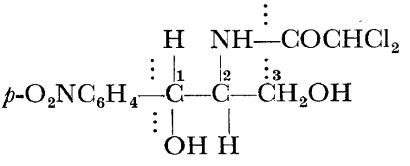
SYNTHETIC APPROACH TO THE STRUCTURE–ACTIVITY RELATION OF SOME ANTIBIOTICS

M. M. Shemyakin and M. N. Kolosov

Institute for Chemistry of Natural Products, U.S.S.R., Academy of Sciences, Moscow

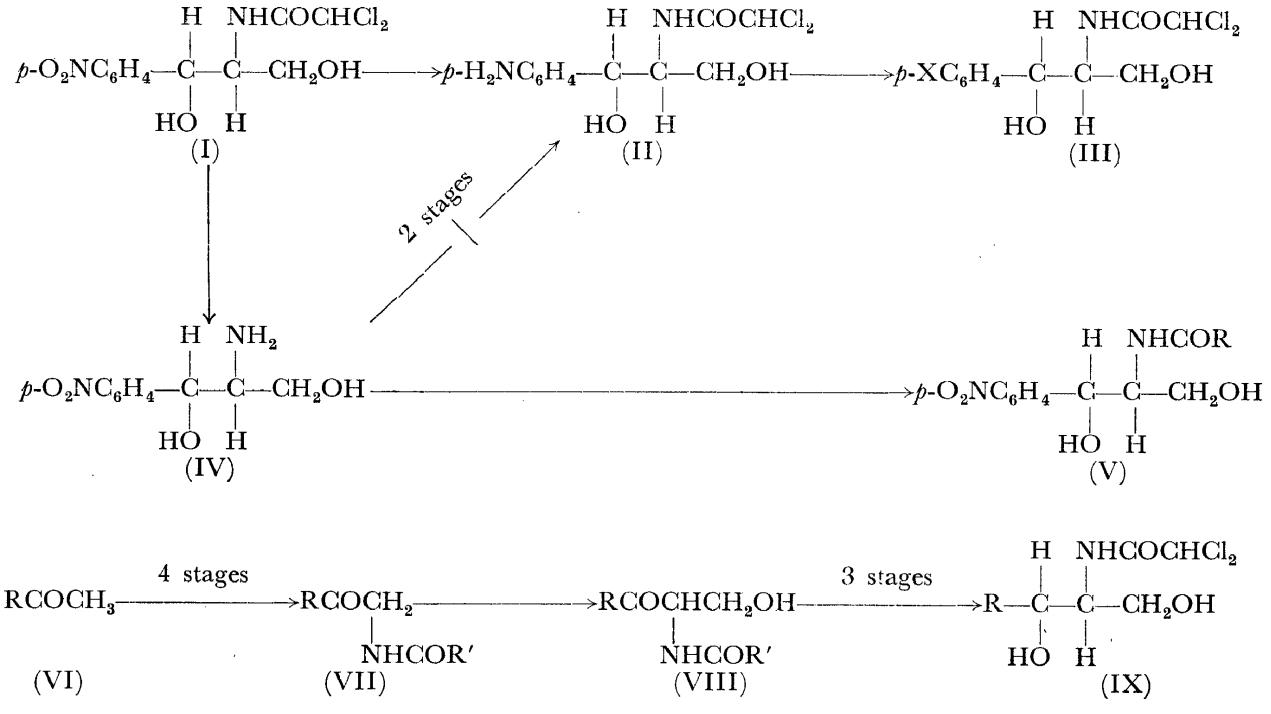
A major objective in antibiotics research (as in the case of other drugs) is to elucidate the rôle played by each of the structural elements in the manifestation of the biological activity of the molecules. Such study not only facilitates the search for new, more valuable therapeutic agents, but is a necessary step in ascertaining the mode of antibiotic action, *i.e.* in solving the problem of how the antibiotic molecule interacts with a particular enzyme or other substance of the microbial cell, inhibiting this or that reaction in the normal metabolic sequence. Quite obviously, one may determine which functional groups of the antibiotic are responsible for its activity and acquire knowledge of the nature of its interaction with a given constituent of the microbial cell by thorough study of the chemistry, especially the stereospecific synthesis, of the antibiotic and of its analogues.

Since one might expect (although not always) more rapid results in the case of simpler molecules, our attention was first centred upon chloramphenicol (I) of which the synthesis of various analogues with predetermined configuration presented no great difficulties. Three types of analogues were required: with an altered aminopropanediol chain, with an altered *p*-nitrophenyl radical and with an altered dichloroacetyl residue.



(I)

Manifold analogues of the first type have been synthesized in widely different ways by numerous investigators since 1949 (for references see the monograph[1]). For analogues of the second type (with altered *p*-nitrophenyl radical) we[2–6] have developed a general synthetic route (I or IV) → (II) → (III), affording compounds with known configuration: here the amino group in compound (II) may be easily substituted by the most varied functions *via* the diazo group, condensation reactions, and other means. Another method of preparing these analogues (VI) → (VII) → (VIII) → (IX) was first proposed by Long and Troutman[7]. This is more general than the first method but the configurations of the resultant compounds are less certain. As for the third type of chloramphenicol analogues (with modified acyl residue), their synthesis (IV) → (V) presents no difficulties[1].


H NHCOCHCl2
p-O2NC6H4—C—C—CH2OH
HO H
(I)
p-H2NC6H4—C—C—CH2OH
(II)
p-XC6H4—C—C—CH2OH
(III)
2 stages
H NH2
p-O2NC6H4—C—C—CH2OH
(IV)
H NHCOR
p-O2NC6H4—C—C—CH2OH
(V)
4 stages
RCOCH3
RCOCH2
NHCOR'
(VI)
(VII)
RCOCHCH2OH
NHCOR'
(VIII)
3 stages
R—C—C—CH2OH
(IX)

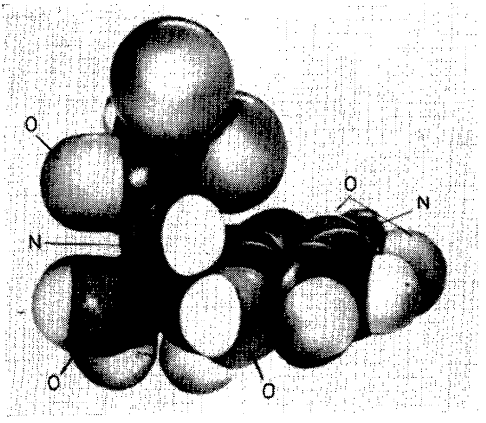


Figure 1. Model of chloramphenicol molecule

The synthesis of a large number of chloramphenicol analogues and investigation of their antibacterial activity[1] allowed some generalities to be made concerning the causes and nature of the effect of the particular groups in the chloramphenicol molecule on its antibiotic properties.

Clearly, the aminopropanediol chain is, biologically, the most specific fragment of the chloramphenicol molecule and any variation in this chain, whether structural or stereochemical, causes a considerable fall in antibiotic activity or an abrupt change in its nature. Indeed, the enantiomer and both diastereomers of the antibiotic are almost or totally inactive. Chloramphenicol analogues with alkyl groups substituted for the hydrogens at the C-atoms of the aliphatic chain are also inactive. Alkylation or acylation of the NH or OH groups, substitution of the OH groups by hydrogen, by alkyl residues or by an SH group, conversion of the primary alcohol group into a carboxyl or carbalkoxyl group—all such modifications are also accompanied by the loss of antibiotic properties. Only when the hydroxyl groups are substituted by chlorine, or when the secondary alcohol group is oxidized to a ketonic group, is the activity retained. These examples, however, are not contradictory to what has been said about the high specificity of the aliphatic chain structure of chloramphenicol, because chlorine is comparable in polarity to hydroxyl, while the transformation of chloramphenicol to the corresponding ketone is accompanied by profound changes in the antibiotic spectrum and apparently in the mode of action.

When one bears in mind the high specificity manifested by the aminopropanediol chain of chloramphenicol, it is only natural to infer that this fragment serves to bind the molecule to the active centres of enzymes of vital importance for the normal metabolism of micro-organisms. This conclusion was first drawn in 1952 by Collins *et al.*[8]. In a further extension of this hypothesis the English workers made a number of statements regarding the structure–activity relations of chloramphenicol, which, however, proved to be inaccurate, having been made on the basis of erroneous information concerning the non-activity of a number of *N*-acyl analogues of the antibiotic and also of a wrong (erythro instead of threo) Stuart model.

If one starts with the correct model of chloramphenicol, and also bears in mind the stability of the *cis*-conformation, one can easily discern a highly characteristic structural feature of this compound, namely, the very close proximity, and at the same time accessibility, of the acylamino and hydroxy groups, which enables them to interact strongly with the peptide groupings of proteins. We therefore thought it quite probable[6] that the amide and both hydroxyl groups of chloramphenicol form the active centre (*Figure 1*), which is responsible for the binding of the antibiotic to the specific centres of the enzymes or other cellular substances, thereby excluding them from participation in the metabolism of the micro-organisms.

The specific electronic structure of the *p*-nitrophenyl radical and strong polarizing action on the aminopropanediol grouping greatly affects the chloramphenicol activity. The dimensions of this part of the molecule, however, unlike the aliphatic chain, are not of great importance. This viewpoint, first expressed by us already in 1954–55[4, 6], readily explained a number of important relationships between the structure of this part of the chloramphenicol molecule and the antimicrobial activity of the latter.

The validity of these ideas follows from the fact that the nitro group can be shifted along a conjugated system without causing any essential decrease in activity (III; X = *p*-$O_2NC_6H_4N$ = N or *p*-$O_2NC_6H_4CH$ = N), while violation of the conjugation leads to weakly active or totally inactive analogues (III; X = *p*-$O_2NC_6H_4CONH$ or *p*-$O_2NC_6H_4O$). Further, we have found that in conformity with this hypothesis, the activity of chloramphenicol analogues of type (III) usually decreases as the electron acceptor properties of the substituent X are reduced; so that according to their effect on the activity these substituents may be arranged in an order approximately corresponding to their electronegativities:

$$O_2N > CN > CO_2Me > Cl(Br) > SO_2Me > SO_2NH_2 > H > Me$$

Of course, this order is somewhat conditional, because the inhibition of different micro-organisms by somewhat differing, though analogous, antibacterial agents may proceed in different ways*. In this connection one should give due regard to the interesting, although as yet unexplained, fact that certain representatives of type (III) analogues with *p*-substituents (MeO, MeS or MeSe) devoid of strong electron-acceptor properties are very active. Also noteworthy is the high activity of a number of analogues with unsubstituted or substituted biphenyl residues in place of the *p*-nitrophenyl radical.

Similar views regarding the influence that the polarizing effect of the nitrophenyl radical exerts on chloramphenicol activity were expressed independently in 1956 by Hahn and co-workers[9]. At the same time, in order to account for the exceptions, Hahn postulated that the activity of chloramphenicol is associated with the appreciable planar surface, formed by the aromatic radical and the side chain groups coplanar with it, and that the role of the *p*-substituent is to maintain the coplanarity by stabilizing the *p*-quinonoid resonance structure of the phenyl residue. This we believe to be erroneous. Indeed, it follows from Hahn's hypothesis that the direction of polarization of the phenyl radical (and hence the character of the polarization of C_1) is of no importance, *i.e.* it makes no difference whether the *p*-substituent is an electron-acceptor or electron-donor, a fact which is in contradiction with the aforementioned tendency for the activity to decrease with increase in electron-donor properties of the substituent. One may cite as a further example that, of the *p*-fluoro-, *p*-chloro- and *p*-bromo-substituted type (III) analogues of chloramphenicol, the first is the least active, whereas of all halogens it is fluorine that possesses the greatest +M effect.

Investigation of the rôle played by the *p*-nitrophenyl radical led to the discovery of still another interesting relationship[6]. It was found that substitution of the nitro group in chloramphenicol by various ions or ionogenic groupings always affords almost or totally inactive substances (III; X = SO_3H, SO_2H, CO_2H, AsO_3H_2, $N(CH_3)_3^+$, OH), irrespective of the strength and direction of the polarizing action of these substituents. Apparently the

* For instance, the analogue (III; X = SO_2Me) is about 1/10 as active *in vitro* as chloramphenicol against *Staphylococcus aureus*, *Escherichia coli* and *Salmonella typhosa*, but it is three times as active against *Brucella abortus*. At the same time, *in vivo*, it is more active than chloramphenicol against *Salmonellae* and has for this reason even found practical application under the trade name Thiocymetin.

appearance of an ionic charge in the molecule greatly changes its ability to adsorb onto proteins, thus changing also its ability to penetrate biological media and combine with the specific bacterial enzymes. This rule makes it clear that it is pointless to look for highly active chloramphenicol analogues among compounds with ionogenic groupings.

The chloroacetyl residue of chloramphenicol is located in close proximity to those active groups of the molecule that are responsible for its attachment to the specific centres of the bacterial enzymes. Hence the acyl residue should not only exert a certain polarizing effect on the aminopropanediol chain, but should also satisfy definite geometric requirements[6, 9, 10].

The requirement of a certain optimal polarity of the acyl moiety of the molecule follows from the high activity of chloramphenicol analogues of type (V) with R = $CHBr_2$ or CH_2CN and the abrupt fall in activity in the same analogues when R = CCl_3 or CH_3. At the same time, the high activity of compounds (V; R = $C_6H_4CHCl_2$-*p*) shows that the dichloromethyl group, like the nitro group, may be shifted along a conjugated system, and that consequently not only the polarity, but the polarizing action of this group is necessary for the display of antibiotic activity by the molecule. A similar, though less lucid, picture is revealed on comparing the three analogues of type (V) with R = CCl_3, CH = $CHCCl_3$ and $CH_2CH_2CCl_3$. The second of these compounds is only slightly less active than the first, whereas the last is already markedly less active. On the other hand, compounds (V) with R = $C(CH_3)Cl_2$ and CH = $CHCHCl_2$ proved to be considerably less active than chloramphenicol, although the incorporation of a methyl radical or the shift of the dichloromethyl radical by one vinyl group should exert no essential change in the electronic character of the amide group. Apparently the 1,1-dichloroethyl and 3,3-dichloropropenyl (as well as many other) radicals do not possess the appropriate dimensions, shielding the active centre of the molecule or changing its conformation and thus depriving the aminopropanediol chain of the possibility of specifically interacting with the peptide groupings of the bacterial proteins or with other substances of the bacterial cell. It follows, therefore, that the geometry of the *N*-acyl residue is at least as important as its polarizing effect.

Thus, the high antibiotic activity of chloramphenicol is simultaneously determined by three factors:

(*i*) strictly defined dimensions and the appropriate conformation of the aminopropanediol chain;

(*ii*) a strong polarizing effect of the *p*-nitrophenyl radical the size of which is not important, and

(*iii*) a strong polarizing effect of the dichloroacetyl residue which should also comply with definite geometrical requirements.

The polarizing action of the *p*-nitrophenyl radical considerably affects the electronic character of the C_1 atom of the aliphatic chain and the hydroxyl group attached to this atom, while the same action of the dichloroacetyl residue bears primarily on the electronic character of the nitrogen atom, a decrease in electronic density taking place in both cases.

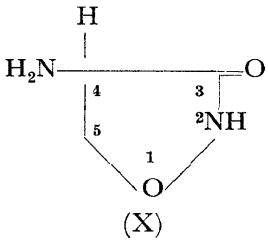
These effects, in combination with the steric proximity, and at the same time accessibility, of the acylamino and hydroxy groups create the conditions for the strong interaction of the antibiotic with specific groups of certain

enzymes or other substances of the microbial cell, ultimately causing the breakdown of its metabolism.

Since the required decrease in electron density at the N- and O-atoms of the acylamino and hydroxy groups may be brought about by the polarizing effect of various substituents, it becomes clear why the *p*-nitrophenyl and dichloroacetyl residues are not very specific and can be replaced by other radicals of similar polarizing effect. The reason why these radicals should not contain ionogenic groups is now also clear, for the conversion of the polar molecule into an ion (no matter whether positive or negative) leads to non-specific association with the proteins due to interaction of the ionic charges.

The relations between structure and activity in chloramphenicol must necessarily be borne in mind when attacking the problem of its mode of action. The majority of investigators now agree that this antibiotic exerts its effect mainly by suppression of protein biosynthesis. Regrettably, a deeper insight into the mode of action is hindered by the absence of detailed knowledge concerning protein synthesis, so that it is as yet difficult to indicate just which stage of bacterial protein synthesis is interrupted by chloramphenicol, let alone to specify the nature of its participation in this act.

Interesting results have been obtained in recent years in the study of another important antibiotic, cycloserine (X), discovered in 1955. In this case the synthetic approach also made it possible to elucidate certain structural features of the molecule, important for the manifestation of its antibiotic activity; although it has not as yet revealed the niceties in the structure–activity relation that have become clear in the case of chloramphenicol. At the same time, our knowledge of the mode of action of cycloserine has advanced more than in the case of chloramphenicol.



(X)

Owing to the simplicity of the cycloserine structure, the antibiotic itself, the L-antipode, and also a number of analogues proved to be readily available synthetically. As a result, the effect of substitution in the isoxazolidine ring and in the amino group of cycloserine could be ascertained, as could also the significance of the configuration for antibiotic activity. Due to the researches of Folkers, Kochetkov, Plattner, Šorm and their co-workers (see the review[11]) in particular, it was established that elimination of the NH_2 group, as well as its acylation or alkylation, transforms cycloserine into completely inactive compounds. Incorporation of an alkyl or aryl group in position 5 is accompanied, if not by complete, by considerable inactivation, the greatest fall in activity being caused by substituents *cis*-oriented with respect to the NH_2 group. As a result it was shown that the free amino group is structurally a

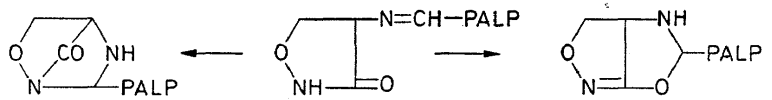
biologically specific element of the antibiotic molecule. On the other hand a very important detail was discovered: the D-configuration is not a prerequisite for the antibiotic activity of cycloserine, and the L-antipode even surpasses the natural antibiotic in activity towards a number of micro-organisms, including *Mycobacterium tuberculosis*, *Salmonella paratyphi*, *S. typhosa etc.*

At the same time it was found that cycloserine is a potent selective inhibitor of biochemical transamination and other reactions that take place with the participation of phosphopyridoxal enzymes[11] and which are known[12, 13] to proceed *via* azomethine intermediates of the type (XIa) ⇄ (XIb).

$$\underset{\text{(XIa)}}{\text{Sub—N=CH—PALP}} \rightleftarrows \underset{\text{(XIb)}}{\text{Sub'=N—CH}_2\text{—PALP}}$$

Sub = substrate, PALP = pyridoxal phosphate enzyme residue

This led to the proposal that the mode of action of the antibiotic is based on interaction of the amino group with the aldehyde group of pyridoxal phosphate, blocking the phosphopyridoxal enzymes and, thereby, suppressing the biosynthesis of amino-acids and consequently of the specific nucleoproteids constituting the major component of the total protein of *Mycobacterium tuberculosis*[14]. The irreversible binding of PALP enzymes by cycloserine was interpreted as the result of stabilization of the originally formed azomethines (XIII) in the form of fused ring structures such as (XII) or (XIV)[15].

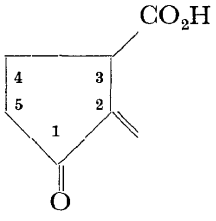


(XII) (XIII) (XIV)

Further study showed, however, that the affinity for pyridoxal phosphate is not the decisive factor determining the antibiotic properties of cycloserine. Indeed, the antibiotic proved to be non-stereospecific, the L-form being more active than the natural product not only against numerous micro-organisms, but also with respect to a number of L-transaminases. Further, a synergistic action was discovered for combinations of the antibiotic and its L-antipode, indicating a fundamental difference in the modes of action of these two compounds. Finally, it was established that D-cycloserine inhibits the synthesis of mucopeptides of the bacterial cell wall by suppressing the incorporation of D-alanine, apparently owing to its structural similarity with this substrate, and to competition with the latter for the specific contact sites in the active centres of the corresponding enzymes. Evidently, the antibacterial activity of this antibiotic is due to this circumstance[15–19].

Hence, synthetic investigations in the field of cycloserine not only provided some insight into the mode of action of this group of compounds, but led to the discovery of a new antibacterial substance, namely DL-cycloserine.

The antitumour-antibiotic sarkomycin which is (−)-2-methylenecyclopentanone-3-carboxylic acid (XV) possesses a still simpler structure than cycloserine and like the latter is readily accessible for synthetic study.



(XV)

Owing to this, shortly after the structure of sarkomycin had been established, syntheses were achieved of the antibiotic itself, its (+)-antipode, the racemate, a number of derivatives, various isomers, and manifold alicyclic and aliphatic analogues. For instance, racemic sarkomycin (as the semicarbazone) was synthesized by us[20–22] according to Scheme A, starting with ethyl 2-methylcyclopentanone-3, 3-dicarboxylate (XVI).

This antibiotic was obtained at the same time by Japanese investigators[23, 24], using a shorter route (Scheme B). They discovered that ethyl cyclopentanone-3-carboxylate (XVIII; R = Et) on interaction with formaldehyde and dialkylamine forms 2-dialkylaminoketo-ester (XVII), which could then be converted into sarkomycin. Both the Japanese workers and ourselves simultaneously found that in the case of the free acid (XVIII; R=H) the Mannich reaction takes place at position 5 and in this way isosarkomycin (XIX) was obtained[25, 26]. Employing such methods, the (−), (×) and (±)-isomers not only of sarkomycin (XV) but also of isosarkomycin (XIX) were prepared.

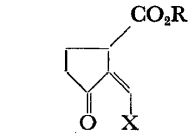
The synthesis of other compounds of this type is also straightforward and there is no need to dwell upon it here. The main difficulty in the synthetic studies in this field is associated with the instability of sarkomycin and those of its analogues which contain the vinylketone grouping. Some of these compounds, including sarkomycin itself, could not be isolated as such.

The most interesting of the sarkomycin analogues are given in *Figure 2**.

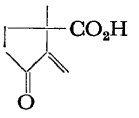
The available data on the antibacterial and antitumour properties of these compounds allow one to conclude that neither the configuration nor the structure of sarkomycin are specific for its biological activity. Thus, the (+)-antipode displays approximately the same activity as the natural (−)-sarkomycin (XV); a similar phenomenon exists in the case of (+) and (−)-isosarkomycins (XXIIIa; R=H). Even the opening of the five-membered ring of the antibiotic is not associated with disappearance of its antibacterial or anti-tumour properties. The acyclic analogues (XXVIII), (XXIX) and (XXX) are highly active, the free acid (XXIXa) and the methyl esters (XXIXa) and (XXIXb) even surpassing sarkomycin in activity.

* See the monograph[27] and some more recent papers[28–30].

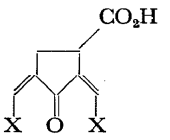
Scheme A
CO2Et
CO2Et
CH3
O
(XVI)
5 stages
CO2H
CO2H
CH2NMe2
Sem
4 stages
CO2−
CH2+NMe3
Sem
CO2H
Sem
(XV)
Sem = H2NCONHN=
Scheme B
(XV)
2 stages
CO2Et
CH2NR2
O
(XVII)
CO2R
O
(XVIII)
CO2H
R2NCH2
O
3 stages
CO2H
O
(XIX)



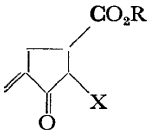
(XXa): X = Me
(XXb): X = OAc
(XXc): X = SEt
(XXd): X = NC_5H_{10}



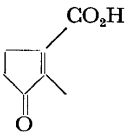
(XXI)



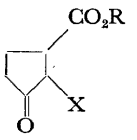
(XXIIa): X = C_6H_5
(XXIIb): X = C_6H_4OMe-4
(XXIIc): X = $C_6H_4NMe_2$-4
(XXIId): X = $C_6H_3O_2CH_2$-3,4



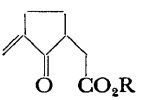
(XXIIIa): X = H
(XXIIIb): X = Me



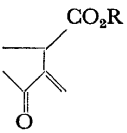
(XXIV)



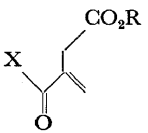
(XXVa): X = H
(XXVb): X = Me
(XXVc): X = Et
(XXVd): X = CH_2NMe_2
(XXVe): X = CH_2S-]$_2$



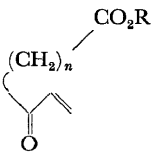
(XXVI)



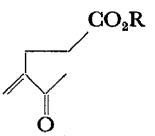
(XXVII)



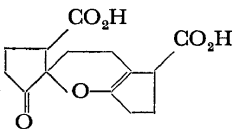
(XXVIIIa): X = Me
(XXVIIIb): X = Ph



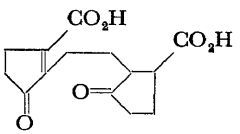
(XXIXa): $n = 2$
(XXIXb): $n = 3$
(XXIXc): $n = 8$
(XXIXd): $n = 9$



(XXX)



(XXXI)



(XXXII)

Figure 2

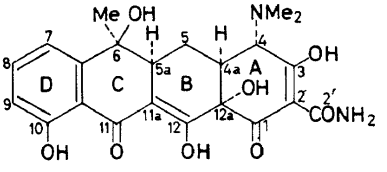
As for cyclopentane compounds, although cyclopentanone-3-carboxylic acid (XXVa; R=H) is biologically inactive, many of its analogues with substituents at positions 2 or 5 exert an effect similar to that of sarkomycin. At the same time these compounds are active only when they contain a free keto group and no endocyclic double bond. Thus, the 2,4-dinitrophenylhydrazone of sarkomycin, the acid (XXIV), isomeric with the antibiotic, and the dimer of sarkomycin (XXXII) are completely devoid of both antibacterial and anti-tumour properties, and sarkomycin B (XXXI) is only very slightly active. However, exocyclic methylene is not necessary. There are numerous compounds without this grouping (the bromide and hydrazide derivatives of sarkomycin, sarkomycin S_1 (XXVe; R=H) and the analogue (XXVd; R=Et) that are almost as efficient anti-tumour agents as sarkomycin itself. If should, however, be mentioned that the antimicrobial

spectrum of sarkomycin S_1 and the anti-tumour spectrum of the isonicotinoylhydrazide derivative of sarkomycin differ somewhat from the corresponding spectra of the antibiotic. On the other hand, the replacement of a hydrogen atom in the exocyclic CH_2-group of sarkomycin by an acetoxyl, ethylmercaptyl, or *N*-piperidyl residue (analogues XXb–XXd) leads to complete disappearance of the biological activity. Substitution of the methylene group in position 2 by a methyl group, *i.e.* transformation of sarkomycin into dihydrosarkomycin (XXVb; R=H) is associated with loss of anti-bacterial activity; as for the anti-tumour properties of the dihydrocompound, the available data are very contradictory.

The position of the methylene group at C–2 is also not a very specific factor in the biological action of sarkomycin, isosarkomycin (XXIIIa; R=H) being at least as active as the natural antibiotic, and the compound (XXVI) also possessing both anti-bacterial and anti-tumour properties. The anti-tumour action is retained on incorporation of a methyl group in position 3 of sarkomycin (analogue XXI), whereas it disappears completely when this group is incorporated in position 2 of isosarkomycin (analogue XXIIIb; R=H). At the same time the ethyl ester of 2-methylisosarkomycin (XXIIIb; R=Et) is not much inferior to isosarkomycin (XXIIIa) in activity. In general, esterification of the carboxyl group usually leads to a marked increase in activity (sometimes five- or six-fold) both in the case of cyclic and aliphatic analogues of sarkomycin, but no such effect has been noted for the antibiotic itself.

Hence, despite the simplicity of structure of sarkomycin, the synthesis and study of the biological properties of numerous analogues did not reveal the structural elements responsible for its activity. Furthermore, no analogues of sarkomycin have yet been found that are of practical value as antitumour agents, almost all of them, including the most active, being much more toxic than the antibiotic itself.

Compared with the antibiotics we have discussed, tetracycline (XXXIII) and related antibiotics (XXXIIIa)–(XXXIIIg) are of much more involved



(XXXIII)

(XXXIII) Tetracycline

(XXXIIIa) Aureomycin (7-chlorotetracycline)

(XXXIIIb) Bromotetracycline (7-bromotetracycline)

(XXXIIIc) Terramycin (5-hydroxytetracycline)

(XXXIIId) Demethyltetracycline (6-demethyltetracycline)

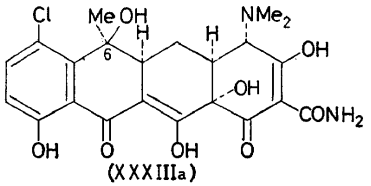
(XXXIIIe) Dechlomycin (6-demethyl-7-chlorotetracycline)

(XXXIIIf) 2-Decarboxamido-2-acetyl-5-hydroxytetracycline

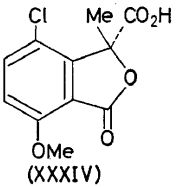
(XXXIIIg) 5a(11a)-Dehydro-7-chlorotetracycline

structure. All of them excepting compound (XXXIIIg) possess high antimicrobial activity and a broad spectrum.

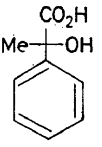
A series of chemical and X-ray studies* which began in 1952 gradually led to the establishment of the relative configuration of the tetracyclines; their absolute configuration was determined by us in 1962[37] by correlation of the C–6 asymmetric centre of aureomycin (XXXIIIa) *via* its oxidation product (XXXIV) with (−)-D-atrolactinic acid (XXXV).



(XXXIIIa)

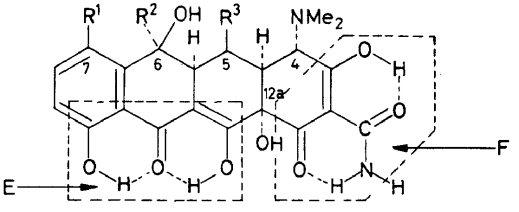


(XXXIV)



(XXXV)

Not one of these antibiotics has as yet been totally synthesized. However, a considerable number of closely related analogues was obtained by modifying the naturally-occurring products of this group and this made it possible to shed light on some important aspects of the structure–activity relation†. Based on the data obtained from these studies we concluded[31] that the most important structural elements of tetracyclines, necessary for their antibiotic activity, are the phenoldiketone fragment E, and the carboxamidodiketone fragment F, which form two characteristic systems of chelate bonds as shown in Formula (XXXVI). The other substituents in positions 4, 5, 6, 7 and 12a of tetracyclines apparently are of minor significance.



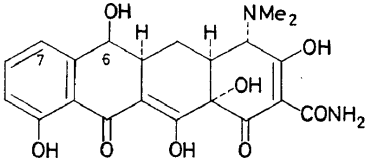
(XXXVI)

Indeed, the majority of the structural changes at C–5, C–6 and C–7 of the tetracycline molecule do not cause any important changes in the antimicrobial properties. Thus, high activity is possessed by the naturally occurring 5-hydroxy and 7-halogen derivatives of tetracycline (XXXIIIc, XXXIIIa, and XXXIIIb), and by 6-demethyltetracyclines (XXXVII, and 7-Cl-XXXVII) as well as by such partially synthesized compounds as 7-substituted 5-hydroxytetracyclines (7-Br, I, NO_2, or ArN_2 derivatives of XXXIIIc), various 6-deoxytetracyclines (XXXVIII and its 7-Cl, Br, I, NO_2, or NH_2, and 9-NH_2 derivatives; 6-Me-XXXVIII and its 7-Cl, Br, I,

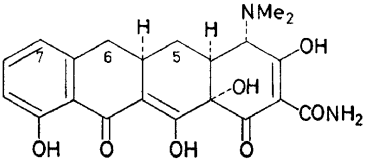
*For a review of the literature see[31] and also the more recent publications[32–36].

† See the monograph[31], where a bibliography up to 1960 has been presented, and also some recent papers[33–35, 38–43].

or NO_2, and 5-OH or 9-NH_2 derivatives; 9-NH_2-7-Br and 9-NH_2-7-NO_2-XXXVIII) and 6-methylenetetracyclines (6-CH_2-XXXVIII and its 5-OH- and 7-Cl-5-OH-derivatives). Some of these compounds, such as (XXXIIIa), (7-NO_2-XXXVIII) and (7-Cl-5-OH-6-CH_2-XXXVIII), are even more markedly active against a number of micro-organisms than tetracycline (XXXIII) itself.

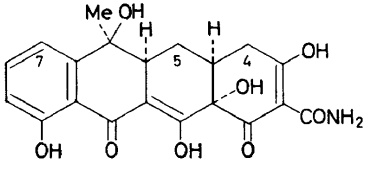


(XXXVII)

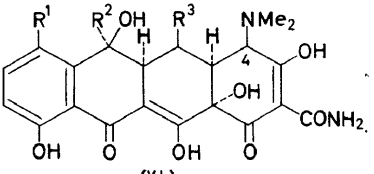


(XXXVIII)

The dimethylamino group in position 4 apparently may be removed without loss of antimicrobial activity, a fact which made possible the suggestion by Stephens *et al.* in 1958 that the entire "upper periphery" (C_4—C_5—C_6—C_7) of the tetracyclines is non-specific[44]. However, epimerization of the asymmetric centre at C–4, or quaternization of the dimethylamino group, causes a sharp change in antimicrobial properties of the compound. For instance, it has been reported (see review[31]) that 4-dedimethylaminotetracyclines (XXXIX; 5-OH-XXXIX; 7-Cl- or 7-Br-XXXIX) are highly active *in vitro* against many organisms, whereas the quaternary salts of the natural tetracyclines, and the 4-epitetracyclines (quatrimycins) (XL), are completely, or almost completely, devoid of this property.

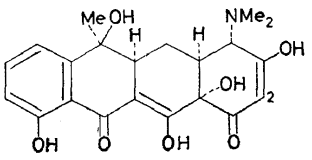


(XXXIX)

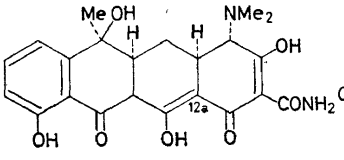


(XL)

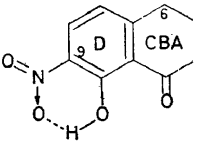
Some structural changes are also permissible at C–2 and C–12a of the tetracyclines. For example, the Mannich base (2-$CONHCH_2NR_2$-XLI) prepared from the unsubstituted or 5- or 7-substituted tetracyclines are comparable in activity to the original antibiotics, and one of the former compounds, called reverin (2-$CONHCH_2N(CH_2)_4$-XLI), has even been recommended as a highly active tetracycline derivative for parenteral administration. The O-12a esters of the tetracyclines also possess high antimicrobial activity. However, substitution of the carboxamide group of the tetracyclines by a nitrile or acetyl group (transition from (XXXIII) to (2-cyano-XLI) and from (XXXIIIc) to (2-acetyl-5-hydroxy-XLI) causes a fall in activity to 1/10 or 1/20 the original value, and when the 12a-hydroxyl is eliminated (transition from (XXXIII) to (XLII)), the activity drops to about 1/50 that of the antibiotic.



(XLI)

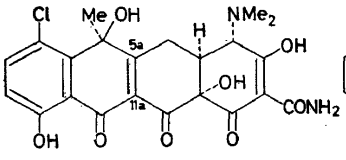


(XLII)

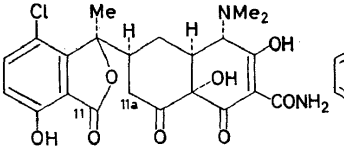


(XLIII)

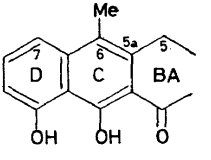
Finally, considerable inactivation is caused by epimerization of the asymmetric centre at C–5a, by dehydrogenation at 5a(11a), or cleavage of the molecule between C–11 and C–11a (transition from aureomycin (XXXIIIa) to dehydrochlorotetracycline (XLIV) and isoaureomycin (XLV)), or by incorporation of a nitro group in position 9 of 6-deoxytetracycline (XXXVIII) giving rise to a new chelate structure (XLIII) in the molecule. Dehydration of tetracyclines into 5a,6-anhydrotetracyclines (XLVI; 5-hydroxy-XLVI; 7-chloro-XLVI *etc.*) is accompanied by fundamental changes in the anti-microbial spectrum, apparently due to a change in the mode of action.



(XLIV)



(XLV)



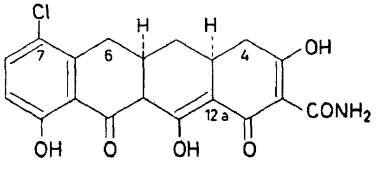
(XLVI)

It seems likely that in most of the above mentioned cases inactivation is due to alteration of the characteristic chelate systems E and F (XXXVI) of the tetracycline antibiotics caused by a change in conformation of the molecule, or by a different distribution of the electron density, or sometimes by a change in direction of enolization of the carbonyl groups.

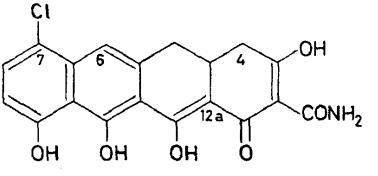
One of the most generally accepted hypotheses regarding the chemical nature of tetracycline activity is that these antibiotics inhibit protein synthesis by binding into stable chelates the multivalent cations necessary for the normal functioning of certain enzyme systems. This hypothesis explains why such structural changes in the tetracycline molecule as epimerization of the asymmetric centre at 5a, quaternization of the tertiary amino group, the substitution of the carboxamido group by a cyano or acetyl residue, aromatization of ring C, dehydrogenation at position 5a, 11a, *etc.*, obviously disrupting the intramolecular hydrogen bond system of the E and F fragments (XXXVI), lead either to considerable loss in activity or to far-reaching changes in the nature of the activity.

The part played by each of the chelate systems of the tetracyclines could be ascertained to some extent by a biochemical study of simpler compounds containing such structures. However, synthesis of even the simpler members of such compounds involves considerable experimental difficulties and, at present, only a few synthetic analogues of the tetracyclines are available and, furthermore, data on the biological properties of most of them are regrettably

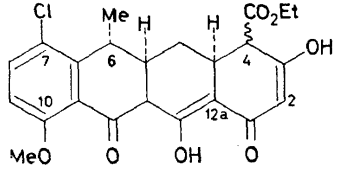
lacking. The most interesting of the analogues are some compounds obtained in the search for routes to the total synthesis of the tetracyclines and their anhydro derivatives. Deserving special attention are the compounds synthesized by Boothe *et al.*[45–48], namely, (±)-4-dedimethylamino-6,12a-dideoxy-6-demethyl-7-chlorotetracycline (XLVII) and (±)-4-dedimethylamino-12a-deoxy-6-demethyl-7-chloroanhydrotetracycline (XLVIII). The compounds synthesized by Muxfeldt and co-workers[49–51], (±)-2-decarboxamido - 4 - dedimethylamino - 4 - carbethoxy - 6,12a - dideoxy - 10 - O - methyl-7-chlorotetracycline (XLIX) and (±)-4-dedimethylamino-12a-deoxy-7-chloroanhydrotetracycline (L) are also of considerable interest. However, synthesis of such, even highly simplified and therefore appreciably stable, analogues is as yet very difficult. Thus, despite the absence of at least three functional groups (4-NMe_2, 6-OH and 12a-OH) present in the natural tetracyclines, the syntheses of the analogues (XLVII)–(L) is characterized not only by the difficulties involved in certain stages but also by the large number of stages (up to 25).



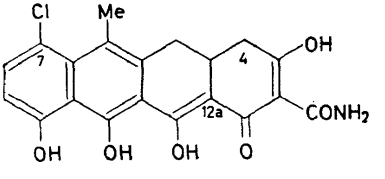
(XLVII)



(XLVIII)



(XLIX)

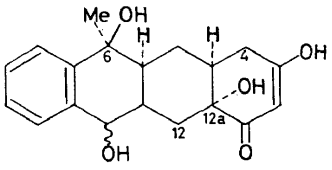


(L)

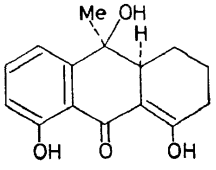
A more distant hydronaphthacene analogue of the tetracyclines (LI) which, however, has hydroxy groups in positions 6 and 12a was prepared by us in a search for methods of synthesizing 4-dedimethylamino-12-deoxotetracyclines, but this synthesis also involves a large number of stages[52–61]. Thus a detailed study of the structure–activity relation in the field of tetracyclines is still considerably hampered by the absence of rational methods for their synthesis*. True, the simpler analogues may in some cases be of considerable interest, for example, the tricyclic dihydroxydiketone (LII)[62] and the bicyclic dimethylaminohydroxydiketone (LIII)[61] which we have prepared recently. The former imitates the DCB ring system,

* Recently, Woodward reported the preparation of a highly active tetracyclines analogue, namely racemic 6-demethyl-6-dehydroxytetracycline (XXXVIII); this is a great synthetic achievement[83].

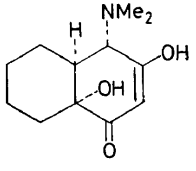
the latter the A ring system of tetracyclines. The tricyclic dihydroxydiketone (LII) was found to possess considerable antibiotic activity (1/10–1/20 that of tetracycline) and an antibacterial spectrum similar to that of the tetracyclines.



(LI)



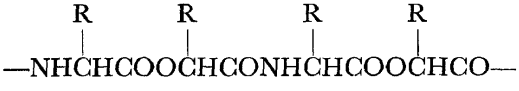
(LII)



(LIII)

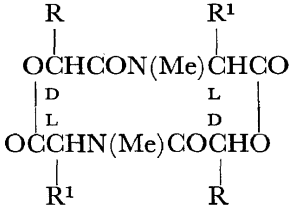
One may expect that further synthetic studies of tetracyclines will in the very near future lead to convenient routes both to the antibiotics themselves, as well as to numerous analogues, and that these compounds will help to throw light on the nature and mode of action of this important class of therapeutic agents.

A group of natural products that has expanded rapidly in recent years is the depsipeptides, compounds built up of hydroxy and amino-acid residues joined together by ester and amide bonds (LIV).



(LIV)

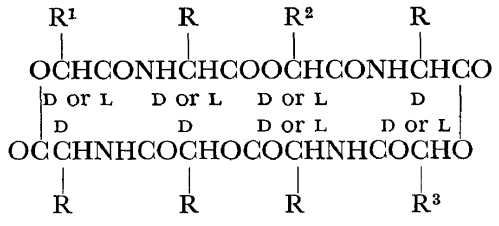
The depsipeptides include not only a number of antibiotics—enniatins A, B and C, amidomycin, valinomycin and esperin (LV–LXI), but also several alkaloids (ergot alkaloids), certain proteins and other types of naturally occurring substances, for instance, sporidesmolide I (LXII) (see refs. 63–65 for a comprehensive survey of the literature). A characteristic feature of all the known depsipeptide antibiotics is that they are of a cyclic structure. However, as in the case of the peptide antibiotics, here too one can observe no regularities in the hydroxy and amino-acid constituents.



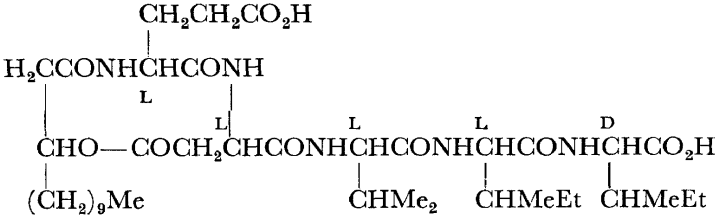
Enniatin A (LV): $R = CHMe_2$, $R^1 = CHMeEt$

Enniatin B (LVI): $R = R^1 = CHMe_2$

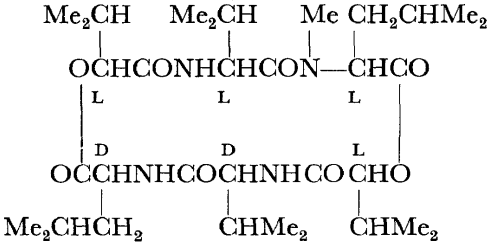
Enniatin C (LVII): $R = CHMe_2$, $R^1 = CH_2CHMe_2$



Amidomycin (LVIII): $R = R^1 = R^2 = R^3 = CHMe_2$
Valinomycin (LIX): $R^1 = R^3 = Me$, $R = R^2 = CHMe_2$
(LX): $R^1 = R^2 = Me$, $R = R^3 = CHMe_2$



Esperin (LXI)



Sporidesmolide I (LXII)

In order to study the relation between the structure and antibacterial (antituberculotic) action of the depsipeptide antibiotics we developed general methods for the synthesis first of linear (LXIV) and then cyclic (LXIII) depsipeptides with regularly and non-regularly alternating residues of optically active hydroxy and amino-acids[64–66]*. These methods are in principle similar to those employed for the synthesis of the analogous peptides.

The linear tetra- and octa-depsipeptides which we prepared (for instance LXIV; $n=1$ or 3, $X=X'=H$, $Y=OH$, $R=CHMe_2$) proved to be inactive with respect to *Mycobacterium phlei*. When, however, with the aid of the methods we had developed we were able to synthesize the cyclodepsipeptides corresponding to formulae (LV)–(LIX) ascribed to enniatins

* The synthesis of linear depsipeptides with racemic α-hydroxy acid residues was described by other workers[67–69].

(OCHRCONHCHRCO)n
(OCCHRNHCOCHRO)n
(LXIII)
XX'NCHRCO(OCHRCONHCHRCO)nOCHRCOY
(LXIV)
X + X' = C6H4(CO)2; X = H X' = Cbo or O2NCbo; X = X' = H
Y = OCH2Ph, OBut, OCH2CN, Cl, OH
XX'NCHRCOOCHRCOOH(COCl)
XX'NCHRCOOCHRCOOH + HOCHRCOY
XX'NCHRCOOCHRCOY
H2NCHRCOOCHRCOY
XX'NCHRCOOCHRCONHCHRCOOCHRCOY

A, B and C[70, 71], amidomycin[72] and valinomycin[73, 74] the resultant compounds were found to differ greatly in physical and biological properties from the naturally occurring antibiotics. Hence the latter do not possess structures (LV)–(LIX) which had been assigned to them[75–79*]. Only in the case of sporidesmolide I did the synthesis confirm the validity[80] of the formula (LXII) proposed for this substance[81]. At present, work is in progress to test the correctness of formula (LXI) for esperin[82] and the unsymmetric formula (LX) for valinomycin[73, 74] proposed together with formula (LIX).

Hence synthetic studies within this group of antibiotics have as yet been rewarded only by the knowledge that the problem as a whole is much less clear than it was formerly thought to have been.

One may see from the facts presented in this report how synthetic studies can help (chloramphenicol, cycloserine) or correct (depsipeptide antibiotics) investigations into the structure–activity problem, as well as how in certain cases (sarkomycin) such studies do not advance the problem to any extent, and in others (tetracyclines) how solution of the problem must await general methods of synthesis of the given group of antibiotics.

References

[1] M. M. Shemyakin, A. S. Khokhlov, M. N. Kolosov, L. D. Bergleson, and V. K. Antonov. *Khim. Antibiotikov* (Chemistry of Antibiotics), Vol I, p. 337–429, Publ. House Acad of Sci. USSR, Moscow (1961).

[2] M. M. Shemyakin, E. M. Bamdas, E. I. Vinogradova, M. G. Karapetyan, M. N. Kolosov, A. S. Khokhlov, Yu. B. Shvetsov, and L. A. Shchukina. *Doklady Akad. Nauk SSSR* **86**, 565 (1952); *Zhur. Obshcheĭ Khim.* **23**, 1854 (1953).

[3] M. M. Shemyakin, E. M. Bamdas, E. I. Vinogradova, M. A. Guberniev, V. N. Orekhovich, A. S. Khokhlov, Yu. B. Shvetsov, and L. A. Shchukina. *Doklady Akad. Nauk SSSR* **94**, 257 (1954); *Zhur. Obshcheĭ Khim.* **24**, 2076 (1954).

[4] M. M. Shemyakin, M. N. Kolosov, M. G. Karapetyan, E. M. Bamdas, Yu. B. Shvetsov, E. I. Vinogradova, and L. A. Shchukina. *Zhur. Obshcheĭ Khim.* **25**, 1199 (1955).

[5] M. M. Shemyakin and M. Yu. Lur'e. *Zhur. Obshcheĭ Khim.* **29**, 2531 (1959).

[6] M. M. Shemyakin, M. N. Kolosov, M. M. Levitov, K. I. Germanova, M. G. Karapetyan, Yu. B. Shvetsov, and E. M. Bamdas. *Doklady Akad. Nauk SSSR* **102**, 953 (1955); *Voprosy Med. Khim.* **1**, 350 (1955); *Zhur. Obshcheĭ Khim.* **26**, 773 (1956).

[7] L. M. Long and H. D. Troutman. *J. Am. Chem. Soc.* **71**, 2473 (1949).

[8] R. J. Collins, B. Ellis, S. B. Hansen, H. S. Mackenzie, R. J. Moualim, V. Petrow, O. Stephenson, and B. Sturgeon. *J. Pharm. and Pharmacol.* **4**, 693 (1952).

[9] F. E. Hahn, J. E. Hayes, C. L. Wisseman, H. E. Hopps, and J. E. Smadel. *Antibiotics & Chemotherapy* **6**, 531 (1956).

[10] B. N. Feitelson, J. T. Gunner, R. J. Moualim, V. Petrov, O. Stephenson, and S. W. Underhill. *J. Pharm. and Pharmacol.* **3**, 149 (1951).

[11] Ref. 1, vol. II, p. 861–871.

[12] A. E. Braunshteĭn and M. M. Shemyakin. *Doklady Akad. Nauk SSSR* **85**, 1115 (1952); *Biokhimya* **18**, 393 (1953).

[13] D. E. Metzler, M. Ikawa, and E. E. Snell. *J. Am. Chem. Soc.* **76**, 648 (1954).

[14] N. K. Kochetkov, R. M. Khomutov, M. Ya. Karpeĭskiĭ, E. I. Budovskiĭ, and E. S. Severin. *Doklady Akad. Nauk SSSR* **126**, 1132 (1959).

[15] A. E. Braunshteĭn. *Proc. 5th Internl. Congr. of Biochemistry*, Symposium IV, **4**, p. 3, Moscow (1961).

[16] A. Buogo, A. DiMarco, M. Ghione, A. Migliacci, and A. Sanfilippo. *Giorn. microbiol.* **6**, 131 (1958).

[17] J. Ciak and F. E. Hahn. *Antibiotics & Chemotherapy* **9**, 47 (1959).

[18] J. L. Strominger, E. Ito, and R. H. Threnn. *J. Am. Chem. Soc.* **82**, 998 (1960); *Biochim. et Biophys. Acta* **33**, 280 (1959).

[9] Yu. O. Sazykin. *Antibiotiki* **6**, 453 (1961).

* M. M. Shemyakin, Yu. A. Ovchinnikov, A. A. Kiryushkin and V. T. Ivanov have recently synthesized two copolymer homologues of compound (LVI)—the corresponding cyclohexa- and cyclo-octadepsipeptide. Of these the first was found to be identical with enniatin B and the second to possess antibiotic activity commensurate with the first.

[20] M. M. Shemyakin, G. A. Ravdel, E. S. Chaman, Yu. B. Shvetsov, and E. I. Vinogradova. *Chem. & Ind. (London)* 1320 (1957); *Izvest. Akad. Nauk SSSR, Otdel. Khim. Nauk* 1007 (1957); 2188 (1959).
[21] M. M. Shemyakin, L. A. Shchukina, E. I. Vinogradova, M. N. Kolosov, R. G. Vdovina, M. G. Karapetyan, V. Ya. Rodionov, G. A. Ravdel, Yu. B. Shvetsov, E. M. Bamdas, E. S. Chaman, K. M. Ermolaev, and E. P. Semkin. *Zhur. Obshcheĭ Khim.* **27**, 742 (1957).
[22] M. M. Shemyakin, G. A. Ravdel, E. S. Chaman, Yu. B. Shvetsov, E. I. Vinogradova, R. G. Vdovina, K. M. Ermolaev, and E. M. Bamdas. *Izvest. Akad. Nauk SSSR, Otdel. Khim. Nauk* 2177 (1959).
[23] K. Toki, H. Wada, Y. Suzuki, and C. Saito. *J. Antibiotics (Japan) Ser. A.* **10**, 35, 226 (1957).
[24] K. Toki. *Bull. Chem. Soc. Japan* **30**, 450 (1957); **31**, 333 (1958).
[25] S. Umezawa and H. Kinoshita. *J. Antibiotics (Japan) Ser. A* **9**, 194 (1956); *Bull. Chem. Soc. Japan* **30**, 267 (1957).
[26] M. M. Shemyakin, M. N. Kolosov, M. G. Karapetyan and V. Ya. Rodionov. *Zhur. Obshcheĭ Khim.* **28**, 2068 (1958).
[27] Ref. 1, vol. I, p. 133–141.
[28] E. Takeuchi, K. Nitta, T. Hikiji, M. Kinoshita, and H. Umezawa. *J. Antibiotics (Japan) Ser A.* **14**, 54 (1961) and references cited therein.
[29] R. Giuliano, M. DiFonzo, and A. Ermili. *Ann. chim. (Rome)* **49**, 1607 (1959); **50**, 750 (1960).
[30] V. B. Piskov. *Zhur. Obshcheĭ Khim.* **30**, 1390 (1960); **31**, 2937 (1961).
[31] Ref. 1, vol. I, p. 180–269.
[32] S. Hirokawa, Y. Okaya, F. M. Lovell, and R. Pepinsky. *Z. Krist.* **112**, 439 (1959).
[33] A. Green and J. H. Boothe. *J. Am. Chem. Soc.* **82**, 3950 (1960).
[34] R. K. Blackwood, H. H. Rennhard, and C. R. Stephens. *J. Am. Chem. Soc.* **82**, 745, 5194 (1960).
[35] R. K. Blackwood, J. J. Beereboom, H. H. Rennhard, M. Schach von Wittenau, and C. R. Stephens. *J. Am. Chem. Soc.* **83**, 2773 (1961).
[36] H. H. Rennhard, R. K. Blackwood, and C. R. Stephens. *J. Am. Chem. Soc.* **83**, 2775 (1961).
[37] V. N. Dobrynin, A. I. Gurevich, M. G. Karapetyan, M. N. Kolosov, and M. M. Shemyakin. *Tetrahedron Letters* 901 (1962).
[38] J. J. Beereboom, J. J. Ursprung, H. H. Rennhard, and C. R. Stephens. *J. Am. Chem. Soc.* **82**, 1003 (1960).
[39] J. H. Boothe, J. J. Hlavka, J. P. Petisi, and J. L. Spencer. *J. Am. Chem. Soc.* **82**, 1253 (1960).
[40] J. R. D. McCormick, E. R. Jensen, P. A. Miller, and A. P. Doerschuk. *J. Am. Chem. Soc.* **82**, 3381 (1960).
[41] F. A. Hochstein, M. Schach von Wittenau, F. W. Tanner, and K. Murai. *J. Am. Chem. Soc.* **82**, 5934 (1960).
[42] W. E. Clapper and R. Proper. *Antibiotics & Chemotherapy* **10**, 473 (1960).
[43] E. Jeney and T. Zsolnai. *Naturwiss.* **47**, 136 (1960).
[44] C. R. Stephens, K. Murai, H. H. Rennhard, L. H. Conover, and K. J. Brunnings. *J. Am. Chem. Soc.* **80**, 5324 (1958).
[45] J. H. Boothe, A. S. Kende, T. L. Fields, and R. G. Wilkinson. *J. Am. Chem. Soc.* **81**, 1006 (1959).
[46] T. L. Fields, A. S. Kende, and J. H. Boothe. *J. Am. Chem. Soc.* **82**, 1250 (1960); **83**, 4612 (1961).
[47] A. S. Kende, T. L. Fields, J. H. Boothe, and S. Kushner. *J. Am. Chem. Soc.* **83**, 439 (1961).
[48] R. G. Wilkinson, T. L. Fields, and J. H. Boothe. *J. Org. Chem.* **26**, 637 (1961).
[49] H. Muxfeldt. *Chem. Ber.* **92**, 3122 (1959).
[50] H. Muxfeldt, W. Rogalski, and K. Striegler. *Angew. Chem.* **72**, 170 (1960).
[51] H. Muxfeldt and A. Kreutzer. *Chem. Ber.* **94**, 881 (1961).
[52] M. M. Shemyakin, M. N. Kolosov, M. G. Karapetyan, and E. S. Chaman. *Doklady Akad. Nauk SSSR* **112**, 669 (1957).
[53] M. M. Shemyakin, M. N. Kolosov, Yu. A. Arbuzov, V. V. Onoprienko, and G. A. Shatenshteĭn. *Izvest. Akad. Nauk SSSR, Otdel. Khim. Nauk* 794 (1958).
[54] M. M. Shemyakin, M. N. Kolosov, Yu. A. Arbuzov, M. G. Karapetyan, E. S. Chaman, and A. A. Onishchenko. *Zhur. Obshcheĭ Khim.* **29**, 1831 (1959).
[55] M. M. Shemyakin, M. N. Kolosov, Yu. A. Arbuzov, Se Yuĭ-yuan, Shen Huaĭ-yuĭ, K. A. Sklobovskiĭ, and A. I. Gurevich. *Doklady Akad. Nauk SSSR* **128**, 113 (1959).
[56] M. M. Shemyakin, Yu. A. Arbuzov, M. N. Kolosov, G. A. Shatenshteĭn, V. V. Onoprienko, and Ju. V. Konnova. *Zhur. Obshcheĭ Khim.* **30**, 542 (1960).
[57] M. M. Shemyakin, M. N. Kolosov, Ju. A. Arbuzov, V. V. Onoprienko, and Se Yuĭ-yuan. *Zhur. Obshcheĭ Khim.* **30**, 545 (1960).
[58] M. M. Shemyakin, M. N. Kolosov, Yu. A. Arbuzov, and Yu. A. Berlin. *Doklady Akad. Nauk SSSR* **128**, 744 (1959).

[59] M. M. Shemyakin, Yu. A. Arbuzov, M. N. Kolosov, and Yu. A. Ovchinnikov. *Doklady Akad. Nauk SSSR* **133**, 1121 (1960).
[60] Yu. A. Arbuzov, A. A. Kiryushkin, M. N. Kolosov, Yu. A. Ovchinnikov, and M. M. Shemyakin. *Doklady Akad. Nauk SSSR* **137**, 1106 (1961).
[61] Yu. A. Arbuzov, Yu. A. Berlin, Yu. P. Volkov, M. N. Kolosov, Yu. A. Ovchinnikov, Se Yuĭ-yuan, Tao Chzhen-e, and M. M. Shemyakin. *Antibiotiki* **6**, 585 (1961).
[62] M. N. Kolosov, S. A. Popravko, and M. M. Shemyakin. *Ann.* (in press).
[63] Ref. 1, vol. II, p. 1161–1172.
[64] M. M. Shemyakin. *Angew. Chem.* **71**, 741 (1959); **72**, 342 (1960).
[65] M. M. Shemyakin. *Uspekhi Khim.* **31**, 269 (1962).
[66] M. M. Shemyakin, E. I. Vinogradova, M. Yu. Feĭgina, N. A. Aldanova, V. A. Oladkina, and L. A. Shchukina. *Doklady Akad. Nauk SSSR* **140**, 387 (1961).
[67] H. Gibian and K. Lübke. *Chimia (Switz.)* **14**, 380 (1960); *Angew. Chem.* **72**, 523 (1960); *Ann.* **644**, 130 (1961).
[68] R. Schwyzer and J. P. Carrion. *Helv. Chim. Acta* **43**, 2101 (1960).
[69] I. Ugi and U. Fetzer. *Angew. Chem.* **73**, 621 (1961).
[70] P. A. Plattner and U. Nager. *Experientia* **3**, 325 (1947).
[71] P. A. Plattner and U. Nager. *Helv. Chim. Acta* **31**, 665, 2192, 2203 (1948).
[72] L. C. Vining and W. A. Taber. *Can. J. Chem.* **35**, 1109 (1957).
[73] H. Brockmann and G. Schmidt-Kastner. *Chem. Ber.* **88**, 57 (1955).
[74] H. Brockmann and H. Geeren. *Ann.* **603**, 216 (1957).
[75] M. M. Shemyakin, Yu. A. Ovchinnikov, A. A. Kiryushkin, and V. T. Ivanov. *Tetrahedron Letters* 301 (1962).
[76] M. M. Shemyakin, Yu. A. Ovchinnikov, A. A. Kiryushkin, and V. T. Ivanov. *Izvest. Akad. Nauk SSSR, Otdel. Khim. Nauk* 2154 (1962).
[77] Yu. A. Ovchinnikov, V. T. Ivanov, A. A. Kiryushkin, and M. M. Shemyakin. *Izvest. Akad. Nauk SSSR, Otdel. Khim. Nauk* 1497 (1962).
[78] M. M. Shemyakin, Yu. A. Ovchinnikov, V. T. Ivanov, and A. A. Kiryushkin. *Tetrahedron* (in press).
[79] M. M. Shemyakin, E. I. Vinogradova, M. Yu. Feĭgina, and N. A. Aldanova. *Tetrahedron Letters* (in press).
[80] M. M. Shemyakin, Yu. A. Ovchinnikov, V. T. Ivanov, and A. A. Kiryushkin. *Izvest. Akad. Nauk SSSR, Otdel. Khim. Nauk*, 1699 (1962).
[81] D. W. Russell. *Biochim. et Biophys. Acta* **45**, 411 (1960).
[82] T. Ito and H. Ogawa. *Bull. Agr. Chem. Soc. Japan* **23**, 536 (1959).
[83] R. B. Woodward. Lecture read at 2nd Internl. Symp. on the Chem. of Natural Products, Prague (1962) *Pure Appl. Chem.* **6**, (4), in press.
L. H. Conover, K. Buttler, J. D. Johnston, J. J. Korst, and R. B. Woodward. *J. Am. Chem. Soc.* **84**, 3222 (1962).