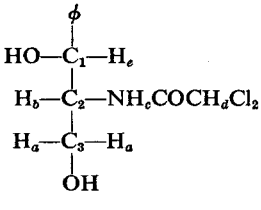
IN Part I[1] an attempt was made to define broadly the structural features associated with antibiotic activity in the chloramphenicol series. Since then additional studies have been carried out in this and other laboratories which permit further elaboration of the concepts developed in the earlier publication. The present investigation is concerned largely with the preparation of some analogues of chloramphenicol (I) in which the hydrogen atoms marked *a*, *b* and *c* are replaced by methyl groups. Interpretation of the results obtained is included in the discussion.

Experiments on the synthesis of 3-methylchloramphenicol (V; R = $-CHCl_2$; R′ = –Me) in which H*a* of (I) is replaced by Me, have been recorded by Rebstock,[2] who obtained a compound of unknown configuration, hereafter termed the "α"-isomer, by reduction of *iso*nitrosobenzoylacetone, followed by nitration and dichloroacetylation of the resulting amino-alcohol. The asymmetric configuration of the structural type (V), however, provides for the existence of 4 pairs of enantiomorphs, of which 2 pairs may be regarded as derived from *erythro*- and two from *threo*-2-acetamido-1-*p*-nitrophenylpropane-1 : 3-diol. We have, therefore, carried out experiments in order to prepare further compounds of this type.



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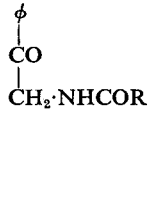

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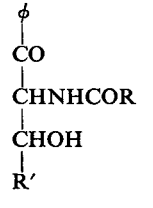

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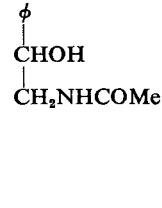

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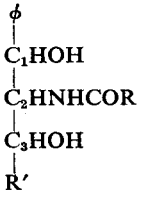

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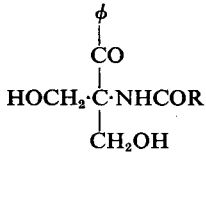

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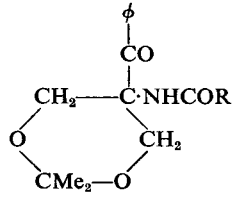

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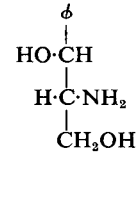

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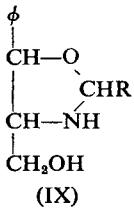
* *This Journal*, 1951, 3, 149, is considered as Part I.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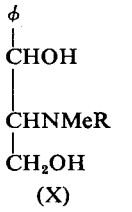
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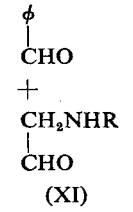
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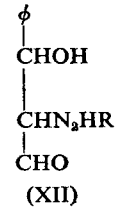
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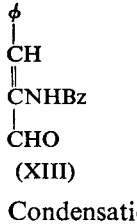
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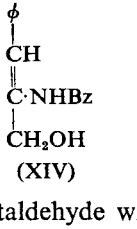
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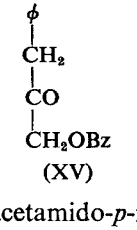
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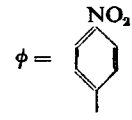

Condensation of acetaldehyde with ω-acetamido-*p*-nitroacetophenone (II; R = Me) in aqueous ethanol in the presence of sodium bicarbonate led to the formation of 1-acetamido-2-hydroxy-*p*-nitrobutyrophenone (III; R = R′ = Me) in *ca.* 60 per cent. yield. The product thus formed proved less stable than the corresponding formaldehyde condensation product (III; R = Me; R′ = H) in that on Ponndorf reduction in boiling *iso*propanolic solution it underwent smooth fission and reduction to give 2-acetamido-1-*p*-nitrophenylethan-1-ol (IV)[4] in excellent yield. Reduction at room temperature was next examined, when it was hoped that fission of (III; R = R′ = Me) into its component parts might be avoided. The complex mixture obtained, however, still contained substantial quantities of (IV) admixed, in this instance, with *ca.* 10 per cent. of a new isomer of (V; R = R′ = Me) which we designate "β"-2-acetamido-1-*p*-nitrophenylbutane-1:3-diol. Hydrolysis of this compound with 6 N hydrochloric acid furnished "β"-2-amino-1-*p*-nitrophenylbutane-1:3-diol, from which "β"-2-dichloroacetamido-1-*p*-nitrophenylbutane-1:3-diol ("β"-3-methylchloramphenicol) (V; R = $-CHCl_2$; R′ = Me) was obtained by reaction with methyl dichloroacetate.

Attempts to simplify the preparation of "β"-3-methylchloramphenicol by employing ω-dichloroacetamido-*p*-nitroacetophenone[5,6] (II; R = $-CHCl_2$) as starting material proved unsuccessful. Reaction of (II; R = $-CHCl_2$) with acetaldehyde in alkaline solution offered no difficulty, 1-dichloroacetamido-2-hydroxy-*p*-nitrobutyrophenone (III; R = $-CHCl_2$; R′ = Me) being readily obtained in 50 per cent. yield. Ponndorf reduction of this product at room temperature, however, led to a complex mixture from which only (II; R = $-CHCl_2$) was isolated in *ca.* 25 per cent. yield.

It is, of course, not possible to decide the stereochemical configuration of "β"-3-methylchloramphenicol from its mode of synthesis alone. At the same time it seems likely that the compound has the *erythro*-configuration at C_1:C_2 on the basis of the following evidence. Whereas Ponndorf reduction of 1-(acet)-amido-2-hydroxy-*p*-nitropropiophenone (III; R = Me; R′ = H) gives DL-*threo*-2-(acet)-amido-1-*p*-nitrophenylpropane-1:3-diol[4] (≡ DL-chloramphenicol base) with only small quantities of the *erythro*-isomers, Ponndorf reduction of 1-acetamido-2-*acyloxy*-

p-nitropropiophenone gives products in which the *erythro*-forms predominate (Ellis and Sturgeon, *unpublished observations*). Increase in molar volume of the terminal $-CH_2OH$ group of (III; R = Me; R′ = H) is thus seen to favour production of *erythro*-forms. Extending this concept further, it seems reasonable to conclude that the terminal methyl group (R′) of (III; R = R′ = Me) may likewise exert a comparable steric effect on the Ponndorf reduction of this compound with formation of an *erythro*-isomer of (V; R = R′ = Me). Again, *erythro*-compounds of type (I) are known to undergo conversion into the corresponding *threo*-derivatives under the action of thionyl chloride.[7] As this reagent converts "β"-3-methylchloramphenicol into a new isomer, "γ"-3-methylchloramphenicol, it seems likely that the latter compound is a *threo*-form of (V; R = $-CHCl_2$; R′ = Me) (relative to C_1 and C_2), and that the "β"-isomer is its *erythro*-analogue.

The second *threo*-enantiomorph of (V; R = $-CHCl_2$; R′ = Me) is probably represented by the "α"-3-methylchloramphenicol of Rebstock.[2] This view derives from consideration of the von Auwers-Skita generalisation[8,9] that catalytic hydrogenation (of *iso*nitrosobenzoylacetone[2]) in neutral media may be expected to lead to the formation of *trans*- (*i.e.*, *threo*)-isomers.

In addition to the foregoing series of transformations, we have also studied the condensation of (II; R = Me) with chloral, benzaldehyde, and *p*-nitrobenzaldehyde, hoping thereby to obtain chloramphenicol analogues (V) in which R′ = $-CCl_3$, -Ph, and $-C_6H_4NO_2(p)$. Reaction between ω-acetamido-*p*-nitroacetophenone (II; R = Me) and chloral readily occurred in the presence of sodium bicarbonate as condensing agent to give 1-acetamido-2-hydroxy-3:3:3-trichloro-*p*-nitrobutyrophenone (III; R = Me; R′ = $-CCl_3$). Ponndorf reduction of this compound gave 2-acetamido-1-*p*-nitrophenyl-4:4:4-trichlorobutane-1:3-diol (V; R = Me; R′ = $-CCl_3$), hydrolysed by dilute hydrochloric acid to the corresponding amine. Dichloroacetylation of the latter furnished 2-dichloroacetamido-1-*p*-nitrophenyl-4:4:4-trichlorobutane-1:3-diol (V; R = $-CHCl_2$; R′ = $-CCl_3$) of presumed *threo*-configuration, as it was recovered unchanged after treatment with thionyl chloride. Employment of benzaldehyde and *p*-nitrobenzaldehyde in the above reaction with (II; R = Me) led to the formation of 2-phenyl-(III; R = Me; R′ = -Ph) and 2-*p*-nitrophenyl-1-acetamido-2-hydroxy-*p*-nitropropiophenone (III; R = Me; R′ = $-C_6H_4NO_2(p)$). Ponndorf reduction of these compounds to the corresponding amino-diols (V; R = Me; R′ = Ph and $-C_6H_4NO_2$) could not be accomplished, however, as fission occurred in both cases with regeneration of the component parts, which severally underwent reduction in the normal way (see Experimental).

Attention was next directed to the preparation of 2-methyl-chloramphenicol (I; H_b replaced by -Me) from 1-acylamidopropiophenone by the general procedure of Long and Troutman.[10] Before this objective could be attained, however, a publication appeared by Huebner and Schultz[11] which not only covered essentially the same ground, but also revealed that the desired compound was inactive antibacterially. Our

own experiments were therefore discontinued. Interest in a 2-substituted chloramphenicol was nevertheless revived shortly afterwards when a publication (dated 1950) by Šorm, Gut, Suchý and Reichl[6] became available, in which it was claimed that "introduction of a second hydroxymethyl group on carbon atom 2 does not alter or even enhances the activity" (p. 508). As it seemed difficult to reconcile this result with conclusions reached from a study of 2-methylchloramphenicol, an attempt was made to repeat and hence confirm the startling and challenging claim put forward by the Czech workers.

2-Acetamido-2-*p*-nitrobenzoylpropane-1:3-diol (VI; R = Me) was prepared in *ca.* 40 per cent. yield by hydroxymethylation of ω-acetamido-*p*-nitroacetophenone (II; R = Me) by short warming with excess of formaldehyde in ethanolic solution in the presence of sodium bicarbonate as catalyst. Alternatively, the "monohydroxymethylation product" (III; R = Me; R′ = H) could be employed as starting material. The corresponding dichloroacetyl- (VI; R = $-CHCl_2$) (Šorm *et al*[6]) and propionyl- (VI; R = –Et) derivatives were readily prepared in the same way but in somewhat better yields, as the intermediate monohydroxymethylation products were more soluble in the reaction media employed than in the case of (II; R = Me), thereby facilitating introduction of the second hydroxymethyl-group. Ponndorf reduction of the acetyl-compound (VI; R = Me) employing the experimental conditions used by Šorm *et al.*[6] for the dichloroacetyl analogue (*vide infra*) led to the formation of a gummy product smelling strongly of formaldehyde from which no crystalline material could be obtained. Elimination of a hydroxymethyl group as formaldehyde was likewise observed during reduction of the propionyl-analogue (VI; R = Et) when only DL-*threo*-1-*p*-nitrophenyl-2-propionamidopropane-1:3-diol (V; R = Et; R′ = H) was isolated in low yield. The constitution assigned to this compound was confirmed by comparison with an authentic specimen prepared by propionylation of DL-*threo*-1-*p*-nitrophenylpropane-1:3-diol. Ponndorf reduction of 2-dichloroacetamido-2-*p*-nitrobenzoylpropane-1:3-diol (VI; R = $-CHCl_2$) (Šorm *et al.*[6]) followed an identical pattern with elimination of one hydroxymethyl group and formation of DL-chloramphenicol in 35 per cent. yield. No evidence for concomitant production of "2-hydroxymethylchloramphenicol" was obtained.

The facility with which compounds of type (VI) lose one molecule of formaldehyde during Ponndorf reduction leads us to doubt the validity of the Czech claims. Their identification of "2-hydroxymethylchloramphenicol" was based solely upon analyses for carbon, hydrogen, and nitrogen. The analytical figures for "2-hydroxymethylchloramphenicol," however, are not markedly different from those required by chloramphenicol and thus cannot serve alone to distinguish between the two compounds:—

	Per cent.		
	C	H	N
Hydroxymethylchloramphenicol, $C_{12}H_{14}O_6N_2Cl_2$ ..	40·8	4·0	7·9
Chloramphenicol, $C_{11}H_{12}O_5N_2Cl_2$	40·9	3·8	8·7
Product obtained by Šorm *et al.*	40·5	3·6	7·9

In addition, the melting point of their so-called "hydroxymethylchloramphenicol" was the same as that of chloramphenicol itself, with which their product is almost certainly identical. The term "hydroxymethylchloramphenicol" should not, therefore, be applied to the Ponndorf reduction product of (VI; R = $-CHCl_2$) unless evidence confirming this designation becomes available. Attempts to prevent elimination of formaldehyde from (VI) during Ponndorf reduction by stabilising the structure as the *iso*propylidene derivatives (VII) proved unsuccessful, (VII; R = –Me or –Pr) being recovered unchanged after treatment with aluminium *iso*propoxide in the usual way. No acetone was detectable in the distillates.

Experiments to prepare *N*-methylchloramphenicol (I; H_c replaced by –Me) by extension of the Long and Troutman procedure[10] to *N*-methylphenacylamine[12] and *p*-nitro-*N*-methylphenacylamine proved impracticable, as we were unable to obtain these alternative starting materials in more than minute yield by reaction of methylamine with the corresponding ω-bromoacetophenone. Attention was therefore directed to the *N*-methylation of DL-*threo*-2-amino-1-*p*-nitrophenylpropane-1:3-diol (VIII). Reaction of (VIII) with conventional methylating agents proved unsuccessful, intractable gums being obtained. Employment of methyl *p*-toluenesulphonate gave what appeared to be a dimethyl-product. In an attempt to control the methylation, (VIII) was condensed with anisaldehyde to give the oxazolidine [XI; R = $-C_6H_4OMe$ (*p*)]. The latter passed readily into the quaternary salt on treatment with methyl iodide, but attempts to decompose this product with water led only to liberation of anisaldehyde without concomitant production of the *N*-methylated base. Success was achieved by condensing (VIII) with formaldehyde, when the oxazolidine (IX; R = H) was presumably formed, followed by reduction with formic acid. The latter was then removed and the residue treated with one molar proportion of alkali to saponify the *O*-formyl esters present. DL-*threo*-2-Form-methylamido-1-*p*-nitrophenylpropane-1:3-diol (X; R = –CHO) thus obtained was hydrolysed with hydrochloric acid to give (X; R = H), which was characterised as the picrate and hydrochloride.

Dichloroacetylation of (X; R = H) presented initial difficulty, as boiling with ethyl dichloroacetate in ethanolic solution for 30 hours led to the formation of DL-*threo*-2-dichloroacetmethylamido-1-*p*-nitrophenylpropane-1:3-diol (X; R = $-COCHCl_2$) in only 10 per cent. yield. Pentachloroacetone, previously employed by Fritsch[13] to dichloracetylate aniline, proved more satisfactory, reacting rapidly with (X; R = H) to give DL-*N*-methylchloramphenicol (X; R = $-COCHCl_2$) in very high yield.

Inter alia we examined the possibility of preparing chloramphenicol by aldol condensation between *p*-nitrobenzaldehyde and derivatives of aminoacetaldehyde (XI) to give (XII), followed by Ponndorf reduction to (I). Unfortunately, it proved impossible to stop the initial condensation at the aldol stage (XII), dehydration to (XIII) invariably taking place. 2-Benzamido-*p*-nitrocinnamaldehyde (XIII), obtained in this way,

was reduced by the Ponndorf method to the corresponding cinnamyl alcohol (XIV). Hydrolysis of the latter compound with hydrochloric acid led to the formation of 2-keto-3-*p*-nitrophenylpropyl benzoate(XV), together with a second compound, $C_{17}H_{16}O_4N_2$, which was not fully characterised.

Biological study of the above compounds by Dr. S. W. F. Underhill and his staff failed to reveal marked antibiotic activity.

Discussion

Antibiotic action is now generally thought to be due to interference by the antibiotic in an enzyme system essential to the cellular organism. Enzymes themselves are specific, catalytically-active proteins, which often require association with relatively simple molecules, known as prosthetic groups or coenzymes, for their activity. The terms "prosthetic groups" and "coenzyme" are not, strictly speaking, synonymous. The former designation is usually applied to those groups which are sufficiently firmly bound to the protein to be considered as part of the enzyme molecule. The term "coenzyme," in contrast, is reserved for those cases in which the association is so loose that in solution the enzyme exists largely in the free state. The spatial relationship between the enzyme and its related coenzyme or prosthetic group has been discussed by Pauling, who assumes that the protein molecule forms a polypeptide chain, rolled up and packed and held in a specific shape by hydrogen bonding and other forces between the polypeptide groups and other polar functions. Embedded in this protein matrix lie the coenzyme molecules at such points where a transfer of hydrogen ions to and from the peptide chain can be effected, and closely associated with these lie strategically placed "cavities" which represent the active centres through which the enzymes perform their catalytic functions.

The mechanism of enzyme action may then be pictured as taking place in the following sequence: (i) approach of the metabolite molecule or substrate to the active centre or "cavity," (ii) combination between the metabolite molecule and the active centre, which is preceded by close juxtaposition between certain polar groups present in the substrate with complementary groups present in the enzyme, (iii) occurrence of the specific enzyme reaction, and (iv) release of the modified metabolite. In order to interfere with such an enzyme system, an antimetabolite must fulfil certain conditions. Firstly, it must show close spatial correspondence to at least that part of the metabolite molecule which is in close juxtaposition to complementary polar groups present in the "cavity" of the enzyme. Secondly, it must be able to form a complex with the enzyme which is either irreversible, or else cannot be converted into the normal metabolite transformation product. Compounds which function in this way by blocking the approach of the metabolite to its particular "cavity" in the enzyme molecule are known as "structural analogues antagonistic to metabolites," or more simply, as "antimetabolites." Thus chloramphenicol is thought to function as an antimetabolite by interfering with enzymic transformations undergone by an essential metabolite probably

related to phenylalanine.[14,15] The exact structure of the metabolite has yet to be determined. It is, nevertheless, possible from the present evidence to draw certain conclusions regarding the nature of the spatial relationship between the antibiotic and the active enzymic centre or "cavity" to which its unique structure is so delicately adjusted. Combination between chloramphenicol and enzyme involves all the polar groups of (I) (Part I[1]) which must be in close juxtaposition to complementary groups within the enzyme matrix. Such a situation obtains if the molecule assumes the planar form shown in Figures 1 and 1*a*, the face represented in Figure 1 lying in contact with the enzyme. In this position all the polar groups of (I) are available for complex formation with the enzyme, whilst H_a, H_c, H_d, and H_e are all directed away from the enzyme surface (cf. Fig. 1*a*).

Pronounced regularities in biological activity are often evident in closely related series of compounds. If, therefore, Figure 1 and 1*a* represent the spatial relationship between the antimetabolite and the enzyme matrix, it follows that replacement of H_a, H_c, or H_d, respectively, by methyl should lead to homologues with biological activity. Work outlined in the preceding section, however, shows clearly that replacement of H_a, H_b, H_c, or H_d by methyl leads, in all cases, to loss of antibiotic action. These facts point to the conclusion that combination between antimetabolite and enzyme is such that H_a, H_b, H_c, and H_d are *embedded within* the protein matrix. If this is indeed the case, it follows that their replacement by methyl must hinder close juxtaposition between antimetabolite and enzyme, and thus destroy the basis upon which antibiotic action depends.

It is, of course, possible to picture the situation described in the foregoing paragraph in terms of the spatial configuration represented in Figures 1 and 1*a*. At the same time the facts are better accommodated by means of the "buckled" structure represented in Figures 2 and 2*a*, in which the propane side chains of the antibiotic, and hence H_a, H_b, H_c, and H_d are assumed to lie within the body of the enzyme. The conclusions thus reached on biological grounds gain added significance from studies described by Dunitz[16] on the crystal structure of chloramphenicol. His results show that (I) exists in the crystal state in a spatial form almost identical with that shown in Figures 2 and 2*a* and that this configuration is especially suitable for interaction with the polar groups of a protein chain.

Somewhat different considerations apply to the *p*-nitrophenyl portion of the molecule. In contrast to the propanediol side chain, important but limited changes in structure may be effected in this part of the molecule with retention of biological activity. Thus replacement by *p*-chlorophenyl-,[17] *p*-dichloroacetylamido-,[18] 4′-nitro-4-biphenyl-,[19] 4-nitro-1-naphthyl-,[20] etc., leads to compounds with some antibiotic action. Whilst, therefore, the propanediol side chain represents a *specific* pharmacodynamical portion of the molecule, the nitro(phenyl)-group represents a relatively *non-specific* pharmacodynamical structure. The relative non-specificity of the *p*-nitro(phenyl)-portion of the molecule is best

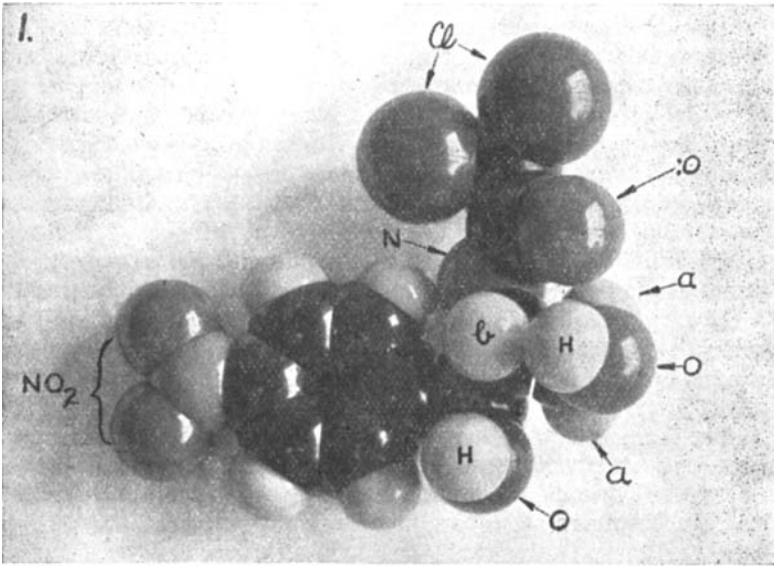

FIG. 1.

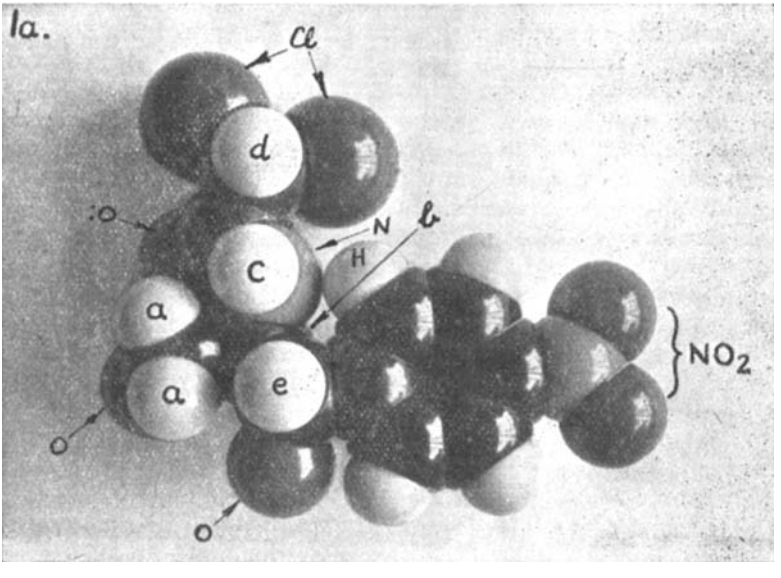

FIG. 1*a*.

interpreted by the assumption that this part of the antimetabolite is not embedded within the protein matrix but held upon its surface. The *p*-nitro(phenyl) group may therefore be likened to a grappling hook

which holds the antibiotic to the surface of the enzyme. Its structural requirements will therefore be less rigid than those required by the propanediol side-chain which lies embedded in the protein matrix. The

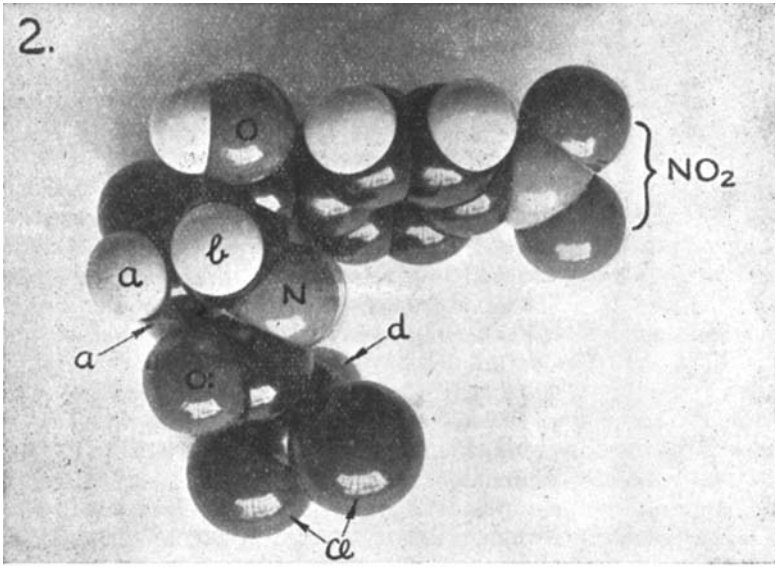

FIG 2.

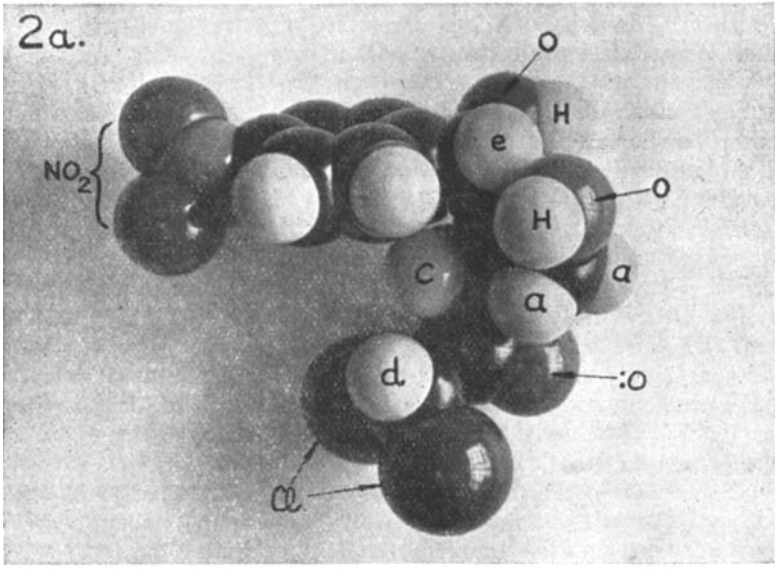

FIG. 2*a*.

concepts formulated above thus imply a fundamental difference in the functions associated with specific and non-specific pharmacodynamical groups. It is therefore suggested that these contrasting types be differentiated by use of the prefixes σ (specific) and υ (non-specific).

EXPERIMENTAL

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

1-*Acetamido*-2-*hydroxy*-p-*nitrobutyrophenone* (III; R = R′ = Me).—A mixture of ω-acetamido-*p*-nitroacetophenone (20·0 g.), ethanol (95 per cent.) (80 ml.), acetaldehyde (8·0 g.) and water (16 ml.) was stirred mechanically at 35° C. when sodium bicarbonate (1·3 g.) was added. Stirring at 35° C. was continued for a further 2 hours, when the mixture was cooled to 5° C. The separated solids were collected and crystallised from ethanol to give 1-*acetamido*-2-*hydroxy*-p-*nitrobutyrophenone*, needles, m.pt. 192° to 194° C. Found: C, 54·1; H, 5·2; N, 10·5. $C_{12}H_{14}O_5N_2$ requires C, 54·1; H, 5·3; N, 10·5 per cent.

Ponndorf reduction of 1-*acetamido*-2-*hydroxy*-p-*nitrobutyrophenone*.—A suspension of the foregoing compound (98 g.) in *iso*propanol (1900 ml.) containing redistilled aluminium *iso*propoxide (120 g.) was stirred for 5 days at room temperature. The red mixture was treated with water (400 ml.), stirred for 24 hours, filtered through a pad of filter aid, and the filtrate concentrated *in vacuo*. Separation of yellow granular solids occurred when the volume had been reduced to *ca.* 150 ml. The material (*Fraction A*; 19 g., m.pt. 145° C.) was removed, the filtrate taken to dryness, and the residue treated with ethyl acetate (100 ml.). The insoluble portion (*Fraction B*; 8·7 g., m.pt. 180° C.) was collected and the mother liquors concentrated to half bulk to give more solids (*Fraction C*; 4·5 g., m.pt. 150° C.).

Purification of *Fraction A* from ethyl acetate gave needles of 2-acetamido-1-*p*-nitrophenylethan-1-ol, m.pt. 160° C. (not depressed in admixture with an authentic specimen[4]). Found: C, 53·8; H, 4·7; N, 12·9. Calculated for $C_{10}H_{12}O_4N_2$: C, 53·6; H, 5·4; N, 12·5 per cent.

Fraction B was treated with hot ethyl acetate (60 ml.) and the insoluble portion crystallised from methanol-ethyl acetate-light petroleum. "β"-2-*Acetamido*-1-p-*nitrophenylbutane*-1:3-*diol* (7·7 g.) was obtained in plates, m.pt. 195° C. Found: C, 53·2; H, 6·1; N, 10·4. $C_{12}H_{16}O_5N_2$ requires C, 53·7; H, 6·0; N, 10·4 per cent.

Fraction C was extracted with hot ethyl acetate (40 ml.). Purification of the insoluble fraction gave a further quantity (2·5 g.) of the compound, m.pt. 195° C. The ethyl acetate extract, on cooling, deposited the compound m.pt. 160° C.

"β"-2-*Amino*-1-p-*nitrophenylbutane*-1:3-*diol*.—The acetyl-derivative (1·5 g.) in 6N hydrochloric acid (10 ml.) was heated under reflux for 1 hour and the solution (charcoal) taken to dryness *in vacuo*. The residue was dissolved in water (5 ml.) and treated with 2N sodium hydroxide to *p*H 12. Crystallisation of the precipitate from water gave

"β"-2-*amino*-1-p-*nitrophenylbutane*-1:3-*diol* as needles, m.pt. 155° to 156° C. Found: C, 53·0; H, 6·0; N, 12·4. $C_{10}H_{14}O_4N_2$ requires C, 53·1; H, 6·2; N, 12·4 per cent.

"β"-2-*Dichloroacetamido*-1-p-*nitrophenylbutane*-1:3-*diol* crystallised from ethyl acetate-light petroleum in plates, m.pt. 109° to 110°C. Found: C, 42·6; H, 4·5; N, 8·0; Cl, 19·1. $C_{12}H_{14}O_5N_2Cl_2$ requires C, 42·7; H, 4·2; N, 8·3; Cl, 21·0 per cent.

1-*Dichloroacetamido*-2-*hydroxy*-p-*nitrophenylbutyrophenone* (III; R = $-CHCl_2$; R′ = Me), formed prismatic needles, m.pt. 170° to 172° C. after crystallisation from methanol. Found: C, 42·9; H, 3·6; N, 8·0; Cl, 19·9. $C_{12}H_{12}O_5N_2Cl_2$ requires C, 43·0; H, 3·6; N, 8·4; Cl, 21·2 per cent.

Ponndorf reduction at room temperature yielded 1-dichloroacetamido-*p*-nitroacetophenone (II; R = $-CHCl_2$), needles, m.pt. 146° to 147° C., after crystallisation from ethyl acetate-hexane. Found: C, 41·5; H, 3·0; N, 9·3; Cl, 23·8. Calculated for $C_{10}H_8O_4N_2Cl_2$: C, 41·2; H, 2·7; N, 9·6; Cl, 24·4 per cent. The m.pt. was not depressed in admixture with an authentic specimen.[21]

"γ"-2-*Amino*-1-p-*nitrophenylbutane*-1:3-*diol*.—A solution of "β"-2-acetamido-1-*p*-nitrophenylbutane-1:3-diol (V; R = R′ = –Me) (5 g.) in redistilled thionyl chloride (15 ml.) was kept for 1 hour at room temperature, when it was treated with water (15 ml.) added drop by drop. The mixture was heated for 1 hour on the steam bath and the solution (charcoal) made alkaline with 20 per cent. aqueous sodium hydroxide. The crystalline solids (2·0 g.) were purified from hot water giving "γ"-2-*amino*-1-p-*nitrophenylbutane*-1:3-*diol* in needles, m.pt. 109° to 110° C. Found: C, 53·4; H, 6·3; N, 12·9. $C_{10}H_{14}O_4N_2$ requires C, 53·1; H, 6·2; N, 12·4 per cent.

"γ"-2-*Dichloroacetamido*-1-p-*nitrophenylbutane*-1:3-*diol* formed rhombs, m.pt. 128° C. after crystallisation from ethanol-light petroleum. Found: C, 42·6; H, 4·4; N, 8·8; Cl, 21·2. $C_{12}H_{14}O_5N_2Cl_2$ requires C, 42·7; H, 4·2; N, 8·3; Cl, 21·0 per cent.

1-*Acetamido*-2-*hydroxy*-3:3:3-*trichloro*-p-*nitrobutyrophenone* (III; R = –Me; R′ = $-CCl_3$), after crystallisation from methanol, was obtained (60 per cent.) in glistening needles, m.pt. 180° to 181° C. (decomp.). Found: C, 39·0; H, 3·2; N, 7·5; Cl, 28·3. $C_{12}H_{11}O_5N_2Cl_3$ requires C, 39·0; H, 3·0; N, 7·6; Cl, 28·8 per cent.

2-*Acetamido*-1-p-*nitrophenyl*-4:4:4-*trichlorobutane*-1:3-*diol* (V; R = –Me; R′ = $-CCl_3$) was obtained (25 per cent.) by Ponndorf reduction of the foregoing compound. After crystallisation from ethyl acetate it formed plates, m.pt. 188° to 189° C. Found: C, 39·1; H, 3·5; N, 7·6; Cl, 28·4. $C_{12}H_{13}O_5N_2Cl_3$ requires C, 38·8; H, 3·5; N, 7·5; Cl, 28·7 per cent.

2-*Amino*-1-p-*nitrophenyl*-4:4:4-*trichlorobutane*-1:3-*diol* was obtained by hydrolysis of the foregoing compound with 7·5 per cent. hydrochloric acid for 3 hours on the steam bath. After crystallisation from ethyl acetate-hexane it formed needles, m.pt. 149° to 150° C. Found: C, 36·8; H, 3·6; N, 8·5; Cl, 31·7. $C_{10}H_{11}O_4N_2Cl_3$ requires C, 36·4; H, 3·3; N, 8·5; Cl, 32·3 per cent.

2-*Dichloroacetamido*-1-p-*nitrophenyl*-4:4:4-*trichlorobutane*-1:3-*diol* (V; R = $-CHCl_2$; R′ = $-CCl_3$), prismatic needles, m.p. 174° to 175° C., after crystallisation from ethyl acetate. Found: C, 32·9; H, 2·5; N, 5·6; Cl, 40·3. $C_{12}H_{11}O_5N_2Cl_5$ requires C, 32·7; H, 2·5; N, 6·3; Cl, 40·3 per cent.

2-*Phenyl*-1-*acetamido*-2-*hydroxy*-p-*nitropropiophenone* (III; R = Me; R′ = Ph) was purified from methanol to give (25 per cent.) prismatic needles, m.pt. 172° to 173° C. Found: C, 62·8; H, 4·9; N, 9·0. $C_{17}H_{16}O_5N_2$ requires C, 62·2; H, 4·9; N, 8·5 per cent.

2-p-*Nitrophenyl*-1-*acetamido*-2-*hydroxy*-p-*nitropropiophenone* (III; R = Me; R′ = $-C_6H_4NO_2$) separated from ethanol in needles, m.pt. 175° C. Found: C, 54·0; H, 4·0; N, 11·4. $C_{17}H_{15}O_7N_3$ requires C, 54·7; H, 4·0; N, 11·3.

2-p-*Nitrophenyl*-1-*dichloroacetamido*-2-*hydroxy*-p-*nitropropiophenone* (III; R = $-CHCl_2$; R′ = $-C_6H_4NO_2$), after crystallisation from a large volume of ethanol, formed pale yellow leaflets, m.pt. 211° C. Found: C, 47·0; H, 2·7; N, 9·3. $C_{17}H_{13}O_7N_3Cl_2$ requires C, 46·2; H, 2·9; N, 9·5 per cent.

ω-Dichloroacetamidoacetophenone was prepared from the tin salt complex of ω-aminoacetophenone by the procedure of Long and Troutman.[10] Recrystallisation from light petroleum afforded needles, m.pt. 122° to 123° C. Found: C, 49·1; H, 3·5; N, 5·6; Cl, 28·7. $C_{10}H_9O_2NCl_2$ requires C, 48·8; H, 3·7; N, 5·7; Cl, 28·9 per cent.

1-*Dichloroacetamido*-2-*hydroxy*-2-p-*nitrophenylpropiophenone*, prepared by treating ω-dichloroacetamidoacetophenone (600 mg.) and *p*-nitrobenzaldehyde (370 mg.) in ethanolic solution with 1 drop of piperidine, was purified from methanol to form crystals (80 per cent.), m.pt. 174° to 175° C. Found: C, 51·5; H, 3·8; N, 6·4; Cl, 16·6. $C_{17}H_{14}O_5N_2Cl_2$ requires C, 51·4; H, 3·5; N, 7·0; Cl, 17·9 per cent.

1-*Acetamido*-2-*hydroxy*-2-p-*nitrophenylpropiophenone* formed yellow crystals, m.pt. 171° to 172° C. Found: C, 61·9; H, 5·0; N, 8·7. $C_{17}H_{16}O_5N_2$ requires C, 62·2; H, 4·9; N, 8·5 per cent.

2-*Acetamido*-2-p-*nitrobenzoylpropane*-1:3-*diol* (VI; R = Me).—ω-Acetamido-*p*-nitroacetophenone (22·2 g.) in hot ethanol (100 ml.), was treated with 36 per cent. solution of formaldehyde (100 ml.; *ca.* 12 mole), the solution cooled to 45° C., and sodium bicarbonate (1·0 g.) added. After standing for 2 days at room temperature, the dark-coloured mixture was saturated with salt and the product extracted with ethyl acetate. Crystallisation from ethanol-light petroleum (b.pt. 60° to 80° C.) furnished 2-*acetamido*-2-p-*nitrobenzoylpropane*-1:3-*diol* as needles, m.pt. 151° to 154° C. Found: C, 50·7; H, 5·3; N, 9·9. $C_{12}H_{14}O_6N_2$ requires C, 51·1; H, 5·0; N, 9·3 per cent. The m.pt. varies somewhat with the rate of heating.

The iso*propylidene* derivative (VII; R = Me) was prepared by shaking the foregoing compound (2·2 g.) in dry acetone with phosphorus pentoxide (2·0 g.) for 30 minutes at room temperature. After decanting from the gum and shaking with solid sodium carbonate until neutral, the mixture was concentrated and diluted with water. The product,

after crystallisation from methanol, formed needles, m.pt. 199° to 200° C. Found: C, 56·1; H, 5·7. $C_{15}H_{18}O_6N_2$ requires C, 55·9; H, 5·6 per cent.

ω-Propionamido-p-*nitroacetophenone* (II; R = Et). ω-Amino-*p*-nitroacetophenone hydrochloride (from 200 g. of *p*-nitroacetophenone) was thoroughly stirred with water (2 l.) for 10 minutes when propionic anhydride (260 g.) followed by sodium acetate trihydrate (240 g.) in water was added. The *product*, collected after stirring for 2 hours, was recrystallised from ethyl acetate forming cream needles (132 g.), m.pt. 128° C. Found: C, 55·5; H, 5·1; N, 12·1. $C_{11}H_{12}O_4N_2$ requires C, 55·9; H, 5·1; N, 11·9 per cent.

2-Hydroxy-1-propionamido-p-*nitropropiophenone*, prepared from the foregoing compound, was purified from chloroform-light petroleum, forming prismatic needles, m.pt. 116° C. Found: C, 54·0; H, 5·3; N, 10·1. $C_{12}H_{14}O_5N_2$ requires C, 54·1; H, 5·3; N, 10·5 per cent.

2-p-*Nitrobenzoyl-2-propionamidopropane-1:3-diol* (VI; R = Et), prepared (48 per cent.) by heating ω-propionamido-*p*-nitroacetophenone (5 g.), ethanol (20 ml.), solution of formaldehyde (7 ml. of 37 per cent.; *ca.* 4 moles) and sodium bicarbonate (200 mg.) for 2 hours at 40° C., crystallised from benzene-methanol in prismatic needles, m.pt. 172° to 174° C. Found: C, 53·3; H, 5·2; N, 9·5. $C_{13}H_{16}O_6N_2$ requires C, 52·7; H, 5·4; N, 9·5 per cent.

The iso*propylidene* derivative, after crystallisation from ethanol-light petroleum, formed rosettes of felted needles, m.pt. 169° to 170° C. Found: C, 57·2; H, 5·6; N, 8·3. $C_{16}H_{20}O_6N_2$ requires C, 57·2; H, 5·8; N, 8·3 per cent.

Ponndorf reduction of (VI; R = Et) gave DL-*threo*-1-p-*nitrophenyl-2-propionamidopropane-1:3-diol*, fibrous needles, m.pt. 131° to 132° C., after crystallisation from ethyl acetate. Found: C, 53·3; H, 5·8. $C_{12}H_{16}O_5N_2$ requires C, 53·7; H, 6·0. The compound thus obtained gave no depression of m.pt. in admixture with a sample prepared in the following way. DL-*threo*-2-Amino-1-*p*-nitrophenylpropane-1:3-diol (3·8 g.), suspended in a mixture of ethyl acetate (12 ml.) and ethanol (3·8 ml.), was treated with propionic anhydride (9·6 ml.) and warmed to boiling point. After allowing to cool, the product was collected and crystallised from ethyl acetate, to give needles, m.pt. 132° C. Found: C, 53·3; H, 5·8; N, 10·3. $C_{12}H_{16}O_5N_2$ requires C, 53·7; H, 6·0; N, 10·5 per cent.

2-Dichloroacetamido-2-p-*nitrobenzoylpropane-1:3-diol* (VI; R = $-CHCl_2$) was recrystallised from benzene-methanol to give feathery needles, m.pt. 136° to 137° C. Found: C, 41·2; H, 3·6; N, 7·8; Cl, 20·2. Calc. for $C_{12}H_{12}O_6N_2Cl_2$: C, 41·0; H, 3·4; N, 8·0; Cl, 20·2 per cent. Šorm *et al.*[6] give m.pt. 134° C.

Ponndorf reduction of (VI; R = $-CHCl_2$) furnished DL-chloramphenicol, identified by m.pt. and mixed m.pt. with an authentic specimen.

DL-threo-1-p-*Nitrophenyl*-2-p-*toluenesulphonamidopropane-1:3-diol*, prepared by heating the DL-*threo*-amine (6·3 g.), tosyl chloride (6·0 g.), sodium acetate (3·0 g.) and ethanol (40 ml.) under reflux for 1 hour, was crystallised from ethyl acetate-methanol to give silvery platelets,

m.pt. 210° C. Found: C, 51·9; H, 4·9; N, 7·8; S, 8·6. $C_{16}H_{18}O_6N_2S$ requires C, 52·5; H, 4·9; N, 7·7; S, 8·7 per cent.

*O-Formyl-*DL*-chloramphenicol*, prepared by heating DL-chloramphenicol (1 g.) with formic acid (10 ml.) under reflux for 1 hour, crystallised from aqueous ethanol in flat needles, m.pt. 146° C. Found: C, 41·0; H, 3·4; N, 8·1; Cl, 20·0. $C_{12}H_{12}O_6N_2Cl_2$ requires C, 41·0; H, 3·4; N, 7·9; Cl, 20·2 per cent.

4-p-*Nitrophenyl-5-dichloroacetamido-*1 : 3-*dioxane* was prepared by heating DL-chloramphenicol (1 g.) with solution of formaldehyde (2 ml. of 36 per cent.) and formic acid (10 ml.) for 1 hour on the steam bath. After crystallisation from aqueous ethanol it formed long flat needles, m.pt. 147° to 148° C. Found: C, 42·9; H, 3·5; N, 8·5; Cl, 21·1. $C_{12}H_{12}O_5N_2Cl_2$ requires C, 43·0; H, 3·6; N, 8·4; Cl, 21·2 per cent.

DL-threo-2-*Form-methylamido-*1-p-*nitrophenylpropane-*1 : 3-*diol* (X; R = -CHO).—The DL-*threo*-amine (10 g.) was covered with ethanol and then treated with solution of formaldehyde (5 ml. of 36 per cent., 1 mol.), when immediate reaction occurred with dissolution. After heating for 10 minutes at 100° C., formic acid (50 ml.) was added and heating continued for a further 90 minutes. After evaporation of most of the formic acid the residue was dissolved in water (50 ml.) and neutralised with potassium carbonate. Potassium hydroxide (2·5 g.) was then added and the mixture heated to saponify any *O*-formates present. After allowing to cool the product was collected and crystallised from ethyl acetate-methanol. DL-threo-2-*Form-methylamido-*1-p-*nitrophenylpropane-*1 : 3-*diol* (3·2 g.) formed cream prisms, m.pt. 196° C. Found: C, 51·4; H, 5·4; N, 11·1. $C_{11}H_{14}O_5N_2$ requires C, 52·0; H, 5·5; N, 11·0 per cent.

DL-threo-2-*Methylamino-*1-p-*nitrophenylpropane-*1 : 3-*diol hydrochloride*, prepared by hydrolysis of the foregoing compound (2·8 g.) with 4 N hydrochloric acid (30 ml.) for 1 hour at 100° C., formed needles (2·2 g.), m.pt. 176° to 177° C., after crystallisation from ethyl acetate-methanol. Found: C, 45·3; H, 5·7; N, 10·5; Cl, 13·6. $C_{10}H_{14}O_4N_2 \cdot HCl$ requires C, 45·7; H, 5·7; N, 10·7; Cl, 13·5 per cent.

The corresponding *base* (X; R = H) was crystallised from ethyl acetate forming prisms, m.pt. 107° C. Found: C, 53·1; H, 6·2; N, 12·4. $C_{10}H_{14}O_4N_2$ requires C, 53·1; H, 6·3; N, 12·4 per cent. Its *picrate* separated from water in flat yellow needles, m.pt. 186° to 187° C. Found: C, 42·1; H, 4·0; N, 15·1. $C_{10}H_{14}O_4N_2$ requires C, 42·2; H, 3·7; N, 15·4 per cent.

DL-threo-2-*Dichloroacetmethylamido-*1-p-*nitrophenylpropane-*1 : 3-*diol* (X; R = $-COCHCl_3$) was prepared by treating the corresponding amine (1·0 g.), suspended in cold ethanol (10 ml.), with pentachloroacetone/tetrachloroacetone (3·5 g. of 33 per cent. pentachloroacetone; 1 mole) when a vigorous reaction took place. After 15 minutes, light petroleum (20 ml.) was added and the product collected after 24 hours at 0° C. Purification from ethanol-light petroleum gave prisms, m.pt. 141° to 142° C. Found: C, 42.6; H, 4·0; N, 8·0; Cl, 20·6. $C_{12}H_{14}O_5N_2Cl_2$ requires C, 42·7; H, 4·2; N, 8·3; Cl, 21·1 per cent.

Phthalimido-acetal.—Potassium phthalimide (50 g.), bromoacetal (50 g.)

and potassium iodide (50 g.) were heated together in ethylene glycol (150 ml.) for 4 hours at 145° C. The mixture was poured into water and extracted with ether. The residue left after evaporation partially crystallised. The crystalline material was separated with the aid of light petroleum and the filtrate distilled. After a small forerun of bromoacetal, *phthalimido-acetal* was collected at 180° C./0·5 mm. It was combined with the crystalline material and purified from light petroleum to give plates (30·0 g.), m.pt. 69° C. Found: C, 63·9; H, 6·5. $C_{14}H_{17}O_4N$ requires C, 64·0; H, 6·5 per cent.

Phthalimidoacetaldehyde.—(i) The foregoing compound (10 g.) and N hydrochloric acid (60 ml.) were heated at 100° C. for 20 minutes with stirring. The product crystallised on cooling. It was collected (7 g.) and purified from chloroform-light petroleum, m.pt. 112° C.

(ii) Phthalylglycyl chloride (100 g.), dry xylene (500 ml.), 5 per cent. palladium-barium sulphate catalyst and quinoline-sulphur catalyst poison[22] (1 ml.) were heated under reflux with stirring in a stream of hydrogen for 10 hours. The solution was filtered hot and cooled to 0° C. *Phthalmidoacetaldehyde* (45 g.) separated, a second crop (19 g.) being obtained by evaporation, m.pt. 111° C. Total yield 75 per cent. (cf. Radde[23]).

Phthalimidoacetaldehyde diacetate, prepared by treating the aldehyde (500 mg.) in cold acetic anhydride (5 ml.) with concentrated sulphuric acid (2 drops) for 12 hours at room temperature, was crystallised from chloroform-light petroleum to give prisms, m.pt. 128° to 129° C. Found: N, 4·7. $C_{14}H_{13}O_6N$ requires N, 4·8 per cent.

Acetamidoacetaldehyde.—A cooled solution of aminoacetal (6·6 g.) in ether (30 ml.) was treated with acetic anhydride (6·5 g.) in ether (10 ml.) with stirring. After 1 hour the solution was evaporated to dryness under reduced pressure and the residue distilled at 10 mm. Acetamidoacetal (7·3 g.) was obtained as a colourless oil, b.pt. 136° to 138° C./10 mm. Found: C, 54·6; H, 9·6; N, 8·2. $C_8H_{17}O_3N$ requires C, 54·9; H, 9·7; N, 8·0 per cent.

Addition of a small quantity of acetamidoacetal to 2:4-dinitrophenylhydrazine in 5 N hydrochloric acid led to separation of *acetamidoacetaldehyde 2:4-dinitrophenylhydrazone*, soft golden needles, m.pt. 182° C., after crystallisation from ethanol. Found: C, 42·9; H, 3·9; N, 25·0. $C_{10}H_{11}O_5N_5$ requires C, 42·7; H, 3·9; N, 24·9 per cent.

Attempts to liberate acetamidoacetaldehyde from its acetal proved unsuccessful owing to decomposition. Attempts to condense the crude material with *p*-nitrobenzaldehyde likewise proved unsuccessful.

Toluenesulphonamidoacetal was prepared by treating aminoacetal (2·6 g.) in dry pyridine with *p*-toluenesulphonylchloride (3·8 g.) added in portions with ice-cooling, followed by reaction at room temperature overnight. After crystallisation from ether-light petroleum, it formed flat needles, m.pt. 68° to 69° C. Found: C, 53·4; H, 6·8; N, 5·0; S, 11·5. $C_{13}H_{21}O_4NS$ requires C, 54·0; H, 7·3; N, 4·9; S, 11·1 per cent.

Toluenesulphonamidoacetaldehyde 2:4-dinitrophenylhydrazone, after

crystallisation from ethanol, formed yellow leaflets, m.pt. 184° to 185° C. Found: N, 17·3. $C_{15}H_{15}O_6N_5S$ requires N, 17·8 per cent.

Pivalyl-aminodiethylacetal.—Aminodiethylacetal (5·4 g.) in ether (35 ml.) was treated with 3 ml. of a solution of potassium hydroxide (6·5 g.) in water (15 ml.) with stirring and cooling at 0°. Pivalyl chloride (5 g.) and the remainder of the potassium hydroxide solution were added drop by drop simultaneously over 10 minutes with cooling and stirring. The mixture was stirred at room temperature for 4 hours. The ethereal layer was separated, washed twice with saturated sodium chloride solution, dried and the ether removed. The residual solids (8·04 g.) were crystallised from light petroleum (b.pt. 40° to 60° C.) at −70° C. to give pivalyl-aminodiethylacetal, needles, m.pt. 43° to 44° C. Found: C, 60·6; H, 10·6; N, 6·5. Calc. for $C_{11}H_{23}O_3N$: C, 60·8; H, 10·6; N, 6·5 per cent.

Attempts to hydrolyse the acetal to the corresponding aldehyde proved unsuccessful.

p-*Nitrobenzylidene-phthalimidoacetaldehyde* (cf. XIII).—Phthalimido-acetaldehyde (950 mg.) and *p*-nitrobenzaldehyde (750 mg.), dissolved in ethanol (8 ml.), were treated with 2 drops of piperidine. After allowing to evaporate spontaneously at room temperature for 7 days, the product (90 mg.) was collected and purified from ethanol. *p-Nitrobenzylidene-phthalimidoacetaldehyde* formed cream needles, m.pt. 176° to 177° C. Found: C, 63·3; H, 3·2; N, 8·7. $C_{17}H_{10}O_5N_2$ requires C, 63·3; H, 3·1; N, 8·4 per cent.

Replacement of piperidine by other basic catalysts failed to give products which could be identified.

2-*Benzamido*-p-*nitrocinnamaldehyde* (XIII).—Benzamidoacetaldehyde[24] (820 mg.) and *p*-nitrobenzaldehyde (750 mg.) in ethanol (5 ml.) containing collidine (4 drops) were heated under reflux for 7½ hours. After allowing to cool the separated solids (56 per cent.) were collected and purified from chloroform. 2-*Benzamido*-p-*nitrocinnamaldehyde* formed yellow micro-crystals, m.pt. 193° C. Found: C, 64·8; H, 4·2; N, 9·6. $C_{16}H_{12}O_4N_2$ requires C, 64·9; H, 4·1; N, 9·5 per cent.

The *semicarbazone*, after crystallisation from methanol, formed felted needles, m.pt. 210° to 225° C. (decomp.). Found: N, 19·6. $C_{17}H_{15}O_4N_5$ requires 19·8 per cent.

2-*Benzamido*-p-*nitrocinnamyl alcohol* (XIII) was prepared by Ponndorf reduction of the foregoing compound. After crystallisation from benzene it formed microneedles, m.pt. 175° C. Found: C, 64·3; H, 4·8. $C_{16}H_{14}O_4N_2$ requires C, 64·4; H, 4·7 per cent.

The *acetate* was crystallised from aqueous ethanol to form silky needles, m.pt. 145° C. Found: C, 63·6; H, 4·5; N, 8·1. $C_{18}H_{16}O_5N_2$ requires C, 63·5; H, 4·7; N, 8·2 per cent.

Hydrolysis of (XIII).—the foregoing compound (1·0 g.) in ethanol was heated under reflux with concentrated hydrochloric acid (0·5 ml.) for 30 minutes. After allowing to cool the separated solids were removed (*fraction A*) and the filtrate neutralised with sodium bicarbonate when a precipitate (*fraction B*) was obtained.

Purification of *fraction A* from aqueous acetone gave 2-*keto*-3-p-*nitrophenylpropyl benzoate*, plates, m.pt. 162° to 163° C. Found: C, 64·7; H, 4·8; N, 4·3. $C_{16}H_{13}O_5N$ requires C, 64·2; H, 4·3; N, 4·7 per cent. The compound formed an oxime, m.pt. 121° to 123° C., after crystallisation from aqueous ethanol. Alkaline hydrolysis led to the formation of benzoic acid.

Purification of *fraction B* from aqueous methanol gave brown prisms, m.pt. 121° C. Found: C, 65·3; H, 4·6; N, 8·9. $C_{17}H_{16}O_4N_2$ requires C, 65·4; H, 5·1; N, 9·0 per cent.

Summary and Conclusions

1. The preparation and biological activity of some methyl-homologues of chloramphenicol are described.

2. It is concluded that the pharmacodynamical groups present in the antibiotic fall into two categories (i) specific, and (ii) relatively non-specific, designated herein as σ- and ν-groups respectively.

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DISCUSSION

The paper was presented by DR. F. HARTLEY.

MR. D. E. SEYMOUR (Welwyn) asked for more details of the biological screening tests which had been carried out to determine the activity of the various compounds. He thought it would also be interesting to know whether the reduction of the nitro-group of chloramphenicol would alter its activity, particularly to *M. tuberculosis*.

DR. A. H. BECKETT (London) welcomed the authors' emphasis on the stereochemical configuration, and asked whether the physicochemical properties of the substances had been dealt with before the stereochemical mechanism was postulated.

DR. F. HARTLEY, in reply, said that in Part I of this series of papers the general procedure for the biological examination of the compounds had been indicated. The majority had been tested against *M. tuberculosis* as well as *Entamœba histolytica*. The range of organisms in the later work had been extended to include *B. coli*, *B. dysenterica*, and *B. typhosus*. In each case the activity fell from about 1 in 125,000 to 1 in 4,000 (bacteriostatic) and from about 1 in 20,000 to about 1 in 1000 (bactericidal). With regard to the nitrogroup, many would like to see some means of removing this radical. In acylated reduced nitro-compounds some activity was retained and also in the iodo-compound, but not in the corresponding chloro- and bromo-compounds. The stereochemical problem was intriguing. The puzzling feature was that 3-methylchloramphenicol had 4 possible DL forms, but only 3 had so far revealed themselves. The form isolated in America had, in the present authors' view, a "*threo*" configuration on the basis of the von Auwers-Skita generalisation regarding catalytic hydrogenation. The physico-chemical properties of the β-isomer now isolated were in line with those expected of an "*erythro*" form, in particular the solubility was greater than that of the γ-stereoisomer resulting on treatment with thionyl chloride. Although the "*erythro*" compounds in this series were in general more soluble than the "*threo*" compounds it was not easy to see why that should be so.