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RESOLUTION OF ASYMMETRICALLY SUBSTITUTED MYOINOSITOLS INTO OPTICAL ANTIPODES

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Abstract—A method of resolving asymmetrically substituted derivatives of myoinositols (MI) *via* their diastereomeric orthoacetates with D-mannose followed by isolation of enantiomers from separated diastereomeric orthoesters is described. Starting with optically active MI a number of natural compounds and/or their fragments involving MI have been obtained.

INTRODUCTION

Asymmetrically substituted optically active MI, such as (+)- and (−)-bornesitols (3- and 1-O-methyl-sn-MI), are common natural compounds‡.[2]

Optically active asymmetrically substituted MI is a structural component of an important class of natural phospholipids such as inositolphosphatides.[2] One reason for an intensive synthetic study of various MI derivatives lies in the ever growing interest in this class of compounds.[2]

Among fundamental investigations of the chemistry and biochemistry of MI derivatives are those by Angyal *et al.* on the role of different groupings as selective protectors of MI hydroxyl groups and on the synthesis of MI derivatives as fragments of natural compounds or as starting compounds for their synthesis.[3–5] Ballou,[6–8] Dawson,[9] Hawthorne[10] and other workers have elucidated the structure of the most essential natural compounds of MI, in particular of mono-, di-, tri-, and mannophosphoinositides. We have synthesised mono- and diphosphoinositides[12,13] as well as triester phosphoinositides replicating the possible natural structure.[12,14]

Molotcovsky has recently obtained phosphatidylinositol containing unsaturated fatty acids,[15] the synthetic routes to optically inactive phosphotadylinositol have been discussed.[16]

Compared to these advances those in the synthesis of optically active derivatives of asymmetrically substituted MI and natural compounds with optically active MI are very modest evidently due to the difficulties involved.

Two approaches to this problem are, (i) that of a partial synthesis of optically active asymmetrically substituted MI with a required structure from natural optically active compounds containing MI and (ii) that of a total synthesis of optically active MI derivatives by resolving the corresponding racemic compounds obtained from MI. One of the early examples of the first approach is the work by Ballou[17] on the synthesis of sn-MI 3-phosphate from galactinol. The scheme proposed by these workers cannot however be extended to natural phosphorus esters of MI such as inositolphosphatides with the phosphate group in position 1. The same limitation holds for schemes worked out by Gero[18] to synthesise 1,2,4,5,6-penta-O-benzoyl-sn-MI and sn-MI 3-phosphate.

Our attempted synthesis of optically active MI from natural compounds resulted in the conversion of 1-O-methyl-sn-MI, (−)-bornesitol to pentasubstituted MI with a free 1-OH-group, that is 2,3,4,5,6-penta-O-tosyl-sn-MI.[19] Unfortunately our scheme involves the introduction of protecting groups which are stable to even vigorous demethylation conditions.

The second approach dealing with the resolving of various racemic derivatives of MI appears therefore to be more promising. Attempts to resolve racemic asymmetrically substituted MI have been unsuccessful to date. Thus Gigg *et al.*[20] reported the failure to resolve pentabenzyl ether of MI through the ester of hydrogen monophthalate and monosuccinate followed by the formation of diastereomeric salts with optically active bases. It might be presumed that both this procedure and the classical method used to obtain diastereomeric esters with optically active acids are of little value for derivatives of asymmetrically substituted MI.¶ On the other hand there was an indication[22] that

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‡The configuration of asymmetrically substituted optically active MI is described in terms of stereospecific nomenclature proposed by two of the present authors and N. A. Preobrazhenskii.[1]

¶Molotcovsky and Bergelson[21] have recently reported to have obtained 1,2,4,5,6- and 2,3,4,5,6-penta-O-acetyl-sn-MI by resolving the racemic pentaacetate through diastereomeric salts of MI pentaacetate hydrogen oxalate with chinidine or α-phenetylamine. The physico-chemical constants of their enantiomeric pentaacetates are close to those of synthetic optically active pentaacetates already described.[34]

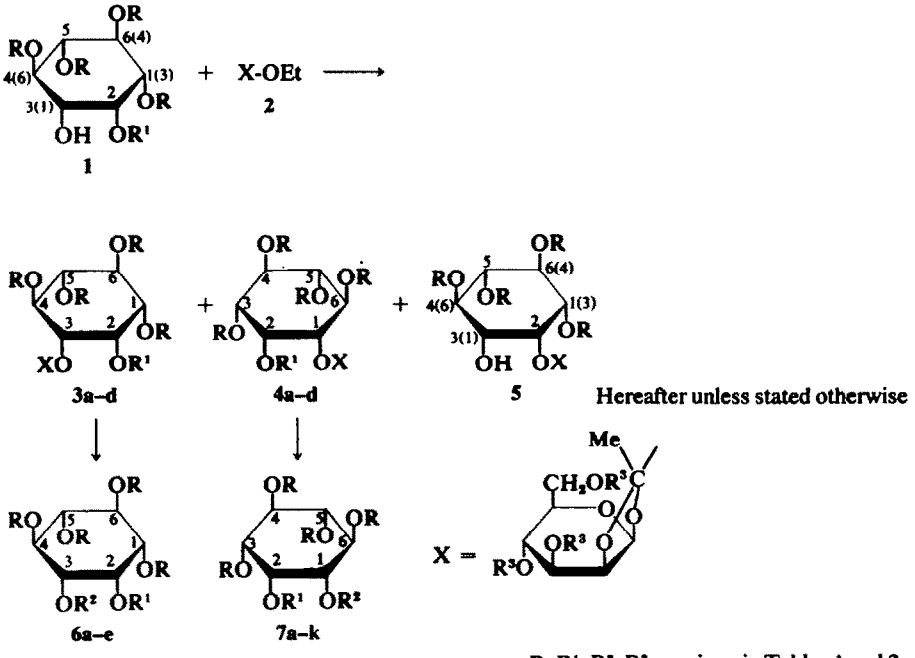
diastereomeric 1- and 3-mannopyranosyl-sn-MI could be separated by crystallisation. The authors did not however attempt to use their diastereomers for preparing optically active MI derivatives owing to insufficient quantities of available glycosides. Literature reports few cases when carbohydrate derivatives were used as asymmetrical agents in resolving racemic alcohols. This may be accounted for by the facts that diastereomeric glycosides are obtained in low yields and the isolation of optically active alcohols from separated glycosides requires rigid conditions or the presence of enzymatic systems. It is to be mentioned that Neuberg has obtained enantiomers of amyl alcohol, menthol, and borneol through respective D-glycosides,[23] and Helferich has resolved *trans*-cyclopentadiol-1,2 through D-glycosides.[24] The ready separation of diastereomeric mannosides of MI[22] encouraged us[25] to increase their yield by synthesising MI mannosides by means of the orthoester procedure.[26] In spite of the two-fold increase in yield of mannosyl-MI these compounds still remain not readily available. The preparation of optically active asymmetrically substituted MI from MI-mannosides requires further conversions (protection of hydroxyl groups and hydrolysis of the glycoside bond).

It may however be suggested that not only glycosides but other diastereomeric derivatives of MI and D-mannose (in particular orthoesters) also possess physico-chemical properties different enough to enable their separation to be effected. Studies in this country[26] and in Canada[27] of monosaccharide orthoesters, not previously used as asymmetric reagents for resolving racemic alcohols, have made them available. It is also advantageous to use orthoesters rather than glycosides because they provide a convenient means of regenerating the optically active alcohol from separated diastereomeric orthoesters on mild acid hydrolysis.

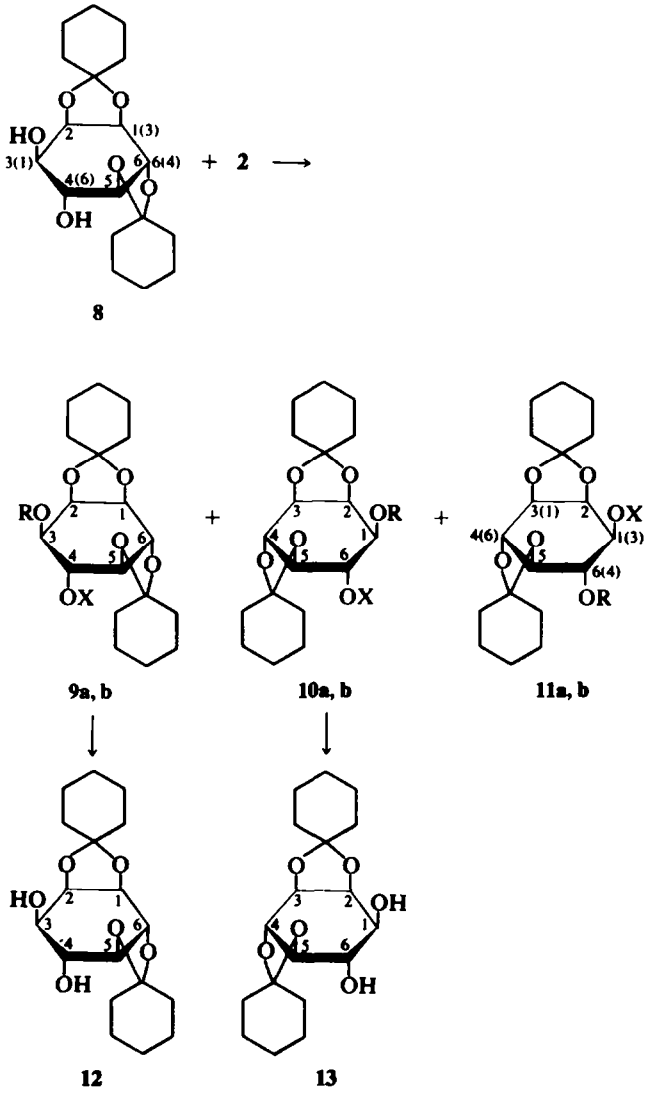
The present report is concerned with our method of resolving racemic derivatives of asymmetrically substituted MI and with the synthesis of a number of optically active natural compounds containing MI and of their fragments.

RESULTS AND DISCUSSION

The proposed method is based on the reaction of racemic mixtures of MI with the orthoacetate of D-mannose and ethanol under conditions of reesterification followed by separation of the resulting diastereomeric orthoesters of D-mannose and MI by chromatography or crystallisation and isolation of enantiomers from separated orthoesters by mild acid hydrolysis or reverse reesterification with methanol (Schemes 1 and 2). Reesterification was carried out under standard conditions[29] in boiling dichloroethane in the presence of TsOH (0·02–0·25 M per mole of II) for 3–8 hr with aseotropic distillation of ethanol. No marked formation of isomeric glycosides was thereby observed. Physico-chemical characteristics of resulting diastereomeric orthoesters are given in Table 1.



SCHEME 1



SCHEME 2

Diastereomeric orthoesters were isolated by crystallisation or chromatography on Al_2O_3. To obtain orthoesters **9a** and **10a** a two-fold molar excess of the starting orthoacetate **2** was used to raise the yield.[30] The structures of D-mannose orthoesters and of asymmetrically substituted MI were proved by means of IR and PMR spectra. The latter spectra of all orthoesters revealed the presence of a singlet peak for protons of the C—Me group over the δ 1·7–1·8 p.p.m. This fact substantiates the data[29] that reesterification reactions lead essentially to endo-C—Me orthoester isomers because nucleophilic attack on the intermediate acyloxonium ion by sterically hindered alcohols represented by the asymmetrically substituted MI proceeds more favourably from the *trans*(*exo*) position in respect to the pyranose ring.

The isolation of enantiomers of asymmetrically substituted MI from respective separated orthoesters was achieved, usually, by acid hydrolysis. Thus, tetrabenzyl[31] and pentabenzyl[32–33] ethers of MI-**6a,b** and **7a,b** were obtained by hydrolysis respectively of **3a**, **4a**, **3b**, and **4b** with 0·1 N H_2SO_4 in 90% aqueous acetone, and 1,2,4,5,6- and 2,3,4,5,6-penta-O-acetyl-*sn*-MI (**6d**,' **7h**) were isolated by heating orthoesters **3d**, **4d** with 80% AcOH.[34] Acid hydrolysis proved unsuitable for the isolation of optically active diketals of MI **12** and **13** because under acidic conditions the stability of the orthoester and cyclohexylidene groups is very similar so that they are hydrolysed simultaneously. Therefore, in the case of 1,2:5,6- and 2,3:4,5-di-O-

Table 1. Monosaccharide and asymmetrically substituted myoinositol orthoacetates

ortho-ester	R	R¹	R³	Yield %	M.p. °C (solvent)	$[\alpha]_D^{20}$ (c0·15–0·6, $CHCl_3$)	R_f[a]	δ C–Me p.p.m.
3a	Bz	H	Ac	9·68[b]	135·0–137·0 (EtOH)	+0·54	0·4 (A)	1·75
3b	Bz	Bz	Bz	66·2 (from **3a**)	—[c]	—[c]	0·65 (B)	—[c]
3c	Ac	H	Ac	48·2[b]		—	0·50 (C)	—[c]
3d[d]	Ac	Ac	Ac	58·9 (from **3c**)	amorphous	−9·24	0·61 (D)	1·62
4a	Bz	H	Ac	18·6[b]	155·5–156·0 (EtOH)	+9·8	0·52 (A)	1·75
4b	Bz	Bz	Bz	71·3 (from **4a**)	112·0–113·0 (EtOH)	+47·1	0·31 (B)	1·78
4c	Ac	H	Ac	36·1[b]	—[c]	—[c]	0·28 (C)	—[c]
4d	Ac	Ac	Ac	58·0 (from **4c**)	amorphous	−0·52	0·64 (D)	1·62
5	Bz	H	Ac	8·68[b]	151·0–155·0 (EtOH)	—[c]	0·63 (A)	1·72
9a	H	—	Ac	56·1[b]	amorphous	+8·6	0·46 (A)	1·74
9b	Me	—	Ac	80·7 (from **9a**)	150·0–160·0 (Et_2O-light petroleum, 1:10)	+16·7	0·82 (E)	1·74
10a	H	—	Ac	24·6[b]	172·0–174·0 (Et_2O)	+2·0	0·27 (A)	1·81
10b	Me	—	Ac	72·3 (from **10a**)	154·0–155·8 (light petroleum)	−4·25	0·81 (E)	1·80
11a	H	—	Ac	10·7[b]	amorphous	—[c]	0·12 (A)	1·77
11b	Me	—	Ac	75·1 (from **11a**)	148·0–155·0 (Et_2O-light petroleum, 1:10)	—[c]	0·76 (E)	1·75
14a	Bz	H	Ac	46·2[b]	amorphous	—[c]	0·32 (A)	1·66
14b	Bz	Me	Ac	76·2 (from **14a**)	amorphous	—[c]	0·33 (B)	1·68
15a	Bz	H	Ac	23·3[b]	amorphous	—[c]	0·60 (A)	1·72
15b	Bz	Me	Ac	51·2 (from **15a**)	amorphous	—[c]	0·43 (B)	1·73
16a	H	—	Ac	14·9[b]	amorphous	—[c]	0·23 (A)	1·72
16b	Me	—	Ac	54·2 (from **16a**)	145·0–147·0 (Et_2O)	—[c]	0·79 (F)	1·74
17a	H	—	Ac	17·9[b]	amorphous	—[c]	0·41 (A)	1·73
17b	Me	—	Ac	61·6 (from **17a**)	136·0–138·0 (Et_2O)	—[c]	0·70 (F)	1·73
18	—	—	Ac	10·9[b]	211·0–212·5 (Et_2O)	—[c]	0·62 (A)	1·76

[a]TLC was carried on neutral alumina of the activity III after Brockman, the solvents systems: $CHCl_3$:Me_2CO, 9:1(A), $CHCl_3$(B), $CHCl_3$:Me_2CO, 3:2 (C), $CHCl_3$:Me_2CO, 4:1(D), $CHCl_3$:Me_2CO, 95:5(E), $CHCl_3$:Me_2CO, 20:1(F).

[b]In the reactions of reesterification yields are given in consideration of reacting derivative MI.

[c]Compounds not in analytically pure state.

[d]Reesterification of 1,2,4,5,6-penta-O-acetyl-MI (1, R = R′ = Ac) with **2** resulted in a diastereomeric mixture of **3d** and **4d**; yield 63·2%, amorphous, R_f 0·6–0·65(D), δ_{C-Me} 1·62 p.p.m.

[e]Diastereomeric mixtures.

cyclohexylidene-sn-MI (12, 13) regeneration was effected by the "reverse" reesterification of orthoesters **9a** and **10a** with a double amount of methyl alcohol.[30] The physico-chemical characteristics of enantiomers of asymmetrically substituted MI are listed in Table 2. The enantiomorphic relations existing in every pair of antipodes were confirmed by the shape of the ORD curves.

The preparation of a number of optically active asymmetrically substituted MI provided the opportunity to study synthetic routes to phosphoinositides with natural structure. It appeared at first that the most suitable compounds to be investigated were pentasubstituted MI derivatives with a free group at C_1 (**7e,h,9a**). However the negligible yield of pentabenzyl ethers and pentaacetyl esters from **7e,h**[32,34] on resolving compounds **1**, R=R′=Bzl and **1**, R=Ac, R′=H proved a handicap for using them to produce the phosphoinositide structure. A change in the synthetic scheme was therefore made and 2,3,4,5,6-penta-O-benzyl-sn-MI (**7e**) was prepared from the available orthoester **4a** using the orthoacetate of D-mannose both as an asymmetric reagent to resolve tetrabenzyl ether **1**, R=Bzl, R′=H and as a temporary protection of the hydroxyl group at C_1.[33]

Orthoester **4a** was benzylated with benzyl chloride in the presence of alkali, as the orthoester group is stable under alkaline conditions.[35] The IR spectra of the benzylated orthoester **4b** revealed no νOH at 3560 cm^{-1} and νC=O in $COOCH_3$ at 1750, 1730 cm^{-1} which points to the complete benzylation of both the hydroxyl group and those resulting from acetate saponification. The proton singlet at δ 1·78 p.p.m. in the PMR spectrum of **4b** showed that benzylation did not affect the orthoacetate group. Hydrolysis of **4b** gave 2,3,4,5,6-penta-O-benzyl-sn-MI (**7e**) in an overall 65% yield on **4a**. Enantiomer **6b** was obtained similarly in a total 50% yield (with no separation of the intermediate orthoester **3b**).

Preparation of optically active MI pentaacetates

Table 2. Optically active asymmetrically substituted mycinositols

Compound	R	R¹	R²	Yield %	M.p. °C (solvent)	$[\alpha]_D^{20}$ (c, solvent)	R_f[a]
6a	Bz	H	H	81·2 (from **3a**)	140·2–142·1 (MeOH)	+25·0 (0·18, $CHCl_3$)	0·16 (A)
6b	Bz	Bz	H	50·3 (from **3b**)	59·0–60·0 (light petroleum)	+13·9 (0·3, $CHCl_3$)	0·25 (B)
6c	H	H	Me	55·4 (from **9b**)	202·0–204·0 (MeOH)	+32·8 (2·4, water)	1·4 (H)[b]
6d[c]	Ac	Ac	H	74·5 (from **3d**)	186·0–188·0 (water)	+11·5 (0·1, $CHCl_3$)	0·73 (G)
6e	Ac	Ac	Bz	49·5 (from **7d**)	181·0–183·0 (EtOH)	+28·7 (0·1, $CHCl_3$)	0·9 (B)
7a	Bz	H	H	86·3 (from **4a**)	141·0–143·0 (MeOH)	−24·3 (1·3, $CHCl_3$)	0·16 (A)
7b	Bz	H	Me	25·3 (from **7a**)	115·0–116·0 (light petroleum)	−1·5 (0·2, $CHCl_3$)	0·58 (A)
7c	Bz	Me	H	5·8 (from **7a**)	137·0–138·0 (light petroleum)	−69·4 (0·18, $CHCl_3$)	0·8 (A)
7d	H	H	Me	71·4 (from **7b**)	204·0–205·0 (MeOH)	−33·2 (0·2, water)	1·4 (H)[b]
7e	Bz	Bz	H	75·5 (from **4a**)	59·1–60·0 (light petroleum)	−13·5 (0·3, $CHCl_3$)	0·25 (B)
7f	Bz	Bz	$OP(O)(OPh)_2$	68·5 (from **7e**)	64·0–64·5 (hexane)	−11·0 (0·6, $CHCl_3$)	0·3 (B)
7g	H	H	$OP(O)(OH)_2 . 2C_6H_{11}NH_2$	77·6 (from **7f**)	202·0–204·0 (Me_2CO-water)	+3·4 (1·0, water, pH9)	1·31 (I)[b]
7h[c]	Ac	Ac	H	64·1 (from **4d**)	188·0–189·0 (water)	−11·2 (0·05, $CHCl_3$)	0·7 (G)
7i	Ac	Ac	Bz	45·3 (from **6a**)	180·0–182·5 (EtOH)	−26·55 (0·08, $CHCl_3$)	0·9 (B)
7j	H	H	—[d]	48·5 (from **7k**)	169·0–172·0 ($CHCl_3$-Me_2CO)	+7·48 (0·3, $CHCl_3$-MeOH, 2:1)	0·6[e]
7k	Bz	Bz	—[f]	33·2 (from **7e**)	53·0–54·0 (EtOH)	−7·76 (1·4, $CHCl_3$)	0·65[g]
12	—	—	—	72·3 (from **9a**)	153·0–156·0 (C_6H_6-light petroleum)	−7·4 (0·5, C_6H_6)	0·08 (G)
13	—	—	—	79·8 (from **10a**)	153·2–156·0 (C_6H_6-light petroleum)	+7·5 (0·5, C_6H_6)	0·08 (G)

[a]TLC was carried out on neutral alumina of the activity III after Brockman, the solvent systems: $CHCl_3$:Me_2CO, 1:1(G); the rest systems are given in Table 1.

[b]The descending chromatography on Whatman 1, or "Quick Leningrad" paper, was carried out in the solvents systems: Me_2CO:water, 4:1 (H), *i*-PrOH-NH_4OH-water, 7:1:2 (I); R_f values are given in ratio of the length run of MI.

[c]**6d** and **7e** were also obtained by hydrogenolysis of **6e** and **7i** over palladium black, yields 79·4 and 77·1%, accordingly; constants were analogous.

[d]$OP(O)(O^-\overset{+}{N}H_4)OCH_2 . CH(OOCC_{15}H_{31}) . CH_2(OOCC_{15}H_{31})$.

[e]TLC on silica gel KSK, $CHCl_3$:MeOH:4N NH_4OH, 9:7:2.

[f]$OP(O)(OPh)OCH_2CH(OOCC_{15}H_{31})CH_2(OOCC_{15}H_{31})$.

[g]TLC on silicic, "aqueous," acid, Et_2O:hexane, 1:1.

6d, **7h** to be used as starting compounds for synthetic unsaturated phosphatidylinositols[21,36] obtained through D-mannose orthoacetates and 1,4,5,6-tetra-O-acetyl-MI (**3c** and **4c**) followed by their complete acetylation to **3d** and **4d** and acid hydrolysis of the two latter compounds was extremely laborious and did not result in sufficiently high yields of the end products.[34] To overcome this drawback we proposed[34] to obtain 2,3,4,5,6-penta-O-acetyl-sn-MI (**7h**) from 1,4,5,6-tetra-O-benzyl-sn-MI (**6a**) by its acetolysis to 1-O-benzyl-2,3,4,5,6-penta-O-acetyl-sn-MI (**7i**) followed by hydrogenolysis of the latter compound to pentaacetate **7h**. In a similar way 1,2,4,5,6-penta-O-acetyl-sn-MI (**7d**) was prepared from 3,4,5,6-tetra-O-benzyl-sn-MI (**7a**).[34]

To prove the structure, optical purity and absolute configuration of the antipodes of asymmetrically substituted MI, additional syntheses were undertaken which gave a number of natural compounds involving MI and their fragments (Scheme 1,2; Table 2).

(−)-Bornesitol (7d).[31] **7a** was made to react with MeI in the presence of alkali to give 1- and 2-O-methyl-3,4,5,6-tetra-O-benzyl-sn-MI (**7b** and **7h**) separated by chromatography on Al_2O_3. The structure of isomeric methyl ethers **7b** and **7c** was proved by comparison with those of respective racemic compounds.[37] Hydrogenolysis of **7b** led to 1-O-methyl-sn-MI (**7d**) shown to be identical with the natural (−)-bornesitol* by means of TLC and PC, the m.p. of the mixed sample, ORD curves, and IR spectra.

(+)-Bornesitol (6c).[30] **9a** was methylated by MeI in the presence of Ag_2O to give **9b** whose structure was determined by means of IR spectra (no νOH) and PMR spectra (δO—CH_3 3·44 p.p.m.). **9b** was hydrolysed with 80% AcOH at 100°. These conditions led to the abstraction of the orthoester and

*Samples of natural (−)- and (+)-bornesitols were kindly provided by Prof. L. Anderson (Madison, USA).

both cyclohexylidene groups, 3-O-methyl-sn-MI (6c) obtained by crystallisation was proved to be identical with (+)-bornesitol* by direct comparison.

Similarly orthoacetate 10a gave (−)-bornesitol (7d).

sn-MI 1-Phosphate (7g).[32] Pentabenzyl ether 7e was phosphorylated by diphenylphosphochloridate in pyridine to give 1-diphenylphosphate 2,3,4,5,6-penta-O-benzyl-sn-MI (7f). Hydrogenolysis of 7f in the presence of palladium black and then of Adams catalyst resulted in sn-MI 1-phosphate, obtained as bis-cyclohexylammonium salt (7g), shown to be identical with bis-cyclohexylammonium salt of sn-MI 1-phosphate isolated from soya bean phosphatides by a modified procedure.[6]

Phosphatidylinositol 7e.[34,38] Syntheses of enantiomers of various MI derivatives resulted in the first chemical synthesis of phosphatidylinositol with natural structure 7j starting from 2,3,4,5,6-penta-O-benzyl-sn-MI (7e) (Scheme 1, Table 2).

The synthesis of 1-O-(1,2-di-O-palmitoyl-sn-glyceryl-3-O-phosphoryl)-sn-MI (7j) was based on condensation of 7e with phenylphosphodichloridate and 1,2-di-O-palmitoyl-sn-glycerol that led to 1-O-[1,2-di-O-palmitoyl-sn-glyceryl-3-O-(phenyl)-phosphoryl]-2,3,4,5,6-penta-O-benzyl-sn-MI (7k). Hydrogenolysis of 7k on palladium black and platinic oxide gave rise to phosphoinositide 7j isolated as its ammonium salt. The synthetic phosphatidylinositol (7j) was shown to have IR, ORD curve and chromatographic data similar to those of natural monophosphoinositides.[39,40]

The method proposed for the resolution of racemic MI derivatives may be considered as general for obtaining antipodes of asymmetric cyclitoles. The resolving effect appears to depend in each particular case on the correct choice of the monosaccharide orthoester used. In order to elucidate the resolving effect of monosaccharides we have attempted to resolve 1, R=Bzl, R′=H by means of the orthoester of another monosaccharide, 3,4,6-tri-O-acetyl-1,2-O-tert.-butylorthoacetyl-α-D-glucopyranose.[41]

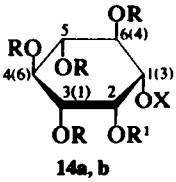
Reesterification of the orthoester with tetrabenzyl ether 1, R=Bzl, R′=H gave rise to diastereomeric orthoester mixtures at C_1 and C_2 of MI, 14a and 15a, whose methylation of MeI in the presence of Ag_2O resulted in 3,4,6-tri-O-acetyl-1,2-O-(1,4,5,6-tetra-O-benzyl-2-O-methyl-MI-3-O-orthoacetyl)-α-D-glucopyranose (14b) and its 3-O-methyl isomer 15b which yielded on acid hydrolysis methyl ether 1, R=Bzl, R′=Me and its 3-O-methyl isomer identical with synthetic racemic samples.[37] The latter conversions pointed to the absence of resolving with D-glucose orthoacetate used as asymmetric reagent. Orthoesters 14a and 15a are positional isomers.

On the other hand the difference in physicochemical properties of diastereomeric orthoacetates of D-mannose and MI derivatives is greatest when the MI molecule has a free hydroxyl group vicinal to the bonding site of the orthoester residue. Thus, for example, 1,4,5,6-tetra-O-substituted MI (1,R=H) is resolved quite readily[30,33] whereas orthoesters with pentasubstituted MI(1) reveal very similar physico-chemical properties.[32,34]

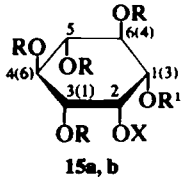
It proved also possible to resolve readily 1,2:5,6-diketal of MI (8) with free vicinal hydroxylic groups.[30] It was in this respect of interest to investigate the possibility of resolving 1,2:4,5-di-O-cyclohexylidene-MI whose free hydroxylic groups are not vicinal.

Reesterification of orthoester 2 and 1,2:4,5-di-O-

*Samples of natural (−)- and (+)-bornesitols were kindly provided by Prof. L. Anderson (Madison, USA).



14a, b



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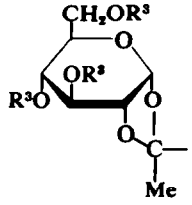

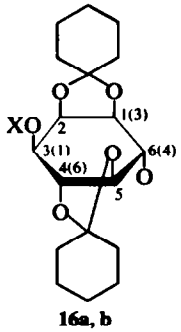

16a, b 17a, b 18

cyclohexylidene-MI gave rise to diastereomeric orthoester mixtures in 1(3) **16a** and 4(6) positions **17a**, as well as to diorthoester derivative **18**.[42] The structures of orthoacetates **16a** and **17a** were established by their methylation,[30,31,41] to corresponding methylated orthoesters **16b**, **17b** followed by their hydrolysis with 80% AcOH, resulting in (±)-ononitol (4-O-methyl-MI)[43] and (±)-bornesitol (1-O-methyl-MI),[37] respectively. Hence, in the case of 1,2:4,5-di-O-cyclohexylidene-MI, whose hydroxylic groups are isolated, no essential difference in chromatographic mobility of diastereomeric D-mannose orthoacetates was observed in mixtures of **16a** and **17a**.

The possibility to prepare optically active derivatives of asymmetrically substituted MI following the method proposed in this work provides a practical basis for synthetic studies of various natural phosphoinositides that are of considerable biochemical interest.

EXPERIMENTAL

The m.ps were all determined in open capillaries and are uncorrected. The rotation angles and ORD curves were measured by Cary-60 and SPU-M automatic spectropolarimeters. IR spectra were taken on a Perkin-Elmer, 257 in nujol or KBr discs. PMR spectra were measured on a Perkin-Elmer R-12 in $CDCl_3$ or $CHCl_3$ solution; signals recorded in δ (p.p.m.) relative to HMDS as zero.

The column chromatography and TLC were carried on alumina of activity 3 after Brockman, on silica gel KSK or silicic, "aqueous", acid in solvents listed in Tables 1 and 2. The spots were detected on slides by spraying them with conc. H_2SO_4 followed by carbonisation at 300–350°. The descending chromatography on Whatman 1, or "Quick Leningrad" paper, was effected in the solvent systems shown in Table 2. Spots were detected with alkaline $AgNO_3$ or periodate-benzidine reagent. The compounds prepared all gave satisfactory elementary analyses (Table 3).

Reesterification of 3,4,6-*tri*-O-*acetyl*-1,2-O-*ethylorthoacetyl*-β-D-*mannopyranose* (**2**; R^3=Ac) *with* 1,4,5,6-*tetra*-O-*benzyl*-MI (**1**; R=Bzl, R^1=H). A solution of orthoacetate (**2** R^3=Ac) (5·95 g)[28] and of 1,4,5,6-tetra-O-benzyl MI (**1** R=Bzl, R′=H) (4·6 g) in $C_2H_4Cl_2$ (50 ml) was boiled with 0·055 g of TsOH for 4 hr, $C_2H_4Cl_2$ was distilled and solvent simultaneously added to retain constant volume of the mixture. Several drops of Py were then added, the solution evaporated and the product treated with Et_2O (50 ml). The precipitate was filtered, washed with Et_2O (4 × 10 ml), recrystallised from EtOH (15 ml) to give 3,4,6-tri-O-acetyl-1,2-O-(3,4,5,6-tetra-O-benzyl-*sn*-MI-1-O-orthoacetyl)-β-D-mannopyranose (**4a**); 0·75 g, 18·6%, m.p. 155·5–156°, $[\alpha]_D^{20}$ +9·8°, R_f 0·52 (A); ν max: 3560 (OH), 3085, 3060, 3015, 1600, 1495 (benzene rings), 1750, 1730 (C=O in acetates) cm^{-1}; PMR, δ 1·75 (C—Me), 2·00 (3 MeCO).

The combined ether extract was evaporated and the compound (9·12 g) chromatographed on Al_2O_3 (450 g) in CCl_4:$CHCl_3$(1:1). Fractions containing orthoacetate **5** were repeatedly purified by prep TLC (A) and crystallised from EtOH (2 ml), 0·35 g, 8·68%, m.p. 151–155°, R_f 0·63 (A); ν max: 3500 (OH), 3090, 3060, 3015, 1495 (benzene rings), 1740 (C=O in acetates) 1065 (C—O—C) cm^{-1}; PMR, δ 1·72 (C—Me).

Fractions containing orthoester **3a** were evaporated to dryness, the residue (0·92 g) purified by prep TLC (A) and crystallised from EtOH (2 ml) as orthoacetate **3a**, 0·39 g, 9·68%, m.p. 135–137°, $[\alpha]_D^{20}$ +0·54°, R_f 0·4 (A); ν max: 3560 (OH), 3085, 3060, 3015, 1600, 1495 (benzene

Table 3. Analytical data

Compound	Formula	Calc. % C	Calc. % H	Found % C	Found % H	Compound	Formula	Calc. % C	Calc. % H	Found % C	Found % H
3a	$C_{48}H_{54}O_{15}$	66·19	6·25	66·24	6·50	**7j**	$C_{41}H_{82}O_{13}NP^c$	59·47	9·98	59·00	9·74
3d	$C_{30}H_{40}O_{20}$	49·99	5·59	50·09	5·55	**7k**	$C_{82}H_{113}O_{13}P^d$	73·62	8·51	73·57	8·64
4a	$C_{48}H_{54}O_{15}$	66·19	6·25	66·03	6·41	**9a**	$C_{33}H_{46}O_{15}$	57·30	6·91	57·46	7·37
4b	$C_{70}H_{72}O_{12}$	76·06	6·56	75·88	6·43	**9b**	$C_{33}H_{48}O_{15}$	57·88	7·06	58·07	7·34
4d	$C_{30}H_{40}O_{20}$	49·99	5·59	50·37	5·15	**10a**	$C_{32}H_{46}O_{15}$	57·30	6·91	56·98	7·00
5	$C_{48}H_{54}O_{15}$	66·19	6·25	65·58	6·70	**10b**	$C_{33}H_{48}O_{15}$	57·88	7·06	57·86	7·25
6a	$C_{34}H_{36}O_6$	75·53	6·71	75·23	6·84	**11a**	$C_{32}H_{46}O_{15}$	57·30	6·91	57·29	7·06
6b	$C_{41}H_{42}O_6$	78·07	6·70	78·26	6·81	**11b**	$C_{33}H_{48}O_{15}$	57·88	7·06	58·00	7·47
6c	$C_7H_{14}O_6$	43·29	7·22	43·44	7·15	**12**	$C_{18}H_{28}O_6$	63·51	8·29	63·54	8·23
6d	$C_{16}H_{22}O_{11}$	49·22	5·67	49·08	5·65	**13**	$C_{18}H_{28}O_6$	63·51	8·29	63·44	8·18
6e	$C_{25}H_{38}O_{11}$	57·49	5·87	57·34	5·42	**14a**	$C_{48}H_{54}O_{15}$	66·19	6·25	65·74	6·67
7a	$C_{34}H_{36}O_6$	75·53	6·71	75·53	6·84	**14b**	$C_{49}H_{56}O_{15}$	66·50	6·37	66·74	6·45
7b	$C_{35}H_{38}O_6$	75·79	6·90	75·40	6·75	**15a**	$C_{48}H_{54}O_{15}$	66·19	6·25	65·92	6·26
7c	$C_{35}H_{38}O_6$	75·79	6·90	75·35	6·74	**15b**	$C_{49}H_{56}O_{15}$	66·50	6·37	66·15	6·42
7d	$C_7H_{14}O_6$	43·29	7·22	43·01	7·26	**16a**	$C_{32}H_{46}O_{15}$	57·30	6·91	57·09	7·12
7e	$C_{41}H_{42}O_6$	78·07	6·70	77·99	6·57	**16b**	$C_{33}H_{48}O_{15}$	57·88	7·06	57·96	7·15
7f	$C_{53}H_{51}O_9P^a$	73·77	5·95	73·75	6·08	**17a**	$C_{32}H_{46}O_{15}$	57·30	6·91	57·51	6·63
7g	$C_{18}H_{39}O_9N_2P^b$	—	—	—	—	**17b**	$C_{33}H_{48}O_{15}$	57·88	7·06	57·44	6·94
7h	$C_{16}H_{22}O_{11}$	49·22	5·67	49·41	5·77	**18**	$C_{46}H_{64}O_{24}$	55·19	6·44	55·05	6·70
7i	$C_{23}H_{28}O_{11}$	57·49	5·87	57·24	5·63						

[a]Calc. %: P 3·59. Found %: P 3·60.
[b]Calc. %: N 6·11; P 6·75. Found %: N 5·98; P 6·79.
[c]Calc. %: N 1·70; P 3·74. Found %: N 1·78; P 3·81.
[d]Calc. %: P 2·31. Found %: P 2·24.

rings), 1760, 1740 (C=O in acetates) cm^{-1}; PMR, δ 1·75 (C—Me), 1·95 (MeCO).

Similarly, reesterification of orthoacetate 2 R^3=Ac with respective racemic asymmetrically substituted MI[3,11] led to orthoesters **3c**, **4c**, **9a**, **10a**, **11a**, diastereomeric mixtures of **3d**, **4d**, **16a**, **17a**, **18**, reesterification of 3,4,6-tri-O-benzyl-1,2-O-ethylorthoacetyl-β-D-mannopyranose (2 R^3=Bz)[32] with 1 R=R^1=Bz[32] resulted in orthoacetates **3b**, **4b**, and reesterification of 3,4,6-tri-O-acetyl-1,2-O-t-butylorthoacetyl-α-D-glucopyranose[44] with 1; R=Bz, R'=H gave rise to diastereomeric mixture **14a**, **15a** (Table 1). For orthoesters **9a**, **10a**, **11a**, **16a**, **17a**, **18** a twofold excess of orthoacetate 2 R^3=Ac was used.

The sample of resulting orthoester was completely hydrolysed[26] by 0·1 N H_2SO_4 in 90% aqueous Me_2CO for 30 min at 18–20°.

3,4,6-*Tri*-O-*benzyl*-1,2-O-(2,3,4,5,6-*penta*-O-*benzyl*-sn-MI-1-O-*orthoacetyl*)-β-D-*mannopyranose* (**4b**). A mixture of orthoacetate **4a** (0·8 g) and powdered KOH (0·52 g) in $PhCH_2Cl$ (0·07 g) and 25 ml THF was boiled for 8·5 hr. Excess $PhCH_2Cl$ and dibenzyl ether were removed by steam distillation and the residue extracted with C_6H_6 (3 × 20 ml). The organic layer was washed with H_2O (3 × 10 ml), dried (Na_2SO_4) and evaporated. The product crystallised from EtOH (10 ml) as **4b**. 0·724 g, 71·3%, m.p. 112–113°, $[\alpha]_D^{20}$ +47·1°, R_f 0·31 (B); ν max: 3095, 3060, 3040, 1605, 1500 (benzene rings) cm^{-1}; PMR, δ 1·78 (C—Me). Similarly, orthoacetate **3a** resulted in **3b** (Table 1).

3,4,6-*Tri*-O-*acetyl*-1,2-O-(1,2,4,5,6-*penta*-O-*acetyl*-sn-MI-3-O-*orthoacetyl*)-β-D-*mannopyranose* (**3d**). A solution of orthoacetate **3c** (0·052 g) was kept in Py (2 ml) and Ac_2O (0·5 ml) for 48 h at 20°. The mixture was evaporated, MeOH (10 ml) added and again evaporated three times. Orthoacetate **3d** was obtained by TLC, 0·033 g, 58·9%, $[\alpha]_D^{20}$ −9·24°, R_f 0·61 (D); ν max: 1750 cm^{-1} (C=O in acetates); PMR, δ 1·62 (C—Me), 1·8–2·22 (7 MeCO). Similarly, compound **4c** gave orthoester **4d** (Table 1).

3,4,6-*Tri*-O-*acetyl*-1,2-O-(1,2:5,6-di-O-*cyclohexylidene*-3-O-*methyl*-sn-MI-4-O-*orthoacetyl*)-β-D-*mannopyranose* (**9b**). To the solution of orthoester **9c** (0·67 g) in MeI (2 ml), freshly supplied Ag_2O (0·464 g) and drierite (0·19 g) were added. The mixture was boiled for 10 hr, additional 0·5 ml of MeI and 0·232 g of Ag_2O were added and the reaction continued for 20 hr. Solid was filtered, washed with $CHCl_3$ (4 × 10 ml) and solvent evaporated. Prep TLC of the residue (E) yielded 0·185 g of orthoester **9a**, R_f 0·38, and 0·485 g (80·7%) of **9b**, R_f 0·82. After crystallisation from 15 ml of Et_2O/light petroleum (1:10) **9b** had: m.p. 150–160° (deformation at 85–88°), $[\alpha]_D^{20}$ +16·7°; ν max: 1750 cm^{-1} (C=O in acetates); PMR, δ 1·74 (C—Me), 2·01 (2 MeCO), 2·07 (MeCO), 3·44 (OMe). Similarly, methylation of respective orthoesters led to **10b**, **11b**, **14b**, **15b**, **16b**, **17b** (Table 1).

1,4,5,6-*Tetra*-O-*benzyl*-sn-MI (**6a**). Orthoester **3a** (0·06 g) was treated with of 0·1 N H_2SO_4 (110 ml) in 90% aqueous Me_2CO for 1 hr at 20°. The mixture was mixed with amberlite IRA −400 (OH-form) for 10 min at 20°, filtered and the resin washed with Me_2CO (2 × 30 ml). Solvent was evaporated and the product (0·055 g) crystallised from MeOH (1 ml), dried for 6 hr at 70–80° at 0·01 mm to obtain tetrabenzyl-MI (**6a**); 0·03 g, 81·2%, m.p. 140·2–142·1°, $[\alpha]_D^{20}$ +25·0°, R_f 0·16 (A); ν max: 3460, 3350 (OH), 3090, 3070, 1605, 1500 (benzene rings) cm^{-1}. Similarly, respective orthoesters gave **6b**, **7a**, and **7e** (Table 2).

3-O-*Methyl*-sn-MI, (+)-*bornesitol* (**6c**). Orthoester **9b** (0·14 g) was heated in 80% AcOH (2 ml) for 3 hr at 100°. The mixture was evaporated to dryness and 5 ml of MeOH added to the residue. The product was crystallised from MeOH (0·2 ml) to give 3-O-methyl-sn-MI (**6c**), identical with natural (+)-bornesitol as evidenced by m.m.p., IR, TLC and PC. Yield 0·022 g, 55·4%, m.p. 202–204°, $[\alpha]_D^{20}$ +32·8°, R_f 1·4 (H); ν max: 3410, 3330, 3200 (OH), 2800 (MeO) cm^{-1}. Similarly, hydrolysis of respective orthoesters **3d** and **4d** gave pentaacetates **6d** and **7h** (Table 2).

1,2:5,6-*Di*-O-*cyclohexylidene*-sn-MI (**12**). The solution of orthoester **9a** (0·335 g) in $C_2H_4Cl_2$ (2 ml) was boiled, evaporating 5–10 ml of solvent and adding simultaneously $C_2H_4Cl_2$ to retain constant volume. To the reaction MeOH (0·04 ml) and TsOH (0·002 g) were added and the mixture brought to boiling in 2–3 min. 2–3 drops of Py were added to the cooled mixture which was evaporated. The residue on prep TLC (E) gave: (i) 3,4,6-tri-O-acetyl-1,2-O-methylorthoacetyl-β-D-mannopyranose (0·129 g, 79·5%);[25] (ii) the starting orthoester **9a** (0·035 g); (iii) diketal **12** (0·11 g, 72·3%). After twice crystallisation from 5 ml of the C_6H_6-light petroleum mixture (2:3) **12** had m.p. 153–156°, $[\alpha]_D^{20}$ −7·4°, R_f 0·08 (G); νmax: 3500, 3320, 3160 (OH). Similarly, reesterification of orthoacetate **10a** with MeOH resulted in 2,3:4,5-di-O-cyclohexylidene-sn-MI (**13**) (Table 2).

2,3,4,5,6-*Penta*-O-*acetyl*-1-O-*benzyl*-sn-MI (**7i**). A mixture of 1,4,5,6-tetra-O-benzyl-sn-MI (**6a**) (0·5 g) and acetolysis mixture[5] (3 ml) was kept for 60 hr at 0° and poured into glacial H_2O (50 ml) with $NaHCO_3$ (pH 9) and $CHCl_3$ extracted. The $CHCl_3$ solution was water washed (3 × 20 ml) and dried (Na_2SO_4). Solvent was removed and the product crystallised from EtOH (3 ml). 0·113 g, 45·3%, m.p. 180–182·5°, $[\alpha]_D^{20}$ −26·56°, R_f 0·9 (B); ν max: 3060, 3040, 1590 (benzene rings), 1750 (C=O in acetates) cm^{-1}; PMR, δ 2·00 (4 MeCO), 2·18 (MeCO), 3·62 (J = 2·7 and 8 Hz, 1-H).[5] Similarly, 3,4,5,6-tetra-O-benzyl-sn-MI (**7a**) led to **6e** (Table 2).

2,3,4,5,6-*Penta*-O-*acetyl*-sn-MI (**7h**). 0·08 g of **7h** was hydrogenated in glacial AcOH (10 ml) in the presence of palladium black (0·25 g) at 20°. Catalyst was filtered, washed with glacial AcOH (3 × 2 ml), the solvent evaporated, and the residue crystallised from H_2O (0·5 ml) to yield (0·05 g, 77·1%) pentaacetate **7h** identical with that obtained from orthoester **6d** (Table 2).

Similarly, **6e** resulted in pentaacetate **6d** (79·4%) identical with that prepared from orthoacetate **3d** (Table 2).

1-O-*Methyl*-3,4,5,6-*tetra*-O-*benzyl*-sn-MI (**7b**). To a solution of **7a** (0·5 g) in C_6H_6 (20 ml) MeI (0·07 ml) and powdered KOH (6·5 g) were added. The mixture was stirred for 2 hr at 100°, cooled, diluted with H_2O (20 ml) and C_6H_6 (3 × 10 ml) extracted. Solvent was removed and the product crystallised from light petroleum (150 ml). Prep TLC produced an analytically pure sample of **7b**. 0·13 g, 25·3%, m.p. 115–116°, $[\alpha]_D^{20}$ −1·5°, R_f 0·58 (A); ν max: 3400 (OH), 3100, 3075, 3040, 1610, 1500 (benzene rings), 2805 (MeO) cm^{-1}.

The mother liquor was concentrated and the prep TLC of the residue gave 2-O-methyl-3,4,5,6-tetra-O-benzyl-sn-MI (**7b**) 0·03 g, 5·8%, m.p. 137–138°, $[\alpha]_D^{20}$ −69·4°, R_f 0·8 (A); ν max: 3360, 3300 (OH), 3090, 3060, 3035, 1610, 1500 (benzene rings), 2805 (MeO) cm^{-1}.

1-O-*Methyl*-sn-MI, (−)-*bornesitol* (**7e**). Methyl ether **7b** (0·06 g) was hydrogenated in the presence of palladium black (0·01 g) in glacial AcOH (2 ml) for 5 hr and catalyst separated and washed with glacial AcOH (5 ml). The filtrate was evaporated, MeOH (2 ml) added to the residue which was again evaporated. The residue was crystallised

from MeOH (0·5 ml) to give **7d**, identical with the natural (−)-bornesitol (m.m.p., IR, TLC, PC). Yield 0·015 g, 71·4%, m.p. 204–205·5°, $[\alpha]_D^{20}$ −33·2°, R_f 1·4 (H).

Bis-cyclohexylammonium salt of sn-MI 1-*phosphate* (**7g**). To a solution of pentabenzyl ether (0·32 g) in Py (2 ml) at 0°, $(PhO)_2P(O)Cl$ (0·15 g) was added with stirring. The mixture was agitated for 16 hr at 20° and poured into glacial H_2O (100 ml), extracted (4 × 25 ml) and the extract washed with glacial H_2O (4 × 15 ml) and dried (Na_2SO_4). Solvent was evaporated and the product crystallised from 8 ml of hexane to give 1-diphenylphosphate 2,3,4,5,6-penta-O-benzyl-sn-MI (**7f**). 0·3 g, 68·5%, m.p. 64–64·5°, $[\alpha]_D^{20}$ −11°, R_f 0·3 (B); ν max: 3090, 3070, 3015, 1600, 1500 (benzene rings), 1270, 1260 (P═O), 950 (P—O—C) cm^{-1}.

Compound **7f** (0·15 g) in EtOH (15 ml) was hydrogenated in the presence of PtO_2 (0·01 g) and then palladium black (0·02 g). Conventional treatment[6] resulted in phosphate **7g** identical with the bis-cyclohexylammonium salt of natural sn-MI 1-phosphate obtained by modified procedure[6] from soya bean phosphatides. 0·062 g, 77·6%, m.p. 202–204°, $[\alpha]_D^{20}$ +3·4°, R_f 1·31 (7); ν max: 3400–3000 (OH, NH, $\overset{+}{N}H_3$), 1640, 1510 ($\overset{+}{N}H_3$), 1230 (P═O), 1080 (P—O^-), 1030 (P—O—C) cm^{-1}.

1-O-[1,2-*Di*-O-*palmitoyl*-sn-*glyceryl*-3-O-(*phenyl*)*phosphoryl*]-2,3,4,5,6-*penta*-O-*benzyl*-sn-MI (**7k**). To **7e** (0·7 g) in Py (2 ml) was added $PhOP(O)Cl_2$ at 0° and stirred for 24 hr at 20°, with 1,2-di-O-palmitoyl-sn-glycerol (0·63 g)[45] then added in Py (3 ml) and $CHCl_3$ (3 ml) at 0°. The mixture was agitated for 24 hr at 20°, H_2O (2 ml) added and $CHCl_3$ (3 × 40 ml) extracted after 30 min. The extract was H_2O washed (3 × 30 ml), dried (Na_2SO_4) and evaporated. The product consisted, according to TLC, of intact **7e** and 1,2-diglyceride, phenylphosphatidic and phenyl-bis-phosphatidic acids and of phosphoinositide **7k** was separated by chromatography on silicic acid to obtain **7k**. 0·49 g, 33·2%, m.p. 53–54° (EtOH), $[\alpha]_D^{20}$ −7·76°, R_f 0·65 (silicic acid, Et_2O-hexane, 1 : 1); ν max: 3100, 3040, 1595, 1500 (benzene rings), 1745 (C═O in COOR), 1215 (P═O) cm^{-1}.

1-O-(1,2-*Di*-O-*palmitoyl*-sn-*glyceryl*-3-O-*phosphoryl*)-sn-MI (**7j**, *ammonium salt*). **7k** (0·4 g) in EtOH (40 ml) was shaken in H_2 in the presence of PtO_2 at 18–20° until the phenyl group was completely removed (TLC). The catalyst was separated and washed with EtOH (10 ml). The filtrate was hydrogenated in the presence of palladium black to completely remove the benzyl groups (TLC, IR). Catalyst was separated and 4N NH_4OH added to the alcoholic solution to pH 7–8, the solvent was distilled *in vacuo* at 30–40°. The residue was dissolved in $CHCl_3$ (5 ml), Me_2CO (10–15 ml) was added and the mixture left to stand for 18–20 hr at 0°. The precipitate was filtered, washed with Me_2CO (5 ml) to result in phosphatidylinositol **7j**, 0·12 g, 48·5%, m.p. 169–172°, $[\alpha]_D^{20}$ +7·48°, R_f 0·6 (silica gel KSK, $CHCl_3$-MeOH-4N NH_4OH, 9 : 7 : 2);* max: 3350 (OH), 3150, 3060, 1425 ($\overset{+}{N}H_4$), 1740 (C═O in COOR), 1325, 1300, 1280, 1260, 1235 (CH_2), 1215 (P═O), 1060 (P—O—C, C—O—C) cm^{-1}.

*Spots on slides were detected by the reagent for phosphoinositides.[46]

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