THE DEVELOPMENT OF BIOORGANIC CHEMISTRY IN THE U.S.S.R. DURING THE LAST DECADE

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ABSTRACT

A brief review has been made of selected work in bioorganic chemistry carried out in the Soviet Union in the past decade. Most consideration has been given to the chemistry of peptide and protein substances, including structural, synthetic, stereochemical and structure–functional studies. The most important results have been touched upon, obtained from chemical study of nucleic acids, carbohydrates and mixed biopolymers and also of bioregulators (enzymes, hormones, antibiotics, etc). Special attention has been paid to the structure of biological membranes and the physical chemistry of their functioning, in particular, to studies of transmembrane ion transport.

Beginning with the middle of this century progress in the natural sciences has become closely identified with our deepening knowledge of the physicochemical basis of life. The outstanding achievements marking progress in the solution of this greatest of Nature's enigmas have been the direct result of the ingraining into biology of the concepts and methods of chemistry and physics. As the result of this development we are now, in this area, thinking on a molecular level.

The chemistry of natural products has also not escaped fundamental changes particularly in the last decade. First and foremost, this field has become intimately linked with the study of biological function, a union which has rapidly transformed the classical, largely descriptive natural product chemistry into modern bioorganic chemistry, a major branch in the vigorously developing complex of sciences that have given contemporary biology its physicochemical footing.

It is only natural that bioorganic chemistry has developed hand in hand with advances in biochemistry and biophysics and that many of the most important types of compounds, biochemical processes and biological phenomena are simultaneously the subject of these three closely allied sciences.

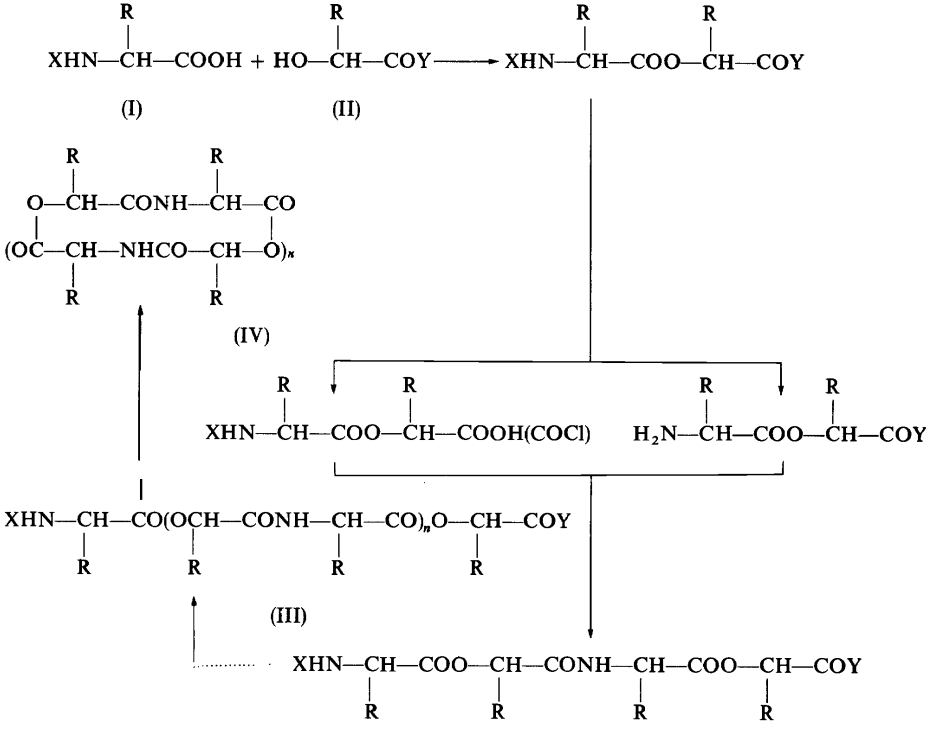
In the course of the transition into modern bioorganic chemistry, not only the objectives, but the objects themselves and the methods of their study have undergone fundamental change. Primary attention is now being given to those biopolymers (proteins, nucleic acids, lipids, carbohydrates), whose conversions comprise the chemical essence of vital activity, and to those bioregulators (enzymes, hormones, antibiotics, etc.) which induce these conversions or effect

their control. As for the methods the rapid industrialization of laboratory techniques has led to such acceleration of the tempo of research that elucidation of the structure of a substance, or its synthesis, has become no longer an end in itself, but a cognitive means.

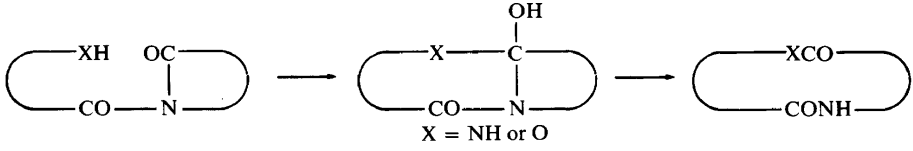
It is in such a direction that bioorganic chemistry has been developing in the Soviet Union in the last decade. The main results of this development are the subject matter of the present lecture.

PEPTIDES AND PROTEINS

Much of the Soviet work in the chemistry and biochemistry of peptides and proteins has, as in other parts of the world, been connected with problems of their structure–function relations. Along these lines atypical compounds have attracted much interest.



X = $PhCH_2OCO$, *p*-$O_2NC_6H_4CH_2OCO$, *t*-BuOCO, *o*-$O_2NC_6H_4S$
Y = $PhCH_2O$, *p*-$O_2NC_6H_4CH_2O$, *t*-BuO



(V) (VI) (VII)

Among the atypical peptides, of particular interest are depsipeptides, because their constituent ester and amide groups, despite structural differences, are very similar sterically and electronically. This circumstance has created unique conditions for a directed structure–function study of depsipeptides, particularly by way of comparison with the corresponding peptide systems.

Intensive growth of depsipeptide chemistry in the Soviet Union was induced by the development of simple, and at the same time general, methods for the synthesis of optically active linear and cyclic depsipeptides of types (III) and (IV)[1,2]. The basic method involves the stepwise condensation of protected hydroxy and amino acids (I) and (II). A fundamentally different method is based on hydroxy- and amino-acyl incorporation into amides acylated by hydroxy and amino acid residues (V)→(VII), proceeding via cyclols (VI). A large number of naturally occurring depsipeptides and their analogues, including a number of antibiotics, have been synthesized by these two procedures. Some of the stable oxa- and aza-cyclol intermediates (VI) were also subjected to detailed study from another angle[3–5], since they are unique examples of orthoamide systems usually regarded as intermediates in various nucleophilic reactions (including enzymic) of carboxylic acid derivatives.

The synthetic studies provided the means for a deeper insight into various aspects of the structure–function relation of depsipeptides and peptides. The first significant result in this respect was the establishment of the mutual replaceability of an ester and an amide group in the naturally occurring depsipeptide and peptide molecules without their loss of biological activity. In particular, it was shown (*Table 1*)[6,7] that replacement of one or two ester

Table 1. Antibiotic activity of valinomycin and its analogues

No.	*Compound*	*Minimal growth inhibiting concentration* (γ/ml)			
		Staph. aureus u.v. 3	Sarcina lutea	Myco-bact. phlei	Cand. albi-cans.
(VIII)	[(D-Val-L-Lac-L-Val-D-HyIv)$_3$] (Valinomycin)	0.8	1.5	0.3	0.8
(IX)	[D-Val-L-Ala-L-Val-D-HyIv-(D-Val-L-Lac-L-Val-D-HyIv)$_2$]	1.0	2.0	0.4	4.5
(X)	[(D-Val-L-Ala-L-Val-D-HyIv-)$_2$D-Val-L-Lac-L-Val-D-HyIv]	2.0	2.0	2.0	2.0

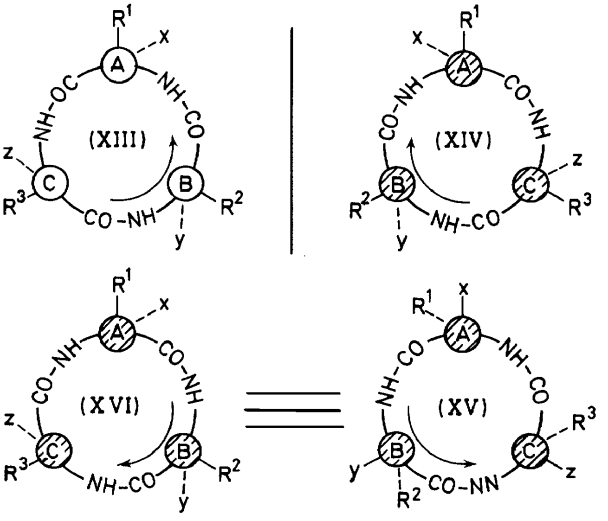
groups by amide groups in valinomycin (VIII), leading to the analogues (IX) and (X), has little effect on its antimicrobial properties. On the other hand the replacement of amide groups by ester groups has afforded a number of biologically interesting analogues of bradykinin (XI), angiotensin II (XII) and other peptide hormones. Thus, the analogue (XIa) was found to have a higher hypotensic action than bradykinin itself and the substitution of an amide group by an ester group in the angiotensin molecule (XII) has led to differentiation of its biological properties, in analogues (XIIa and XIIb) the pressor activity peculiar to this hormone being lowered to a considerably less degree than its accompanying oxytocic activity, which practically disappears.

These studies served as starting point for elaborating new principles of

	Bradykinin (1 2 3 4 5 6 7 8 9)	Dimunution of blood pressure (g/kg)
(XI)	H-Arg-Pro-Pro-Gly-Phe-Ser-Pro-Phe-Arg-OH	1.0×10^{-7}
	(XIa) $Phe^8 \rightarrow PhL^8$	4.0×10^{-7}

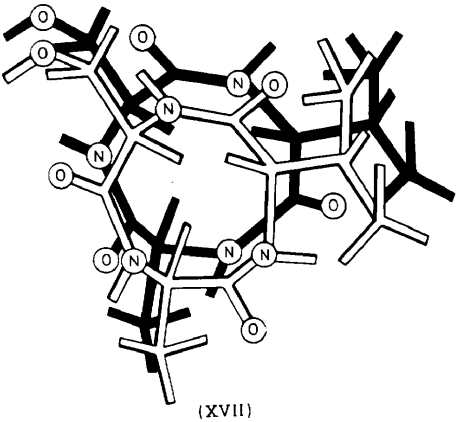
	Angiotensin II (1 2 3 4 5 6 7 8)	Pressor activity (g/ml)	Oxytocin activity (i.u.)
(XII)	H-Asn-Arg-Val-Tyr-Val-His-Pro-Phe-OH ..	1.0×10^{-9}	54
	(XIIa) $Val^5 \rightarrow HyIv^5$	3.0×10^{-8}	0.05
	(XIIb) $Phe^8 \rightarrow PhL^8$	1.0×10^{-8}	0.05

rational search for analogues of these compounds[7] resulting in the topochemical principle of their transformation whereby the molecule as a whole is modified and yet the resultant analogue is quite similar to the initial compound both sterically and with respect to the electronic nature of the functional groups, so as to comply with the pre-requisites for manifestation of biological activity. Among the rational, topochemical transformations of cyclic peptide and depsipeptide systems are: inversion of the configuration of all active centres [enantioisomers of types (XIII) and (XIV)], inversion of acylation direction of the amino and hydroxy acid residues [retro-isomers of the type (XV), equivalent to (XVI)], replacement of amide groups by ester groups (and vice versa) etc. Such



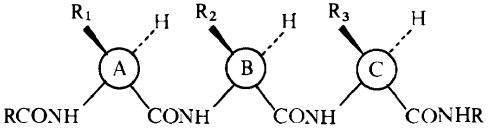
topochemical analogues could play the role of 'doubles' in biological processes (XVII) since they would have very similar biological properties.

A similar approach can also be applied to linear peptides with equalized N-

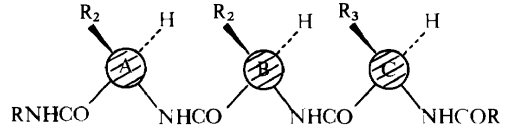


(XVII)

and C-termini. Thus compounds of type (XVIII) and (XIX) differ only in the inverse arrangement of atoms in the amide groupings. It is therefore no wonder that the topochemical analogue of the protected dipeptide (XX), namely its



(XVIII)



(XIX)

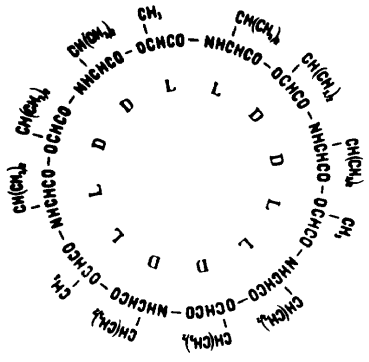
CH_3CO-L-Leu-L-Tyr-$NHCH_3$ CH_3CO-D-Tyr-D-Leu-$NHCH_3$

(XX) (XXI)

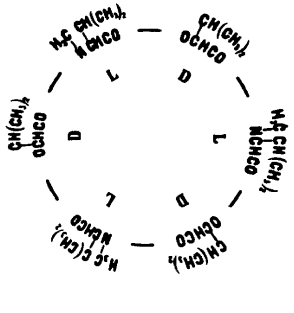
retro-enantiomer (XXI), proved to be an effective competitive inhibitor of pepsin, whereas all other stereoisomers, not being topochemical analogues, displayed a mixed type of inhibition.

The most considerable advances in structure–function studies of the depsipeptides have been made in the last few years in the course of their use for investigating trans-membrane ion transport, bringing us to the threshold of comprehending the essence of both the relation and the biological phenomenon itself[8–14].

A distinctive feature of many macrocyclic depsipeptides belonging to the group of valinomycin (VIII) and enniatin antibiotics (XXIIa, b) is their ability greatly to enhance the permeability of artificial (for instance bilayer phospho-



Valinomycin (VIII)



Enniatin B (XXIIb)

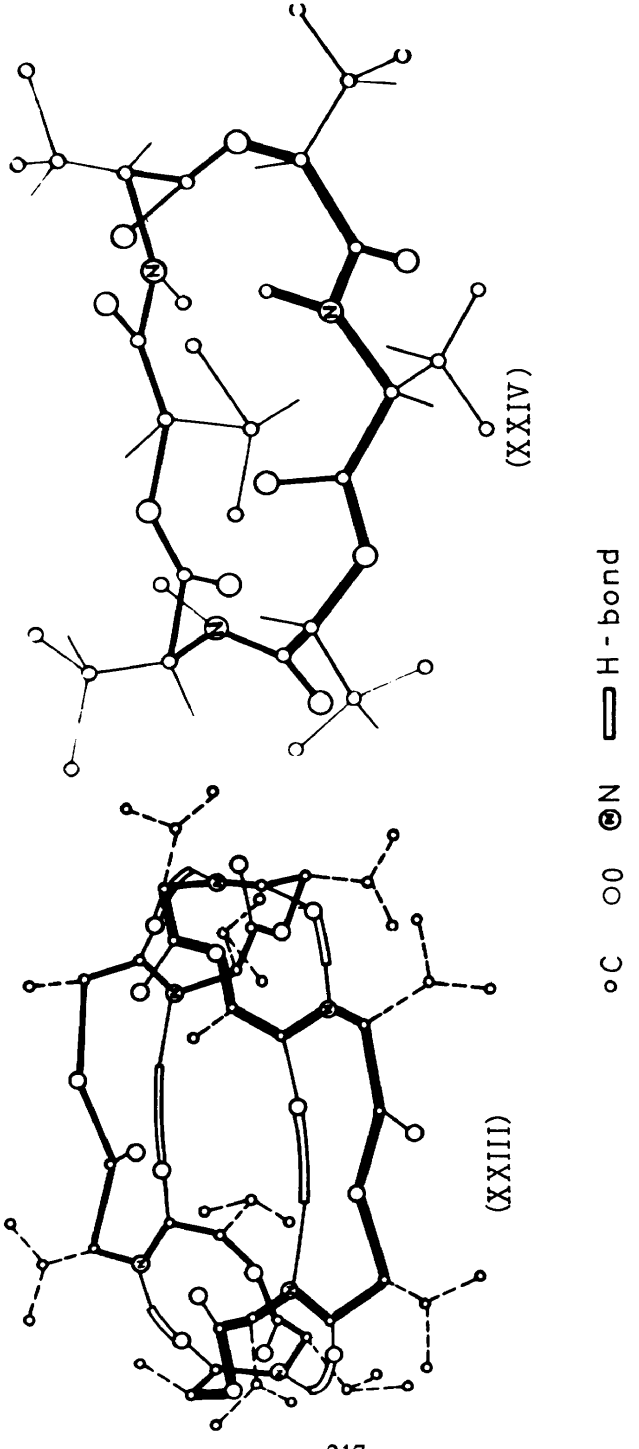
Table 2

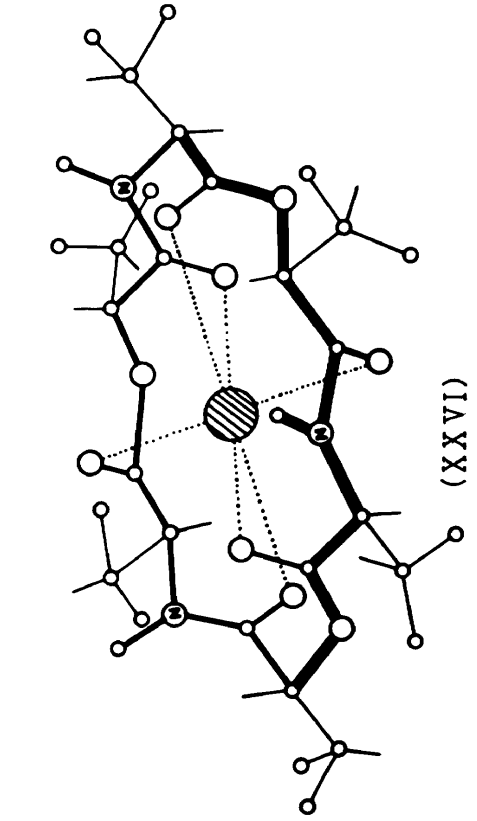
The complexing ability of valinomycin and enniatin B and their effect on transmembrane ion transport

	Complexation $K \times 10^{-3}$ L/mole EtOH, 25°		Bi-ionic potential (mV)* on egg lecithin bilayer membranes	Relative K^+ transport induction in mitochondria
	Na^+	K^+		
Valinomycin (VIII)	0	2 000	140	100
Enniatin B (XXIIb)	2.6	6.5	8.5	1.3

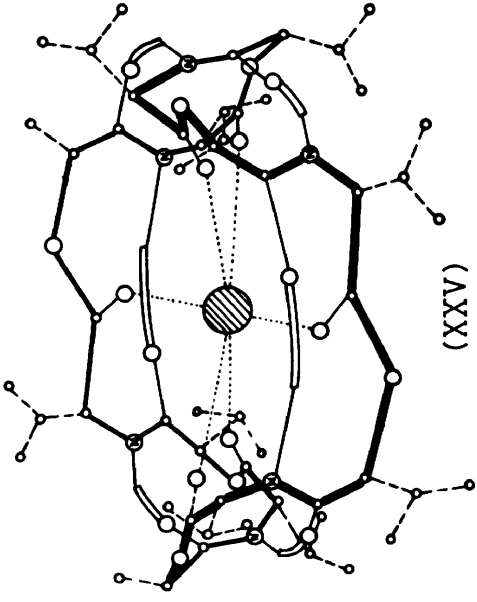
* Membranes separated 0.1 M NaCl and 0.1 M NaCl + 0.1 M KCl solution.

lipid) and biological (for instance, mitochondrial) membranes, an effect often associated with a strikingly high ion selectivity (see the data in *Table 2*). This property is the result of the ability of the cyclodepsipeptides to bind the alkali metal ions into a lipophilic cation complex. It was shown by a combination of physical methods that such complexes are unstable in water, forming only in low polar media since in these latter the depsipeptides are in a quite rigid conformation, like a bracelet (XXIII) in the case of valinomycin and like a disc (XXIV) in the case of the enniatins. 'Internal solvation' of the cation occurs in the complexes (XXV) and (XXVI) owing to strong ion–dipole interaction with the ester and amide carbonyls, whereas the lipophilicity necessary for incorporation of the complexes into the membrane is provided by the peripheral hydrocarbon sidechains. Valinomycin and enniatin analogues with unstable bracelet or disc conformations are devoid of both the ability to form complexes and to induce ion transfer through artificial and biological membranes. The ion selectivity of the cyclodepsipeptides depends upon the conformational rigidity of their molecules. With the rigid bracelet conformation of valinomycin the selectivity [binding of only K^+, but not Na^+; cf. formulas (XXVII) and (XXVIII)] is determined by the correspondence of the ion size and the internal cavity of the bracelet. On the other hand enniatin compounds possessing greater


(XXIV)
(XXIII)
C O N H - bond

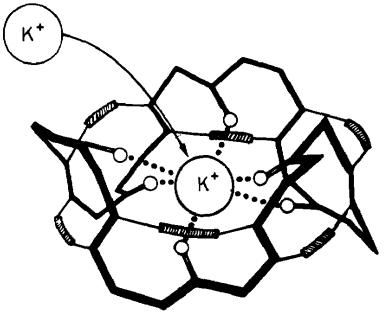


(XXVI)

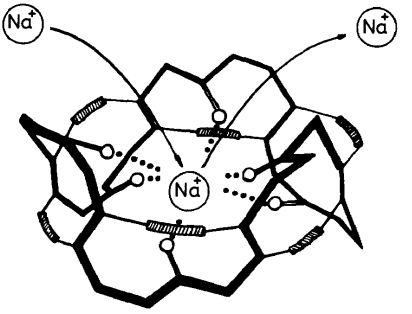


(XXV)

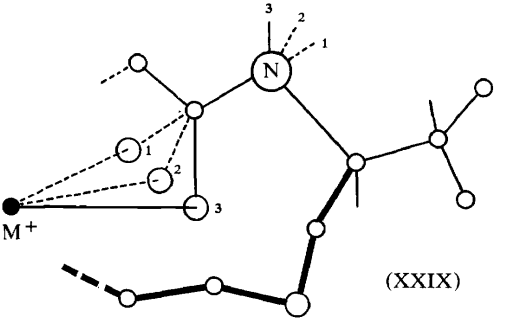
°C ○O ⊗N ● K ═ H-bond



(XXVII)

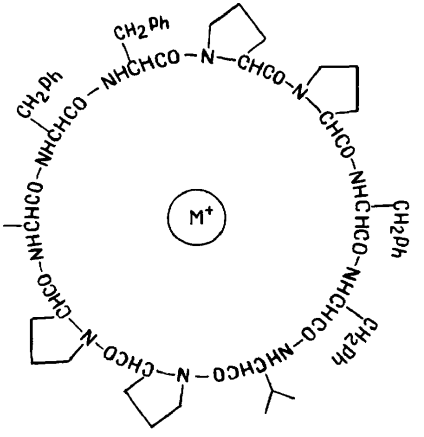


(XXVIII)



(XXIX)

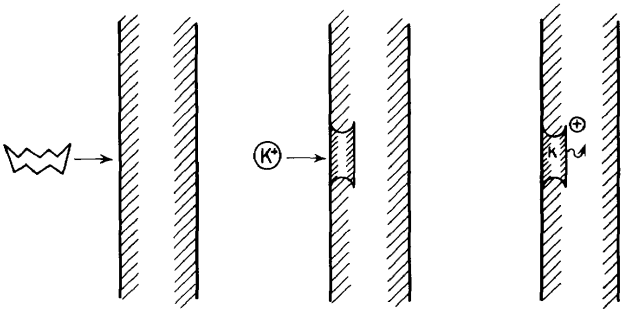
Dependence of enniatin conformation on the effective radius of the complexing cation



Antamanide (XXX)

conformational flexibility, are capable of adapting their conformation to the size of the cation ['induced fit', see (XXIX)], which thus explains their lower ionic selectivity (binding of both K^+ and Na^+). Similar structure–functional relations are also observed among cyclopeptides, such as, for instance antamanide, capable of binding alkali metal (preferably sodium) ions in low polar media (XXX).

Thus the trans-membrane-ion-transport-inducing depsipeptide first penetrates the lipophilic region of the membrane and only then, acquiring the suitable, sufficiently rigid conformation, entraps a cation from the aqueous phase, to become converted into the lipophilic complex cation, capable of



(XXXI) (XXXII) (XXXIII)

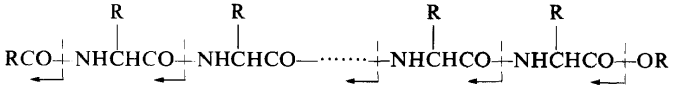
moving within the membrane (XXXI)→(XXXII)→(XXXIII). In this way the depsipeptide molecule can function either as a direct trans-membrane ion carrier, or ion transport can be accomplished by a mechanism involving relaying of the ion from the complex to another (or other) depsipeptide molecule.

The studies just described have brought us to the threshold of comprehending the mode of polypeptide promoted ion transport through biological membranes (see p. 247).

Turning to protein chemistry, mention must be made of the development of a mass spectrometric method for determining the amino acid sequence in peptides [15–17]. This was achieved after preliminary elucidation of the general and particular relationships in the mass spectrometric fragmentation of acylpeptide esters containing all the ordinary amino acids. It was shown that the basic type of fragmentation of the acylpeptide esters, on which the determination of the amino acid sequence rests, is the amino acid type of fragmentation (XXXIV). Particularly fruitful for sequence studies of proteins is mass spectrometry in combination with preliminary Edman cleavage of the peptide chain from the N-terminus or its cleavage by carboxypeptidase from the C-terminus (XXXV). Such a combination opens up new possibilities for rapid primary structure determination of proteins and is being successfully used at present for elucidating the structure of enzymes (aspartate transaminase, pepsin, etc.).

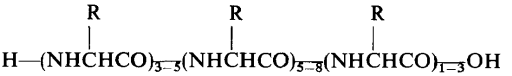
Of the large number of studies devoted to the three dimensional structure of proteins, special attention should be given to the new approach combining x-ray

Amino acid type of fragmentation in acylpeptide esters



(XXXIV)

Determination of amino acid sequence in peptides

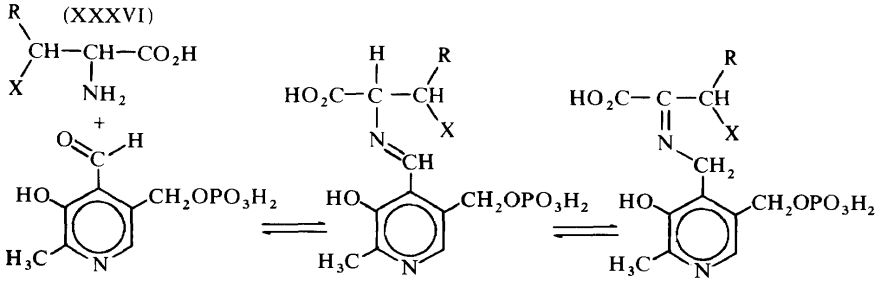


(XXXV)

By Edman's method | By mass spectrometry | By carboxypeptidase

analysis with theoretical conformational analysis[18–20]. It was demonstrated that the steric structure of the amino acid residues in the protein can be described by a limited number of conformations, corresponding to potential energy minima, that could be utilized as a conformational code. With the aid of this code from x-ray data of only the principal steric arrangement of the polypeptide chain in the protein one may approximately calculate the tertiary structure of the molecule.

In recent years the study of the chemical basis of enzymic catalysis has received an ever increasing impetus in the USSR. In this field predominant attention is being allotted to the structure–function relationships. These studies have as their source the earlier classical researches in enzymology of V. A. Engelhardt and A. E. Braunstein.



(XXXVII) (XXXVIII) (XXXIX)

(XXXVIa) L-$RCH(NH_2)CO_2H \rightleftharpoons$ D-$RCH(NH_2)CO_2H$

(XXXVIb) $RCH(NH_2)CO_2H + R'COCO_2H \rightleftharpoons RCOCO_2H + R'CH(NH_2)CO_2H$

(XXXVIc) $RCH(NH_2)CO_2H \rightleftharpoons RCH_2NH_2 + CO_2$

(XXXVId) $RCH(X)CH(NH_2)CO_2H \rightleftharpoons RCH(SR')CH(NH_2)CO_2H$

(XXXVIe) $RCH(X)CH(NH_2)CO_2H \rightleftharpoons RCH_2COCO_2H + HX + NH_3$

(XXXVIf) $RCH(X)CH(NH_2)CO_2H \rightleftharpoons RCHO + H_2NCH_2CO_2H$

(XXXVIg) $RCH_2CH(NH_2)CO_2H \rightleftharpoons CH_2(Y)CH(NH_2)CO_2H$

(XXXVIh) $CH_3(X)CHCH(NH_2)CO_2H \rightleftharpoons CH_3CH_2COCO_2H + XH + NH_3$

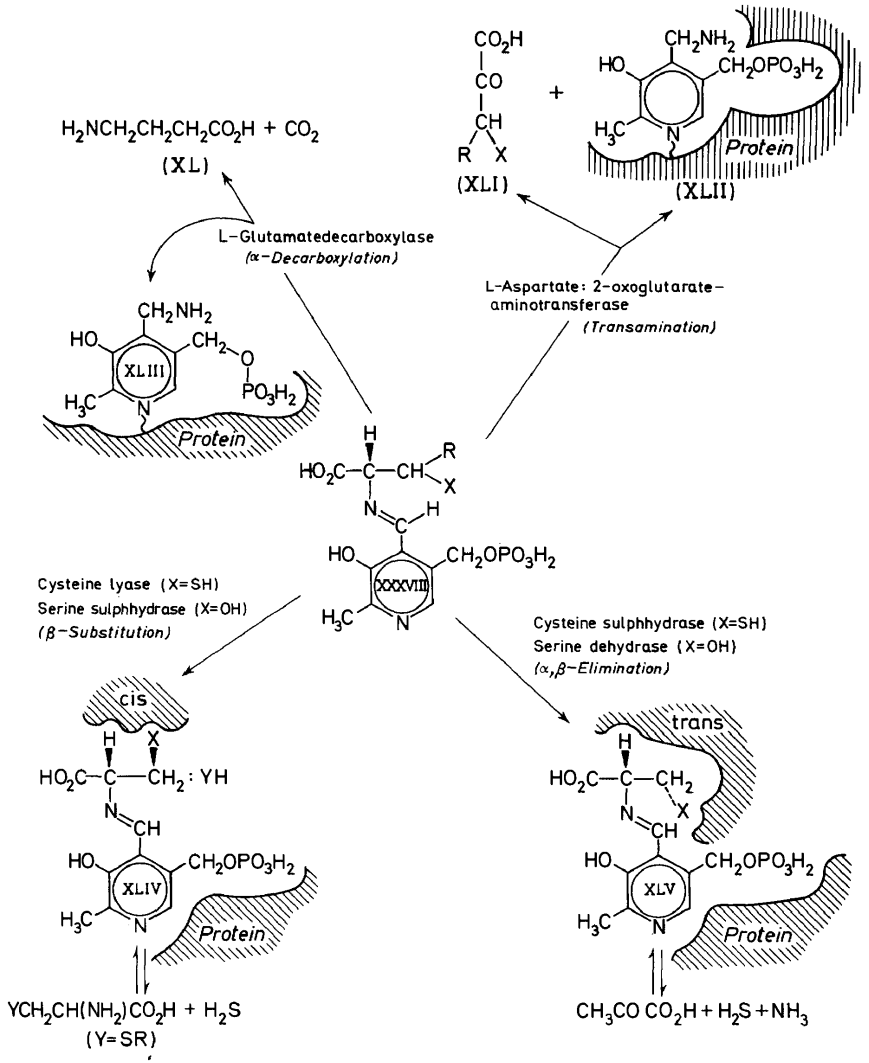
X = OH; SH; OR''; SR''

Y = H; OH

Of the work carried out in the past decade detailed discussion is merited by the series of studies devoted to biological and chemical pyridoxal catalysis.

As early as 1952–1953 Soviet scientists developed a theory of metabolic reactions of amino acids catalysed by pyridoxal phosphate dependent enzymes[21]. According to this theory the key stage in such processes is the formation of Schiff bases (XXXVIII)⇌(XXXIX) from the amino acid (XXXVI) and pyridoxal phosphate (XXXVII). Further conversion of these Schiff bases is determined by the nature of the protein moiety of the enzyme. Similar concepts were published in the U.S.A. in 1954[22].

The basic principles of this theory, and the predictions following from it,



were confirmed in detailed studies on individual enzymes conducted under the direction of A. E. Braunstein[23, 25–32]. The main objects of these studies, which utilized a wide variety of physical and chemical methods, were enzymes catalysing the transamination or decarboxylation of glutamic acid, and also enzymes effecting substitution or elimination of polar β-substituents in α-amino acids [reactions (XXXVIb)–(XXXVIe)].

From a comparative study of individual pyridoxal phosphate dependent lyases which catalyse transformations of cysteine and serine it was found that the enzymes can be divided into subgroups, depending upon whether they induce only α,β-elimination [reaction (XXXVIII)→(XLV)], only β-substitution [reaction (XXXVIII)→(XLIV)], or reactions of both types.

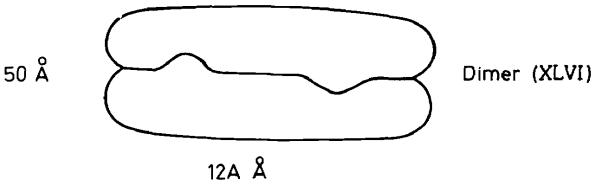
The reaction specificity is determined by the conformation around the C_α—C_β bond of the amino acid in the enzyme-substrate aldimine fixed in the active centre of the enzyme: *cis* for β-substitution [compound (XLIV)], or *trans* for α,β-elimination [compound (XLV)][27].

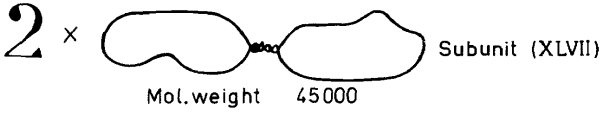
The study of enzymatic decarboxylation of L-glutamic acid [reaction (XXXVIII)→(XL)] revealed the chemical nature of one of the mechanisms of physiological inactivation of pyridoxal enzymes. It was found that when glutamate decarboxylase acts on a variety of quasisubstrates, or on the normal substrate, the enzyme becomes gradually inactivated owing to an abortive transamination reaction, as a result of which the coenzyme is transformed into the amino form, catalytically inactive for this enzymic reaction [reaction (XXXVIII)→(XLIII)]. Such an abortive reaction is in principle possible for any pyridoxal enzyme and an ever increasing number of inactivations by this mechanism has been detected[26].

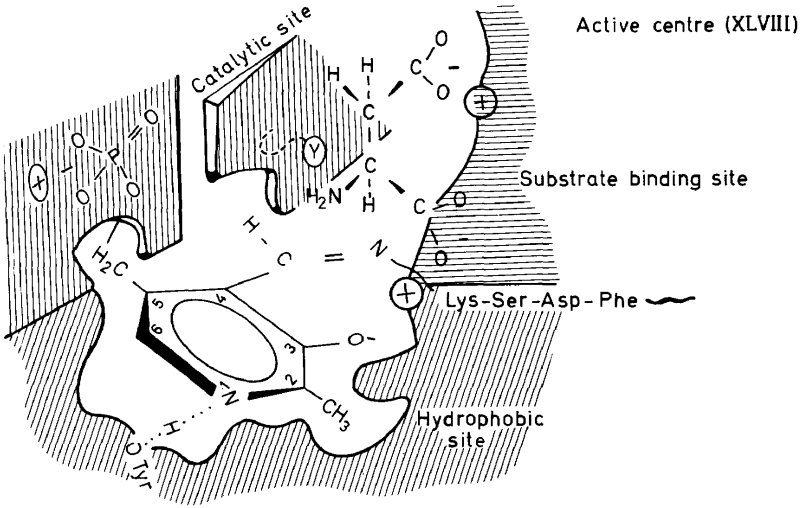
The enzyme investigated in greatest detail was L-aspartate: 2-oxoglutarateaminotransferase [the transamination reaction (XXXVIII)→(XLI)+(XLII)][24, 25]. The study has acquired particular importance insofar as it has provided a deeper insight into the physicochemical basis of enzymic pyridoxal catalysis.

This enzyme is a dimeric globular protein (XLVI). It consists of two identical subunits; each subunit (XLVII) contains one molecule of pyridoxal phosphate and consists of one polypeptide chain folded in space into the shape of a dumb-bell. The dimer and monomer possess approximately equal catalytic activity[28]. Pyridoxal phosphate is rigidly fixed in the active centre of the enzyme (XLVIII) by multipoint attachment to the protein. Its pyridine nitrogen is hydrogen bonded to a tyrosyl hydroxyl, the 2-methyl group is in a hydrophobic environment, the phenol 3-hydroxyl interacts with a positively charged site of the substrate area, the 4-formyl group is linked by an aldimine bond to the ε-amino group of a lysyl residue; electrostatic interaction of the phosphate group with the protein makes a major contribution to the binding energy of pyridoxal phosphate with the apoenzyme. All these conclusions are based on analysis of the interactions and reaction products of pyridoxal phosphate and its analogues with the apoenzyme[30].

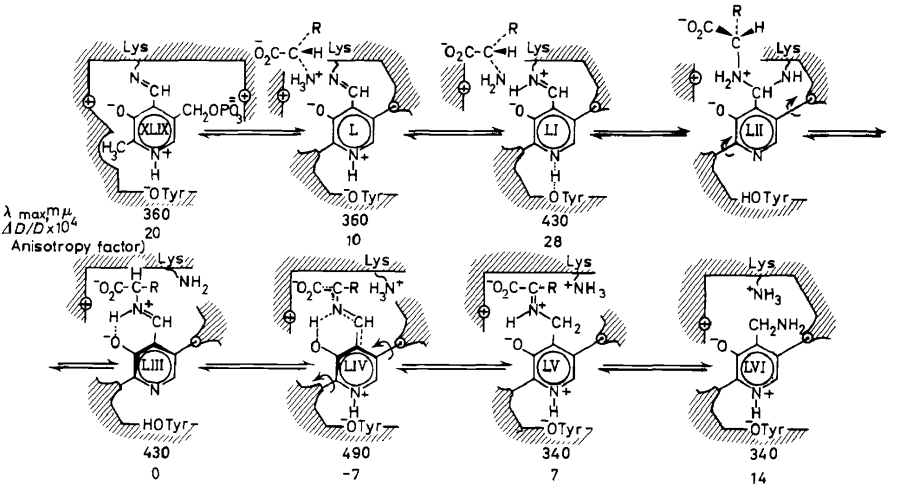
A general idea about the results of mechanistic studies of enzymic transamination[30] can be obtained from the schematic presentation of conversions (XLIX) to (LVI). The use of various quasisubstrate and conformationally rigid inhibitors (substrate analogues[31] made it possible to fix single 'frozen' reaction stages and to obtain an insight into the structures and spectral







characteristics of a number of the enzyme–substrate intermediates. By making use of the induced optical activity of enzyme–bond pyridoxal phosphate discovered in 1963[32, 33], not only did it become possible to distinguish between the complexes (XLIX) and (L) (initial binding of the substrate in the active centre) and (LV) and (LVI) (transformation of the final ketimine into the amino form of the enzyme), but also to uncover the existence of structural reorganization in the active centre during the catalytic act: change in orientation of the pyridine ring in the complexes (LI) and (LIII)[30].



Owing to multipoint attachment in the active centre, the components of the enzyme–substrate intermediates are fixed at each reaction stage in the state which is most reactive for the given reaction step and, moreover, are oriented along the reaction coordinate required for the given step. At the stage of nucleophilic addition of the substrate, this is complex (LI) containing the protonated hydrogen-bonded coenzyme aldimine and an amino acid with a non-protonated amino group [reactions (L)→(LI)→(LII)]. At the stage of prototropic shift it is complex (LIII) which contains the hydrogen bonded enzyme–substrate aldimine and the deprotonated ε-amino group of lysine [reactions (LIII)→(LIV)→(LV)], etc. A distinctive feature of this scheme is that optimal conditions for each of the sequential reaction steps are provided as the result of compulsory structural modifications in the preceding stage [formation of complex (LI) from (XLIX), complex (LIII) from (LI) and complex (LVI) from (LIV)].

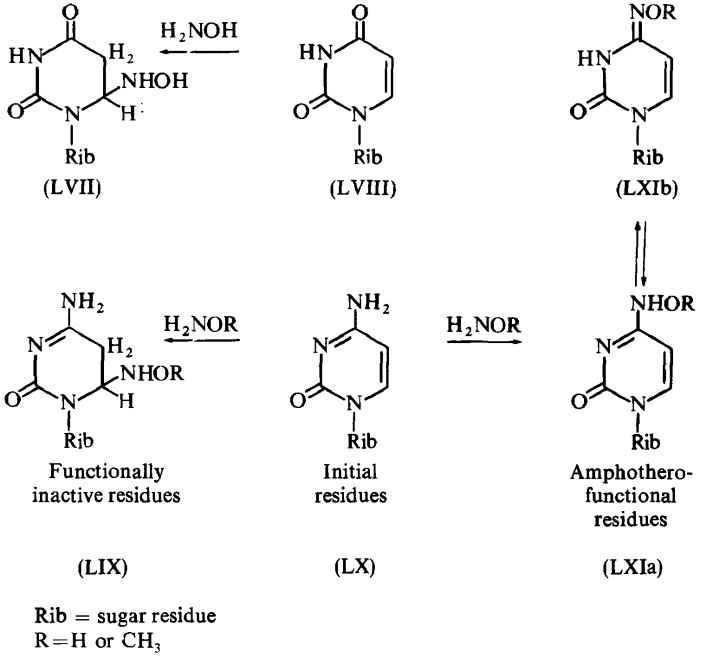
Hence, the high catalytic efficiency of enzymes can be rationalized in terms of ordinary chemical reactions, with due consideration of optimum conditions varying at each step of the multistage enzymic reaction.

NUCLEIC ACIDS

Chemical and biochemical investigations of nucleic acids in the U.S.S.R. were initiated by A. N. Belozersky who, as far back as in the 'thirties, demonstrated the presence of DNA in higher plants[34, 35], and later showed that the broad variations in nucleotide composition of microbial DNAs can be utilized as a taxonomic characteristic[36, 37]. In the course of further studies it was found that a still more specific feature of DNA is the distribution of purines and pyrimidines, including methylated bases[38, 39]. This is a species-specific characteristic which reflects the nucleotide sequence and is of considerable importance for biological classification and for the study of evolutionary relations[36, 39].

Among other researches of Soviet scientists reported during the last decade mention will be made primarily of those which have fundamentally contributed to elucidation of structure–function interrelations in nucleotide coenzymes, polynucleotides and nucleoproteins. These studies demonstrate the possibilities afforded by various approaches to the mode of functioning of biochemical systems containing nucleic acids and nucleotide coenzymes as components. The data accumulated clearly show that the extraordinary variety and high specificity of biological systems are determined by diverse combinations of simple chemical principles.

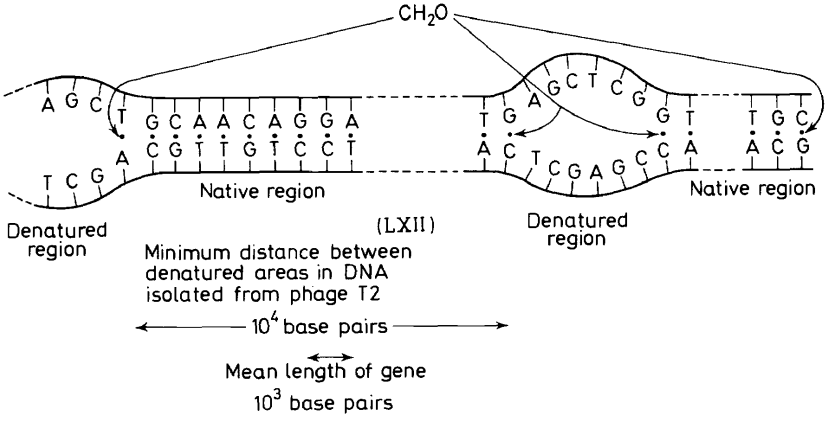
In the course of investigations into the reactivity of nucleic acid constituents towards nucleophilic agents, a study was made of the reaction of nucleic bases with hydroxylamine and *O*-methylhydroxylamine, and also of the properties of the modified nucleoside residues[40–42]. The modified uridine (LVII) and cytidine (LIX) residues are functionally inactive, whereas the modified cytidine residues (LXIa,b) possess ambivalent functionality, i.e. they can replace either cytidine



or uridine (thymidine) residues in template-dependent biosynthesis[43, 44]. This appears to be the cause of transitions (mutations with base substitution) in hydroxylamine or *O*-methylhydroxylamine induced mutagenesis. The data obtained make it possible to correlate the chemical and genetic changes in polynucleotides caused by chemical mutagens.

Important information on the higher levels of nucleic acid and nucleoprotein structure was derived from studies concerning the relative reactivity of their nucleoside residues. Utilizing the enhanced reactivity of bases in denatured segments and at the termini of native segments, a kinetic formaldehyde method

was devised for determining interdefect distances in the secondary structure of DNA (LXII). Thus it was shown that the interdefect distances in the secondary structure of isolated phage T2 DNA exceeds 10^4 base pairs, i.e. is tenfold the average length of a gene. This lends support to the opinion that the gene

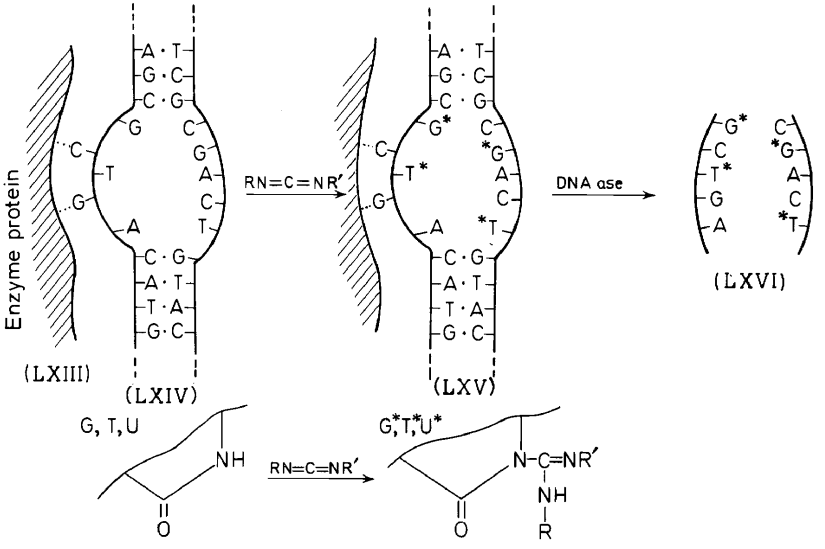


boundaries are determined by the unique nucleotide sequences rather than by the secondary structure of DNA[45, 46].

In functioning DNA (LXIV), as opposed to native two-strand DNA, part of the chain is readily accessible to modification, for instance, by soluble carbodiimide [(LXIV)→(LXV)][47]. This is because nucleoside links in the functioning areas of DNA are involved in dynamic complexes with enzyme proteins (LXIII), which gives rise to local 'denaturation' and thereby facilitates modification in these areas[47, 48]. Subsequent nuclease treatment (LXV)→(LXVI) does not affect the carbodiimide-modified zones (LXVI) which can be isolated for study[49]. When involved in the formation of functioning nucleoprotein complexes, the protein molecule also undergoes structural changes, as can be seen, for example, from the increased stability of RNA polymerase towards a variety of agents upon its binding to the DNA template and onset of RNA synthesis[50].

The two-stranded DNA is not completely in the double-helix form either in the dynamic or in the static nucleoprotein, such as phage particles, as was shown recently by means of a number of chemical and physical methods[51, 52]. Comparison of the reactivity of the nucleoside units in non-helical zones of the phage particles towards various types of reagent showed that in these zones the bases are involved in non-covalent interaction with the protein[53–55]. When phage (LXVII) is treated with *O*-methylhydroxylamine (LXVII)→(LXVIII) such non-covalent interactions facilitate covalent bonding of the bases with XH substituents (X=NH, S, O) of the amino acid residues of the peptide chain (LXVIII)→(LXIX)[54, 55]. The formation (LXVII)→(LXVIII)→(LXIX), and subsequent cleavage (LXIX)→(LXX) of covalent bonds between the polynucleotide and the protein in nucleoproteins, is a promising approach to elucidation of the mode of interactions between nucleic acids and proteins.

Determination of functioning DNA segments

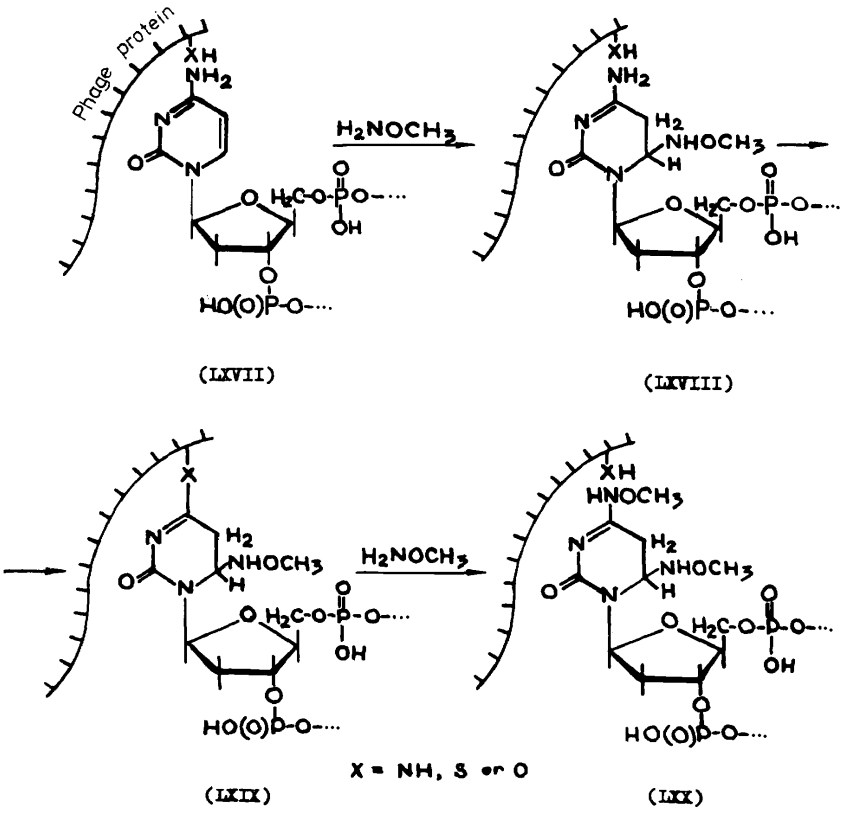


A few years ago nucleic acid fragments covalently bound to peptides were isolated from natural sources[56]. By comparison of the natural nucleopeptides with model compounds it was shown that the former possess phosphoamide bonds of types (LXXI) and (LXXII)[57–59]. The peculiar chemical properties of the P—N bond [stability in alkaline medium and capacity for phosphate transfer on protonation (LXXII)→(LXXIII)] suggest that activation of the nucleotide phosphorus might occur via intermediate formation of phosphoamide bonds between the nucleotide residues and amino groups of the enzyme.

Despite the non-covalent nature of the tertiary and quaternary structures of polynucleotides and nucleoproteins, these structures are quite stable and of high biological specificity even in the case of relatively small molecules such as tRNAs. Thus the halves and even smaller fragments of $tRNA_1^{Val}$ (LXXIV) whose primary structure was determined in 1967[60], were shown to associate readily in solution, and some associates, e.g. (LXXV) and LXXVI), undergo enzymic aminoacylation to just the same degree as the intact tRNA molecule (LXXIV). Moreover, even fragments deprived of some of the nucleotide units are capable of associating, for example the associate (LXXVII)–(LXXXI), and some of them (LXXVII)–(LXXIX) can undergo enzymic aminoacylation[61, 62]. This circumstance is of fundamental importance in structure–functional studies, since it affords new approaches to the identification of segments of the tRNA molecule that are essential for its specific functioning. For example, by means of this procedure two segments [formula (LXXXII)] were detected which are necessary for the binding of $tRNA_1^{Val}$ to the corresponding aminoacyl–tRNA synthetase[63].

Since the components of nucleoprotein systems are synthesized separately in the cell, they must undergo self-assembly in order to acquire functional activity.

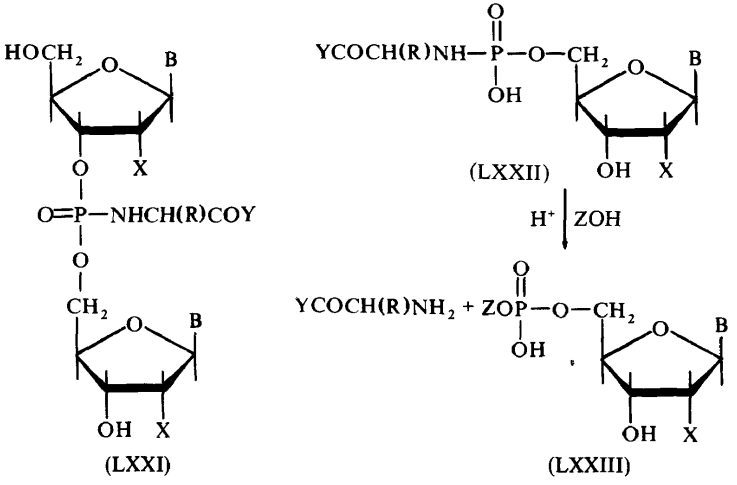
Reversible covalent bonding of polynucleotides and proteins in nucleoproteins



In many cases such self-assembly could be accomplished *in vitro*. Self-assembly was investigated on ribosomes which, as far back as in 1963[64], were shown to consist of compactly coiled nucleoprotein strands with the ribosomal proteins fixed on to a polynucleotide carcass. The strand may be uncoiled (LXXXIII)→(LXXXIV)→(LXXXV), and its refolding leads to recovery of the biological function[64, 65]. Further, it was shown that stepwise removal of the ribosomal proteins does not cause irreversible inactivation, so that when the 'half-stripped' ribosome is subsequently associated *in vitro* with the missing proteins restitution of the biological activity can be achieved[66–69].

It was found several years ago[70, 71] that, at least in animal cells, messenger RNA and its nuclear precursor (LXXXVI) exist in the form of nucleoprotein complexes (LXXXVIII), which are likewise formed by self-assembly (LXXXVI)+(LXXXVII)→(LXXXVIII)[72–75] and are involved in the control of protein synthesis at the translation level.

Finally, we may consider the formation of cyclic polynucleotides as a



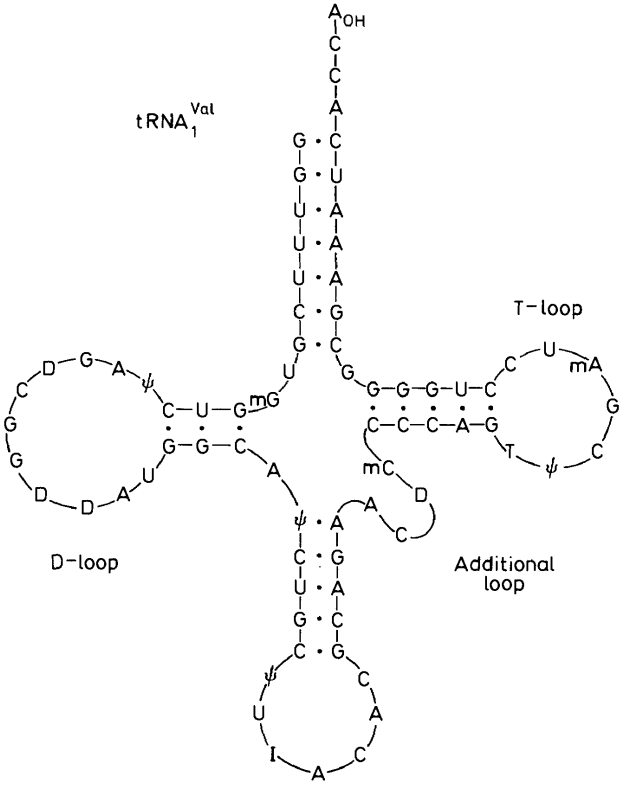
B = Pyrimidine or purine base
X = H or OH
Y = OH, OAlk or NHCH(R)COOH
Z = $(OH)_2OP$, Alk

peculiar type of self-assembly, completed by enzymic formation of a covalent bond between the chain termini. This phenomenon was previously known only for DNA. However, a recent study of the properties of the replicative form of encephalomyocarditis virus RNA indicates that this too has a cyclic structure with the ring closed covalently[76]. The formation of cyclic RNA implies the existence of RNA ligases, a fact of considerable importance, particularly for the genetics of RNA-containing viruses.

A very convenient object for the study of specific interaction of nucleic acids with proteins is a system for DNA-dependent synthesis of RNA. In 1962 it had been established that, in the process of intracellular reproduction of bacteriophage T2, initially mainly 'early' RNAs (LXXXIX) are being synthesized, and that only later does the synthesis of 'late' RNAs (XCI) set in; i.e. different groups of genes of the phage chromosome are successively involved in the synthesis[77]. In the initial stage, RNA synthesis is controlled by bacterial RNA polymerase which selects on the phage DNA only the initial sites of 'early' genes (XC)[78]. In the recognition of the specific nucleotide sequences of these sites a particular role is played by one of the RNA–polymerase subunits, whereas another subunit directly carries out the template-dependent synthesis of RNA[79, 80].

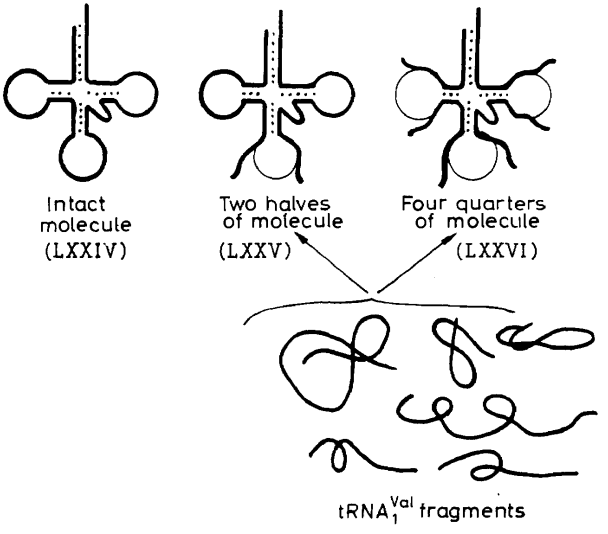
Thus, recent investigations have shed light on a number of structure–functional problems of interest from both chemical and biological aspects.

Worthy of mention also are practically important findings concerning the ability of nucleases to inhibit the reproduction of a variety of pathogenic viruses in human and animal cells. Such ability is due to the fact that on penetration into the cell, viral nucleic acids exist for a while without the protective protein coat;

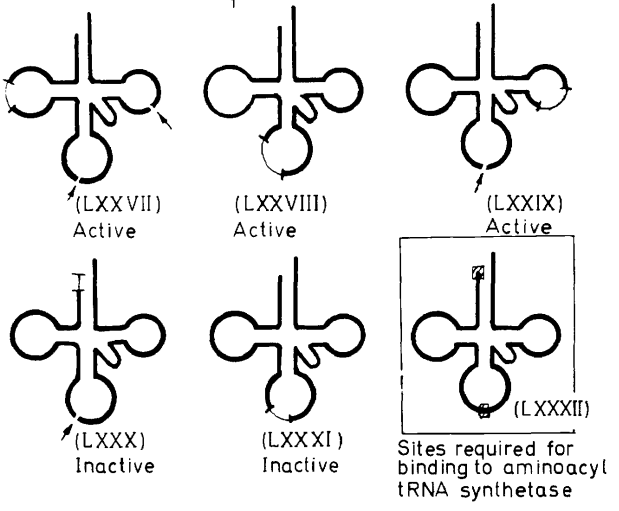


(LXXIV)

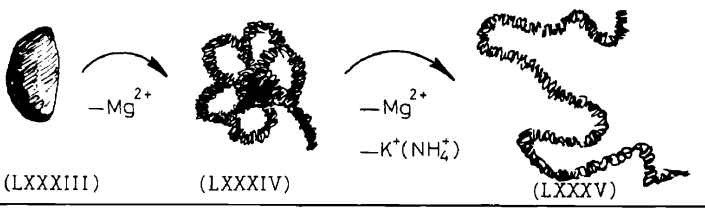
Self-assembly of $tRNA_1^{Val}$ molecule and restitution of its ability to undergo enzymic aminoacylation



Capacity for enzymic aminoacylation of nucleotide deficient $tRNA_1^{Val}$ molecules



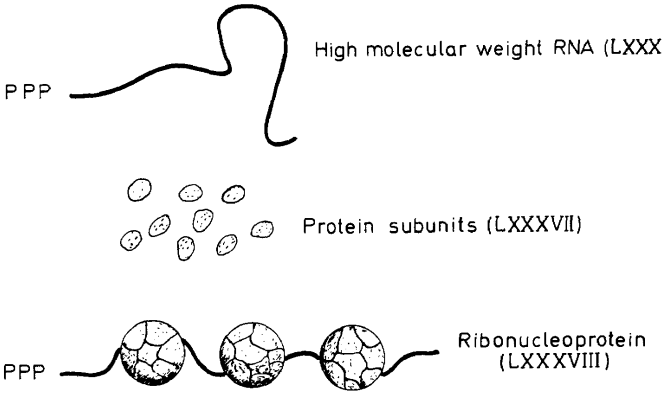
Unfolding of ribosomes



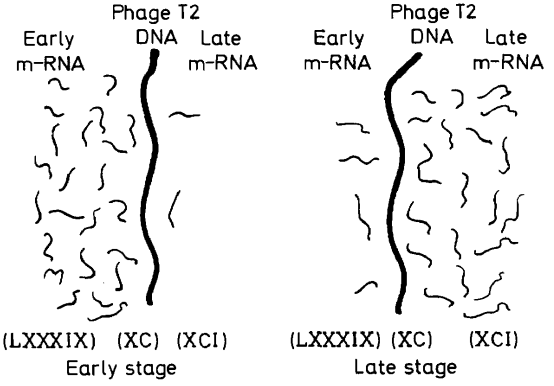
	(LXXXIII)	(LXXXIV)	(LXXXV)
$S_{20,w}$	50S	35S	22S
Mol.wt	1.7×10^6	1.7×10^6	1.7×10^6
ρ g/cm^3	1.64	1.64	1.64
Protein content per cent	35	35	35

Disassembly and self-assembly of ribosomes

	Ribosomal particles	Intermediate RNP particles		Minimal RNP particles	RNA
$S_{20,w}$	50S → (5–6 proteins)	43S → (3–4 proteins)	36S	32S → 28S	23S
			5S RNA	7–9 proteins	15–20 proteins
$S_{20,w}$	30S → (3–4 proteins)	28S → (3–4 proteins)	23S → (3–4 proteins)	20S → (~10 proteins)	16S
Protein content, per cent	35	30	25	15–20	0
ρ g/cm^3	1.64	1.67	1.7	1.75	1.9



Transcription patterns of phage T2 DNA in early and late phage development in *E. coli*

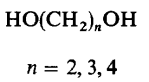


in this state they readily succumb to attack by the nucleases. These observations provided the basis for application of purified nucleases in the treatment of a number of viral diseases such as herpetic and adenoviral affections of the eye, herpes of the skin and of nerve trunks, viral encephalitis and meningitis[81–83].

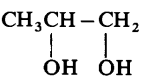
LIPIDS

Traditional investigations of the composition of fats and oils have been carried out in the U.S.S.R. for many years. Considerable contributions were also made to the synthesis of lipids[84–86]. Such studies are also being carried out at present, an example being the synthesis of glycosphingolipids[87] and of bacterial lipoamino acids[88]. At the same time, the beginning of the 'sixties was

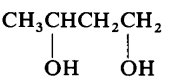
Diols



(XCII)

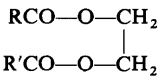


(XCIII)

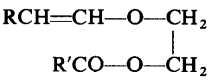


(XCIV)

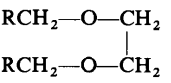
Diol lipids



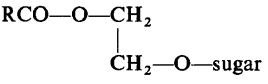
(XCV)



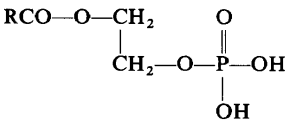
(XCVI)



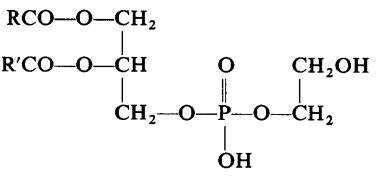
(XCVII)



(XCVIII)



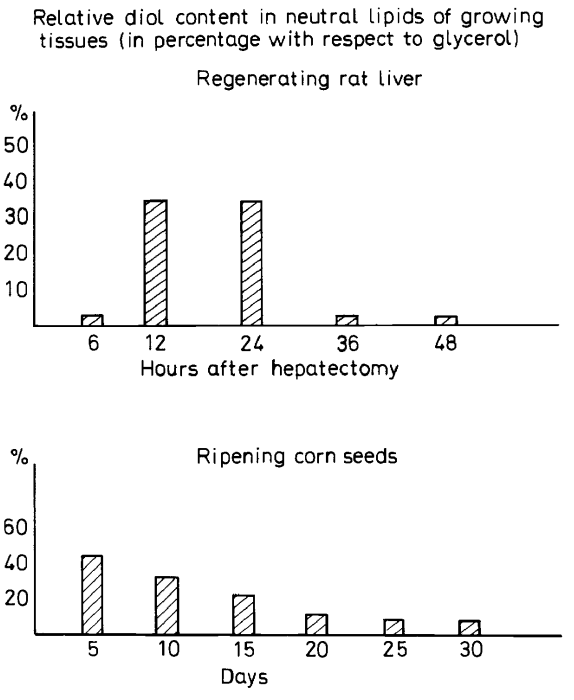
(XCIX)



(C)

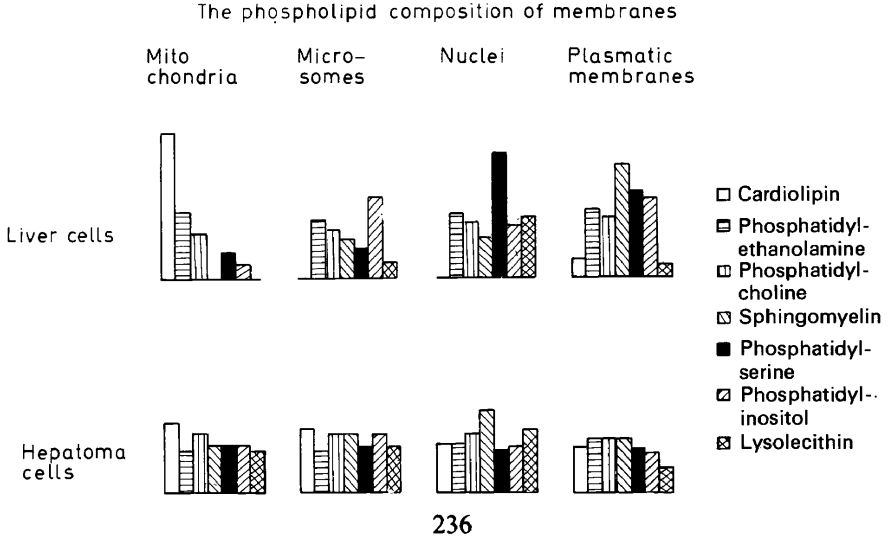
marked by new trends in lipid chemistry. For instance, modern techniques, in particular, chromato-mass spectrometry, have revealed in a number of animals, micro-organisms and plants unusual lipids built on the basis of diols (XCII)–(XCIV)[89, 90]. Diol lipids were found in neutral fats (XCV)–(XCVII), in glycolipids (XCVIII) and in phosphatides (XCIX), (C). They are only minor components of normal cells, but their content can increase immensely under conditions of rapid cell growth. Thus large amounts of them were found in regenerating rat liver[91] and also in seeds in the early stages of ripening[92]. A similar increase in the diol lipids content was observed in the logarithmic growth phase of yeast. The formation of diol lipids during rapid growth of such widely varying organisms is an intriguing fact pointing to the existence of heretofore unknown aspects of lipid metabolism in the growing cell.

Interesting results were also obtained in studies of the structure–functional part played by lipids as components of cellular membranes. Comparative studies of the lipid composition of the various membranes in normal and cancer cells disclosed a new kind of regulator impairment, displayed by at least certain species of tumour cells (rat and mouse hepatomas, sarcomas): whereas in normal cells each type of membrane has its own characteristic lipid composition, the latter differs very little between the different membranes of cancer



cells[93]. Hence degeneration of normal cells into malignant tumour cells is accompanied by dedifferentation of the lipid composition of the membranes.

These data not only provide a new approach to problems of malignant growth, but also have a certain bearing on the structural organization of the cell.



Attacks on this problem were begun at the end of the last century. But they were pointless until the concrete structural material of the cells was known. As our knowledge increased, it was found that there exist certain general principles governing the structural organization of the lipid core; namely all the various membranes are built up of the same lipid 'units', the individuality of the membranes being the result of various proportions and combinations of these units.

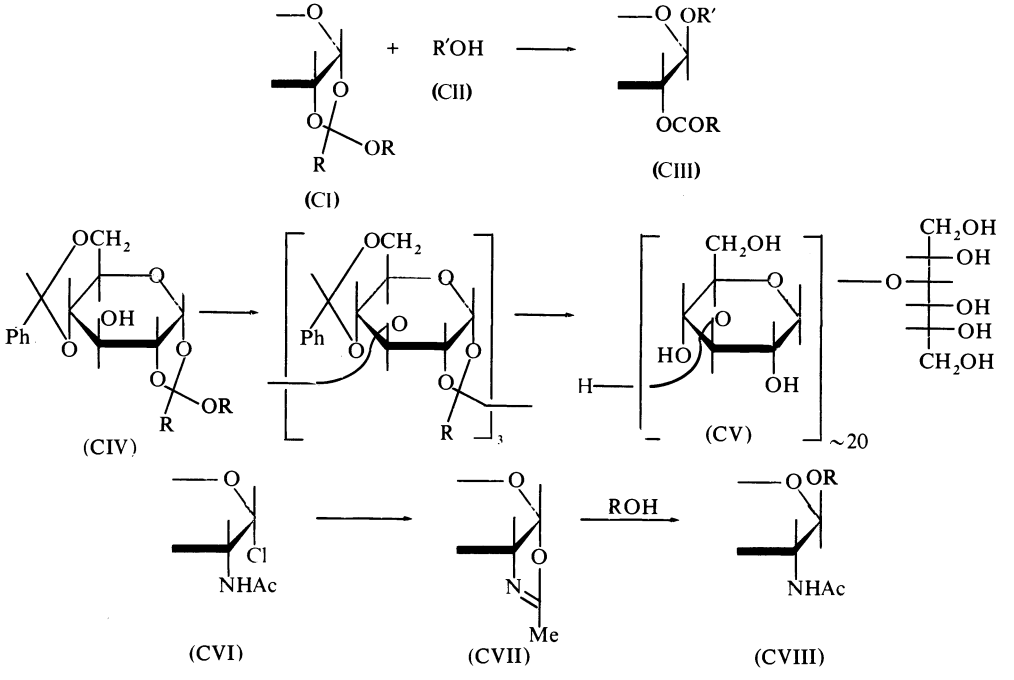
CARBOHYDRATES

The latter years have witnessed considerable intensification of the chemical studies on biologically important carbohydrates.

With the objective of elaborating structure–analytical methods for carbohydrates, the mass spectrometry of mono- and oligo-saccharides has been investigated. This has made possible the microdetermination of the ring size and hydroxyl group positions in monosaccharides, and of the position of the glycoside links in the monosaccharide units of oligosaccharides[94, 95].

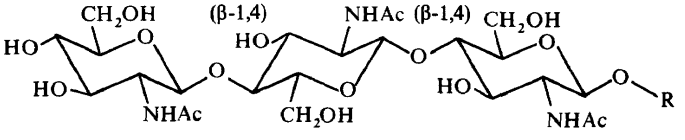
Noteworthy among the syntheses developed during that time are the general orthoester and oxazoline methods of making glycoside bonds.

The orthoester route to glycosides (CIII) is based on reaction of sugar orthoesters (CI) with an alcohol component (CII)[96]. The method, characterized by structural and steric specificity, is of universal applicability with respect to both the glycosylating agent (CI) and the compound undergoing glycosylation (CII). It opens the way for the synthesis of polysaccharides by polymerization

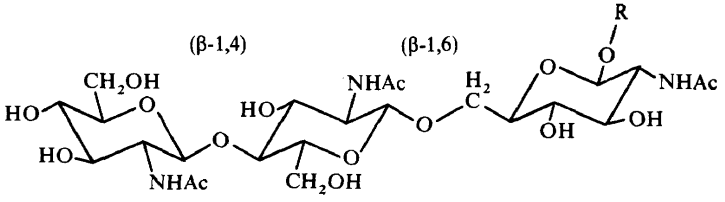


or polycondensation of the orthoesters of mono- or oligo-saccharides that contain both a glycosylating and glycosylable function. Thus, starting with the orthoester (CIV), β-1,3-glucan (CV) related to naturally occurring laminaran has been synthesized[97].

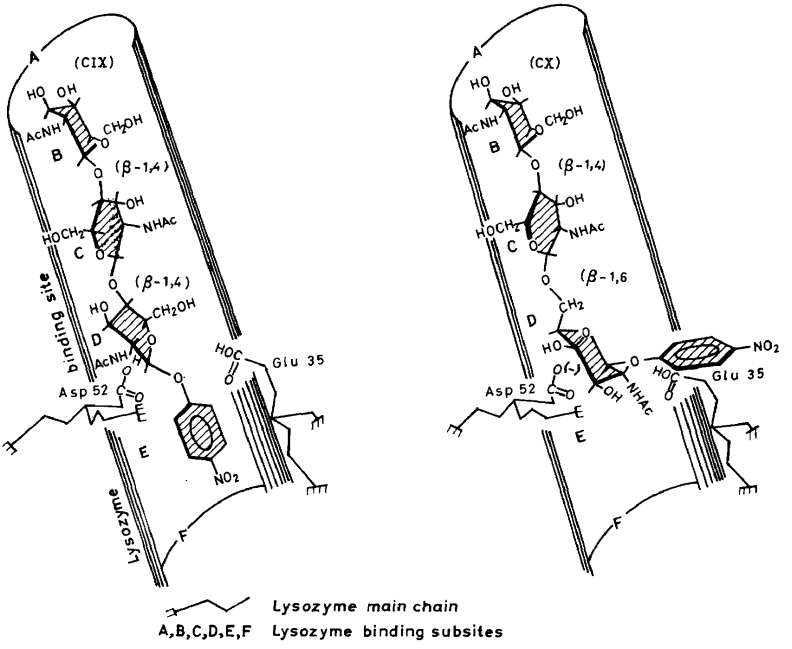
The second method, formation of glycoside bonds via an oxazoline, (CVI)→(CVII)→(CVIII), owes its origin to the development of a simple, general route to glyco-oxazolines (CVII)[98], that has made these glycosylating agents readily available. The efficacy and universality of the oxazoline synthesis



(CIX)



(CX)

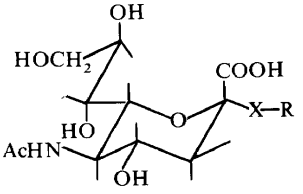


Interaction of nitrophenyltriazide substrates (CIX) and (CX) of lysozyme with its active centre

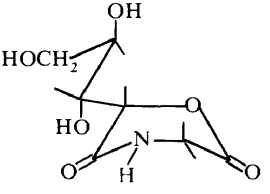
of glycosaminides (CVIII) has been demonstrated by preparation of a large number of oligosaccharides and their derivatives with predetermined glycoside bond combinations: β-1,3; β-1,4; β-1,6. The synthesis of these compounds [for instance (CIX) and (CX)], substrates of glycosidases (lysozyme, chitinase, *N*-acetyl-β-D-glucosaminidase, etc.), was undertaken in connection with studies of the specificity and mode of action of the enzymes[99, 100].

While studying the interaction of lysozyme with these substrates it was found in particular, that the nitrophenyltriosides (CIX) and (CX), although differing in both structure and conformation, possess a very similar affinity for the active centre of the enzyme[101] (cf. previous page). This indicates that the x-ray determined conformation of the lysozyme active centre is apparently not the only one possible.

To further the mechanistic studies of different neuraminidases it was found necessary first to devise methods for synthesizing sialic acid derivatives,



(CXI): X = O, S or NH, R = ϕNO_2
(CXIIa): X = O, R = 6-D-Glu or 3-D-Glu
(CXIIb): X = O, R = 6-D-Gal or 3-D-Gal



(CXIII)

including the *O*-, *S*- and *N*-ketosides of *N*-acetylneuraminic acid (CXI), and *N*-acetylneuraminic acid-terminating disaccharide derivatives, such as those of type (CXII). The lactone (CXIII), an active inhibitor of neuraminidases, was also synthesized[102, 103].

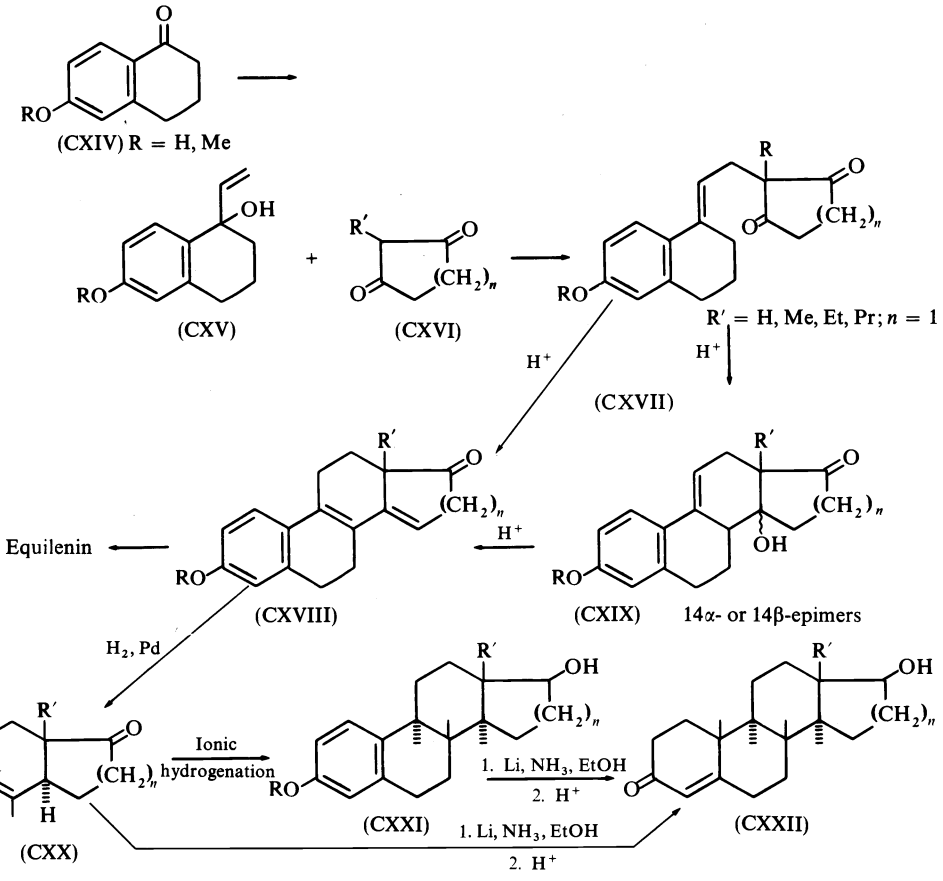
Compounds (CXI)–(CXIII) were used in studying the specificity of neuraminidases. In particular, it was found that compounds (CXI; X=S or NH) and (CXIII) differ in their inhibiting effect on neuraminidases of various strains of the influenza virus; this fact was utilized as a new means of differentiating the latter.

STEROIDS

Among the numerous chemical and biochemical studies of steroids carried out in the U.S.S.R, special mention should be made of the following.

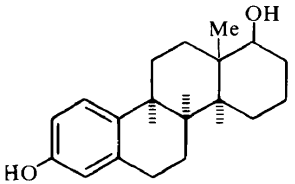
A novel synthetic route to the steroid system has been devised[104, 105], utilizing two new reactions, namely condensation of the vinylcarbinol (CXV) with the cyclic β-diketones (CXVI), and intramolecular cyclization of the resultant tricyclic diketones (CXVII) into the steroid ketones (CXVIII) or ketols (CXIX). By this means the most varied derivatives of estrane, D-homoestrane, and their heterocyclic analogues, have been synthesized and a number of their structure–functional relations have been investigated.

Since the starting ketones of type (CXIV) are readily available, this method

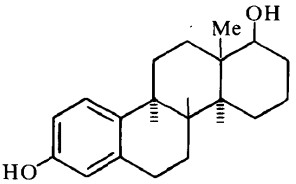


has been found to be so convenient that it is being exploited commercially in a number of countries. The over-all yield of 19-nortestosterone and 19-nor-D-homotestosterone by the scheme (CXVIII)→(CXX)→(CXXII) is as high as 20 per cent, and that of estradiol by the scheme (CXVIII)→(CXX)→(CXXI), even reaches 35 per cent. The method can also be used to prepare the difficultly accessible 14-hydroxysteroids[106, 107] from the ketols (CXIX) formed by intramolecular aldol condensation of the diketones (CXVII). In the D-homo series both 14α- and 14β-epimers are formed, whereas in the normal steroid series only 14 β-hydroxy derivatives are obtained.

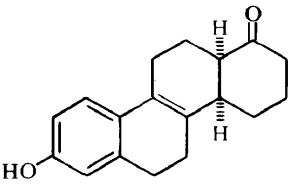
D-Homosteroids were found to be very interesting from a structure–functional standpoint. Up to very recently it was the widely held belief that oestrogenic activity is characteristic only of substances with the naturally occurring anti-*trans* configuration. However, it turned out that such activity in 8-iso-D-homo-estradiol (CXXIII) is by an order of magnitude higher than that of D-homoestradiol (CXXIV) with the 'natural' configuration. With D-homosteroids it was also found possible to discriminate between the hormonal



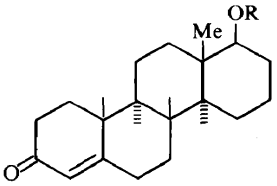
(CXXIII)



(CXXIV)

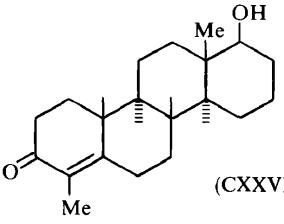


(CXXV)



(CXXVI)

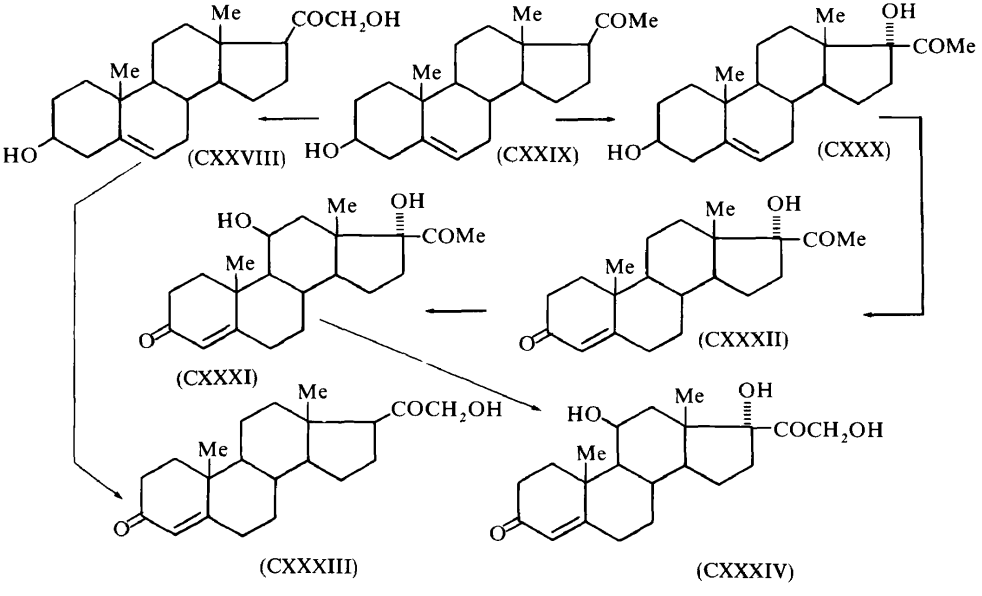
R = H, $COCH_3$, $COCH_2CH_2Ph$



(CXXVII)

effect and their other physiological functions. Thus, certain of the D-homo-oestrone derivatives [for instance compound (CXXV)] are almost devoid of oestrogenic action, while they inhibit the growth of transplantable tumours in animals[108]. In the 19-norsteroid series which had been investigated for anabolic activity, the highest potency was found in D-homo-19-nortestosterone (CXXVI) and its esters, whereas separation of androgenic and anabolic activities was observed most dramatically in 4-methyl-D-homo-19-nortestosterone (CXXVII)[109].

Biochemical studies of steroid metabolism yielded the important finding that 21-hydroxy- and 17α-hydroxycorticoids are formed not only via progesterone

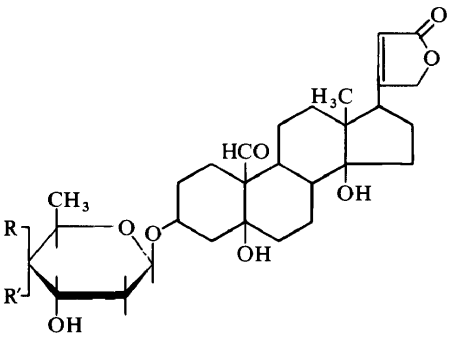


but also via pregnenolone (CXXIX). The latter is first converted into 17α-hydroxypregnenolone (CXXX) and then oxidized to 17α-hydroxyprogesterone (CXXXII), which by the corresponding hydroxylases is converted via compound (CXXXI) into cortisol (CXXXIV). It was found that up to one third of the cortisol in the mammalian organism is formed by this pathway[110, 111]. Pregnenolone (CXXIX) may also be hydroxylated into the ketodiol (CXXVIII) which is then converted into cortexone (CXXXIII).

PLANT SUBSTANCES

In the past decade many studies were devoted to biologically active substances of plant origin. It should be noted, however, that this branch of natural products chemistry is at a level when the question arises whether such a study can yield any fundamentally new types of substances. The attraction of such investigations lies in the possibility of finding substances of practical value, in particular, of medical interest.

The investigations of plant glycosides in the Soviet Union concerned three main groups: cardiac glycosides, triterpene saponins and flavonoid glycosides.

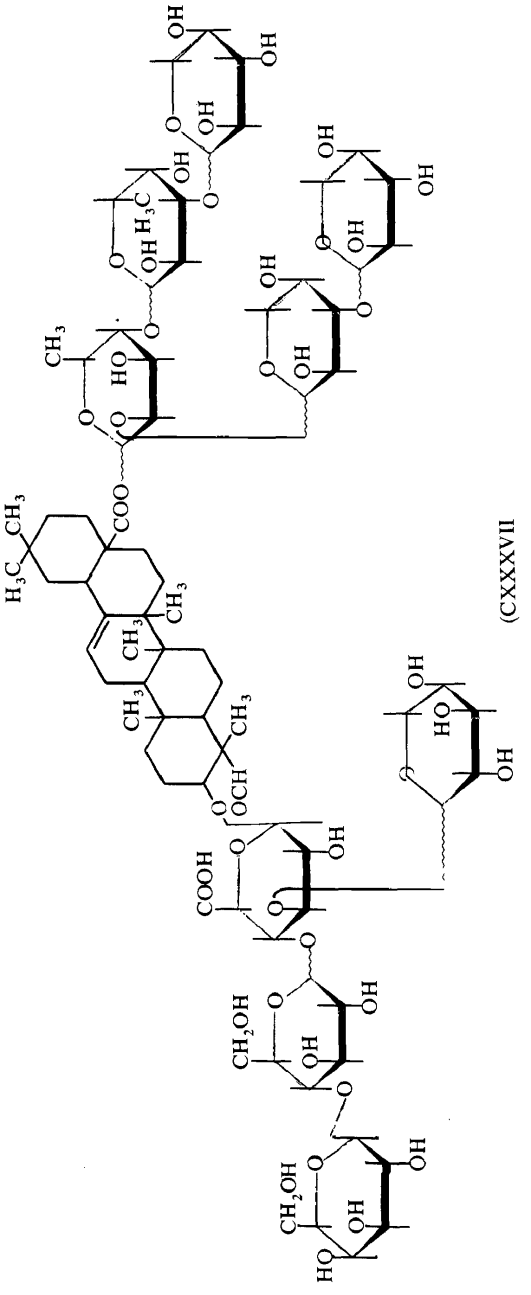


(CXXXV): R′ = H, R = β-D-Glu1 → : Olitoriside from *Corchorus olitorius* L.
(CXXXVI): R′ = H, R = α-D-Glu1 → : Erysimoside from *Erysimum diffusium* Ehrh.

New cardiac glycosides were discovered of which olitoriside (CXXXV) and erizimoside (CXXXVI), highly potent drugs, are now being produced commercially[112, 113].

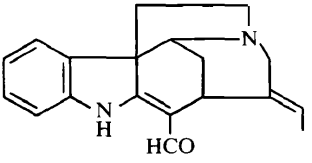
Among the glycosides, a new type of interesting substances comprises the compounds with carbohydrate moieties containing up to eleven monosaccharide residues. These compounds, discovered in studies of biologically active triterpene glycosides, have received the name of oligosides. General methods have been elaborated for their isolation and structure determination, and their structural characteristics were elucidated. A typical example of an oligoside is gypsoside (CXXXVII)[114]. Soviet workers have determined the structures of over 30 triterpene glycosides during the past five to six years (see, for instance refs 115–117); the accumulated experimental material provides evidence of the widespread occurrence of oligosides in Nature.

The study of alkaloid-bearing plants, in particular those growing in Central

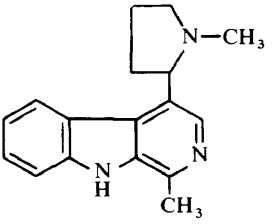


(CXXXVII

Gypsoside from *Gypsophila pacifica* Kom.



Vincanine (CXXXVIII)



Brevicolline (CXXXIX)

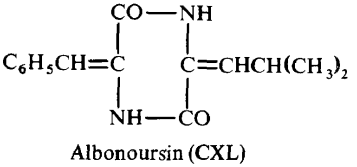
Asia, has been proceeding on a broad front. In this decade the structures of over 130 new alkaloids belonging to various groups have been determined[118]. Among these are a number of compounds displaying interesting medical properties and having found application in medical practice. Examples include vincanine (CXXXVIII)[119] (stimulant of the central nervous system) and brevicolline (CXXXIX)[120] (stimulant of uterine contraction).

In Central Asia, where cotton is one of the basic farming products, the chemical composition of the cotton plant has been thoroughly investigated. This has resulted in the isolation of about 100 compounds belonging to various classes, some of the compounds being of practical importance[121–123].

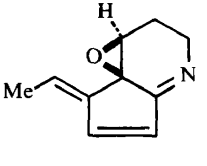
MICROBIAL PRODUCTS

In contrast to the higher plants, microbes are at present one of the richest sources of new naturally occurring compounds, of interest chemically, as tools in the study of biochemical processes, or from a practical standpoint.

This can be illustrated by a number of recent Soviet studies. Thus arylidene- and alkylidene-substituted diketopiperazines such as albonoursin (CXL) and its analogues have been found in a number of actinomycetes[124]. Another new type of compound is represented by the antibiotic abikoviromycin (CXLI) which manifests selective antiviral activity[125].

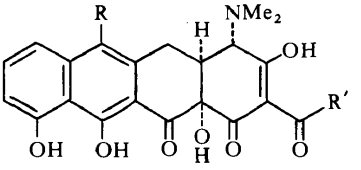


Albonoursin (CXL)



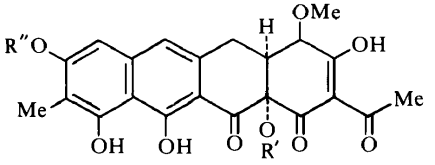
Abikoviromycin (CXLI)

Recently an interesting microbial metabolite chromocyclomycin (CXLIIIa) has been isolated and investigated in detail[126]. This compound can be regarded as a connecting link between two types of antibiotics—the tetracyclins and a group of antitumor substances which includes the olivomycins and chromomycins and which has been now called the aureolic acid group after the first discovered compound[127]. The structure and stereochemistry of aureolic acid



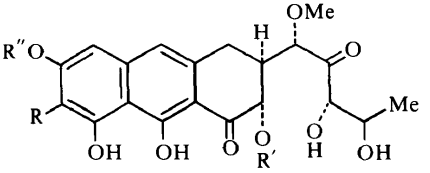
R = H or Me, R′ = NH_2 or Me

Anhydrotetracyclins (CXLII)



R′ and R″ = sugar chains

Chromocyclomycin (CXLIIIa)
[Chromocyclin (CXLIIIb): R′ = R″ = H]

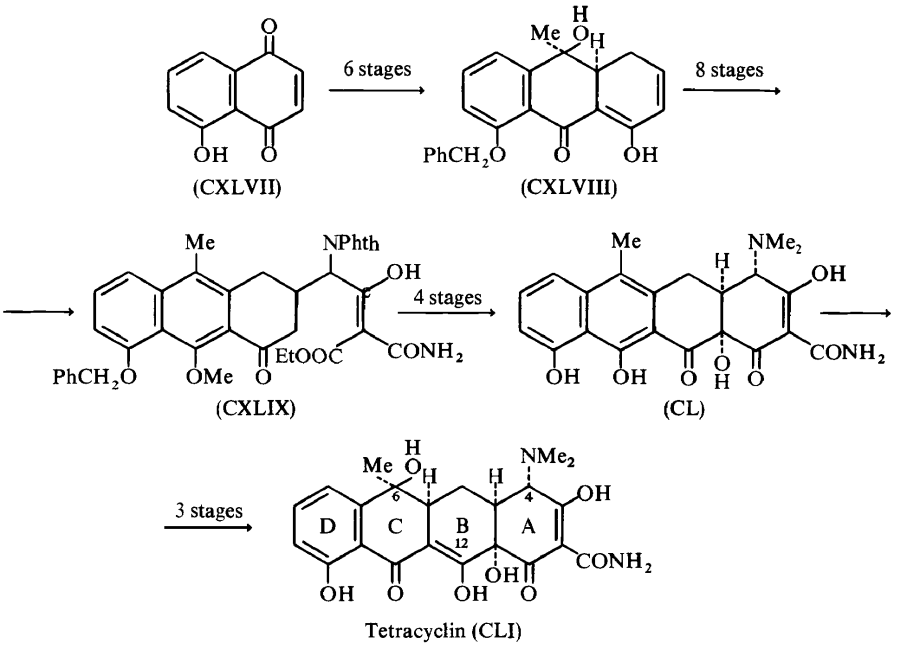


R′ and R″ = sugar chains

Aureolic acid (CXLIV): R = Me
Olivomycins (CXLV): R = H
Chromomycins (CXLVI): R = Me

(CXLIV)[128] and of the olivomycins (CXLV)[129, 130] was elucidated by Soviet workers, whereas that of the chromomycins (CXLVI) was established by a Japanese team[131, 132]. Soviet scientists have determined the absolute configuration of the tetracyclins[135] and have achieved the first total synthesis of a naturally occurring tetracyclin (CLI) according to the scheme: (CXLVII)→(CXLVIII)→(CXLIX)→(CL)→(CLI)[134]. The structural kindship of chromocyclomycin (CXLIIIa) to the antibiotics of the aureolic acid family (CXLIV)–(CXLVI) on the one hand, and to the tetracyclins on the other, comes particularly to the fore in a comparison with anhydrotetracyclins (CXLII), precursors of the tetracyclin antibiotics.

A large series of studies has been devoted to the chemistry of depsipeptide microbial products. Based on earlier devised synthetic methods (p. 213) a large number of this class of compounds has been prepared, resulting in the confirmation, correction, or rejection of formulae proposed for several naturally occurring cyclodepsipeptides[1, 2]. Thus final proof has been afforded of the structure of



the sporidesmolides (I–IV) [formulae (CLII)–(CLV)], of angolide (CLVI), and of the enniatin A (XXIIa) and B (XXIIb), valinomycin (VIII) and serratamolide (CLVII) antibiotics; and on the other hand, it has been shown that the formulae proposed for the antibiotics esperin and amidomycin are erroneous.

cyclo-(L-HyIv-X-D-Leu-Y-L-Val-Z)

X = D-Val; Y = L-HyIv; Z = L-MeLeu	Sporidesmolide I (CLII)
X = D-aIle; Y = L-HyIv; Z = L-MeLeu	Sporidesmolide II (CLIII)
X = D-Val; Y = L-HyIv; Z = L-Leu	Sporidesmolide III (CLIV)
X = D-Val; Y = L-HyIc; Z = L-MeLeu	Sporidesmolide IV (CLV)

cyclo-(L-HyIv-L-Ile-L-HyIv-D-aIle)

Angolide (CLVI)

cyclo-(L-*N*-MeIle-D-HyIv)$_3$

Enniatin A (XXIIa)

cyclo-(L-*N*-MeVal-D-HyIv)$_3$

Enniatin B (XXIIb)

cyclo-(D-Val-L-Lac-L-Val-D-HyIv)$_3$

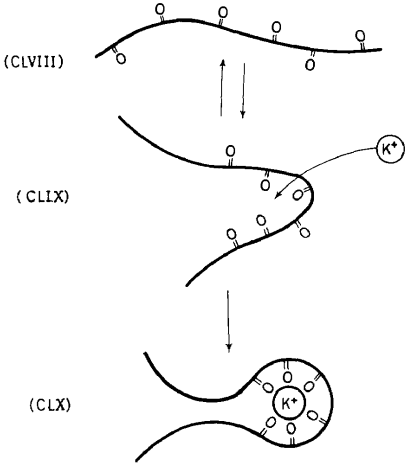
Valinomycin (VIII)

cyclo-(L-Ser-D-β-HyDec)$_2$

Serratamolide (CLVII)

The depsipeptide antibiotics and their analogues belonging to the valinomycin and enniatin series have acquired special importance lately after their value had been shown as chemical tools for the study of ion transport through artificial and biological membranes. A detailed structure–function study of this group of cyclodepsipeptides has considerably promoted our knowledge of the physicochemical basis of trans-membrane ion transport (p. 220), and this in turn has provided an approach to the problem of the mode of the antibiotic action of these compounds. The latter was found to be intimately related to their ability to effect selectively an immense increase in potassium ion transport across the cytoplasmic membrane of the bacterial cell, the direct result of the selective binding of the cation by a conformationally ordered system of ester and/or amide carbonyls in the cyclodepsipeptide molecule [formulae (XXV) and (XXVI)].

From this it is only natural to make further assumptions concerning the mechanism of ion transport through biological membranes in general. Since as the result of conformational changes in the peptide chain of the protein molecule carbonyl ligands can become ordered into an arrangement similar to that characteristic of the macrocyclic depsipeptides and peptides, they may acquire the ability to bind selectively metal ions and thus provide the conditions



for subsequent transport of the latter (CLVIII)→(CLIX)→(CLX). It therefore appears plausible that such a mechanism could be involved in the functioning of the ion exchanging sites in transport ATP-ases, and in other structural elements of the biological membranes responsible for the transport of metal ions[14]. The problem of the recognition of the ion by the membrane, and of the mode of its transport from the aqueous solution through the latter, thus acquires a fundamentally new physicochemical aspect.

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