805. *Cyclitols. Part X.*[1] *Myoinositol Phosphates.*

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Syntheses of myoinositol 4- and 5-phosphate, and of the 1,4-, 1,6-, and 4,5-diphosphate, are described. Heating in N-hydrochloric acid causes phosphate migration from all positions, the migration being faster between *cis*- than between *trans*-hydroxyl groups.

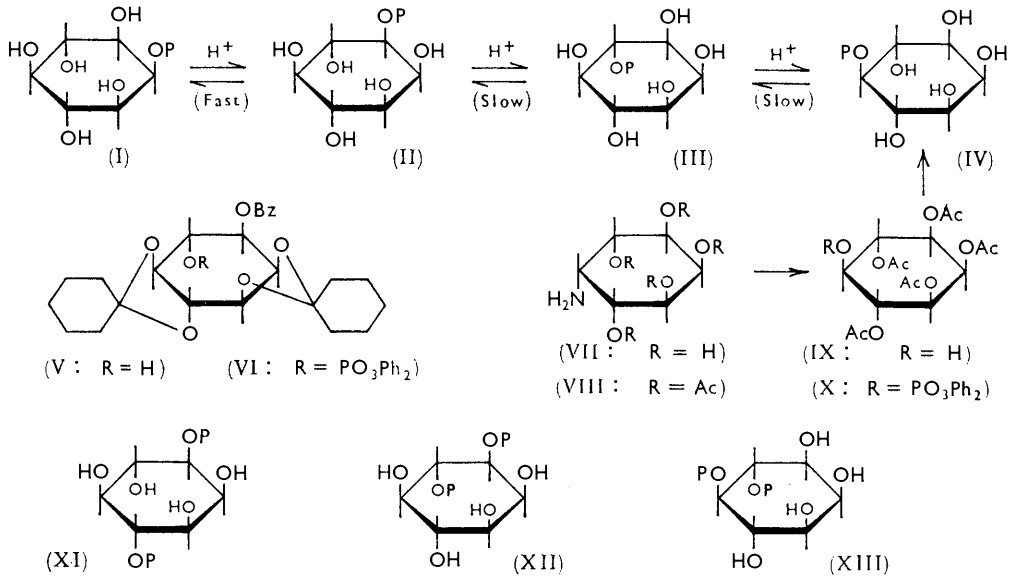
Phospholipids containing myoinositol, often called phosphoinositides, form a diverse group of products widely distributed in Nature.[2] On hydrolysis they yield, amongst other compounds, myoinositol and a monophosphate of myoinositol. In the case of a phospholipid isolated from brain, it was claimed by Folch [3] that a myoinositol diphosphate is obtained by hydrolysis; Grado and Ballou [4] have recently found that, in fact, a mixture is produced from which they separated two triphosphates, two diphosphates, and a monophosphate of myoinositol. In order to identify these products, and to study their behaviour under the conditions employed for the hydrolysis of phospholipids, we have synthesised those myoinositol monophosphates which were not previously known; three diphosphates have also been synthesised.*

Myoinositol 2-phosphate (I) has been known for a long time. It has been prepared [6] by the phosphorylation of 1,3,4,5,6-penta-*O*-acetylmyoinositol, and by the partial hydrolysis of phytic acid (myoinositol hexaphosphate) by chemical [7] and by enzymic means.[8] Contrary to earlier claims,[9] it is now well established [10,11] that each of these methods yields the same monophosphate. Hydrolysis of liver or soya-bean phospholipids gives a monophosphate which differs from the 2-phosphate, but the difference was not clearly recognised until Pizer and Ballou [10] showed that this phosphate is optically active. By synthesising its enantiomorph, Ballou and Pizer proved [12] that the product obtained from natural sources is (1*R*)-myoinositol 1-phosphate (II). Its racemate was prepared from the 2-phosphate by phosphate migration in acid solution,[13,14] and by the formation and opening of the cyclic 1,2-phosphate.[10,15]

* A preliminary account of part of this work has appeared.[5]

[1] Part IX, Angyal, Tate, and Gero, preceding paper.
[2] For a review, see Hawthorne, *J. Lipid Res.*, 1960, **1**, 255.
[3] Folch, *J. Biol. Chem.*, 1949, **177**, 497, 505.
[4] Grado and Ballou, *J. Biol. Chem.*, 1960, **235**, PC23.
[5] Angyal, Murdoch, and Tate, *Proc. Chem. Soc.*, 1960, 416.
[6] Iselin, *J. Amer. Chem. Soc.*, 1949, **71**, 3822.
[7] Courtois and Masson, *Bull. Soc. chim. biol.*, 1950, **32**, 314; Desjobert, *ibid.*, 1954, **36**, 1293.
[8] McCormick and Carter, *Biochem. Prep.*, 1952, **2**, 65.
[9] Fleury, Desjobert, and Lecocq, *Bull. Soc. Chim. biol.*, 1954, **36**, 1301.
[10] Pizer and Ballou, *J. Amer. Chem. Soc.*, 1959, **81**, 915.
[11] Brown and Hall, *J.*, 1959, 357.
[12] Ballou and Pizer, *J. Amer. Chem. Soc.*, 1960, **82**, 3333.
[13] Posternak, *Helv. Chim. Acta*, 1959, **42**, 390.
[14] Brown, Hall, and Letters, *J.*, 1959, 3547.
[15] Posternak, *Helv. Chim. Acta*, 1958, **41**, 1891.

The synthesis of inositol phosphates depends on the availability of myoinositol derivatives in which only one hydroxyl group is unsubstituted. Apart from the previously mentioned 1,3,4,5,6-penta-acetate, no such derivative of myoinositol has been described. The preceding paper contains the preparation of 3-*O*-benzoyl-1,2:5,6-di-*O*-cyclohexylidene-myoinositol (V); this compound served as an intermediate in the synthesis of myoinositol



[P = ·PO(OH)$_2$.]

[Compounds are racemates unless otherwise stated.]

4-phosphate. Treatment with diphenyl phosphorochloridate in pyridine gave the corresponding 4-(diphenyl phosphate) (VI). On catalytic hydrogenation, this compound lost the phenyl groups, and the acidity of the resulting free phosphate caused a partial loss of the cyclohexylidene groups as well. The ketal groups were completely removed by mild acid hydrolysis, and the benzoate group by alkaline hydrolysis; (±)-myoinositol 4-phosphate (III) was isolated as the biscyclohexylammonium salt. This isomer was found to be structurally identical with the optically active monophosphate obtained from brain phosphoinositide by Grado and Ballou.[4]

In an analogous manner, 4-*O*-benzoyl-1,2:5,6-di-*O*-cyclohexylidenemyoinositol was converted into the corresponding 3-(diphenyl phosphate); but this route to the synthesis of the 1-phosphate was not pursued further as the starting material is not readily prepared in quantity.

Many unsuccessful attempts have been made to prepare from myoinositol a derivative in which only the 5-hydroxyl group was unsubstituted. A round-about way was finally found which utilises 2-amino-2-deoxyneoinositol (VII) as starting material. This inosamine has been synthesised [16] but is more readily available by the hydrolysis of the antibiotic hygromycin A.[17] On treatment with nitrous acid the inosamine (VII) is partially converted into myoinositol,[17] the replacement of the amino- by a hydroxyl group occurring exclusively by inversion. We found that the penta-*O*-acetate (VIII) of the inosamine gives 1,2,3,4,6-penta-*O*-acetylmyoinositol (IX) in 33% yield on treatment with nitrous acid. (The deamination of inosamines is being studied and will be the subject of a forthcoming communication.)

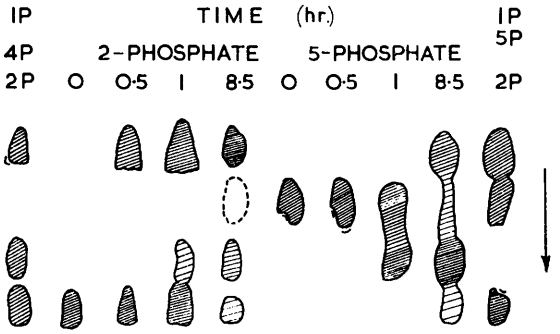
[16] Allen, *J. Amer. Chem. Soc.*, 1956, **78**, 5691.

[17] Patrick, Williams, Waller, and Hutchings, *J. Amer. Chem. Soc.*, 1956, **78**, 2652; Mann and Woolf, *ibid.*, 1957, **79**, 120.

Phosphorylation of the penta-acetate (IX) with diphenyl phosphorochloridate gave the corresponding 5-(diphenyl phosphate) (X); after hydrogenolysis and deacetylation, myoinositol 5-phosphate (IV) was isolated as its biscyclohexylammonium salt.

The melting points of the myoinositol phosphates, or of their salts, are not suitable criteria for their characterisation [10] since they vary with the rate of heating, and melting is accompanied by decomposition. The isomers can be distinguished, however, by paper chromatography in a mixture of propan-2-ol and concentrated ammonia solution (4 : 1 v/v). The R_F values are low and the movement of the phosphates is slow but after several days' irrigation the four isomers are clearly separated.

When attempts are made to determine, in a phosphoinositide, the position of the phosphate–inositol linkage, the possibility of phosphate migration during hydrolysis must be taken into account. This migration, well-known in the case of the glycerol phosphates,[18] occurs generally in polyols if they contain a free hydroxyl group in a sterically suitable position. Myoinositol 1- and 2-phosphate are thus interconvertible [13,14] and form an



Chromatographic study showing phosphate migration for myoinositol 2- and 5-phosphate in N*-hydrochloric acid at* 80—85°.

equilibrium mixture in acid solution; migration therefore takes place between *cis*-hydroxyl groups in myoinositol. Availability of the other isomers enabled us to show that migration occurs between *trans*-hydroxyl groups as well, albeit more slowly. Boiling 80% acetic acid, suitable for the interconversion of 1- and 2-phosphates, appears to cause no significant amount of *trans*-migration; but N-hydrochloric acid at 80—85° does so. Under these conditions, equilibration of the 1- and the 2-phosphate is virtually complete in 30 minutes; *trans*-migration is noticeable after 1 hour and all the isomers are detected after 8 hours. Fig. 1 illustrates chromatographic evidence of the isomerisation of the 2- and of the 5-phosphate. Migration occurs, therefore, as indicated in formulæ (I) to (IV), right around the cyclitol ring.

Inositol phosphates suffer no migration under alkaline conditions; but alkaline hydrolysis of a phosphoinositide may be accompanied by migration owing to the formation of an intermediate cyclic phosphate.[19] Such migration was observed on alkaline hydrolysis of a sample of an inositol phosphatide isolated from peas,[20] kindly supplied by Dr. H. E. Carter; a mixture of inositol 1- and 2-phosphate was formed similar to that obtained from the inositides of soya bean, beef liver, horse liver, etc. For these materials, attachment of the phosphate group to the 1-position of myoinositol is now well established.[10,21]

There has been some interest in the action of inositol phosphate as a growth factor for micro-organisms. Woolley [22] and McKibbin [23] have both recorded that (structurally not defined) inositol phosphate had partial activity when used to replace myoinositol as

[18] Baer and Kates, *J. Biol. Chem.*, 1950, **185**, 615.
[19] Brown and Higson, *J.*, 1957, 2034.
[20] Wagenknecht, Lewin, and Carter, *J. Biol. Chem.*, 1959, **234**, 2265.
[21] Brown, Clark, Hall, and Letters, *Proc. Chem. Soc.*, 1960, 212.
[22] Woolley, *J. Biol. Chem.*, 1941, **140**, 461.
[23] McKibbin, *J. Biol. Chem.*, 1956, **220**, 537.

a growth factor, but Germanier [24] found the 2-phosphate to be inactive. With the availability of all isomers, the biological activity of the phosphates could be investigated. Dr. Laurens Anderson, of the University of Wisconsin, kindly tested the four phosphates against *Saccharomyces carlsbergensis* and reported that they all had very low activities, between 0·7 and 1·5% of an equivalent amount of myoinositol. Dr. F. D. Collins, of the University of Melbourne, obliged us with a test against " inositol-less " *Neurospora crassa* and found very similar low activities. In each case the 2-phosphate was the least active. Whatever activity the phosphates appear to possess may be caused by myoinositol formed through hydrolysis; and the earlier reports of positive results may have been due to contamination be myoinositol. These results are in accord with the present view that myoinositol phosphates are *not* intermediates in the biosynthesis of phosphoinositides.[25]

Eagle and his co-workers found [26] that myoinositol is necessary for the growth of normal and malignant human cells in tissue culture; they reported different activities for two different samples of inositol phosphates. Dr. Eagle has now tested the four pure isomers and has found that all have activities similar to that of myoinositol, except that the 2-phosphate is somewhat less active. Apparently the tissue cultures contain a phosphatase which liberates myoinositol from all isomers; the axially substituted 2-phosphate would be hydrolysed more slowly.

Because of interest in the " diphosphoinositide " occurring in brain, several myoinositol diphosphates have been synthesised. 1,2:4,5-Di-*O*-cyclohexylidenemyoinositol [1] was readily phosphorylated at both free hydroxyl groups, and removal of the protecting groups then gave myoinositol 1,4-diphosphate (XI). Dr. Ballou has informed us that this compound is structurally identical with one of the optically active diphosphates obtained by hydrolysis of the brain phosphoinositide.

The other dicyclohexylidene ketals reported in the preceding paper [1] have vicinal hydroxyl groups and the outcome of their phosphorylation appeared doubtful. With diphenyl phosphorochloridate, 1,4,5,6-tetra-*O*-acetylmyoinositol [10,15] and 1,2:5,6-di-*O*-isopropylidene-(−)-inositol [27] both give cyclic phosphates, instead of diphosphates; the monophosphate formed initially suffers displacement of a phenyl group by the adjacent hydroxyl group. In the former case, the two hydroxyl groups are in *cis*-relation to each other but in the latter they are in *trans*-relation; nevertheless, distortion of the cyclitol ring by two *cis*-attached ketal rings brings these hydroxyl groups in close proximity,[28] as shown by their ability to form a ketal with acetone. In 1,2:5,6- and in 1,2:3,4-di-*O*-cyclohexylidenemyoinositol, however, the two free *trans*-hydroxyl groups appear to be at the usual distance from each other: phosphorylation gives the diphosphates, myoinositol 1,6-diphosphate (XII) and myoinositol 4,5-diphosphate (XIII). The latter, as Dr. Ballou has informed us, proved to be structurally identical with the second diphosphate obtained from the brain phosphoinositide.

Experimental

M. p.s are corrected.

(±)-3-O-*Benzoyl*-1,2:5,6-*di*-O-*cyclohexylidenemyoinositol* 4-(*Diphenyl Phosphate*) (VI).—3-*O*-Benzoyl-1,2:5,6-di-*O*-cyclohexylidenemyoinositol [1] (4·5 g.) was dissolved, by heat on the steam bath, in a mixture (35 ml.) of diphenyl phosphorochloridate and pyridine (1 : 9 v/v). After being kept overnight, the mixture was heated on the steam bath for 2·5 hr., then cooled and poured on ice. The precipitated *diphenyl phosphate* crystallised from ethanol in needles (4·7 g., 73%), m. p. 153°; recrystallisation from ethanol raised the m. p. to 155° (Found: C, 65·75; H, 6·3. $C_{37}H_{41}O_{10}P$ requires C, 65·65; H, 6·1%).

(±)-*Myoinositol* 4-(*Dihydrogen Phosphate*) (III).—The above diphenyl ester (4·5 g.) was

[24] Germanier, *Arch. Mikrobiol.*, 1959, **33**, 337.
[25] Agranoff, Bradley, and Brady, *J. Biol. Chem.*, 1958, **233**, 1077; Paulus and Kennedy, *ibid.*, 1960, **235**, 1303.
[26] Eagle, Oyama, Levy, and Freeman, *J. Biol. Chem.*, 1957, **226**, 191.
[27] Kilgour and Ballou, *J. Amer. Chem. Soc.*, 1958, **80**, 3956.
[28] Angyal and Macdonald, *J.*, 1952, 686.

shaken with Adams platinum oxide catalyst (250 mg.) in ethanol (500 ml.) under hydrogen until no more hydrogen was consumed. On evaporation *in vacuo*, the solution gave an oil the weight (3·83 g.) of which indicated partial hydrolysis of the ketal groups. The oil was dissolved in boiling methanol (35 ml.), the solution was filtered, and N-methanolic barium methoxide solution (30 ml.) was added; after 10 min. the mixture, which had set to a jelly, was heated on a steam bath for 30 min. After addition of water (100 ml.) and removal of the methanol by distillation, the mixture was cooled and freed from barium by gradual addition of N-sulphuric acid. The solution (pH approx. 2) was filtered and extracted with ether (4 × 30 ml.) in order to remove benzoic acid. Cyclohexylamine (2 ml.) was added, the solution evaporated *in vacuo*, and the residue was crystallised several times from water–acetone to give needles (0·94 g., 30%) of *myoinositol* 4-(*biscyclohexylammonium phosphate*), m. p. 206—207° (decomp.) (Found: C, 46·5; H, 8·7; N, 6·15. $C_{18}H_{39}O_9N_2P,\frac{1}{2}H_2O$ requires C, 46·15; H, 8·6; N, 6·0%). The material was chromatographically homogeneous. The mother-liquors yielded two more crops of slightly impure material (0·78 and 0·62 g., total yield, 78%).

(±)-4-O-*Benzoyl*-1,2:5,6-*di*-O-*cyclohexylidenemyoinositol* 3-(*Diphenyl Phosphate*).—4-*O*-Benzoyl-1,2:5,6-di-*O*-cyclohexylidenemyoinositol [1] (52 mg.) was heated on the steam bath for 6 hr. with a solution (0·4 ml.) of diphenyl phosphorochloridate in pyridine (1 : 9 v/v). The mixture was then poured on ice, and the precipitated *diphenyl phosphate* was crystallised twice from ethanol: needles (26 mg., 33%), m. p. 153°, were obtained (Found: C, 65·3; H, 6·4. $C_{37}H_{41}O_{10}P$ requires C, 65·65; H, 6·1%).

1,2,3,4,6-*Penta*-O-*acetylmyoinositol* (IX) (with Mr. J. S. Murdoch).—A mixture of finely powdered 5-amino-5-deoxyneoinositol hydrochloride (9·8 g.), acetyl bromide (35·4 g.), and chloroform (25 ml.) was stirred under a reflux condenser at 50° until the evolution of hydrogen bromide had ceased (*ca.* 2 hr.). The mixture was then evaporated to dryness *in vacuo* to give penta-*O*-acetyl-5-amino-5-deoxyneoinositol hydrobromide (20·8 g.) as a white powder. To a solution of this compound (20 g.) in water (340 ml.), a solution of sodium nitrite (3·2 g.) in 0·05N-hydrochloric acid (10 ml.) was added dropwise with stirring at 0°: nitrogen was evolved and a sticky precipitate separated in a few minutes. After 1·5 hr. the mixture was extracted with chloroform (4 × 100 ml.), and the extracts were dried (Na_2SO_4) and evaporated. The remaining yellow syrup was dissolved in the minimum amount of benzene and set aside at 0°: 1,2,3,4,6-*penta*-O-*acetylmyoinositol* (5·1 g., 33%) crystallised. After one recrystallisation from ethyl acetate–light petroleum it had m. p. 178° (Found: C, 49·3; H, 5·7. $C_{16}H_{22}O_{11}$ requires C, 49·2; H, 5·7%).

Myoinositol 5-(*Dihydrogen Phosphate*) (IV).—A mixture of 1,2,3,4,6-penta-*O*-acetylmyoinositol (1·09 g.), diphenyl phosphorochloridate (2·25 g.), and dry pyridine (3·1 ml.) was heated for 10 hr. on the steam bath. On cooling, crystals appeared; methanol (5 ml.) was added, and after 1 hr. the 1,2,3,4,6-*penta*-O-*acetylmyoinositol* 5-(*diphenyl phosphate*) (X) (1·22 g., 70%), m. p. 166—167°, was filtered off. Recrystallisation from anhydrous ethanol raised the m. p. to 168° (Found: C, 53·9; H, 5·0. $C_{28}H_{31}O_{14}P$ requires C, 54·0; H, 5·0%).

The diphenyl ester (1·09 g.) was hydrogenated in anhydrous ethanol (25 ml.) in the presence of Adams platinum oxide catalyst (65 mg.): 8·3 mol. of hydrogen were taken up, mostly in the first hour. The solution was filtered and evaporated to give a colourless solid (0·81 g., 98%), m. p. 229° (decomp.). Crystallisation from ethanol (3 ml.) gave 1,2,3,4,6-*penta*-O-*acetylmyoinositol* 5-(*dihydrogen phosphate*) as needles, m. p. 229° (Found: C, 39·7; H, 5·1. $C_{16}H_{23}O_{14}P,H_2O$ requires C, 39·35; H, 5·1%).

The penta-acetate (0·54 g.) was dissolved in hot methanol (20 ml.), and methanolic N-barium methoxide (4·5 ml.) was added, causing immediate precipitation. After 5 minutes' heating on the steam bath, water (10 ml.) was added and the solution was concentrated *in vacuo*. The residue was redissolved in water (10 ml.), a solution of cyclohexylammonium sulphate (0·66 g.) in water (10 ml.) was added and, after a short period of heating to coagulate the barium sulphate, the solution was filtered and evaporated to dryness *in vacuo*. The residue was dissolved in water (2 ml.) containing a few drops of cyclohexylamine, and acetone (3 ml.) was added: on storage at 0° needles (0·39 g., 79%), m. p. 207—209° (decomp.), were deposited. Recrystallisation from water (1·5 ml.) by addition of acetone (10 ml.) gave the *myoinositol* 5-(*biscyclohexylammonium phosphate*), m. p. 209—211° (decomp.) (Found: C, 44·95; H, 8·6; N, 6·5. $C_{18}H_{39}O_9N_2P,H_2O$ requires C, 45·3; H, 8·7; N, 5·9%).

(±)-*Myoinositol* 1,4-*Bis*(*dihydrogen Phosphate*) (XI).—1,2:4,5-Di-*O*-cyclohexylidenemyoinositol [1] (1·7 g.) was heated on the steam bath with diphenyl phosphorochloridate (2·0 ml.) and

anhydrous pyridine (6 ml.) until it dissolved. The mixture was then kept for 2 days, whereupon it set solid. After addition of ice and water, the mixture was filtered, and the solid product (3·5 g.) was dissolved in hot ethanol (300 ml.). The filtered solution was concentrated to 150 ml. *in vacuo* and stored at 0°, whereupon it deposited 1,2:4,5-*di*-O-*cyclohexylidenemyoinositol* 3,6-*bis*(*diphenyl phosphate*) (2·17 g., 54%), m. p. 181—182°. Recrystallisation from ethanol raised the m. p. to 183° (Found: C, 62·25; H, 5·65. $C_{42}H_{46}O_{12}P_2$ requires C, 62·7; H, 5·75%).

When only one equiv. of phosphorylating agent was used the bisphosphate was obtained in 34% yield but no monophosphate could be isolated.

The bisphosphate (204 mg.) was hydrogenated in ethanol (50 ml.) in the presence of Adams catalyst (102 mg.) for 64 hr.; 18 mol. of hydrogen were taken up (which is the theoretical value if it is assumed that the liberated cyclohexanone was also reduced). After filtration, cyclohexylamine (0·5 ml.) was added and the solution was set aside at 0°, whereupon crystals (49 mg.) separated. Addition of acetone produced a second crop (88 mg., total yield 83%). Recrystallisation from water by addition of acetone to incipient turbidity gave needles of the *triscyclohexylammonium salt* of *myoinositol* 1,4-*bis*(*dihydrogen phosphate*), decomp. 186—197° (Found: C, 44·65; H, 8·65; N, 5·85, 6·1. $C_{24}H_{53}O_{12}N_3P_2,\frac{1}{2}H_2O$ requires C, 44·6; H, 8·4; N, 6·5%).

(±)-*Myoinositol* 1,6-*Bis*(*dihydrogen Phosphate*) (XII).—A mixture of 1,2:5,6-di-*O*-cyclohexylidenemyoinositol [1] (340 mg.) and a 20% (v/v) solution of diphenyl phosphorochloridate in pyridine (3 ml.) was set aside for 18 hr. and then poured on ice. The resulting gum, which was washed with water (5 × 20 ml.), did not crystallise. It was hydrogenated in ethanol (80 ml.) in the presence of Adams catalyst (0·2 g.) for 72 hr. at 20°; 496 ml. of hydrogen were taken up. Addition of cyclohexylamine and acetone to the filtered solution caused precipitation of a colourless solid (0·48 g.) which crystallised from water–acetone in needles, m. p. 196—197°. Paper chromatography and analysis showed that one cyclohexylidene group was still present; the compound is presumably the *triscyclohexylammonium salt* of 1,2-O-*cyclohexylidenemyoinositol* 3,4-*bis*(*dihydrogen phosphate*) (Found: C, 47·2; H, 8·6; N, 5·6; P, 8·3. $C_{30}H_{61}O_{12}N_3P_2,3H_2O$ requires C, 46·9; H, 8·4; N, 5·45; P, 8·05%). A solution of this compound (180 mg.) in water (4 ml.) was passed through a column of ZeoKarb 225 and then heated for 30 min. at 100°. Addition of cyclohexylamine and acetone gave crystals of the *tricyclohexylammonium salt* of *myoinositol* 1,6-*bis*(*dihydrogen phosphate*) (115 mg.) which recrystallised from water–acetone and then melted with decomposition at 161—163° (Found: C, 42·9; H, 8·65; N, 6·4. $C_{24}H_{53}O_{12}N_3P_2,2H_2O$ requires C, 42·8; H, 8·45; N, 6·25%).

It is noteworthy that under the conditions described above the cyclohexylidene groups can be removed without simultaneous phosphate migration. The product was chromatographically homogeneous.

(±)-*Myoinositol* 4,5-*Bis*(*dihydrogen Phosphate*) (XIII) (with Mr. S. D. Gero).—A mixture of 1,2:3,4-di-*O*-cyclohexylidenemyoinositol [1] (350 mg.) and a 20% (v/v) solution of diphenyl phosphorochloridate in pyridine (3 ml.) was kept for 18 hr., and then poured on ice. The resulting gum can be crystallised from ethanol; it was not isolated, however, but was hydrogenated in ethanol with Adams catalyst (275 mg.) for 75 hr. at 20°: 315 ml. of hydrogen were taken up. The filtered solution was boiled for 30 min. and then evaporated; the residue was dissolved in water (4 ml.). Addition of cyclohexylamine (1 ml.) and then of acetone (80 ml.) gave crystals of the *triscyclohexylammonium salt* of *myoinositol* 4,5-*bis*(*dihydrogen phosphate*) (312 mg., 45%) which melted at 182—183° (with decomposition) after recrystallisation from water–acetone (Found: C, 42·8; H, 8·6; N, 6·2. $C_{24}H_{53}O_{12}N_3P_2,2H_2O$ requires C, 42·8; H, 8·45; N, 6·25%).

Paper Chromatography of Phosphates.—Descending chromatograms were run on Whatman No. 1 paper essentially by the method of Pizer and Ballou.[10] Control of temperature is desirable because the solvent composition required for optimum separation depends on it; higher temperatures (30—40°) require a higher proportion of propan-2-ol. The slower-moving diphosphates are better separated by a solvent mixture containing less (50—60%) propan-2-ol. Separation requires 3—4 days. The monophosphates were applied as 2%, the diphosphates as 3% solutions of their cyclohexylammonium salts. Alkaline silver nitrate [29] was used for detection of the spots; the sensitivity was increased, when necessary, by holding the paper over a steam bath for a short time after application of the ethanolic sodium hydroxide solution. Movement of the phosphates is given relative to that of myoinositol (R_M) in the following two examples:

(i) Propan-2-ol–ammonia (*d* 0·88) (4 : 1 v/v), 28°, 96 hr.; myoinositol was found 43·0 cm.

[29] Anet and Reynolds, *Nature*, 1954, **174**, 930.

from the origin. R_M values: myoinositol 1-phosphate, 0·26; 2-phosphate, 0·34; 4-phosphate, 0·32; 5-phosphate, 0·29.

(ii) Propan-2-ol–ammonia (6 : 4), 26°, 39 hr.; myoinositol was found 38 cm. from the origin. R_M values: myoinositol 1-phosphate, 0·68; 2-phosphate, 0·78; 4-phosphate, 0·73; 5-phosphate, 0·71; 1,4-diphosphate, 0·38; 1,6-diphosphate, 0·46; 4,5-diphosphate, 0·56.

Phosphate Migration.—The monophosphates (3 mg.) were heated in N-hydrochloric acid (0·1 ml.) at 80°. Samples were removed at intervals and were chromatographed in propan-2-ol–ammonia (7 : 3) at 16—22° for 14 days. Some of the results are shown in Fig. 1. The 4-phosphate showed the presence of the 1- and the 5-isomer after 1 hr., and all isomers were discernible after 8·5 hr.

When the 1,4-diphosphate was treated with refluxing 80% acetic acid for 1 hr., a new spot appeared at R_M 0·49 in addition to the original one at 0·41 (propan-2-ol–ammonia–water, 5 : 4 : 1, 22°, 72 hr.). Since *trans*-migration does not occur to any significant extent under these conditions, the new compound is presumably myoinositol 2,4-diphosphate.

Hydrolysis of the Phosphoinositide from Peas.—The inositide (19 mg.) was heated under reflux with 2N-potassium hydroxide (1 ml.) for 1 hr.[10] The cooled solution was treated with ZeoKarb 225, and the acid eluate was extracted with ether, made alkaline with cyclohexylamine, and evaporated to dryness. The residue was extracted with ethanol; on chromatography both the extract and the insoluble residue showed the presence of myoinositol and its 1- and 2-phosphate. There was no detectable amount of 4- or 5-phosphate.

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