MEETING REPORT

ANTIBIOTICS: CHEMISTRY AND MODE OF ACTION

Report of a Pre-Symposium held in Riga, U.S.S.R., on June 19 and 20, 1970, within the scope of the VII. International Symposium on the Chemistry of Natural Products

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For more than a quarter of a century antibiotics have unceasingly attracted the attention of numerous researchers in different branches of science. For those working in the fields of medicine and agriculture these substances are of importance primarily from a practical standpoint as compounds of remarkable biological activity. To chemists their interest lies in their unusual structures and reactions. Biochemists and molecular biologists regard them first of all as tools for studying specific biological processes and structures in the cell. It is, therefore, feasible to organize meetings of the widely differing specialists in the field of antibiotics to review achievements and discuss the areas and problems urgently in need of elucidation.

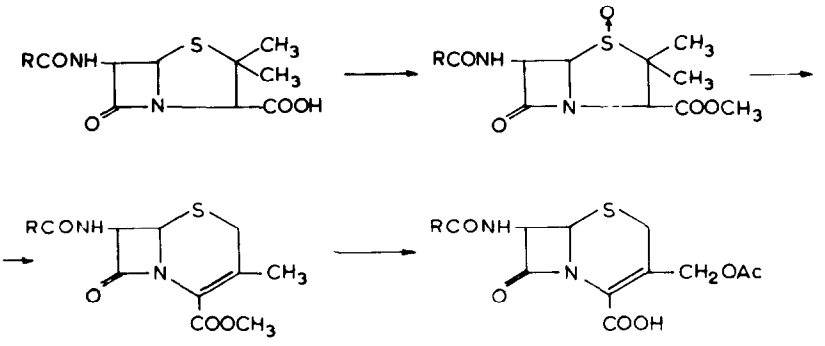
Opening the Pre-Symposium, its Chairman, A.S. Khokhlov briefly outlined its objectives and the manner of its procedure. His introductory remarks were followed by the reading and discussion of sixteen invited papers, which will be briefly reviewed here.

The papers can be divided into two groups. Most of them (those by Sheehan, Sensi, Shemyakin, Boothe, Kolosov, Borowski, Thrum, Khokhlov, Yonehara, Bognar and Gross) were devoted to critically reviewing the present state and future trends of the chemistry and molecular biology of large groups of antibiotics important from either an applied or theoretical point of view. The other lectures (Strominger, Weisblum, W. Kersten, Hahn and H.Kersten) were devoted to general problems directly connected with the mode of action of different antibiotics.

J.Sheehan (Cambridge, USA) discussed in detail studies on the synthesis and chemical transformations of the penicillins and cephalosporins which led to substances that in one way or another were superior to the naturally occurring antibiotics. He briefly discussed data on the most important semisynthetic penicillins. The author devoted his main attention, however, to the total synthesis of cephalosporins and their preparation by expansion of the thiazolidine ring of the penicillins to the corresponding 6-membered ring of the cephalosporins. Such a method involves the conversion of phenoxymethylpenicillin into phenoxymethylcephalosporin (scheme 1).

In the discussion of this lecture A.S.Khokhlov (Moscow, USSR) spoke about modification of penicillins at positions other than those mentioned by Sheehan. In particular, he pointed to the synthesis of homo- and bishomopenicillins, compounds possessing antipenicillinase activity. S.A.Giller (Riga, USSR) discussed the preparation of semisynthetic penicillins containing nitrofuran residues. The latter also impart antifungal activity to the penicillins.

Similar in spirit in its synthetic part to Sheehan's paper was the report by Sensi (Milan, Italy) on the rifamycins in which he described the long search for methods of modifying the naturally occurring rifamycin B so as to obtain derivatives with properties excelling those of the parent compound. Study of the biological properties of numerous such products showed that the enhanced *in vitro* activity (e.g. in case of quinoneimine analogs) does not necessarily correlate with *in vivo* data. Also (for instance on modification of the rifamycin B chromophore to give pienazine, phenoxazine or indole compounds) when compounds



Scheme 1

highly active *in vivo* were obtained, they were often poorly absorbed or produced considerable side effects. Consequently only a few products were found fit for clinical use and of these the best one turned out to be 3-(4-methyl-1-piperazinyl-iminomethyl)-rifamycin SV (rifampicin). This compound is successfully employed in the oral treatment of tuberculosis and a number of diseases caused by Gram-positive bacteria. According to preliminary data it is also effective against trachoma and leprosy.

Studies of the mode of action of rifamycins showed them to inhibit the DNA-dependent bacterial RNA polymerase, forming stable complexes with it. Rifamycin attacks the β-subunits of this enzyme. These antibiotics do not affect the RNA polymerase of mammalia, which explains their low toxicity.

E.Ya.Gren (Riga, USSR) reported on the preparation of a number of new products of the transformation of rifamycin B.

Recently research has been attracted more and more to those antibiotics whose action is associated with increases in the permeability of biological membranes to alkali metal ions. The best known members of this class are the macrocyclic depsipeptides (valinomycin, enniatins) the chemistry of which had been studied mainly in the laboratory of the late M.M. Shemyakin (Moscow), whose death occurred suddenly while he was honorary president of the Symposium. Shemyakin's paper at the present Pre-symposium was devoted to structure-function relations of valinomycin and allied cyclodepsipeptides. Investigation of the physicochemical properties of valinomycin and its synthetic analogs in solution and correlation of these properties with the metal binding capacity of these compounds made it possible to establish the rigidity limits of the so-called "bracelet" conformation characteristic of valinomycin and to outline ways for the synthesis of compounds with altered ionic selectivity. The study of the complexing properties of the cyclodepsipeptides and cyclopeptides has become of special interest because the ion-dipole type of cation binding by a spatially ordered system of ligand groups (amide and/or ester carbonyls) is apparently also characteristic of the selective cation binding sites of proteins (e.g. transport ATPases).

Shemyakin devoted much of his report to the mode of action of the cyclodepsipeptides. Many of the valinomycin analogs which bind K^+ ions in solution and which increase the K^+ permeability of model phospholipid membranes are devoid of antibiotic activity. This had been earlier ascribed to the specific structure of bacterial membranes, limiting the possible structural variations of the active cyclodepsipeptide. Similar proposals had also been made with respect to the membranes of valinomycin resistant microorganisms. However, it turned out that antibiotically inactive compounds increase the K^+ permeability of the microbial cell (*Streptococcus faecalis, Staphylococcus aureus*), their effect with respect to valinomycin being about the same as that for model membranes. If the cyclodepsipeptide-induced shifts of the ionic composition of the cells are increased by changing the salt composition of the medium, sensitivity to valinomycin and its "inactive" analogs can be observed among formerly "resistant" microorganisms. In the case of the *S. aureus* 209-P such an effect was achieved by increas-

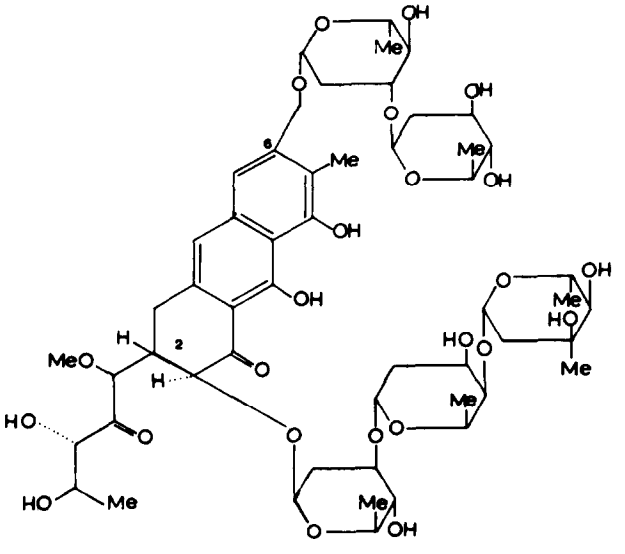
ing the K^+ ion concentration in the medium from 10 mM to 100 mM. The above experiments were reported in detail by A.M.Shkrob (Moscow, USSR).

J.Boothe (Pearl River, USA) discussed in detail the structure-activity relations of the important tetracycline group. He made a number of generalizations about chemical transformations of naturally occurring antibiotics, work undertaken to improve the properties of the parent compounds. Of greatest interest was 2-dimethylamino-6-deoxy-6-demethyltetracycline (minocycline) whose potency excelled that of the other tetracyclines. Particularly interesting is its ability to inhibit the growth of strains resistant to the other membranes of this group. Moreover, resistance to it develops much more slowly than to the other tetracyclines.

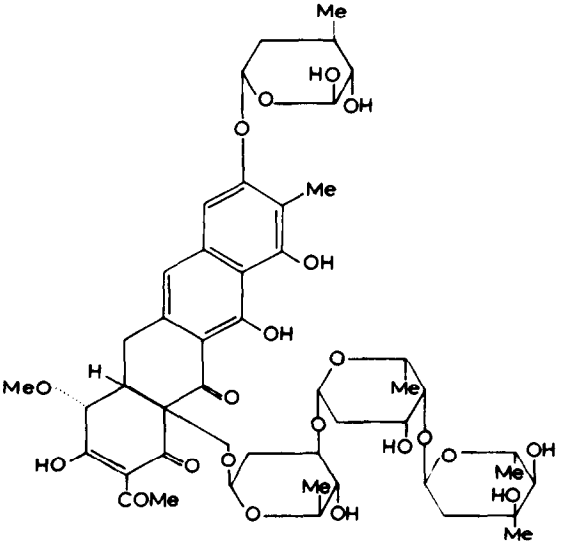
M.N.Kolosov (Moscow, USSR) described chemical studies of the aureolic acid group of antibiotics. The author led a programme of research that established the structures of the antitumor antibiotic aureolic acid (mithramycin) and the allied substances olivomycins, chromomycins and aburamycins. All these compounds are polyhydroxy hydroanthracenic glycosides, whose aglycones are olivin or its methyl homolog chromomycinone and which contain two carbohydrate chains in the 2 and 6 positions. The most active members of this group (aureolic acid, olivomycins A and B and chromomycins A_2 and A_3) have, in position 2, a side chain of 3 carbohydrate residues ending with a branched sugar mycarose or acylolivomycose. The corresponding tetrosides lacking this sugar (olivomycin D, chromomycin A_4) are much less biologically active and may be regarded as unfinished antibiotics. Of all these substances olivomycin A has the best therapeutic index. Besides aureolic acid, an accompanying biologically inactive metabolite, chromocyclomycin, was isolated and its structure elucidated. Its aglycone, chromocycline, is biogenetically close to chromomycinone on the one hand, and to the tetracycline antibiotics on the other (scheme 2).

G.B.Lokshin (Moscow, USSR) reported the structure of a new member of the above group of substances, antibiotic 6604-9A.

Although polyene macrolides are among the most



Aureolic acid



Chromocyclomycin

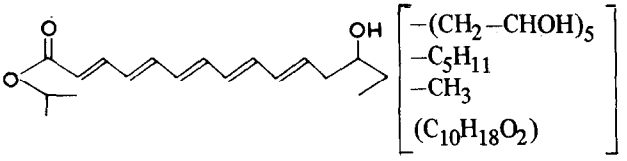
Scheme 2

important of the antibiotics their chemical study proceeded very slowly for a long time. Only in recent years has a much more lively progress in this field been noted, yielding the structures of rimocidin, pimaricin, lucensomycin, the tetrins A and B, hexamycin and other compounds. Generalizations about studies in this field were the subject matter of the papers by E.Borowski (Gdansk, Poland) and H.Thrum and R.Schlegel (Jena, GDR).

Borowski noted that improved methods of separating polyenes have made it possible to isolate new substances from previously described preparations, for instance, the isolation of perimycins A, B and C from perimycin. The author presented a refined classification of heptaene antibiotics dividing them into 4 sub-groups depending upon the nitrogen-containing components: (1) non-aromatic compounds containing the amino sugar mycosamine (candidin, candidinin, candioin, amphotericin B, mycoheptyne); (2) aromatic compounds containing mycosamine and *p*-aminoacetophenone (candicidines, trichomycins, levorins, hamycin etc.); (3) aromatic compounds containing mycosamine and *p*-*N*-methylaminoacetophenone (aureofungin, hedamycin); (4) aromatic compounds containing the amino sugar perosamine and *p*-*N*-methylaminoacetophenone (perimycins A and C). Using the accumulated data, the author developed a general scheme for elucidating the structures of polyene antibiotics such as was used in establishing the structure of candidin and amphotericin B and in correcting the formula of nystatin.

The author also dwelt on some of the biological properties of polyene antibiotics, in particular their ability to form complexes with cholesterol, making it possible to vary the cholesterol content in the organism (experiments on dogs).

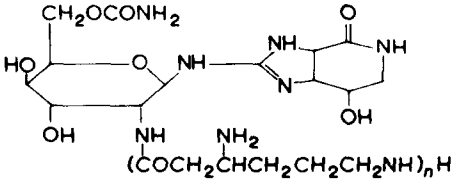
H.Thrum and R.Schlegel described their studies of the peculiar group, the mycoticin–flavomycin polyenes, the distinctive feature of which is the conjugation of a pentaene grouping with a carbonyl. There are at least three different antibiotics in this group: mycoticin A (flavofungin "a"), mycoticin B (flavofungin "b") and flavomycoin. They differ in optical rotation, molecular weights and other properties. The structures of the first two have already been reported and for flavomycoin ($C_{41}H_{68}O_{10}$) the authors propose the following partial formula:



Dermostatin also belongs to this type of compounds, although it has a hexaene grouping.

L.A.Vetlugina (Alma-Ata, USSR) reported on the antibiotic roseofungin which differs in properties from all other substances of the flavomycoin-mycoticin-flavofungin group.

A.S.Khokhlov (Moscow, USSR) described the studies of the streptothricins carried out in the last years under his guidance. It was found that six individual substances (streptothricins A-F) can be isolated from partially purified streptothricins. Their structures are formulated below, they differ in the number of β-lysine residues they contain. A seventh antibiotic of this type (streptothricin X) is obtained by directed fermentation in the presence of large amounts of α-aminoadipic acid.



F: $n = 1$ B: $n = 5$
E: $n = 2$ A: $n = 6$
D: $n = 3$ X: $n = 7$
C: $n = 4$

Streptothricin biosynthesis differs fundamentally from the biosynthesis of proteins and is not suppressed by chloramphenicol. All streptothricins are formed by the same route, streptothricin F is formed first and β-lysine residues are added to it. The β-lysine residues are formed from α-lysine, but not by transamination.

The streptothricins strongly suppress protein biosynthesis in a Nierenberg cell-free system containing *E. coli* B ribosomes. Their activity is commensurate

Scheme 3

with that of chloramphenicol, e.g. protein biosynthesis is inhibited by 98–99% by 400 μM chloramphenicol and by 800 μM streptothricin B. Antibacterially inactive products of mild acid-inactivation of the streptothricins show an effect on protein biosynthesis similar to that of the parent antibiotics.

H.Yonehara (Tokyo, Japan) in his paper on the development of antibiotics for agriculture use in Japan, discussed the results of work begun in 1952 primarily concerned with the search for antifungal agents for the treatment of rice. The antibiotics antimycin (blastmycin), blasticidin S, kasugamycin and the polyoxins proved effective and the last three have found practical application. The antimycins have a strong toxic effect on fish and therefore cannot be used. The author also presented methods for isolation of blasticidins A and S and described the properties of these antibiotics. The possibility of chemical or microbial synthesis of blasticidin S from the products of its splitting, blastidic acid and cytosinine, is of great interest. This allows the preparation of new analogs with altered amino acid moieties. Study of polyoxins showed the presence of about 30 compounds in a crude preparation. Their structure and structure-activity relationships are under investigation.

R.Bognar (Debrecen, Hungary) devoted his lecture "Oligosaccharide moieties of some antibiotics" to a number of very important problems confronting modern organic chemistry. Sugars, many hitherto unknown, have been found as constituents of several dozen antibiotics. Bognar reviewed available data on the structures of the oligosaccharide fragments of streptomycin, neomycin, paromomycin, olivomycin, chromomycin, amycetin, everninomycin, saturated macrolide antibiotics, the anthracycline antibiotics and many others. He paid considerable attention to the oligosaccharide moiety of the ristomycin antibiotics (ristocetins) and in particular showed how the rhamnose and mannose residues were attached to the glucose residue in ristomycin A. N.N.Lomakina (Moscow, USSR) reported data on a new amino sugar from the ristomycins.

E.Gross (Bethesda, USA) spoke about antibiotics containing as component parts α,β-unsaturated α-amino acids, substances that have lately attracted much attention. The author first listed a numer of naturally occurring compounds with such amino acids (albonoursin, telomycin, stendomycin, etc.), but dwelt mostly on nisin, an antibiotic of highly diversified biological activity. He had found conditions for the selective cleavage of nisin with cyanogen bromide (under ordinary conditions such treatment led to a very complicated mixture of products), isolated and characterized the *N*- and *C*-terminal fragments and established the structure of the *N*-terminal undecapeptide shown in scheme 3.

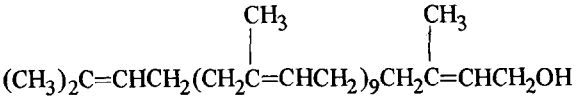
The part played by α,β-unsaturated α-amino acids in creating a definite structure of the antibiotic molecule necessary for its biological activity was also considered.

P.D.Reshetov (Moscow, USSR) presented data on the purification and properties of two other antibiotics belonging to the nisins: subtilin and cinnamycin.

In his lecture J.Strominger (Cambridge, USA) reviewed the work aimed at elucidating the mechanism by which the penicillins, cephalosporins and other antibiotics inhibit the formation of the bacterial cell walls. The author regarded the wall as a gigantic three-dimensional molecule built up of glycan strands composed of alternating acetylglucosamine and acetylmuramic acid residues, the strands being cross linked by peptide bridges.

In a consideration of the biosynthesis of the polyglycan of cell wall, three fundamental stages can be distinguished. The first stage, proceeding in the cell cytoplasm, consists of the biosynthesis of uridine nucleotide precursors and leads to the formation first of UDP-acetyl glucosamine and UDP-acetylmuramyl tripeptide (containing L-alanine, D-glutamic acid and L-lysine) and then, by addition of D-alanyl-D-alanine, of the corresponding UDP-acetylmuramyl-pentapeptide. Important, in the last step, are the two enzymes, alanine racemase and D-alanyl-D-alanine synthetase. Both enzymes are inhibited by D-cycloserine thus explaining its antibiotic action.

The second stage proceeds in the cell membrane. Here a number of processes occur. First there is formed a disaccharide of the pentapeptide attached to the lipid through a pyrophosphate bridge. This is followed by the addition of several amino acids and finally the resultant molecule is built into the growing cell wall with simultaneous formation of a C_{55} alcohol pyrophosphate.



Ristomycin and vancomycin interfere with transfer of the molecule formed to the bacterial wall while bacitracin inhibits the dephosphorylation of the C_{55} alcohol pyrophosphate and interrupts the return of the lipid carrier into the cycle.

The final stage consists of the formation of cross links between two peptidoglycan strands by transpeptidation and simultaneous cleavage of the terminal D-alanine. This stage occurs on the external surface of the membrane. It is here that the penicillins and cephalosporins exert their inhibiting action, reacting with two enzymes catalyzing the above reactions, namely peptidoglycan transpeptidase and D-alanine carboxypeptidase. The first enzyme is inactivated irreversibly, the second, reversibly, its activity being restored by treatment, for instance, with hydroxylamine or ethylmercaptane.

In a discussion of this paper G.Peck (Moscow, USSR) noted that in this excellent work where it was possible to pinpoint the site of action of the penicillins on bacteria, the part played by the side chain of these compounds is worthy of attention. N.M.R. and other physicohemical studies seemed to show that the side chain does not affect the bicyclic penicillin ring system in the ground state, although it is known to have a profound effect on the stability of these compounds. The side chain must therefore apparently play a definite role in the activated complex and should be taken into consideration in mechanistic treatment of the splitting of the β-lactam ring although of course the enzymatic reactions may proceed differently.

B.Weisblum (Madison, USA) reported on his discovery of the ability of erythromycin to modify *Staphylococcus aureus* ribosomes, as a result of which he was able to obtain strains resistant to three different types of the 50S ribosome subunits (macrolides, lincosaminides and antibiotics of the type of streptogramine B). The resistant cultures again become antibiotic-susceptible when cultivated for 90 min in the absence of erythromycin. The induction of resistance is suppressed by chloramphenicol (inhibitor of protein biosynthesis) and by streptovaricin (inhibitor of the RNA formation) but not by novobiocin (inhibitor of DNA biosynthesis). Further study revealed two types of induced resistance. Ribosomes isolated from both types of resistant cells were changed in their functional properties and also probably in their structure.

F.Hahn (Washington, USA), W.Kersten (Erlangen, GFR) and H.Kersten (Erlangen, GFR) devoted their lectures to various aspects of the interaction between antibiotics and nucleic acids. The first two authors studied the reactions *in vitro*, whereas the last author was concerned with the properties of nucleic acids after the action of antibiotics on the microbial cell.

F.Hahn showed that antibiotics and other drugs forming complexes with DNA can be divided into two classes according to the binding mechanism. Members of one group such as spermine, distamycin etc. complex with groups on the periphery of the double helix, whereas with most antibiotics and other pharmaceutical preparations (for example, atebrin, chloroquine, quinine) complexing is accompanied by intercalation.

In both cases increases in rigidity of the double helix occurs hindering its unfolding. The binding by DNA of chloroquine, atebrin and quinine inhibits the polymerization reactions not only of DNA, but also of RNA.

W.Kersten showed that in the formation of complexes with DNA the chromophore grouping of the anthracycline antibiotics undergoes conformational changes. Daunomycin and nogalamycin differ from the chromomycins and mithramycin in the mode of their interaction with DNA. The two latter compounds preferentially bind GC pairs whereas the former two react also with AT pairs.

Granaticin inhibits RNA synthesis which may be ascribed to binding of the sulphydryl groups of proteins controlling the synthesis of RNA.

H.Kersten dwelt on the significance of methyl groups for the functioning of RNA. Pactamycin, an antibiotic inhibiting protein synthesis, stimulates the formation of RNA. Pactamycin- (and also tetracycline-) treated ribosomal and messenger RNA from *B. subtilis*

contain relatively fewer methyl groups than do those from normal cells.

In a cell free model with polyU as mRNA, 70 S ribosomes from pactamycin treated cells were found to be less active in the synthesis of polyphenylalanine. The acceptor activity of tRNA from the pactamycin-treated cells is reduced towards phenylalanine, but not towards lysine, valine or alanine. All these data are of considerable interest from the point of view of the functional role of methyl groups in the synthesis of RNA and of proteins.

Closing the Pre-symposium D.Gottlieb (Urbana, USA) stressed the importance of studying the interaction of antibiotics with the living cell for comprehending the principles of its structure and functioning. He noted the timeliness and good organization of this meeting.