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MASS SPECTRA OF *O*-ACETYL AND *O*-TRIMETHYLSILYL DERIVATIVES OF GLYCALS: ISOTOPIC LABELLING BY SELECTIVE INTRODUCTION OF ACETYL AND DEUTERIOACETYL GROUPS

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ABSTRACT

The mass spectra of the *O*-acetyl and *O*-trimethylsilyl derivatives of D-glucal and D-xylal have been determined. A study of compounds substituted with acetyl and acetyl-d_3 groups at specific positions permitted elucidation of the fragmentation mechanisms. Fragmentation processes in the spectra of the *O*-trimethylsilyl derivatives have been explained with the aid of data from the spectra of the perdeuteriotrimethylsilyl analogues.

INTRODUCTION

The principal features in the mass-spectrometric fragmentations of carbohydrate derivatives have been reviewed by Kochetkov and Chizhov[1]. Per-*O*-acetyl derivatives have been used extensively in the mass spectrometry of sugars, because of their good gas-chromatographic properties, which render them amenable to g.l.c.–m.s., and because their preparation is both simple and quantitative down to the submilligram level. Characteristic processes in the mass spectra of *O*-acetyl derivatives of sugars involve sequential eliminations of acetic acid (CH_3COOH) accompanied by losses of ketene ($CH_2C{=}O$) and/or acetal radicals ($CH_3\dot{C}{=}O$). Postulation of reasonable fragmentation schemes and structural assignments to fragment ions was made possible by comparison of the mass spectra of per-*O*-acetyl and per-*O*-deuterioacetyl derivatives[2,3]. Since the most significant fragmentation processes involve the acetyl group, it seemed that more-definitive fragmentation schemes could be deduced from the study of mixed acetyl/acetyl-d_3 derivatives of known structure. A similar approach has been described using mixed trimethylsilyl and perdeuteriotrimethylsilyl derivatives of steroids[4,5].

We now report on the mass spectra of specifically labelled *O*-acetyl/*O*-acetyl-d_3 derivatives of D-glucal and D-xylal and, for comparison purposes, the *O*-trimethylsilyl derivatives. Data on the mass spectrometry of unsaturated carbohydrates are

relatively scarce. Moreover, there is a need to study unsaturated carbohydrates, as they may be regarded as intermediates in the fragmentation of the corresponding saturated analogues[6]. There has been an increased interest in unsaturated carbohydrates, as a result of the finding that blasticidin S, an antibiotic active against rice blast disease[7,8], contains an unsaturated sugar moiety. Related pyranosyl-2,3-unsaturated nucleosides can be prepared by fusion of tri-*O*-acetyl-D-glucal with purine bases in the presence of acid[9].

RESULTS AND DISCUSSION

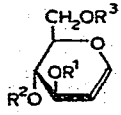
Acetyl derivatives. — Table I contains the principal peaks in the mass spectra of the 3,4,6-tri-*O*-acetyl (**1**), 3,4,6-tri-*O*-acetyl-d_3 (**2**), 3,6-di-*O*-acetyl-4-*O*-acetyl-d_3 (**3**), and 6-*O*-acetyl-3,4-di-*O*-acetyl-d_3 (**4**) derivatives of D-glucal. On the basis of these data, the fragmentation pattern given in Scheme 1 was developed. The usefulness of the selectively labelled derivatives **3** and **4** is apparent on examination of the proposed

TABLE I

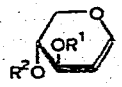
PRINCIPAL PEAKS IN THE MASS SPECTRA OF *O*-ACETYL/*O*-ACETYL-d_3 DERIVATIVES OF D-GLUCAL AND D-XYLAL.

1 m/e	*Relative intensity*	**2** m/e	**3** m/e	**4** m/e	**5** m/e	*Relative intensity*	**6** m/e
213	0.9	219	216	216	200	0.2	206
199	0.1	205	202	205	170	5.5	176
187	0.3	191	[a]	[a]	157	1.8	163
170	2.1	176	173(6)[b]; 176(1)[b]	173(1); 176(6)	140	10.0	143
153	3.5	156	153(4); 156(1)	153(4); 156(1)	128	3.0	132
152	6.2	155	152(1); 155(1)	152(1); 155(3)	115	5.0	119
139	18.5	142	139(7); 142(1)	139(1); 142(10)	98	59.5	99
128	9.5	132	129(2); 131(3)	132	86	7.0	88
110	20.9	111	110(1); 111(1)	110(1); 111(3)	81	30.0	81
97	80.2	98	97(2); 98(1)	97(1); 98(1.6)	43	100.0	46
86	10.0	88	87	88			
81	7.5	81	81	81			
43	100.0	46	43(1); 46(1)	43(1); 46(1)			

[a]Isotopic scramble prohibits assignment of correct mass-shift. [b]Figures in parentheses refer to relative ratios of indicated peaks.

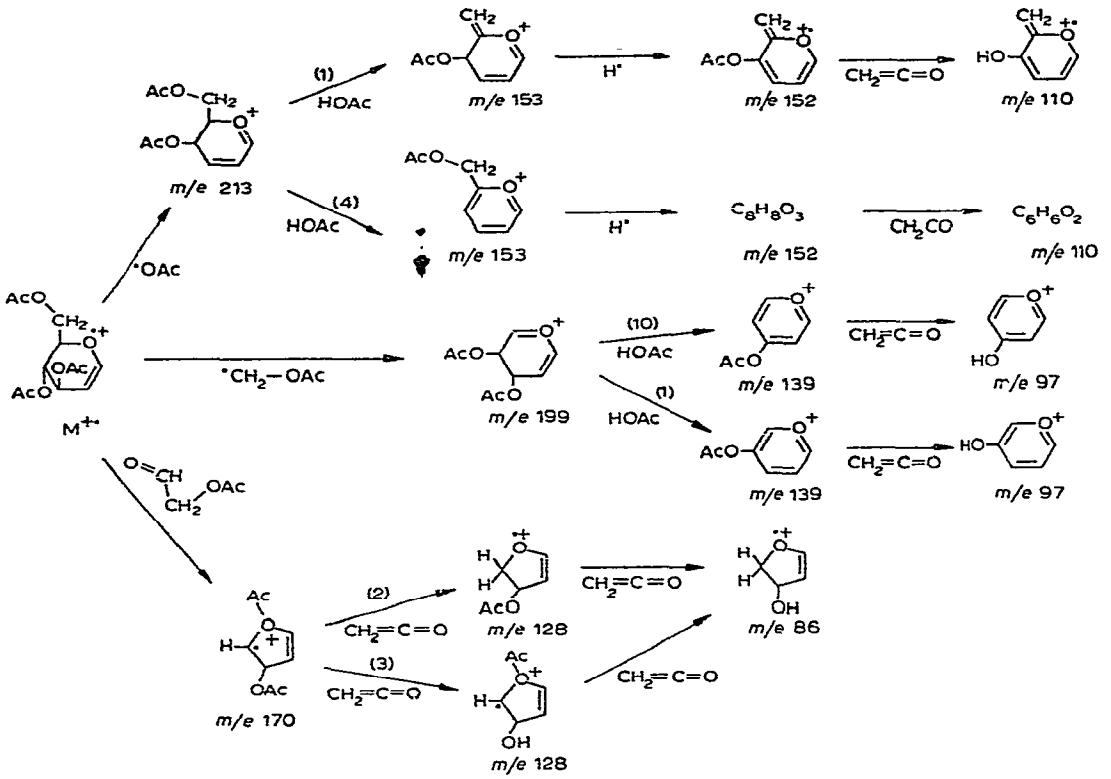


1 $R^1 = R^2 = R^3 = Ac$
2 $R^1 = R^2 = R^3 = CD_3CO$
3 $R^1 = R^3 = Ac$, $R^2 = CD_3CO$
4 $R^1 = R^2 = CD_3CO$, $R^3 = Ac$



5 $R^1 = R^2 = Ac$
6 $R^1 = R^2 = CD_3CO$

structures for the various fragment ions. For example, formation of the *m/e* 213 ion involves loss of an acetate radical from position 3, whereas *m/e* 153 consists of two isomeric fragment ions in the ratio 4:1. Similarly, the ion *m/e* 139 consists of two isomeric species formed by elimination of CH_3COOH from *m/e* 199. The isotopic-labelling data support the preferential formation (10:1) of the ion containing AcO-3. Further elimination of ketene from *m/e* 139 yields the stable fragment ion *m/e* 97. Hydrogen scramble apparently precedes the formation of the latter ion, because of the lack of complete shift to *m/e* 98 in the spectrum of **4**. A similar situation of hydrogen scramble is also encountered during the formation of the *m/e* 110 fragment ion, judging from the incomplete shift (75%) to *m/e* 111 in the spectrum of **4**.



Scheme 1. Fragmentation pattern for 3,4,6-tri-*O*-acetyl-D-glucal

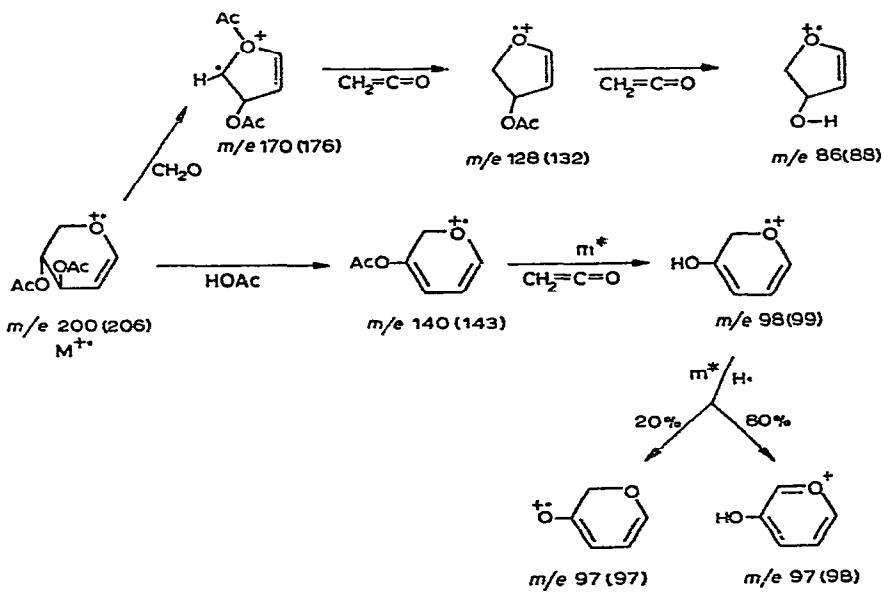
It was previously suggested[6] that *m/e* 97 further fragments by loss of an oxygen radical to an ion of *m/e* 81 (**7**). From the isotopic-labelling data, at least a partial shift to *m/e* 82 would be anticipated in the spectra of **1–3**. This shift does not occur, which suggests that formation of ion **7** is not preceded by an acetyl-hydrogen transfer, but probably involves a direct cleavage of the C-5–C-6 and C-3,C-4–OAc bonds.

Loss of the side chain results in formation of the fragment ion at *m/e* 170, which

subsequently eliminates CH_2=C=O, with a 3:2 preference from position 3 compared with position 4, to yield *m/e* 128. In either case, the latter ion further eliminates CH_2=C=O to give *m/e* 86.

7 (*m/e* 81)

Many of the types of ions present in the spectrum of **1** are also prominent in that of 3,4-di-*O*-acetyl-D-xylal (**5**). The principal peaks for **5** and the 3,4-di-*O*-acetyl-d_3 analogue **6** are given in Table I and illustrated in Scheme 2. In addition to the aromatic fragment-ion **7**, the most-notable peaks are those of *m/e* 170, 128, 97, and 86. Comparison of the spectra of the protium and deuterium derivatives makes possible the quantitative differentiation of isomeric ions and thus the distinction of parallel fragmentation processes, such as the loss of a hydrogen radical from *m/e* 98 which arises from competition between a ring hydrogen and an acetyl hydrogen transferred during elimination of CH_2=C=O.



Scheme 2. Fragmentation pattern for 3,4-di-*O*-acetyl-D-xylal.

O-Trimethylsilyl derivatives. — Trimethylsilylation has been employed extensively in the study of carbohydrates by mass spectrometry[10]. Me_3Si derivatives of monosaccharides[11], disaccharides[12], and trisaccharides[13] have been studied, and

more recently the analysis of partially methylated glucosides as their Me_3Si derivatives by g.l.c.–m.s. has been reported[14]. Table II summarizes the *m/e* values of the principal ions in the mass spectra of the Me_3Si and Me_3Si-d_9 derivatives of D-glucal and D-xylal. As the principal fragmentation processes involve the trimethylsilyl moiety, perdeuteriotrimethylsilyl derivatives[15] were prepared to help elucidate the mechanism of formation of the major fragment ions. Based on the data in Table II, the fragmentation pathways outlined in Schemes 3 and 4 were developed.

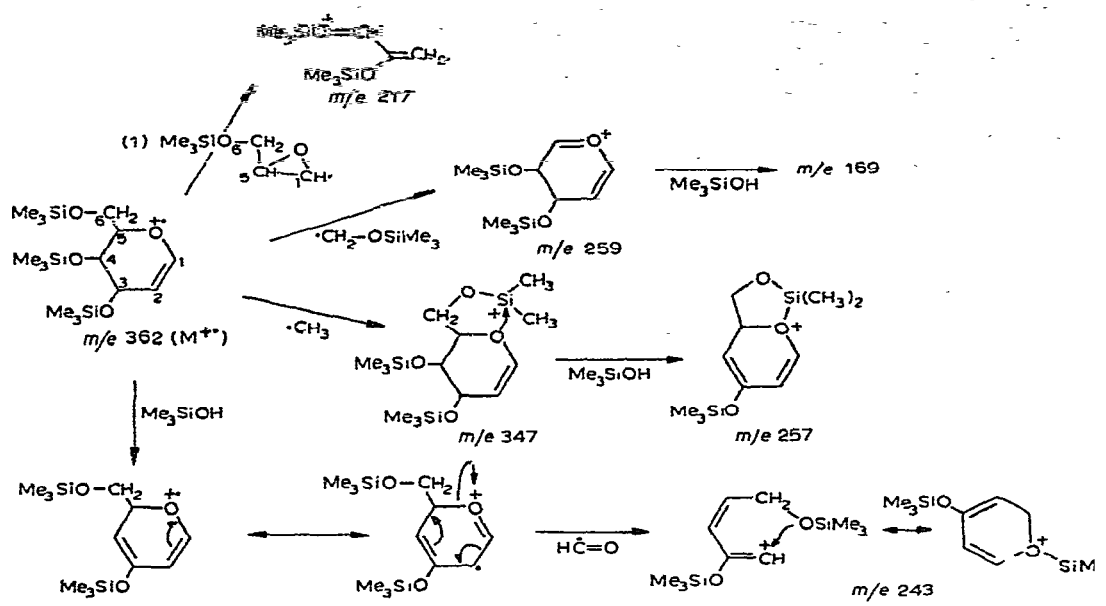
TABLE II

PARTIAL MASS SPECTRA OF 3,4,6-TRIS-*O*-(TRIMETHYLSILYL)-D-GLUCAL (8), 3,4-BIS-*O*-(TRIMETHYLSILYL)-D-XYLAL (9), AND THEIR *O*-(TRIDEUTERIOMETHYLSILYL) DERIVATIVES 10 AND 11

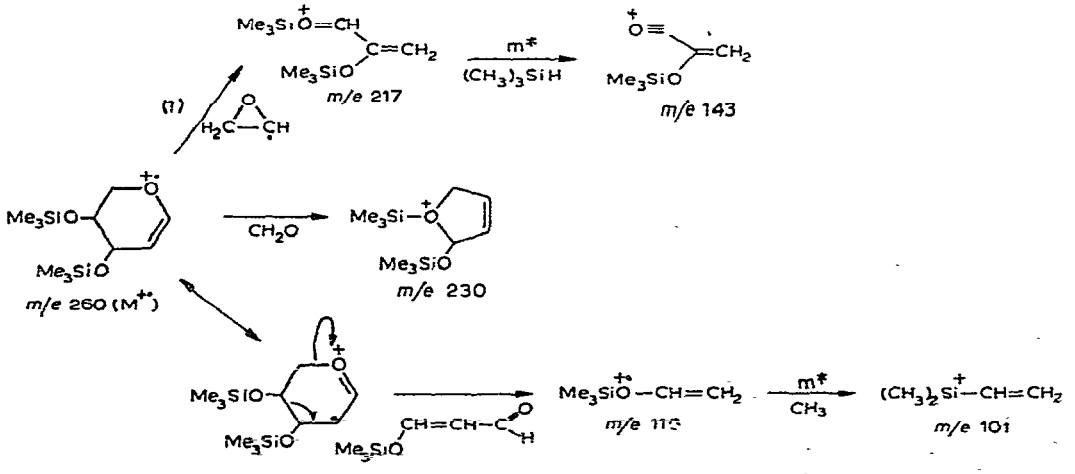
8 m/e (*Relative intensity*)	10 m/e	9 m/e	11 m/e
347(1.2)	371	260(0.5)	278
257(2.5)	272	245(2.1)	260
243(1.6)	261	230(2.6)	248
230(4.2)	248	217(90.8)	235
218(51.0)	236	147(59.8)	162
217(100)	235	143(19.8)	152
203(11)	218	129(15.1)	135
191(20.5)	209	116(60)	125
169(6)	178	101(100)	107
147(76.2)	162	73(110)	82
129(30)	138(1); 135(1)		
103(11)	112		
73(225)	82		

Stepwise elimination of trimethylsilanol from the molecular ion and/or $[M-15]^+$, a common occurrence in the mass spectra of the Me_3Si derivatives of alicyclic compounds, can be used to rationalize the formation of many of the ions in the spectra of the D-glucal and D-xylal derivatives (Schemes 3 and 4). Some of the major cleavages involve the ring system, as exemplified by process 1 in Schemes 3 and 4.

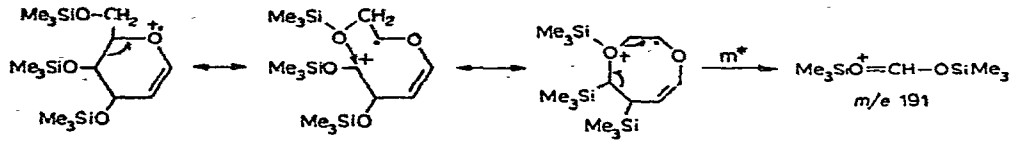
Typical of Me_3Si derivatives, the ion of *m/e* 73 (Me_3Si) dominates the 70-eV spectra (Table II). In addition, the rearrangement ion of *m/e* 147 ($Me_3Si–\overset{+}{O}–SiMe_3$), the relative abundance of which is often representative of the stereochemical orientation of trimethylsilyloxy groups[16,17], is very abundant. Characteristic of the $CH_2–O–SiMe_3$ group in the D-glucal derivative **8** is the ion at *m/e* 103 ($CH_2=\overset{+}{O}–SiMe_3$), as well as the weak, but structurally significant, complementary ion at *m/e* 259 $[M-103]^+$. Another characteristic rearrangement ion, often found in the mass spectra of Me_3Si derivatives of alicyclic compounds, is observed at *m/e* 191 in the spectrum of the D-glucal derivative **8**, but not in that of the D-xylal analogue **9**. Thus, it is reasonable to assume that *m/e* 191 is formed by an interaction involving the Me_3SiO-6 function *via* a ring-expansion process[18], as shown in Scheme 5.



Scheme 3. Fragmentation pattern of 3,4,6-tris-*O*-(trimethylsilyl)-D-glucal

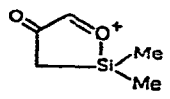


Scheme 4. Fragmentation pattern of 3,4-bis-*O*-(trimethylsilyl)-D-xylal



Scheme 5

An ion of *m/e* 129 ($Me_3\overset{+}{S}iO{=}CH{-}CH{=}CH_2$) is characteristic of 3-(Me_3SiO)-Δ^5-steroids or 17-(Me_3SiO)-steroids[19]. This ion exhibits a shift of 9 a.m.u. in the spectra of the perdeuteriotrimethylsilyl derivatives, and this labelling technique has permitted the recognition of a second ion of the same nominal mass exhibiting a shift of 6 a.m.u., to which structure **12** has been assigned[20].



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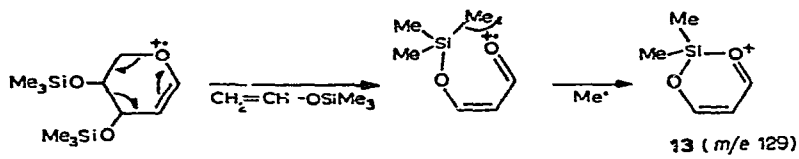
The isotopic-labelling data indicate that all of the *m/e* 129 ion in the spectrum of the D-xylal derivative **9**, and 50% of it in the spectrum of the D-glucal analogue **8**, contains two of the three Me_3Si groups. A fragmentation mechanism consistent with this shift of 6 a.m.u. is depicted in Scheme 6. It is significant that the resulting ion **13** has a markedly different structure from the ion (**12**) of identical elemental composition.



Scheme 6

EXPERIMENTAL

3,4,6-Tri-*O*-acetyl-D-glucal and 3,4-di-*O*-acetyl-D-xylal were prepared conventionally[21,22]. Me_3Si derivatives were obtained by reaction of D-glucal and D-xylal with *N,O*-bis(trimethylsilyl)trifluoroacetamide at room temperature. Acetyl-d_3 derivatives were synthesized by reaction of acetic anhydride-d_6–pyridine with D-glucal and D-xylal.

Selectively labelled acetyl derivatives of D-*glucal.* — A solution of 3,4,6-tri-*O*-acetyl-D-glucal (2.72 g) in methanol (10 ml) and *N,N*-dimethylamine (0.45 g) was kept for 3 h at room temperature, and then concentrated *in vacuo*. This process was repeated 5 times with the addition of methanol (5 ml) before concentration. T.l.c. (Kieselgel G–Merck, ethyl ether–light petroleum–ethanol, 20:1:1) revealed two

major components (R_F 0.51 and 0.79), which were isolated by column chromatography and shown to be 3,6-di-*O*-acetyl-D-glucal (**14**) and 6-*O*-acetyl-D-glucal (**15**) on the basis of n.m.r. and chemical data. The n.m.r. data [80 MHz, $CDCl_3$, internal $(Me_3Si)_2O$] are shown in Table III. Compound **15** also consumed 1.1 mol. of periodate.

TABLE III

RING-PROTON SIGNALS (τ) OF 3,4,6-TRI-*O*-ACETYL-D-GLUCAL, **14**, AND **15**

Compound	*H-1*	*H-2*	*H-3*	*H-4*	*AcO*
3,4,6-Tri-*O*-acetyl-D-glucal	3.59	5.21	4.68	4.85	7.99, 8.03 (3 AcO)
14	3.62	5.32	4.76	5.60	7.97 (2 AcO)
15	3.75	5.33	5.61	5.74	7.96 (1 AcO)

Treatment of **14** and **15** with acetic anhydride-d_6–pyridine gave the selectively labelled derivatives **3** and **4**, respectively.

Mass spectrometry. — Mass spectra were recorded on a Nuclide 12-90-G mass spectrometer, equipped with a capillary g.l.c. inlet. Mass spectra of selectively labelled acetyl derivatives were recorded on a Varian MAT 711 mass spectrometer at 70 eV. The samples were introduced into the spectrometer *via* the g.l.c. inlet (SE-30 column) at 120°, with a Biemann–Watson separator at 150° and an ion-source temperature of 150°.

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