

H. Gobind Khorana

**Some Recent  
Developments  
in the Chemistry  
of  
Phosphate Esters  
of Biological  
Interest**

# Some recent developments in the chemistry of phosphate esters of biological interest

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## Preface

This book is based on a series of lectures given at the Rockefeller Institute in the spring of 1959 and covers a discussion of some selected aspects of the chemistry of phosphate esters — in particular, of nucleotides and related compounds. Although the selection of topics was largely determined by my own research interests, the coverage of recent work in this field is essentially comprehensive in the individual chapters. The book is offered in the hope that it may be of interest not only to research workers in specialized fields but to chemists and biochemists in general.

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*Madison, Wisconsin*  
*July 1961*

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# 1

## General introduction: classification of phosphate esters of biological interest

### Contents

Single Substitution on Phosphoric Acid

Monoesters of Pyrophosphoric Acid

Monoesters of Triphosphoric Acid

Dieters of Pyrophosphoric Acid

Dieters of Phosphoric Acid

References

Since the first demonstration in 1905 by Harden and Young (1) of the dependence of alcoholic fermentation on the presence of inorganic phosphate, biochemical research has uncovered an amazing variety of the manifestations and functions of organic phosphate esters in living processes. There is hardly anything that goes on in the cell in which esters of phosphoric acid, in one form or another, are not involved at some stage. However, although the organic structures associated with the phosphate esters cover an extremely wide spectrum in type and complexity, the categories, considered on the basis of substitution on the phosphoric or pyrophosphoric acid molecules, are relatively few. An attempt is therefore made here to introduce the large family of phosphate esters of biological interest by classifying them as shown in Table 1.\*

\* Inorganic meta- and polyphosphates are also found in large amounts in cells (see, e.g., 2, 3). Of the organic structures containing phosphorus, as far as the author is aware, all are derived from orthophosphoric acid, except for the very recent report of the

isolation of the crystalline 2-aminoethanephosphonic acid  $[H_2NCH_2CH_2-\overset{\overset{O}{\parallel}}{P}-(OH)_2]$  from rumen protozoa (4).

TABLE 1  
General Classification of Phosphate Esters of Biological Interest

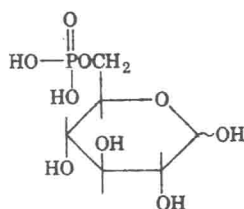
Substitution on Ortho- Pyrophosphoric Acid, etc.	Nature of Substituent(s)	Customary Designation of Compounds	Examples
I. $\begin{array}{c} \text{O} \\ \parallel \\ \text{X}-\text{P}-\text{OH} \\   \\ \text{OH} \end{array}$	(1) $\text{X}- = \text{RO}-$	Monoesters of phosphoric acid	Sugar phosphates, glycerol phosphate, mononucleotides
	(2) $\begin{array}{c} \text{Z} \\ \parallel \\ \text{X}- = \text{R}-\text{C}-\text{O}- \\ \text{(unsaturated component)} \end{array}$	Enol phosphates	Phosphoenolpyruvic acid, acetyl phosphate
	(3) $\text{X}- = \text{RNH}-$	Phosphoramidates	Creatine phosphate, arginine phosphate
II. $\begin{array}{c} \text{O} \quad \text{O} \\ \parallel \quad \parallel \\ \text{X}-\text{P}-\text{O}-\text{P}-\text{OH} \\   \quad   \\ \text{OH} \quad \text{OH} \end{array}$	$\text{X}- = \text{nucleoside-5'}$		Nucleoside-5' diphosphates, thiamine pyrophosphate



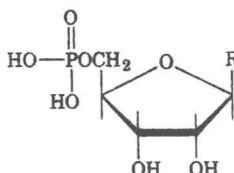
III. $\begin{array}{c} \text{O} & \text{O} & \text{O} \\ \parallel & \parallel & \parallel \\ \text{X}-\text{P}-\text{O}-\text{P}-\text{O}-\text{P}-\text{OH} \\   &   &   \\ \text{OH} & \text{OH} & \text{OH} \end{array}$	X— = nucleoside-5'	Adenosine-5' triphosphate
IV. $\begin{array}{c} \text{O} & \text{O} \\ \parallel & \parallel \\ \text{X}-\text{P}-\text{O}-\text{P}-\text{Y} \\   &   \\ \text{OH} & \text{OH} \end{array}$	X— = nucleoside-5' Y— = RO—	Nucleotide coenzymes
V. $\begin{array}{c} \text{O} \\ \parallel \\ \text{X}-\text{P}-\text{Y} \\   \\ \text{OH} \end{array}$	(1) X— = nucleoside-5' Y— = acyloxy group (2) X— = RO— Y— = R'O—	Acyl adenylates, "active sulfate" Diesters of phosphoric acid Nucleic acids, phospholipids, cell wall polymers

## Single Substitution on Phosphoric Acid (Class I, Table 1)

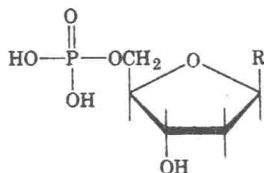
In the first major category there is a single substitution on the phosphoric acid molecule. When the substituting group is a "hydroxylic" compound, monoesters of phosphoric acid result, and this class embodies easily the largest number of phosphate compounds. The main groups are the phosphorylated derivatives of carbohydrates such as glucose-6 phosphate (I) (Chapter 2 and refs. 5, 6) and the mononucleotides, such as ribo- (II) and deoxyribonucleoside-5' phosphates (III) (Chapters 2, 4 and ref. 7).



I  
Glucose-6 phosphate



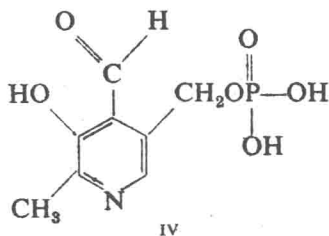
II  
Ribonucleoside-5'  
phosphate



III  
2'-Deoxyribonucleoside-5'  
phosphate

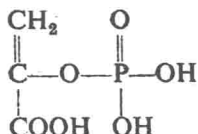
II, III: R = purine or pyrimidine

A third group of phosphomonoesters is that of the phosphoproteins, in which the hydroxyl groups of serine and threonine may be esterified. In addition to these major groups a number of vitamins exercise their "coenzymic" function as phosphorylated derivatives, for example, pyridoxal phosphate (IV) and riboflavin-5' phosphate (Chapters 2, 4). The list of phosphomonoester-type compounds is by no means complete.

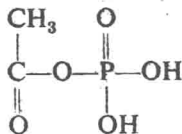


IV

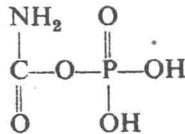
The group I.2 in Table 1 consists of a number of high-energy (8) phosphate esters, which may be called mixed anhydrides. Well-known



V



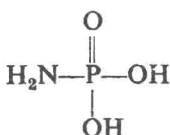
VI



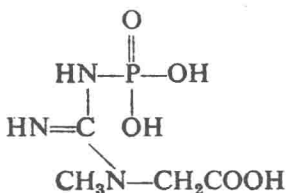
VII

examples of anhydrides are phosphoenolpyruvic acid (V), acetyl phosphate (VI) (see Chapter 4), and carbamyl phosphate (VII) (Chapter 4).

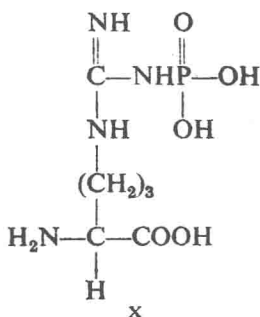
Phosphoramidic acid (VIII) (I.3, Table 1), creatine phosphate (IX), and arginine phosphate (X) are members of another group of the high-energy phosphate compounds.



VIII



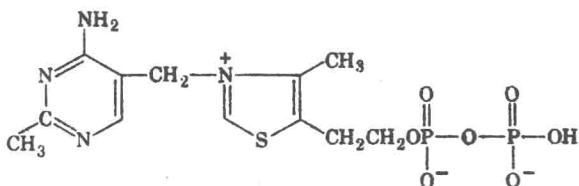
IX



X

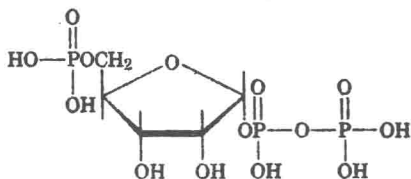
### Monoesters of Pyrophosphoric Acid (Class II, Table 1)

Although the classical example of this class is adenosine-5' pyro(di)-phosphate, the complete series of diphosphates corresponding to all the commonly occurring nucleosides has been discovered (Chapter 4). Another well-known example is the coenzyme, coenzyme A, which is the pyrophosphate ester of vitamin B<sub>1</sub> (thiamine). The structure of the coenzyme is represented by XI.



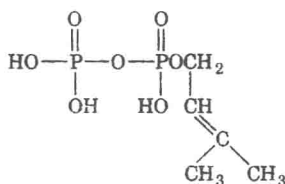
XI: Thiamine pyrophosphate

5-O-Phosphoryl- $\alpha$ -D-ribofuranose-1 pyrophosphate (XII) (refs. 9-11 and Chapter 2), the biological precursor of ribonucleoside-5' phosphates, is

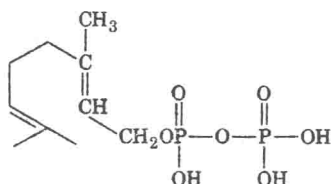


XII

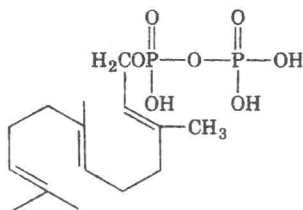
another example of this group, and then there is a family of compounds (XIII–XV) which have recently been shown to be involved in the biosynthesis of a host of polyisoprenoid compounds (squalene, steroids, rubber) (see, e.g., 12, 13).



XIII: Isopentenyl pyrophosphate



XIV: Geranyl pyrophosphate



XV: Farnesyl pyrophosphate

### Monoesters of Triphosphoric Acid (Class III, Table 1)

This group includes nucleoside-5' triphosphates, adenosine-5' triphosphate being the classical example (Chapter 4).

### Diesters of Pyrophosphoric Acid (Class IV, Table 1)

This group is comprised of a variety of nucleotide coenzymes and related pyrophosphates whose structures and synthesis are discussed in Chapter 4. Related to these in chemical structure is the group of compounds that are properly called mixed anhydrides of different acids and the phosphoric acid group of nucleotides, mostly adenosine-5' phosphate. The compounds (Class V.1 of Table 1) are also discussed in Chapter 4.

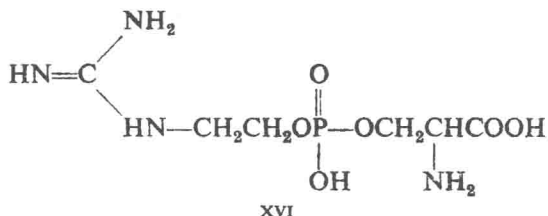
### Diesters of Phosphoric Acid

This class covers the widest variety of organochemical structures, both for molecular size and chemical structure. It is a remarkable fact that definitive information on the exact structures, for all the groups of compounds encompassed by this category, has been forthcoming during the

last ten or eleven years. The single most important development contributing to this progress has been the understanding of the hydrolytic behavior of diesters of phosphoric acid bearing suitably placed vicinal hydroxyl groups. Although the latter topic is discussed at length in Chapter 3, a detailed description of the structural derivation of the different naturally occurring diesters of phosphoric acid is not within the scope of the present writing. Only examples of the typical structures involved are given with selected references to articles dealing with more detailed discussions. Furthermore, no claim is made concerning all-inclusiveness of representatives of different subcategories.

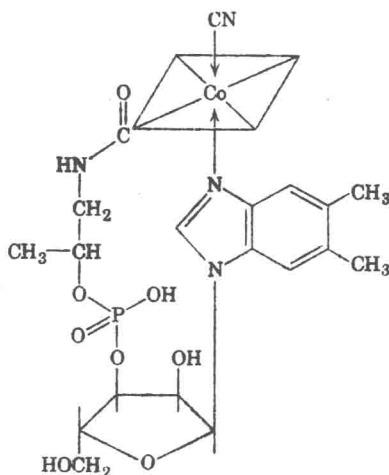
#### LOMBRICINE

Lombricine (XVI) from earthworms (14) and the O-phosphodiester between D-serine and ethanolamine (15) are, perhaps, the simplest of the structures of this group.



#### VITAMIN B<sub>12</sub>

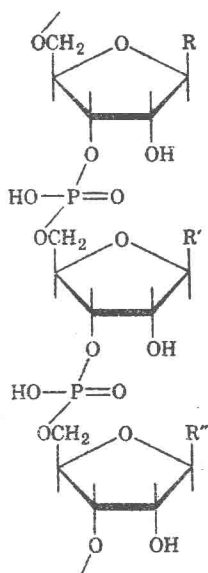
Vitamin B<sub>12</sub> and its relatives all contain a phosphodiester bridge as shown in the typical partial structure (XVII).



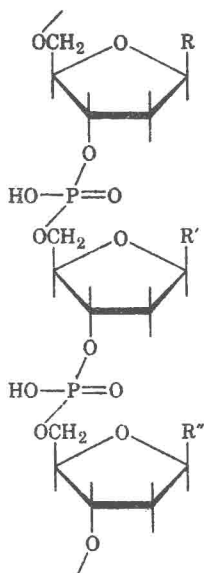
XVII: Partial structure, vitamin B<sub>12</sub>

## THE NUCLEIC ACIDS

Both the ribo- and deoxyribonucleic acids are now known with certainty to contain polynucleotide chains in which the individual ribo- or deoxyribonucleosides are joined together by repeating  $C_3-C_5'$  phosphodiester bonds. The repeating pattern is shown in the trinucleotide segments given in structures XVIII and XIX. The full development of the chemistry of the phosphodiester (internucleotide) bonds in the nucleic acids has been reviewed (16).



XVIII  
Segment of a  
ribopolynucleotide  
chain



XIX  
Segment of a  
deoxyribopolynucleotide  
chain

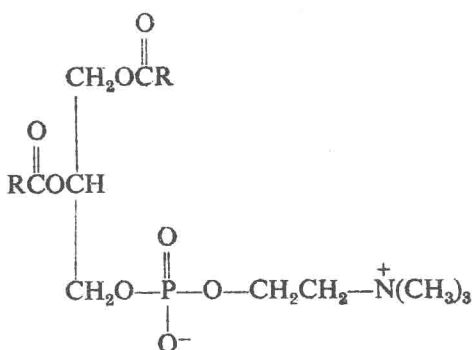
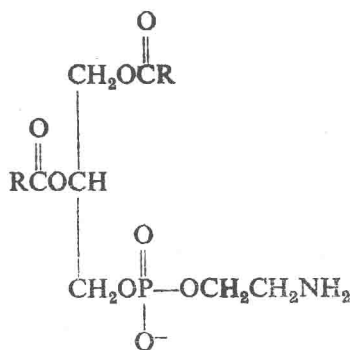
R, R', R'' = purine or pyrimidine

## PHOSPHOLIPIDS

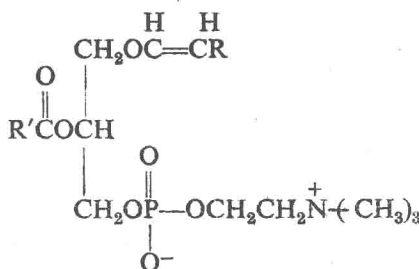
*Lecithins* and *cephalins* have the general structures shown respectively by XX and XXI (17).

*Plasmalogins* (18) resemble the phosphatidylcholines (lecithins), except that one fatty ester is replaced by an  $\alpha,\beta$  unsaturated ether. Although the exact orientation of the ester and the ether linkages cannot be considered to be settled, current opinion (19) favors the general structure (XXII).

*Phosphoinositides* (20), in particular, have recently been the focus of intensive research in a number of laboratories, and generally accepted

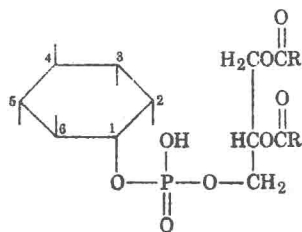
XX  
L- $\alpha$ -LecithinsXXI  
L- $\alpha$ -Cephalins

RC— = long-chain saturated or unsaturated fatty acyl groups



XXII: R, R' = long-chain aliphatic group

structures of some simpler members (monophosphoinositides) of this group have emerged. The structure (XXIII) (di-O-acylglycerol L-myoinositol-1 phosphate) has been elucidated for the monophosphoinositide from

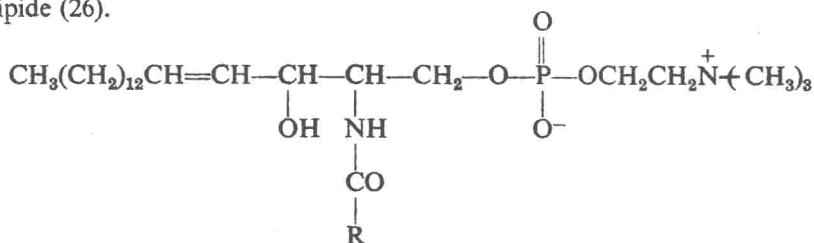


XXIII

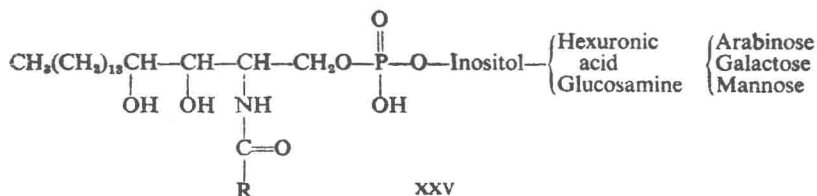
soybean (21, 22) and from horse liver (23; see also ref. 24). The structures of the more complex di-(?) (20) or triphosphoinositides (25) have not as yet been elucidated. Other complex inositol-containing lipids are referred to later.

*Sphingomyelins, phytoglycolipides, and phytosphingolipide.* Sphingomyelins (general structure, XXIV) are the best known among the sphingosine

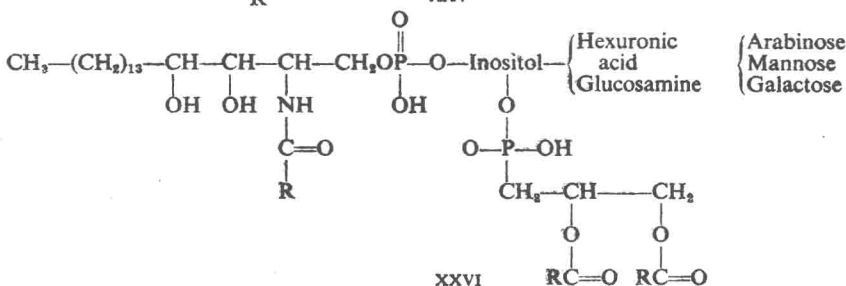
derivatives, but recently evidence for the corresponding ethanolamine compound has been given (26). Although much work remains to be done on the characterization and complete structural elucidation of many more complex lipids, two partial structures (XXV and XXVI) have been proposed, respectively, for a phytylglycolipide (27) and a phytosphingolipide (26).



XXIV: R = long aliphatic chain



xxv



xxvi

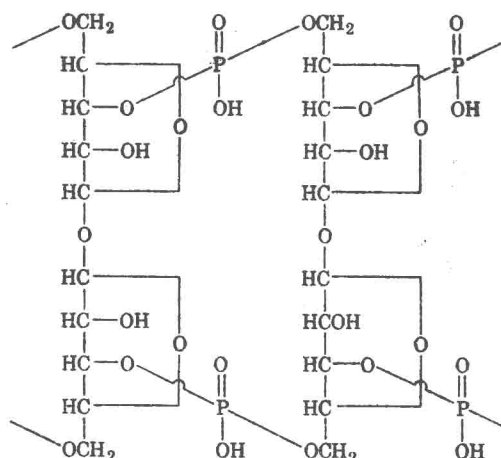
#### POLY(RIBOSE PHOSPHATE)

*Poly(ribose phosphate)* is the type-specific substance of *Hemophilus influenzae*, type b (28), to which the structure XXVII has been ascribed. It appears that other carbohydrate phosphate polymers specific to other types of this organism also exist (29).

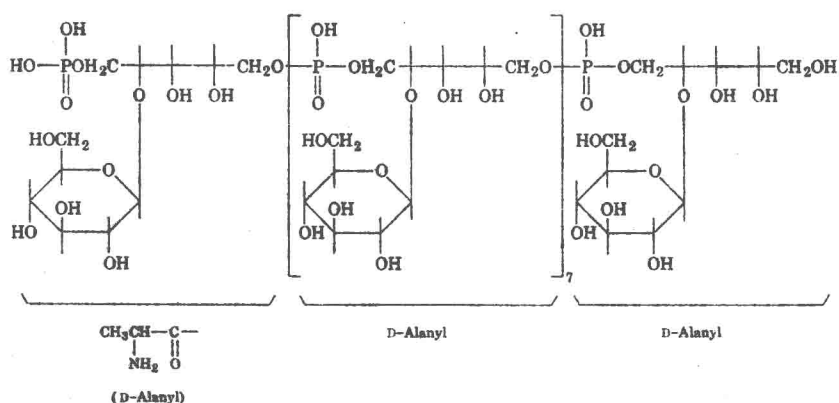
#### CELL WALL POLYMERS (TEICHOIC ACIDS)

Polymers formed from glycerol phosphate and D-ribitol phosphate have been isolated from the walls of a number of bacteria, and the structures of these polymers, designated teichoic acids, have been studied (30-32). The structure of one such polymer (D-ribitol phosphate polymer from *Bacillus subtilis*) has recently been largely elucidated and is represented by XXVIII (32).





XXVII

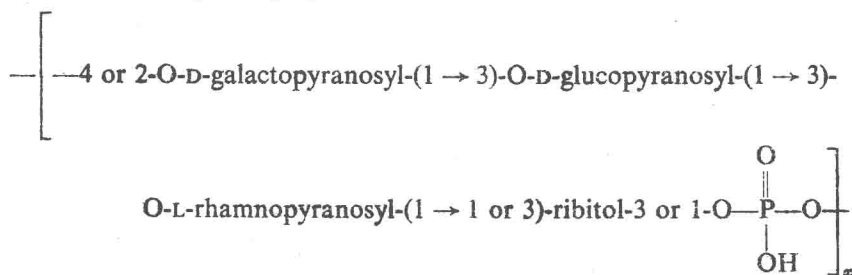


XXVIII

D-Ribitol teichoic acid from *Bacillus subtilis* (32)

## SPECIFIC POLYSACCHARIDE OF TYPE VI PNEUMOCOCCUS

A recent investigation (33) has assigned the following tentative structure (XXIX) to the specific polysaccharide.



XXIX