[CONTRIBUTION FROM THE CHEMISTRY DIVISION OF THE BRITISH COLUMBIA RESEARCH COUNCIL]

Cyclic Phosphates. III. Some General Observations on the Formation and Properties of Five-, Six- and Seven-membered Cyclic Phosphate Esters

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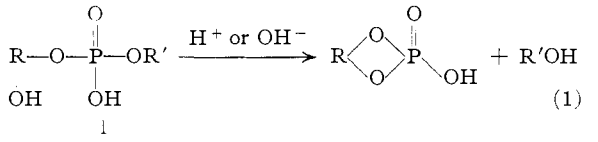
Using the reagent dicyclohexylcarbodiimide (DCC) a general procedure has been developed for the formation of five-, six- and seven-membered cyclic phosphates from phosphate esters containing suitably placed hydroxyl functions. Five-membered cyclic phosphates may be distinguished from their six and seven-membered counterparts by their further reaction with DCC to form N-phosphorylureas. The stereochemical requirements for cyclic phosphate formation are discussed with particular reference to the sugar phosphates and possible uses of the present technique in structural analysis are indicated. Finally, the stabilities of typical five-, six- and seven-membered cyclic phosphates to acid and alkali are compared.

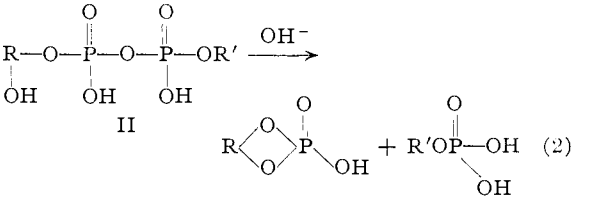
In connection with work in progress in this Laboratory on the synthesis of biologically interesting phosphate esters, especially phosphorylated sugars, we have extended our study[1] of the formation and properties of cyclic phosphate esters. The present communication records the observations we have made recently on these compounds.

While their implication in phosphoryl group migration had been suggested by a number of early workers,[2] only in recent years have the cyclic phosphate esters[3] been studied closely. Thus, their production from the ribonucleic acids and a number of nucleotide coenzymes has been reported and the chemical synthesis of a variety of these compounds has been recorded. In general, five or six-membered cyclic phosphates may be formed by *transesterification* from compounds of the type (I), where the component R bears a suitably-placed hydroxyl function (eq. 1). Examples of the base-catalyzed transesterification are the formation of ribonucleoside 2′,3′-cyclic phosphates (five-membered) from the ribonucleic acids,[4] pantoic acid 2,4-cyclic phosphate derivatives[5a] and 1,2-*O*-isopropylidene-D-xylofuranose 3,5-cyclic phosphate[5b] (six-membered cyclic phosphates) from the appropriate acyclic intermediates (see also Experimental). The above reactions, at least those resulting in the formation of five-membered cyclic phosphates, may also be catalyzed by acid[6] as has now been shown by the conversion of ribonucleoside 2′,(3′)-mono-alkyl phosphates to the corresponding cyclic phosphates (see Experimental).



(1)

Cyclic phosphates also may be formed from pyrophosphates of the nucleotide coenzyme type under alkaline conditions, whenever a suitably placed hydroxyl function is present. This process may be termed *intramolecular phosphorylation* and may be generally represented by eq. 2, where the component R of the molecule II is shown to possess the required hydroxyl group. Examples of this type of reaction are: the formation of glucose 1,2-cyclic phosphate[7] from uridine diphosphate glucose, riboflavin 4′,5′-cyclic phosphate[8] from flavin–adenine dinucleotide, pantothenic acid 2′,4′-cyclic phosphate[9] from coenzyme A and 5-phosphoryl-D-ribose 1,2-cyclic phosphate[10] from 5-phosphoryl-D-ribose α-1-pyrophosphate.



(2)

Practical methods for the synthesis of cyclic phosphate esters from hydroxylic compounds have utilized either phosphorylation with polyfunctional reagents,[5,8,11] when the cyclic ester is formed directly, or, phosphorylation with a monofunctional reagent, for example diphenylphosphorochloridate (ref. 5 and see Experimental), followed by a transesterification reaction, a method which appears to be particularly suitable for the preparation of the six-membered cyclic phosphates. Where monoalkyl-phosphate esters[12] are available, *e.g.*, ribonucleoside 2′(3′)-phosphates, cyclization may be brought about by reaction with trifluoroacetic anhy-

(1) (a) C. A. Dekker and H. G. Khorana, THIS JOURNAL, **76**, 3522 (1954); (b) G. M. Tener and H. G. Khorana, *ibid.*, **77**, 5348 (1955).

(2) (a) P. A. Levene and A. L. Raymond, *J. Biol. Chem.*, **107**, 75 (1934); (b) M. Bailly, *Compt. rend.*, **208**, 443 (1939); (c) P. E. Verkade, J. C. Stoppelenburg and W. D. Cohen, *Rec. trav. chim.*, **59**, 886 (1940); (d) E. Chargaff, *J. Biol. Chem.*, **144**, 455 (1942); (e) E. Baer and M. Kates, *ibid.*, **175**, 79 (1948).

(3) See, however, the early work of O. Bailly (*Bull. soc. chim. France*, **31**, 848 (1922)) who records a preparation of glycerol 1,3-cyclic phosphate.

(4) R. Markham and J. D. Smith, *Biochem. J.*, **52**, 552 (1952); D. Lipkin and P. T. Talbert, *Chemistry and Industry*, 143 (1955).

(5) (a) J. Baddiley and E. M. Thain, *J. Chem. Soc.*, 903 (1953); (b) J. G. Moffatt and H. G. Khorana, THIS JOURNAL, **79**, in press.

(6) The lability to acid of adenosine 2′(3′)-monobenzyl phosphates in contrast with the stability of the 5′-analog (D. M. Brown and A. R. Todd, *J. Chem. Soc.*, 44 (1952)) is strong presumptive evidence that hydrolysis in the former case occurs *via* transesterification.

(7) A. C. Paladini and L. F. Leloir, *Biochem. J.*, **51**, 426 (1952).

(8) H. S. Forrest and A. R. Todd, *J. Chem. Soc.*, 3295 (1950).

(9) J. Baddiley and E. M. Thain, *ibid.*, 3783 (1952).

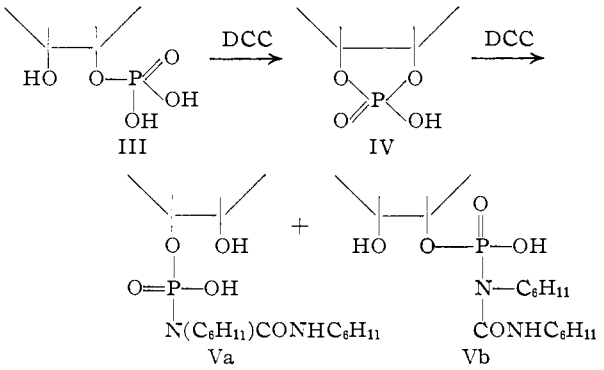
(10) C. N. Remy, W. T. Remy and J. M. Buchanan, *J. Biol. Chem.*, **217**, 885 (1955).

(11) (a) H. S. Mosher, J. Reinhart and H. C. Prosser, THIS JOURNAL, **75**, 4899 (1953); (b) J. Baddiley, J. G. Buchanan and L. Szabo, *J. Chem. Soc.*, 3826 (1954).

(12) Yet another method for the synthesis of cyclic phosphate esters utilizes the salts of chloroalkyl phosphates. This method was used by Bailly[3] in perhaps the earliest recorded synthesis of a cyclic phosphate. The method is of great practical importance wherever the necessary intermediates are available. Its application in the synthesis of ethylene glycol 1,2-cyclic phosphate has been recorded very recently by Lecocq.[15]

dride[13,14] or dicyclohexylcarbodiimide[1,14] (hereafter abbreviated to DCC). The latter reagent offers the marked advantages of simplicity of operation and mildness of conditions and has been used exclusively in the present work. It has been possible to formulate a simple general technique for studying the cyclization process and the results obtained using it may be conveniently described separately under five-, six- and seven-membered cyclic phosphates.

Five-membered Cyclic Phosphates.—It has already been shown[1] that ribonucleoside 2′(3′)-phosphate esters which possess an adjacent *cis*-hydroxyl function react with DCC in aqueous pyridine at room temperature to form the five-membered ribonucleoside 2′,3′-cyclic phosphates and that the latter react further to form N-phosphorylureas (partial formulas, III → IV → V). The reaction sequence may be followed conveniently by paper

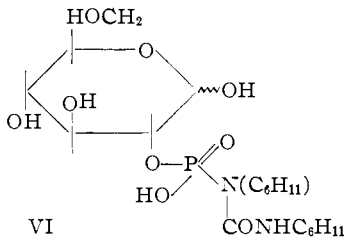


chromatography, using the solvent system isopropyl alcohol–ammonia–water, the R_f's of the products following, invariably, the order V > IV > III. It has now been found that ethylene glycol (two primary hydroxyl functions) and propane-1,2-diol (one primary and one secondary hydroxyl function) monophosphates also react with DCC according to the above sequence. In the former case it was possible to obtain further support for the reaction IV → V by treating ethylene glycol 1,2-cyclic phosphate, prepared from an authentic sample of the crystalline calcium salt,[15] with DCC under identical conditions. From the examples studied it seems reasonable to conclude that the formation of phosphorylureas (V) on treatment with DCC is a completely general reaction of the five-membered phosphate esters. This reaction which, as shown below, does not occur with the six- or the seven-membered cyclic phosphates, clearly is a consequence of the strain present in the five-membered phosphate ring (*cf.* their great lability under acidic and alkaline conditions, which is discussed later).

Useful structural information may sometimes be obtained by following the reaction of phosphate esters, especially sugar phosphates, with DCC. For example, in the anomeric D-ribofuranose 1-phosphates, it is clear that because of the more or less planar nature of the furanose ring, only the α-1-phosphate is able to form a 5-membered ring with the hydroxyl group at C_2 and, indeed, cyclization was found to occur only in the case of the dextrorotatory, enzymatically-prepared anomer.[16,17] The technique has, thus, provided a direct confirmation of the configurations of the natural and the earlier synthesized[16] samples.

The method is now used routinely in this Laboratory for ascertaining rapidly the configurations of the phosphate groups in synthetic pentofuranose 1-phosphates.[18] It has also been applied to D-ribofuranose 1,5-diphosphate,[19] the coenzyme for the phosphoribomutase, and has indicated the configuration of the phosphate group at C_1 to be also α.

By studying some well-known hexopyranose 1-phosphates it has been possible to draw general conclusions regarding the conditions under which cyclization may occur in pyranose derivatives. Glucose α-1-phosphate (Cori ester) and glucose β-1-phosphate[20] both reacted with DCC in aqueous pyridine according to the reaction sequence (III → IV → V), thus indicating the formation of five-membered cyclic phosphates. That the reaction indeed involved the hydroxyl groups at C_2[21] was confirmed by studying the rates of hydrolysis in 0.1 *N* hydrochloric acid at 100° of the ultimate products (presumably, VI, in both cases). These rates were



very similar to that recorded for glucose 2-phosphate[6,22] and different from that of glucose 6-phosphate. Since the anomeric D-glucose 1-phosphates probably exist in the stable Cl conformation,[23] the results show that cyclization is possible, both when

(13) (a) D. M. Brown, D. I. Magrath and A. R. Todd, *J. Chem. Soc.*, 2708 (1952); (b) T. Ukita, N. A. Bates and H. E. Carter, *J. Biol. Chem.*, **216**, 867 (1955).

(14) Both these reagents bring about activation of the phosphate group with consequent internal esterification, similar in principle to that observed in the breakdown of nucleotide coenzymes (eq. 2).

(15) J. Lecocq, *Compt. rend.*, **242**, 1902 (1956). We are grateful to Dr. Lecocq for a generous gift of the crystalline calcium salt of ethylene glycol 1,2-cyclic phosphate.

(16) R. S. Wright and H. G. Khorana, THIS JOURNAL, **78**, 811 (1956).

(17) The possibility of cyclization on to the 5-hydroxyl group in the case of β-D-ribofuranose 1-phosphate was considered, especially since 2,3,6-*O*-orthobenzoyl-β-D-fructofuranose has been prepared recently. (B. Helferich and L. Bottenbruch, *Chem. Ber.*, **86**, 651 (1953); B. Helferich and W. Schulte-Hurmann, *ibid.*, **87**, 977 (1954)). The cyclization has not been observed under the conditions used in the present work.

(18) (a) G. M. Tener, R. S. Wright and H. G. Khorana, THIS JOURNAL, **78**, 441 (1956); (b) R. S. Wright and H. G. Khorana, *ibid.*, in press.

(19) H. Klenow, *Arch. Biochem. Biophys.*, **46**, 186 (1953). We are grateful to Dr. Klenow for the gift of ribose 1,5-diphosphate.

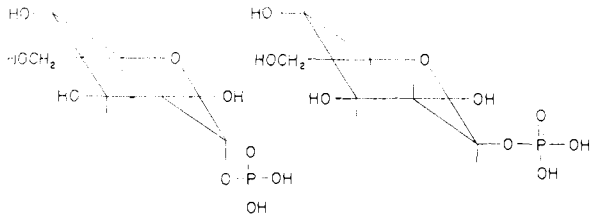
(20) Kind gift of Dr. W. Z. Hassid, Department of Plant Biochemistry, University of California, Berkeley.

(21) If glucose 1-phosphate attained the highly unfavorable IC conformation, then the hydroxyl groups at either carbon 3 or 6 could be involved in the cyclization reaction. The evidence presented here definitely excludes the participation of the hydroxyl group at C_6. The alternative possibility involving the hydroxyl group at C_3 also appears to be highly unlikely since this would lead to the formation of a six-membered cyclic phosphate, which would not be expected to react further with DCC to form fast-travelling material of the type VI.

(22) K. R. Farrar, *J. Chem. Soc.*, 3131 (1949).

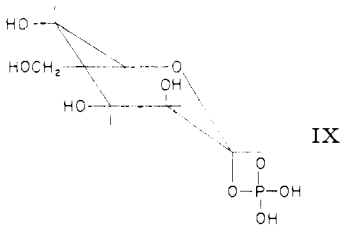
(23) R. E. Reeves, THIS JOURNAL, **72**, 1499 (1950).

the relationship of a phosphate group to the adjacent hydroxyl function is axial[24] to equatorial[24] (*cis*) (as in VII) and when this relationship is equatorial to equatorial[25] (*trans*) (as in VIII). Apparently, in the latter situation, the large size of the phosphorus atom, as compared with that of the carbon atom, enables the cyclization reaction to occur, since neither acyl group migration[26] nor isopropylidene derivative formation[27,28] has ordinarily been observed when the hydroxyls are *trans* (equatorial to equatorial).



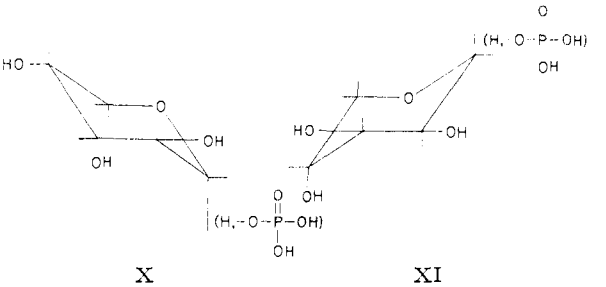
VII, α-D-glucose 1-phosphate VIII, β-D-glucose 1-phosphate

Obviously, cyclization will not be possible when the phosphate and the hydroxyl group on the adjoining carbon atom are both axial (*trans*). This situation is present in α-D-mannose 1-phosphate[29] (stable conformation, Cl, IX) and this substance did not form a cyclic phosphate on treatment with DCC.[30]



IX

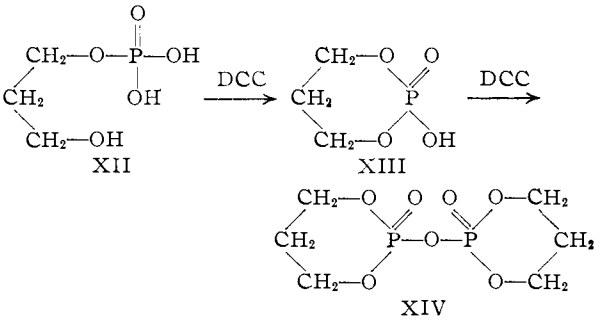
From the above it is clear that the cyclization test in pyranose 1-phosphates can give only limited information regarding the conformation and configuration of these compounds. Thus, both the synthetic D-ribopyranose[16] and D-arabinopyranose 1-phosphates[18b] gave, first, the cyclic phosphates and then the phosphorylureas. This finding excludes only the axial–axial (*trans*) relationship between the phosphate and the C_2-hydroxyl groups. If, as is likely, these two substances have, respectively, the Cl (X) and $1C^{23}$ (XI) conformations, then the hydroxyl groups at C_2 will be equatorial and cyclizations will occur, both when the phosphate groups have the α- or the β-configuration.



X XI

The possibilities of six-membered cyclic phosphate formation in pyranose phosphates are discussed below.

Six-membered Cyclic Phosphates.—The simplest example studied in detail has been that of 3-hydroxypropyl monophosphate (XII). This substance on treatment with DCC in *ca.* 20% aqueous pyridine rapidly gave the six-membered cyclic phosphate (XIII), which could be isolated as the



XII XIII XIV

crystalline cyclohexylammonium salt. While in aqueous pyridine XIII was the ultimate product, in anhydrous media, the free acid XIII reacted further with DCC to form the crystalline pyrophosphate XIV, in a quantitative yield. The significant observation that six-membered cyclic phosphate esters (XIII and see below), unlike five-membered cyclic phosphates, do not form phosphoryl ureas but instead yield the corresponding pyrophosphates (*cf.* simple non-cyclic diesters of phosphoric acid[33]) is in agreement with the known general stability of the six-membered cyclic phosphate ring.

The synthesis of the six-membered D-glucose 4,6-cyclic phosphate has been reported recently by Baddiley and co-workers,[11b] and, in the present work, it was considered of interest to examine the possible formation of this substance directly by the reaction of glucose 6-phosphate with DCC. Were this ester to exist in aqueous pyridine in the furanose or in the open-chain form, then a five-membered cyclic phosphate would be the initial product of the reaction. On the other hand, if, as might be expected, the ester exists in the pyranose form (stable Cl conformation, XV) then cyclization with the equatorial hydroxyl function on C_4 would be favored. A stable cyclic phosphate ester was the sole ultimate product of the reaction of glucose 6-phosphate with DCC in *ca.* 25% aqueous pyridine and the evidence presented below leaves little doubt

(24) D. H. R. Barton, O. Hassel, V. Prelog and K. S. Pitzer, *Nature*, **172**, 1095 (1953).

(25) See also the reaction of D-xylose 3-phosphate, which is discussed below.

(26) See, *e.g.*, H. B. Wood and H. G. Fletcher, Jr., THIS JOURNAL, **78**, 207, 2849 (1956).

(27) O. Hassel and B. Ottar, *Acta Chem. Scand.*, **1**, 929 (1947).

(28) S. J. Angyal and C. G. Macdonald, *J. Chem. Soc.*, 686 (1952).

(29) Kind gift of Professor T. Posternak, University of Geneva.

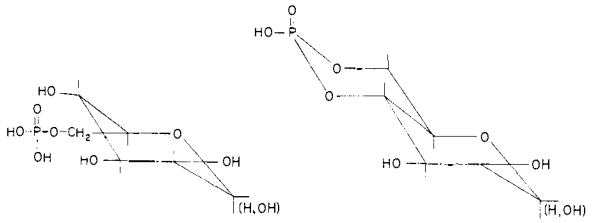
(30) Buchanan, *et al.*,[31] have pointed out the difference in the hydrolytic behavior of uridine diphosphate glucose and guanosine diphosphate mannose.[32] In contrast with uridine diphosphate glucose, which forms glucose 1,2-cyclic phosphate,[7] guanosine diphosphate mannose, apparently, does not form a mannose cyclic phosphate. This finding indicates the α-configuration at the glycosidic center in mannose, since then the phosphate and the hydroxyl group at C_3 would be axial–axial (see above).

(31) J. G. Buchanan, *et al.*, "Phosphorus Metabolism," Vol. II, The Johns Hopkins Press, Baltimore, Md. 1952, p. 450.

(32) E. Cabib and L. F. Leloir, *J. Biol. Chem.*, **206**, 779 (1954).

(33) H. G. Khorana and A. R. Todd, *J. Chem. Soc.*, 2257 (1953).

that this product is, indeed, the six-membered 4,6-cyclic phosphate[34] XVI. The chromatographic

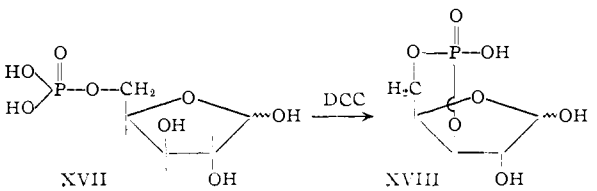


XV XVI

properties in several solvent systems were very close to those reported[11b] for XVI. The analytical data were in agreement with the cyclic phosphate formulation and an electrometric titration showed the absence of a secondary phosphoryl dissociation. On treatment with periodic acid, the substance consumed two molecular equivalents of the oxidant.

Other possibilities for cyclization in pyranose phosphates obviously exist: for example, when an axial hydroxyl is β to an axial phosphate group in a chair conformation[35] or when a sugar phosphate assumes a boat conformation. However, suitable examples are not as yet available to test these possibilities experimentally.

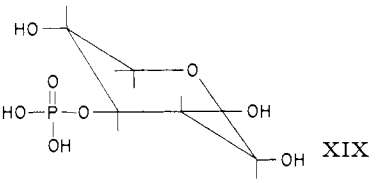
To summarize the above discussion of five and six-membered cyclic phosphates, if the reaction of a phosphate ester with DCC in aqueous pyridine gives a cyclic phosphate as the ultimate product, the latter probably consists of a six- (or higher, see below) membered ring. The reaction sequence (III → IV → V), on the other hand, is indicative of five-membered ring formation. A striking illustration of the use of this technique in studying the properties of sugar phosphates is provided by D-xylose 5- and 3-phosphates.[36] The 5-isomer XVII reacts with DCC in aqueous pyridine to form the six-membered D-xylofuranose 3,5-cyclic phosphate (XVIII). D-Xylose 3-phosphate, on the other hand, forms five-membered cyclic phosphate(s)[37]



XVII XVIII

and then N-phosphorylureas. It is therefore clear that whereas D-xylose-5-phosphate exists in solution in the furanose form XVII, the 3-phosphate is present in the pyranose form XIX, (stable conformation C1).

Where the formation of both five- and six-membered cyclic phosphates is possible, the five-membered ring is favored.[38] Thus, α-glycerophosphate forms, on treatment with DCC, a cyclic phosphate (and then phosphorylureas[39]) which is acid- and alkali-labile. It is, apparently, identical with glycerol 1,2-cyclic phosphate described by Ukita, *et al.*,[13b] and different from the stable glycerol 1,3-cyclic phosphate recorded by Bailly.[3]



XIX

Seven-membered Cyclic Phosphate.—It was considered of interest to extend the present studies to the preparation of a seven-membered cyclic phosphate ester, no ester of this type having been reported previously in the literature.[40] The preparation of butane-1,4-diol cyclic phosphate (XXI) was therefore undertaken. Butane-1,4-diol was phosphorylated with diphenyl phosphorochloridate in pyridine to give an excellent yield of the oily 4-hydroxybutyl diphenyl phosphate from which the phenyl groups were removed by hydrogenolysis in the presence of platinum. 4-Hydroxybutyl phosphate (XX) was thus obtained as the highly crystalline barium salt.

A study of the reaction of XX with DCC in aqueous pyridine showed that a substance with R_f 0.62 was the major ultimate product; however, a weak spot (R_f 0.52) was present sometimes and, in addition, a very faint spot just ahead of the starting material (R_f 0.15) could be detected. The major spot (R_f 0.62) was purified by chromatography on paper sheets and isolated as the highly crystalline cyclohexylammonium salt. From its chromatographic behavior, elemental analysis and an electrometric titration (absence of any phosphoryl dissociation in the *p*H range 4–9), this substance was concluded to be butane-1,4-diol cyclic phosphate[41] (XXI).

The two alternative reactions which may occur when phosphate esters such as XX are treated with DCC are: (1) cyclization to form XXI and (2) anhydride formation to form XXII. In order to provide further support for the formulation of the product obtained above as XXI it was decided to prepare XXII in an authentic manner. The synthesis of XXII was accomplished by (1) acetylation of XX to give 4-acetoxybutyl phosphate (XXIII), (2) conversion of the latter to the corresponding pyrophosphate (XXIV) by treatment with DCC,[42] and finally (3) deacetylation under mildly alkaline conditions. The product XXII was

(34) Incidentally, the quantitative formation of this product confirms that at least in aqueous pyridine glucose 6-phosphate exists entirely in the pyranose form (C1 conformation).

(35) As perhaps in the hypothetical α-D-altrose 1-phosphate (C1 conformation).

(36) J. G. Moffatt and H. G. Khorana, THIS JOURNAL, in press.

(37) Clearly, in (XIX) cyclization may occur with the hydroxyl group on C_3 as well as on C_4.

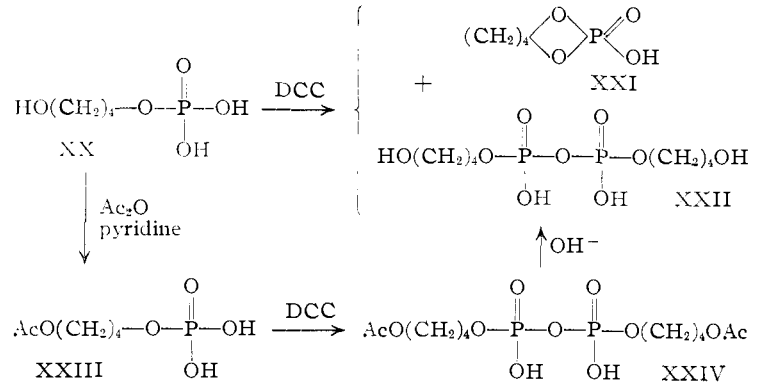
(38) *Cf.* the formation of riboflavin 4',5'-cyclic phosphate from flavin-adenine dinucleotide.[8]

(39) Unpublished work of Dr. Charles A. Dekker, University of California, and present work.

(40) It has been suggested by Baddiley, *et al.* (ref. 11b), that the cyclic phosphate ester obtained by the phosphorylation of methyl α-D-glucoside using monoanilino phosphorodichloridate (Zeile and Kruckenberg, *Ber.*, **75**, 1127 (1942)) is actually a six- and not a seven-membered cyclic phosphate.

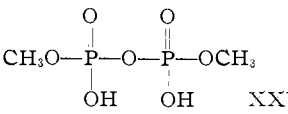
(41) It may be mentioned in passing that the preparation of this cyclic ester from the neutral 4-hydroxybutyl diphenyl phosphate ester *via* transesterification (ref. 5) was not possible because of the great tendency, especially under alkaline conditions, of the neutral ester to decompose to diphenyl phosphate ion and tetrahydrofuran.

(42) H. G. Khorana, THIS JOURNAL, **76**, 3517 (1954).

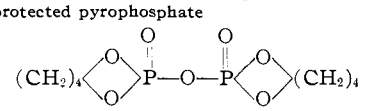


purified on paper sheets and was isolated as the crystalline bis-cyclohexylammonium salt. The analytical data checked well for this formulation and an electrometric titration showed the absence of any secondary phosphoryl dissociation. Paper chromatographic examination showed that this product (R_f 0.54) was distinctly different from XXI and was, in fact, identical with the minor product which was sometimes obtained during the reaction of 4-hydroxybutyl phosphate (XX) with DCC. Further, it was found that the treatment of the pure synthetic pyrophosphate XXII with DCC resulted in the formation of XXI. It is therefore clear that both reactions, cyclization and anhydride formation, occur when 4-hydroxybutyl phosphate (XX) is treated with DCC and that the pyrophosphate XXII is also converted, subsequently, to the cyclic phosphate[43] XXI.

The hydrolytic behavior of the pyrophosphate (XXII) deserves mention. Both under acidic and alkaline conditions, this substance hydrolyzed to form 4-hydroxybutyl phosphate (XX) and inorganic phosphate and under no conditions was the stable seven-membered cyclic phosphate[44] XXI formed as an intermediate. The "intramolecular phosphorylation" which was discussed above in connection with the alkaline hydrolysis of pyrophosphates (general formula, II) is thus limited to the formation of five-[7,8,10] and six-membered[9] cyclic phosphates. In contrast with the great alkaline lability of the pyrophosphates[7-10] which hydrolyze *via* cyclic phosphates (eq. 2), the substance XXII was found to be very stable to alkali, requiring 8 hours in 1 N sodium hydroxide at 100° for complete hydrolysis[45] to the monophosphate XX.



(43) Presumably this occurs in two steps; (1) cyclization of XXII to the fully protected pyrophosphate



and (2) decomposition of the latter to XXI.

(44) As shown below, the cyclic ester is stable under the conditions found necessary for the complete hydrolysis of the pyrophosphate XXII (*ca.* 8 hours at 100° in 1 N sodium hydroxide).

(45) These are the very conditions which will hydrolyze P^1P^2-dimethyl pyrophosphate[46] (XXV) to methyl phosphate.

(46) Prepared from methyl phosphate, using DCC.[42]

(Some inorganic phosphate also was formed under these conditions.)

Relative Stabilities of Five-, Six- and Seven-membered Cyclic Phosphates.—It is known that five-membered cyclic phosphates are highly labile to acid and alkali. The six-membered cyclic phosphates are, on the other hand, very stable under these conditions, resembling the simple dialkyl phosphates. Our own observations reported herein have confirmed these general conclusions and, further, have shown that the seven-membered cyclic ring is even more stable than the six-membered one. Thus, the five-membered ethylene glycol cyclic phosphate was found to have a half-life of only 5–10 minutes in 0.5 N sodium hydroxide at room temperature. Heating at 100° in 1 N sodium hydroxide was necessary to open the six- and seven-membered phosphate rings. The results obtained using these conditions were as follows: 1,2-isopropylidene-D-xylofuranose 3,5-cyclic phosphate, 50% hydrolysis in 4 hours, 90% in 12 hours; propane-1,3-diol cyclic phosphate, 20% hydrolysis in 24 hours; 36% in 48 hours. Butane-1,4-diol cyclic phosphate (XXI) was completely unaffected in 6 hours. Also under acidic conditions this substance was found to be more stable than propane-1,3-diol cyclic phosphate. Thus, on being heated in 0.5 N hydrochloric acid at 100° for 7.5 hours the six-membered cyclic phosphate was converted largely to 3-hydroxypropyl phosphate whereas butane-1,4-diol cyclic phosphate was practically unaffected.

Experimental

Acid-catalyzed Transesterification of Uridine 2′(3′)-Monoalkyl Phosphates to Uridine 2,3-Cyclic Phosphate.—Ammonium uridine 2′(3′)-monobenzyl (*n*-propyl) phosphates[1b] (15–20 mg.) were suspended in anhydrous dioxane (10 ml.) and the bulk of the solvent was distilled off at normal pressure with exclusion of moisture. More dioxane was added and the process repeated to ensure complete removal of traces of moisture from the mixture. To the suspension in *ca.* 2 ml. of dioxane (the suspension at this stage was shown to be completely free from the cyclic phosphate) were then added a few drops of freshly-distilled trifluoroacetic acid and the sealed reaction mixture shaken mechanically. Aliquots were removed at intervals, added immediately to an excess of 50% aqueous pyridine and the resulting solutions examined by paper chromatography in isopropyl alcohol–ammonia–water.[47] After a three-hour reaction period, a spot corresponding to the uridine 2,3-cyclic phosphate and a weak spot corresponding to uridylic acid had appeared, with *ca.* 60–70% of the starting material being present. After 18 hours, the alkyl ester had largely disappeared and was replaced by strong spots corresponding to the cyclic phosphate and uridine 2′(3′)-monophosphate (traces of moisture, complete exclusion of which is difficult on the small scale experiment, obviously are responsible for the formation of the free nucleotide).

General Method for the Reaction of Phosphate Esters with Dicyclohexyl Carbodiimide.—Pyridinium salts of the phosphate esters were used in all cases. Wherever barium or cyclohexylammonium salts were available, these (5–10 mg.) were converted to the pyridinium salts by passing them through pyridinium Dowex 50 (or IR-120) resin and evaporating the total effluent and washings to dryness. Pyridine (0.5–1 ml.) containing 10–20% water (by volume) was added, followed by 25–50 mg. of DCC. At intervals (usually 1, 2, 5 and 20 hours) 0.1-ml. aliquots were removed.

(47) R. Markham and J. D. Smith, *Biochem. J.*, **52**, 552 (1952); D. M. Brown and A. R. Todd, *J. Chem. Soc.*, 2040 (1953).

diluted with an equal volume of water and the mixture extracted three times with ether. The aqueous solution was then examined by paper chromatography (descending technique) using Whatman No. 4 paper. The solvent system employed throughout the present work was isopropyl alcohol–ammonia–water (70-10-20, v./v.).[47] The R_f's of the compounds were in the ranges indicated below: the starting materials, sugar phosphates, etc., 0.05–0.20; cyclic phosphates, 0.45–0.65; N-phosphorylureas, 0.85–0.95.

Diphenyl 3-Hydroxypropyl Phosphate.—To a cooled solution (5°) of an excess of propane-1,3-diol (30 ml.) in 100 ml. of anhydrous pyridine was added dropwise 12.4 g. of diphenylphosphorochloridate and the mixture left at room temperature for 12 hours. The excess of solvent and propane-1,3-diol was removed *in vacuo*, the oily residue taken up in ether (200 ml.) and the ethereal solution washed with water (4 × 200 ml.). Evaporation of the dried ethereal solution gave 3-hydroxypropyl diphenyl phosphate as a viscous oil in practically quantitative yield.

Phenylpropane-1,3-diol Cyclic Phosphate.—On long standing in contact with a small amount of aqueous pyridine the above oil deposited a crystalline material, which was recrystallized first from water and then from an ether–petroleum ether mixture to give colorless needles, m.p. 76–76.5°. *Anal.* Calcd. for $C_9H_{11}O_4P$: C, 50.52; H, 5.09; P, 14.47. Found: C, 51.08; H, 5.22; P, 14.8.

3-Hydroxypropyl Phosphate.—This was prepared from diphenyl 3-hydroxypropyl phosphate either by hydrogenation in methyl alcohol in the presence of a platinum catalyst, or by alkaline treatment to form propane-1,3-diol cyclic phosphate (see below), followed by hydrolysis under its own *p*H (1.5) for 16 hours. The product was isolated as the highly crystalline barium salt. *Anal.* Calcd. for $C_3H_7O_5PBa \cdot 1\ H_2O$: C, 11.64; H, 2.93; P, 10.00. Found: C, 12.24; H, 2.85; P, 10.00.

Propane-1,3-diol Cyclic Phosphate (XIII). (a) From 3-Hydroxypropyl Phosphate by Treatment with DCC.—A solution of the barium salt (1 g.) of 3-hydroxypropyl phosphate was passed through a Dowex 50-pyridinium column (5 cm. × 1 cm.) and the total effluent and washings evaporated. The resulting gum was taken up in a mixture of water (2 ml.) and pyridine (10 ml.). DCC (3 g.) was then added and the two phase mixture shaken mechanically overnight. Paper chromatography showed only one spot (R_f 0.55), corresponding to propane-1,3-diol cyclic phosphate. The mixture was then diluted with water, the solution filtered from urea and the clear filtrate extracted three times with ether. The aqueous layer was concentrated to a few ml. and the concentrate passed slowly through an IR 120 (H^+) column (10 cm. × 1 cm.). The total acidic effluent and washings were neutralized with aqueous cyclohexylamine to *p*H 4.5 and the solution evaporated to a gum. Ethyl alcohol was added and evaporation repeated. The gum crystallized from a mixture of ethyl alcohol (2 ml.) and acetone (15 ml.) to give 350 mg. of pure cyclohexylammonium propane-1,3-diol cyclic phosphate. A further crop (150 mg.) was obtained from the mother liquor after concentration and addition of ether. A sample was recrystallized from a mixture of ethyl alcohol and acetone; m.p. 177.5–178° after shrinkage at 173°. *Anal.* Calcd. for $C_9H_{20}NO_4P$: C, 45.60; H, 8.46; N, 5.90. Found: C, 45.40; H, 8.49; N, 5.81.

(b) From Diphenyl-3-hydroxypropyl Phosphate.—The neutral ester (7 g.) was dissolved in a mixture of dioxane (25 ml.) and 2 *N* sodium hydroxide (75 ml.) and the solution kept at room temperature for 48 hours in a polyethylene flask. The excess of alkali was neutralized by the gradual addition of Dowex 50 (H^+) resin. The latter was removed by filtration and washed with small portions of water. The combined filtrate and washings (*p*H 5) were extracted with ether to remove phenol. Paper chromatography at this stage showed complete conversion to the cyclic phosphate. The aqueous solution was concentrated to a small volume and then passed through a Dowex 50 (H^+) column (25 cm. × 2 cm.). The total acidic effluent was evaporated to a gum *in vacuo* below 25°. Some dioxane was added to the gum and the mixture re-evaporated. The residual oil was dissolved in warm tetrahydrofuran (*ca.* 15 ml.) and ether (10 ml.) was added. The crystalline propane-1,3-diol cyclic phosphate (2 g.) was collected by filtration and washed with ether. The acid was recrystallized from a mixture of acetonitrile and ether, m.p. 102–102.5°. *Anal.* Calcd. for $C_3H_7O_4P$: C, 26.10; H, 5, 11; P, 22.45. Found: C, 25.92; H, 5.21; P, 22.35. An electrometric titration showed the absence of a secondary phosphoryl dissociation in the *p*H range 4–9 and gave a figure of 147 for the neutralization equivalent (titration of the primary phosphoryl dissociation). Calcd. for propane-1,3-diol cyclic phosphate, 138.1.

The free acid is readily soluble in warm acetonitrile, soluble in hot tetrahydrofuran and only very sparingly soluble in ether, benzene and chloroform.

Preparation of Bis-(propane-1,3-diol cyclic)-pyrophosphate (XIV).—Propane-1,3-diol cyclic phosphate (278 mg., 2 mmoles of free acid) was dissolved in 9 ml. of anhydrous acetonitrile and to the solution was added DCC (226 mg.; 1.1 mmoles), dissolved in 1 ml. of acetonitrile. The mixture was shaken at room temperature with exclusion of moisture, and after one hour dicyclohexylurea (214 mg.; theoretical 245 mg.) was removed by filtration and washed with ether. The total filtrate was evaporated *in vacuo*, the crystalline residue taken up in 1 ml. of acetonitrile and the solution filtered from a small amount of urea. On the cautious addition of ether to the solution, XIV crystallized and was collected after one hour; yield 230 mg. (theoretical 260 mg.), m.p. after recrystallization (long needles) from a mixture of acetonitrile and ether, 137–137.5°. *Anal.* Calcd. for $C_6H_{12}O_7P_2$: C, 27.93; H, 4.69. Found[48]: C, 28.71; H, 4.91.

This substance dissolves readily in cold water to give a neutral solution which, on brief heating or on standing at room temperature, develops acidity, indicating hydrolysis to propane-1,3-diol cyclic phosphate.

D-Glucose 4,6-Cyclic Phosphate.—Barium glucose 6-phosphate (heptahydrate, 1.0 g.) was converted to the free acid by suspending it in water and adding IR-120 H^+ resin. The clear solution was evaporated *in vacuo* and the sirup taken up in 5 ml. of water and pyridine added until an oil began to separate. To the solution was added 3 g. of DCC in 20 ml. of pyridine and the resulting mixture shaken vigorously for three days at room temperature. Water (20 ml.) was then added, the crystalline urea filtered off and the aqueous filtrate extracted three times with ether. Paper chromatography showed that the conversion to glucose 4,6-cyclic phosphate was essentially complete. The product was purified by chromatography[49] on a cellulose powder column (35 cm. × 2 cm.) using isopropyl alcohol–pyridine–water (70:5:25) according to the procedure described earlier.[1a] The combined fractions (total volume 50 ml.) containing glucose 4,6-cyclic phosphate (appearing after 100 ml. of eluent had passed through the column) were concentrated and neutralized to *p*H 8 with saturated barium hydroxide solution. The slight precipitate was removed by centrifugation and 2 volumes of acetone then added to the clear supernatant. The oil which separated solidified on trituration with ethyl alcohol. After one further precipitation from water (1 ml.) and ethyl alcohol (6 ml.), the barium salt was washed with acetone and dried *in vacuo;* wt. 485 mg. *Anal.* Calcd. for $C_6H_{10}O_8P \cdot {}^1/_2\ Ba \cdot {}^1/_2\ H_2O$: C, 22.56; H, 3.48. Found: C, 22.57; H, 3.74. $[\alpha]^{20}D$ +11.1° (*c* 4.79 in water). (Baddiley, *et al.*,[11b] quote for this substance $[\alpha]^{20}D$ +16.1°.)

4-Hydroxybutyl Phosphate.—To a mixture of freshly distilled anhydrous butane-1,4-diol (30 ml.) and pyridine (10 ml.) was added gradually diphenyl phosphorochloridate (10 ml.) with exclusion of moisture (total time of addition, *ca.* 15 minutes) and the mixture allowed to stand at room temperature for 18 hours. Water (100 ml.) was then added and the oil which separated was extracted into ether. The ether layer was washed repeatedly with water (6 ×), dilute hydrochloric acid solution (2 ×) and again with water. Evaporation of the dried ether solution gave 4-hydroxybutyl diphenyl phosphate as a colorless viscous oil (12 g.). Hydrogenation of this product (4.3 g.) in methyl alcohol in the presence of a platinum catalyst gave an oil which was neutralized with saturated barium hydroxide solution to *p*H 8. The precipitate (largely barium phosphate, 0.53 g.) was removed by centrifugation and the clear supernatant evaporated to a small volume. On the

(48) The higher values obtained probably indicate a trace contamination of dicyclohexylurea in the product.

(49) The cyclic phosphate may also be freed from traces of the starting material by fractional precipitation of the barium salt from water–ethyl alcohol mixtures.

addition of ethyl alcohol (4 volumes) to the concentrate, the barium salt of butane-1,4-diol monophosphate crystallized as rosettes of needles. It was collected by centrifugation, washed with ethyl alcohol and then ether; wt. 2.93 g. A portion was recrystallized from aqueous ethyl alcohol. *Anal.* Calcd. for $C_4H_9O_5P \cdot Ba \cdot 2H_2O$: C, 14.08; H, 3.81. Found: C, 13.59; H, 3.35.

Preparation of Butane-1,4-diol Cyclic Phosphate.—Preliminary experiments on the reaction of butane-1,4-diol monophosphate with DCC in pyridine containing 5–20% water showed the formation of one major spot (R_f in solvent system A, 0.62), a weaker spot (R_f 0.54) and another very faint spot travelling just ahead of the starting material.

In a large scale experiment, the pyridinium salt of the monophosphate (prepared from 0.9 g. of the barium salt) was dissolved in a mixture of pyridine (16 ml.) and water (2 ml.) and to the solution was added 2.5 g. of DCC. After 18 hours the reaction mixture was worked up in the usual manner and the major product (R_f 0.62) was purified by descending paper chromatography using two Whatman 3 MM sheets. The aqueous solution of the product obtained after elution from the paper sheets was passed through a Dowex 50 (H^+) column (5 cm. × 1 cm.) and the acidic effluent and washings neutralized with aqueous cyclohexylamine to *p*H 4. Evaporation gave 250 mg. of a crystalline residue which was recrystallized first from a mixture of ethyl alcohol and ether and then from tetrahydrofuran; m.p. 152–153°. *Anal.* Calcd. for $C_{10}H_{22}NO_4P$: C, 47.8; H, 8.79; N, 5.51. Found: C, 47.63; H, 8.74; N, 5.58.

An electrometric titration showed the absence of a secondary phosphoryl dissociation in the *p*H range 4–9.

Preparation of P^1P^2-Bis-4-hydroxybutyl Pyrophosphate (XXII).—Butane-1,4-diol monophosphate (free acid, prepared by passing 1 g. of the barium salt through a Dowex 50 (H^+) column) was dissolved in 5 ml. of pyridine and to the solution was added 2 ml. of acetic anhydride. The reaction mixture was allowed to stand overnight and then diluted with water. The solution was then evaporated and the residual acetic acid and pyridine were removed by repeated evaporation in the presence of dioxane. Paper chromatography showed the presence of a major spot corresponding, presumably, to 4-acetoxy-butyl phosphate (R_f, 0.37) (XXIII) and a weak spot corresponding to the P^1P^2-bis-4-acetoxybutylpyrophosphate (R_f 0.68) (XXIV). The total mixture was treated with DCC (5 g.) in 10% aqueous pyridine[41] (20 ml.) for 24 hours when paper chromatography revealed roughly 50–60% conversion of the starting material (R_f, 0.37) to the corresponding pyrophosphate (R_f 0.68) (XXIV). After working up, the mixture of the products was treated with 1 *N* sodium hydroxide solution for 1.5 hours at room temperature to remove the acetyl groups. The resulting mixture, consisting mainly of P^1P^2-4-hydroxybutyl pyrophosphate (R_f, 0.54) and 4-hydroxybutyl phosphate (R_f, 0.15) was separated on Whatman 3 MM sheets. The pyrophosphate band was eluted, passed through a Dowex 50 (H^+) column and the solution of the free acid neutralized with aqueous cyclohexylamine to *p*H 4. Evaporation of the aqueous solution gave a residue which readily crystallized. The bis-(cyclohexylammonium) salt was recrystallized by dissolving in a minimum amount of ethyl alcohol and adding acetone to turbidity; m.p. 150–154°. Paper chromatography gave a single strong spot with R_f, 0.54. An electrometric titration showed the absence of a secondary phosphoryl dissociation. *Anal.* Calcd. for $C_{20}H_{46}N_2O_9P_2$: C, 46.15; H, 8.91; N, 5.38. Found: C, 45.76; H, 8.84; N, 5.43.

Treatment of a small amount of the free acid prepared by passing the crystalline bis-(cyclohexylammonium) salt through Dowex 50 (H^+) with DCC in aqueous pyridine resulted in conversion to a slightly faster travelling spot (R_f, 0.62), corresponding to butane-1,4-diol cyclic phosphate.

Hydrolysis of Cyclic Phosphates.—Paper chromatography was employed mostly for studying the hydrolysis of cyclic phosphate esters. In the case of alkaline solutions, the excess of alkali was neutralized with Dowex 50 (H^+) before applying on paper strips.

The rates of alkaline hydrolysis (ring-opening) were also followed quantitatively by following the production of secondary phosphoryl dissociation (*p*H range 4–8). The procedure is illustrated by the following experiment on the ring-opening of 1,2-isopropylidene-D-xylofuranose 3,5-cyclic phosphate[36] to form a mixture of 1,2-isopropylidene-D-xylofuranose 3- and 5-phosphates.[36] Cyclohexylammonium 1,2-isopropylidene-D-xylofuranose 3,5-cyclic phosphate (137 mg.) was dissolved in 4 ml. of 1 *N* sodium hydroxide in a polyethylene tube and the solution was heated at 100° using a reflux condenser fitted with a soda-lime tube. At intervals 1-ml. aliquots were removed, passed slowly through a small column (4 cm. × 0.6 cm.) of Dowex 50 (H^+) resin and the acidic effluent titrated electrometrically to *p*H, using 0.101 *N* sodium hydroxide. The results obtained are given in Table I. Column A shows the amount of alkali (ml.) required for neutralization of the acidic solutions to *p*H 4 (primary phosphoryl dissociation). Column B shows the amount of alkali (ml.) required for neutralization from *p*H 4 to *p*H 8 (secondary phosphoryl dissociation). The ratio of B to A will indicate the extent of hydrolysis, since at 100% ring-opening B:A will be 1:1.

TABLE I

Time (hr).	A	B	% ring-opening
1	0.95	0.18	19
5	0.94	.59	62.8
11.5	1.02	.91	89.2
23	0.76	.77	100

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