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DIRECT GAS-CHROMATOGRAPHIC RESOLUTION OF DL-*myo*-INOSITOL 1-PHOSPHATE AND OTHER SUGAR ENANTIOMERS AS SIMPLE DERIVATIVES ON A CHIRAL CAPILLARY COLUMN*

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ABSTRACT

A commercially available, chiral stationary-phase was used to separate sugar enantiomers. Derivatives commonly used for the gas chromatography of carbohydrates were employed. The most generally useful was found to be the per(heptafluorobutanoyl) derivative, in which form D- and L-arabinose, fucose, xylose, mannose, and *chiro*-inositol were well resolved. The D and L enantiomers of glucose were best separated as 6-*O*-trimethylsilyl-α-glucofuranose 1,2:3,5-bis(methaneboronate). DL-*myo*-Inositol 1-phosphate was well resolved as the 2,3,4,5,6-penta-*O*-(trimethylsilyl)-1-dimethylphosphate. Other derivatives were less well resolved, and some sugar enantiomers could not be separated by the means used.

INTRODUCTION

Previously reported gas-chromatographic separations of sugar enantiomers have utilized diastereomeric derivatives which were separated on conventional packed or capillary columns. The use of optically active alcohols esterified to sugar acids[1–3] has been described, as well as glycosides of reducing sugars[4,5]. In these examples, the sugars were gas-chromatographically resolved as the acetyl[1,2,5] or trimethylsilyl[3,4] co-derivatives. Another technique is to form bis(ethyl L-lactate) acetals from aldose diethyl dithioacetal peracetates[6].

A more attractive approach to the separation of sugar enantiomers is the chromatography of common derivatives on a capillary column coated with an optically active phase. These derivatives are generally produced in quantitative yield by simple procedures, and many have been developed that contain moieties which enhance their detection. In addition, many substances have been mass-spectrometrically evaluated in the form of derivatives prepared for gas chromatography.

We became interested in the use of chiral gas chromatography in order to

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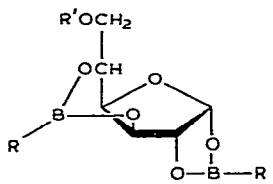
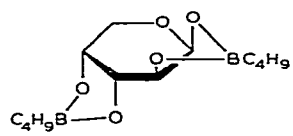
determine the enantiomeric composition of *myo*-inositol 1-phosphate in rat brain[7]. We found that the optical isomers of the inositol phosphate could be separated as the per(trimethylsilyl)-dimethylphosphate derivative on a commercially available, glass capillary column coated with a copolymer of *N-tert*-butyl-L-valinamide and an organosiloxane (Chirasil-Val)[8,9]. We now report our evaluation of the general usefulness of the Chirasil-Val capillary column for the direct separation of enantiomers of other inositol phosphates and of other sugars as simple, gas-chromatographic derivatives.

RESULTS

Separation of aldose enantiomers. — Tables I and II list those sugar derivatives for which enantiomeric separations have been achieved. In general, the heptafluorobutanoyl (Hfb) derivative was found to be the most effective. DL-Arabinose (**1**),





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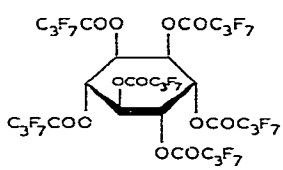
6 R = CH_3, R' = Me_3Si
7 R = C_4H_9, R' = COC_3F_7
8 R = C_4H_9, R' = Me_3Si
9 R = C_4H_9, R' = Ac
10 R = C_4H_9, R' = Ac
11 R = C_8H_{17}, R' = Ac



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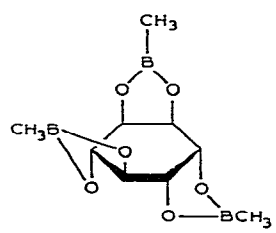

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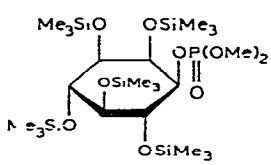

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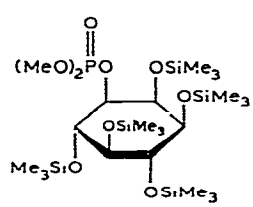

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TABLE I

ENANTIOMERIC SEPARATION OF HEPTAFLUOROBUTANOATE DERIVATIVES[a]

Per(heptafluorobutanoyl) derivative of	*Retention time (min)*		*Separation factor*[b]	*Resolution*[c]	*Column temperature (°C)*
	L *enantiomer*	D *enantiomer*			
Arabinose (1)	6.12	6.41	1.05	0.71	105
Fucose (2)	7.01	6.76	1.04	0.50	100
Mannose (3)	3.13	3.38	1.08	1.19	125
Xylose (4)	14.84	14.38	1.03	0.3[d]	95
Glucose (5)	5.93	5.96	1.01	0.2[d]	120
chiro-Inositol (16)	3.72	3.40	1.09	1.03	115

[a]Gas chromatography on a 20-m Chirasil-Val capillary column at 15 lb.in^{-2} as the per(heptafluorobutanoyl) derivative. [b]Greater retention-time (uncorrected) divided by lesser retention-time. [c]Resolution, except where noted, as defined by $R = 2d/w_1 + w_2$, where d is the distance between the two peaks and w is the width of each peak at baseline. [d]An approximate resolution equivalent to the depth of the valley between two peaks divided by the average peak-height.

TABLE II

ENANTIOMERIC SEPARATION OF BORONATE DERIVATIVES[a]

	Retention time (min)		*Separation factor*[b]	*Resolution*[c]	*Column*[d] *temperature (°C)*
	L *enantiomer*	D *enantiomer*			
Glucose Meb-Me_3Si (6)	5.64	5.75	1.02	0.8	130
Glucose Bub-Hfb (7)	6.09	6.22	1.03	0.8	180
Glucose Bub-Me_3Si (8)	10.18	10.35	1.02	0.6	170
Glucose Meb-Ac (9)	14.65	15.01	1.02	0.2	110
Glucose Bub-Ac (10)	15.04	15.28	1.02	0.2	170
Glucose Octb-Ac (11)	19.45	19.65	1.01	0.4	175[e]
Arabinose Bub (12)	11.64	11.82	1.02	0.3	140
chiro-Inositol Meb (17)	2.92	3.00	1.03	0.4	150

[a]Gas chromatography on a 25-m Chirasil-Val capillary column as methaneboronates (Meb), butaneboronates (Bub) or the octaneboronate (Octb), sometimes coderivatized with a trimethylsilyl, heptafluorobutanoyl (Hfb), or acetyl (Ac) group. [b]Uncorrected R_L/uncorrected R_D. [c]An approximate resolution obtained from the depth of the valley between two enantiomers divided by the average peak-height. [d]Column-head pressure of 20 lb. in.$^{-2}$ in each case, except for 17 which was determined at 18 lb. in.$^{-2}$. [e]Temperature programmed: 125° for 4 min, (isothermal) and then 10°/min to 175°.

DL-fucose (2), and DL-mannose (3) were all well resolved (Fig. 1, Table I) as the per(Hfb) esters. DL-Xylose heptafluorobutanoate (4) was less-well resolved, and the per(Hfb) derivative of DL-glucose (5) was nearly unresolved by this column (Table I). The best separations of the glucose enantiomers (Table II) were obtained by using the methaneboronate-Me_3Si derivative, which has the structure 6-*O*-trimethylsilyl-

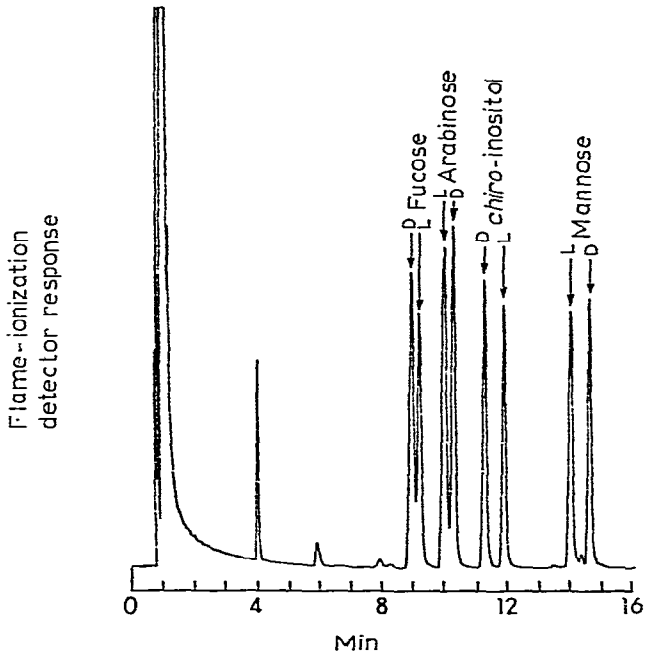

Fig. 1. Gas chromatogram of the per(heptafluorobutanoic) esters of the mixed enantiomers of fucose, arabinose, *chiro*-inositol, and mannose. The peak at 4 min is an unknown of variable occurrence related to the derivatization procedure. Each peak represents ~10 ng of a sugar (underivatized weight) on the column. Column: 25-m, 0.3-mm (i.d.) open-tubular glass capillary coated with Chirasil-Val. Helium carrier, 15 lb. $in.^{-2}$; split injection; temperature program: 95° for 8 min and then 3° per min. Flame-ionization detection.

α-D-glucofuranose 1,2:3,5-bis(methaneboronate)[10] (**6**), and the bis(methaneboronate) heptafluorobutanoate (**7**), which probably has the analogous structure[11]. As Table II shows, the butaneboronate-Me_3Si derivative (**8**) as well as 6-*O*-acetyl-α-DL-glucofuranose 1,2:3,5-bis(methane-, butane- and octaneboronates), (**9**, **10**, **11**) all showed poorer separation of enantiomers than either **6** or **7**. DL-Arabinose butaneboronate (**12**), Table II, was slightly resolved as the methaneboronate (**13**), with a resolution of <0.2 ($R = 2d/w_1 + w_2$). Fucose as the butaneboronate (**14**) was very slightly resolved ($R < 0.1$, 33 min retention time 120°, 12 $lb.in^{-2}$) as was mannose as the methaneboronate Me_3Si-coderivative (**15**) ($R < 0.1$, 20 min, 20 $lb.in^{-2}$, 95°).

No enantiomeric separation was achieved with α-DL-xylofuranose 1,2:3,5-bis(butaneboronate), tetra-*O*-(trimethylsilyl)-DL-arabinose, DL-fucose bis(methaneboronate), or with the bis(butaneboronate)-acetate and trimethylsilyl coderivative of DL-mannose.

The anomeric composition of Hfb derivatives of aldoses. — When pure α- and β-D-glucose were derivatized with heptafluorobutanoylimidazole and chromatographed on a non-polar methylpolysiloxane capillary column (DB-1, J. & W. Scientific), following overnight equilibration at room temperature, the α anomer accounted for 89% of the total peak area in each case. The retention time of the α anomer is

0.59 relative to the β form. When the anomeric mixture of per(Hfb) D-glucose was subjected to gas chromatography on the Chirasil-Val column, only a single peak was observed. Each enantiomer of fucose, arabinose, and mannose also emerged from the chiral phase as a single peak as the per(Hfb) derivative. On the DB-1 column, the Hfb-aldoses chromatographed as two peaks the area percentages of which were: D-fucose, peak 1, 7%, peak 2, 93% (relative retention time, 0.86); L-arabinose peak 1, 9%, peak 2, 91% (relative retention, 0.86); L-mannose, peak 1, 9%, peak 2, 91% (relative retention 0.88).

Inositol and inositol phosphates. — The only optically active diastereomer of the unsubstituted inositols is *chiro*-inositol. Fig. 1 and Table I show that these enantiomers may be separated as the hexakis(heptafluorobutanoic) esters (**16**). *chiro*-Inositol 1:2,3:4,5:6 tris(methaneboronate)[12] (**17**) was partially resolved by a 25-m column (Table II), however, the enantiomers did not separate as the per(trimethylsilyl) ethers. *myo*-Inositol forms the (±)-1,2:3,5:4,6-tris(butaneboronate) on treatment with butaneboronic acid in pyridine[12]. This ester, although a racemic mixture, does not separate on the chiral column.

Of the *myo*-inositol monophosphates, three can form enantiomeric pairs: *myo*-inositol 1-phosphate, *myo*-inositol 4-phosphate, and *myo*-inositol 1,2-cyclic phosphate. We have examined each of these as the trimethylsilyl ether-methyl ester derivatives and find that only *myo*-inositol 1-phosphate undergoes enantiomeric separation on Chirasil-Val, when chromatographed as the 2,3,4,5,6-pentakis(*O*-trimethylsilyl)-1-(dimethylphosphate) **18**. Separation factors of 1.03–1.07 (corrected R_t of D form/L form) were obtained at temperatures of from 220 to 160°. Resolution was also excellent (R = 0.8 at 220°, 1.14 at 190°, and 2.08 at 160°). In the latter case, the L enantiomer was separated from the D form by almost 3 min (average R_t 42 min).

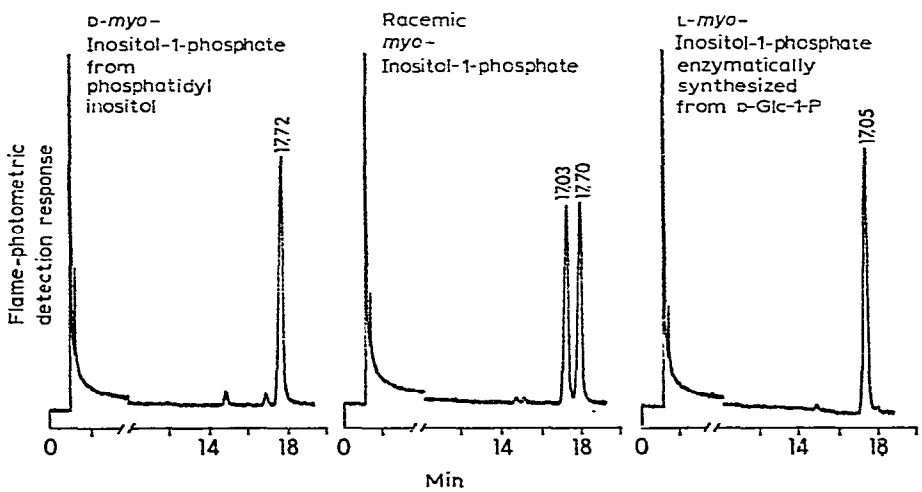

Fig. 2. Gas chromatogram on the same column as Fig. 1 of *myo*-inositol 1-phosphates as the 2,3,4,5,6-penta-*O*-(trimethylsilyl)-1-*O*-dimethylphosphate derivative. Each peak represents ~12 ng of the free acid on a 20-m Chirasil-Val column. Split injection, helium carrier 20 lb. in.$^{-2}$; 190° isothermal. Flame-photometric detection in phosphorus-selective mode.

The analogous derivatives of racemic mixtures of the other two cyclitol phosphates did not separate. None of the per(trimethylsilyl) derivatives of these three inositol phosphates underwent satisfactory chromatography on the chiral phase. Each produced broad peaks and low recovery, based on the detector response.

An application of the chiral separation of *myo*-inositol 1-phosphate is the determination of which enantiomer of this cyclitol phosphate is formed by *myo*-inositol-1-phosphate synthase (E.C. 5.5.1.4) of bovine testis. The synthase from rat testis has been shown to convert D-glucose 6-phosphate into L-*myo*-inositol 1-phosphate[13]. In our laboratory, we have purified the inositol phosphate synthase from bovine testis to homogeneity[14]. The chiral identity of the product of the bovine enzyme had not been established prior to our development of the separation reported in this paper. Fig. 2 shows that the product of the bovine testis synthase has chirality opposite to that of the *myo*-inositol-1-phosphate produced on hydrolysis of soybean phosphatidyl inositol[15]. The latter inositol phosphate has been shown to have an absolute configuration[16] that has been designated as the D enantiomer[17]. Therefore, the bovine testis synthase produces L-*myo*-inositol 1-phosphate, as does the rat enzyme.

Miscellaneous carbohydrates. — The only alditol enantiomers tested were those of arabinitol, which did not separate as either the Me_3Si or the heptafluorobutanoate derivatives. The aldonolactones DL-galactono-1,4-lactone and DL-gulono-1,4-lactone were unresolved as the heptafluorobutanoates, the only derivative tested. DL-Glycero-aldehyde 1-phosphate was subjected to gas chromatography as the butaneboronate trimethylsilyl ether without enantiomeric separation.

DISCUSSION

All of the enantiomeric separations previously described on the chiral chromatographic phase used in this study were with such nitrogen-containing substances as amino acids and 2-amino-1-phenylethanols[7,8]. In the case of lactic acid, the nitrogen was introduced by formation of the cyclohexylamide[8]. Each of these compounds was derivatized by esterification with pentafluoropropanoic acid. The separations of the nitrogen-containing isomers were uniformly achieved with the D enantiomer being eluted before the L form. This order was attributed to the better association of the L enantiomer with the chiral chromatographic phase, which contains *N-tert*-butyl-L-valine in amide linkage to a polysiloxane. Our work shows that the nitrogen moiety is not necessary for the chiral separation and that the fluoroacyl derivative is not the only one suitable for these separations.

The carbohydrate derivatives we have tested show considerable variability with respect to the chiral separation. For example, DL-mannose is well separated chromatographically as the heptafluorobutanoate (3, Table I, Fig. 1), but is almost unresolved as the methaneboronate-trimethylsilyl derivative (15). Just the opposite is the case with glucose, which is only slightly resolved as the perfluorobutanoate (5), but is well separated as the methaneboronate-Me_3Si derivative (6). Thus, for this

application, use of the chiral column must be approached empirically, but with wide scope for derivative selection.

We have observed what we interpret to be interactions of some sugar esters with the chiral column phase. Whenever trimethylsilyl esters of phosphorylated substrates were chromatographed, severe tailing occurred. Often, sample loss was evident from the diminished detector-response. These problems were eliminated by forming the methylphosphates and were absent in Me_3Si derivatives not containing ester moieties. We believe that the chromatographic effects arise from interaction between the liquid phase and the highly reactive (see Experimental) trimethylsilyl ether, phosphate ester groups. *chiro*-Inositol was also found to be chromatographed poorly on the chiral phase as the alkaneboronate derivative, whereas the *myo*-inositol alkaneboronates did not behave in this way. This result may, again, be attributable to reactivity, as we have observed that *chiro*-inositol tris(alkaneboronates), which have one *trans* cyclic boronic ester, are less stable than the all-*cis* esters[12].

EXPERIMENTAL

Heptafluorobutanoic esters. — Preparation. The sugar (0.1–0.5 mg) is suspended in 0.1 mL of heptafluorobutanoylimidazole (Pierce) and heated for 30–60 min at 60° with occasional mixing. The resulting, clear solution may be chromatographed directly or extracted with hexane, the hexane chilled (−20°) to remove the excess of reagent, and the sample then concentrated prior to chromatography. Heptafluorobutanoic anhydride caused extensive degradation of aldose samples.

Structure. Tetra-*O*-(heptafluorobutanoyl)arabinopyranose (**1**), mol. wt. 934, had a mass spectrum compatible with a pyranoid structure. Ions were observed at *m/z* 721 ($M^+ - C_3F_7CO_2\cdot$) (6%), 507 (*m/z* 721 − $C_3F_7CO_2H$) (7%), 478 ($M^+ - [C_3F_7CO_2H]_2 - CO$) (73%), 465 $[C_3F_7CO_2CH)_2CH]^+$ (39%), 452 $[C_3F_7CO_2CH)_2]^+$, and 293 (*m/z* 721 − $[C_3F_7CO_2H]_2$) (100%). Furanose peracetates[18] and pertrifluoroacetates[19] fragment readily with cleavage of the C-4–C-5 bond to form a stable ion containing the furan ring. This ion is absent or of very low abundance in acetates and trifluoroacetates of aldopyranoses. In the case of heptafluorobutanoated aldose, the ion occurs at *m/z* 707. No *m/z* 707 ion is observed in this spectrum, suggesting that the derivative is tetra-*O*-(heptafluorobutanoyl)arabinopyranose. One principal (>95%) gas-chromatographic peak is observed for each optical isomer on Chirasil-Val and only a single peak is observed on a DB-1 fused-silica capillary column (J & W Scientific, Rancho Cordova, CA).

Tetra-*O*-(heptafluorobutanoyl)fucopyranose (**2**), mol. wt. 948, also had a mass spectrum compatible with a pyranoid structure. Ions were found at *m/z* 735 ($M^+ - C_3F_7CO_2\cdot$) (5%), 690 ($M^+ - CH_3CHO$) (9%), 521 (*m/z* 735 − $C_3F_7CO_2H$) (2%), 492 ($M^+ - [C_3F_7CO_2H] - CO$) (14%), 465 $[(C_3F_7CO_2CH)_2CH]^+$ (40%), 452 $[(C_3F_7CO_2CH)_2]^+$ (12%), and 307 (*m/z* 735 − $[C_3F_7CO_2H]_2$) (100%). Evidence for the pyranose form is, again, the absence of *m/z* 707 from the spectrum.

Again, >90% of the derivative was chromatographed as a single g.l.c. peak on Chirasil-Val (single enantiomers) and on the DB-1 capillary column.

Penta-*O*-(heptafluorobutanoyl)mannopyranose (**3**), mol. wt. 1160, also had a mass spectrum compatible with a pyranoid structure, again because of the absence of *m/z* 707. Ions were found at *m/z* 733 ($M^+ - C_3F_7CO_2 \cdot - C_3F_7CO_2H$) (3%), 519 (*m/z* 733 — $C_3F_7CO_2CH_2$) (10%), 453 $[C_3F_7CO\ CH_2CHOCC_3F_7]^+$ (14%), and 452 $[C_3F_7CO_2CH)_2]^+$ (18%). One gas-chromatographic peak was observed (>99%) on Chirasil-Val (single enantiomer) and on the DB-1 capillary column.

Hexa-*O*-(heptafluorobutanoyl)-*chiro*-inositol (**16**), mol. wt. 1356, showed the highest mass-spectrometric ion at *m/z* 714 ($M^+ - [C_3F_7CO_2H]_3$) (13%). Other ions had *m/z* 519 (13%), 501 (20%), 305 (23%), 277 (28%), and 249 (12%).

Each enantiomer of the foregoing sugars had identical spectra, within experimental variation. Heptafluorobutanoyl derivatives generally show *m/z* 169 $[C_3F_7]^+$ as major ion.

The heptafluorobutanoyl derivatives of xylose (**4**) and glucose (**5**) were also prepared, but not examined mass spectrometrically.

Boronic esters. — *6-O-trimethylsilyl-α-glucofuranose 1,2:3,5-bis(methaneboronate)* (**6**) and the homologous butaneboronate (**8**) were made by the method of Reinnold *et al.*[10].

6-O-Acetyl-α-glucofuranose 1,2:3,5-bis(methaneboronate) (**9**) and the homologous *n*-butaneboronate (**10**) and *n*-octaneboronate (**11**) were made by the method previously described[11]. *n*-Octaneboronic acid was prepared as described earlier[12].

6-O-(Heptafluorobutanoyl)-α-glucofuranose 1,2:3,5-butaneboronate (**7**) was prepared by the treatment of glucose with butaneboronic acid[11] followed by the addition of 100 μL of heptafluorobutanoylimidazole per mg of sugar. This reaction gave a complex mixture, as judged by the chromatogram; however, the enantiomers eluted free of adjacent peaks.

β-Arabinopyranose 1,2:3,4-bis(butaneboronate) (**12**) and *methaneboronate* (**13**) have been previously described[10] as has α-L-*fucopyranose 1,2:3,4-di(butaneboronate)* (**14**).

The mannose methaneboronate trimethylsilyl coderivative (**15**) should have the structure 1-*O*-trimethylsilyl-α,β-mannofuranose 2,3:5,6-bis(methaneboronate), based on studies with the acetate coderivative[11], however, the 2,3:4,6-diboronate has been suggested[10].

chiro-Inositol 1,2:3,4:5,6-tris(methaneboronate) (**17**) was prepared as earlier described[12].

Inositol phosphates. — *2,3,4,5,6-penta-O-trimethylsilyl-myo-inositol-1-dimethylphosphate* could be prepared by treating a methanolic solution of the inositol phosphate as the free acid with diazomethane and then with trimethylsilylating reagent[20]. In our hands, this procedure gave mainly the 1-phosphate in admixture with other phosphorylated products, apparently the result of phosphate migration. The dimethyl ester of the inositol phosphate was best prepared by taking advantage of the high reactivity of the trimethylsilyl phosphate moieties. The inositol phosphate was per(tri-

methylsilylated) with 9:1:10 (v/v) *N,O*-bis(trimethylsilyl)trifluoroacetamide–chlorotrimethylsilane–pyridine, taken to dryness under a stream of nitrogen, and then dissolved in 1 mL of 10% anhydrous methanol in diethyl ether at 0°. This procedure removes the trimethylsilyl groups in ester linkage with phosphate while leaving the trimethylsilyl ether groups intact. After 5 min, ethereal diazomethane (from Diazald, Aldrich) was added dropwise until the color persisted. After a further 5 min at 0°, the solvents and the excess of diazomethane were removed in a stream of nitrogen at 50° and the residue taken up in the trimethylsilylating reagent. Under these conditions, the samples obtained were less complex than with direct diazomethane treatment. The structure of this derivative was confirmed by chemical-ionization mass spectrometry with ammonia as reagent gas. The protonated molecular ion, *m/z* 649, of the inositol phosphate-$(Me_3Si)_5Me_2$, was observed.

D-*myo*-Inositol 1-phosphate was prepared by hydrolysis of phosphatidyl inositol and purification by high-pressure liquid chromatography[21]. L-*myo*-inositol 1-phosphate was prepared, by the method of Burton and Wells[22], from D-glucose 6-phosphate, by the action of *myo*-inositol 1-phosphate synthase obtained from bovine testis. DL-*myo*-Inositol 4-phosphate was the gift of Professor S. J. Angyal, University of New South Wales. DL-*myo*-Inositol cyclic-1,2-phosphate was prepared from *myo*-inositol 2-phosphate (Sigma) by treatment with *N,N*-dicyclohexylcarbodiimide[15].

Identity of the elution order of enantiomers. — This order was established with each compound by gas chromatography of the pure optical isomer on Chirasil-Val. Each enantiomer gave predominantly one peak under these conditions.

Gas chromatography and gas chromatography–mass spectrometry. — A Varian 3700 gas chromatograph with Varian capillary column accessories was used with a Varian flame-photometric detector in phosphorus-selective mode, or a flame-ionization detector. The column used was a 20–25-m Chirasil-Val 0.3-mm i.d. open tubular glass capillary (Applied Science Laboratories).

G.l.c.–m.s. conducted with an LKB-9000 instrument using packed columns with electron ionization at 70 eV. Capillary g.l.c.–m.s. was performed on a Finnigan model 3300 chemical-ionization instrument with helium as carrier and ammonia as reagent gas.

Sources of enantiomeric sugars. — Arabinose, D-, Calbiochem, L-, Sigma; fucose, D-, Sigma, L-, Pfanstiehl; mannose, D-, Fisher, L-, Sigma; *chiro*-inositol, D-, Calbiochem, L- was the gift of Professor Laurens Anderson, University of Wisconsin; D- and L-xylose, Pierce; glucose, D-, Fisher, L-, Sigma: DL-glyceraldehyde, Sigma; D- and L-arabinitol, Pierce; gulono-1,4-lactone, D-, Pfanstiehl, L-, Nutritional Biochemicals Corp; galactono-1,4-lactone, D-, Pierce, L-, General Biochemicals, Inc.

NOTE ADDED IN PROOF (1 April 1982)

While this manuscript was under review, two articles[23] were brought to our attention that describe the enantiomeric separation of many of the sugars reported in this study, using the trifluoroacetyl derivative and a new chiral stationary phase.

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