# FORMATION OF PARTIALLY TRIMETHYLSILYLATED DERIVATIVES OF CARBOHYDRATES. THE TRIMETHYLSILYL TRIS-O-TRIMETHYL-SILYL-D-GLUCOPYRANOSIDES AND TETRAKIS-O-TRIMETHYLSILYL-

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### INTRODUCTION

**D-GLUCOSE** 

Simple, versatile methods have been described for the separation and estimation of carbohydrates and related polyhydroxy compounds by gas-liquid chromatography (g.l.c.). In our own work, we have used poly-(trimethylsilyl) ethers<sup>1</sup>, which are readily prepared on a small scale and can be chromatographed with relative ease. The identification of an unknown compound can often be made by comparing the g.l.c. retention time of a derivative with those of authentic reference compounds under standardized conditions. Since a given partition coefficient is by no means exclusively characteristic of only one compound, such data must be interpreted with caution. It is usually expedient to compare the behavior of the unknown, on more than one type of liquid phase, with established retention data of standards.

Preparation of the trimethylsilyl ethers of sugars with a limited amount of reagent yields a mixture of partially substituted products, in addition to the usual completely substituted form. This observation led to a new type of qualitative g.l.c. analysis of carbohydrates in which the resolving power of the g.l.c. column was used to produce a complex array of peaks from a single sugar<sup>2</sup>. The collection of retention times, thus obtained, reduces to a minimum the possibility of a mistaken identification of an unknown substance. The value of this method of "partial derivative analysis" for identifications of carbohydrates is based on the premise that the number and relative proportions of partially substituted trimethylsilyl derivatives are dependent on the conformation and stereochemistry of the molecule. Although subtle structural differences may not be sufficiently great to allow any separation of the completely substituted trimethylsilyl derivatives of two compounds, it is expected that one or more of the partial derivatives will have a different retention time or will constitute different proportions of the whole mixture. This view was substantiated by the finding of qualitative and quantitative differences in the mixtures obtained after brief exposure of a number of simple sugars to hexamethyldisilazane<sup>2</sup>. Similar observations on the

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formation of partially substituted derivatives of bile acids were made some time ago by Sjövall<sup>3</sup> and more recently by Briggs and Lipsky<sup>4</sup>.

The preparation and properties of partial trimethylsilyl derivatives of carbohydrates have received very little attention. The present work was, therefore, undertaken as part of a general study of the major partial trimethylsilyl derivatives obtained by the reaction of a carbohydrate with a limited amount of the silylation reagent. It was hoped that the work would provide some information on the relative reactivities of carbohydrate hydroxyl groups, and that ultimately it might be possible to propose a favored pathway for the sequence of substitution reactions which lead to the completely trimethylsilylated derivatives.

#### **RESULTS AND DISCUSSION**

In our early studies, partial trimethylsilyl derivatives were obtained by reaction of various carbohydrates with an excess of the standard silylating mixture for very short periods of time<sup>2</sup>. It was, however, later found more convenient to prepare the partial derivatives by using limited amounts of a trimethylsilyl group donor, as described by Birkofer *et al.*<sup>5</sup>. Under such conditions, g.l.c. revealed many more components in the reaction mixtures than had been detected previously. When the reaction was performed with  $\beta$ -D-glucose and hexamethyldisilazane, in the molar ratio 4.6:1 mixed with catalytic amounts of chlorotrimethylsilane and with pyridine as solvent



Fig. 1. Gas-liquid chromatogram of the mixture of partially trimethylsilylated derivatives obtained from 150 mg of  $\beta$ -D-glucose and 250  $\mu$ l of silylation reagent under the conditions described in the text. The column was 3% XE-60 at 170°. In 1A, the chromatogram was obtained immediately after the end of the reaction period. In 1B, the chromatogram was obtained after allowing the mixture to stand for three days at room temperature.

the complex chromatogram shown in Fig. 1A was obtained. Analysis of the mixture was performed immediately after the 5-minute reaction period. It showed the presence of at least 18 components. Further reactions occurred in such mixtures when they were kept longer. For example, the chromatogram shown in Fig. 1B was obtained after the mixture was kept for 3 days at room temperature. Some new peaks are evident, and the amounts of other components have decreased. These changes are attributed to transsilylation reactions.

In order to simplify the identification problem, we have, in the first place, prepared partial derivatives under conditions where the number of products is limited. Secondly, we have studied the synthesis of partial derivatives having four trimethylsilyl groups. For such work, an obvious requirement was a protective group which could subsequently be removed by a method which would leave intact the trimethylsilyl ethers. The availability of benzyl  $\alpha,\beta$ -D-glucopyranoside and of 2-, 3-, 4-, and 6-O-benzyl-D-glucose, and the possibility of cleavage of the benzyl groups by nonhydrolytic methods, suggested that 2,3,4,6-tetrakis-O-trimethylsilyl-D-glucose (1) and the trimethylsilyl glycosides of the four possible tris-O-trimethylsilyl ethers might be prepared. When anomeric mixtures of benzyl D-glucoside and the four benzyl ethers were treated with an excess of hexamethyldisilazane, the expected benzyl 2, 3, 4, 6-tetrakis-O-trimethylsilyl-D-glucoside, and the trimethylsilyl O-benzyl-tris-O-trimethylsilyl-D-glucosides were obtained quantitatively. G.l.c. showed that the products contained, as expected, two major components, which are attributed to the presence of the  $\alpha$ -D and  $\beta$ -D-pyranoside forms. In Table I are given the relative retention times of all of the mono-O-benzyl-tetrakis-O-trimethylsilyl-derivatives of  $\alpha$ - and  $\beta$ -D-glucose. The reaction mixtures usually contained small amounts of other materials in addition

# TABLE I

Relative retention times of benzyl and trimethylsilyl ethers of benzyl and trimethyl-silyl  $\alpha$ ,  $\beta$ -d-glucopyranosides<sup>a</sup>

Compound	Relative retention
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Trimethylsilyl 2,3,4,6-tetrakis-O-trimethylsilyl- $\beta$ -D-glucoside	1.00
Benzyl 2,3,4,6-tetrakis-O-trimethylsilyl-a-D-glucoside	2.93
Benzyl 2,3,4,6-tetrakis-O-trimethylsilyl- $\beta$ -D-glucoside	3.49
Trimethylsilyl 2-O-benzyl-3,4,6-tris-O-trimethylsilyl-a-D-glucoside	2.65
Trimethylsilyl 2-O-benzyl-3,4,6-tris-O-trimethylsilyl-β-D-glucoside	3.43
Trimethylsilyl 3-O-benzyl-2,4,6-tris-O-trimethylsilyl-a-D-glucoside	2.24
Trimethylsilyl 3-O-benzyl-2,4,6-tris-O-trimethylsilyl-β-D-glucoside	3.26
Trimethylsilyl 4-O-benzyl-2,3,6-tris-O-trimethylsilyl-a-D-glucoside	2.37
Trimethylsilyl 4-O-benzyl-2,3,6-tris-O-trimethylsilyl-β-D-glucoside	3.48
Trimethylsilyl 6-O-benzyl-2,3,4-tris-O-trimethylsilyl-a-D-glucoside	3.26
Trimethyisilyl 6-O-benzyl-2,3,4-tris-O-trimethylsilyl-β-D-glucoside	3.97

<sup>a</sup>The retention times were determined on a 6-foot column of 2.5% SE-30; temperature, 220°. The anomeric assignments are based on the assumption of a longer retention time for the  $\beta$ -D anomer in each case<sup>1</sup>.

to the two major components. Small amounts of a third material, possibly possessing a furanose structure, were present even in trimethylsilyl 6-O-benzyl-2,3,4-tris-Otrimethylsilyl-D-glucoside (2) purified by distillation. Evidence that the reaction conditions did lead to the formation of the tetrakis substituted compounds, was obtained by determining the mass spectrum of trimethylsilyl 6-O-benzyl-2,3,4-tris-O-trimethylsilyl- $\beta$ -D-glucopyranoside (3). As shown in Fig. 2, a peak for the molecular ion was



Fig. 2. Mass spectrum of benzyl 2,3,4,6-tetrakis-O-trimethylsilyl- $\beta$ -D-glucopyranoside.

not obtained, but the molecular weight was clearly indicated by peaks at m/e 468 and 379 for (M-90)<sup>+</sup> and (M-90-89)<sup>+</sup>. Peaks at m/e 91(-CH<sub>2</sub>C<sub>6</sub>H<sub>5</sub>)<sup>+</sup> and m/e 147(-CH = CHCH<sub>2</sub>OCH<sub>2</sub>C<sub>6</sub>H<sub>5</sub>)<sup>+</sup> provided conclusive evidence for the presence of a benzyl group. It was not possible, however, to deduce the location of the benzyl group from inspection of the mass spectrum.

The anomeric mixtures of the monobenzyltetrakis-O-trimethylsilyl derivatives were subjected to hydrogenolysis with palladium on charcoal as the catalyst. The removal of the benzyl groups was followed by the appearance of new peaks on the gas chromatogram. In most cases\*, two new major peaks appeared, which where assumed to be the two anomers of the trimethylsilyl tris-O-trimethylsilyl-D-glucoside. That this was the case was shown by isolation of the peaks by preparative gas chromatography, followed by further silylation of the liberated hydroxyl group to give the known trimethylsilyl tetrakis-O-trimethylsilyl- $\alpha$ - or  $\beta$ -D-glucoside (4 or 5, respectively). In this way, assignment of the anomeric configuration could be made for the tetrakis derivatives. The relative retention times for the trimethylsilyl  $\alpha$ -D- and  $\beta$ -D- glycosides of 2,3,6- (6,7), 2,4,6- (8,9), 3,4,6- (10,11), and 2,3,4-tris-O-trimethylsilyl-D-glucose (12,13) are shown in Table II. The rate of hydrogenolysis was surprisingly low. After 48 h at room temperature and atmospheric pressure, the yields of the trimethylsilyl tris-O-trimethylsilylglucosides ranged from 40 to 70%.

The product of hydrogenolysis of benzyl tetrakis-O-trimethylsilyl- $\alpha$ ,  $\beta$ -D-glucopyranoside (14) was not the expected 1. It consisted mainly of a mixture of trimethyl-

<sup>\*</sup>When 3-O-benzyl-D-glucose was the starting material, only one new major peak appeared, consistent with the fact that the starting material was essentially the  $\beta$ -D anomer. When 3-O-benzyl-Dglucose was heated in pyridine prior to the silylation reaction, two new major peaks were observed (see Experimental section).

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Position of trimethylsliyl ether group	Position of free OH group	Relative re XE-60	etention time SE-30	Yields f α-D-glu	rom hexa cose <sup>c</sup> and	methyldis β-D-glu	ilazane <sup>a</sup> cose <sup>d</sup>	Yields f œ-D-gluc	rom N-(trim :ose <sup>c</sup> and	ethylsilyſ)ac β-D-gluc	cetamide <sup>b</sup> ose <sup>c</sup>
				4%	в Х	%		4 %	B %	А %	B %
Trimethylsilyl B-D-slucopyranoside											
2,3,4,6	None	1.00	1.00			5.9	(2.3)	0.8	8.6	3.8	1.2
2,3,6	4	1.35	0,61			31.3	(5.4)	12.6	11.5	11.5	24.7
2,4,6	e	0.88	0,43			23.1	(2.8)	15.3	12.7	12.6	15.3
3,4,6	7	1.24	0.61			10.1	(0,8)	6.0	4.3	2.7	4.0
2,3,4	6	1.85	0.76			10.2	(3.8)	5.3	1.9	6.2	5.9
Trimethylsilyl ¤-D-glucopyranoside											
2,3,4,6	None	0.58	0.61	38.9	29.0			5.2	14.0	8.4	1.3
2,3,6	4	0.95	0.46	32.0	36.4			32.1	22.4	11.8	26.1
2,4,6		0.72	0.37	14.0	18.8			9.1	12.0	17.8	12.4
3,4,6	61	0.90	0.49								
2,3,4	9	1.46	0.64					5.1	1.8	3.6	1.4
Unidentified				15.1	15.8	19.4	(0'9)	8.5	10.8	21.6	7.7
<sup>a</sup> The reactions with hexi Freshly prepared reaction for N-trimethylsilylacetan means for four runs. The	amethyldisilazan 1 mixtures were nideglucose, as figures in parer	te were perf used in cach described in ttheses are ti	formed with a new preserve of the representation of the text.	molar ra cactions w plicate ru	tio of 1.' vith N-tri ns, A and	5:1 for gl methylsily d B, were	lucose:hex vlacetamic carried or	amethyld le were p ut in thes	isilazane, a erformed w e cases. <sup>d</sup> T	is described vith a molar the values r	d in the text. r ratio of 4.4:1 ecorded are the

silyl tetrakis-O-trimethylsilyl- $\alpha$ - and  $\beta$ -D-glucoside (4 and 5). It is believed that the anomeric hydroxyl groups are too reactive to remain unsubstituted under the conditions used for hydrogenolysis, and that intermolecular transsilylation therefore occurs. This phenomenon was also observed to a minor extent during hydrogenolysis of trimethylsilyl 2-O-benzyl-tris-O-trimethylsilyl-D-glucoside (15), although the major product, as expected, was a mixture of the anomers of trimethylsilyl 3,4,6-tris-O-trimethylsilyl-D-glucoside (10,11).

Of the two liquid phases used for g.l.c., the polar cyanomethyl silicone (XE-60) gave more widely separated peaks than did the nonpolar methyl silicone (SE-30). For example, a mixture of 7, 11, and 4 was not resolved on SE-30 columns, but 3 well-separated peaks were obtained with an XE-60 column (see Figs. 3 and 4). On an XE-



Fig. 3. Gas-liquid chromatogram of a mixture of partially trimethylsilylated derivatives obtained from 100 mg of  $\beta$ -D-glucose and 500  $\mu$ l of silylation reagent under the conditions described in the text. The column was 2.5% SE-30 at 170°; (D 9; (E) mixture of 11, 7, and 4; (F) 13; (G) 5.



Fig. 4. Gas-liquid chromatogram of a mixture of partially trimethylsilylated derivatives prepared from  $\beta$ -D-glucose, on 3% XE-60 at 170°. The sample was prepared in the same way as that of Fig. 2; (A) 4; (B, 9; (C, 5; (D) 11; (E) 7; (G) 13.

60 column, the trimethylsilyl tris-O-trimethylsilyl- $\alpha$ -D-glucopyranosides had retention times longer than that of 4; similarly, with the exception of 9 the trimethylsilyl tris-O-trimethylsilyl- $\beta$ -D-glucopyranosides had retention times longer than that of the fully trimethylsilylated glucoside 5. The partial derivatives showed shorter retention times on SE-30 than those of the corresponding compounds 4 and 5, with the exception of 12. On XE-60, the retention times of the partial derivatives increased in the order, 8 < 10 < 6 < 12, for the  $\alpha$ -D series, and in the order 9 < 11 < 7 < 13, for the  $\beta$ -D series. The order of retention times was essentially the same on SE-30 except for the fact that the pairs, 10 and 6, and 11 and 7, were scarcely separated.



Prior to our work, little attention had been directed to the identification of partially substituted trimethylsilyl derivatives of sugars. In 1956, Schwarz *et al.*<sup>6</sup> described the preparation of tris- and tetrakis-O-triethylsilyl derivatives of glucopyranose, but the positions of substitution were not determined. Birkofer *et al.*<sup>5</sup> reported the synthesis of trimethylsilyl 2,4,6-tris-O-trimethylsilyl-D-glucopyranoside (mixture of anomers?) from the 3-O-benzyl derivative by hydrogenolysis with palladium on charcoal. They also reported that trimethylsilyl 2,3,4-tris-O-trimethylsilyl-D-glucopyranoside (4.4 moles) in pyridine. Our experiments, discussed below, indicate that complex mixtures are obtained under these conditions.

Other approaches were also considered in attempting to establish structures for the partial derivatives. One possibility was to convert individual products, isolated by preparative g.l.c., into known derivatives. In contrast to the properties of partially trimethylsilylated derivatives of methyl  $\alpha$ -D-glucopyranoside<sup>7</sup>, the derivatives of D-glucopyranose were found to be insufficiently stable to survive conditions for the conversion of free hydroxyl groups into acetyl, methyl, or benzyl derivatives. For example, additions of acetic anhydride and dry pyridine to any of the partially trimethylsilylated derivatives always resulted in the formation of penta-O-acetyl-Dglucose, exclusively. Methylations and benzylations were equally unsuitable. In attempts to prepare pure sodium salts of partially substituted derivatives, with freshly cut sodium and a solution of the substance in absolute diethyl ether, transsilylations occurred. Under these conditions, 9 was converted into 7 (53% of 9 at equilibrium).

It is important to note that isomerization of individual, partially trimethylsilylated derivatives during passage through the g.l.c. column (SE-30 or XE-60) did not occur to a significant extent. When single components, collected by preparative g.l.c., were rechromatographed, only one peak, having the same retention time as that originally found in the mixture, was observed. It is probable, therefore, that the number and relative proportions of partial derivatives, formed under limiting conditions, reflect differences in the rates of silylation of various hydroxyl groups, and that g.l.c. records of the mixture provide reasonably accurate data on the composition of these mixtures.

The availability of the reference compounds, reported here, allows some conclusions to be drawn concerning the composition of the partial mixtures obtained from trimethylsilylation of  $\alpha$ - and  $\beta$ -D-glucose. Conditions were so selected that the composition was less complex than that of the mixture shown in Fig. 1A. With 0.66 mole of hexamethyldisilazane per mole of  $\beta$ -D-glucose, the products contained predominantly four and five trimethylsilyl groups, as shown in Figs. 3 and 4. The major compounds were identified as 9 and 7 (peaks B and E in Fig. 4) on the basis of their relative retention times. One substantial component (peak F in Fig. 4) and about three minor components remained unidentified. When  $\alpha$ -D-glucose was used under the same conditions, the mixture contained a larger proportion of the fully trimethylsilylated glucoside, as shown by the results recorded in Table II. With  $\alpha$ -D-glucose were best obtained using a value of 1.85:1 for the molar ratio, glucose to hexamethyldisilazane. The reaction was not easy to control, and considerable variability in the relative proportions of the components was observed from run to run (see Table II).

An example of the mixture produced by reaction of N-(trimethylsily)acetamide and  $\alpha$ -D-glucose under the conditions of Birkofer *et al.*<sup>5</sup> is shown in Fig. 5. In this case, the major compounds were again 8 and 6. In this reaction, considerable anomerization had apparently taken place, since peaks with the retention times of 9, 11, and 7 were also present (peaks E, F, and G in Fig. 5). There were also four unidentified materials (peaks A, H, I, and J in Fig. 5). Quantitative data on the reaction of both  $\alpha$ - and  $\beta$ -D-glucose with N-(trimethylsilyl)acetamide are given in Table II. In all cases,

there was considerable anomerization, and considerable variation in the proportions observed in duplicate reactions. The highest yield of the combined  $\alpha$ - and  $\beta$ -D-glucosides having substitution at O-2, O-3, and O-4 was about 10%. It should be noted that this observation is contrary to that of Birkofer *et al.*<sup>5</sup>. Furthermore, trimethylsilyl 2,3,4-tris-O-trimethylsilyl-D-glucopyranoside was only a minor product in the reaction of  $\beta$ -D-glucose with hexamethyldisilazane (peak G in Fig. 4; see also Table II) and could not be observed at all in the same reaction with  $\alpha$ -D-glucose (Table II).



Fig. 5. Gas-liquid chromatogram of a mixture of partially trimethylsilylated derivatives of  $\alpha$ -D-glucose, prepared with N-(trimethylsilyl)acetamide as described in the text. The column was XE-60 at 170°; (B) 4; (C) 8; (D) 6; (E) 9; (F) 11; (G) 7.

Our work provides some information on the relative reactivities of carbohydrate hydroxyl groups toward trimethylsilylation. Such reactivity differences with other reagents have been widely studied<sup>8</sup>. Thus, in some reactions (*e. g.* glycoside formation), only the anomeric hydroxyl group reacts. Other reactions exhibit a marked selectivity for the primary hydroxyl groups (*e. g.*, tosylation, tritylation). In reactions requiring alkaline conditions, the hydroxyl group at C-2 may be particularly reactive as a result of the electron-withdrawing effect of the anomeric center. Under the conditions described in this paper, the reaction of glucose with either hexamethyldisilazane or N-(trimethylsilyl)acetamide always yields, as the major tetrasubstituted products, trimethylsilyl glycosides substituted at positions 2,3,6 and 2,4,6. It is therefore clear that the hydroxyl groups at C-1, C-2, and C-6 are particularly reactive in this process.

Although insufficient information is available to distinguish the most reactive of these three positions, it seems likely that the early reactions occur primarily with hydroxyl groups at C-1 and C-2 of the glucose molecule; in later steps steric factors may control the nature of products formed. Assuming that trimethylsilylation gives first a trimethylsilyl glycoside substituted at O-2, the third substitution reaction could occur at O-3, O-4, or O-6. This step could yield three different derivatives, by random substitution, or differences in rates or steric factors might limit the number of trisubstituted products. If either one of the hydroxyl groups at C-3 and C-4 has been substituted, it is presumed that, because of steric hindrance, the fourth trimethylsilyl group would be linked to O-6 rather than to the remaining position 3 or 4. This might explain why the trimethylsilyl 2,3,6- and 2,4,6-tris-O-trimethylsilylglucosides are the major products from  $\alpha$ -D-glucose and  $\beta$ -D-glucose.

It is apparent that considerable work will be necessary to gain a complete understanding of the reaction sequence involved in the formation of a trimethylsilyl tetrakis-O-trimethylsilylhexoside. As one possible approach, we plan to obtain kinetic data on the relative rates of trimethylsilylation of compounds containing four trimethylsilyl groups.

# EXPERIMENTAL

General. — Commercially available preparations of  $\alpha$ -D-glucose and  $\beta$ -D-glucose were used. Hexamethyldisilazane and chlorotrimethylsilane were obtained from Applied Science Laboratories, Inc., State College, Pa. Pyridine, used as solvent in preparing trimethylsilyl derivatives, was of reagent grade, and was stored over KOH pellets. Palladium on charcoal (10%), used for hydrogenolysis, was purchased from Matheson, Coleman & Bell, East Rutherford, N. J. N-(Trimethylsilyl)acetamide was prepared by the method of Birkofer *et al.*<sup>9</sup>.

For g.l.c., the instruments used were the F & M Models 400 and 402, each of which was equipped with flame ionization detectors. Two U-shaped glass columns (6 ft  $\times$  1/8 in i.d.) were employed; one was packed with 3% XE-60 on acid-washed, silanized Gas Chrom S (100 to 120 mesh), the other with 2.5% SE-30 on the same support. Column packings were prepared from toluene solutions of SE-30 or acetone solutions of XE-60, with the solution coating technique<sup>10</sup>. The liquid phases and packings were obtained from Applied Science Laboratories, Inc., State College, Pa. Both columns were conditioned for several h at 300° without gas flow, and then for an additional period of several h at 250° with normal gas flow. Analyses were performed at 170° with an injection port temperature of 210°.

Mass spectra were recorded with an LKB 9000 combined gas chromatographmass spectrometer, in which chromatographic effluents are conducted directly into the ion chamber of the mass spectrometer after removing helium preferentially with a jet-type molecular separator of the Becker–Ryhage type<sup>11</sup>. All spectra were recorded with an electron impact energy of 20 e.v., 3500 volts accelerating voltage, and ion source temperature of 250°.

Preparation of partially trimethylsilylated derivatives of D-glucose. — Several procedures were used in the synthesis of partially trimethylsilylated derivatives of D-glucose. In one series of experiments, amounts of glucose ranging from 5 to 150 mg were trimethylsilylated as follows. A portion (250  $\mu$ l) of a mixture of hexamethyl-disilazane, chlorotrimethylsilane, and pyridine (2:1:10, v/v) was diluted to 1.5 ml with

pyridine. The solution was warmed to 70°, D-glucose was added, and the mixture was shaken intermittently for 5 min. An appropriate aliquot was then analyzed directly by g.l.c. The complexity of the sample increased as the molar ratio, glucose to hexamethyldisilazane, was changed from 0.15:1 to 4.5:1. With the ratio, 0.15:1, only two peaks corresponding to 4 and 5 were obtained. Fig. 1A shows a chromatogram of the complex mixtures obtained when the ratio was 4.5:1.

To prepare less complex mixtures, in which it was hoped that compounds with four trimethylsilyl groups would predominate, the following conditions were used. To solid  $\beta$ -D-glucose (0.1 g) was added a mixture (0.5 ml) of hexamethyldisilazane, chlorotrimethylsilane, and pyridine (2:1:10) and a further portion of pyridine (4 ml). The molar ratio, glucose to hexamethyldisilazane, was 1.5:1. The mixture was refluxed for 5 min, after which pyridine and silane components were removed by distillation (10 mm pressure), and the residue was dissolved in abs. ethyl acetate (10 ml). The white precipitate, consisting of ammonium chloride and unreacted glucose, was removed by filtration, and aliquots of the ethyl acetate solution were used for g.l.c. analyses. Figs. 3 and 4 show typical chromatograms of this mixture on columns of XE-60 and SE-30. The identifications recorded in these figures were made on the basis of the retention times of known compounds, synthesized by the methods subsequently described. A similar mixture of partially substituted derivatives was prepared from  $\alpha$ -D-glucose in the same way. In this case, however, the sugar (0.1 g) was mixed with the mixed silylation reagent (0.4 ml) to diminish the formation of **4**.

Mixtures prepared with N-trimethylsilylacetamide were also investigated. A chromatogram of the mixture obtained by the reaction of  $\alpha$ -D-glucose and N-trimethyl-silylacetamide in the molar ratio 0.22:1 according to Birkofer *et al.*<sup>5</sup>, is shown in Fig. 5.

Benzyl  $\alpha,\beta$ -D-glucopyranoside and benzyl ethers of D-glucose. — Benzyl  $\alpha,\beta$ -D-glucopyranoside<sup>12</sup>, 2-O-benzyl-<sup>13</sup>, 3-O-benzyl-<sup>14</sup>, and 4-O-benzyl-D-glucose<sup>15</sup> were prepared as described in the literature. When small samples of the benzyl derivatives were trimethylsilylated under our usual conditions<sup>1</sup>, two peaks were obtained in all cases, indicating that the original samples were mixtures of the anomeric forms. With 3-O-benzyl-D-glucose, the second component was very small but it was increased considerably by first refluxing briefly a suspension of the solid in dry pyridine. The major component of the solid 3-O-benzyl-D-glucose was shown to be the  $\beta$ -D anomer by the method subsequently described (conversion into trimethylsilyl 3-O-benzyl-2,4,6-tris-O-trimethylsilyl- $\beta$ -D-glucoside, and thence to the known 5).

The compound, N-(3,4,6-tris-O-acetyl-D-glucopyranosyl)piperidine, which was required as an intermediate in the synthesis of 2-O-benzyl-D-glucose, was prepared as follows, with slight modification of the method described by Hodge and Rist<sup>16</sup>. Penta-O-acetyl- $\alpha$ -D-glucopyranose (39 g, 0.1 mole) and piperidine (25.8 g, 0.3 mole) were mixed first with manual, then later with mechanical stirring. After a short time, the mixture became fluid; stirring was continued until a thick crystalline mass formed in the flask (about 30 min). After addition of diethyl ether (150 ml), and stirring for an additional hour, the mixture was chilled in the refrigerator, and the crystalline product was removed by filtration. Recrystallization from abs. ethyl al-

cohol gave a material having m.p. 149° (42% yield). When the reaction was repeated under the same conditions with penta-O-acetyl- $\beta$ -D-glucopyranose, the product had m.p. 130°. The two products showed identical retention times on g.l.c. analysis, and both were dextrorotatory,  $[\alpha]_D^{23} + 31.7^\circ$  (c 2, chloroform). Since these m.ps. fell outside the range of 121–126° (dependent on the manner and rate of heating) reported previously<sup>16</sup>, further evidence was sought for the correctness of the assigned structure. The product was treated with hexamethyldisilazane<sup>1</sup> to trimethylsilylate the remaining free hydroxyl group. Mass spectrometry of the product, N-(3,4,6-tri-Oacetyl-2-O-trimethylsilyl-D-glucopyranosyl)piperidine, confirmed the identity of the synthetic material. Peaks were observed at m/e 445=molecular ion, 430=M-15 (loss of CH<sub>3</sub>), 402=M-43 (loss of CH<sub>3</sub>CO), 370=M-75 (loss of CH<sub>3</sub> and CH<sub>3</sub>COOH), and 84 (C<sub>5</sub>H<sub>10</sub>N)<sup>+</sup>; the most intense peak was at m/e=114 (CH(OCOCH<sub>3</sub>)=CH-CHO)<sup>+</sup>.

6-O-Benzyl-D-glucose was prepared by the following method: To 3,5-O-benzylidene-1,2-O-isopropylidene-D-glucofuranose<sup>17</sup> (2.9 g) were added N,N-dimethylformamide (50 ml, dried over BaO and freshly redistilled) and benzyl bromide (15 ml). After the solution was cooled briefly, dried silver oxide (15 g) was added in small portions over a period of about 10 min. The flask was stoppered tightly, and the mixture was stirred overnight. The silver salt was removed, and the filtrate was concentrated *in vacuo* to a thick syrup. Methanol (50 ml) and 2N sulfuric acid (30 ml) were added, and the mixture was refluxed for 1 h. Methanol was removed by distillation, and the conc. solution was refluxed for 4 h. The water-insoluble portion which formed was removed in a separatory funnel, and the aqueous solution was neutralized with excess barium carbonate. The aqueous solution of 6-O-benzyl-D-glucose, free of sulfate ions, was concentrated *in vacuo* to a dry residue which was used without further manipulation, for preparation of the trimethylsilyl derivative.

Trimethylsilylation of the benzyl derivatives of D-glucose. — In most cases, 10 ml of the "2:1:10" silylation mixture was added to 500 mg of the required benzyl compound. After a reflux of 5 min, the volatile materials were removed by distillation, abs. ethyl acetate (30 ml) was added, and the precipitate was removed by filtration. This reaction gave a mixture of the trimethylsilyl mono-O-benzyl-tris-O-trimethyl-silyl- $\alpha$ - and  $\beta$ -D-glucopyranosides in quantitative yield. For preparation of the 6-O-benzyl derivative, the dry residue of 6-O-benzyl-D-glucose, obtained as described earlier, was treated with 60 ml of the 2:1:10 silylation mixture. After a reflux of 5 min, the precipitate was filtered off, and the volatile materials were removed by distillation at atmospheric pressure. Trimethylsilyl 6-O-benzyl-2,3,4-tris-O-trimethylsilyl-D-glucoside (anomeric mixture, 2) was recovered in 80% yield (3.5 g) by high-vacuum distillation: b.p. 156°/0.03 mm.

Hydrogenolysis of benzyl tetrakis-O-trimethylsilyl- $\alpha$ - $\beta$ -D-glucoside (14) and trimethylsilyl mono-O-benzyl-tris-O-trimethylsilyl-D-glucopyranosides. — To the ethyl acetate solution of compound 14 or the mono-O-benzyl-tris-O-trimethylsilyl-Dglucosides (usually prepared from 500 mg of O-benzyl derivative) was added 10% palladium-charcoal catalyst (1 g). Dry hydrogen was bubbled through the solution

with continuous stirring. After 48 h, the mixture was filtered, and aliquots of the ethyl acetate solution were analyzed by g.l.c. The various trimethylsilyl derivatives of D-glucose were recovered in yields ranging from 40 to 70%.

For the assignment of anomeric configuration, the two peaks obtained in each case were separately collected as they were eluted from a preparative SE-30 column (10%, 250°). Small samples of the pure anomeric forms were then converted by further trimethylsilylation<sup>1</sup> into the completely substituted derivatives (4 or 5). Configurations were assigned by comparison of the retention times of these derivatives with those of the known 4 or 5.

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### SUMMARY

When mixtures of the  $\alpha$  and  $\beta$  anomers of benzyl D-glucopyranoside, and of 2-, 3-, 4-, and 6-O-benzyl-D-glucose were treated with hexamethyldisilazane, the tetrakis-O-trimethylsilyl derivatives of the benzyl glycosides and the tris-O-trimethylsilyl ethers of the trimethylsilyl glycosides were obtained (anomeric mixtures). On hydrogenolysis of the trimethylsilyl 2-, 3-, 4-, and 6-O-benzyl-tris-O-trimethylsilyl glycosides, the  $\alpha$ - and  $\beta$ -anomers of the trimethylsilyl tris-O-trimethylsilyl-D-glucosides having the following substitution patterns were obtained, respectively: 3,4,6; 2,4,6; 2,3,6; and 2,3,4. The anomers were separated by gas-liquid chromatography. Hydrogenolysis of benzyl tetrakis-O-trimethylsilyl- $\alpha$ ,  $\beta$ -D-glucopyranoside gave mainly a mixture of trimethylsilyl tetrakis-O-trimethylsilyl- $\alpha$ - and  $\beta$ -D-glucosides. Direct trimethylsilylation of  $\alpha$ - and  $\beta$ -D-glucose with limiting amounts of trimethylsilyl donor [hexamethyldisilazane or N-(trimethylsilyl)acetamide] yielded complex mixtures of partially trimethylsilylated derivatives. The composition of such mixtures has been determined by g.l.c. The major components were the trimethylsilyl tetrakis-Otrimethylsilyl and the 2,3,6- and 2,4,6-tris-O-trimethylsilyl-D-glucosides.

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