Analysis of Trimethylsilyl Derivatives of Carbohydrates by Gas Chromatography and Mass Spectrometry[1]

D. C. DeJongh,[2a,b] T. Radford,[2a] J. D. Hribar,[2a] S. Hanessian,[2c] M. Bieber,[2d] G. Dawson,[2d] and C. C. Sweeley[2d]

Contribution from the Department of Chemistry, Wayne State University, Detroit, Michigan 48202; Department of Chemistry, University of Montreal, Montreal, Quebec, Canada; and the Department of Biochemistry, Michigan State University, East Lansing, Michigan 48823. *Received October 25, 1968*

Abstract: The mass spectra of the trimethylsilyl ethers of α-D-glucose and its 1,2,3,4,5,6,6-d_7, 1-d, and 6,6-d_2 analogs, of methyl α-D-glucopyranoside, of ethyl β-D-galactofuranoside, and of methyl 3-acetamido-3-deoxy-α-D-glucopyranoside and its $NHCOCD_3$ analog are presented. The fragmentations of these derivatives are discussed in detail, using deuterium-labeling and exact-mass data from high-resolution measurements to support the interpretations. The mass spectra of these model compounds are used in the identification of minor components formed in the trimethylsilylation of equilibrium mixtures of D-galactose, D-glucose and three of its deuterated analogs, 3-*O*-methyl-D-glucose, and 2-acetamido-2-deoxy-D-galactose. The same approach was used to determine the products from glycosidation of D-galactose and D-glucose. The mass spectra of these minor components were obtained from a coupled gas chromatograph–mass spectrometer. Furanose and furanoside structures can be assigned to the minor components.

In recent years, classical methods of structure determination in carbohydrate chemistry have been supplemented by newer techniques. The application of gas–liquid partition chromatography (glpc) to carbohydrate derivatives introduced a convenient method for the examination and small-scale separation of complex mixtures, such as those resulting from hydrolysis of polysaccharides.[3] Methyl ethers,[4] acetates,[5,6] and trimethylsilyl (TMSi) ethers[7] of carbohydrates have been shown to be amenable to this method of analysis. Trimethylsilyl ethers appear to be the most suitable both with regard to their ease of preparation and to the excellent separations which have been achieved.[7]

The successful use of TMSi derivatives in mass-spectral investigations of hydroxy steroids,[8-11] and the recent demonstrations of the utility of TMSi ethers in the investigation of complex carbohydrate-containing antibiotics[12-16] and nucleotides and related substances,[17] suggest that the technique might generally be applied to the field of carbohydrate chemistry. In addition, the requirement of submicrogram amounts of material for the determination of a mass spectrum and the possibility of analysis with a gas chromatograph directly coupled to a mass spectrometer present obvious advantages for the study of trimethylsilyl ethers.[18]

Mass-spectral data from TMSi ethers were first reported in 1957 when derivatives of aliphatic alcohols were examined.[19] It was found that several types of rearrangements occurred and structures were proposed for the principal fragment ions. Recent labeling studies have confirmed many of these assignments, but indicate in some cases that revisions should be made.[20,21]

Preliminary investigations of the mass spectrometry of TMSi derivatives of carbohydrates have already been made, among which are the studies of carbohydrate-containing antibiotics.[12-16] The mass spectra of other trimethylsilyl ethers of carbohydrates, *e.g.*, benzyl 2,3,4,6-tetra-*O*-trimethylsilyl-β-D-glucopyranoside,[22] 2,3,4-tri-*O*-trimethylsilyl-D-glucosan,[23] and the methyl glycoside of methyl 4,7,8,9-tetra-*O*-trimethylsilyl-D-neuraminate[24] have been published. A mass-spectrometric study of aldonolactones as trimethylsilyl ethers,[25] a use of mass

(1) Presented in part at the "Symposium on Newer Aspects of Spectroscopy in Carbohydrate Chemistry," D. C. DeJongh, J. D. Hribar, and S. Hanessian, 153rd National Meeting of the American Chemical Society, Miami Beach, Fla., April 1967, Abstract C-27.

(2) (a) Wayne State University. (b) Alfred P. Sloan Foundation Research Fellow. (c) University of Montreal. (d) Michigan State University. A portion of the work by this group was carried out at the University of Pittsburgh.

(3) For a review see W. W. Wells, C. C. Sweeley, and R. Bentley in "Biomedical Applications of Gas Chromatography," H. A. Szymanski, Ed., Plenum Press, New York, N. Y., 1964.

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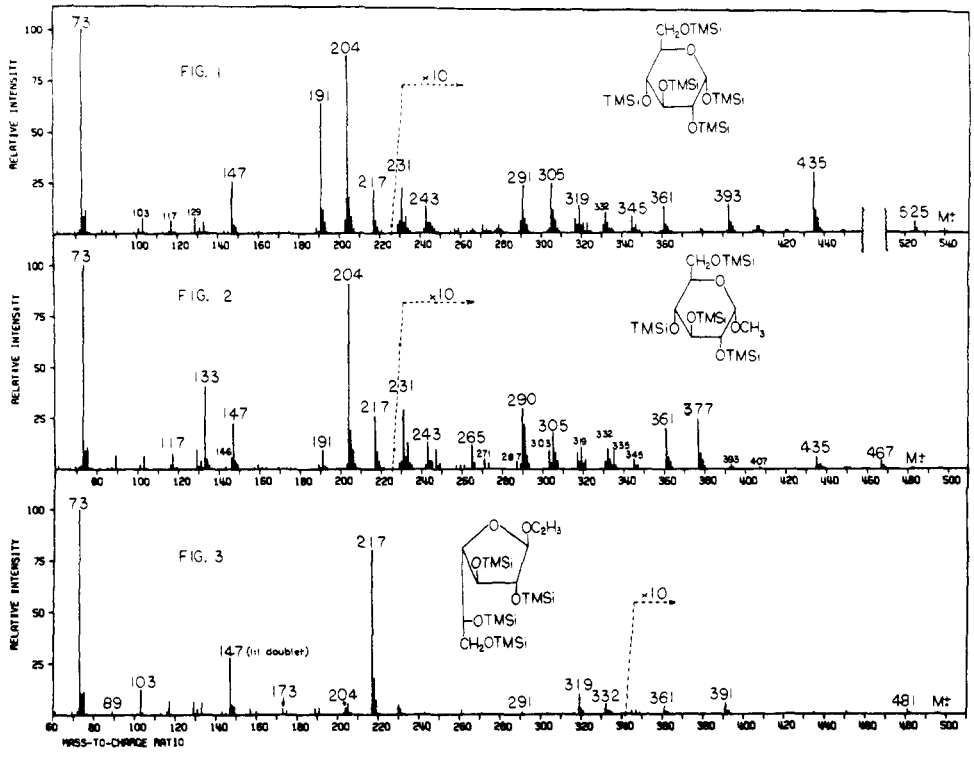


Figure 1. The 70-eV mass spectrum of 1,2,3,4,6-penta-*O*-trimethylsilyl-α-D-glucopyranose (**1**); molecular weight, 540.

Figure 2. The 70-eV mass spectrum of methyl 2,3,4,6-tetra-*O*-trimethylsilyl-α-D-glucopyranoside (**12**); molecular weight, 482.

Figure 3. The 70-eV mass spectrum of ethyl 2,3,5,6-tetra-*O*-trimethylsilyl-β-D-galactofuranoside (**16**); molecular weight, 496.

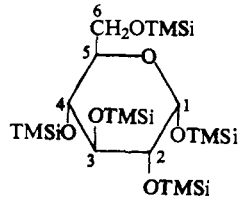
spectrometry to determine the number and position of methoxyl groups in trimethylsilyl ethers of methylated aldopentoses,[26] and a publication emphasizing characteristic differences among mass spectra of trimethylsilyl ethers of disaccharides having different types of linkages[27] have also appeared.

We have undertaken a detailed investigation of TMSi ethers of carbohydrates. Mass spectra were determined with TMSi derivatives of purified crystalline sugars and in certain cases labeling studies and high-resolution measurements were performed. The fragmentations of these model compounds are discussed in detail.

When TMSi ethers are made from free sugars, more than one product can be obtained, due to the presence of various anomeric and ring forms. Combined glpc–mass spectrometry was used to record the mass spectra of the components of such reaction mixtures. The combination of glpc retention behavior with mass-spectral data, provides a powerful tool for structural elucidation of carbohydrates on the submicrogram scale.

Results and Discussion

The Mass Spectrum of Penta-*O*-trimethylsilyl-α-D-glucopyranose (1). The trimethylsilyl ethers of α-D-glucose (**1**) and of D-glucose-1,2,3,4,5,6,6-d_7 (**2**), D-glucose-1-*d* (**3**), and D-glucose-6,6-d_2 (**4**) were prepared as previously reported[7] and were purified by gas–liquid partition chromatography. Their mass spectra are given in Table I; the mass spectrum of **1** is shown in bargraph form in Figure 1. The relative intensities have not been corrected for contributions from carbon, hydrogen, oxygen, and silicon isotopes. Table I also contains elemental compositions of selected peaks, calculated from exact-mass measurements.



2, 1,2,3,4,5,6,6-d_7
3, 1-*d*
4, 6,6-d_2

It has been shown by gas–liquid partition chromatography that two major components result from trimethylsilylation of D-glucose, and these have been assigned to α and β anomers by comparisons of their retention times with those of the TMSi ethers of pure α- and β-D-glucose.[7] The mass spectra of penta-*O*-trimethylsilyl-α- and -β-D-glucopyranoses are identical.

The data in Table I and Figure 1 were obtained from an AEI MS 902 high-resolution mass spectrometer, equipped with a direct-insertion probe. Mass spectra obtained from an Atlas CH4 mass spectrometer and obtained directly, *i.e.*, without prior collection, from a LKB-9000 gas chromatograph–mass spectrometer are essentially the same as those from the AEI MS 902, except for minor relative-intensity differences. Interpretation of fragmentations involving ions in the high-mass region are hindered by the absence of metastables. However, analogies with

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Table I. Relative-Intensity Data from the Mass Spectra of **1–4**[a]

m/e	α-D-Glucose	Elemtl compstn	d_7	α-D-Glucose 1-d	6, 6-d_2	m/e	α-D-Glucose	Elemtl compstn	d_7	α-D-Glucose 1-d	6, 6-d_2
547			0.1			347	0.4			0.4	0.9
542					0.1	346	0.2			0.8	0.3
541				0.1		345	0.8	$C_{14}H_{29}Si_3O_4$		0.2	
540	0.1					341			0.1		
535			0.2			340			0.2		
534			0.4			339			0.4		
533			0.7			338			0.2		
532			1.3			337			0.6		
531			0.1			336	0.1		0.1	0.1	0.3
529				0.1	0.2	335	0.2		0.2	0.1	0.6
528	0.1			0.2	0.4	334	0.2			0.3	0.8
527	0.2			0.3	0.6	333	0.5			0.6	0.3
526	0.2			0.6		332	1.0	$C_{14}H_{32}Si_3O_3$		1.2	0.2
525	0.5	$C_{20}H_{49}Si_5O_6$				331	0.4			0.3	
452					0.2	329			0.2		
451				0.1		328			0.4		
450	0.1			0.1		327			1.3		
449	0.1					326			0.6	0.1	
445			0.1			325	0.1		0.3	0.2	0.5
444			0.3			324	0.1		0.4	0.5	
443			0.7			323	0.5		1.0	0.1	0.5
442			1.3			322	0.1		0.8	0.2	
441			3.1			321	0.5		0.2	0.6	0.8
440			0.2	0.1	0.3	320	0.4			0.7	0.6
439	0.1			0.3	0.8	319	1.3	$C_{13}H_{31}Si_3O_3$		1.7	1.4
438	0.2			0.8	1.6	318	0.4			0.6	0.4
437	0.7			1.6	3.4	317	0.7	$C_{13}H_{29}Si_3O_3$		0.5	
436	1.1			2.8	0.4	312			0.1		
435	2.9	$C_{17}H_{39}Si_4O_5$		0.7	0.2	311			0.3		
428			0.1			310			0.3		
422	0.1			0.1		309	0.1		0.7	0.1	
421	0.1					308	0.2		0.9	0.4	0.4
415			0.1			307	0.6		1.5	0.9	0.8
414			0.3			306	1.1		0.2	1.4	1.2
413			0.7			305	2.4	$C_{12}H_{29}Si_3O_3$		2.2	2.5
412			0.7			304	0.2			0.3	
411			0.1	0.1		303	0.1			0.1	
410	0.1		0.1	0.2	0.2	296			0.4		
409	0.1		0.1	0.3	0.4	295			0.9		
408	0.3			0.2	0.3	294			2.5		
407	0.3			0.2		293	0.4		1.3	0.6	0.5
406	0.1			0.1		292	0.7			1.5	0.7
405	0.1			0.1		291	2.3	$C_{11}H_{27}Si_3O_3$		1.4	2.1
400			0.1			290	0.6			0.8	0.8
399			0.4			279	0.4	$C_{10}H_{27}Si_3O_3$	0.7	0.5	0.7
398			0.7			276			0.4		
397			1.8	0.1		272				0.4	
396	0.1		0.1	0.3	0.2	271	0.4	$C_{12}H_{23}Si_2O_3$			
395	0.3			0.7	0.4	267			0.4		
394	0.5			1.7	0.7	266			1.4	0.7	
393	1.4	$C_{15}H_{37}Si_4O_4$		0.2	1.9	265	1.0	$C_9H_{25}Si_3O_3$		0.7	1.1
392	0.1					249			0.4		
382			0.1			248			1.0		
381			0.1	0.1		245	0.5			0.5	1.0
380	0.1			0.1		244	0.5			0.7	0.5
379	0.2	$C_{14}H_{35}Si_4O_4$		0.1	0.2	243	1.3	$C_{11}H_{23}Si_2O_2$		1.1	
370			0.2			237			0.4		
369			0.2			236			1.1		
368			0.4			235			2.4		
367			1.3			234			0.8		0.8
366			0.1			233	0.8			0.7	1.1
365	0.1			0.1	0.3	232	0.5			0.9	2.1
364	0.1		0.1	0.3	0.6	231	2.2	$C_9H_{19}Si_2O_3$		2.6	0.6
363	0.3		0.1	0.6	1.7	230	0.6			0.7	0.6
362	0.4			1.4	0.2	229	0.4				
361	1.3	$C_{15}H_{33}Si_3O_4$		0.3		224			0.4		
360	0.1			0.1		223			1.5		
359	0.1					222			4.4	0.4	0.7
354			0.1			221	1.5		6.5	1.7	2.5
353			0.2			220	0.5		23.6	1.0	2.8
352			0.2			219	3.1		1.1	4.1	3.4
351			0.3			218	6.2			8.9	6.4
350			0.7	0.1		217	21.1	$C_9H_{21}Si_2O_2$	0.6	26.0	21.4
349	0.1			0.1	0.2	210			0.4		
348	0.1			0.4	0.3	209			1.5		

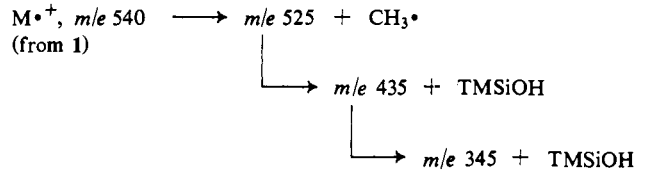
Table I (*Continued*)

m/e	α-D-Glucose	Elemtl compstn	α-D-Glucose d_7	α-D-Glucose 1-*d*	α-D-Glucose 6, 6-d_2	m/e	α-D-Glucose	Elemtl compstn	α-D-Glucose d_7	α-D-Glucose 1-*d*	α-D-Glucose 6, 6-d_2
208			9.9	0.5	0.7	131	2.7	2:3 doublet $C_5H_9SiO_2$ and $C_6H_{13}SiO$	2.2	2.9	4.3
207	2.0		19.5	2.5	2.6	130	1.1			2.3	1.3
206	8.1		92.0	10.6	9.9	129	7.9			7.5	5.6
205	17.2		2.6	25.0	21.5	127	0.4				
204	86.2	$C_8H_{20}Si_2O_2$	1.1	100.0	100.0	122			0.4		
203	6.4			1.8	1.2	121			0.9		
195			1.1	0.9		120			6.7		0.6
194	0.7		8.5	6.7	0.9	119	0.8		1.1	1.0	5.6
193	5.5		18.4	15.6	7.9	118	0.7		1.2	1.1	0.8
192	11.5		96.0	82.0	15.7	117	6.6	$C_4H_9SiO_2$	0.8	6.4	1.5
191	63.2	$C_7H_{19}Si_2O_2$	4.1	12.5	80.0	116	1.2		0.5	1.1	0.9
190	0.9		0.4	1.5	1.1	115	0.5			0.4	0.5
189	2.5	$C_7H_{17}Si_2O_2$		2.2	2.1	114				0.5	
179			0.4			113	0.7				0.5
178			0.8			106			0.9		0.5
177	0.5		0.6	0.4	0.7	105	0.4		6.7	0.6	4.0
176				0.6		104	0.8		3.0	2.4	1.0
175	0.7			0.6	0.6	103	7.3	$C_4H_{11}SiO$	1.7	6.4	4.7
173			0.5		0.4	102	0.4		0.6	1.2	0.6
170				0.4		101	2.4			2.0	1.5
169	0.6			0.4	0.5	99	0.5			0.4	0.5
161	0.6		1.3	0.7	0.6	93			0.4		
160			0.5			92			1.3		
159	0.4				0.5	91			0.4		1.3
157	0.7			0.5	0.6	90			0.4		
155	0.5					89	1.7	C_3H_9SiO		1.4	0.6
151			0.4			87			0.4		
150	0.4		1.3	0.5	0.6	86			0.7		
149	3.3		3.2	3.1	3.2	85	1.2				
148	4.3		5.8	4.6	4.7	83	2.0				0.5
147	25.3	$C_5H_{15}Si_2O$	27.6	27.8	25.7	81	0.6				0.4
146			0.4			78			0.4		
145	0.8		0.9	0.6	0.6	77	0.5		0.7	0.7	0.7
143	1.3			1.0	1.0	76	0.7		4.7	1.8	1.5
135	0.5		0.8	0.5	0.6	75	11.5		6.5	8.4	9.8
134	0.8		2.7	0.9	1.0	74	8.6		11.5	9.4	21.5
133	5.4		9.2	5.1	5.6	73	100.0	C_3H_9Si	100.0	100.0	100.0
132	0.6		2.5	0.6	0.9	72	2.1		2.1	1.9	2.1
						71	0.4				0.6

[a] Peaks of relative intensity < 0.4 were omitted below *m/e* 300.

the fragmentations exhibited by methyl ethers of monosaccharides are evident.[28,29]

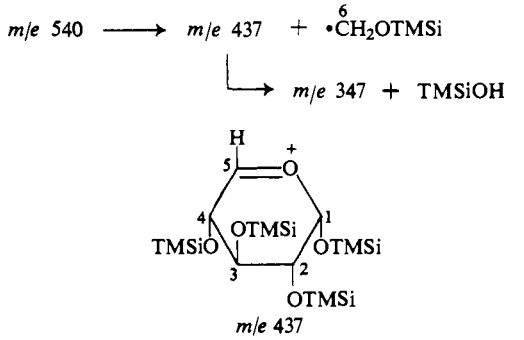
Molecular ions are observed in the mass spectra of **1–4**, although their relative intensities are low. Generally, a high sample pressure must be maintained in the ion source if a molecular ion is to be seen. A more intense peak is present for the loss of $CH_3\cdot$ from one of the trimethylsilyl groups; the molecular weight can therefore be determined from this if no molecular-ion peak is present. This "M − 15" ion fragments further by a stepwise elimination of two molecules of trimethylsilanol (TMSiOH).



A trimethylsilyloxy radical and trimethylsilanol are also lost from the molecular ion: *m/e* 450, $M\cdot^+$ − TMSiOH; *m/e* 361, $M\cdot^+$ − TMSiOH − TMSiO·; *m/e* 271, $M\cdot^+$ − 2TMSiOH − TMSiO·.

(28) N. K. Kochetkov, N. S. Wulfson, O. S. Chizhov, and B. M. Zolotarev, *Tetrahedron*, **19**, 2209 (1963).
(29) N. K. Kochetkov and O. S. Chizhov, *ibid.*, **21**, 2029 (1965).

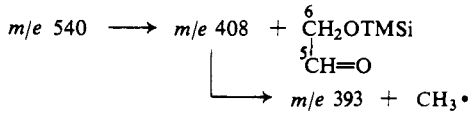
A series of low-intensity peaks is present, resulting from the loss of C-6 and its substituent followed by TMSiOH.

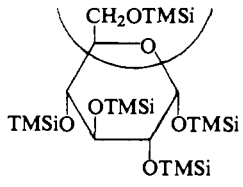


Most of the relative intensities of *m/e* 437 and part of 347 in Figure 1 are due to isotopes of *m/e* 435 and 345.

Fragments retaining one, two, three, and four carbon atoms of the ring are prevalent in the mass spectrum of **1**. Some of these ions have been reported previously,[1,25-27] and we have confirmed the assignments with deuterium-labeling and exact-mass measurements.

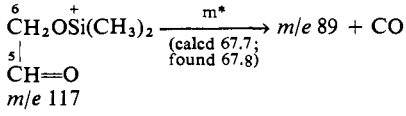
A small peak at *m/e* 408 arises from the cleavage of the molecule into a fragment retaining C-1 through C-4. The peak at *m/e* 393 corresponds to a further loss of $CH_3\cdot$. Structure **5** illustrates which part of the molecule is





5

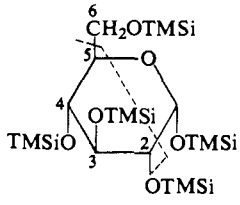
eliminated in the formation of *m/e* 408. A fragment retaining C-5, C-6, and the ring oxygen and losing C-1 through C-4 is found at *m/e* 117 (**6**). Carbon monoxide is subsequently expelled from *m/e* 117, a transition supported by a metastable peak.



6

The ring also fragments by elimination of C-1 and the ring oxygen. Structure **7** illustrates the part of the molecular ion which is lost. In addition, peaks are present for prior or subsequent eliminations of $CH_3\cdot$ and of TMSiO· and TMSiOH (Scheme I).

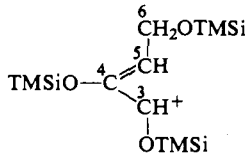
The fragment at *m/e* 319 retains four carbon atoms of the glucose molecule and three TMSiO groups. Structures **8** and **9** summarize two possibilities for this peak. Also, the peak at *m/e* 319 might result from a fragmentation



8

m/e 319

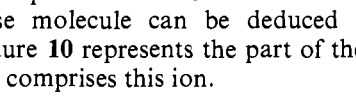
C-2–C-5



9

m/e 319

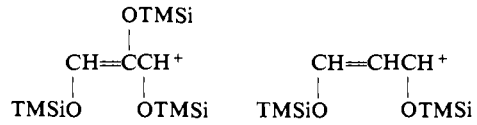
C-3–C-6



C-1–C-4

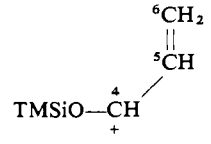
pathway involving loss of TMSiO· from *m/e* 408. The elemental compositions of these three possibilities are the same. The shifts of the *m/e* 319 region in the mass spectra of the deuterated forms are complex; however, labeling data do indicate that C-1 and C-6 are not present. Thus, structure **8** is the major contributor to *m/e* 319.

Fragments at *m/e* 305 and 217 are common from trimethylsilyl derivatives of carbohydrates.[1,25–27] These peaks retain C-2, C-3, and C-4, mainly. However, their general occurrence suggests that they can form from other portions of the molecule, sometimes with rearrangement.



m/e 305 *m/e* 217

High-resolution measurements on *m/e* 129 show that it is a doublet, approximately two-thirds of which is a $C_6H_{13}OSi$ ion. From the shifts in the mass spectra of **2–4**, the presence of C-6 and four of the hydrogens of the glucose molecule can be deduced for this fragment. Structure **10** represents the part of the original molecule which comprises this ion.

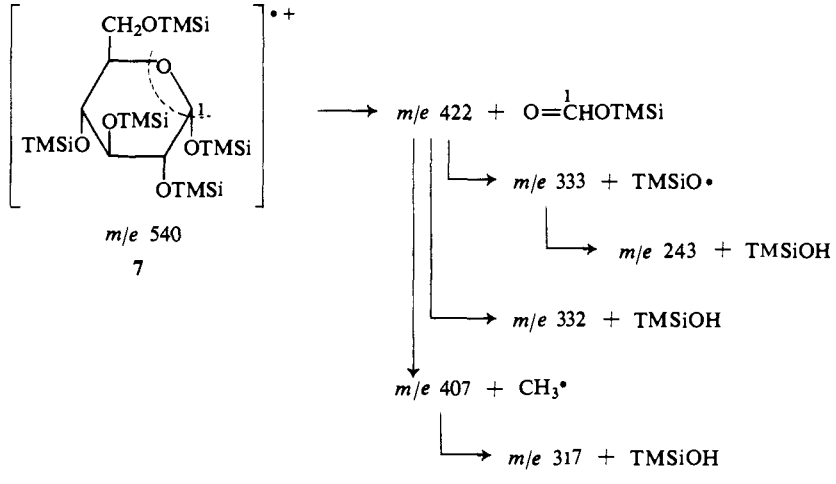


10

m/e 129 ($C_6H_{13}OSi$)

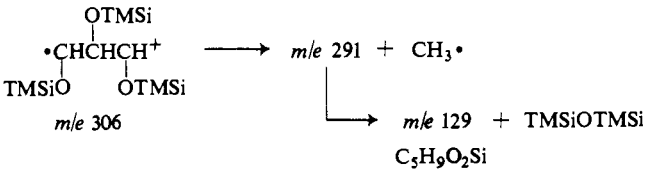
Even though *m/e* 306 is of low intensity and largely an isotope of *m/e* 305, the exact-mass data and the shifts of

Scheme I



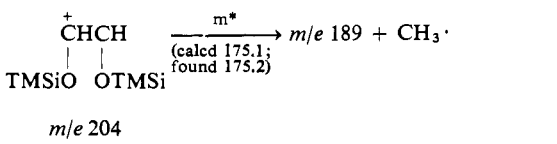
7

m/e 291 and 129 in the spectra of the deuterated analogs support the following scheme for the formation of the

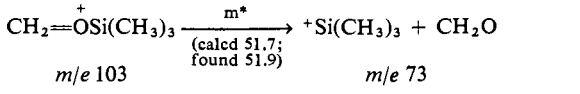


remaining one-third of m/e 129. However, no metastable peaks were found for these transitions and no other important pathways were found to involve elimination of TMSiOTMSi. Therefore this assignment is tenuous.

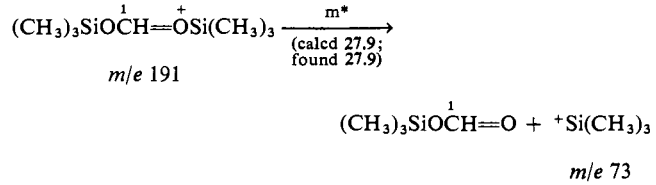
The two-carbon fragment, m/e 204, is a common, characteristic fragment from TMSi ethers of carbohydrates.[1,25-27] It originates, for the most part, from C-2–C-3 and C-3–C-4. A metastable peak is present for its further fragmentation to m/e 189.



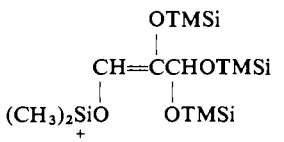
The peak at m/e 103 retains one carbon atom of the glucose molecule. This fragment could arise directly by cleavage of the C-5–C-6 bond with charge retention on C-6. However, the mass spectrum of the 6,6-d_2 derivative **4** shows that this accounts for only half of the intensity of m/e 103. Therefore, it must also form by rearrangement of a hydrogen atom to one of the carbons of the ring. A metastable peak indicates that m/e 73 (C_3H_9Si) arises by elimination of formaldehyde from m/e 103.[20]



Another fragment which is formed *via* a rearrangement is found at m/e 191.[1,25-27] It is composed of two TMSiO groups and one C–H of the glucose molecule; about 90% of m/e 191 retains C-1. One of these TMSiO groups originates from C-3 and is rearranged to C-1. This peak in the mass spectrum of tetra-*O*-trimethylsilyl-3-*O*-methyl-D-glucose is found at m/e 133, due to the rearrangement of the 3-*O*-methyl group to C-1. This fragment is also found at m/e 133 in the mass spectrum of the methyl α-D-glucopyranoside derivative. A metastable peak is present for the decomposition of m/e 191 to 73. A similar C-3–C-1 rearrangement is observed in the mass spectra of *O*-methyl ethers.[28,29]

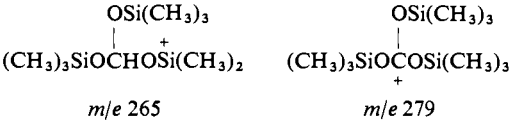


Rearrangements are common in the mass spectra of trimethylsilyl ethers of carbohydrates, a factor which must be taken into account when interpreting their spectra. Three small, but interesting, peaks which are found at m/e 379, 279, and 265 illustrate how extensive rearrangement can be. The first of these peaks retains three ring carbons, two ring hydrogens, and four TMSiO groups, at least one of which must be rearranged. An elemental composition of $C_{14}H_{35}O_4Si_4$ is consistent with the exact-mass data. A possible structure is **11**.

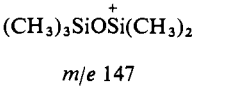


11, m/e 379

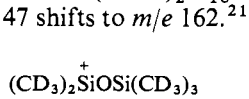
The peak at m/e 265 contains three TMSiO groups and only one carbon atom of the sugar; approximately half of these ions retain C-1. A similar peak is present at m/e 279. Possible structures are



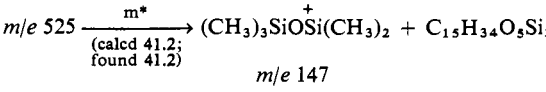
The ion at m/e 147 is present in the mass spectra of all TMSi derivatives of carbohydrates reported to date.[1,25-27] Its origin has been discussed in detail in relation to the



mass spectra of TMSi derivatives of aliphatic glycols.[30] A metastable peak is present for the formation of m/e 147 from ($M\cdot^+ - CH_3\cdot$) ions in the mass spectra of the aliphatic glycol's TMSi ethers[30] and in the mass spectra of the TMSi derivative of 3-hydroxy-4,5-dihydroxymethyl-2-methylpyridine hydrochloride.[31] McCloskey, *et al.*, have compared the mass spectra of 1,10-decanediol-$(TMSi)_2$ ether and 1,10-decanediol-$(TMSi)_2$-d_{18} ether and have observed that m/e 147 shifts to m/e 162.[21] A metastable



peak is present in the mass spectrum of **1** for the formation of m/e 147 from m/e 525 ($M\cdot^+ - CH_3\cdot$).



The Mass Spectra of the TMSi Derivatives of Glycosides. The mass spectrum (Figure 2) of methyl 2,3,4,6-tetra-*O*-trimethylsilyl-α-D-glucopyranoside (**12**), prepared from authentic methyl α-D-glucopyranoside and purified by gas–liquid partition chromatography, was obtained *via* the direct probe of the AEI MS 902 mass spectrometer. Selected ions are listed in Table II, along with the corresponding ions from the 1,2,3,4,5,6,6-d_7 (**13**), 1-*d* (**14**), and 6,6-d_2 (**15**) analogs. The fragmentations of **12** are the same as those of **1** except that some peaks are shifted by 58 mass units, which can be accounted for by the difference between CH_3O and $(CH_3)_3SiO$ at C-1 (Scheme II).

Fragments which have lost C-1 and the ring oxygen are present at the same m/e location as they were in the mass

(30) J. Diekman, J. B. Thomson, and C. Djerassi, *J. Org. Chem.*, 33, 2271 (1968).

(31) W. Richter, M. Vecchi, W. Vetter, and W. Walther, *Helv. Chim. Acta*, **50**, 364 (1967).

Scheme II

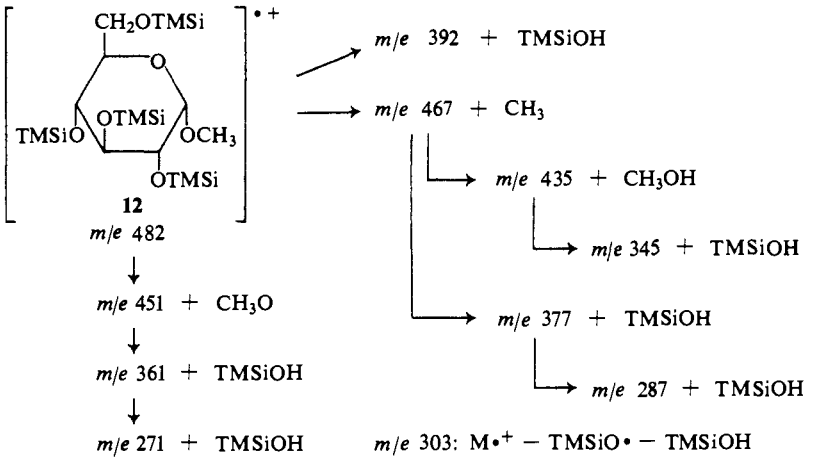


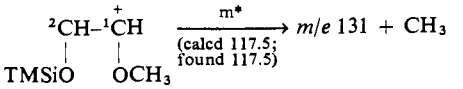
Table II. Selected Peaks from the Mass Spectrum of **12** and Their Location in the Mass Spectra of **13–15**

12 *m/e*	(d_7, **13**) *m/e*	(1-d, **14**) *m/e*	(6, 6-d_2, **15**) *m/e*	Elemntl compstn
482	489	483	484	
467	474	468	469	
451	458	452	453	
435	441	436	437	$C_{17}H_{39}O_5Si_4$
407	413	407	409	
393	397	393	395	$C_{15}H_{37}O_4Si_4$
392	398	393	394	
377	383	378	379	$C_{15}H_{33}O_5Si_3$
361	367	362	363	
345	350	346	347	
335	339	336	335	$C_{13}H_{31}O_4Si_3$
332	337	332	334	
319	323	319	319	
305	307	305	305	
303	309	304	305	
287	292	288	289	
271	276	272	273	$C_{12}H_{23}O_3Si_2$
217	220	217	217	
204	206	204	204	
191	192	191	191	
147	147	147	147	
146	148	147	146	$C_6H_{14}O_2Si$
133	134	134	133	$C_5H_{13}O_2Si$
131	133	132	131	
117	120	117	119	
89	92, 89	89	91, 89	C_3H_9OSi
73	73	73	73	

spectrum of **1** (see **7**; 1-OCH_3): *m/e* 422, $M^{\cdot+}$ − $O{=}\overset{1}{C}HOCH_3$; *m/e* 407, *m/e* 422 − CH_3; *m/e* 332, *m/e* 422 − TMSiOH. The origin of the peaks at *m/e* 73, *m/e* 117 → *m/e* 89, *m/e* 217, *m/e* 305, and *m/e* 319 can be explained as described in the previous section, in relation to the mass spectrum of **1**.

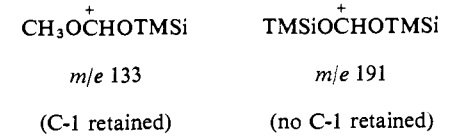
The peak at *m/e* 335 in Figure 2 corresponds to *m/e* 393 in the mass spectrum of **1** (see **5**), except that a methoxyl group has replaced one of the TMSiO groups. A very small *m/e* 393 is present also in Figure 2 and has the same elemental composition as in Figure 1. Since its formation from the molecular ion of **12** must involve rearrangement, it can be concluded that *m/e* 393 is a general ion.

The relatively small peak at *m/e* 146 retains C-1 and C-2. A metastable peak is present for the formation of *m/e* 131 by loss of CH_3 from *m/e* 146. The peak at *m/e* 191,



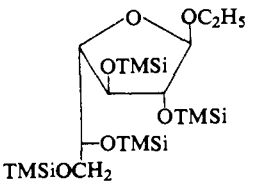
m/e 146

discussed in the previous section in relation to rearrangements, is largely (~80%) shifted to *m/e* 133 in the spectrum of **12**. This gives an indication of the extent to which C-1 is retained in this rearrangement process.

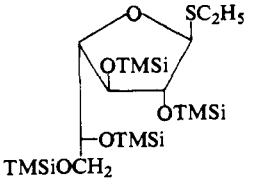


Approximately 75% of the peak at *m/e* 89 in Figure 2 remains at *m/e* 89 in the mass spectrum of the d_7 derivative **13**. This portion of *m/e* 89 can be accounted for by an ion analogous to *m/e* 147: $CH_3OSi^+(CH_3)_2$, *m/e* 89. The 25% of *m/e* 89, which shifts to *m/e* 92 in the spectrum of **13**, is the result of loss of CO from *m/e* 117 (**6**). Both ions have the same elemental composition.

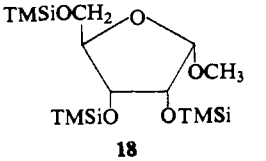
The relative intensity of the fragment at *m/e* 204, which is the second most intense peak in the mass spectra of **1** and **12**, is drastically reduced in the mass spectra of glycofuranosides. For example, the relative intensity of *m/e* 204 is 5.4% in the mass spectrum of ethyl 2,3,5,6-tetra-*O*-trimethylsilyl-β-D-galactofuranoside (**16**, Figure 3), 3.1% in the mass spectrum of the 1-thioethyl analog (**17**) of **16**, and only 1.9% in the mass spectrum of methyl 2,3,5-



16



17



18

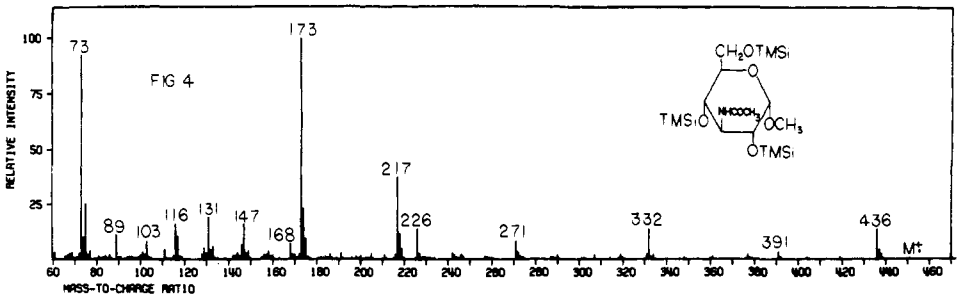
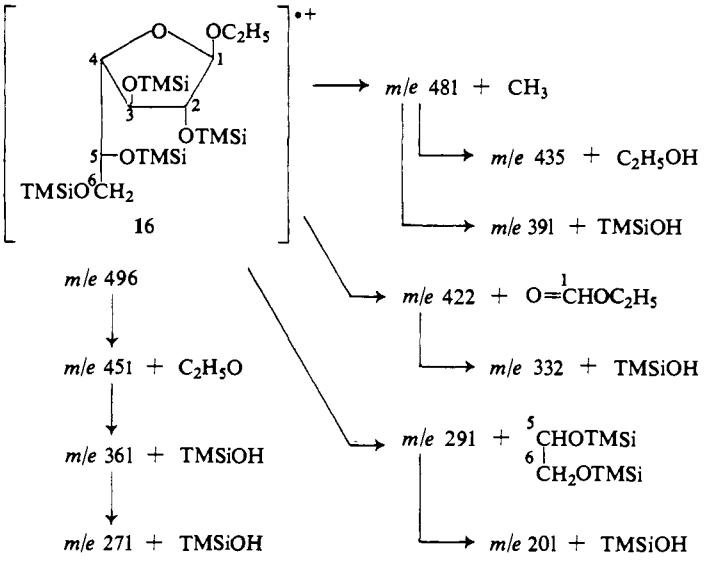
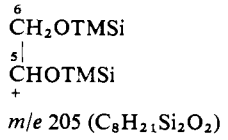


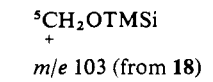
Figure 4. The 70-eV mass spectrum of methyl 3-acetamido-3-deoxy-2,4,6-tri-*O*-trimethylsilyl-α-D-glucopyranoside (**20**); molecular weight, 451.

Scheme III



tri-*O*-trimethylsilyl-α-D-ribofuranoside (**18**). The intensity of *m/e* 205 is greater in the mass spectra of the hexofuranosides (6.3% for **16**) than in the mass spectrum of the pentofuranoside (0.7% for **18**), which can be accounted for in the former cases by cleavage of the bond between C-4 and C-5 with charge retention on C-5. Similarly, *m/e*

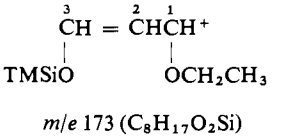




103 is enhanced in the mass spectrum of pentofuranoside **18** as compared to the spectra of the hexofuranosides, the pyranosides, and the pyranoses.

The small peak at *m/e* 291 in Figure 3 is a 2/3:1 doublet of $C_{12}H_{27}O_4Si_2$ and $C_{11}H_{27}O_3Si_3$. The 12-carbon fragment corresponds to loss of 205 mass units, C-5–C-6. The 11-carbon fragment has the same elemental composition as *m/e* 291 which is present in Figure 1 and which has been discussed above.

The peak at *m/e* 217 is the base peak in the mass spectra of furanosides **16** and **18** and is the second most intense peak in the spectrum of **17**. The peak at *m/e* 173 (2.9%) in Figure 3 can be accounted for by retention of the ethoxy group on C-1 and a TMSiO group on C-2 or C-3 (**19**).

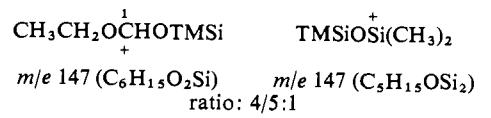


19

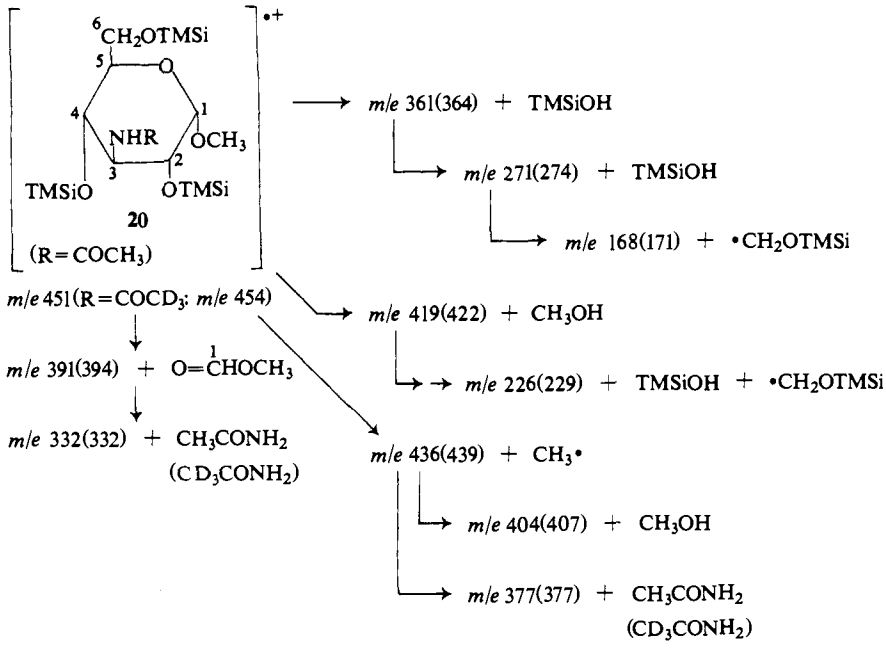
This fragment is found at *m/e* 159 (CH_3O at C-1) and *m/e* 189 (C_2H_5S at C-1) in the mass spectra of **18** and **17**, respectively.

A particularly intense peak at *m/e* 319 appears to be characteristic of the hexofuranosides. Its relative intensity is 13% in the mass spectrum of **16** (Figure 3), and 11% in the mass spectrum of **17**. In contrast, it is 1.3% in the mass spectrum of hexopyranose **1** (Figure 1), 1.1% in the mass spectrum of hexopyranoside **12** (Figure 2), and only 0.1% in the case of pentofuranoside **18**. The origin of *m/e* 319 will be discussed later, in conjunction with its usefulness in identifying furanose forms of hexoses.

In Figure 3, the peak resulting from rearrangement of a TMSiO substituent to C-1 is found at *m/e* 147, along with $C_5H_{15}OSi_2$.



Scheme IV



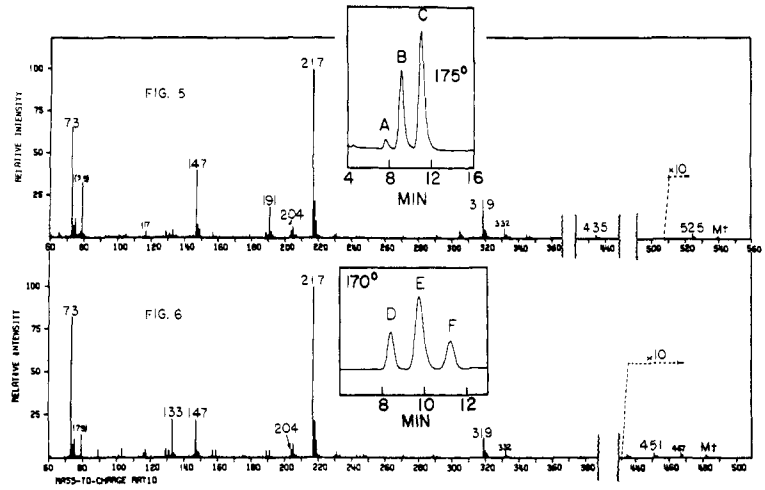


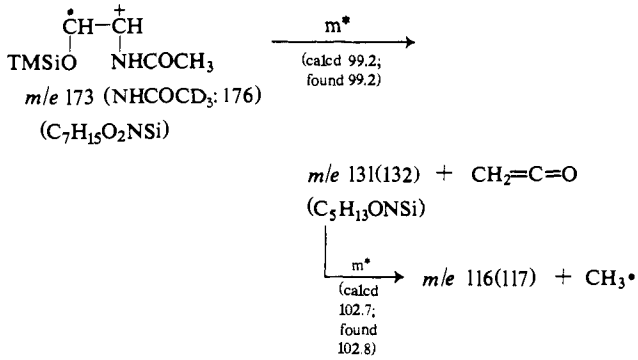
Figure 5. The gas chromatogram of the product from trimethylsilylation of D-galactose and the mass spectrum of component A.

Figure 6. The gas chromatogram of the product from the trimethylsilylation of the mixture of methyl D-galactosides resulting from reaction of D-galactose with methanolic HCl; the mass spectrum of component D.

Scheme III summarizes other fragmentations of the molecular ion of **16**.

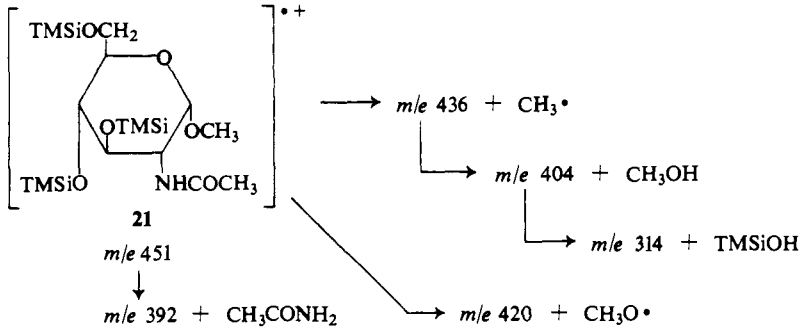
Mass Spectra of Trimethylsilyl Ethers of Methyl 2-Acetamido-2-deoxy-α-D-glucopyranoside and of Methyl 3-Acetamido-3-deoxy-α-D-glucopyranoside. The mass spectra (Figure 4) of methyl 3-acetamido-3-deoxy-2,4,6-tri-*O*-trimethylsilyl-α-D-glucopyranoside (**20**) and of its 3-NHCOCD$_3$ analog contain fragmentation pathways similar to those found with the TMSi ethers of free sugars and the methyl glycosides. However, the presence of the acetamido group modifies the fragmentation to some extent (see Scheme IV). The values in parentheses in Scheme IV are the locations of the peaks in the mass spectrum of the 3-NHCOCD$_3$ analog.

Of significance in Figure 4 is the absence of *m/e* 305 and 204 which were discussed in connection with the mass spectrum of **1**. However, an ion at *m/e* 173 is intense. Metastable ions for the loss of ketene followed by CH$_3$· from *m/e* 173 are present.



In contrast to the presence of relatively intense re-

Scheme V



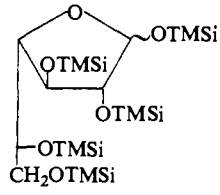
arrangement ions at *m/e* 191 in the mass spectra of free-sugar derivatives such as **1** and at *m/e* 133 in the mass spectra of methyl glycoside derivatives such as **12**, only a very small rearrangement ion is present in the mass spectrum of **20** for the transfer of the 3-acetamido group to C-1: $CH_3CONHC^+HOCH_3$, *m/e* 102 (105). The peak at *m/e* 102 is only 2.5% relative intensity, but it shifts to *m/e* 105 as expected in the spectrum of the deuterated compound.

The gross features of the mass spectrum (not shown) of methyl 3,4,6-tri-*O*-trimethylsilyl-2-acetamido-2-deoxy-α-D-glucopyranoside (**21**) are similar to those of the 3-acetamido derivative. Several significant differences can be distinguished, however. For example, the 2-acetamido derivative eliminates CH_3CONH_2 from the molecular ion, whereas the 3-acetamido compound does not (Scheme V).

The ion at *m/e* 173 (100%) is again present and, as indicated by metastable peaks, fragments to *m/e* 131 and 116. The ion at *m/e* 204 is present with 12% relative intensity. The ion at *m/e* 217, 56% in the spectrum of **20**, is only 9.5% in the spectrum of **21**. The C-3–C-1 rearrangement ion is, as expected, at *m/e* 133 (14.7%).

Studies Using Combined Gas–Liquid Partition Chromatography–Mass Spectrometry. When a free sugar is dissolved in water, an equilibrium mixture of isomeric forms is produced. This equilibrium mixture can be trimethylsilylated to give a mixture of TMSi ethers which can be separated by glpc. The major components of these mixtures of TMSi ethers can be assigned to α- and β-pyranose forms by comparing their retention times with those of authentic samples prepared from pure anomers. A third component is present in certain cases and has been referred to as the "γ" form.[7] Its nature has not been established although furanose and open-chain forms have been suggested as the most likely possibilities.[7,32]

We have examined by glpc–mass spectrometry the products which result from trimethylsilylation of equilibrium mixtures of D-galactose and D-glucose and its 1,2,3,4,5,6,6-d_7, 1-*d*, and 6,6-d_2 analogs in order to determine the structures of the minor isomers in these cases. The gas chromatogram of the products obtained from trimethylsilylation of D-galactose is given in Figure 5 along with the mass spectrum of component A. The mass spectra of components B and C are identical and exhibit only minor intensity differences when compared with the mass spectrum in Figure 1; this confirms the assignment[7] of B and C as pyranose anomers. The mass spectrum of the minor component A (Figure 5) is similar to the mass spectrum of **16** (Figure 3). For example, *m/e* 319 is 16.7% relative intensity in Figure 5 compared with 13% in the mass spectrum of **16**, and only 1.3% in the mass spectrum of **1**. The intensities of *m/e* 204 (4.3%) and *m/e* 205 (6.5%) are also similar to those found for the corresponding peaks in the spectrum of **16**, but differ significantly from the values recorded for **1**. These comparisons support the formulation of component A as a furanose form, penta-*O*-trimethylsilyl-α- or -β-D-galactofuranose (**22**).



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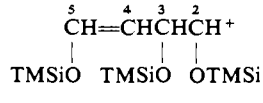
Similar treatment of D-glucose also gives two major and one minor component. The mass spectra of the two major components are identical with the mass spectrum of **1**. The mass spectrum of the minor component contains a peak at *m/e* 204 of 5% relative intensity and a peak at *m/e* 319 of 16% intensity. The mass spectra of the minor components obtained from trimethylsilylation of the deuterated analogs of D-glucose show the following peak shifts (d_7, 1-*d*, 6,6-d_2): *m/e* 319: 323, 319, 319; *m/e* 205: 208, 205, 207; *m/e* 204: 206, 204, 204. From these data, it can be concluded that *m/e* 319 retains C-2–C-5; **23** is a possible representation of this ion. The shift of



m/e 319

23

m/e 205 to 207 in the mass spectrum of the D-glucose-6,6-d_2 derivative supports the contention that the fragment of *m/e* 205 forms by cleavage of the C-4–C-5 bond with charge retention on C-5. Thus, the minor component from trimethylsilylation of D-glucose can be assigned to penta-*O*-trimethylsilyl-α- or -β-D-glucofuranose.

It should be noted that the minor components discussed above are present in the reaction products in relatively low concentrations. As a consequence, the mass spectra obtained are less intense than the mass spectra of the

(32) R. Bentley and N. Botlock, *Anal. Biochem.*, **20**, 312 (1967).

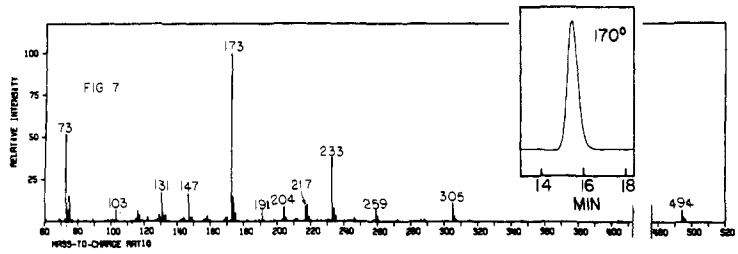


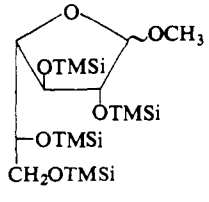
Figure 7. The gas chromatogram of the product from the trimethylsilylation of 2-acetamido-2-deoxy-D-galactose and the mass spectrum of the component.

major components. At times, background peaks are of significant intensity; for example, both the intense peak at *m/e* 79 and the weak peak at *m/e* 499 in Figure 5 are incompatible with structure **22**. Similarly, low intensity peaks are present at *m/e* 494 and *m/e* 509 in the mass spectrum of the minor component from D-glucose. The fact that these peaks do not shift in the mass spectra of the deuterated derivatives indicates that they cannot be attributed to a derivative of D-glucose and are therefore due to background.

Although only three components attributed to carbohydrate derivatives are discernible in the gas chromatogram of the trimethylsilylation products of D-galactose and D-glucose, it is likely that the second anomeric furanose component is obscured by the pyranose peaks.

The same approach was used to determine the products from glycosidation of D-galactose and D-glucose. Trimethylsilylation of the mixture of methyl D-galactosides resulting from reaction of D-galactose with methanolic hydrogen chloride gave three components, D, E, and F. The gas chromatogram is given in Figure 6, along with the mass spectrum of D.

The mass spectra of E and F closely resemble the mass spectrum of **12** (Figure 2), and these two components represent the anomeric methyl tetra-*O*-trimethylsilyl-D-galactopyranosides. The mass spectrum (Figure 6) of the minor component D is typical of the mass spectra of the furanosides **16** (Figure 3), **17**, and **18**, and of the mass spectrum of the furanose **22** (Figure 5). In particular, the peak at *m/e* 319 is 12% relative intensity and *m/e* 204 and 205 are 4.6 and 8.2%, respectively. This suggests that D is methyl tetra-*O*-trimethylsilyl-α- or -β-D-galactofuranoside (**24**).



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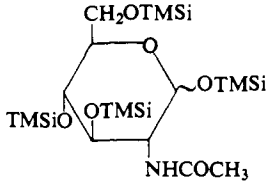
The results from treatment of D-glucose with methanolic hydrogen chloride followed with trimethylsilylation are similar. On the basis of their mass spectra, the major components can be assigned to methyl tetra-*O*-trimethylsilyl-α- and -β-D-glucopyranosides and the minor component can be assigned to methyl tetra-*O*-trimethylsilyl-α- or -β-D-glucofuranoside.

Trimethylsilylation of 2-acetamido-2-deoxy-D-galactose gives one component on the glpc trace.[33] The base peak in the mass spectrum (Figure 7) of this component is *m/e* 173; this is also the base peak in the mass spectra of **21** and **20** (Figure 4). The parallel trends discernible in Figures 4 and 7 and discussed for the mass spectrum of **21** allow the assignment of a pyranose structure (**25**) to the TMSi ether obtained from 2-acetamido-2-deoxy-D-glucose.



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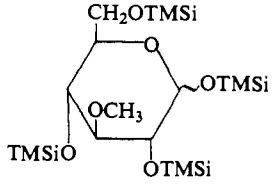
The gas chromatogram resulting from trimethylsilylation of 3-*O*-methyl-D-glucose is shown in Figure 8 along with partial mass spectra of the three components. The major components G and I can be assigned to the pyranose anomers (**26**) on the bases of the similarity of their spectra and the high relative intensity of the ion of *m/e* 146, which



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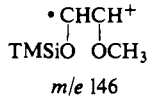
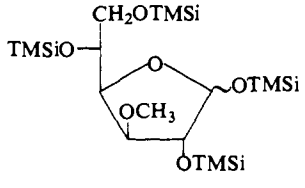


m/e 146

27



28

may be assigned structure **27**. In contrast, *m/e* 146 is not predominant in the mass spectrum of component H. The peak at *m/e* 261 is probably an analog (**29**) of *m/e* 319 (**23**). Thus, the mass spectrum of H is consistent with a furanose

(33) J. R. Clamp, G. Dawson, and L. Hough, *Biochim. Biophys. Acta*, **148**, 342 (1967).

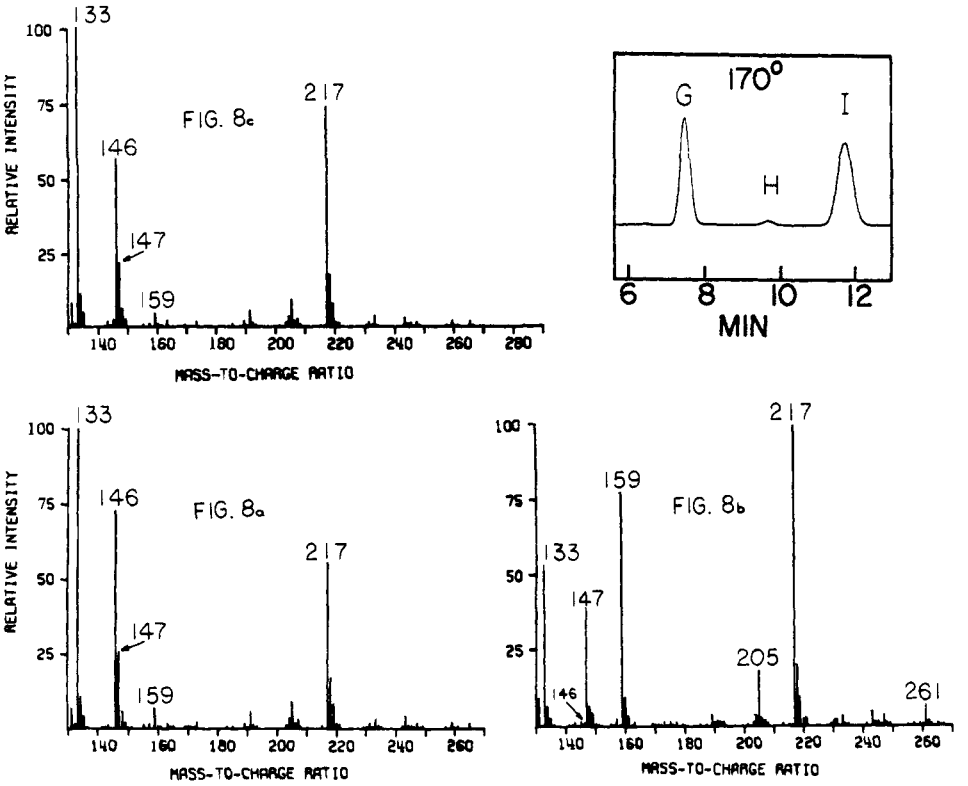
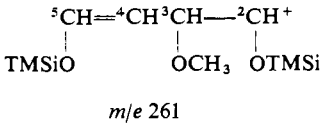


Figure 8. The gas chromatogram of the product from the trimethylsilylation of 3-*O*-methyl-D-glucose and partial mass spectra, 8a, 8b, and 8c, of components G, H, and I, respectively.



m/e 261

29

structure and H is best represented as tetra-*O*-trimethylsilyl-3-*O*-methyl-α- or -β-D-glucofuranose (**28**).

The peak at *m/e* 159 in the mass spectrum of component H is the 3-*O*-methyl analog of *m/e* 217, with CH_3O replacing TMSiO, a difference of 58 mass units. The peak at *m/e* 159 is not as intense in the mass spectra of pyranose components G and I, indicating that the 3 substituent is more readily eliminated from the pyranose forms than from the furanose forms.

Summary

These results indicate that the mass spectra of TMSi derivatives of sugars can be interpreted in terms of molecular structures. It is possible to recognize ring size from the mass spectra. For example, the fragmentation involved in the formation of *m/e* 319 (or its equivalent) is much more prominent in the mass spectra of the furanoses and furanosides than in the mass spectra of the pyranoses and pyranosides. The reverse is true of *m/e* 204 (or its equivalent): it is more prominent in the mass spectra of the six-membered ring isomers. It is also possible to derive the degree and position of substitution. For example, the shift of *m/e* 204 to 173 is characteristic of the acetamido group and the shift of *m/e* 204 to 146 is characteristic of the methoxy group. The presence and location of the peak due to the C-3 to C-1 rearrangement ion can be used to deduce substitution on C-3 and C-1: it is found at *m/e* 191 in the mass spectra of the free sugar derivatives and mainly at *m/e* 133 in the mass spectra of derivatives of methyl glycosides and 3-*O*-methylglucose.

The combination of glpc–mass spectrometry provides a method of assigning full or partial structures to the components of mixtures of sugars using submicrogram quantities of material. Comparisons of retention times and mass spectra of model compounds with those of the unknown components reveal considerable information about the unknown.

A potential application of this approach is the investigation of mutarotation phenomena. Values determined by glpc analysis for the composition of aqueous equilibrium mixtures of aldoses are in general agreement with data obtained by other methods.[32] This probably results from the very rapid rate of the trimethylsilylation reaction. It would appear that periodic determinations of the constitution of the mutarotation mixture by glpc–mass spectrometry of the TMSi derivatives offers an alternative means of following the mutarotation process.

Experimental Section

Mass Spectra. The mass spectra in Figures 1–4 were determined using the direct inlet system of an AEI MS 902 high-resolution mass spectrometer, at an ionizing potential of 70 eV, an ionizing current of 100 μA, and a resolution of approximately 1000 (10% valley). The ion source was not heated beyond the temperature maintained by the filament. High-resolution measurements were determined under the same conditions, but with a resolution of 10,000 (10% valley). Mass spectra were also obtained, for purposes of com-

parison, using the high temperature inlet system of an Atlas CH4 mass spectrometer at an ionizing potential of 70 eV and an ionizing current of 30 μA.

The exact-mass data were obtained by peak matching against heptacosafluorotributylamine. For the calculation of elemental compositions, all values were within ±2.5 millimass units, and 73% were within ±1.0 mmμ, of the theoretical value. Above *m/e* 146, all values were within 10 ppm of the theoretical value.

Combined Glpc–Mass Spectrometry. The combined glpc–mass spectrometric studies were conducted with an LKB-9000 gas chromatograph–mass spectrometer using an ionizing energy of 70 eV. The ion source block was maintained at 190° and the molecular separator at 170°. The glpc column consisted of coiled glass (6 ft × 1/8 in. i.d.) packed with 3% OV-1. The column was operated isothermally (see figures). The inner gas flow was maintained at 40 cc/min.

Deuterated D-glucose derivatives were obtained from Merck Sharp and Dohme of Canada, Limited. **2-*O*-Methyl-D-glucose** was prepared by the procedure of Brigl and Schinle, mp 158–159° (lit.[34] mp 158°). **3-*O*-Methyl-D-glucose** was obtained from CalBiochem Co., mp 161–162° (lit.[35] mp 161°). **Ethyl β-D-galactofuranoside** was prepared by the method of Green and Pascu.[36] It was used as a syrup; the TMSi derivative gave a single component on the gas chromatogram. **Methyl 3-acetamido-3-deoxy-α-D-glucopyranoside** was prepared by the method of Baer,[37] mp 213–214° (lit.[37] mp 214–215°). **Methyl 2-acetamido-2-deoxy-α-D-glucopyranoside** was prepared by the procedure of Kuhn and coworkers,[38] mp 186–187° (lit.[38] mp 187–188°). **Methyl β-D-ribofuranoside** was prepared by the method of Barker and Fletcher,[39] mp 74–76° (lit.[39] mp 79–80°). Trimethylsilyl derivatives were prepared in the usual manner[7] and purified by preparative glpc using a column of SE-30 (2.5%) adsorbed on Chromosorb W.

Acknowledgment. The support of the Alfred P. Sloan Foundation and of the Public Health Service of the Department of Health, Education, and Welfare, through Grants AI 07570 and GM 12328 is gratefully acknowledged by D. C. DeJongh. The AEI MS 902 high-resolution mass spectrometer was purchased with funds awarded to Wayne State University under the National Science Foundation's Higher Education Facilities Program. The support of the Public Health Service through Grant/AM 04308 and AM 12434 is acknowledged by C. C. Sweeley; the LKB-9000 gas chromatograph–mass spectrometer was purchased under Grant FR 00273.

(34) P. Brigl and R. Schinle, *Ber.*, **63B**, 2884 (1930).
(35) J. C. Irvine and T. P. Hogg, *J. Chem. Soc.*, **105**, 1386 (1914).
(36) J. W. Green and E. Pascu, *J. Am. Chem. Soc.*, **59**, 1205 (1937).
(37) H. H. Baer, *Chem. Ber.*, **93**, 2865 (1960).
(38) R. Kuhn, F. Zilliken, and A. Gauhe, *ibid.*, **86**, 466 (1953).
(39) R. Barker and H. G. Fletcher, Jr., *J. Org. Chem.*, **26**, 4605 (1961).

Effects of Substituents on the Exchange of Iodine with Benzoyl Iodides in Nonpolar Solvents

Donald W. Hamilton[1] and Richard M. Noyes

Contribution from the Department of Chemistry of the University of Oregon, Eugene, Oregon 97403. *Received September 30, 1968*

Abstract: Substituted benzoyl iodides undergo isotopic exchange with elementary iodine at conveniently measurable rates in solvents of low dielectric constant near room temperature. Rates in 1,2-dichloroethane (dielectric constant 10) are about 100 times those in hexane (dielectric constant 2). The rates contain kinetic contributions from parallel paths containing zero, one, and two molecules of iodine in the transition state, but photochemically generated iodine atoms do not contribute significantly to the exchange. In hexane at 25°, unsubstituted benzoyl iodide exchanges by the single iodine molecule path 43 times as rapidly as *p*-chlorobenzoyl iodide does. This difference is consistent with a Hammett ρ of −7, which is larger in magnitude than has been claimed for any other reaction of neutral molecules. Although some anomalies remain unexplained, these exchange reactions obviously involve very polar transition states even in solvents of low dielectric constant and may be useful for studying effects of solvent on chemical reactivity.

Benzoyl iodide, C_6H_5COI, exchanges easily with isotopically labeled elementary iodine in nonpolar solvents near room temperature.[2] Most of the rate is consistent with parallel paths having one and two molecules of elementary iodine in the respective transition states. In hexane, the entropies of activation for both paths are about −50 cal/(mol deg), and the rate in 1,2-dichloroethane (dielectric constant 10) is about 60 times that in hexane (dielectric constant 2). These observations suggest that the transition states involve so much separation of charge that they approximate ion pairs even in hexane.

If this mechanistic conclusion is correct, the rate of exchange should be very sensitive to different substituents on the aromatic ring. If the reaction develops almost a full unit of charge, electron-donating and -withdrawing substituents can greatly affect the charge distribution in the aromatic system. Any redistribution of electrical charge caused by a substituent will be opposed by polarization of the surrounding solvent. Since such polarization will be minimal for a solvent like hexane, substituent effects on ion-pair reactions should be maximized.

The experiments reported here were undertaken to test the above prediction and to obtain information concerning substituent and solvent effects on the rate of this exchange.

Experimental Section

Materials. ***p*-Chlorobenzoyl iodide** was prepared by passing hydrogen iodide into neat *p*-chlorobenzoyl chloride at 50°. The

(1) Based on the Ph.D. dissertation of Donald W. Hamilton.
(2) A. Goldman and R. M. Noyes, *J. Am. Chem. Soc.*, **79**, 5370 (1957).