## 390. *Phospholipids. Part I. The Hydrolysis of Some Esters of* cyclo*Hexanediol Phosphates.*

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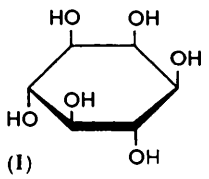
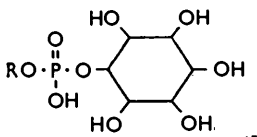
*cis-* and *trans*-2-Hydroxy*cyclo*hexyl phosphate and their benzyl esters are prepared. Hydrolysis of the esters by alkali or acid yields the diol phosphates and in each case proceeds more readily than that of dialkyl phosphates lacking a vicinal hydroxyl function; the evidence indicates that *cis-* and *trans*-1 : 2-*cyclo*hexylidene phosphate are intermediates in the hydrolyses. Such cyclic phosphates have been synthesised and are readily hydrolysed by acid or alkali. *cis*-1 : 3-*cyclo*Hexylidene phosphate, in contrast, is very stable. The results, together with observations already recorded, indicate phosphoryl migration during hydrolysis of the *myo*-inositol-containing phospholipids to inositol mono- or di-phosphate.

Among the phospholipids there exists a widely distributed and apparently diverse group of natural products containing *myo*inositol (I). In addition to phosphate, each individual may contain glycerol, long-chain fatty acids, sugars, and nitrogenous bases as constituent residues although, since it is not clear whether any one of these lipids has been obtained pure, contaminants may account for some of the constituents reported. Discovered first by Anderson [1] in the lipids of the tubercle bacillus, phosphoinositides have since been isolated from other bacterial, plant, and animal sources.[2] They yield *myo*-inositol monophosphate or, in the case of the brain lipid, inositol diphosphate, on hydrolysis.

Substances isolated from wheat germ,[3] liver,[4,5] heart muscle,[6] and possibly soya bean [7] appear to be relatively simple in constitution. They contain fatty acids, glycerol, inositol, and phosphate in the ratio 2 : 1 : 1 : 1 and on hydrolysis yield inositol monophosphate. The structure (II; R = diacylglycerol residue) has therefore been suggested. Others, more complex, may contain (II) as a structural unit with further substituents on the inositol fragment, for example glycosyl [8,9] or phosphorylated residues.[10]




(I)



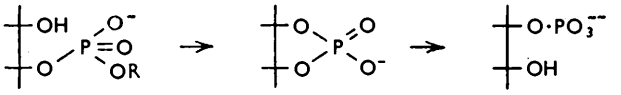

(II)

The inositol phosphates isolated after hydrolysis of phosphoinositides have not been investigated in detail and little comment seems to have been made on the possibility of phosphoryl migration on the inositol residue during hydrolysis. Thus Folch [11] has assumed that the orientation of the phosphate groups in the brain inositide is the same as that in the so-called " inositol meta-diphosphate " isolated from it on hydrolysis by mineral acid. Malkin and Poole [9] mention the possibility of phosphate migration without further comment. Hawthorne and Chargaff [10] regard phosphoryl migration as

[1] Anderson, *J. Amer. Chem. Soc.*, 1930, **52**, 1607.
[2] For refs. see Folch and LeBaron, *Canad. J. Biochem. Physiol.*, 1956, **34**, 305.
[3] Faure and Morelec-Coulon, *Compt. rend.*, 1953, **236**, 1104.
[4] McKibbin, *Fed. Proc.*, 1954, **13**, 262; *J. Biol. Chem.*, 1956, **220**, 537.
[5] Hawthorne, *Biochem. J.*, 1955, **59**, ii.
[6] Faure and Morelec-Coulon, *Compt. rend.*, 1954, **238**, 411.
[7] Okuhara and Nakayama, *J. Biol. Chem.*, 1955, **215**, 295; see however ref. 8.
[8] Hawthorne, *Biochim. Biophys. Acta*, 1955, **18**, 389.
[9] Malkin and Poole, *J.*, 1953, 3470.
[10] Hawthorne and Chargaff, *J. Biol. Chem.*, 1954, **206**, 27.
[11] Folch, *J. Biol. Chem.*, 1949, **177**, 505.

unlikely on the grounds that the oxygen–oxygen distances in polyhydroxy*cyclo*hexane systems are too great to allow of its occurrence. This is, however, unjustified, in view of the preparation of *iso*propylidene [12] and cyclic sulphite [13] derivatives of both *cis*- and *trans-cyclo*hexane-1 : 2-diol. It seemed to us that clarification of this point was essential before any rational approach could be made, based on hydrolytic evidence, to the structures of the intact phosphoinositides.

Migration of phosphate was first recognised during hydrolysis of alkyl esters of glycerol phosphate, by Bailly and Gaumé,[14] and later in phospholipids of lecithin and kephalin type by Baer and Kates [15] who discussed a mechanism for the process. The ribonucleic acids were then shown [16] to contain analogous systems and studies of the hydrolysis of alkyl esters of the 2′- and 3′-monoribonucleotides (partial formula III; R = alkyl) have permitted a clearer view of the hydrolytic process.[17,18] This, briefly, is considered to proceed, in the case of alkaline hydrolysis, by attack of the neighbouring hydroxyl group



(III) (IV) (V)

on phosphorus with expulsion of $RO^-$, to give the cyclic diester (IV) which then breaks down to the two isomeric monoesters (V). Participation by the vicinal function results in (a) much greater lability of the system by comparison with normal dialkyl phosphates which are extremely stable,[19] and (b) retention of the phosphate group on the hydroxyalkyl residue, that is, with no significant production of $RO{\cdot}PO_3H_2$ from (III).[20] Acid hydrolysis, likewise, proceeds *via* the intermediate cyclic phosphate (IV) and moreover migration of the alkylphosphoryl group to the vicinal position can also occur without loss of the R group.[18]

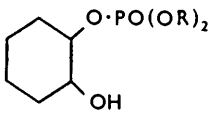
Because of the complexity of the inositol phosphate derivatives it was decided to study, first, the chemistry of their simple analogues, the hydroxy*cyclo*hexyl phosphates and their esters. Dibenzyl 2-hydroxy*cyclo*hexyl phosphate (VI; $R = CH_2Ph$), evidently the *trans*-isomer from its mode of formation from *cyclo*hexene oxide and dibenzyl phosphate, was converted by debenzylation with 4-methylmorpholine into *trans*-2-hydroxy*cyclo*hexyl benzyl phosphate [21] (VII; $R = CH_2Ph$), which on hydrogenolysis afforded *trans*-2-hydroxy*cyclo*hexyl dihydrogen phosphate (VIII).

For the preparation of members of the *cis*-series, *cis-cyclo*hexane-1 : 2-diol was treated with dibenzyl phosphorochloridate. The crystalline, but unstable *cis*-(dibenzyl phosphate) (VI) obtained could be debenzylated with lithium chloride or by partial hydrogenolysis to the monobenzyl ester (VII), whence further hydrogenolysis afforded *cis*-2-hydroxy*cyclo*hexyl dihydrogen phosphate (VIII). The last substance was more conveniently prepared by direct phosphorylation of *cis-cyclo*hexane-1 : 2-diol with phosphoryl chloride.

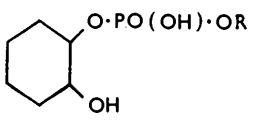
The cyclic *cis*- and *trans*-1 : 2-*cyclo*hexylidene phosphates (IX) were also prepared since they were considered to be possible intermediates in the hydrolysis of the *cis*- and the *trans*-monobenzyl ester (VII). *cis*-2-Hydroxy*cyclo*hexyl dihydrogen phosphate (VIII), when treated with trifluoroacetic anhydride,[22] was about half converted into another

[12] Christian, *Canad. J. Chem.*, 1951, **29**, 911.
[13] Price and Berti, *J. Amer. Chem. Soc.*, 1954, **76**, 1211.
[14] Bailly and Gaumé, *Bull. Soc. chim. France*, 1935, **2**, 354.
[15] Baer and Kates, *J. Biol. Chem.*, 1948, **175**, 79; 1950, **185**, 615.
[16] Brown and Todd, *J.*, 1952, 44, 52.
[17] *Idem*, *Ann. Rev. Biochem.*, 1955, **24**, 311.
[18] Brown, Magrath, Neilson, and Todd, *Nature*, 1956, **177**, 1124.
[19] Plimmer and Burch, *J.*, 1929, 279.
[20] Cf. however, Fleury, Lecocq, and le Dizet, *Compt. rend.*, 1956, **242**, 420.
[21] Baddiley, Clark, Michalski, and Todd, *J.*, 1949, 818.
[22] Brown, Magrath, and Todd, *J.*, 1952, 2708.

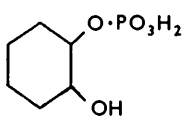
substance running faster on chromatograms. The same substance was obtained in high yield by the action of di*cyclo*hexylcarbodi-imide on the acid (VIII) in dimethylformamide.[23,24] Both reagents have been used previously for the preparation of cyclic esters in the ribonucleotide series. Applied to the *trans*-diol phosphate the latter method afforded a



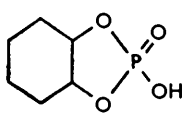

(VI)



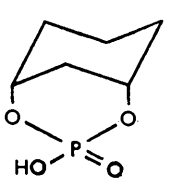

(VII)




(VIII)




(IX)




(X)

substance with very similar chromatographic characteristics. Both the *cis*- and the *trans*-derivative formed a crystalline *cyclo*hexylamine salt and on electrometric titration showed no buffering action in the pH range 5—8 associated with secondary phosphoryl dissociation. The easy hydrolysis of the substances both by acid and base, characteristic of other 5-membered cyclic phosphates,[22] together with the other evidence leads us to formulate them as the *cis*- and *trans*-isomers of the cyclic compound (IX), rather than as pyrophosphates which can arise when the carbodi-imide reagent is applied to alkyl phosphates lacking the vicinal hydroxyl function.[25]

Some phosphorylated derivatives of the *cyclo*hexane-1 : 3-diols have been prepared and their hydrolysis studied. When *cis-cyclo*hexane-1 : 3-diol was treated with dibenzyl phosphorochloridate a mixture of mono- and di-phosphorylated materials was obtained. These could not be separated nor was it possible to purify the partially debenzylated products. Complete hydrogenolysis permitted the isolation of the crystalline *cyclo*hexylamine salt of *cis*-1 : 3-*cyclo*hexylidene bisphosphate. The monophosphate, present in the mother-liquors was obtained chromatographically pure but in quantity insufficient for characterisation. *trans*-3-Hydroxy*cyclo*hexyl dihydrogen phosphate, however, was obtained by phosphorylation of *trans*-3-benzoyloxy*cyclo*hexanol followed by removal of protecting groups.

Phosphorylation of the *cis*-1 : 3-diol with phosphoryl chloride gave in high yield the *cis*-1 : 3-*cyclo*hexylidene phosphate.[26] This cyclic phosphate was extremely stable;[27] it was unaffected by 30% aqueous sodium hydroxide at 100° and was only slowly degraded, with formation of phosphoric acid, by 3N-hydrochloric acid at this temperature. It formed a crystalline methyl ester of considerable stability which with N-hydrochloric acid reverted to the cyclic phosphate (X) only, but with N-alkali yielded an additional substance stable to more prolonged treatment. This product which could not be obtained pure was almost certainly *cis*-3-hydroxy*cyclo*hexyl methyl hydrogen phosphate. Its high stability together with that of the 1 : 3-cyclic phosphate (X) is a strong indication that no participation by a *cis*-3-hydroxyl group is effective in the hydrolysis of *cyclo*hexyl alkyl phosphates. Since cyclic ester formation requires a 1 : 3-diaxial conformation it is even less likely to occur in the *myo*inositol (I) series where five oxygen atoms would then require to be axially disposed.

[23] Brown, unpublished results, quoted by Khorana (*Chem. Rev.*, 1953, **53**, 145).
[24] Dekker and Khorana, *J. Amer. Chem. Soc.*, 1954, **76**, 3522.
[25] Khorana and Todd, *J.*, 1953, 2259.
[26] Cf. Stetter and Steinacker, *Chem. Ber.*, 1952, **85**, 451.
[27] Cf. Baddiley, Buchanan, and Szabo, *J.*, 1954, 3826.

The hydrolysis of the *cyclo*hexane-1 : 2-diol phosphate derivatives was studied in more detail. Both *cis*- and *trans*-1 : 2-*cyclo*hexylidene phosphate (IX) underwent complete hydrolysis to the diol phosphate in less than 18 hr. in N-acid or -alkali at room temperature and in less than 10 min. at 100°. The rate in base for the *trans*-isomer was about twice that for the *cis*-isomer, consistently with the greater strain to be expected in a *trans*-fused 5 : 6-bicyclic system. The *cis*- and the *trans*-2-hydroxy*cyclo*hexyl benzyl phosphate (VII) were more stable than the cyclic phosphates, as expected. Thus the *trans*-isomer was recovered unchanged after two days at room temperature in N-sodium hydroxide, although the *cis*-isomer had undergone partial hydrolysis. At 100° the *cis* ester was half-hydrolysed in about 1 hr. and was completely converted in 5 hr. into a product which was isolated and characterised as *cis*-2-hydroxy*cyclo*hexyl phosphate. The *trans*-ester likewise yielded only the *trans*-2-hydroxy*cyclo*hexyl phosphate on complete hydrolysis; in neither case was a detectable amount of benzyl phosphate produced. By contrast, neither dibenzyl phosphate nor di*cyclo*hexyl phosphate was affected by N-alkali at 100° during 7 hr.

The monobenzyl esters (VII) were also hydrolysed by acid with loss of the benzyl group, the rates again being in the order *cis*- greater than *trans*-isomer; N-acid led to complete hydrolysis of the former in 20 min. whereas 2 hr. were required for the *trans*-isomer. These results are more equivocal since benzyl phosphates are known [28] to be more susceptible than other alkyl phosphates to acid hydrolysis but the large difference in rates between the *cis*- and the *trans*-isomer does indicate that the vicinal function is involved.

Taken together, these experiments strongly suggest that the hydrolysis of the monobenzyl ester of both *cis*- and *trans*-2-hydroxy*cyclo*hexyl phosphate (VII) occurs by the mechanism, discussed above, which involves the corresponding 1 : 2-*cyclo*hexylidene phosphate (IX). Alkaline hydrolysis proceeds much faster than that of normal dialkyl phosphates and only in the direction of formation of the diol phosphate (VIII). In addition the greater rate observed in the *cis*- than in the *trans*-series can be accommodated in terms of deformational strain in the *cyclo*hexane ring due to formation of the bicyclic intermediate.[29,30] The ready synthesis of the cyclic esters (IX) is also an argument in favour of the proposed hydrolytic mechanism. It is to be noted, however, that the benzyl esters (VII) are much more stable than their nucleotide counterparts (III), but this is to be expected since the stereochemistry associated with the ribofuranose residue of the latter is much more favourable to participation of the vicinal group in the ester hydrolysis.

Further experiments with glycerol esters and more fully hydroxylated *cyclo*hexyl phosphates are in progress, but the present results indicate that phosphate migration, in the absence of evidence to the contrary, must be expected to occur during hydrolysis of phosphoinositides based on (II). Moreover, during acid hydrolysis, a method hitherto used extensively in degradative work in this field, the *myo*inositol phosphate produced is probably subject to further acid-catalysed phosphate migration. Relevant to this is the recent observation [31] that such migration does occur in the case of the 3-phosphate of shikimic acid (a trihydroxy*cyclo*hexenecarboxylic acid).

## Experimental

*Paper Chromatography of Some* cyclo*Hexyl Phosphates.*—Paper chromatography was used throughout for identification and as a criterion of purity of products. Authentic specimens were run concurrently, where possible, and spots were detected with a phosphate spray reagent. The Table contains the $R_F$ values recorded for the substances studied; Whatman No. 1 paper and two solvent systems were used, namely, (A) propan-2-ol–water–ammonia (*d* 0·880) (7 : 2 : 1 v/v) and (B) butan-1-ol–acetic acid–water (6 : 2 : 3 v/v). No significant differences between the $R_F$ values of *cis*- and *trans*-isomers was noted.

[28] Kumamoto and Westheimer, *J. Amer. Chem. Soc.*, 1955, **77**, 2515.
[29] Angyal and Macdonald, *J.*, 1952, 686.
[30] Eliel and Pillar, *J. Amer. Chem. Soc.*, 1955, **77**, 3600.
[31] Weiss and Mingioli, *ibid.*, 1956, **78**, 2894.

| Substance | $R_F$ values in solvent A | B |
|---|---|---|
| Dibenzyl 2-hydroxy*cyclo*hexyl phosphate | 0·90 | 0·95 |
| Benzyl 2-hydroxy*cyclo*hexyl hydrogen phosphate | 0·75 | 0·81 |
| 2-Hydroxy*cyclo*hexyl dihydrogen phosphate | 0·33 | 0·45 |
| 1 : 2-*cyclo*Hexylidene hydrogen phosphate | 0·60 | 0·70 |
| Benzyl dihydrogen phosphate | 0·40 | 0·50 |
| *trans*-3-Hydroxy*cyclo*hexyl dihydrogen phosphate | 0·20 | 0·14 |
| 1 : 3-*cyclo*Hexylidene hydrogen phosphate | 0·50 | 0·50 |
| 1 : 3-*cyclo*Hexylidene methyl phosphate | 0·82 | 0·85 |
| *cis*-1 : 3-*cyclo*Hexylidene bis(dihydrogen phosphate) | 0·05 | — |

trans-2-*Hydroxy*cyclo*hexyl Dihydrogen Phosphate.*—*cyclo*Hexylammonium *trans*-2-hydroxy-*cyclo*hexyl benzyl phosphate [21] (1·03 g.) was hydrogenolysed at room temperature and pressure in ethanol (25 c.c.) over 5% palladised charcoal. After 9 hr. uptake of hydrogen was complete and the catalyst was removed by filtration. Evaporation of the solvent gave cyclo*hexyl-ammonium* trans-2-*hydroxy*cyclo*hexyl hydrogen phosphate* (0·884 g.) which formed colourless needles (from ethanol), m. p. 208—211°, softening at 180° (Found, in material dried at 80°/0·5 mm. : C, 48·8; H, 9·0. $C_{12}H_{26}O_5NP$ requires C, 48·8; H, 8·9%).

A solution of the above salt was percolated through a column of Dowex-50 (H form). Percolate and washings were evaporated to dryness; the residue crystallised from dioxan. trans-2-*Hydroxy*cyclo*hexyl dihydrogen phosphate* formed colourless needles, m. p. 164—168° (Found, in material dried at 100°/0·5 mm. : C, 36·2; H, 6·3. $C_6H_{13}O_5P$ requires C, 36·7; H, 6·6%).

*Dibenzyl* cis-2-*Hydroxy*cyclo*hexyl Phosphate.*—*cis-cyclo*Hexane-1 : 2-diol (4·05 g.), dried over phosphoric oxide at 0·5 mm., was dissolved in dry pyridine (10 c.c.), and the solution cooled at −20°. Dibenzyl phosphorochloridate [32] [from dibenzyl phosphite (5·1 g.)] in carbon tetrachloride was added dropwise with rigid exclusion of moisture during 0·5 hr. After being kept at −20° to −15° for 3 hr. and at 0° overnight the solution was washed with dilute sodium hydrogen carbonate solution, twice with potassium hydrogen sulphate solution, and then with water. After being dried ($Na_2SO_4$), the solution was evaporated at 35°/12 mm., finally with additions of ethanol to remove pyridine. The crude product (5·02 g.; 62%) was crystallised from light petroleum (b. p. 60—80°) and gave *dibenzyl* cis-2-*hydroxy*cyclo*hexyl phosphate* in needles, m. p. 64—67° (Found, in material dried at 30°/0·2 mm. for 48 hr. : C, 62·8; H, 6·7. $C_{20}H_{25}O_5P$ requires C, 63·8; H, 6·9%). Three other apparently similar preparations gave only yellow oils.

The compound and the corresponding *trans*-isomer [31] were converted within 10 min. into the monobenzyl ester when heated at 100° in N-sodium hydroxide; no dibenzyl phosphate was formed.

*Benzyl* cis-2-*Hydroxy*cyclo*hexyl Hydrogen Phosphate.*—To the above dibenzyl phosphate (0·49 g.) in redistilled ethyl cellosolve (15 c.c.), freshly fused lithium chloride (0·5 g.) was added and the solution then heated on the water-bath for 2 hr. To the cooled solution N-sodium hydroxide (30 c.c.) was added and unchanged starting material extracted with ether. The aqueous layer was acidified with concentrated hydrochloric acid and extracted with chloroform. The chloroform solution was dried ($Na_2SO_4$) and evaporated and the residual, oily benzyl *cis*-2-hydroxy*cyclo*hexyl hydrogen phosphate converted into its cyclo*hexylamine* salt by addition of *cyclo*hexylamine to its aqueous solution, to pH 10, and then evaporation to dryness. It crystallised from ethanol–ethyl acetate in needles, m. p. 187—193° (0·19 g.) (Found : C, 59·4; H, 8·4; N, 3·7. $C_{19}H_{32}O_5NP$ requires C, 59·2; H, 8·3; N, 3·6%).

The same monobenzyl phosphate was obtained as its *cyclo*hexylamine salt (0·91 g.) after (slow) hydrogenolysis of a non-crystalline sample of the above dibenzyl phosphate (1·0 g.) over palladium–charcoal.

cis-2-*Hydroxy*cyclo*hexyl Dihydrogen Phosphate.*—(*a*) The above crystalline dibenzyl ester (0·43 g.) was hydrogenated smoothly over 10% palladium–charcoal, in 95% ethanol (20 c.c.). Filtration and then evaporation gave the product as a colourless glass. The cyclo*hexylamine hydrogen phosphate* formed needles (0·23 g.) (from ethanol), m. p. 192—204° (Found, in material dried at 100°/0·5 mm. : C, 48·8; H, 8·7; N, 4·7. $C_{12}H_{26}O_5NP$ requires C, 48·5; H, 8·8; N, 5·0%).

The *cyclo*hexylamine salt (0·32 g.) was converted into the *disilver salt* by treating it in hot

[32] Atherton, Openshaw, and Todd, *J.*, 1945, 382.

water (10 c.c.) with excess of silver nitrate solution. A white granular precipitate was formed, which was collected after cooling to 0° in the dark. The amorphous product (0·35 g.) was light sensitive (Found, in material dried at 100°/0·5 mm. for 2 hr.: C, 17·8; H, 2·9; P, 7·8. $C_6H_{11}O_5Ag_2P$ requires C, 17·6; H, 2·7; P, 7·6%). Attempts to prepare the monosilver salt by using 1 mol. of silver nitrate yielded only the disilver salt.

(*b*) Pure phosphoryl chloride (0·5 c.c.) was added to dry pyridine (10 c.c.), and 8 c.c. of this solution were added dropwise with shaking to a solution of *cis-cyclo*hexane-1 : 2-diol (0·5 g.) in pyridine (5 c.c.), cooled in ice. After being set aside overnight at 0°, precipitated pyridine hydrochloride was filtered off and the pyridine removed *in vacuo*. The residue was dissolved in water, and barium hydroxide solution added to pH 10, then excess of reagent neutralised with carbon dioxide. After filtration from barium phosphate, the solution was passed through a column of Dowex-50 (H form) resin, and the acidic percolate and washings were combined and evaporated, finally at 0·5 mm. over potassium hydroxide. The crystalline residue (0·45 g.) of cis-2-*hydroxy*cyclo*hexyl dihydrogen phosphate* recrystallised from acetone as needles, m. p. 134—135° (Found, in material dried at 80°/0·5 mm.: C, 36·8; H, 6·7. $C_6H_{13}O_5P$ requires C, 36·7; H, 6·6%).

Paper chromatography of the reaction mixture showed that the 1 : 2-cyclic phosphate was the main product initially, and that this was hydrolysed during working up.

cis-1 : 2-cyclo*Hexylidene Hydrogen Phosphate*.—(*a*) Dry *cyclo*hexylammonium *cis*-2-hydroxy-*cyclo*hexyl hydrogen phosphate (115 mg.), dissolved in trifluoroacetic anhydride (5 c.c.), was set aside for 2 days with rigid exclusion of moisture. Evaporation *in vacuo* at 35° left a colourless oil. Paper chromatography in the propan-2-ol–ammonia–water solvent showed that the product was present to the extent of 50% and had a higher $R_F$ value (0·65) than the starting material (0·33). The oil was dissolved in 3% aqueous ammonia, and sufficient propan-2-ol added to give a clear solution. This was applied to a sheet of washed seed-testing paper at the origin as a band and then chromatographed with the propan-2-ol–ammonia–water system. The product was clearly separated from starting material, and the appropriate section of the paper was cut out, eluted with water (2 × 5 c.c.), 1 : 1 aqueous ethanol (5 c.c.), and ethanol (5 c.c.). The combined eluates were filtered and evaporated *in vacuo*, and contaminating ammonium trifluoroacetate removed by microsublimation at 75°/0·5 mm. for 2 days, leaving *ammonium* cis-1 : 2-cyclo*hexylidene phosphate* (Found: C, 36·2; H, 7·2; N, 7·0. $C_6H_{14}O_4NP$ requires C, 36·9; H, 7·2; N, 7·2%).

(*b*) *cis*-2-Hydroxy*cyclo*hexyl dihydrogen phosphate prepared by hydrogenation of its dibenzyl ester (345 mg.) was dried at room temperature and 1 mm. over phosphoric oxide for 48 hr. and then dissolved in pure dry dimethylformamide (5 c.c.). Di*cyclo*hexylcarbodi-imide (217 mg.) in dimethylformamide (5 c.c.) was added slowly with shaking and the whole set aside for 2 days. *NN'*-Di*cyclo*hexylurea was filtered off and the filtrate was evaporated *in vacuo*. The residue was extracted with water, and the solution brought to pH 10 with *cyclo*hexylamine. Evaporation gave the crystalline cyclo*hexylamine salt* (91 mg., 75%), m. p. 182—188° (Found: C, 51·0; H, 8·4; N, 5·3. $C_{12}H_{24}O_4NP$ requires C, 52·0; H, 8·7; N, 5·1%).

Electrometric titration showed that no group in the molecule was titrated at pH 5—8.

trans-1 : 2-cyclo*Hexylidene Hydrogen Phosphate*.—Preparation (*b*), above, was repeated from, as starting material, *trans*-2-hydroxy*cyclo*hexyl dihydrogen phosphate (120 mg.), prepared by passing its *cyclo*hexylamine salt through a short column of Dowex-50 (H form) resin and evaporating the washings to dryness. cyclo*Hexylammonium* cis-1 : 2-cyclo*hexylidene phosphate* (89 mg.) was obtained after two crystallisations from alcohol–ether. The salt did not melt sharply, but was chromatographically homogeneous (Found, in material dried at 80°/0·5 mm.: C, 51·4; H, 8·9; N, 5·2. $C_{12}H_{24}O_4NP$ requires C, 52·0; H, 8·7; N, 5·05%). Electrometric titration demonstrated the absence of secondary phosphoryl dissociation.

cis-1 : 3-cyclo*Hexylidene Bis*(*dihydrogen Phosphate*).—*cis-cyclo*Hexane-1 : 3-diol [33] (5·0 g.) in pyridine was treated in the usual way with dibenzyl phosphorochloridate (from 11·5 g. of dibenzyl phosphite) in carbon tetrachloride. A yellow oil (12·34 g.) was obtained. The oil (1·8 g.) was hydrogenated in ethanol over 10% palladium–charcoal. After filtration and evaporation *in vacuo*, the residual oil was dissolved in water and brought to pH 10 with barium hydroxide, and excess of barium removed by carbon dioxide. After filtration, evaporation gave a colourless residue which was dissolved in the minimum of cold water and the solution filtered. On warming, the solution deposited microcrystalline *dibarium* cis-1 : 3-cyclo*hexylidene bisphosphate* (0·11 g.). For analysis two further recrystallisations were carried out (Found, in

material dried at 50°/0·5 mm.: C, 11·2; H, 3·2; P, 9·6. $C_6H_{10}O_8P_2Ba_2,4H_2O$ requires C, 11·4; H, 2·9; P, 9·9%).

The barium salt was converted into the free acid, obtained as an oil, by treatment with Dowex-50 (H form), and then into the *di*(cyclo*hexylamine*) *salt* which crystallised from aqueous acetone and had m. p. 230—245° with darkening (Found: C, 45·4; H, 8·3; N, 6·3. $C_{18}H_{40}O_8P_2N_2$ requires C, 45·6; H, 8·5; N, 5·9%).

The mother-liquors from the crystallisation of the above barium salt contained another material, faster running on chromatograms, which was evidently the *cyclo*hexane-1 : 3-diol dihydrogen monophosphate. It was separated from residual diphosphate by chromatography on a cellulose column with the propan-2-ol–ammonia solvent. Although chromatographically pure the ammonium salt from the column gave no consistent analyses and there was insufficient material for further purification.

Experiments with *trans-cyclo*hexane-1 : 3-diol showed that analogous mono- and diphosphates were formed but these could not be obtained analytically pure.

cis-1 : 3-cyclo*Hexylidene Hydrogen Phosphate.*—Pure phosphoryl chloride (1·0 c.c.) was dissolved in dry pyridine (9 c.c.). 3·9 c.c. of this solution were added dropwise with shaking to an ice-cold solution of *cis-cyclo*hexane-1 : 3-diol (0·465 g.) in pyridine. The solution, protected from moisture, was set aside overnight at room temperature and then pyridine hydrochloride was filtered off. The solution was poured into water, solvents were removed *in vacuo*, and inorganic phosphate was removed as the barium salt. The solution was freed from barium ions by means of Dowex-50 (H form) and then evaporated to dryness, finally over potassium hydroxide at 0·5 mm. to remove hydrochloric acid. cis-1 : 3-cyclo*Hexylidene hydrogen phosphate* (0·645 g.) remained as a crystalline residue. A sample was purified by crystallisation twice from water and had m. p. 170—172° (Found, in material dried at 100°/0·5 mm.: C, 40·4; H, 6·3. $C_6H_{11}O_4P$ requires C, 40·3; H, 6·2%).

The cyclo*hexylamine salt* crystallised from aqueous acetone (Found: C, 51·8; H, 8·45; N, 5·2. $C_{12}H_{24}O_4NP$ requires C, 52·0; H, 8·45; N, 5·0%).

Electrometric titration demonstrated the absence of buffering by the hydrogen phosphate in the pH range 5—8. The substance, as judged by paper chromatography, was stable to 30% sodium hydroxide solution at 100° but was slowly hydrolysed by 3N-hydrochloric acid at 100° with liberation of inorganic phosphate.

cis-1 : 3-cyclo*Hexylidene Methyl Phosphate.*—The above acid (74 mg.), in methanol (5 c.c.), was treated in the cold with ethereal diazomethane until a permanent yellow colour remained. After 1 hr. solvents were removed at 35° and the residue was crystallised from light petroleum (b. p. 40—60°). The *methyl ester* formed rosettes of needles (50 mg.), m. p. 120—127° (Found: C, 43·7; H, 6·5. $C_7H_{13}O_4P$ requires C, 43·7; H, 6·8%).

The ester was hydrolysed by aqueous-methanolic N-hydrochloric acid at 100° in 1 hr. to 1 : 3-*cyclo*hexylidene phosphate. With N-sodium hydroxide it formed the cyclic phosphate (1 part) and another compound (2 parts), probably *cis*-3-hydroxy*cyclo*hexyl methyl hydrogen phosphate, which travelled slightly faster on chromatograms and was stable to further treatment with alkali.

trans-3-*Hydroxy*cyclo*hexyl Dihydrogen Phosphate.*—*trans-cyclo*Hexane-1 : 3-diol monobenzoate[33] (4·0 g.) was phosphorylated in the usual way with dibenzyl phosphorochloridate (1 mol.). The neutral product was isolated as a yellow oil (4·5 g.), presumably crude 3-benzoyloxy*cyclo*hexyl dibenzyl phosphate. It (1·0 g.) was hydrogenolysed in ethanol (50 c.c.) over palladised charcoal. Removal of catalyst and solvent left an oil, which had $R_F$ 0·30 in solvent system A ($R_F$ of the dibenzyl phosphate 0·94), presumably 3-benzoyloxy*cyclo*hexyl dihydrogen phosphate. Removal of the benzoyl group was effected by heating the oil in 0·5N-sodium hydroxide for 1·5 hr. under reflux. After cooling, the solution was stirred with excess of Dowex-50 (H form). Benzoic acid was removed by ether-extraction and the aqueous layer was neutralised with *cyclo*hexylamine. The solid salt was triturated with ethanol. An insoluble residue of *cyclo*hexylamine phosphate was removed and di*cyclo*hexylamine *trans*-3-hydroxy*cyclo*hexyl phosphate separated from the filtrate on concentration. It formed needles of the *dihydrate*, m. p. 200—203° (from ethanol), $R_F$ (in solvent A) 0·19 (Found, in material dried at 80°/0·5 mm.: C, 50·6; H, 9·9; N, 6·5. $C_6H_{13}O_5P,2C_6H_{13}N,2H_2O$ requires C, 50·3; H, 10·0; N, 6·5%).

When the above crude benzoyloxy*cyclo*hexyl dibenzyl phosphate was hydrolysed by 5%

[33] Clarke and Owen, *J.*, 1950, 2103.

aqueous-alcoholic sodium hydroxide at 100° for 1·5 hr., it gave rise to a product with $R_F$ 0·83 in solvent A, which was stable to further alkali treatment. The substance, presumably *trans*-3-hydroxy*cyclo*hexyl benzyl hydrogen phosphate, could not however be obtained pure.

*Hydrolysis of Some* cyclo*Hexyl Phosphate Esters.*—(*a*) trans-2-*Hydroxy*cyclo*hexyl benzyl hydrogen phosphate.* The *cyclo*hexylammonium salt [20] (155 mg.) was heated in N-sodium hydroxide (10 c.c.) under reflux for 25 hr. Cations were removed by treatment with Dowex-50 resin (H form), and the solution was then taken to dryness. The residue was dissolved in ethanol, and *trans*-2-hydroxy*cyclo*hexyl dihydrogen phosphate was isolated as its *cyclo*hexylamine salt (102 mg.), identified by comparison of its infrared spectrum and m. p. with that of an authentic sample.

(*b*) cis-2-*Hydroxy*cyclo*hexyl benzyl hydrogen phosphate.* The *cyclo*hexylamine salt (55 mg.) was treated as above for the *trans*-isomer. The product, isolated as its *cyclo*hexylamine salt, was identified as *cis*-2-hydroxy*cyclo*hexyl dihydrogen phosphate, as above.

A chromatographic study of the hydrolysis of the above compounds showed that for the *cis*-isomer in N-sodium hydroxide at 100° half-hydrolysis occupied about 1 hr., reaction being complete in $<5$ hr. The *trans*-isomer was only about half-hydrolysed in 2·25 hr. With N-hydrochloric acid at 100° hydrolysis of the *cis*-isomer was complete in 20 min., but 2 hr. were required for the *trans*-isomer. No benzyl phosphate was produced under any of these conditions. A more exact study of hydrolysis rates of these and other related phosphate esters is in progress.

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