142. *The Oxidation of Cyclic Phosphites to Cyclic Phosphates.*

By L. Keay and E. M. Crook.

The occurrence and synthesis of cyclic phosphates and the factors influencing ring formation, ring size, and stability are discussed with special reference to the degree of lability of the esters. Present methods for synthesis and their limitations are assessed. Ethyl esters of cyclic phosphates with five- and six-membered rings were prepared by the oxidation of the corresponding phosphites with mercuric oxide or dinitrogen tetroxide. The cyclic phosphates were also prepared by oxidation of cyclic phosphorochloridites and hydrolysis without isolation of the intermediate phosphorochloridate.

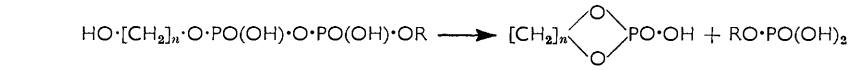
It has long been known that a neighbouring hydroxyl group renders phosphate esters more susceptible to alkaline hydrolysis [1] and leads to migration of the phosphate group in acid or alkaline conditions,[1-5] reaction proceeding through a cyclic phosphate.[2] The hydrolysis of ribonucleic acid and of pyrimidine nucleotide 3′-benzyl or -methyl ester by alkali or bovine pancreatic ribonuclease also proceeds through a cyclic phosphate [3,4] which has been isolated by Markham and Smith.[5] In these reactions five-membered rings are formed. Study of the cyclization of phosphate esters by Khorana and his colleagues [6] strongly suggests that this is the preferred form and is always formed if the stereochemical conditions permit; indeed these workers propose that the reaction has structural diagnostic implications. Nevertheless, larger rings can be made [6] and once formed resist hydrolysis to some extent by acid or alkali, the stability increasing rapidly with the ring size.[6-11] The stability of seven-membered ring compounds approaches that of simple dialkyl phosphates which have been shown to be hydrolysed 10^6 times more slowly than the five-membered ring compounds in alkaline solution.[12] The relative stabilities are very similar in acid solution and the recent preparation of adenosine 3′,5′-phosphate, which was shown to be fairly stable in both acid and alkali,[9,10] indicates that these findings must be of fairly general applicability.

The greater stability of six-membered than of five-membered rings to hydrolysis with acid was quantitatively confirmed during the course of the present work: the latter were hydrolysed at least 10^4 faster than the former in 0·05M-solution by 0·05N-hydrochloric acid.

Most syntheses of cyclic phosphates depend upon the availability of phosphate mono- or di-esters or pyrophosphate esters. Both five- and six-membered rings may be formed from diesters $-CH(OH)\cdot CH[O\cdot PO(OH)\cdot OR]-$ under alkaline conditions.[4,5,13,14] Five-membered rings may also be made by this reaction under acid conditions; *e.g.*, uridine 2′(3′)-(benzyl hydrogen phosphate) can be converted into uridine 2′,3′-phosphate in

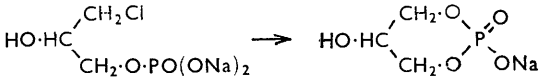
[1] Bailly and Gaume, *Bull. Soc. chim. France*, 1936, **3**, 1396; Baer and Kates, *J. Biol. Chem.*, 1948, **175**, 79; 1950, **185**, 615; Leloir, *Arch. Biochem. Biophys.*, 1951, **33**, 186; Chargaff, *J. Biol. Chem.*, 1942, **145**, 455.
[2] Brown and Todd in " The Nucleic Acids," ed. Chargaff and Davidson, Academic Press, New York, Vol. I, 1955, p. 414.
[3] Brown and Todd, *J.*, 1952, 52.
[4] Brown, Dekker, and Todd, *J.*, 1952, 2715.
[5] Markham and Smith, *Biochem. J.*, 1952, **52**, 552.
[6] Khorana, Tener, Wright, and Moffat, *J. Amer. Chem. Soc.*, 1957, **79**, 430.
[7] Baddiley and Thain, *J.*, 1952, 3783.
[8] Bailly, *Bull. Soc. chim. France*, 1922, **31**, 848.
[9] Cook, Lipkin, and Markham, *J. Amer. Chem. Soc.*, 1957, **79**, 3607.
[10] Rall and Sutherland, *J. Biol. Chem.*, 1958, **232**, 1077.
[11] Rall and Sutherland, *J. Amer. Chem. Soc.*, 1957, **79**, 3608; Baddiley, Buchanan, and Szabó, *J.*, 1954, 3826.
[12] Kumamoto, Cox, and Westheimer, *J. Amer. Chem. Soc.*, 1956, **78**, 4858.
[13] Lipkin and Talbert, *Chem. and Ind.*, 1955, 143.
[14] Baddiley and Thain, *J.*, 1953, 903.

anhydrous trifluoroacetic acid.[6] Similarly, pyrophosphate diesters undergo ring closure



under alkaline conditions; *e.g.*, pantothenic acid 2′,4′-phosphate is obtained from coenzyme A,[9] and glucose 1,2-phosphate from uridine diphosphate glucose.[15] These methods usually give poor yields even under carefully controlled conditions and the starting materials are rarely readily accessible.

If the starting materials are the monoesters, some type of cyclising reagent must be used, although it is possible to esterify the phosphate group with the adjacent hydroxyl group in small yields by use of anhydrous trifluoroacetic acid.[16] The earliest method [8] of effecting cyclization was by the use of a halogenoalkyl phosphate, *e.g.*, (I) ⟶ (II), but this route [17] has the disadvantages of poor yields, difficulty in obtaining the halogenoalkyl phosphate, and possible inversion of configuration. More useful reagents include trifluoroacetic acid,[18] tetraphenyl pyrophosphate,[19] carbodi-imides,[6,20] and ethyl chloroformate,[21] and Crook *et al.*[16] have recorded valuable modifications. Ring sizes up to seven have been formed thus,[8] although five-membered rings are always formed if there is a free adjacent hydroxyl group in the correct steric orientation even when other hydroxyl groups are free.



(I) (II)

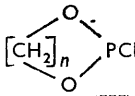
In the absence of suitable phosphate monoesters it has been possible to synthesise certain cyclic phosphates directly. For instance Baddiley and his colleagues used phosphoryl chloride in moist pyridine to form the cyclic phosphate of pantetheine,[14] and prepared methyl α-D-glucoside 4,6-phosphate by the use of phenyl phosphorodichloridate in pyridine with subsequent hydrogenation.[11] These do not appear to be general methods, however, and the yields are poor and the products difficult to purify.

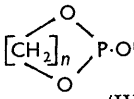
In considering alternative routes from unphosphorylated starting materials we noted that cyclic phosphorochloridites (III) and cyclic phosphite esters (IV) can readily be prepared in good yield,[22–24] and studied their conversion into cyclic phosphates. In agreement with previous experience,[23] attempts to form cyclic phosphorochloridates by treating the cyclic phosphite esters with halogens caused ring fission and gave halogenoalkyl phosphorochloridates. However, cyclic phosphites reacted vigorously with sulphur, to form cyclic thiophosphate esters, although the cyclic phosphorochloridites failed to react with sulphur.[12,25] These results suggested an investigation of the oxidation of cyclic phosphites to phosphates as a general synthesis. A wide range of oxidising agents had previously been used to oxidise phosphines, phosphites, and phosphonites to phosphine oxides, phosphates, and phosphonates respectively,[26] but aqueous solutions, especially acidic or alkaline ones, rapidly cleave many cyclic phosphates. Preliminary experiments

[15] Paladini and Leloir, *Biochem. J.*, 1952, **51**, 426.
[16] Crook, Mathias, and Rabin, *Biochem. J.*, 1960, **74**, 230.
[17] Atherton, Openshaw, and Todd, *J.*, 1945, 382; Lecocq, *Compt. rend.*, 1956, **242**, 1902; cf. refs. 8 and 12.
[18] Brown, McGrath, and Todd, *J.*, 1952, 2708; Ukita, Bates, and Carter, *J. Biol. Chem.*, 1955, **216**, 867.
[19] Forrest, Mann, and Todd, *J.*, 1952, 2530.
[20] Moffat and Khorana, *J. Amer. Chem. Soc.*, 1957, **79**, 1194.
[21] Michelson, *J.*, 1959, 3655.
[22] Arbusov and Zorostrova, *Izvest. Akad. Nauk S.S.S.R., Otdel. khim. Nauk*, 1948, 208.
[23] Rossuiskaya and Kabachnick, *Izvest. Akad. Nauk S.S.S.R., Otdel. khim. Nauk*, 1947, 509.
[24] Lucas, Mitchell, and Scully, *J. Amer. Chem. Soc.*, 1950, **72**, 5491
[25] Yamasaki and Sato, *Sci. Reports Tôhoku Univ.*, 1954, **6**, *A*, 384; 1956, **8**, *A*, 45.
[26] Kosolapoff, " Organophosphorus Compounds," John Wiley & Co., New York, 1950.

were therefore carried out with yellow mercuric oxide to oxidise non-cyclic phosphites in acetone: triethyl, tri-isopropyl, and triphenyl phosphate were readily obtained in high yields.

The same simple procedure was used to prepare ethyl trimethylene phosphate and ethyl ethylene phosphate (V and VI; R = OEt), the yield of the latter being lower. The boiling points of these cyclic phosphates are much higher than those of similar open chain phosphates (ethyl trimethylene phosphate, b. p. 114—115°/0·1 mm.; triethyl phosphate, b. p. 106—108°/24 mm.) and the possibility of pyrophosphate formation was suspected. Infrared spectroscopy [27] showed that the cyclic phosphate possessed the


(III)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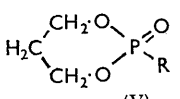
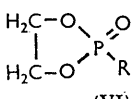

(V)


(VI)

strong absorption of the phosphoryl group at 1290 cm.$^{-1}$ and did not exhibit the P–O–P absorption at 870—970 or the hydroxyl absorption at 3400—3600 cm.$^{-1}$. Further, on mild hydrolysis with barium hydroxide, barium ethyl 3-hydroxypropyl phosphate was isolated; this could only arise from the cyclic phosphate or a symmetrical *PP'*-diethyl *PP'*-di-(3-hydroxypropyl)pyrophosphate, the latter being excluded by the spectroscopic evidence.

When yellow mercuric oxide was added to diethyl phosphite in acetone there was no apparent reaction as indicated by the lack of temperature and colour changes.

Recently, dinitrogen tetroxide was used to prepare aromatic aldehydes [28] and to oxidise sulphides and phosphines to their oxides.[29] We found dinitrogen tetroxide to oxidise triethyl phosphite to the phosphate in a slightly higher yield than did yellow mercuric oxide and to involve an even simpler procedure. Dinitrogen tetroxide in chloroform is green at low temperature and can be used as a self-indicating system. The reaction with trialkylphosphites is vigorous and external cooling is required. This method also gave ethyl ethylene and trimethylene phosphate in yields higher than did mercuric oxide. By both methods, the yield of the compound with the five-membered is somewhat lower than of that with the six-membered ring. Phenyl trimethylene phosphate [30] was also prepared by this method.

Because the cyclic phosphate group is more labile than other ester linkages hydrolysis of the cyclic triester cannot be considered a general synthesis of the cyclic hydrogen phosphates (V and VI; R = OH), and the oxidation of phosphorochloridites to phosphorochloridates was examined. Diethyl phosphorochloridate was obtained by using dinitrogen tetroxide; but when cyclic phosphorochloridites were oxidised, decomposition accompanied distillation at reduced pressure and only viscous polymers were obtained. As the oxidation appeared to have proceeded normally, immediate hydrolysis of the unisolated cyclic phosphorochloridates (V and VI; R = Cl) to the cyclic hydrogen phosphates (R = OH) was attempted. The main difficulty was to separate the product from the hydrochloric acid produced during hydrolysis. The solution had to be neutralised immediately to prevent hydrolysis of the cyclic phosphate, and the salts fractionated. Trimethylene hydrogen phosphate was best isolated as the guanidinium salt; dimethylene hydrogen phosphate was isolated as the silver salt and then converted into the guanidinium salt.

During this work, Cox and Westheimer [31] described the use of dinitrogen tetroxide for conversion of triphosphites and cyclic phosphorochloridites into the corresponding

[27] Corbridge, *J. Appl. Chem.*, 1956, 456; McIvor, Grant, and Hubley, *Canad. J. Chem.*, 1956, **34**. 1611.
[28] Field and Grundy, *J.*, 1955, 1110.
[29] Addison and Sheldon, *J.*, 1956, 2705.
[30] Ayres and Rydon, *J.*, 1957, 1109.
[31] Cox, jun., and Westheimer, *J. Amer. Chem. Soc.*, 1958, **80**, 5441.

triphosphates and cyclic phosphorochloridates. However, their yields were low and they were not able to hydrolyse the cyclic phosphorochloridate to the corresponding cyclic phosphate without opening the ring.

Experimental

Materials.—Acetone ("AnalaR") was dried with phosphoric anhydride and distilled. Chloroform was dried ($CaCl_2$) and distilled over anhydrous magnesium sulphate.

Dinitrogen tetroxide was prepared by Partington and Park's method.[32] Diethyl phosphorochloridite was prepared by the reaction of two equiv. each of ethanol and diethylaniline with one of phosphorous trichloride in ether at 0°; after filtration, the product was isolated by distillation (b. p. 54—56°/25 mm.). Ethylene phosphorochloridite, b. p. 48°/20 mm., trimethylene phosphorochloridite, b. p. 68—69°/19 mm., ethyl ethylene phosphite, b. p. 57—58°/19 mm., and ethyl trimethylene phosphite, b. p. 68—69°/16 mm., were prepared by the method of Lucas, Mitchell, and Scully.[24] Phenyl trimethylene phosphite, prepared from trimethylene phosphorochloridite and phenol in the presence of diethylaniline in ether at 0°, had b. p. 81—82°/0·3 mm. Other organophosphorus compounds used were available commercially.

Oxidation of Triethyl Phosphite.—(a) *With mercuric oxide.* To triethyl phosphite (16·6 g.), dissolved in anhydrous acetone (75 ml.), yellow mercuric oxide (35 g.) was added in small portions. The reaction is exothermic and external cooling was used. After several hours at room temperature, the mixture was filtered and evaporated, and the residual oil distilled, to yield triethyl phosphate (15·5 g., 85%), b. p. 106—108°/24 mm. (lit., b. p. 103°/25 mm.) (Found: C, 39·9; H, 8·3; P, 16·7. Calc. for $C_6H_{15}O_4P$: C, 39·6; H, 8·2; P, 17·0%). In similar experiments tri-isopropyl phosphite gave an 81% yield of tri-isopropyl phosphate, b. p. 100—102°/18 mm. (lit., b. p. 95°/10 mm.) (Found: C, 48·6; H, 9·1; P, 14·1. Calc. for $C_9H_{21}O_4P$: C, 48·2; H, 9·4; P, 13·8%), and triphenyl phosphite a 61% yield of triphenyl phosphate, m. p. 49—50° (lit., 50°) (from ethanol) (Found: C, 65·6; H, 4·5; P, 9·8. Calc. for $C_{18}H_{15}O_4P$: C, 66·3; H, 4·6; P, 9·5%).

(b) *With dinitrogen tetroxide.* To triethyl phosphite (16·6 g.) in dry chloroform (50 c.c.), cooled in acetone–solid carbon dioxide, a cooled solution of dinitrogen tetroxide in dry chloroform was added in small portions, with stirring and cooling, until a permanent green colour remained. After 1 hr. at room temperature, the solvent was removed at the water-pump and the residual oil distilled, to yield triethyl phosphate (15·7 g., 85%), b. p. 98—99°/19 mm. (lit., 103°/25 mm.) (Found: C, 39·5; H, 8·0; P, 17·2%).

Oxidation of Diethyl Phosphorochloridite with Dinitrogen Tetroxide.—Diethyl phosphorochloridite (15·6 g.) in chloroform (50 ml.) was oxidized as described above, to yield on distillation diethyl phosphorochloridate (10·1 g., 58%), b. p. 94—96°/18 mm. (lit., b. p. 93—95°/18 mm.) (Found: C, 27·5; H, 5·9; P, 18·3; Cl, 20·2. Calc. for $C_4H_{10}ClO_3P$: C, 27·8; H, 5·8; P, 18·0; Cl, 20·6%).

Oxidation of Ethyl Ethylene Phosphite.—(a) *With mercuric oxide.* Ethyl ethylene phosphite (27·2 g.) in acetone (100 ml.) was oxidized by addition of mercuric oxide (75 g.) in small portions with cooling. After attaining room temperature overnight, the mixture was filtered, the solvent removed at the water-pump, and the residual oil distilled *in vacuo*, to yield *ethyl ethylene phosphate* (8·5 g., 28%), b. p. 106—108°/0·7—0·8 mm. (Found: C, 32·2; H, 6·4; P, 20·0. $C_4H_9O_4P$ requires C, 31·6; H, 5·9; P, 20·4%).

(b) *With dinitrogen tetroxide.* To ethyl ethylene phosphite (15·0 g.) in dry chloroform (50 ml.), cooled in ice, a solution of dinitrogen tetroxide was added, dropwise, with stirring until a permanent green colour remained. After 0·5 hr. the solvent was removed and the residual oil distilled, to yield ethyl ethylene phosphate (7·0 g., 42%), b. p. 89—90°/0·3 mm. (Found: C, 32·1; H, 6·1; P, 20·5%).

Oxidation of Ethyl Trimethylene Phosphite.—(a) *With mercuric oxide.* To ethyl trimethylene phosphite (15·0 g.) in anhydrous acetone (100 ml.), mercuric oxide was added in small portions with cooling and shaking until no further reaction occurred. The solids were removed by

[32] Partington and Park, *J.*, 1924, **125**, 74.

centrifugation and the solvent was evaporated from the supernatant liquid. The residual oil was distilled, to yield *ethyl trimethylene phosphate* (8·7 g., 52%), b. p. 114—115°/0·1 mm. (Found: C, 35·6; H, 6·7; P, 18·8. $C_5H_{11}O_4P$ requires C, 36·2; H, 6·6; P, 18·7%).

(b) *With dinitrogen tetroxide.* To ethyl trimethylene phosphite (18·0 g.) in anhydrous chloroform (100 ml.), cooled to −20° in acetone–solid carbon dioxide, dinitrogen tetroxide in chloroform was added dropwise with shaking until a faint permanent green colour remained. After 0·5 hr. at −20°, the mixture was allowed to warm to room temperature during 1 hr. and then evaporated at water-pump pressure. Distillation of the residual oil yielded ethyl trimethylene phosphate (12·8 g., 65%), b. p. 114—116°/0·1 mm. (Found: C, 36·0; H, 6·8; P, 18·3%).

Oxidation of Phenyl Trimethylene Phosphite.—Phenyl trimethylene phosphite (10 g.) was oxidized with dinitrogen tetroxide as described in the previous section. Removal of the solvent yielded a brown oil. The oil was redissolved in chloroform (50 ml.) and shaken with three portions of activated alumina and filtered. Carbon tetrachloride was added and the solution partially evaporated. On cooling, needles (8·2 g., 76%) of phenyl trimethylene phosphate were obtained; they had m. p. 74—75° (lit., m. p. 76—77°) alone or mixed with a sample provided by Professor H. N. Rydon (Found: C, 50·0; H, 5·1; P, 15·2. Calc. for $C_9H_{11}O_4P$: C, 50·5; H, 5·1; P, 14·5%).

Hydrolysis of Ethyl Trimethylene Phosphate.—Ethyl trimethylene phosphate (0·7 g.) was heated at 80° for 10 min. with barium hydroxide octahydrate (0·5 g.) in water (5 ml.); ethanol (5 ml.) was added and the mixture filtered. Acetone (100 ml.) was added with shaking. The precipitate was filtered off, washed with acetone, and dried, to yield *barium ethyl 3-hydroxypropyl phosphate* (0·4 g.) (Found: C, 23·1; H, 5·1; P, 12·1. $C_{10}H_{24}BaO_{10}P_2$ requires C, 23·8; H, 4·8; P, 12·3%).

Preparation of Guanidinium Trimethylene Phosphate.—Trimethylene phosphorochloridite (7·0 g.) in anhydrous chloroform was oxidized with dinitrogen tetroxide as described above. The chloroform was removed under reduced pressure and the residual oil poured into cold water and neutralized with a solution of guanidine carbonate. After being stirred for 1 hr., the solution was brought to pH 7·0 with guanidine carbonate solution. Acetone was added until a permanent turbidity was formed. A colorless oil separated at 0°, but when shaken with more acetone, slowly crystallized, to give *guanidinium trimethylene phosphate* (5·0 g., 52%) (Found: C, 24·2; H, 6·8; N, 20·6; P, 15·7. $C_4H_{12}N_3O_4P$ requires C, 24·4; H, 6·1; N, 21·2; P, 15·7%). Titration showed that no dissociation occurred between pH 4 and 10, *i.e.*, there is no secondary phosphate dissociation.

Preparation of Silver Ethylene Phosphate.—Ethylene phosphorochloridite (6·3 g.) in chloroform was oxidized as above with dinitrogen tetroxide and the solvent removed under reduced pressure. The residual oil was poured into cold water and shaken and a suspension of silver acetate (16·7 g.) in water added. After 1 hour's stirring, the pH was raised to 7·0 by the addition of triethylamine, and the silver chloride was filtered off, the filtrate freeze-dried, and the solid dissolved in hot water (500 ml.), cooled and filtered. The volume was reduced and the solution again filtered off. Finally, the filtrate was freeze-dried, to give *silver ethylene phosphate* (4·0 g., 43%) (Found: C, 10·1; H, 2·1; P, 13·0; Ag, 46·3. $C_2H_4AgO_4P$ requires C, 10·4; H, 1·7; P, 13·4; Ag, 46·7%).

Conversion of Silver Ethylene Phosphate into the Guanidinium Salt.—Silver ethylene phosphate (2·3 g.) in water was added to a solution of guanidine hydrochloride (0·95 g.), and the silver chloride filtered off. The filtrate was freeze-dried, the residual white powder dissolved in boiling methanol, and an excess of anhydrous ether added. At −10°, white crystals were obtained; these were filtered off and washed with ether, to give *guanidinium ethylene phosphate* (1·73 g., 88%) (Found: C, 19·9; H, 5·8; N, 22·2; P, 16·5. $C_3H_{10}N_3O_4P$ requires C, 19·7; H, 5·4; N, 22·9; P, 16·9%). Titration showed that there was no dissociation between pH 4 and 8.

Hydrolysis of Guanidinium Ethylene and Trimethylene Phosphate.—0·1M-Cyclic phosphate solution (5·0 ml.) was mixed with 0·1M-hydrochloric acid (5·0 ml.). An aliquot part was removed immediately and the pH adjusted to 4·5 by the addition of 0·05N-sodium hydroxide, a pH meter being used. The secondary phosphate dissociation present was then estimated by measuring the further volume of sodium hydroxide required to bring the pH to 9·0. The remainder of the solution was then placed in a constant-temperature bath, and further aliquot parts were removed at suitable time intervals and titrated in the same way. The appearance

of secondary phosphate dissociation is expressed in the Table as the percentage hydrolysis of the cyclic phosphate.

Guanidinium ethylene phosphate						Guanidinium trimethylene phosphate				
Min. at 37°	0	5	15	25	360	Hr. at 100°	0	2	5	24
Hydrolysis (%)...	0	27	48	63	100	Hydrolysis (%) ...	0	5	10	25

Paper Chromatography.—Ascending chromatography (see Table) was carried out on Whatman No. 1 paper with 50% v/v propan-2-ol–water as the developing system. Phosphate esters were detected by the method of Hanes and Isherwood,[33] and guanidine by the α-naphthol–biacetyl method.[34] Some samples were incubated with a suspension of *Crotalus adamanteus* venom before chromatography, as this venom is known to hydrolyse phosphate diesters and would therefore indicate the nature of the compound under test.

R_P values.

	Phosphate reagent	Guanidine reagent
Silver ethylene phosphate	0·80	—
Guanidinium ethylene phosphate	0·77	0·78
Venom-treated guanidinium ethylene phosphate	0·60	—
Acid-treated guanidinium ethylene phosphate	0·59	
Guanidinium trimethylene phosphate	0·71	0·71
Venom-treated guanidinium trimethylene phosphate	0·52	—
Acid-treated guanidinium trimethylene phosphate	0·71, 0·51	—

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[33] Hanes and Isherwood, *Nature*, 1949, **164**, 1107.
[34] Smith, "Chromatographic Techniques," Interscience Publ. Inc., New York, 1958, p. 154.