Synthesis in the Study of Nucleotides.

THE PEDLER LECTURE, DELIVERED IN BIRMINGHAM ON FEBRUARY 12TH, 1946, AND IN LONDON ON MARCH 7TH, 1946.

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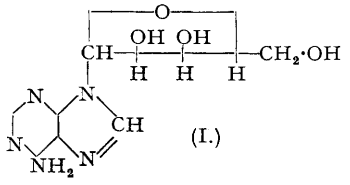
THE term nucleotide covers a very large number of natural substances ranging from such comparatively simple compounds as muscle adenylic acid, through co-enzymes such as adenosine triphosphate important in phosphate transfer or riboflavin-adenine-dinucleotide, the prosthetic group of many flavoproteins, to the nucleic acids (polynucleotides) which, associated with proteins, form the nucleoproteins embracing substances intimately concerned with the reproduction of living cells as well as the causative agents of certain diseases (plant viruses). All these are structurally related and can be regarded as built up of basic units each consisting of a cyclic nitrogenous substance (usually a purine or pyrimidine) combined with a sugar (usually *d*-ribose or 2-deoxyribose) and phosphoric acid. The simplest nucleotides are single units and the others vary in complexity up to the polynucleotides in which the molecule may contain a very large number of units linked together. The importance of the nucleotides in the chemistry of living matter can hardly be over-estimated and it is therefore not surprising that they are increasingly attracting the attention of organic chemists. From the chemist's standpoint the macromolecular nucleic acids present structural problems resembling those encountered in the study of proteins. Whilst proteins, however, frequently contain twenty or more different amino-acid residues, present knowledge suggests that nucleic acids are normally of two types—ribonucleic and deoxyribonucleic—each built up from some four different simple nucleotide units. This makes the problem of their structure at once easier and, in certain directions, more difficult to solve. The structure of the nucleic acids has been the subject of investigations by many workers during the past thirty or forty years. Much has been learnt as a result regarding the fundamental units of some of the more accessible, although of the nature of the polynucleotides themselves our knowledge remains scanty. It is not my purpose to discuss here the degradative work on polynucleotides. I shall, however, touch on the results of such work on the simpler nucleotides where they are relevant to or arise from my main theme, which is an account of an approach to the nucleotide problem using synthetic methods. This approach must obviously begin with the simplest units and work up to the complex, and it has been and remains a major interest in my laboratory. In this lecture I propose to outline to you the pattern and development of our researches as far as they have gone. In so doing I act as spokesman for a group of enthusiastic colleagues with whom it has been my good fortune to be associated and to whom should be accorded the credit for the progress which has been made.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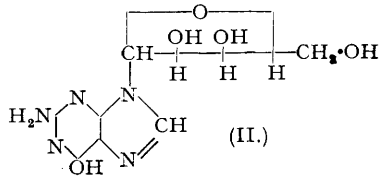
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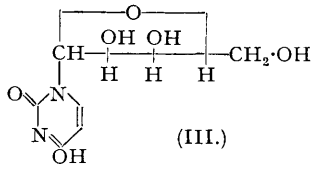
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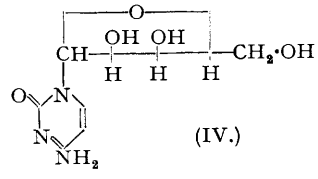
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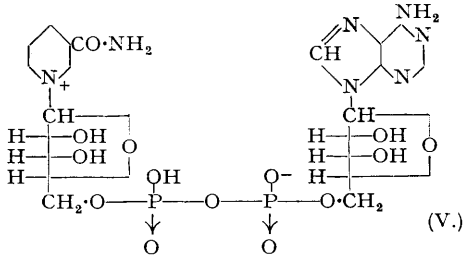
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At this point it may be well to consider the structures, as far as known at the commencement of our researches, of some simple nucleotides obtained by suitable hydrolytic breakdown of polynucleotides.[1] From ribonucleic acids one obtains the 3′-phosphates of the glycosides (ribonucleosides), adenosine (I), guanosine (II), uridine (III) and cytidine (IV). The structures of these compounds have been cleared up in most essentials by analytical methods. In each case the structure of the aglycone is certain, the sugar residue is known to be *d*-ribofuranose, and the position of the phosphate group on C_3 of the sugar chain has been established. In the

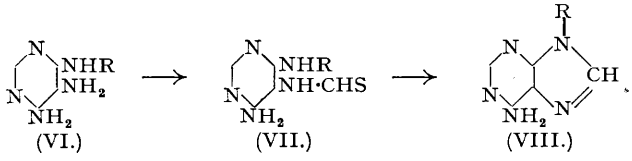
case of the purine derivatives the glycosidic linkage was early shown to be at N_7 or N_9 in the iminazole nucleus, but the only evidence hitherto advanced to distinguish between these possibilities is that of Gulland and his collaborators [2] who concluded on spectroscopic grounds that the sugar residue was attached to N_9—a conclusion which has been generally accepted. In my own view the N_9 location is also rendered highly probable by the fact that this position in the purines corresponds to N_9—the point of attachment of the ribityl residue—in riboflavin, a substance which is probably biogenetically related to the purine nucleosides. In the pyrimidine nucleotides the location of the sugar residue at N_3 was adequately established.[3] Whether the natural compounds are α- or β-glycosides remained unknown.

Of the four deoxyribonucleotides obtained by hydrolytic breakdown of deoxyribonucleic acids less is known, and their formulation rests largely on analogy with the ribonucleotides. They are hydrolysed to 2-deoxyribose, phosphoric acid, and adenine, guanine, cytosine, and thymine respectively. The furanose nature of the glycosidic residue, demonstrated only in the case of thymidine,[4] is generally assumed, as is the location of the phosphate grouping at C_3 in the sugar chain. In the purine deoxyribosides spectroscopic evidence again suggests attachment of the sugar to N_9.[2]

Although any synthetic approach to the nucleotide problem must ultimately lead, if successful, to the nucleic acids, my colleagues and I entered this field with more limited initial objectives. We had been interested in the question of the specificity of B vitamins, several of which are components of nucleotide molecules which exercise co-enzyme function, *e.g.*, cozymase (V), riboflavin-adenine-dinucleotide. We wished to find out more about the factors governing the specificity of such co-enzymes and their linkage with apoenzymes. To do this we decided to investigate the possibility of total synthesis of such substances by methods which would be general and sufficiently flexible to permit the synthesis of variants of the molecule at will. In this way we hoped to obtain a range of compounds which might be of value in furthering the study of co-enzyme function, and possibly in opening the way to the production of highly specific chemotherapeutic agents acting by a competitive mechanism. Taking the molecule of cozymase (V) as an example it is clear that the problem of synthesis at once resolves itself into three parts: (*a*) the synthesis of nucleosides, (*b*) the phosphorylation of sensitive molecules (*i.e.*, nucleotide synthesis), and (*c*) linkage of molecules through phosphate or polyphosphate residues (*i.e.*, polynucleotide synthesis). All three have occupied our attention, but it will be convenient to deal with each in turn commencing with the synthesis of nucleosides. Although there has naturally been some overlap through various lines being pursued simultaneously, the order in which they have been named does correspond roughly to the pattern of our investigations. The nucleoside problem is epitomised in the substance adenosine (I), probably the most important of all the nucleosides, and I propose first to discuss, in the main, experiments directed to the total synthesis of this and related purine glycosides.

Although no natural purine or pyrimidine nucleoside has been synthesised numerous attempts have been made in the past to do so. The nearest approach has been that of Hilbert and Rist who synthesised 3-*d*-ribopyranosidouracil.[5] In the case of the purine nucleosides previous synthetic attempts have been of two types. In the first of these the silver salt of a purine has been caused to react with an acetohalogeno-sugar and the desired substituents introduced into the purine nucleus by subsequent operations. This method was used in the pioneer work of Fischer [6] who was able in this way to synthesise a number of purine glucosides including those of adenine and theophylline in which, on spectroscopic grounds, the glucose residue is considered to be located at N_9 and N_7 respectively. Whilst this method does offer a route to the naturally occurring compounds it would require for its realisation the use of the hitherto unknown acetohalogeno-ribofuranose and it is not unambiguous as regards the location of the sugar residue in the products obtained by its use. The second type of synthesis attempted involved the preparation of a glycoside of a pyrimidine or an iminazole with subsequent completion of the purine nucleus by building on the second ring. All such attempts had failed through inability to obtain the appropriate intermediates, but nevertheless this type of method appeared to be the most suitable for our purpose which demanded unambiguous and flexible procedures.

In our approach to the problem of adenosine synthesis we envisaged the preparation of a 5 : 6-diamino-4-glycosidaminopyrimidine (*e.g.*, VI; R = *d*-ribofuranose) followed by conversion of this into the required purine

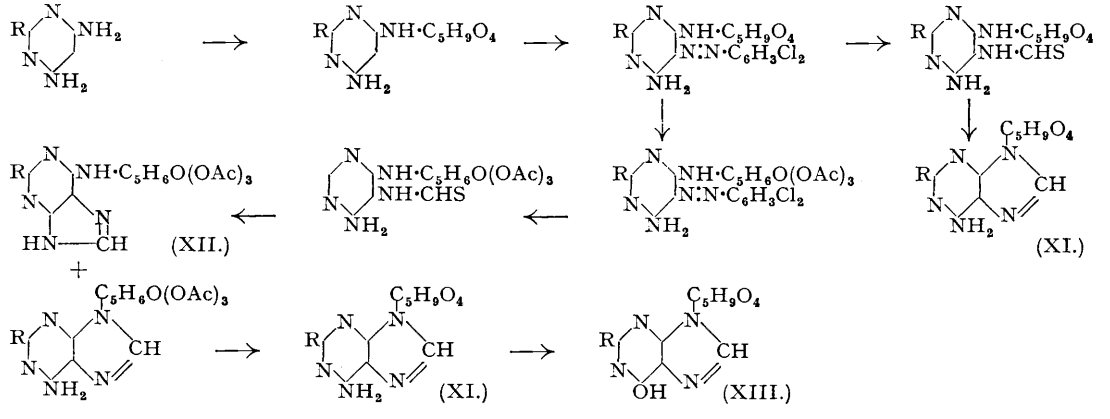


derivative. This method is, of course, based on the Traube synthesis of adenine in which a compound of type (VI; R = H) is heated with formic acid, the initially produced formamido-compound cyclising at higher temperatures by loss of water. The first essential was to devise a milder cyclisation procedure which would

avoid the danger of hydrolysis inherent in the Traube procedure if applied to sensitive glycosides. Such a procedure was found when it was shown that a 5-thioformamido-compound (VII; R = H), prepared from (VI; R = H) by treatment with aqueous sodium or potassium dithioformate, passed into the corresponding purine on heating in dry pyridine, hydrogen sulphide being evolved.[7]

For the synthesis of a 9-substituted adenine derivative by this route an intermediate (VII) would be necessary, and the question at once arises whether cyclisation will proceed in the desired direction to give (VIII) and not (IX). Initially the problem was tackled in model experiments in which R = alkyl, and various readily accessible pyrimidine derivatives analogous to (VII) were employed bearing substituents in the 2-position (*e.g.*, MeS). It seemed to us probable that ring-closure would in fact lead to the desired 9-alkylpurine, since general experience of the behaviour of pyrimidine derivatives suggested that a compound such as (VII; R = alkyl) would tend to react as if it had structure (VIIa) in which case cyclisation by loss of hydrogen sulphide would be expected to give (VIII) and not (IX). This expectation was realised on experiment; cyclisation of (VII) gave (VIII) exclusively and in almost theoretical yield.[7] Assuming that a 4-glycosidamino-group would be analogous to a 4-alkylamino-group the way then seemed clear for the synthesis of a 9-glycosidoadenine.

The next problem to be solved was the synthesis of a 5 : 6-diamino-4-glycosidaminopyrimidine of type (VI) and it was clear that the only practicable method would be preparation of a 6-amino-4-glycosidaminopyrimidine and subsequent introduction of the 5-amino-group. Hitherto 4-glycosidaminopyrimidines had defied all attempts at preparation. For this the principal reason undoubtedly lay in the normal tendency of 4-aminopyrimidines to react in the tautomeric imino-dihydropyrimidine form; in this form glycosidisation could hardly be expected. If, however, we consider the tautomeric possibilities in substituted pyrimidines, it is evident that in 4 : 6-diaminopyrimidines of type (X) where R is H, alkyl, or some substituent other than a group (*e.g.*, OH, SH, NH_2) capable of taking part in prototropic change, one double bond must occupy position 1 : 2 or 2 : 3 and hence one of the amino-groups at positions 4 and 6 should show in considerable degree the behaviour of a true amino-group and as such be capable of glycosidisation. In accordance with this view we were able to condense 4 : 6-diaminopyrimidines fulfilling these conditions with pentoses and hexoses by heating in absolute alcohol in presence of an acidic catalyst under conditions which allowed continuous removal of the water liberated in the reaction.[8] Having obtained by this means the desired 6-amino-4-glycosidaminopyrimidine derivatives it was our original intention to introduce a 5-amino-group by nitrosation in acetic acid solution followed by reduction, but this was possible only in the case of glycosides bearing a methylthio-group in position 2 to facilitate nitrosation. In all other cases nitrosation in position 5 required the use of mineral acids, and the method could not be employed because of consequent hydrolysis of the glycoside. Fortunately a suitable alternative became available when it was found that reactive diazonium compounds such as those from *p*-chloroaniline or 2 : 5-dichloroaniline couple in weakly alkaline solution with 6-amino-4-glycosidaminopyrimidines to give crystalline azo-compounds which can be reduced catalytically to the desired 5-aminoderivatives.[9] The latter can then be thioformylated and cyclised to purine derivatives. The synthetic route thus established was applied successfully to the first unambiguous syntheses of 9-pentosidoadenine derivatives as indicated schematically below :[10]

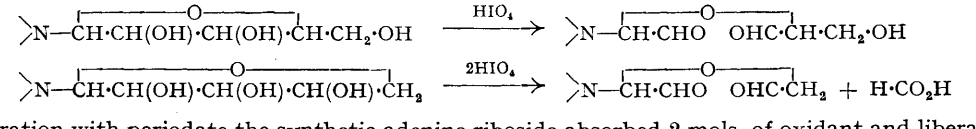


The use of acetylated thioformamido-compounds in the final cyclisation was found to give better yields, but in a number of cases (*e.g.*, in the xylose series when R = H, Me, or MeS) a mixture of the 6-glycosidaminopurine (XII) and the 9-glycosidoadenine (XI) was obtained. These two products are readily distinguished by the solubility of the former in alkali and the insolubility of the latter coupled with its ready deamination to the corresponding 9-glycosidohypoxanthine (XIII). This production of isomeric glycosides never, so far as we know, occurs when acetyl-free thioformamido-compounds are used or when the cyclisation medium—alcoholic sodium alkoxides in our more recent work—is such as to cause preliminary deacetylation. As it is in direct contrast to our experience in the model experiments on the synthesis of 9-alkylpurines, some explanation is required. We believe that the explanation lies in chelation between the glycosidic NH-group and the acetyl group at C_2 in the sugar chain. Such chelation, which is sterically feasible, would clearly increase the basicity

of the glycosidic nitrogen and hence, indirectly, of the nitrogen at position 6 thus enabling it to take part in the cyclisation process.[11, 12] This explanation is supported by all available evidence and is much more satisfactory than any based on steric hindrance. The magnitude of the chelation effect seems to vary considerably from case to case and cannot at present be predicted on theoretical considerations.

The synthetic method illustrated in the above scheme has been applied to a variety of adenine and substituted adenine hexosides and pentosides, but that application has been complicated in two cases—those of the *d*-xylosides of adenine [11] and 2-methylthioadenine [13]—by the occurrence of two series of isomeric intermediates in the earlier stages of the syntheses. These phenomena were for a time a source of confusion but they have been eventually clarified and a satisfactory explanation is now available. The simplest way to deal with this topic is to consider a specific case—that of the synthesis of 9-*d*-xylosido-2-methylthioadenine (XI; R = MeS). Xylosidisation of 4 : 6-diamino-2-methylthiopyrimidine gives a mixture of two isomeric *d*-xylosides which yield distinct 5-nitroso- and 5-arylazo-derivatives. Reduction of these to a 5-amino-compound, however, causes the isomerism to disappear so that only one 5-thioformamido-compound and one 9-*d*-xylosido-2-methylthioadenine can be obtained. Similarly, acetylation of the 5-nitroso-compounds causes disappearance of isomerism. It is clear that this isomerism must have its origin in the sugar residue, *i.e.*, it could be furanose-pyranose or α-β in type. In fact careful study of all the data leads to the conclusion that the two series of intermediates are α-β-isomers—which is perhaps not surprising in view of the analogy between their mode of preparation and Fischer's classical condensation of glucose and methanol to give α- and β-methylglucosides. The reason for the merging of the two isomeric series by interconversion during the course of the purine synthesis has been the subject of much study; it appears to fit into the general behaviour of *N*-glycosides and to be essentially similar to the phenomenon of mutarotation.[14] This is hardly the place to develop lengthy theoretical arguments but, in brief, it is believed that in those cases where separate α- and β-isomers are obtained they are compounds so weakly basic that the velocity of mutarotation is vanishingly small. As soon as the basicity of the glycosidic nitrogen atom is increased, either indirectly by introduction of a 5-amino-group or directly by chelation in the case of acetylated compounds (see above), interconversion (mutarotation) can and does occur and, one isomer being much more stable, it is complete. It has been concluded from optical data that in all the pyrimidine derivatives so far prepared it is the β-glycoside which is the stable form.[14]

A substance of particular interest among those synthesised by the above method is 9-*d*-ribosidoadenine.[15] This synthetic glycoside, although indistinguishable from adenosine in its ultra-violet absorption, is nevertheless not identical with it. Clearly there are three possible reasons any or all of which might account for the difference. First, the synthetic substance might be a pyranoside, the natural nucleoside being a furanoside; secondly, the difference might be due to α : β-isomerism; and finally, although much less probably, the earlier spectroscopic evidence might have been misinterpreted and the sugar residue in the natural compound might be located at some point other than N_9. The obvious first step was to seek some general method for determining lactol ring-structure in purine glycosides which could be rapidly and conveniently carried out on small amounts of material. Such a method became available when it was shown that the periodate oxidation procedure developed by Hudson and his collaborators for the *O*-glycosides could be applied successfully to tertiary *N*-glycosides.[16] The validity of the method was first established using as models theophylline-7-glycopyranosides and natural purine-9-ribofuranosides. As indicated below, oxidation of a pentofuranoside requires an uptake of 1 mol. of periodate whereas a pentopyranoside requires 2 mols. of periodate, and 1 mol. of formic acid is liberated during oxidation:



On titration with periodate the synthetic adenine riboside absorbed 2 mols. of oxidant and liberated 1 mol. of formic acid; it is therefore a 9-*d*-ribopyranosidoadenine.[15] Application of the periodate oxidation method to our other synthetic purine xylosides showed that all of them were pyranosides. The same method could not be applied to determination of lactol ring-structure in the pyrimidine glycosides used as synthetic intermediates, since complete oxidation of the sugar residue occurs in all cases where the glycosidic nitrogen bears a hydrogen atom. A modified method in which lead tetra-acetate was substituted for sodium metaperiodate was also found to be too unreliable for use in such cases. Nevertheless a pyranoside structure may be deduced for these intermediates since otherwise one would have to assume furanose-pyranose interconversion in fully acetylated derivatives during the course of purine synthesis.

The periodate oxidation method has proved a most valuable tool in our investigations and it has been applied to a variety of problems. Some of these concern the nucleotides proper and will be discussed later; for the moment attention will be confined to the determination of configuration at the glycosidic carbon atom in natural and synthetic nucleosides and to the rigid proof of the attachment of sugar at N_9 in the natural purine nucleosides. Knowledge of the glycosidic configuration of the natural nucleosides is of considerable importance from a general viewpoint although it does not appear to have been the subject of any previous investigations. Configuration at the glycosidic centre in the nucleosides must be a major factor in determining the molecular packing in polynucleotides and in governing their linkage with proteins in nucleoproteins. In attacking this problem experimentally we have made the usual assumption that glycosides prepared by the use of *cis*-α-

acetohalogeno-sugars have the β-configuration. Fischer and Helferich,[6] by the interaction of silver dichloro-adenine and α-acetobromoglucose followed by removal of halogen atoms from the product and deacetylation, obtained a glucoside which may be described as adenine 9-β-*d*-glucopyranoside, the location of the sugar resting on spectroscopic evidence. Periodate titration of this substance proceeded with uptake of 2 mols. of oxidant yielding a dialdehyde. Since the configuration at C_4 in *d*-ribose is the same as that at C_5 in *d*-glucose, and since all other asymmetric centres save C_1 are destroyed on periodate oxidation, then if adenosine is a β-glycoside it should on periodate oxidation yield the same dialdehyde. This was found to be the case and it is concluded that adenosine is a β-*d*-ribofuranoside.[17] Since it was also established that synthesis of 9-*d*-glucopyranosidoadenine [18] by our general method gives a product identical with the glucoside of Fischer and Helferich, it follows rigidly that the sugar residue in adenosine is at N_9 and that this nucleoside is correctly described as 9-β-*d*-ribofuranosidoadenine. By an extension of this method a β-configuration has been established for all the purine glycosides synthesised by our general procedure outlined above.

In analogous fashion it has been shown [17] that the natural pyrimidine nucleosides uridine and cytidine can be oxidised to dialdehydes identical with those obtained from 3-β-*d*-glucopyranosidouracil and 3-β-*d*-glucopyranosidocytosine, and it follows that these nucleosides are respectively 3-β-*d*-ribofuranosidouracil and 3-β-*d*-ribofuranosidocytosine.

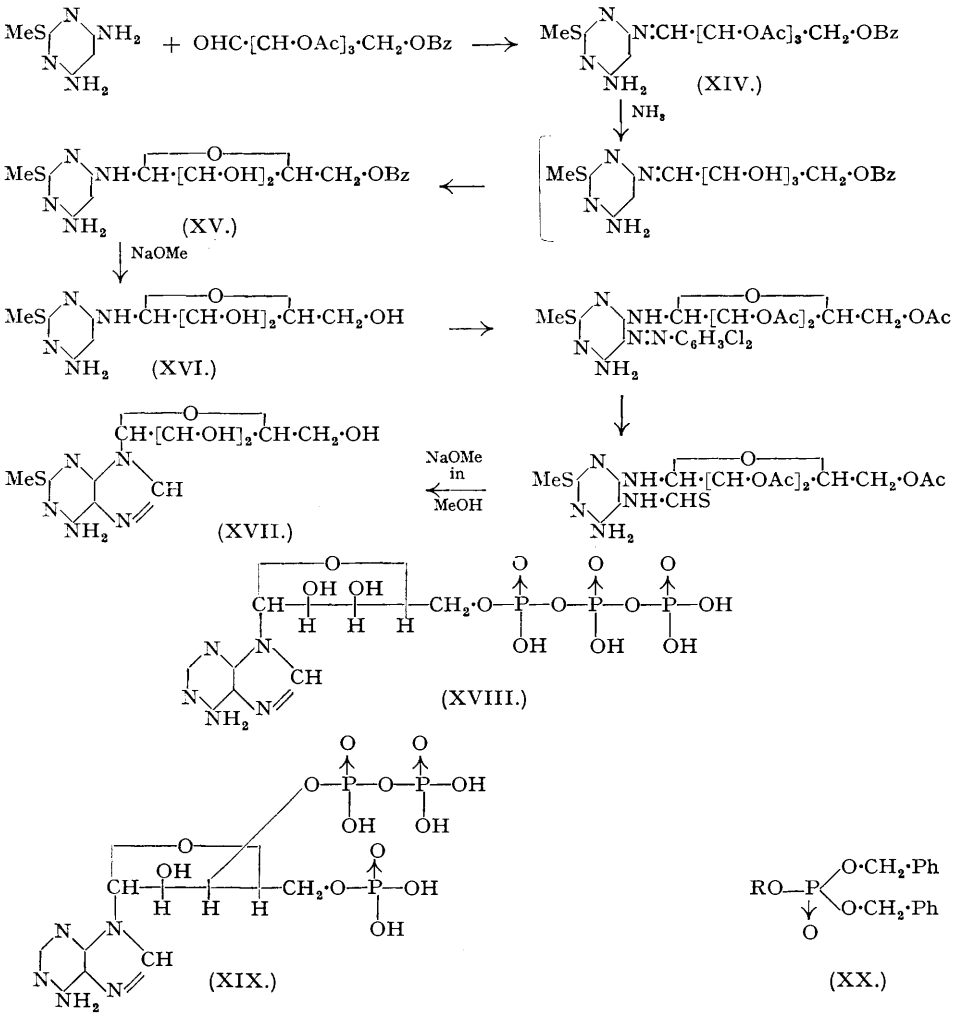
On the basis of the work so far described it may be concluded that a total synthesis of adenosine could be achieved by the synthetic route worked out for the 9-glycopyranosidoadenines provided that a 6-amino-4-*d*-ribofuranosidoaminopyrimidine, necessary as starting material, could be prepared. The preparation of such a compound, however, presented considerable difficulty; the obvious methods—condensation of 4 : 6-diaminopyrimidines with fully acylated furanose sugars or with 5-acylpentofuranoses—failed to yield the desired product. The problem was finally solved by application of a novel glycosidisation procedure.[19] It was first established that the methylthio-group in 4 : 6-diamino-2-methylthiopyrimidine increases the basic character of the amino-groups sufficiently to enable one of them to condense with benzaldehyde to form a Schiff base. In the same way it could be condensed with the aldehydo-sugar 5-benzoyl 2 : 3 : 4-triacetyl *l*-arabinose giving the Schiff base (XIV). Treatment of this product with cold methanolic ammonia removed the acetyl groups leaving the benzoyl group unaffected. The initial product, presumably a Schiff base, isomerised at once to the 5-benzoyl *l*-arabinofuranoside (XV) from which the benzoyl group could subsequently be removed by treatment with sodium methoxide in methanol yielding (XVI). Application of the standard synthetic route using (XVI) as starting material gave as anticipated 9-*l*-arabinofuranosido-2-methylthioadenine (XVII). Since it had previously been shown that replacement of the methylthio-group in 9-glycosido-2-methylthioadenines by hydrogen can be readily effected [13] by means of the elegant desulphurisation method of Mozingo [20] it is evident that the synthesis just described forms an exact model for the synthesis of adenosine. Such a synthesis is now being undertaken and there seems little reason to doubt its success if *d*-ribose proves analogous in behaviour to *l*-arabinose.

Before dealing with experiments bearing on the development of synthetic methods for the nucleotides, *i.e.*, methods of phosphorylation of nucleosides, a point of interest arising from the periodate method for determining lactol ring-structure in purine glycosides may be mentioned. It was observed [16] that yeast adenylic acid (adenosine 3′-phosphate) is unaffected by periodate. Muscle adenylic acid (adenosine 5′-phosphate) on the other hand takes up, like adenosine itself, one molar equivalent of periodate. This at once offered a method for determining unequivocally the structure of adenosine triphosphate, a substance which plays an important rôle in the transfer of phosphate in biological systems. The structure of adenosine triphosphate has hitherto been in some doubt. The titration evidence of Lohmann [21] (recently supported by that of Gulland [22]) clearly favoured the adenosine 5′-triphosphate structure (XVIII). Nevertheless other structures have been proposed by various workers [23] who claimed that it was possible, using certain enzyme preparations, to remove the acid-stable (5′) phosphate residue leaving the acid-labile residues intact; (XIX) may be taken as typical of such structures. It has now been shown that adenosine triphosphate is oxidised by periodate, one molar proportion of oxidant being consumed.[24] This we regard as conclusive proof that structure (XVIII) correctly represents adenosine triphosphate. It also follows that adenosine diphosphate is to be regarded as adenosine 5′-pyrophosphate and that, since cozymase yields adenosine diphosphate by hydrolytic cleavage, further support is given to structure (V) for the former substance.

Hitherto the synthesis of simple nucleotides by phosphorylation of nucleosides, using other than enzymic methods, has not been very practicable owing to the limitations of the methods used. Yeast and muscle adenylic acids have been prepared in very low yield by phosphorylation of adenosine with phosphoryl chloride in the presence of bases, with [25] or without [26] previous protection of other hydroxyl groups in the molecule, while treatment of 2′ : 3′-diacetyladenosine with diphenyl chlorophosphonate and subsequent removal of protecting groups again yielded very small amounts of muscle adenylic acid.[27] It seemed therefore that if our ends were to be achieved some other phosphorylation method would have to be devised. The requirements to be met by such a method would be (*a*) that it should proceed in good yield under mild conditions, (*b*) that no hydrolytic process should be involved which might damage sensitive glycosides, and (*c*) that it should be flexible enough to be capable of extension to the production of polyphosphoric esters.

The search for a convenient method of phosphorylation, fulfilling at any rate the first two requirements, has been pursued intermittently for many years by a considerable number of investigators without any really satisfactory result. Among the reagents which have been proposed are diphenyl chlorophosphonate,[27, 28]

phenyl dichlorophosphinate,[28, 29] anilinophosphonyl and dianilinophosphinyl chlorides,[30] ethyl metaphosphate,[31] and, recently, catechol-oxychlorophosphine.[32] We have made an extended study of this problem and have



selected for particular attention the use of dibenzyl chlorophosphonate.[33] This substance, which can be prepared by the action of chlorine on dibenzyl phosphite dissolved in an inert solvent [33] or by treatment of dibenzyl phosphate with thionyl chloride,[34] reacts readily with alcohols at room temperature in the presence of tertiary bases yielding esters of type (XX). From these products the benzyl groups can be removed smoothly and rapidly by catalytic hydrogenation. This method has been applied to the synthesis of muscle adenylic acid, which can be readily prepared in good yield by treatment of 2 : 3-*iso*propylideneadenosine with dibenzyl chlorophosphonate in pyridine followed by de-benzylation of the product and removal of the acetone residue with very dilute acid.[35]

The use of dibenzyl chlorophosphonate has a particular advantage in that removal of benzyl groups from the reactions by hydrogenolysis has been shown in a model experiment with dibenzyl *iso*amyl phosphate to proceed stepwise, *i.e.*, that partial hydrogenation yields benzyl *iso*amyl phosphate.[33] Since it has also been demonstrated in model experiments that, for example, dialkyl or diaryl chlorophosphonates react with silver dibenzyl phosphate to yield pyrophosphates, it is evident that we have here a potential method for the synthesis of polyphosphates and for the linkage of different molecules through polyphosphate residues. The realisation of this route in the case of nucleotides, in which technical difficulties are admittedly considerable, is at present one of our main preoccupations. Other routes to the same end which seem to offer promise of success I shall not deal with at this stage.

In this lecture I have attempted, however inadequately, to give a picture of the present position of a synthetic attack on the nucleotide problem. The problem is a very large one and it is clear that my colleagues and I have but touched on one aspect and it may well be that the difficulties in the way of our goal may be greater than we realise. Nevertheless we believe that the method of approach I have outlined may, coupled

with those of other chemists and biologists in the field, lead to a better understanding of the nature and rôle of the nucleotides in living matter.

References.

[1] Cf. Reviews by Levene and Bass, "Nucleic Acids," New York, 1931; Bredereck, *Fortschr. Chemie Org. Naturstoffe*, 1938, I, 121; Gulland, *J.*, 1938, 1722; 1944, 208; Lythgoe, *Ann. Reports*, 1944, 200.
[2] *J.*, 1934, 1639; 1936, 765; 1938, 692.
[3] Levene and Tipson, *J. Biol. Chem.*, 1934, **104**, 385.
[4] *Idem, ibid.*, 1934, **105**, 419.
[5] *Ibid.*, 1937, **117**, 371.
[6] Fischer and Helferich, *Ber.*, 1914, **47**, 210.
[7] Baddiley, Lythgoe, McNeil, and Todd, *J.*, 1943, 383.
[8] Baddiley, Lythgoe, and Todd, *J.*, 1943, 571.
[9] Lythgoe, Todd, and Topham, *J.*, 1944, 315.
[10] Baddiley, Lythgoe, and Todd, *J.*, 1944, 318.
[11] Kenner, Lythgoe, and Todd, *J.*, 1944, 652.
[12] Kenner and Todd, *J.*, 1946, in press.
[13] Howard, Lythgoe, and Todd, *J.*, 1945, 556.
[14] Howard, Kenner, Lythgoe, and Todd, *J.*, 1946, in press.
[15] Baddiley, Kenner, Lythgoe, and Todd, *J.*, 1944, 657.
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