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METHYLATION OF METHYL α-D-HEXOPYRANOSIDES WITH DIAZOMETHANE IN THE PRESENCE OF A SMALL AMOUNT OF WATER

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ABSTRACT

The long-time reaction of methyl α-D-gluco-, α-D-manno-, and α-D-galactopyranosides with excess diazomethane–diethyl ether at 25° in the presence of water gave all partially methylated methyl α-D-hexopyranosides which differ in number and position of methyl substitution. The presence of electrolytes, such as potassium or sodium phosphate, in the reaction medium enhanced the degree of methylation, resulting in preferential formation of tri-*O*-methyl derivatives of methyl α-D-hexopyranosides.

INTRODUCTION

It has been reported that the hydroxyl groups in carbohydrates can be methylated with diazomethane to a low degree in dry diethyl ether[1,2]. Previously, we reported that treatment of several sulfated glycosaminoglycans[3,4] or a polyuronide, pectin[5], with diazomethane in diethyl ether in the presence of a small amount of water or phosphate buffer resulted in considerable depolymerization of the polysaccharide chains and concomitant methylation of the hydroxyl groups. We describe herein methylation of several methyl α-D-hexopyranosides by treatment with diazomethane in diethyl ether in the presence of a small amount of water, and the effect of some organic and inorganic electrolytes on the methylation.

RESULTS AND DISCUSSION

A solution of methyl α-D-glucopyranoside in a small amount of water was treated with an excess of diazomethane in diethyl ether with vigorous stirring for 48 h at 25° (see Experimental section). After acetylation, a mixture of the partially methylated products was separated by g.l.c., and all the peaks except Peak No. 5 were characterized by g.l.c.–m.s. with reference to known R_T data[6] (Table I). The two sets of the expected four isomers of trimethyl (Peak Nos. 1–4) and monomethyl derivatives (Peak Nos. 9–12) were characterized; however, the expected

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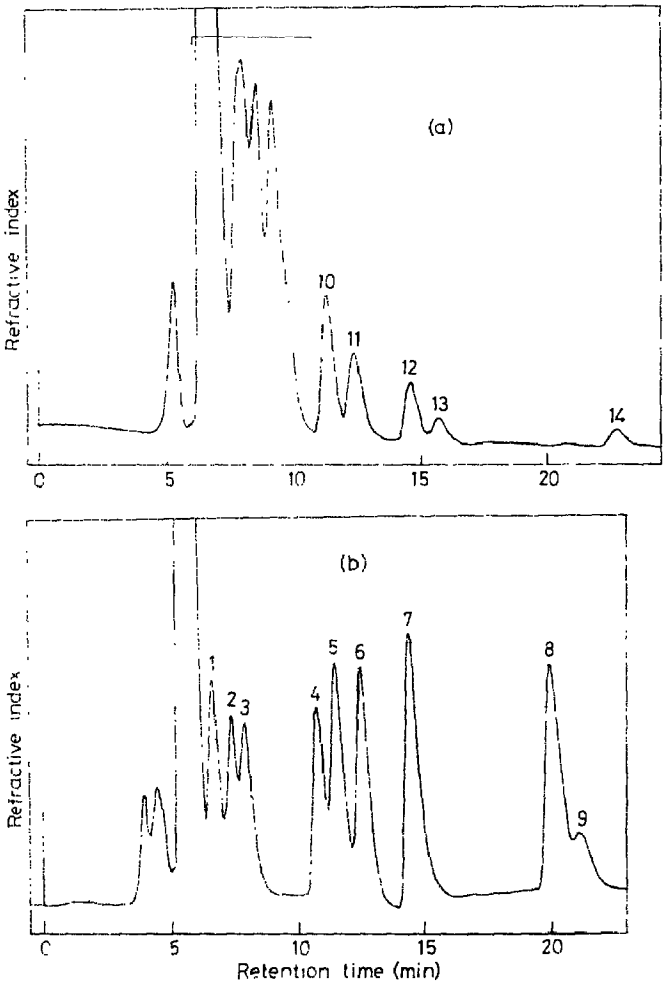

Fig. 1. Liquid chromatography of a mixture of partial methyl ethers of methyl α-D-glucopyranoside on a Hitachi 3056 ODS column with 3:7 methanol–water as eluent (a). The peak materials indicated by the brace in (a) were rechromatographed with 1:9 methanol–water as eluent (b).

six isomers of dimethyl derivatives were resolved into only four peaks (Peak Nos. 5–8) by g.l.c. Since the g.l.c.–m.s. of Peak No. 5 material suggested that it was most likely to be a mixture of many products, a mixture of the partially methylated products was directly subjected to l.c. separation on a Hitachi 3056 ODS column. As shown in Figs. 1a and b. the fourteen peaks were obtained well separated by use of two sets of eluent, 3:7 and 1:9 methanol–water. Each material isolated was acetylated and g.l.c. indicated that Peak No. 5 material was a mixture of Peak Nos. 7, 9, and 10 materials from the l.c. separation (Table I). Each acetylated derivative separated by l.c. was characterized by g.l.c.–m.s. Relative yields of the methyl ethers of methyl α-D-glucopyranoside were calculated from the peak areas of Peak Nos. 1–12 (g.l.c.) and Peak Nos. 7, 9, and 10 (l.c.). Based on these relative

TABLE I

RELATIVE RETENTION TIMES AND RELATIVE YIELDS OF METHYL *O*-ACETYL-*O*-METHYL-α-D-GLUCOPYRANOSIDES OBTAINED BY PARTIAL METHYLATION OF METHYL α-D-GLUCOPYRANOSIDE WITH DIAZOMETHANE IN THE PRESENCE OF WATER, FOLLOWED BY ACETYLATION

Peak No. (g.l.c.)	R_T[a]	*Peak No.*[b] *(l.c.)*	*O-Methyl group*[c]	*Relative yield (%)*[d]
1	12.5	14	3,4,6	1.4
2	19.9	13	2,3,4	2.0
3	21.6	11	2,3,6	7.6
4	38.0	12	2,4,6	6.4
5	43.8	10	4,6	9.3
		7	3,6	15.4
		9	3,4	8.3
6	54.9	8	2,6	14.3
7	57.6	6	2,3	14.1
8	64.0	5	2,4	12.7
9	81.0	4	6	1.5
10	82.7	1	3	3.7
11	88.4	3	4	2.0
12	90.9	2	2	1.5

[a]Relative to the retention time (29.4 min) of methyl α-D-glucopyranoside tetraacetate on an ECNSS-M column (100). [b]See Fig. 1. [c]Position of *O*-methyl groups, determined by g.l.c.–m.s. [d]Calculated from peak areas of gas–liquid and liquid chromatogram; 100% is the sum of all of the partially methylated α-D-glucopyranoside acetates.

yields, the degree of methylation of the four hydroxyl groups in methyl α-D-glucopyranoside was calculated to be 52.2%. As reported previously[3], the long-period (2 days) reaction of heparin with diazomethane at 20° resulted in the cleavage at O-4 of the uronic acid residue to give a mixture of methyl α- and β-glycosides of *N,O*-methyl di-, tetra-, and hexa-saccharides having a 4,5-unsaturated uronic acid, non-

TABLE II

EFFECT OF ADDITION OF ELECTROLYTES ON METHYLATION OF METHYL α-D-GLUCOPYRANOSIDE WITH DIAZOMETHANE IN THE PRESENCE OF WATER

Electrolyte added	*Composition of products (%)*[a]				*Degree of methylation (%)*[b]
	Tetra	*Tri*	*Di*	*Mono*	
None	0	17.4	74.1	8.5	52.2
$C_6H_{11}NHSO_3Na$	0	57.1	42.9	0	64.3
$C_6H_{11}OSO_3Na$	0.6	52.9	46.5	0	63.5
Sodium acetate	1.8	65.5	32.8	0	67.3
KH_2PO_4–Na_2HPO_4 (pH 8.0)	2.6	63.4	34.0	0	67.2
KH_2PO_4–Na_2HPO_4 (pH 8.0, repeated)	10.3	82.4	7.2	0	75.7

[a]Tri, di, and mono-methylated products correspond to Peak Nos. 1–4, 5–8, and 9–12 (g.l.c.) in Table I, respectively. [b]Calculated according to the equation: (4A + 3B + 2C + D)/400 (%), where A, B, C, and D are composition (%) of tetra-, tri-, di-, and mono-methylated products, respectively.

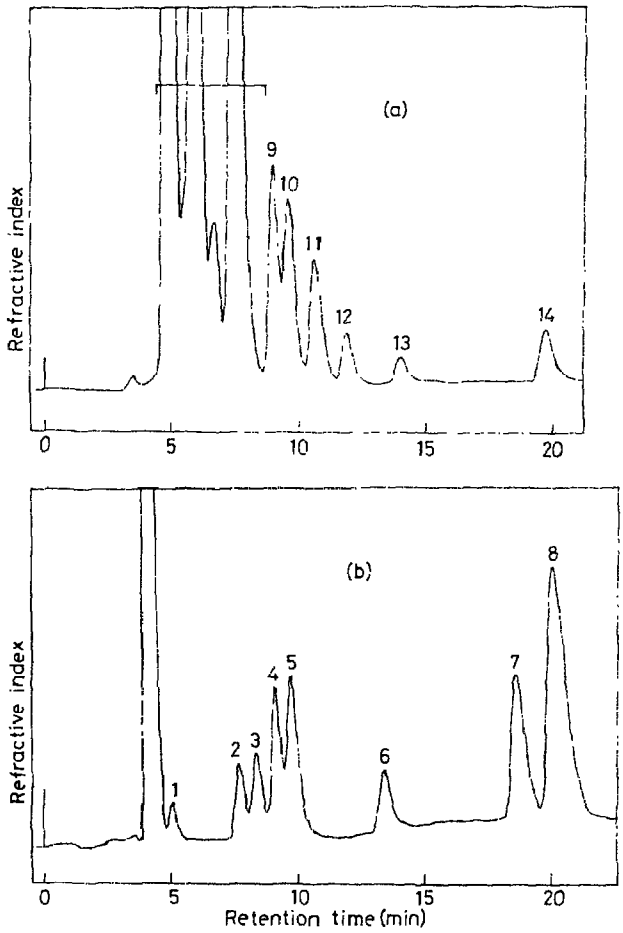

Fig. 2. Liquid chromatography of a mixture of partial methyl ethers of methyl α-D-mannopyranoside on a Hitachi 3056 ODS column with 3:7 methanol–water as eluent (a). The peak materials indicated by the brace in (a) were rechromatographed with 1:9 methanol–water as eluent (b).

reducing end-group. The major disaccharides obtained were almost fully methylated. In the case of the diazomethane treatment of pectin[5], the addition of a small amount of inorganic phosphates accelerated both cleavage and methylation reactions. These observations suggested that the addition of electrolytes which strongly facilitate dissociation may accelerate the methylation of methyl α-D-hexopyranosides with diazomethane. A solution of methyl α-D-glucopyranoside in a small amount of water was treated with an excess diazomethane in diethyl ether with or without electrolytes. In each case, the reaction products were analyzed quantitatively by g.l.c., indicating (Table II) a remarkable effect of these electrolytes, in particular the increase of the yield of trimethyl ethers.

To confirm the absence of side reactions, a portion of each methylated product of methyl α-D-hexopyranosides with diazomethane in the absence of electro-

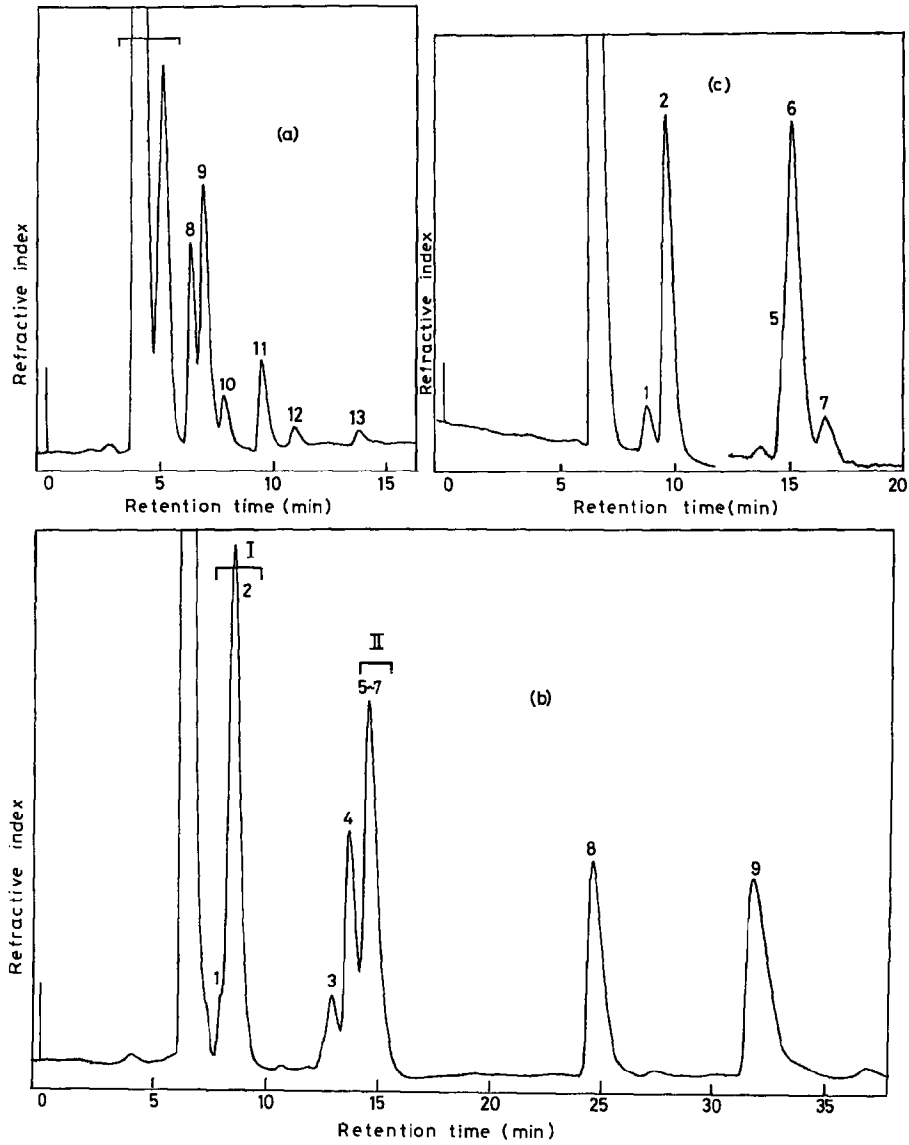

Fig. 3. Liquid chromatography of a mixture of partial methyl ethers of methyl α-D-galactopyranoside on a Hitachi 3056 ODS column with 1:4 methanol–water as eluent (a). The peak materials indicated by the brace in (a) were rechromatographed with 3:47 methanol–water as eluent (b). The peak materials indicated by the braces I and II in (b) were subjected to l.c. with 3:97 methanol–water as eluent, giving Peak Nos. 1 and 2, and 5–7, respectively (c).

lytes was dissolved in dry dichloromethane, and permethylated with diazomethane–boron trifluoride etherate[7]. G.l.c. and t.l.c. analyses indicated the exclusive formation of methyl tetra-*O*-methyl-α-D-hexopyranosides, suggesting the absence of side reactions during the diazomethane treatment (data not shown). When a solution of methyl α-D-glucopyranoside in phosphate buffer (pH 8.0) was treated twice with

TABLE III

RELATIVE RETENTION TIMES AND RELATIVE YIELDS OF METHYL *O*-ACETYL-*O*-METHYL-α-D-MANNOPYRANOSIDES OBTAINED BY PARTIAL METHYLATION OF METHYL α-D-MANNOPYRANOSIDE WITH DIAZOMETHANE IN THE PRESENCE OF WATER, FOLLOWED BY ACETYLATION

Peak No. (g.l.c.)	R_f[a]	*Peak No.*[b] *(l.c.)*	*O-Methyl group*[c]	*Relative yield (%)*[d]
1	7.0	[e]	2,3,4,6	1.1
2	10.1	14	3,4,6	1.3
3	22.5	13	2,3,4	0.5
4	24.2	12	2,4,6	1.0
5	29.7	11	2,3,6	3.7
6	36.5	10	3,4	5.4
		8a[f]	3,6	17.0
7	44.1	9	4,6	7.1
8	54.2	7	2,6	10.5
9	59.7	6	2,4	2.7
		8b[f]	2,3	8.1
10	63.0	5	6	11.2
11	69.6	4	3	11.8
12	83.2	3	4	7.3
13	85.5	2	2	7.0
14	100	1		4.3

[a]Relative to the retention time (37.2 min) of methyl α-D-mannopyranoside tetraacetate (Peak No. 14) on an ECNSS-M column as 100. [b]See Fig. 2. [c]Position of *O*-methyl groups, determined by g.l.c.–m.s. [d]Calculated from peak areas on gas–liquid and liquid chromatogram; 100% is the sum of all of the partially methylated methyl α-D-mannopyranoside acetates. [e]Methyl 2,3,4,6-tetra-*O*-methyl-α-D-mannopyranoside was not eluted from the column of l.c. under the conditions used. [f]Peak No. 8 material of l.c. was separated by g.l.c. into Peak Nos. 6 and 9 of g.l.c.

diazomethane, the yields of tri- and tetra-methyl ethers increased to 82.4 and 10.3%, respectively (Table II). The significant accumulation of the trimethyl ethers as compared to the tetramethyl ethers is closely related to their distribution coefficient between water and diethyl ether in the reaction medium, since the former are fairly soluble in water, but the latter is not.

Methyl α-D-manno- and α-D-galacto-pyranosides were also partially methylated with diazomethane under similar conditions. The reaction products were acetylated and separated by g.l.c. Most of the peaks separated were characterized by g.l.c.–m.s. To analyze the composition of the incompletely separated g.l.c. peak materials (Peak Nos. 6 and 9 of g.l.c. in Table III, and Peak Nos. 7, 9, 10, and 11 of g.l.c. in Table IV), each mixture of the partially methylated products was directly subjected to l.c. separation in an ODS column with two sets of eluent (3:7 and 1:9 methanol–water) for the products of methyl α-D-mannopyranoside, and with three sets of eluent (1:4, 3:47, and 3:97 methanol–water) for the products of methyl α-D-galactopyranoside (Figs. 2a,b and Figs. 3a–c).

The l.c. separation of the partially methylated methyl α-D-mannopyranosides gave fourteen well-separated peaks (Figs. 2a, b). Each peak material obtained was

acetylated and subjected to g.l.c. Peak No. 8 material (l.c.) was resolved into two peaks, Peak Nos. 8a and 8b (g.l.c.), and their retention times coincided with those of Peak Nos. 6 and 9 (g.l.c.), respectively (Table III). G.l.c. and l.c. analyses also revealed that Peak Nos. 6 and 9 materials (g.l.c.) were not homogeneous but contained Peak Nos. 10 and 6 materials (l.c.), respectively (Table III). As described above, all of the theoretically predicted partial methyl ethers of methyl α-D-mannopyranoside were separated and quantitatively determined by g.l.c. and l.c.

The g.l.c. and l.c. separations of the partially methylated methyl α-D-galactopyranosides were not as efficient as those of the two aforementioned, partially methylated methyl α-D-hexopyranosides (Figs. 3a–c and Table IV). Peak Nos. 11–13 (l.c.) did not correlate with any of the Peak Nos. 2–5 (g.l.c.) because of the marginal amounts of compounds (Fig. 3a and Table IV). The elution of Peak No. 5 (l.c.) was detected as an almost imperceptible shoulder of Peak No. 6, and consequently the ratio of areas of Peak Nos. 5 and 6 could not be calculated (Fig. 3c and Table IV). Since the materials of Peak Nos. 5 and 10 (l.c.) were eluted with 3:97 and 1:4 methanol–water, respectively, the ratio of these peak areas could not be calculated (Figs. 3a, c, and Table IV).

TABLE IV

RELATIVE RETENTION TIMES AND RELATIVE YIELDS OF METHYL *O*-ACETYL-*O*-METHYL-α-D-GALACTOPYRANOSIDES OBTAINED BY PARTIAL METHYLATION OF METHYL α-D-GALACTOPYRANOSIDE WITH DIAZOMETHANE IN THE PRESENCE OF WATER, FOLLOWED BY ACETYLATION

Peak No. (g.l.c.)	R_T[a]	*Peak No.*[b] *(l.c.)*	O-*Methyl group*[c]	*Relative yield (%)*[d]
1	6.4		2,3,4,6	0.1
2	15.4	[e]	2,3,6	4.6
3	17.9	[e]	3,4,6	1.5
4	23.9	[e]	2,3,4	0.5
5	26.0	[e]	2,4,6	0.5
6	30.0	9	3,6	11.8
7	39.8	10	4,6	10.9[f]
		5	2,3	
8	52.7	8	2,6	9.7
9	59.1	7	3,4	15.5[g]
		6	6	
10	66.5	2a[h]	3	8.0
		4	2,4	7.3
11	88.1	2b[h]	2	12.5
		1	4	1.7
12	100			15.7

[a]Relative to the retention time (30.8 min) of methyl α-D-galactopyranoside tetraacetate (Peak No. 12) on an ECNSS-M column as 100. [b]See Fig. 3. [c]Position of *O*-methyl groups, determined by g.l.c.–m.s. [d]Calculated from the peak areas on gas–liquid and liquid chromatogram; 100% is the sum of all of the partially methylated methyl α-D-galactopyranoside acetates. [e]Peak Nos. 11–13 of l.c. (Fig. 3a) were not correlated to any of the g.l.c. Peak Nos. 2–5 because of the small amounts of material available. [f]Since Peak Nos. 5 and 10 were eluted with 3:97 and 1:4 methanol–water, respectively, the ratio of these peak areas could not be calculated (see Figs. 3a and c). [g]Since Peak No. 6 contains a small shoulder due to Peak No. 5, the ratio of the areas of Peak Nos. 6 and 7 could not be calculated (see Fig. 3c). [h]Peak No. 2 of l.c. was separated by g.l.c. into two peaks (Peak Nos. 10 and 11 of g.l.c.).

TABLE V

EFFECT OF ADDITION OF INORGANIC PHOSPHATES ON METHYLATION OF METHYL α-D-MANNOPYRANOSIDE AND α-D-GALACTOPYRANOSIDE WITH DIAZOMETHANE IN THE PRESENCE OF WATER

Hexopyranoside and electrolyte added	*Composition of products (%)*[a]					*Degree of methylation (%)*[b]
	Tetra	*Tri*	*Di*	*Mono*	*None*	
Methyl α-D-mannopyranoside						
None	1.1	6.5	50.8	37.4	4.3	40.7
KH_2PO_4–Na_2HPO_4 (pH 8.0)	2.5	38.2	46.4	13.0	0	57.6
KH_2PO_4–Na_2HPO_4 (pH 8.0), repeated	4.6	76.2	19.2	0	0	71.4
Methyl α-D-galactopyranoside						
None	0.1	7.0	39.7[c]	37.7[c]	15.7	34.6
KH_2PO_4–Na_2HPO_4 (pH 8.0)	1.0	33.2		65.8[d]	0	[e]
KH_2PO_4–Na_2HPO_4 (pH 8.0), repeated	1.6	67.7	30.7	0	0	67.7

[a]Tetra-, tri-, di-, mono-, and non-methylated products of methyl α-D-mannopyranoside correspond to Peak Nos. 1, 2–5, 6–9, 10–13, and 14 (g.l.c.) in Table III, respectively. Tetra-, tri-, and non-methylated products of methyl α-D-galactopyranoside correspond to Peak Nos. 1, 2–5, and 12 (g.l.c.) in Table IV, respectively. Composition of di- and mono-methylated products of the latter methyl glycoside was calculated from the relative ratio based on g.l.c. and l.c. peak areas, as shown in Table IV. [b]See footnote *b* to Table II. [c]The relative yield of monomethylated products (37.7%) is that of a mixture containing a dimethyl ether (Peak No. 7 material, Fig. 3c) and, accordingly, that of dimethylated products (39.7%) was obtained by subtracting the dimethyl ether (Peak No. 7) material. [d]Sum of di- and mono-methylated products. Since l.c. separation of the methylated products obtained by the diazomethane treatment in phosphate buffer was not carried out, the relative yields of di- and mono-methylated products were not calculated owing to the overlapping of Peak Nos. 9 and 10 of g.l.c. (see Table IV). [e]Could not be calculated owing to the reason described in the preceding footnote.

The data summarized in Table V indicate that the accelerating effect of the added inorganic phosphates on the methylation of methyl α-D-manno- and α-D-galacto-pyranosides with diazomethane was remarkable, and that repeated diazomethane treatment in phosphate buffer resulted in the accumulation of the trimethyl ethers, as seen earlier for methyl α-D-glucopyranoside (Table II). The usefulness of multiple diazomethane treatments in phosphate buffer for preparing trimethyl ethers of methyl α-D-hexopyranosides is evident when compared to previous attempts to prepare methyl α-D-hexopyranosides with a high degree of methylation[8]. Furthermore, a single diazomethane treatment without electrolytes is a useful method for preparing all of the partially methylated derivatives of methyl α-D-hexopyranosides which differ in number and position of methyl substitution.

EXPERIMENTAL

Materials. — Methyl α-D-glucopyranoside, α-D-mannopyranoside, and α-D-galactopyranoside were purchased from Sigma Chemical Co. (St. Louis, MO

63178). Diazomethane in diethyl ether was prepared from *N*-methyl-*N*-nitroso-*p*-toluenesulfonamide[9]. BF_3 etherate, purchased from Wako Pure Chemical Industries, Ltd., Osaka, was purified by distillation before use.

Gas-liquid chromatography. — A mixture of partially methylated and acetylated methyl α-D-hexopyranosides was analyzed with a Shimadzu gas chromatograph GC-4BM, equipped with a flame-ionization detector and a glass column (0.4 × 200 cm), packed with 3% ECNSS-M on Gas-Chrom Q (100–120 mesh). The chromatograph was operated for 9 min at an initial temperature of 160°, then increased to 200° at 2°/min, and held at 200° until the last peak had been eluted. The carrier gas was N_2 at a flow rate of 40 mL/min (at 160°).

Gas–liquid chromatography–mass spectrometry. — A mixture of partially methylated and acetylated methyl α-D-hexopyranosides was analyzed with a Jeol JMS DX-300 mass spectrometer, coupled to a gas–liquid chromatograph, equipped with the same column as just described. The oven temperature for the column was programmed from 160 to 200° at 2°/min. The ionization voltage was 20 eV.

Liquid chromatography. — Liquid chromatography was performed with a chromatograph equipped with a liquid-delivery pump (NP-DX-5, Nihonseimitsu Co., Tokyo) and refractive-index detector (Shodex RI SE-11), at a pressure of 10 MPa. A mixture of partially methylated methyl α-D-hexopyranosides was separated in a stainless-steel column (4.6 × 250 mm), packed with Hitachi 3056 ODS (4–6 μm) adsorbent, at room temperature, with aqueous methanol as eluent at a flow rate of 0.9 mL/min.

Thin-layer chromatography. — T.l.c. was performed on silica gel plates with 1:19 (v/v) methanol–ethyl acetate as solvent. The plates were sprayed with 0.5% orcinol in 2M H_2SO_4.

Preparation of a mixture of partially methylated methyl α-D-hexopyranosides. — To a solution of methyl α-D-hexopyranoside (20 mg, 0.1 mmol) in water (0.2 mL) was added a solution of diazomethane in diethyl ether (~20 mL), freshly prepared from *N*-methyl-*N*-nitroso-*p*-toluenesulfonamide (3.5 g). The mixture was stirred for 48 h at 25°, and diethyl ether was evaporated. Methanol (1 mL) was added to the residue, and an aliquot (0.1 mL) was transferred to a test tube equipped with a screw cap. The solvent was evaporated to dryness, the residue acetylated with 1:1 (v/v) pyridine–acetic anhydride (0.6 mL) for 1 h at 100°, and analyzed by g.l.c. Another aliquot (0.8 mL) of the mixture was separated by reversed-phase l.c.

Methylation of methyl α-D-glucopyranoside with diazomethane in the presence of electrolytes. — Methyl α-D-glucopyranoside (20 mg) was dissolved in a solution (0.2 mL) containing sodium cyclohexylsulfamate or sodium cyclohexyl sulfate (0.1 mmol), or in 0.5M acetate buffer (pH 8.0, 0.2 mL), or in 0.1M phosphate buffer (pH 8.0, 0.2 mL). Each of the solutions was methylated and acetylated in the same manner as described above, and analyzed by g.l.c.

Repeated diazomethane treatment in the presence of inorganic phosphates. — A mixture (corresponding to 18 mg of starting methyl α-D-hexopyranoside) of par-

tially methylated products, prepared in the presence of inorganic phosphate, was dissolved in 0.1M phosphate buffer (pH 8.0, 0.18 mL) and the solution methylated with diazomethane in diethyl ether (~18 mL) for 48 h at 25°. The mixture was acetylated in the same manner as described above and analyzed by g.l.c.

Permethylation of a mixture of partially methylated methyl α-D-hexopyranosides with diazomethane–boron trifluoride etherate in dichloromethane. — A dried aliquot (corresponding to 2 mg of starting methyl α-D-hexopyranoside) of a mixture of partially methylated methyl α-D-hexopyranosides, prepared in the absence of electrolyte, was dissolved in dichloromethane (0.4 mL). To the solution were added a solution of diazomethane in dichloromethane (3 mL), freshly prepared from *N*-methyl-*N*-nitroso-*p*-toluenesulfonamide (0.5 g), and 40 μL of a catalyst stock solution that contained freshly distilled BF_3 etherate (0.1 mL) in dry dichloromethane (10 mL). The solution was stirred for 30 min at −5°. The reagent and solvent were evaporated, and each residue was analyzed by t.l.c. and g.l.c.

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