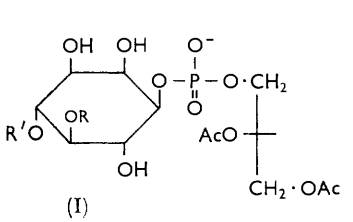
1206. *Hydrolysis of Hydroxyalkyl Phosphate Esters: the Epoxide Route*

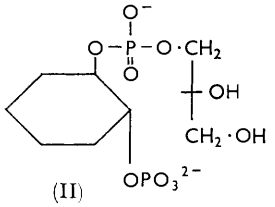
By D. M. Brown and D. A. Usher

The alkaline hydrolysis of the cyclohexyl esters of a variety of 2-hydroxyalkyl phosphates has been shown to proceed by two mechanisms; the normal one in which a cyclic phosphate is first formed, and one in which an epoxide is formed with displacement of cyclohexyl phosphate. The latter route has been established by stereochemical and isotopic studies, and, by suitable substitution in the hydroxyalkyl group, can be made the dominant one.

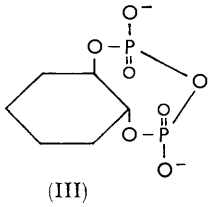
Recent studies on the structure of the naturally occurring phosphoinositides (I) have, in part, been concerned with the products of alkaline hydrolysis. In particular, the product ratio, glycerol phosphate : inositol phosphate, has been used diagnostically.[1,2] Unexpectedly, this product ratio is considerably affected by the presence of additional phosphate residues on the inositol ring.[3] Thus, on hydrolysis of deacylated mono- (I; R = R′ = H), di- (I; R = H, R′ = PO_3H_2), and tri-phosphoinositide (I; R = R′ =



(I)



(II)



(III)

PO_3H_2), glycerol phosphate is formed to the extent of 65, 49, and 25%, respectively. Rapid hydrolysis is observed, and it seems likely that the normal mechanism operates in this series, *via* cyclic phosphate intermediates,[1-3] although, since no rate measurements have been reported, it is not known at present whether these changes in the product ratio are due to an increased rate of formation of glycerol, or a decreased rate of formation of glycerol phosphate, or both. In the case of the glycerol inositol triphosphate, it may be that mutual repulsion of the adjacent, fully ionised phosphate residues distorts the cyclohexane ring, and thus aids the formation of the transition state leading to inositol cyclic phosphate and glycerol.[4*]

* It has been suggested that conformational inversion occurs with *trans*-cyclohexane-1,2-dicarboxylic acid on going from the di-acid to the di-anion. See E. S. Gould, "Mechanism and Structure in Organic Chemistry," Holt, Rinehart, and Winston, Inc., New York, 1959, p. 242—243.

[1] D. M. Brown, G. E. Hall, and R. Letters, *J.*, 1959, 3547; D. M. Brown, B. F. C. Clark, and R. Letters, *J.*, 1961, 3774.

[2] J. N. Hawthorne, *J. Lipid Res.*, 1960, **1**, 255.

[3] C. Grado and C. E. Ballou, *J. Biol. Chem.*, 1961, **236**, 54; D. M. Brown and J. C. Stewart, unpublished work.

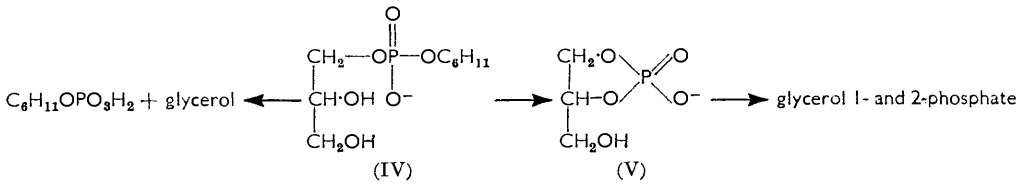
[4] In unpublished work, we find evidence consistent with this view in a study of the optical rotation of (+)-*trans*-cyclohexane-1,2-diol diphosphate as a function of pH.

A consideration of these observations, however, led us to study some cyclohexanediol diphosphate derivatives. First, the glycerol ester of *trans*-cyclohexane-1,2-diol diphosphate (II) was prepared, since, before the structures of the di- and tri-phosphoinositides had been elucidated, it was considered possible that they included a 1,2-diphosphate system, and that an additional hydrolysis pathway which led to loss of glycerol depended on neighbouring phosphate participation, with formation of a 1,2-cyclic pyrophosphate (as III). This compound was synthesised independently by the action of dicyclohexylcarbodi-imide on the diphosphate.

Since no neighbouring hydroxyl group is present on the cyclohexane ring of the model compound (II), the normal hydrolytic pathway should give only glycerol phosphate and the diol monophosphate. Yet cyclohexane-1,2-diol diphosphate was formed to the extent of 30%, the other products being glycerol 1- and 2-phosphates.

However, a mechanism involving formation of (III) in the hydrolysis was made unlikely when it was found that the rate of reaction in 1·0N-sodium hydroxide was about twice that found in 0·5N-hydroxide. Since the phosphate group is fully ionised at both concentrations of alkali, this dependence on hydroxide concentration should not have been found.

In an extension of these experiments, the diphosphates of the *cis*- and *trans*-cyclohexane-1,3- and -1,4-diols were synthesised. That from *cis*-cyclohexane-1,2-diol was also obtained, but in very low yield; evidently steric hindrance in reactions leading to this compound is severe. *trans*-Cyclohexane-1,4-diol diphosphate was converted [5] by glycidol into its glycerol ester, and the hydrolysis of this and of cyclohexyl glycerol phosphate (IV) was studied. Surprisingly, the former gave 72% of 4-hydroxycyclohexyl phosphate and the latter 78% of cyclohexyl phosphate. Thus, the normal reaction to form glycerol phosphate had now become the less important one. The glycerol phosphate formed on



hydrolysis of the cyclohexyl ester contained the 1- and 2-isomers in the same ratio as is found in the hydrolysis of glycerol 1,2-phosphate (V),[5,6] showing that it arose by the usual mechanism. To eliminate any complicating effects of the 3-hydroxyl group of the glycerol residue, cyclohexyl 2-hydroxypropyl phosphate was prepared. Hydrolysis, which was of first order in hydroxide ion and in substrate, gave 86% cyclohexyl phosphate and only 14% of 2-hydroxypropyl phosphate (together with propane-diol and cyclohexanol).

The formation of small amounts of alkyl phosphates on alkaline hydrolysis of related compounds has been reported before. Methyl and benzyl glycerol 1-phosphate gave 8% and 10% of methyl [7] and benzyl phosphate,[8] respectively, whilst dibenzyl *trans*-2-hydroxycyclohexyl phosphate afforded 30% dibenzyl phosphate.[9] Since the last of these was shown to involve epoxide formation, it was suggested [9] that the other two may have, to a small extent, proceeded similarly, although the loss of a phosphate monoester is not expected to be as easy as that of a diester.

In the hydrolysis of the cyclohexyl ester under discussion, three mechanisms which could give rise to cyclohexyl phosphate are: (*a*) epoxide formation, (*b*) displacement by

[5] D. M. Brown, G. E. Hall, and H. M. Higson, *J.*, 1958, 1360.
[6] C. Ukita, K. Nagasawa, and M. Irie, *Pharm. Bull.* (*Japan*), 1957, **5**, 127.
[7] P. F. Fleury, J. Lecocq, and L. Le Dizet-Joly, *Bull. Soc. chim. France*, 1956, 1193.
[8] G. E. Hall, Ph.D. Thesis, Cambridge, 1958.
[9] D. M. Brown and N. K. Hamer, *J.*, 1960, 406.

rearside attack on carbon by hydroxide ion, and (*c*) a similar displacement of glycol by attack on phosphorus. A distinction between these processes has been made in favour of (*a*), in the case of cyclohexyl *erythro*-3-hydroxy-2-butyl hydrogen phosphate (VII). Mechanisms (*a*) and (*c*) are distinguishable from (*b*) by retention of configuration in the resulting glycol. In (*a*), epoxide formation gives an initial inversion, oxide-ring opening a second inversion, and hence overall retention.

2,3-Epoxybutane was prepared from the chlorohydrin, and from the resulting mixture of *cis*- and *trans*-epoxides a sample of the pure *trans*-isomer, containing less than 0·1% *cis*-isomer, was separated by preparative gas–liquid chromatography (g.l.c.). Acid-catalysed opening of the isomeric 2,3-epoxybutanes has been previously shown to proceed by inversion,[10] and this result has been confirmed in the present investigation. Samples of the epoxide containing different amounts of *cis*- and *trans*-isomers were hydrolysed in acid and in base. G.l.c. of the resulting diol [11] showed that the ratio of the *meso*- to DL-isomers was the same, within experimental error (*ca.* ±2%) as the ratio of *trans*- to *cis*-epoxide used in the hydrolysis. The results are shown in Table 1.

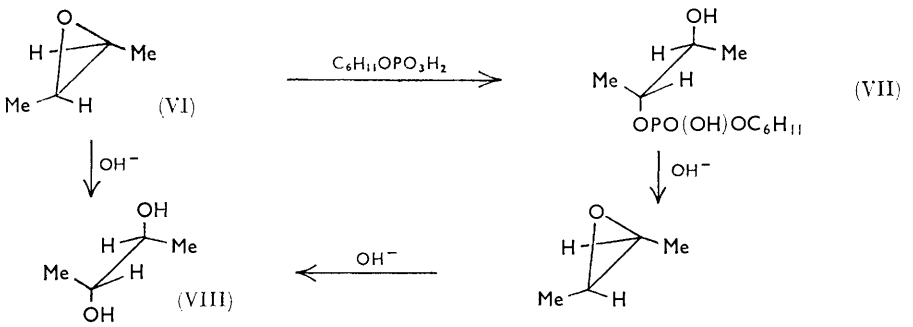
A sample of the pure *trans*-epoxide (VI) (1—2 mol.) with cyclohexyl dihydrogen phosphate in anhydrous chloroform gave cyclohexyl *erythro*-3-hydroxy-2-butyl hydrogen phosphate (VII), isolated as its lithium salt. The same product was obtained in alkaline aqueous solution, but 10—20 equivalents of epoxide were then necessary to achieve a reasonable yield of product. This ester on alkaline hydrolysis afforded cyclohexyl phosphate and hydroxybutyl phosphate (3·7 : 1). The alcohol fraction was examined by g.l.c. and contained only cyclohexanol and *meso*-butane-2,3-diol (VIII) (1 : 3·75), and there was no detectable amount (less than 0·3%) of the DL-isomer. It has been shown

TABLE 1

Hydrolysis of 2,3-epoxybutanes

Fraction	Epoxide (%)		Diol (%) N-NaOH 30 min./100°		N-NaOH 5 hr./100°		0·01N-$HClO_4$ 10 min./25°	
	cis	*trans*	DL	*meso*	DL	*meso*	DL	*meso*
1	21·5	78·5	20	80	24	76	23·5	76·5
2	45	55	45	55	45·5	54·5	—	—
3	87	13	—	—	—	—	88	12

that no further isomeric changes occurred under the reaction conditions, so that mechanism (*b*) is not significant here. (The amount of cyclohexyl phosphate formed by an elimination process is negligible, since the concentration of butane-diol in the hydrolysis reaction mixture was approximately equal to the concentration of cyclohexyl phosphate.)



Attack on phosphorus by solvent hydroxide ion as in mechanism (*c*), should give ^{18}O enrichment in the phosphate when the hydrolysis is conducted in enriched water. The

[10] C. E. Wilson and H. J. Lucas, *J. Amer. Chem. Soc.*, 1936, **58**, 2396.
[11] D. E. Smith and J. R. Coffman, *Analyt. Chem.*, 1960, **32**, 1733.

isolated cyclohexyl phosphate from such an experiment showed no enrichment. The ^{18}O enrichment in the solvent would have been sufficient to detect about 10% of reaction by mechanism (*c*) so this, at present, must be regarded as an upper limit. The reaction is therefore one of epoxide formation with displacement of the alkyl phosphate residue, and it seems likely that the other compounds cited undergo hydrolysis in an analogous fashion.

The conclusion is drawn that in the hydrolysis of esters of 2-hydroxyalkyl phosphates, generally two mechanisms may compete. One, for which many examples are known, involves a displacement on phosphorus with loss of the alkoxy-anion, and the other, in which an alkyl phosphate residue is displaced, involves epoxide formation. It seems evident, qualitatively, that depression of the former pathway can be effected by a change in the esterifying alcohol such that it becomes a poorer leaving group. We discuss this aspect in the accompanying Paper, but meanwhile it is noted that, in this respect, the cyclohexyloxy-anion is to be classed as a poor leaving group, when compared with the anion derived, for example, from the more acidic methanol or glycerol.

The structure of the glycol residue, too, affects the partition between the two hydrolytic pathways. We have made a study of the hydrolysis rates and product ratios of some cyclohexyl glycol phosphates carrying methyl substituents in the glycol residue. These, generally, were prepared by the acid- and base-catalysed reaction of cyclohexyl phosphate with the corresponding epoxide. The hydrolyses were normally carried out in a large excess of N-sodium hydroxide at 100°, aliquot parts removed, run on paper chromatograms, and the phosphate estimated after excision of the spots containing starting material and products. Rate constants and product ratios were thus obtained simultaneously. The data are collected in Table 2 for compounds $C_6H_{11}OPO(OH)\cdot OR$ (IX)—(XIV), in which R is the glycol residue. First-order rate constants, accurate to about ±5%, are given. In the case of compounds (IX), (X), and (XIV) both modes of breakdown are approximately first-order in substrate and in hydroxide ion, at constant ionic strength. In a previous communication [12] second-order rate constants were given for compounds (XI) and (XIII), but although it is likely that these rates (N-NaOH) are first-order in hydroxide ion, this dependence has not been determined experimentally.

TABLE 2

Hydrolysis of some cyclohexyl glycol phosphates: $C_6H_{11}OPO(OH)\cdot OR$

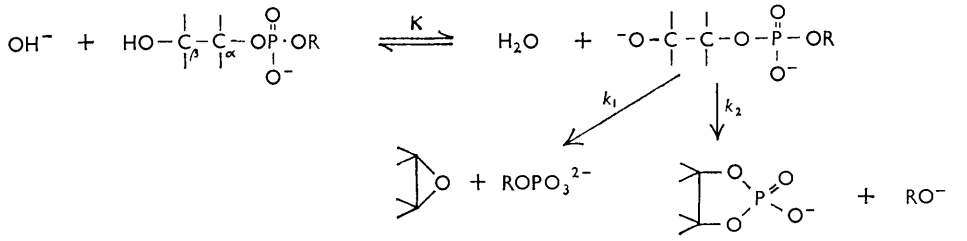
(100°; k in sec.$^{-1}$; $\mu = 1{\cdot}0$)

			Formation of cyclohexyl phosphate		Formation of glycol phosphate	
	R	NaOH (N)	Rate (10^6k)	Mole (%)	Rate (10^6k)	Mole (%)
(IX)	$HO\cdot CH_2CH_2$	1·0	2·9	67	1·4	33
	,,	0·75 *	2·3	64	1·3	36
	,,	0·5 *	1·45	65	0·8	35
(X)	$HO\cdot CHMe\cdot CH_2$	1·0	16·6	86·5	2·6	13·5
	,,	0·5 †	8·2	86	1·35	14
(XI)	$HO\cdot CMe_2\cdot CH_2$	1·0	65	97·5	1·7	2·5
(XII)	$HO\cdot CH_2\cdot CH(OH)\cdot CH_2$	1·0	28·2	78	8·0	22
(XIII)	$HO\cdot CH_2\cdot CMe_2$	1·0	135	26·5	373	73·5
(XIV)	$HO\cdot CHMe\cdot CHMe$	1·0	27·2	76	8·5	24
	,,	0·5 *	14	78	4·0	22

* With added sodium chloride. † With added potassium chloride.

By analogy with the base-catalysed formation of epoxide from the chlorohydrin,[13,14] the hydrolysis of the phosphates may be represented as shown in the annexed scheme.

[12] D. M. Brown and D. A. Usher, *Proc. Chem. Soc.*, 1963, 309.
[13] S. Winstein and H. J. Lucas, *J. Amer. Chem. Soc.*, 1939, **61**, 1576.
[14] A. A. Frost and R. G. Pearson, " Kinetics and Mechanism," Wiley and Sons, New York, 2nd edn., 1961, p. 291.



The epoxide and the cyclic phosphate then react rapidly with hydroxide ion to give the glycol and the glycol phosphate, respectively.

With more acidic hydroxyl groups, or in higher concentrations of alkali, a considerable fraction of the neighbouring hydroxyl group could exist in solution as the alkoxide anion, and the rate of hydrolysis would become between zero- and first-order in hydroxide ion. Since the pK_a values of primary alcohol functions are normally lower than those of secondary or tertiary ones,[15] this effect is most likely to be found with compounds (IX) and (XIII), and it is possible that, in N-sodium hydroxide, ester (IX) is beginning to show this effect, though more accurate rate determinations are needed to resolve this point.

Variation in the substitution, particularly on C-β, could affect the observed rate of hydrolysis or the ratio of products through changes in K, k_1, and k_2.* A change which decreases the equilibrium constant K, such as going from CH_2OH to $CH(OH)CH_3$, would normally also be expected to increase both k_1 and k_2 by making the anion more nucleophilic; hence, the overall effect of such structural changes is not in general predictable.[14] In fact the changes (IX) ⟶ (X) ⟶ (XI) affect the rate of cyclic phosphate formation very little, but increase the rate of formation of epoxide by six and four times for each successive methyl group, so that in (XI) the epoxide route becomes practically exclusive.

In a related series of chlorohydrins,[16] steric crowding in the starting material, which is partially relieved in the transition state leading to the formation of the epoxide, is thought to be the explanation for the increases in rate found in that series on increasing the substitution.[17] This effect, relatively unobscured by changes in K, may be seen by comparing (IX) and (XIII). The rate of formation of cyclohexyl phosphate increased by a factor of 47 as a result of the substitution, whilst the reaction leading to glycol phosphate increased in rate by 260 times.† We assume that these increases are not due to a change in mechanism; similar rate changes accompanying increased substitution have been previously found in other series.[18] Clearly, two methyl substituents on C-α (the carbon carrying the phosphate) are necessary for large rate increases, single substituents on both C-α and C-β as in (XIV) lead only to a very modest rate increment. Molecular models indicate that the methyl groups in (XIII) interfere considerably with the free rotation of the C–OP bond; thus, the entropy decrease on forming the transition state for displacement of cyclohexanol would be expected to be less for (XIII) than for (IX). (XIV) is the *erythro*-isomer, and models show that the formation of the cyclic phosphate may give rise to an unfavorable Me–Me interaction. We have not yet studied the *threo*-isomer, in which

* The measured rate constants (Table 2) include K as a factor.

† It may be calculated from available data[5] that the related ester in which glycerol replaces cyclohexanol would be expected to form glycerol to the extent of over 99% in less than 30 seconds, under the same hydrolytic conditions. A possible method of dephosphorylation may thus be envisaged involving reaction of an alkyl phosphate with 1,2-epoxy-2-methylpropane, then alkali treatment.

[15] P. Ballinger and F. A. Long, *J. Amer. Chem. Soc.*, 1960, **82**, 795; J. Hine and M. Hine, *ibid.*, 1952, **74**, 5266.

[16] H. Nilsson and L. Smith, *Z. phys. Chem.*, 1933, **166***A*, 136.

[17] E. S. Gould, "Mechanism and Structure in Organic Chemistry," Holt, Rinehart, and Winston, Inc., New York, 1959, p. 568.

[18] R. M. Beesley, C. K. Ingold, and J. F. Thorpe, *J.*, 1915, **107**, 1080; G. S. Hammond, "Steric Effects in Organic Chemistry," ed. M. S. Newman, Wiley and Sons, New York, 1956, pp. 460—470; E. Gaetjens and H. Morawetz, *J. Amer. Chem. Soc.*, 1961, **83**, 5049.

this effect should be absent, and in which a rather larger rate increase may therefore be expected.

From both the practical and the theoretical standpoints, it is important to know whether, in diesters having a neighbouring hydroxyl group, a base-catalysed migration of the alkyl phosphoryl group can occur. In a test utilising cytidine-2′- and -3′-alkyl phosphates no migration was observed.[19] The isomeric compounds (XI) and (XIII) constitute useful models, for cyclohexyloxy is a very poor leaving group and a competing migration might now have become observable.

Any migration (XIII) ⟶ (XI) would leave some (XI) still hydrolysing at its own characteristic rate after (XIII) had been reduced to negligible proportions. This was not found to occur, within experimental error (about 1%). Any migration (XI) ⟶ (XIII) would give rise to a fast-hydrolysing compound which gives on breakdown 73% glycol phosphate. However, the hydrolysis products of (XI) were 98% cyclohexyl phosphate and only 2% glycol phosphate. Hence, in these compounds at least, there can be little, if any, migration in either direction. This result is not unexpected, for if we accept that the negative charge on the phosphate anion makes the likely transition state for these displacements an sp^2pd hybrid,[20] then it is difficult to see how a concerted mechanism for attack on phosphorus *could* give rise to migration. By contrast, acid hydrolysis of related compounds has been found to give concomitant migration of the alkylphosphoryl group,[19] though the mechanism of this reaction is still in doubt.

Experimental

Paper Chromatography.—Paper chromatography was used for the separation, identification, and estimation of products. The method of phosphorus estimation, which gives results accurate to better than ±3%, has been described in detail elsewhere.[21] Whatman No. 1, No. 30, and No. 40 papers were used. Phosphate esters were located by the spray reagent of Hanes and Isherwood,[22] as modified by Bandurski and Axelrod.[23] α-Glycols were located by the silver nitrate dip reagent of Anet and Reynolds.[24] Barium, lithium, and sodium salts were converted, before application, into the free acids by treatment with Dowex-50(H^+) resin; use of cyclohexylammonium salts in solvent B gave rise to " double spotting."

Solvent systems used were: (A) propan-2-ol–ammonia (*d*, 0·880)–water (7 : 1 : 2 v/v); (B) nitromethane–pyridine–water (5 : 6 : 4);[25] and (C) butan-1-ol–acetic acid–water (4 : 1 : 5 upper phase).

Hydrolyses of Phosphate Esters.—(*a*) *General.* Except in a single instance, only alkaline hydrolyses were carried out. Hydrolyses in aqueous sodium hydroxide at 60° or above were run in Teflon tubes fitted with tapered stoppers.[26] Borosilicate glass was extensively attacked by the alkali, and polyethylene suffered heat distortion above 60°. De-ionised water was used in preparing all solutions, and care was taken to exclude atmospheric carbon dioxide.

(*b*) *Chromatographic rate method.* The diester (~0·03 mmole), usually as the barium or lithium salt, in water was passed down a column of Dowex-50(H^+). The effluent was freeze-dried and mixed with standard sodium hydroxide (0·5 ml.). The solution was transferred to the Teflon tube, which was supported in a metal cylinder and heated in a thermostat (±0·1°), the required temperature (within 1%) being attained in 2 min. from room temperature. Aliquots portions (50λ, four to six per run) were withdrawn from the tube at intervals, and

[19] D. M. Brown, D. I. Magrath, A. H. Neilson, and A. R. Todd, *Nature*, 1956, **177**, 1124.
[20] R. F. Hudson and M. Green, *Angew. Chem.* (*Internat. Edn.*), 1963, **2**, 11; G. H. Duffey, *J. Chem. Phys.*, 1949, **17**, 196.
[21] D. A. Usher, *J. Chromatog.*, 1963, **12**, 262.
[22] C. S. Hanes and F. A. Isherwood, *Nature*, 1949, **164**, 1107.
[23] R. S. Bandurski and B. Axelrod, *J. Biol. Chem.*, 1951, **193**, 405.
[24] E. F. L. J. Anet and T. M. Reynolds, *Nature*, 1954, **174**, 930.
[25] W. Dierick, J. Stockx, and L. Vandendriessche, *Naturwiss.*, 1956, **43**, 82.
[26] J. Kumamoto, J. R. Cox, and F. H. Westheimer, *J. Amer. Chem. Soc.*, 1956, **78**, 4858.

mixed (in a polyethylene tube) with a slight excess of freshly " blotted-dry " IR-120(H^+) resin. Aliquot portions (5λ) of the resulting solution were applied to strips of Whatman No. 40 paper which were developed in solvent (A). Duplicate chromatograms were invariably run; the results differed negligibly from the original.

The change in hydroxide-ion concentration throughout the hydrolysis was usually sufficiently small to allow first-order plots to be used [log (starting material) *vs* time], otherwise straight lines were obtained by using the standard second-order plot: log (B/A) *vs.* time.[27] Line gradients were obtained by the method of least squares, and from these were calculated the rate constants. Partial rates for the two modes of breakdown were determined from product ratios, which were checked for constancy throughout the hydrolysis. Hydrolyses were taken as far as two or three half-lives. The rate of hydrolysis of cyclohexylammonium benzyl *cis*-2-hydroxycyclohexyl phosphate in N-sodium hydroxide at 60°, was found to be $1{\cdot}6 \times 10^{-5}$ sec.$^{-1}$, in good agreement with the figure found [5] by a titration method ($1{\cdot}5 \times 10^{-5}$ sec.$^{-1}$).

Hydrolysis products were identified by paper chromatography and electrophoresis against known controls, or in the case of cyclohexanol and butane-2,3-diol, by g.l.c. Cyclohexyl phosphate, isolated from the hydrolysis of cyclohexyl 2-hydroxypropyl phosphate, was identified (as the cyclohexylammonium salt) by comparison of its infrared spectrum with an authentic sample.

Nuclear magnetic resonance spectra were obtained at 40 Mc./sec. on a permanent magnet Perkin-Elmer spectrometer.

trans-*P_1-Dibenzyl-P_2-diphenyl Cyclohexane*-1,2-*diol Diphosphate*.—Diphenylphosphorochloridate (5·8 g., 1·3 mol.) in pyridine (25 ml.) was added to dibenzyl *trans*-2-hydroxycyclohexyl phosphate (6·25 g.) [9] in pyridine (35 ml.) at room temperature during 1 hr., and the mixture set aside for 18 hr. Water (1 ml.) was added, and the mixture stirred for 1 hr. The solution was mixed with benzene (200 ml.) and washed at 0° with 25% aqueous sodium hydrogen sulphate (2 × 200 ml.), saturated sodium hydrogen carbonate (60 ml.), and water (3 × 100 ml.).

The benzene solution was dried, and the solvents evaporated; crystals formed slowly in the resulting oil. Yield of crude material (8·5 g., 84%). After three recrystallisations from cyclohexane the *compound* formed colourless crystals, m. p. 71—73° (Found: C, 63·0; H, 6·0. $C_{32}H_{34}O_8P_2$ requires C, 63·2; H, 5·6%).

Synthesis of the Isomeric Cyclohexane-diol Diphosphates; Phosphorylation.—*cis*- [28] and *trans*-Cyclohexane-1,2-diol,[29] *cis*- and *trans*-1,3-diol, and *cis*- [30] and *trans*-1,4-diol [31] were treated with diphenylphosphorochloridate (2·2 mol.) in pyridine (20 ml. per gm. of diol) as described above. Evaporation of the solvents usually left an oil which was hydrogenolysed directly to the diphosphate. The trans-1,4-*bisdiphenylphosphate* was obtained as a colourless solid, which was crystallised from absolute ethanol (yield 93%). Two further crystallisation gave the pure product m. p. 119·5—120° (Found: C, 62·4; H, 5·6; P, 10·6. $C_{30}H_{30}O_8P_2$ requires C, 62·1; H, 5·2; P, 10·7%).

Hydrogenolysis.—The oil was hydrogenolysed in ethanol (10 ml. per 1 g. oil) at room temperature/1 atm. over platinum oxide (0·1 g. per 1 g. oil); uptake of hydrogen ceased at 97—100% of the theoretical (16 mol.).

Addition of cyclohexylamine to the reaction mixture gave the crystalline cyclohexylammonium salt of the diphosphate. In some cases ion-exchange chromatography was necessary to separate the diphosphate from contaminating orthophosphate. The hydrogenolysed material (from 1·4 g. of tetraester) was dissolved in water and run on to a column (18 × 1·6 cm.) of Dowex-1 × 8 (Cl^-) resin, and the phosphates eluted using gradient elution (mixing chamber 500 ml. water; conc. eluant N-LiCl). Fractions (10 ml.) were collected, tubes 17—26 contained orthophosphate and tubes 34—50 the diphosphate. The requisite fractions were evaporated to dryness, the solid extracted with 95% ethanol (2 × 50 ml.), and the remaining solid precipitated from water (15 ml.) by addition of acetone (150 ml.).

trans-*Cyclohexane*-1,2-*diol Tetrahydrogen Diphosphate*.—The *trilithium salt* formed a colourless powder, R_F, 0·04 in solvent (A). (Found: C, 24·2; H, 4·2. $C_6H_{11}Li_3O_8P_2$ requires C, 24·5; H, 3·8%).

[27] Ref. 14, p. 15.
[28] M. F. Clarke and L. N. Owen, *J.*, 1949, 315.
[29] A. Roebuck and H. Adkins, *Org. Synth.*, Coll. Vol. III, p. 217.
[30] T. D. Perrine and W. C. White, *J. Amer. Chem. Soc.*, 1947, **69**, 1542.
[31] L. N. Owen and P. A. Robins, *J.*, 1949, 320.

The optically active diphosphate was prepared similarly from (+)-*trans*-cyclohexane-1,2-diol.[32]

The diphosphate was more conveniently prepared by hydrogenolysis of the dibenzyl-phosphate diphenylphosphate, followed by addition of cyclohexylamine. Two crystallisations of the product from ethanol gave the pure *triscyclohexylammonium salt*, (79%) m. p. 217—226° (decomp.) (Found: C, 50·25; H, 9·6; N, 7·1; P, 10·6. $C_{24}H_{53}N_3O_8P_2$ requires C, 50·25; H, 9·3; N, 7·3; P, 10·8%).

cis-*Cyclohexane*-1,2-*diol Tetrahydrogen Diphosphate*.—This was separated from the major product of phosphorylation, *cis*-2-hydroxycyclohexyl phosphate, [R_F, 0·33 in (A)], by Dowex-1 chromatography, and formed the crystalline *triscyclohexylammonium salt* (0·15%). (Found: C, 50·46; H, 9·4; N, 7·3%). Paper chromatography (solvent A, 4 days) showed that this product contained no *trans*-isomer.

trans-*Cyclohexane*-1,3-*diol Tetrahydrogen Diphosphate*.—This formed a *triscyclohexylammonium salt* (42%) from ethanol–water (Found: C, 50·47; H, 9·5; N, 7·5%).

cis-*Cyclohexane*-1,3-*diol Tetrahydrogen Diphosphate*.—The *dilithium salt* was obtained as the *monohydrate* (48%) (Found: C, 23·8; H, 4·5. $C_6H_{12}Li_2O_8P_2,H_2O$ requires C, 23·6; H, 4·6%).

trans-*Cyclohexane*-1,4-*diol Tetrahydrogen Diphosphate*.—The *tetrakiscyclohexylammonium salt* was obtained as the crystalline *dihydrate* from ethanol–water (69%) (Found: C, 50·75; H, 9·75; N, 7·6. $C_{30}H_{66}N_4O_8P_2,2H_2O$ requires C, 50·84; H, 9·96; N, 7·9%).

cis-*Cyclohexane*-1,4-*diol Tetrahydrogen Diphosphate*.—The *tetrakiscyclohexylammonium salt* was obtained as a *hydrate* by crystallisation from methanol–acetone containing a little water (93%) (Found: C, 51·4; H, 10·2; N, 7·7. $C_{30}H_{66}N_4O_8P_2,1\frac{1}{2}H_2O$ requires C, 51·5; H, 9·9; N, 8·0%).

Cyclohexyl Diphenyl Phosphate.—Cyclohexanol (2·01 g.) was allowed to react with diphenyl phosphorochloridate (6·5 g.) as described in the preparation of *trans*-cyclohexane-1,2-diol dibenzylphosphate diphenylphosphate. The benzene solution was dried and evaporated to leave an oil (6·38 g.), which crystallised on standing at −15° for 24 hr. The solid was recrystallised from light petroleum (5·76 g., 86%). Two further crystallisations from the same solvent gave the pure *product*; m. p. 37·5—38° (Found: C, 65·1; H, 6·2. $C_{18}H_{21}O_4P$ requires C, 65·1; H, 6·4%).

Cyclohexyl Dihydrogen Phosphate.—(*a*) Cyclohexyl diphenyl phosphate was hydrogenolysed over platinum oxide as described above. The compound was isolated as the *biscyclohexylammonium salt*, R_F 0·4 in (A) (Found: C, 54·5; H, 10·6; N, 7·2. $C_{18}H_{39}N_2O_4P,H_2O$ requires C, 54·5; H, 10·4; N, 7·1%).

(*b*) Cyclohexanol (8·0 g.) was treated with crystalline orthophosphoric acid and trichloroacetonitrile as described [33] for the preparation of t-butyl phosphate. The product was free from orthophosphate and gave an i.r. spectrum identical to that given by the monohydrate prepared above (yield 6·25 g., 40%).

trans-*Cyclohexane*-1,2-*diol Cyclic Pyrophosphate*.—Triscyclohexylammonium *trans*-cyclohexane-1,2-diol diphosphate (1·02 g.), in water, was treated with excess Dowex-50(H^+) resin, and the acid solution freeze-dried. The resulting oil (490 mg.) was dissolved in anhydrous dimethylformamide (20 ml.) and dicyclohexylcarbodi-imide (415 mg.) added. After 12 hr. the mixture was filtered, and the solvent evaporated. The oil remaining was extracted with a solution of cyclohexylamine (0·36 g.) in water, and the extract filtered, ether-extracted, and freeze-dried. The pyrophosphate (796 mg.) was freed from residual diphosphate by chromatography on a cellulose column, using as eluant solvent (A). The *biscyclohexylammonium salt* was recrystallised from ethanol–acetone. It has R_F 0·50 in (A) (Found: C, 46·6; H, 8·6; N, 6·0. $C_{18}H_{38}N_2O_7P_2,\frac{1}{2}H_2O$ requires C, 46·5; H, 8·5; N, 6·0%).

The free acid, prepared by treatment of the aqueous solution with IR-120(H^+) resin, showed, on titration, no evidence of secondary phosphoryl dissociation. Treatment with N-sodium hydroxide at 100° gave quantitative hydrolysis to the diphosphate in <5 hr.

Methyl trans-*Cyclohexane*-1,2-*diol Diphosphate*.—The cyclic pyrophosphate (0·5 mmole of the biscyclohexylammonium salt) was shown by chromatography to be unchanged after 12 days at room temperature in 0·3M-methanolic sodium methoxide (25 ml.), but, after 10 hr. under reflux, the only phosphorus-containing compounds present were *trans*-cyclohexanediol

[32] S. Winstein and R. Heck, *J. Amer. Chem. Soc.*, 1952, **74**, 5584; D. M. Brown and B. F. C. Clark, *J.*, 1963, 1475.

[33] F. Cramer, W. Rittersdorf, and W. Böhm, *Annalen*, 1962, **654**, 180.

diphosphate (18%) and methyl *trans*-cyclohexanediol diphosphate (82%). Cyclohexanediol diphosphate was unaffected under these conditions. The products were separated by chromatography on a cellulose column (solvent A), and the *methyl ester* was isolated as the *tricyclohexylammonium salt* (234 mg.), which crystallised from acetone (Found: C, 50·9; H, 9·4; N, 6·7. $C_{25}H_{55}N_3O_8P_2$ requires C, 51·1; H, 9·4; N, 7·1%).

Glycerol trans-*Cyclohexane*-1,2-*diol Trihydrogen Diphosphate*.—Glycidol (50 mmole) and tricyclohexylammonium *trans*-cyclohexane-1,2-diol diphosphate (0·136 mmole) in water (25 ml.) were kept at 30° for 7·1 hr.; the mixture was decationised and freeze-dried, and the products were separated by chromatography on a cellulose column (25 × 2 cm.) in solvent (A). The diphosphate monoglycerol ester was isolated as the *calcium salt*, R_F 0·16 in (A) (Found: C, 25·4; H, 4·7; P, 14·4. $C_9H_{17}Ca_{1·5}O_{10}P_2,H_2O$ requires C, 25·4; H, 4·5; P, 14·6%).

Periodate oxidation, followed spectrophotometrically [34] at 222·5 mμ, indicated the presence of 10% of the glycerol 2-isomer.

Hydrolysis. Hydrolysis at 100° gave a mixture of glycerol phosphate, 2-hydroxycyclohexyl phosphate, and cyclohexanediol diphosphate. In solvent (A) the starting material had about the same R_F (0·17) as glycerol phosphate. It was assumed that the amount of glycerol phosphate formed was equal to the amount of 2-hydroxycyclohexyl phosphate, and that hence, by subtraction, the concentration of starting material could be determined. The resulting first-order plot and constancy of product ratios gave support to this procedure. The rate constant for the production of cyclohexanediol diphosphate was about $2·5 \times 10^{-5}$ sec.$^{-1}$ in 1·0N-alkali, and $1·2 \times 10^{-5}$ sec.$^{-1}$ in 0·5N-alkali.

Glycerol trans-*Cyclohexane*-1,4-*diol Trihydrogen Diphosphate*.—Glycidol (2·0 ml.) and tetrakiscyclohexylammonium *trans*-1,4-cyclohexanediol diphosphate dihydrate (353 mg.) in water (50 ml.) was kept for 21 hr. at 30°. The reaction products were separated on a column of Dowex-2 × 8 (Cl^-) (100—200 mesh) resin using as eluant 0·1—0·3N-lithium chloride.

The fractions which contained the required product were evaporated to dryness at 30°. The resulting solid was extracted by ethanol (95%), and the *trilithium salt* precipitated three times from aqueous solution by ethanol (52·6 mg., chloride-free) (Found: C, 26·8; H, 5·3; $C_9H_{17}Li_3O_{10}P_2,2H_2O$ requires C, 26·8; H, 5·2%).

Cyclohexyl 1-*Glycerol Hydrogen Phosphate*.—Glycidol (1·66 ml.) and biscyclohexylammonium cyclohexyl phosphate monohydrate (500 mg.) in water (20 ml.) were kept at 30° until reaction was complete (5 days). The product was isolated as the *lithium salt* (202 mg.). Three precipitations gave the pure product (Found: C, 39·9; H, 7·15. $C_9H_{18}LiO_6P,\frac{1}{2}H_2O$ requires C, 40·15; H, 7·1%).

Hydrolysis. The ratio of 1- to 2-glycerol phosphate in the hydrolysis products was found to be 43 : 57. Separation was achieved in 2 days on Whatman No. 30 paper using solvent (A), or in 10 hr. using solvent (B). Glycerol was identified in the products by paper chromatography (R_F 0·48; solvent C); and was estimated, by comparison with a standard chromatogram, to be present in concentration approximately equal to that of the cyclohexyl phosphate.

Cyclohexyl 2-*Hydroxypropyl Hydrogen Phosphate*.—1,2-Epoxypropane (12·6 ml.) and biscyclohexylammonium cyclohexyl phosphate monohydrate (1·2 g.) in water (40 ml.) were heated in a sealed tube at 50° for 48 hr. Excess of epoxide was removed *in vacuo* and the solution passed down a column of IR-120(H^+) resin. The acidic effluent was brought to pH 6 with N-aqueous lithium hydroxide and the product isolated by precipitation in the usual way (428 mg.). Two further precipitations gave *lithium cyclohexyl* 2-*hydroxypropyl phosphate* (251 mg.), R_F 0·76 in solvent (A) (Found: C, 44·7; H, 7·6. $C_9H_{18}LiO_5P$ requires C, 44·3; H, 7·4%).

The n.m.r. spectrum showed this product to contain about 10% of the isomeric cyclohexyl 1-hydroxy-2-propyl phosphate. For accurate hydrolysis-product ratios it was necessary to remove this impurity. The lithium salt (1·04 g.) of the mixed isomers was heated for 3 hr. at 100° in 5N-sodium hydroxide (5 ml.);* hydrolysis was about 85% complete. The reaction mixture was diluted with water (20 ml.), and passed down a column of IR-120(H^+) resin. The effluent was neutralised with aqueous lithium hydroxide and the solution concentrated. Addition of ethanol (3 vols.) gave a gelatinous precipitate which was filtered off and discarded.

* The β-isomer (impurity) hydrolyses at least twice as fast as the α-isomer.

[34] J. S. Dixon and D. Lipkin, *Analyt. Chem.*, 1954, **26**, 1092; C. E. Crouthamel, A. M. Hayes, and D. S. Martin, *J. Amer. Chem. Soc.*, 1951, **73**, 82.

Chromatography of the ammonium salts on a cellulose column (solvent A) gave pure cyclohexyl 2-hydroxypropyl phosphate isolated as the lithium salt (89 mg.).

Hydrolysis. Hydrolyses were run in 1·0N- and 0·5N-alkali at 100°, with the ionic strength maintained at 1·0 by the addition of potassium chloride.

Propane-1,2-diol was identified in the reaction products by paper chromatography (R_F 0·76, solvent C).

One hydrolysis was carried out in N-hydrochloric acid at 100° for 3 hr. The phosphorus-containing products were 2-hydroxypropyl phosphate, and about 5% of orthophosphate.

Cyclohexyl 2-Hydroxyethyl Hydrogen Phosphate.—In the same way epoxyethane (4·9 ml.) and biscyclohexylammonium cyclohexyl phosphate monohydrate (0·8 g.) gave *lithium cyclohexyl 2-hydroxyethyl phosphate* (238 mg.). (Found: C, 41·6; H, 7·1. $C_8H_{16}LiO_5P$ requires C, 41·8; H, 7·0%).

Hydrolysis. Ethylene glycol was identified in the products by paper chromatography (R_F 0·67, solvent C) and cyclohexanol by g.l.c. at 100° on a Celite–silicone oil column. The concentration of cyclohexanol in the hydrolysis mixture was found, by comparison with a standard solution, to be 1·2 mg./ml. The amount expected, calculated from the concentration of 2-hydroxyethyl phosphate, was 1·3 mg./ml.

2,3-Epoxybutane.—2-Chlorobutan-3-ol [35] (100 g.) was converted [10,13] into 2,3-epoxybutane and the mixture of *cis*- and *trans*-isomers (46·6 g.) was fractionated at atmospheric pressure through an 18-in. electrically-heated column filled with Lessing rings, using a reflux ratio of 20. Only a partial separation was achieved. Three fractions were collected: (i), b. p. 54—55·5° (12 g.), n_D^{20} 1·3748; (ii) b. p. 56—57° (10 g.), n_D^{20} 1·3771; (iii) b. p. 57—61° (11 g.), n_D^{20} 1·3806. Each was examined by g.l.c. at 20° on a 6 ft. × 4 mm. column packed with 10% silicone oil on Celite, using argon as the carrier gas. An ionisation type detector (I.E. 101) (Gas Chromatography Ltd., Maidenhead, Berks., England) was used. The epoxides were injected as 1% solutions in dioxan. The retention volumes (referred to peak maxima) of the two isomers were *trans*-2,3-epoxybutane (b. p. 53·6—54·1°) [10] 12·4 ml. and *cis*-2,3-epoxybutane (b. p. 59·9—60·4°) [10] 16·6 ml. The peaks were well separated, the distance from the baseline to the minimum between the peaks was, in the case of fraction 2, about 3% of the peak maxima on either side.

The ratios of isomers (±2%) were determined from the relative peak areas (mean of 2 values); it was assumed that the detector had the same sensitivity to each isomer.

Fraction	*trans* (%)	*cis* (%)
(i)	78·5	21·5
(ii)	55	45
(iii)	13	87

From fraction (ii), a sample (0·3 ml.) of the pure *trans*-epoxide, containing less than 0·1% *cis*-isomer, was isolated using a preparative g.l.c. column.

G.l.c. of Butane-2,3-diols. The *meso*- and DL-isomers of butane-2,3-diol were resolved [11] by g.l.c. on a 6 ft. × 5 mm. column packed with Celite containing as stationary phase 15% "polyethylene glycol-400" (L. Light & Co.). The column was maintained at 125°, and the glycols were injected (0·5 μl.) as 10% solutions in acetone. An ionisation detector (I.E. 101) was used. The distance from the baseline to the minimum between peaks was, in the case of 1 : 1, *meso* : DL-mixture, not greater than 2% of the peak maxima on either side. The retention volumes, relative to peak maxima, using argon as carrier gas at a flow rate of 34 ml./min., were: DL-isomer, 500 ml., *meso*-isomer, 604 ml.

Hydrolysis of 2,3-Epoxybutanes. Each sample of epoxide was hydrolysed under the conditions shown in Table 1; the hydrolysis reaction mixture was de-ionised and freeze-dried, and the residue applied to the g.l.c. column as a solution in acetone. Isomer ratios were estimated from the relative peak areas (±2%); no standards were run, but the good correlation with the ratios of the epoxide fractions tends to support this procedure.

Cyclohexyl 3-Hydroxy-2-butyl Phosphate. Cyclohexyl dihydrogen phosphate (540 mg.) and *trans*-2,3-epoxybutane (0·3 ml.) in anhydrous chloroform (10 ml.) were set aside at room temperature for 10 hr., and the solvent was then removed *in vacuo*. Barium hydroxide solution was added, and carbon dioxide was passed into the solution to bring the pH to 7. Ethanol (1 vol.) was added, the precipitate of barium carbonate and barium cyclohexyl phosphate was filtered off, and the filtrate evaporated at 30°. To the resulting oil was added acetone (100 ml.), diethyl

[35] W. T. Somerville and P. E. Spoerri, *J. Amer. Chem. Soc.*, 1950, **72**, 2185.

ether (50 ml.) and light petroleum (b. p. 40—60°; 10 ml.). Barium cyclohexyl 3-hydroxy-2-butyl phosphate was collected by filtration after 10 hr. (Yield 420 mg.) (Found: C, 36·7; H, 6·3. $C_{10}H_{20}Ba_{0·5}O_5P$ requires C, 36·4; H, 6·1%).

Hydrolyses. (*a*) The barium salt (27·3 mg.) was converted into the free acid with Dowex-50(H^+) and heated for 7 hr. at 100° in N-sodium hydroxide (0·5 ml.). The hydrolysis mixture was freeze-dried, the resulting oil triturated with acetone, and the acetone solution was examined by g.l.c.; it contained cyclohexanol and *meso*-butane-2,3-diol, but there was no detectable (less than 0·3%) DL-isomer.

(*b*) The sodium salt (from 10·5 mg. of barium salt) was heated in N-sodium hydroxide (0·5 ml.) for 18 hr. at 100° and the products were examined by g.l.c. and paper chromatography. The ratio of cyclohexyl phosphate to 3-hydroxy-2-butyl phosphate was 3·7 : 1 (±5%). The volatile products were estimated by injection of 1 μl. of the hydrolysis solution on to a 4 ft. Carbowax-1500 column; a hydrogen-flame detector was used. Standard solutions of butane-2,3-diol and cyclohexanol (1 : 1; 2·1 : 1; 4 : 1) in water and in N-sodium hydroxide (with and without heating for 17 hr. at 100°) were also run on the column, and the concentrations of these substances in the hydrolysis mixture were determined by reference to the standard graphs so prepared.

	Butanediol	Cyclohexanol
Concentrations calc. from the phosphate concns....	2·06 ± 0·1 mg./ml.	0·62 ± 0·03 mg./ml.
Found (g.l.c.)	2·3 ± 0·2 mg./ml.	0·7 ± 0·1 mg./ml.
Mole ratios (g.l.c.)	(3·75 ± 0·15) : (1·0)	

Hydrolysis of Cyclohexyl 3-Hydroxy-2-butyl Phosphate in ^{18}O-Enriched Water.—Lithium cyclohexyl 3-hydroxy-2-butyl phosphate (100 mg.) was converted into the free acid, and dissolved in a mixture of 5N-sodium hydroxide (0·6 ml.) and water, (2·4 ml.) containing a low enrichment of ^{18}O. The mixture was heated in a Teflon container for 19 hr. at 100°, then freeze-dried, and the water collected in a trap cooled in liquid nitrogen. Water of the normal isotopic content was added to the semi-solid residue, and to the resulting solution was added barium hydroxide solution (100 mg. of the octahydrate, in water), and ethanol (2 vol.). The resulting precipitate (89 mg.) was stirred with water (30 ml.) for five hr., and the mixture was filtered. To the filtrate was added ethanol (2 vol.), and the resulting precipitate (62 mg.) was shaken for 5 hr. with a mixture of barium carbonate (30 mg.) and water (30 ml.). The mixture was filtered, and ethanol was added to the filtrate; after 3 min. the resulting precipitate was collected by filtration. The barium cyclohexyl phosphate, converted into the free acid by brief treatment with IR-120(H^+) resin, was purified by chromatography on Whatman 3 MM paper (solvent A), and eluted from the paper by water. Addition of the calculated quantity of barium bromide in ethanol gave a precipitate of barium cyclohexyl phosphate which was dried at 60° for 5 hr. and submitted to ^{18}O analysis. This compound was free from 3-hydroxy-2-butyl phosphate.

A control experiment was run in which cyclohexyl phosphate was heated in enriched water under conditions identical to those given above, and was isolated as described, except that the paper chromatography was omitted.

Isotope analysis * (^{18}O atom %)

	Cyclohexyl phosphate	Recovered water
Hydrolysis	0·206	0·410
Control	0·207	0·419
Barium cyclohexyl phosphate; "*ex* bottle"	0·206	—

* The ^{18}O analyses were carried out by Dr. B. Silver of the Weizman Institute of Science, Rehovoth, Israel, through the kindness of Dr. David Samuel of that Institute.

1,2-*Epoxy*-2-*methylpropane.*—1-Chloro-2-methylpropan-2-ol [36] (14·2 g.) was converted into the epoxide using a method similar to that of Wilson and Lucas [10] (yield 7·6; g., 81%). It had n_D^{25} 1·3699 (lit.,[37] 1·3695). The n.m.r. spectrum showed one peak (relative area 3) and one peak (relative area 1) at 1·26 and 2·48 p.p.m., respectively, downfield from the internal standard, tetramethylsilane.

Cyclohexyl 2-Hydroxy-2-methylpropyl Phosphate.—1,2-Epoxy-2-methylpropane (2·7 ml.) and biscyclohexylammonium cyclohexyl phosphate monohydrate (0·4 g.) in water (8 ml.) was heated at 50° in a sealed tube for 34 hr. The solution was filtered from crystals of the amino-alcohol

[36] J. Burgin, G. Hearne, and F. Rust, *Ind. Eng. Chem.*, 1941, **33**, 385.
[37] H. O. House, *J. Amer. Chem. Soc.*, 1955, **77**, 5083.

and the product isolated as the *barium salt* in the usual way (94 mg.) (Found: C, 36·8; H, 6·5. $C_{10}H_{20}Ba_{0·5}O_5P,\frac{1}{2}H_2O$ requires C, 36·5; H, 6·4%).

The n.m.r. spectrum in deuterium oxide indicated an isomeric purity of >99·5%.

Cyclohexyl 1-Hydroxy-2-methyl-2-propyl Phosphate.—Cyclohexyl dihydrogen phosphate (540 mg.) and 1,2-epoxy-2-methylpropane (0·3 ml.) in anhydrous chloroform (6 ml.), were stood for 1 hr., the solvent was removed *in vacuo*, and the residual oil dissolved in a little water. To this solution was quickly added sufficient lithium hydroxide solution to bring the pH to 8.

Ethanol (2 vol.) was added, and the resulting precipitate of unreacted cyclohexyl phosphate filtered off (200 mg.). The filtrate was evaporated to dryness at room temperature, and the residual solid dissolved in the minimum of absolute ethanol. Acetone (50 ml.), diethyl ether (50 ml.), and light petroleum (b. p. 40—60°; 10 ml.) were added, and the seeded mixture was set aside at 0° until precipitation was complete. If a seed was not available, precipitation of the *product* could often be induced by filtering the solution through a Buchner funnel, under vacuum (yield 254 mg.) (Found: C, 46·9; H, 7·8. $C_{10}H_{20}LiO_5P$ requires C, 46·5; H, 7·8%).

When the preparation was carried out as described, there was less than 2% 1-isomer in the product. Allowing the reaction to proceed for 18 hr. at room temperature gave a product containing 6% 1-isomer.

Hydrolyses. Solvent (A) did not separate 2-hydroxy-2-methylpropyl phosphate from 1-hydroxy-2-methyl-2-propyl phosphate (R_F 0·26). Specimens of these compounds were prepared by the reaction of 1,2-epoxy-2-methylpropane with aqueous solutions of disodium hydrogen orthophosphate, and sodium dihydrogen orthophosphate, respectively. These products were identified by n.m.r. spectroscopy.

N.m.r. data for cyclohexyl hydroxymethylpropyl phosphates

(Lithium salts in D_2O)

Compound (R = cyclohexyl)	δ in p.p.m. up-field from HOD: Methyl protons	Methylene protons
$(CH_3)_2C(OPO_3R^-)$–CH_2OH	3·35	1·15
$(CH_3)_2C(OH)$–$CH_2OPO_3R^-$	3·50	Doublet centred on 1·03 $J_{H/P}$ = 6 c./sec.
$(CH_3)_2C(OH)$–CH_2OH	3·55	1·32

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