was placed on the column and a chromatogram developed using the double mixing chamber technique. Several individual (each 25 ml.) or pooled collections, each containing from 3 to 7 mg. of DNA which together amounted to 41% of the original sample, were selected for base analysis. The calculated composition of the solutions used to elute these fractions is given in Table II. The fractions were dialyzed (with less than 10% loss) against distilled water to remove salts and concentrated to small volume (*ca.* 2 ml.) *in vacuo* in a rotating evaporator. The chilled concentrates were adjusted to about 0.1 *M* NaCl by addition of solid NaCl and two volumes of ethanol added to precipitate the DNA. The fibrous precipitates were collected by centrifugation, washed with ethanol and ether and dried. They were hydrolyzed with 88% formic acid for base composition by paper chromatographic analysis[66] and phosphorus estimation.[77] The base analyses are listed in Table II. The average molar recovery based upon phosphorus was 95% (see ref. 18).

The molar base ratios (with adenine taken as 1.00), which are the average of six determinations, show a deviation of less than 2% from the average, and, taken together with the recovery of 95%, the variations in base composition are considered to be experimentally significant. It would appear that the column procedure has effected a separation, from the total sample, of fractions of DNA of varying composition. When the adenine to thymine and guanine to cytosine ratios are computed, significant departures from the widely-accepted (see ref. 18) value of unity are noted (Table II). This procedure would therefore appear to have a basis of separation that is different from those of other published methods[7-9,11,12] all of which yield fractions of progressively changing base composition which, however, still show very little departure from the unity ratio for the base pairs listed above.

(77) C. H. Fiske and Y. SubbaRow, *J. Biol. Chem.*, **66**, 375 (1925).

To ascertain whether the dialysis of the DNA fractions had altered their base compositions, the original DNA was exhaustively dialyzed against distilled water, precipitated and analyzed as above. No significant change was seen in the base composition.

A discussion of the possible significance of these data in terms of the Watson–Crick double helix formulation of DNA[78,79] has appeared.[20] A noteworthy feature of these data is the average ratio *total* purines to *total* pyrimidines, which, for these fractions, is 0.98 ± 0.03.

b. **Other Properties.**—Fractions obtained with eluents of increasing ionic strength and increasing *p*H have been found to show increasing sedimentation coefficients when examined (*cf.* ref. 44, 43) in the analytical ultracentrifuge equipped with ultraviolet optics. Upon alkalinization to *p*H *ca.* 13, such fractions have shown the hyperchromic effects at 260 mμ expected of "undenatured" DNA.[66] The results of these various studies as well as those dealing with the problem of rechromatography will be presented elsewhere. Preliminary accounts of these studies have appeared.[20,40,80,81]

Acknowledgments.—The authors take pleasure in acknowledging the advice and valuable help of Dr. Sam M. Beiser, Dr. George B. Brown, Dr. Giampiero di Mayorca and Dr. C. P. Rhoads.

(78) J. D. Watson and F. H. C. Crick, *Nature*, **171**, 737 (1953); *Cold Spring Harbor Symposia Quant. Biol.*, **18**, 123 (1953).

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(81) H. S. Rosenkranz and A. Bendich, *Federation Proc.*, **17**, 299 (1958).

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Inositol Phosphates: Pinitol 4-Phosphate and (−)-Inositol 3-Phosphate

BY GORDON L. KILGOUR AND CLINTON E. BALLOU

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The synthesis of 3-O-methyl-(+)-inositol 4-phosphate and (−)-inositol 3-phosphate are described. These substances were prepared as model compounds for the study of the properties of inositol phosphates in general.

myo-Inositol occurs in nature both free and in conjugated forms. Of the latter, which may represent up to 90% of the total, the phosphate esters are a major component.[1] Prominent among these phosphate compounds are the hexaphosphoric acid ester (phytic acid) and the widely-distributed inositol-containing phospholipids, from which inositol mono- or diphosphates are obtained on hydrolysis.

myo-Inositol mono- or diphosphates have been isolated (in varying degrees of purity) from inositol phospholipids of brain, heart, liver, wheat germ and soya beans.[2] Others have been obtained from chemical and enzymatic hydrolysis of phytic acid.[3] One compound, believed to be *myo*-inositol 2-phosphate, has been synthesized by the phosphorylation of 1,3,4,5,6-penta-O-acetyl-*myo*-inositol.[4] It has since been found that this pentaacetate undergoes a base-catalyzed acetyl migration from the equatorial 1- or 3-position to the axial 2-position during methylation with methyl iodide and silver oxide.[5] In fact, Angyal, *et al.*, have made use of a similar migration to prepare 1,3-di-O-methyl-*myo*-inositol from the (±)-1,4,5,6-tetraacetate.[6] Since the phosphorylation step in the above synthesis[4] required a high temperature and was carried out in pyridine, there is a possibility that the product is actually the racemic (±)-1-phosphate.[7]

The migration of phosphate groups during the isolation of naturally-occurring inositol phosphates is very likely. The possibility of migration was mentioned by Malkin and Poole[8a] while Hawthorne and Chargaff[8b] discounted the possibility of migra-

(1) P. Fleury and P. Balatre, "Les Inositols," Masson et Cie., Paris, 1947.

(2) See review by J. Folch and L. N. LeBaron, *Can. J. Biochem. and Physiol.*, **34**, 305 (1956).

(3) J. Courtois, *Bull. soc. chim. biol.*, **33**, 1061 (1951); A. Desjobert, *ibid.*, **36**, 1293 (1954); M. H. McCormick and H. E. Carter, *Biochem. Preps.*, **2**, 65 (1952).

(4) B. M. Iselin, THIS JOURNAL, **71**, 3822 (1949).

(5) L. Anderson and A. M. Landel, *ibid.*, **76**, 6130 (1954).

(6) S. J. Angyal, P. T. Gilham and C. G. Macdonald, *J. Chem. Soc.*, 1417 (1957).

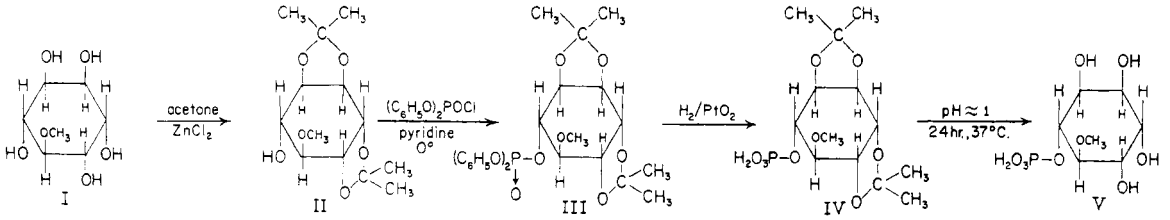
(7) We have, however, found that 1,3,4,5,6-penta-O-acetyl-*myo*-inositol can be heated in pyridine under the conditions of phosphorylation used by Iselin, and can be recovered unchanged. By another procedure we have been able to prepare what is probably (±)-*myo*-inositol 1-phosphate, and shown it to be different from the Iselin phosphate. Thus, his compound must be *myo*-inositol 2-phosphate.

(8) (a) T. Malkin and A. G. Poole, *J. Chem. Soc.*, 3470 (1953); (b) J. N. Hawthorne and E. Chargaff, *J. Biol. Chem.*, **206**, 27 (1954).

tion because of the relatively large oxygen–oxygen distance in *myo*-inositol (chair form). While the current investigation was in progress, Brown and Higson[9] showed that it is possible to make the cyclic phosphates of *cis*- and *trans*-cyclohexanediols, while Khorana, *et al.*,[10] have demonstrated that the phosphate group can cyclize across *trans*-hydroxyl groups in positions 1 and 2 of the glucose molecule. Brown and Higson also presented evidence that the neighboring hydroxyl group in both the *cis* and *trans* forms participates in the hydrolysis of the cyclohexanediol dibenzyl phosphates. Such a conclusion can be drawn, also in the case of the inositol phosphates, from the fact that the alkaline hydrolysis of the glycerol *myo*-inositol phosphate diester from phospholipids yields inositol phosphate as well as glycerol phosphate.[2]

In this paper we are reporting the synthesis of monophosphates of (+)- and (−)-inositol to be used as model compounds for the study of the properties of inositol phosphates in general.

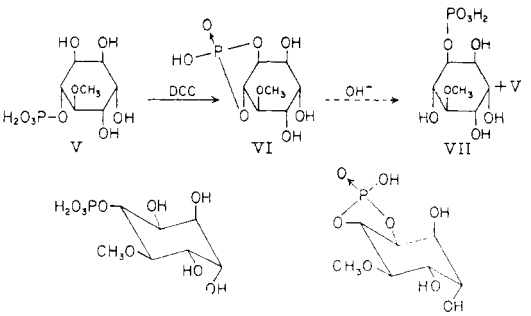
Pinitol 4-phosphate (3-O-methyl-(+)-inositol 4-phosphate) (V) has been prepared, as the crystalline cyclohexylammonium salt, by the route



Phosphorylation of the known 1,2:5,6-di-O-isopropylidene-pinitol (II),[11] obtained by acetonation of pinitol (I), gave 1,2:5,6-di-O-isopropylidene-pinitol 4-O-diphenylphosphate (III). Hydrogenolysis of III with platinum and hydrogen gave 1,2:5,6-di-O-isopropylidenepinitol 4-phosphate (IV), which on mild acid hydrolysis was deacetonated to pinitol 4-phosphate (V). The conditions of acid hydrolysis have been shown not to cause phosphate migration,[12] a result which was confirmed in this case by the fact that the pinitol 4-phosphate (V) consumed exactly three moles of periodate per mole of compound. This compound (V) is, however, well suited to the study of phosphate migrations between "*trans*" equatorial hydroxyls, since any shift in position of the phosphate will result in a product with a decreased uptake of periodate. Such a study is now in progress.

The pinitol 4-phosphate (V) also was used to examine the possibility of preparing new phosphates by "forced migration" of the phosphate group. This involves a cyclization onto a neighboring hydroxyl group, using a carbodiimide reagent,[13] and the subsequent hydrolysis of the cyclic material and separation of the two possible isomeric products. Using dicyclohexylcarbodiimide (DCC), a cyclic product has been prepared from V that is tentatively identified as pinitol 4,5-phosphate (VI). We have been unable to separate the products of base hydrolysis of VI into the two possible pinitol phosphates (V and VII). This difficulty is in accordance with the findings of others[14] that they were unable to separate various isolated *myo*-inositol monophosphates even though the compounds differed as to infrared and X-ray analysis. The most probable conformational forms of V and VI are represented above, and indicate the close proximity of the equatorial hydroxyl groups on carbons 4 and 5, even though they occupy *trans* positions. That the cyclic phosphate VI has a five-membered ring is indicated by the ease of hydrolysis with acid and base to the acyclic product.[10]

We also have carried out the phosphorylation of 1,2:5,6-di-O-isopropylidene-(−)-inositol (VIII)[10b,15] with diphenyl phosphorochloridate. Compound VIII contains a *trans* pair of adjacent hydroxyl groups both in equatorial position. Although we had hoped for mono- and diphosphorylation, depending on the amount of phosphorylating reagent employed, we obtained only the unusual cyclic phosphate IX (1,2:5,6-di-O-isopropylidene-(−)-inositol 3,4-phenylphosphate). This product probably results from an initial phosphorylation



to give the diphenylphosphonate, which then undergoes elimination of phenol by attack on the phosphorus atom by the adjacent free hydroxyl group. Such a reaction was proposed by Brown and Todd[16] to account for the properties of certain phosphate tri-esters. However, to our knowledge the isolation of a 5-membered cyclic intermediate comparable to IX from a phosphorylation reaction has not been previously recorded, although 6-membered cyclic phosphate tri-esters have been

(9) D. M. Brown and H. M. Higson, *J. Chem. Soc.*, 2034 (1957).

(10) H. G. Khorana, G. M. Tener, R. S. Wright and J. G. Moffatt, THIS JOURNAL, **79**, 430 (1957).

(11) A. B. Anderson, D. L. MacDonald and H. O. L. Fischer, *ibid.*, **74**, 1479 (1952); S. J. Angyal and C. G. Macdonald, *J. Chem. Soc.*, 686 (1952).

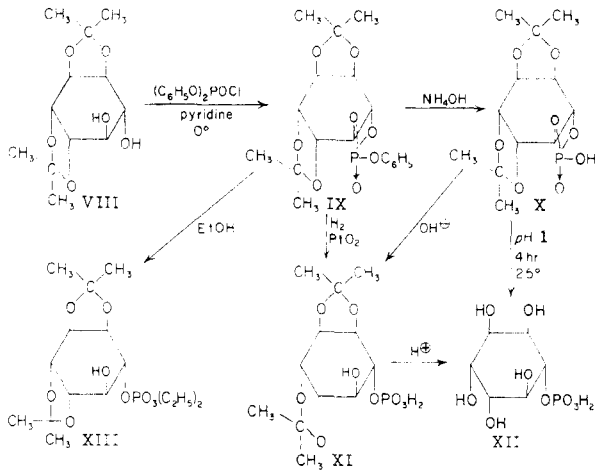
(12) C. E. Ballou and H. O. L. Fischer, THIS JOURNAL, **76**, 3188 (1954).

(13) H. G. Khorana, *Chem. Revs.*, **53**, 145 (1953); C. A. Dekker and H. G. Khorana, THIS JOURNAL, **76**, 3522 (1954); F. M. Huennekens and G. L. Kilgour, *ibid.*, **77**, 6716 (1955).

(14) P. Fleury, A. Desjobert and J. Lecocq, *Bull. soc. chim. biol.*, **36**, 1301 (1954); A. Desjobert and F. Petek, *ibid.*, **38**, 871 (1956).

(15) C. E. Ballou and H. O. L. Fischer, THIS JOURNAL, **75**, 3673 (1953).

(16) D. M. Brown and A. R. Todd, *J. Chem. Soc.*, 52 (1952).



formed by a comparable transesterification.[10] The isolation of IX in this case is a result of its low solubility in pyridine. It crystallizes from the reaction mixture and can be washed free of pyridine and pyridine hydrochloride before the cyclic structure is hydrolyzed. The sensitivity of IX is demonstrated by the observation that attempted recrystallization from absolute ethanol resulted in solvolysis with the formation of the 1,2:5,6-di-O-isopropylidene-(−)-inositol 3-diethylphosphate (XIII).

The phenyl group of IX may be removed by treatment with ammonium hydroxide or by hydrogenolysis. In the first case, 1,2:5,6-di-O-isopropylidene-(−)-inositol 3,4-phosphate (X) is obtained. Mild acid hydrolysis of X results in simultaneous deacetonation and opening of the cyclic phosphate ring to give (−)-inositol 3-phosphate (XII).

The cyclic phosphate structure of X may be opened by alkaline hydrolysis, without deacetonation, to give 1,2:5,6-di-O-isopropylidene-(−)-inositol 3-phosphate (XI). The latter, XI, by mild acid hydrolysis, yields XII; XI was obtained directly from IX during the hydrogenolysis procedure due probably to hydrolysis of the cyclic phosphate structure by the acid produced during hydrogenation. Compound X undoubtedly could be isolated as an intermediate if the hydrogenation were carried out in the presence of a buffer to control the acidity.

Experimental

Phosphorylation of 1,2:5,6-Di-O-isopropylidene-3-O-methyl-(+)-inositol (Diisopropylidenepinitol (II).—Diisopropylidenepinitol[10] (4 g.) was dissolved in 25 ml. of dry pyridine, and the solution was maintained at 5° with an ice-bath. A solution of 6.2 g. of diphenyl phosphorochloridate in 10 ml. of dry pyridine was added over a 20-minute interval with continuous stirring. On standing overnight in the refrigerator, the mixture deposited crystals of pyridine hydrochloride. The solution was filtered, and the crystals were washed with a little dry pyridine. The clear filtrate was chilled to 0°, and a few drops of water were added to decompose the excess phosphorylating reagent. On evaporation of the solution under reduced pressure to about one-fourth volume, crystals appeared. Addition of 200 ml. of water gave a sirup, which crystallized on addition of seeds obtained by treating a small amount of the sirup with methanol. The solid was filtered off, washed extensively with water, and air-dried to yield 7.1 g. (96%) of a white powder (III). Two recrystallizations from a pentane–benzene mixture (50:1) gave 5.3 g. of long needles, m.p. 69°, $[\alpha]^{25}D$ +45.3° (*c* 2, ethanol).

Anal. Calcd. for $C_{25}H_{31}O_9P$ (506): C, 59.3; H, 6.1; P, 6.1; OCH_3, 6.1. Found: C, 59.9; H, 6.1; P, 6.1; OCH_3, 6.4.

1,2:5,6-Di-O-isopropylidenepinitol 4-Phosphate (IV).—Platinum oxide (350 mg.) was suspended in 20 ml. of absolute ethanol and reduced with hydrogen at 25° and atmospheric pressure. Three grams of diisopropylidenepinitol diphenylphosphate (III) in 30 ml. of absolute ethanol was added, and the hydrogenation was continued until the uptake of hydrogen ceased. Approximately 3 hours was required for the consumption of 1160 ml. (calculated 1166 ml.).

The catalyst was filtered off and washed with absolute ethanol. The clear filtrate was taken to *p*H 9 with cyclohexylamine (1.5 ml.) and then was evaporated under reduced pressure. The resulting glass proved soluble in ethanol, ether, acetone and pentane, and finally was crystallized from a hot pentane–heptane mixture (1:1), giving 2.9 g., with m.p. 177–177.5° and $[\alpha]^{25}D$ +2.0° (*c* 1.4, ethanol). It is remarkable that this compound, which is a salt and crystallizes as a monohydrate, is readily soluble in hot pentane.

Anal. Calcd. for $C_{25}H_{49}O_9N_2P \cdot H_2O$ (570): C, 52.6; H, 8.9; N, 4.9; P, 5.4; OCH_3, 5.4; H_2O, 3.2. Found: C, 52.7; H, 8.8; N, 4.7; P, 5.6; OCH_3, 5.7; H_2O, 3.5.

3-O-Methyl-(+)-inositol 4-Phosphate (Pinitol 4-Phosphate) (V).—One gram of diisopropylidenepinitol 4-phosphate cyclohexylamine salt (IV) was dissolved in 30 ml. of distilled water, and 10 ml. of washed IR-120 (H) resin was added. The mixture was swirled for 3 minutes, filtered, and the resin was washed with a total of 10 ml. of water. The combined filtrate was kept at 37° for 20 hours to cause deacetonation at which time it showed a single periodate- and phosphate-positive component on chromatography. Cyclohexylamine (0.5 ml.) was added until the *p*H reached 9, and the solution was then evaporated under reduced pressure to a thin sirup. Addition of ethanol and subsequent evaporation gave an amorphous white residue. This was dissolved in boiling 95% ethanol (100 ml.) and acetone was added to cause incipient turbidity. On leaving the solution in the cold, fine, white crystals were deposited, giving 0.7 g. (82%) with m.p. above 250° and $[\alpha]^{25}D$ +20.5° (*c* 5, water). On oxidation with sodium metaperiodate, V was found to consume exactly 3.0 moles of periodate per mole of compound.

Anal. Calcd. for $C_{19}H_{41}O_9N_2P$ (472): C, 48.3; H, 8.7; N, 5.9; P, 6.6. Found: C, 48.3; H, 8.6; N, 5.8; P, 6.6.

Phosphorylation of 1,2:5,6-Di-O-isopropylidene-(−)-inositol (VIII).—Fifteen grams of VIII was dissolved in 60 ml. of dry pyridine and the solution was maintained at 0° with constant stirring during the dropwise addition of 22 g. of freshly distilled diphenyl phosphorochloridate in 25 ml. of dry pyridine. This required about 30 minutes and resulted in a clear, colorless solution.

After leaving the mixture overnight at 5°, it was almost solid with a crystalline mush. This material was transferred to a sintered glass funnel and filtered under vacuum. The solid on the funnel was washed rapidly and thoroughly with dry benzene to remove excess reagents, and then with water to remove pyridine hydrochloride. The solid product was then triturated in a beaker with 50 ml. of water while the remaining benzene was removed by blowing a current of air across the surface. The fine powdery material was collected by suction filtration and washed with another 100 ml. of water on the funnel. The whole process, beginning with the benzene wash, was repeated and the final product was filtered off on a buchner funnel and dried in air. The yield was 12.4 g. (54%).

For recrystallization, a portion of material was dissolved in hot absolute ethanol, and the solution immediately was chilled in an ice-bath. The product, recovered in a poor yield, was filtered immediately by suction. It melted at 150–200° after softening slowly from 70°. A solvent in which the compound would dissolve readily without decomposition was not found, so its rotation was not determined. On storage the odor of phenol became quite strong. The evidence which follows indicates that this product is a cyclic phosphate monophenyl ester.

Anal. Calcd. for $C_{15}H_{23}O_8P$ (398): C, 54.2; H, 5.8; P, 7.8. Found: C, 53.3; H, 5.8; P, 7.5.

The ultraviolet spectrum of this compound in dioxane showed a maximum at 268 mμ with an extinction of 440. This is characteristic of the phenyl group. Since diisopropylidenepinitol diphenylphosphate (III) had about the same maximum (260) but an extinction of about twice the value (760), it is apparent that IX contains only one phenyl group. The analysis as well as the chromatographic properties of X rule out the possibility of a bis-compound involving two inositol rings and one phosphate.

As will be shown later, the phenyl group in IX can be removed to yield a cyclic phosphate of diacetoneinositol.

Reaction of IX with Ethanol.—The ethanol filtrate from the recrystallization above was allowed to stand at room temperature for 24 hours, at which time it was found to have an acid reaction. On the assumption that the cyclic phosphate had opened, the solution was taken to *p*H 8 by the addition of cyclohexylamine and then was evaporated to a thick sirup under reduced pressure. (Only two drops of cyclohexylamine were required, which was much less than expected on the basis of the amount of IX used). The sirup was dissolved in hot pentane, and on cooling the solution deposited fine needles. After drying, the solid melted at 135°, showed $[\alpha]^{25}D$ −49.7° (*c* 2.3, ethanol), and a solution of the material had no absorption in the ultraviolet region. The product was not acidic, contained no nitrogen and analyzed for diisopropylidene-(−)-inositol 3-diethylphosphate.

Anal. Calcd. for $C_{16}H_{29}O_9P$ (396): C, 48.5; H, 7.3; P, 7.8; OC_2H_5, 21.7. Found: C, 47.7; H, 7.5; N, 0.0; P, 7.8; OC_2H_5, 22.1.

1,2:5,6-Di-O-isopropylidene-(−)-inositol 3,4-Phosphate (X).—Unrecrystallized phosphorylation product IX (5.3 g.) was added to a mixture of 80 ml. of concd. ammonium hydroxide and 80 ml. of water. The suspension was heated to boiling, during which the solid slowly dissolved. After boiling for 5 minutes, the clear solution was evaporated under reduced pressure to about 50 ml., by which time the *p*H was about 6. An equal volume of water was added, and the solution was passed through a column containing 50 ml. of IR-120 resin in the cyclohexylammonium form. The effluent was then extracted twice with two volumes of benzene to remove the phenol. Evaporation of the water layer under reduced pressure gave 5.0 g. (89%) of crystalline material. Recrystallization from a mixture of hot water and acetone gave long fine needles. These did not melt up to 250°, and a water solution showed no specific absorption in the ultraviolet, indicating absence of an aromatic substituent. The titration curve in the range *p*H 3 to 9 showed no secondary phosphate dissociation. The $[\alpha]^{25}D$ was +16.7° (*c* 4, water).

Anal. Calcd. for $C_{18}H_{32}O_8NP$ (421): C, 51.2; H, 7.6; N, 3.3; P, 7.4. Found: C, 50.7; H, 7.6; N, 3.6; P, 7.4.

1,2:5,6-Di-*O*-isopropylidene-(−)-inositol 3-Phosphate (XI) from IX.—One gram of IX was dissolved in 50 ml. of dry dioxane and 200 mg. of platinum oxide catalyst was added. Hydrogenation was carried out at 25° and atmospheric pressure in the presence of hydrogen gas. The uptake of hydrogen (corrected for the uptake by the catalyst) was 220 ml. in 24 hours (calculated 224 ml.). The catalyst was removed by centrifugation, cyclohexylamine was added to the solution to bring the *p*H to about 9, and the solution was evaporated to dryness. Evaporation of this residue from a water solution gave 1.0 g. of crystalline material. Although it was expected that the product would be identical with X, the cyclic phosphate structure had actually been opened due to the acidic conditions existing during the hydrogenation. The substance showed a secondary phosphate dissociation, and on analysis was found to contain a nitrogen to phosphorus ratio of two. It showed $[\alpha]^{25}D$ −2.2° (*c* 1, water), and softened at 195°, but did not melt up to 250°.

Anal. Calcd. for $C_{24}H_{47}O_9N_2P \cdot {}^1/_2H_2O$: C, 52.6; H, 8.6; N, 5.1; P, 5.7; H_2O, 1.7. Found: C, 52.7; H, 8.5; N, 4.5; P, 5.6; H_2O, 1.7.

1,2:5,6-Di-O-isopropylidene-(−)-inositol 3-Phosphate (XI) from X.—Di-O-isopropylidene-(−)-inositol 3,4-phosphate cyclohexylamine salt (X) (1.0 g.) was dissolved in 10 ml. of water, and 2.4 ml. of 5 *N* potassium hydroxide was added. The solution was allowed to stand at room temperature for six hours, and the *p*H then was adjusted to about 7 by the careful addition of IR-120 (H) resin. The solution now was passed through a column containing 10 ml. of IR-120 resin in the cyclohexylammonium form in order to convert all of the product to the cyclohexylamine salt. Evaporation of the solution under reduced pressure yielded a white powder, which was recrystallized from acetone to give 800 mg. of crystals. The compound showed $[\alpha]^{25}D$ −2.0° (*c* 1, water), and softened at 197° but did not melt up to 250°. The compound had identical chromatographic properties with the product produced directly from IX.

Anal. Calcd. for $C_{24}H_{47}O_9N_2P \cdot {}^1/_2H_2O$: C, 52.6; H, 8.6. Found: C, 52.7; H, 8.8.

(−)-Inositol 3-Phosphate (XII).—Diisopropylidene-(−)-inositol 3,4-phosphate (X) (4 g.) was dissolved in 70 ml. of water and the solution was passed through a column containing 20 ml. of IR-120 (H) resin. The effluent, total volume 100 ml., was allowed to stand at room temperature overnight to bring about deacetonation and then was taken to *p*H 9 with 2.2 ml. of cyclohexylamine. The solution was evaporated at reduced pressure to about 20 ml., then heated to boiling and 2 volumes of acetone was added. The product crystallized as a cyclohexylamine salt, which was filtered off, washed with acetone and dried *in vacuo* over calcium chloride to give 3.6 g. (83%) with m.p. above 250°. Titration of the compound, after removal of cyclohexylamine with IR-120 (H) resin, shows a secondary phosphate dissociation with a *pK* of about 6.2, $[\alpha]^{25}D$ −25.6° (*c* 2, water).

Anal. Calcd. for $C_{18}H_{39}O_9N_2P$ (458): C, 47.1; H, 8.5; N, 6.1; P, 6.8. Found: C, 47.2; H, 8.3; N, 6.0; P, 6.7.

TABLE I

CHROMATOGRAPHIC CONSTANTS

Compound	Solvent[a] and R_f I[18]	II[17]	III[18]	IV[18]
myo-Inositol 2-phosphate (Iselin)	0.37	0.27	..	0.34
Pinitol 4-phosphate	.41	.54	0.13	.58
Pinitol 3,4-phosphate	.60	..	..	.48
Diisopropylidenepinitol 4-phosphate	.70	.74	.49	..
(−)-Inositol 3-phosphate	.37	.32	.10	.53
Diisopropylidene-(−)-inositol 3-phosphate	.69	..	..	..
Diisopropylidene-(−)-inositol 3,4-phosphate	.84	.86	.79	..
Inorganic phosphate	..	.33	..	..

[a] Solvent I, *n*-propyl alcohol:ammonia:water (50:40:10); solvent II, methanol:ammonia:water (70:15:15); solvent III, isopropyl alcohol:ammonia:water (60:30:10); solvent IV, ethanol:satd. boric acid:water (80:20:20).

Cyclization of Pinitol 4-Phosphate.—Pinitol 4-phosphate (dicyclohexylamine salt) (1.0 g.) was dissolved in 10 ml. of water and converted to the pyridinium salt by passing the solution through a column containing 10 ml. of IR-120 resin in the pyridinium form. The resulting solution was evaporated to dryness and the solid material was dissolved in 8 ml. of pyridine containing about 0.5 ml. of water. A solution of 3.0 g. of dicyclohexylcarbodiimide in 5 ml. of pyridine was added, and the two-phase mixture shaken mechanically for 24 hours.

The solution was filtered and the insoluble dicyclohexylurea was washed with water. The water–pyridine solution was extracted three times with 5 volumes of ether to remove unreacted DCC and some of the pyridine. The water solution was evaporated to about 25 ml. and then passed through a column containing 10 ml. of IR-120 resin in the cyclohexylamine form. The effluent was evaporated to a small volume and applied to a sheet of Whatman 3MM paper for chromatography in *n*-propyl alcohol:ammonia:water (5:4:1).[18] The band corresponding to the cyclic derivative (R_f 0.60) was cut out and eluted with water. Evaporation of this solution gave a small amount (about

(17) R. S. Bandurski and B. Axelrod, *J. Biol. Chem.*, **193**, 405 (1951).

(18) S. S. Cohen and D. B. M. Scott, *Science*, **111**, 543 (1950).

100 mg.) of amorphous solid. Attempts to crystallize this salt from a variety of solvents were not successful.

A small portion of this material, when treated with hot acid or base at room temperature, gave a compound with chromatographic properties identical with pinitol phosphate. There was no chromatographic evidence for the formation of two isomers.

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[CONTRIBUTION FROM THE DEPARTMENT OF BIOCHEMISTRY, UNIVERSITY OF CALIFORNIA, BERKELEY]

The Preparation and Characterization of Di-O-benzylidene-(−)-inositol

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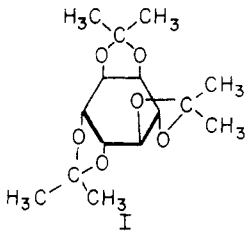
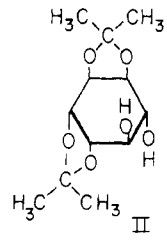
A crystalline di-O-benzylidene-(−)-inositol (V) has been obtained from (−)-inositol when it was treated with benzaldehyde and fused zinc chloride. Lead tetraacetate oxidation of V gave amorphous 2,3:4,5-di-O-benzylidene-L-*manno*-hexodiose (VI) which was characterized as the bis-phenylhydrazone and the bis-*p*-nitrophenylhydrazone; VI was converted to 2,3:4,5-di-O-benzylidene-L-mannitol by reduction. On treatment with ethanethiol, VI yielded the crystalline tetraethyl tetrathioacetal of L-*manno*-hexodiose.

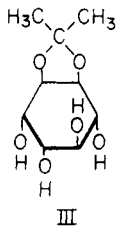
(+)-Inositol[1] and (−)-inositol[2] are readily acetonated in the presence of acetone and zinc chloride under anhydrous conditions at room temperature, yielding a mixture of products consisting mainly of the tri-O-isopropylidene (I) and of the



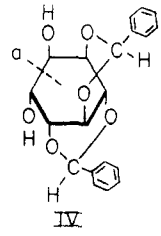
I



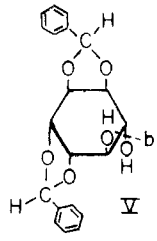
II



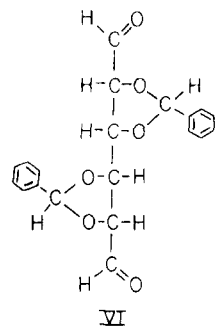
III



IV



V



VI

di-O-isopropylidene (II) derivatives. The tri-O-isopropylidene derivative I can be partially hydrolyzed to yield II and some mono-O-isopropylidene-inositol (III).[2]

According to the modified Hann–Hudson rules for acetal and ketal substitutions, outlined by Barker and Bourne,[3] benzaldehyde derivatives of (+)- or (−)-inositol might be expected to take the (1,3) ring configuration (IV) rather than the (1,2) configuration (V). We have now demonstrated that the reaction of (−)-inositol with benzaldehyde and zinc chloride results in the formation of a di-O-benzylidene derivative. This substance reacts readily with lead tetraacetate, consuming one mole, with the formation of a dialdehyde VI. Treatment of the di-O-benzylidene-L-*manno*-hexodiose (VI) with ethanethiol gives a thioacetal derivative which is enantiomorphic with D-*manno*-hexodiose tetraethyl tetrathioacetal.[1] The reduction of VI gave a dibenzylidene hexitol which yielded L-mannitol on removal of the benzylidene groups. Examination of structures IV and V shows that the only possible combination of benzylidene substituents which would allow fission at (b) to yield L-mannitol involves two *cis*-(1,2) cyclic acetals in positions 1,2 and 3,4 of the (−)-inositol ring. Should the di-O-benzylidene compound have been of structure IV with oxidative splitting at (a), the resulting hexitol would have been D-iditol.

The benzylidene derivatives offer certain advantages as intermediates over the isopropylidene derivatives because the benzylidene radical can be removed readily by catalytic hydrogenation with a palladium catalyst in a neutral medium. The preparation and isolation of free dialdehydes may thus be facilitated.

Experimental

1,2:3,4-Di-O-benzylidene-(−)-inositol.—(−)-Inositol from the demethylation of quebrachitol[4] was treated as follows. Finely ground fused zinc chloride (50 g.) was added to anhydrous benzaldehyde (150 ml.) with mechanical stirring. After a few minutes the mixture became opaque and partially solidified. With continued stirring the viscosity decreased rapidly. After 20 minutes, finely

(1) C. E. Ballou and H. O. L. Fischer, THIS JOURNAL, **75**, 3673 (1953).

(2) S. J. Angyal and C. G. MacDonald, *J. Chem. Soc.*, 686 (1952).

(3) S. A. Barker and E. J. Bourne, *ibid.*, 906 (1952).

(4) Quebrachitol (100 g.) was refluxed with 47% hydriodic acid (250 ml.) for two hours, using an air condenser. The hot solution was poured in boiling absolute ethanol (1600 ml.) and the (−)-inositol crystallized immediately. After 12 hours at 5°, the (−)-inositol was collected on a funnel, washed thoroughly with absolute ethanol and dried *in vacuo* at 80°. The yield was 92.5 g. (99%) and the product melted at 235–237°.