Carbohydrate Research, 67 (1978) 1–15

MASS-SPECTROMETRIC FRAGMENTATION PATHWAYS OF THE *O*-TRIMETHYLSILYL DERIVATIVES OF HEXURONIC ACIDS AND THEIR LACTONES

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(Received October 14th, 1977 accepted for publication, December 13th 1977)

ABSTRACT

Comparison of the mass spectra of the *O*-trimethylsilyl derivatives of the hexuronic acids and their lactones with those for other monosaccharides revealed specific effects of the carboxyl group upon the fragmentation patterns The formation of the odd-electron ion having *m/e* 292 was characteristic of the presence of the C-6 carboxyl group and was due to a McLafferty-type rearrangement of a trimethylsilyl group to the carboxyl group Several other fragments having *m/e* values 14 mass units above those found in the hexoses confirmed the presence of the C-6 structure in the fragment A detailed analysis is presented of the fragmentations of the trimethylsilyl derivatives of β-D-glucopyranuronic acid and α-D-galactofuranuronic acid, as examples of pyranoid and furanoid structures The lactones produced less-complex spectra with the base peak at *m/e* 230, and this odd-electron ion is characteristic of the lactones studied A detailed analysis is presented of the fragmentation pathways of β-D-glucofuranurono-6,3-lactone as its trimethylsilyl derivative

INTRODUCTION

The use of g l c and g l c–m s in qualitative and quantitative analysis of trimethylsilylated carbohydrates has become a routine procedure since the advent of trimethylsilylation[1] These techniques have been used at the nanogram level for the separation and identification of a number of isomeric structures for each monosaccharide as their trimethylsilyl (or methyl) derivatives[2–4]

An understanding of fragmentation patterns is important for predictive purposes, and also for assessment of the usefulness of a particular ion for mass fragmentography Mass fragmentography can be used to give qualitative information at the picogram level, and may provide sufficient specificity for a particular structure to enable a positive identification[5] This technique requires the presence of an internal standard that has an almost identical structure, in order to prevent loss of the low levels of sample *via* adsorption, *etc* Ideally, the internal standard should fragment in a manner similar to that of the compound of interest In addition, it should show either different gas-chromatographic properties, or identical chromatographic properties with the production of a different ion In the latter case, deuterium labelling has become important, but a prerequisite is a known fragmentation pathway, so that an appropriate labelling position may be chosen

The hexuronic acids occur in the presence and absence of many other carbohydrates in many biological systems[6 7], and many methods have been developed for their analysis[6–8] However, the presence of *O*-methyl forms and various glycosiduronic acids makes the problem complex The use of g l c –m s can provide an answer to many of the problems encountered Also, by obtaining a detailed knowledge of the fragmentations, the number and position of substituents can be determined We now report on the fragmentations of the naturally occurring hexuronic acids and their lactones as their trimethylsilyl derivatives induced under conditions of electron-impact mass spectrometry

RESULTS AND DISCUSSION

The mass-spectrometric fragmentations of the trimethylsilyl derivatives of the hexuronic acids have been used to determine the ring size of the various isomers separated by g l c[9] Comparison of this structural information with that obtained from the trimethylsilyl derivatives of the neutral hexoses has now enabled identification of the fragments of trimethylsilyl derivatives of hexuronic acids and a clearer understanding of the breakdown pathways involved

Initial examination of the mass spectra showed only small differences between the various pyranoid forms studied or between the furanoid forms or between the lactone forms Such small differences do not establish a definite trend Differences between these mass spectra and those for the neutral hexoses occurred not only in peak intensity but also in the fragmentation pattern A detailed analysis of three typical mass spectra (for β-D-glucopyranuronic acid, α-D-galactofuranuronic acid, and β-D-glucofuranurono-6 3-lactone) demonstrated the significance of the carboxyl group in fragmentations

β-D-Glucopyranuronic acid (penta-O-trimethylsilyl derivative Table I)

No molecular ion was found in this or any other pyranose spectrum All spectra, however, showed the characteristic M – 15 peak at *m/e* 539, corresponding to the loss of a methyl radical from the molecular ion (Scheme 1)

Many fragments in the spectra were caused by loss of silanol (–90) or the silanyl radical (–89) This loss is common in the mass spectra of carbohydrates, and holds little structural significance The appearance of peaks at *m/e* 464 and 374, however, confirmed the molecular weight of the molecule (554) Similar loss from the ion *m/e* 539 produces the relatively abundant ions *m/e* 449 and 359 All of these fragments have analogues (14 mass units smaller) in the breakdown of the neutral hexoses, due to the hydroxymethyl, rather than the carboxyl, group at C-5

TABLE I

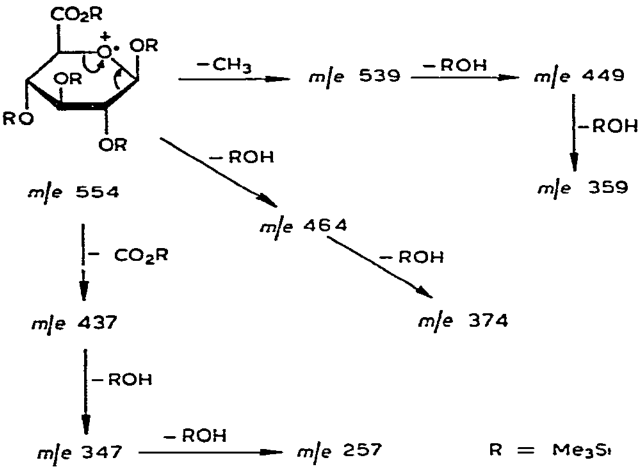
MASS-SPECTRAL DATA FOR THE *O*-TRIMETHYLSILYL DERIVATIVES OF β-D-GLUCOPYRANURONIC ACID (1) AND α-D-GALACTOFURANURONIC ACID (2)

m/e	*Relative intensity* (%)		m/e	*Relative intensity* (%)	
	1	2		1	2
554	—	—	245	2 1	4 0
539	0 6	1 8	243	0 9	0 4
464	0 3	0 03	237	0 06	0 6
449	5 4	0 9	233	8 65	0 4
436	0 06	0 05	232	2 00	0 2
421	0 8	0 8	231	1 00	0 6
408	0 6	0 05	230	0 52	1 0
407	0 2	0 1	217	100	100
393	0 4	0 2	215	0 8	0 7
375	1 5	0 1	204	60 0	4 2
359	2 2	3 1	203	0 8	0 5
347	0 7	1 0	191	23 3	11 8
346	0 3	1 8	189	3 8	2 8
335	2 3	3 3	175	0 6	0 4
333	4 2	0 6	169	0 9	0 5
331	1 4	0 6	157	0 5	0 9
319	0 9	6 5	147	27 9	15 8
317	0 3	0 1	145	0 9	0 4
306	6 5	2 1	143	4 9	1 2
305	23 3	7 0	133	3 8	2 2
292	23 6	24 3	131	1 8	1 2
291	2 2	1 1	129	2 7	2 4
285	0 6	1 3	117	1 4	1 1
279	0 6	0 1	103	2 4	2 7
278	0 3	0 2	95	0 3	2 1
277	0 3	0 2	93	0 4	6 6
265	0 7	0 1	75	9 4	7 3
257	1 4	0 5	73	80 8	58 1

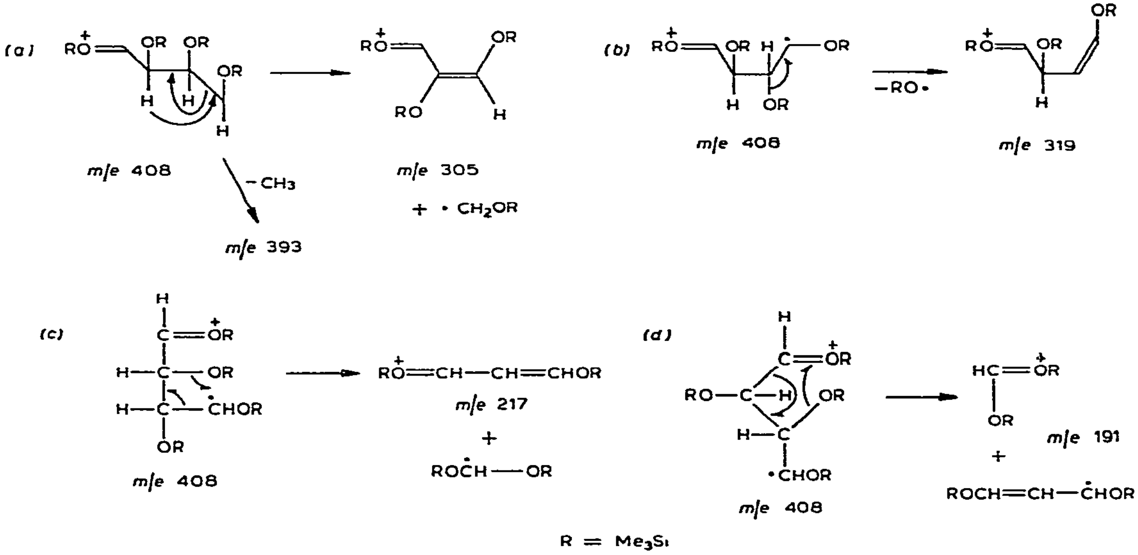
Loss of the C-6 unit produces a series of fragments (*m/e* 437, 436, and 435) common to both hexuronic acid and hexose spectra Sequential loss of silanol produces the fragments *m/e* 347 and 257 Similarly, loss of the C-5,6 unit, to produce the odd-electron ion *m/e* 408, yields a series of common fragments, although their relative abundances are dissimilar The fragment *m/e* 408, only found in very low abundance in the spectra, is a source of the major fragments *m/e* 393, 319, 305 217, and 191 (Scheme 2) However, the presence of the carboxyl group produced shifts of 14 mass units for some of these peaks

The fragment *m/e* 319 is observed in the spectra of the derivatives of the hexuronic acid and of the hexoses, and originates from the C-1–C-4 or C-2–C-5 structures DeJongh *et al* [2] claimed that this fragment contained no C-6 structures In the present studies, however, a significant peak at *m/e* 333 could indicate that at least some of the fragment having *m/e* 319 in the spectra of the hexose derivatives may contain the C-3–C-6 structure (Scheme 3)

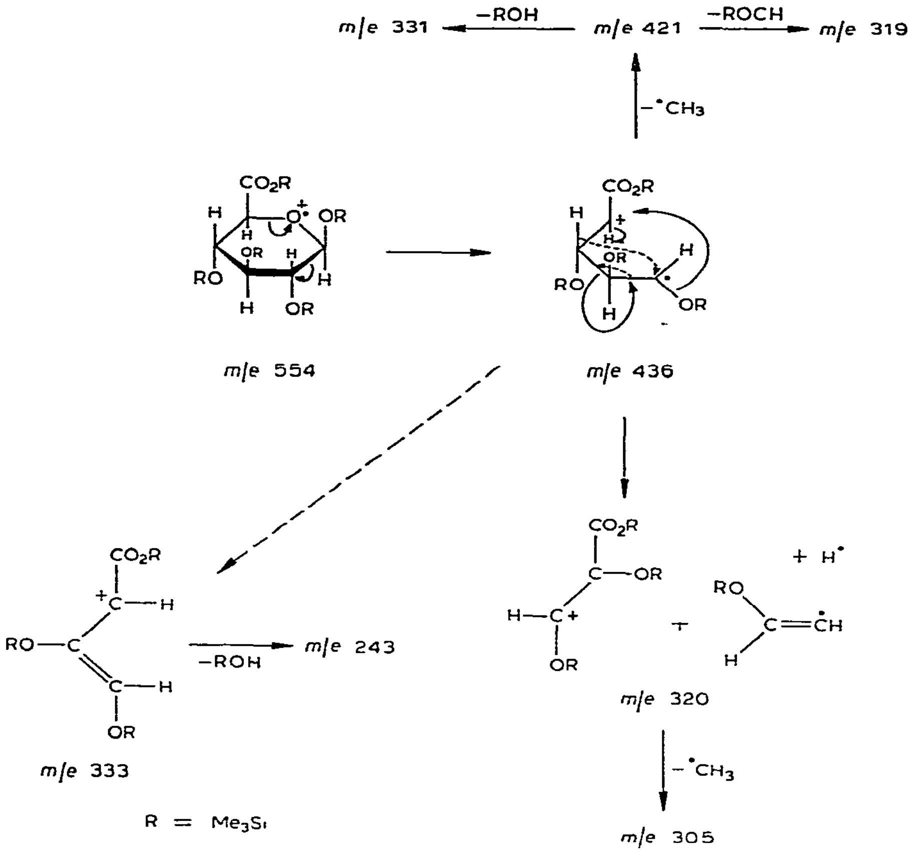
High-resolution measurements of the peak at m/e 305 indicated two components, one corresponding to the C-4–C-6 portion, and the other to that normally found in hexose spectra and arising from the C-2–C-4 and C-3–C-5 portions of the molecule The analogous peak for the C-4–C-6 fragment would be m/e 291 in the hexoses, this peak is found in abundance for hexoses, but is significantly diminished in the hexuronic acid spectra This situation suggests that this fragment can be formed from the C-6 part of the molecule, as well as from other units This contribution to the abundance



Scheme 1 The non-specific fragmentation of the molecular ion in pyranoid structures



Scheme 2 The fragmentation of the m/e 408 ion

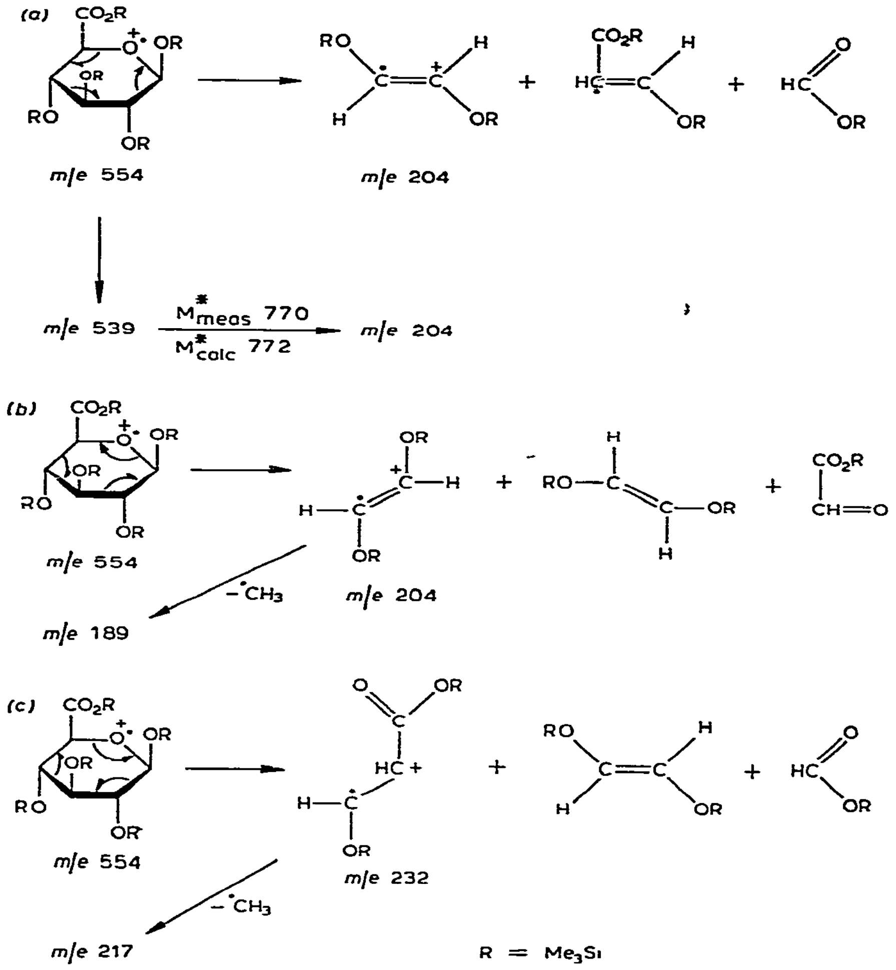


Scheme 3 The fragmentation of the *m/e* 436 ion

of the peak having *m/e* 305 may explain the increased intensity observed in the hexuronic acid spectra (Scheme 3)

The origins of the major fragments *m/e* 204 and 217 are non-specific, production of *m/e* 204 requires a circulation of electrons within the ring which cannot be easily achieved in furanoid structures Also produced in this circulation, but in low abundance, is the ion *m/e* 232 which contains the C-6 structure, and loss of a methyl radical gives rise to the ion *m/e* 217 (Scheme 4) This has a structure different from that of the ion normally produced, and is present as ~ 1% of this peak

The loss of the C-1 unit and O-5, to produce the odd-electron ion *m/e* 436, gives a series of peaks characteristic of the hexuronic acid derivatives Loss of a methyl radical yields the ion *m/e* 421, which subsequently produces the ions *m/e* 331 and 241 by loss of trimethylsilanol (Scheme 3) The major ion *m/e* 245 can also be produced from this parent ion by migration of a trimethylsilyl group from C-4 to C-2 with breakage of the C-2–C-3 bond (Scheme 5) This structure for the ion *m/e* 245 is confirmed by precise mass measurement A major contribution to this ion arises from

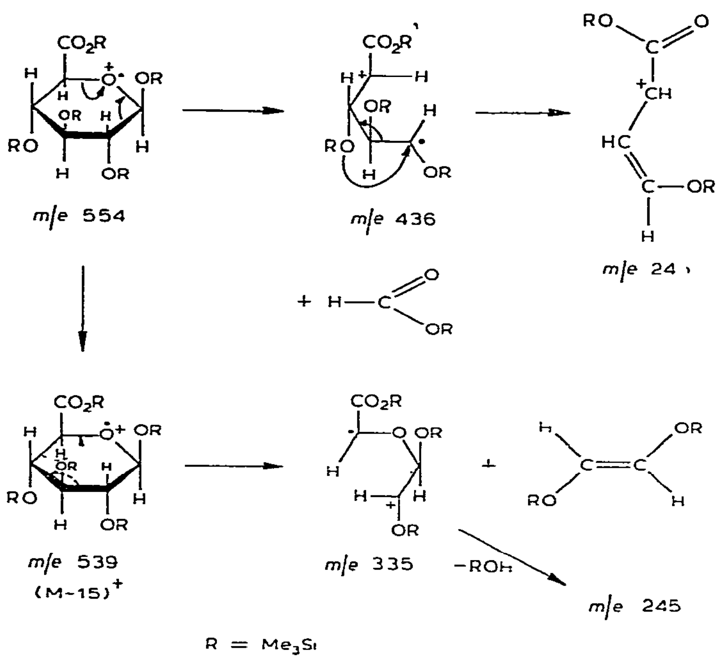


Scheme 4 The formation of the fragments having *m/e* 204 and 232

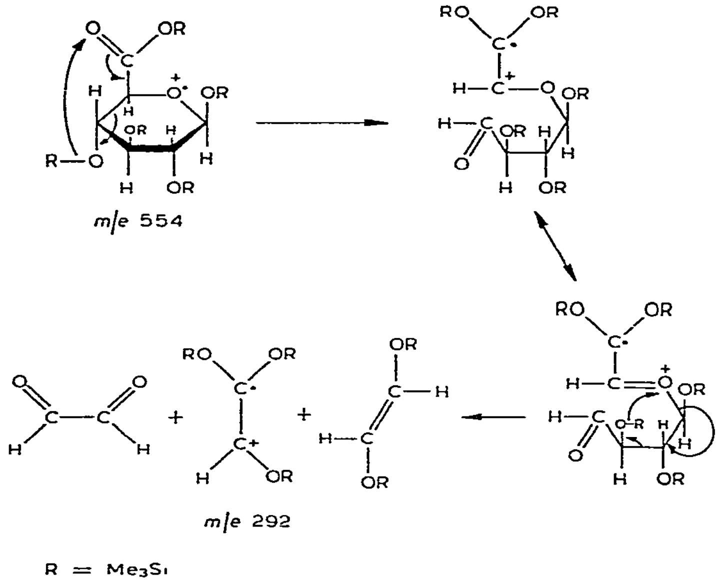
another source, possibly through loss of trimethylsilanol from the ion *m/e* 335 ($C_{12}H_{27}O_5Si_3$ as determined by precise mass measurements) which can originate from the M – 15 ion by loss of C-2–C-3 or C-3–C-4

This group of peaks, *m/e* 331, 333, and 335, although of low abundance, is present at greater intensities than in the hexose spectra Of greater structural significance is the production of the odd-electron ion *m/e* 292 which has been found in the spectra of the trimethylsilyl derivatives of aldonic[10] and aldaric acids[11], following a McLafferty-type rearrangement of a trimethylsilyl group to the carbonyl of the carboxyl group[12] This interpretation cannot provide the whole explanation for such a structure in the case of the hexuronic acid derivatives

Since the *m/e* 292 peak is not found in the mass spectra of the hexose derivatives, its origin seems to be a function of the carboxyl group The presence of a weak, metastable-ion peak at *m/e* 153 8 (calc 153 9) suggests that the origin of the ion



Scheme 5 The formation of the fragment *m/e* 245



Scheme 6 The formation of the fragment *m/e* 292 in pyranoid structures by a McLafferty-type rearrangement of the molecular ion

m/e 292 may be the molecular ion Formation of the *m/e* 292 ion would require migration of a trimethylsilyl group to the carbonyl of the carboxyl group, followed by either a second transfer to the ring oxygen with consequent ring-breakage, or the migration of a trimethylsiloxy group to the C-5 position, again followed by ring breakage (Scheme 6) Since both routes require two rearrangements, it is unlikely that they fully explain the high abundance of this fragment

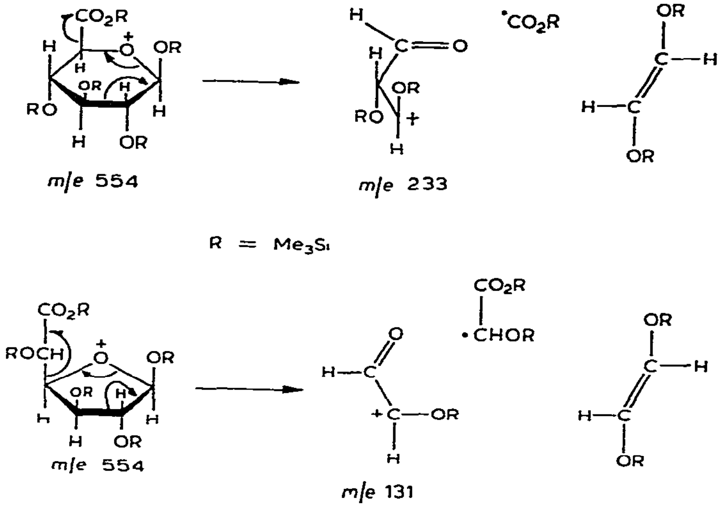
The methyl ester and the methyl ester methyl glycoside of the trimethylsilyl ether of D-galacturonic acid both produced a peak at *m/e* 234 and no fragments at *m/e* 292 or 176 This result demonstrates that the origin of this fragment is the C-5–C-6 portion of the molecule, and that migration, either of trimethylsilyl or trimethylsiloxy groups, was not necessarily from the C-1 position The absence of a peak corresponding to *m/e* 118 in the spectra of the methylated uronic acid tends to support the hypothesis of a McLafferty rearrangement of a trimethylsilyl group[13] Alkyl groups have not been observed to migrate in this way, and hence the methyl groups would "block" the rearrangement However, methoxyl groups should be able to migrate, and the absence of such a migration to produce the ion *m/e* 118 suggests that this route is not important The general occurrence of this fragment (*m/e* 292) throughout the mass spectra of the hexuronic acid derivatives (but not the lactones) is noteworthy

Another series of peaks, not found in the hexose spectra, occurs in the region *m/e* 230–233 The fragment having *m/e* 231, according to precise mass measurements, originates from the C-6 portion of the molecule Its intensity compared to that found for *m/e* 217 was ~1%, thus suggesting that very little of the fragment *m/e* 217 in the hexose spectra arose from the C-4–C-6 region The origin of *m/e* 232 has already been discussed The fragment *m/e* 233 is thought to be formed *via* a breakdown similar to that producing the fragment *m/e* 204 hence its relative importance in the pyranose spectra as compared to the furanose A comparative fragmentation in the furanoid derivative would lead to the fragment *m/e* 131 (Scheme 7) The fragment *m/e* 230 was observed to be the major peak of this group in the furanose spectra, but was of little importance for the pyranose Precise mass measurements indicate two fragments, both of which could have arisen from the loss of a trimethylsilanyl radical from the two proposed forms of the ion *m/e* 319

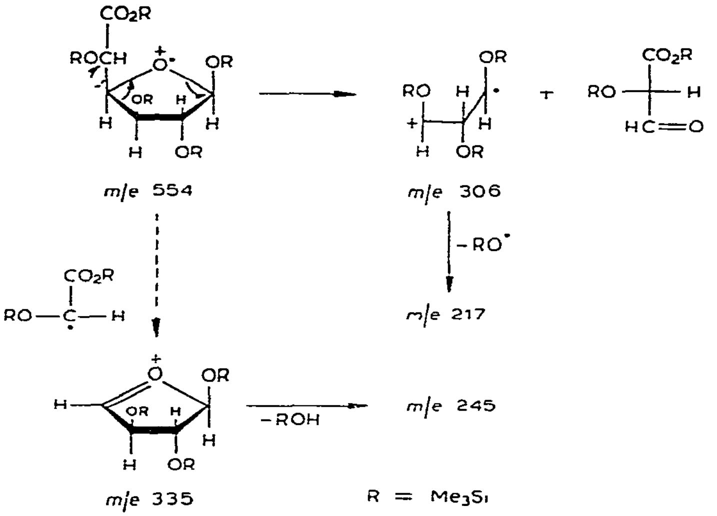
α-D-Galactofuranuronic acid (*Table I*)

Many of the breakdown routes are the same for the furanose as described for the pyranose The inability of the furanose to form easily the fragment *m/e* 408 causes a reduced intensity for peaks originating from this source, such as those having *m/e* 393, 305, and 191 However, the production of *m/e* 306 was facilitated, a peak explained by the higher isotopes of *m/e* 305 in the pyranose spectra Loss of the trimethylsilanyl radical gives rise to the very intense peak having *m/e* 217 (Scheme 8)

An increased production of *m/e* 335 arises from the loss of the C-5–C-6 unit from the molecular ion, which, with a subsequent loss of the trimethylsilanol unit, produces the ion *m/e* 245 The production of *m/e* 204 is significantly less for the

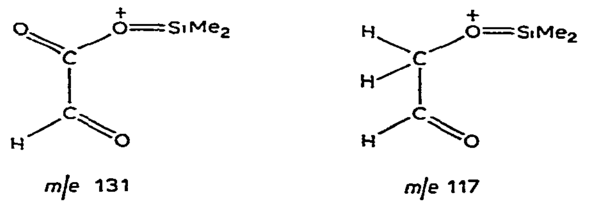


Scheme 7 The comparative production of the fragments *m/e* 233 and 131 in pyranoid and furanoid systems



Scheme 8 Fragmentation of the molecular ion in furanoid structures

furanose than for the pyranose, because of a less favourable circulation of electrons in the ring The products, such as a diradical, are considerably less stable than the pyranoid counterparts and hence this fragmentation is relatively unfavourable Other products from ring circulation of electrons include the ion *m/e* 131, arising from C-3–C-4 For the pyranose, this ion originated from C-5–C-6, supporting the claim of DeJongh *et al* [2] that the ion *m/e* 117 for hexoses was similarly produced (Scheme 9).



Scheme 9 Structures of the fragments *m/e* 131 and 117

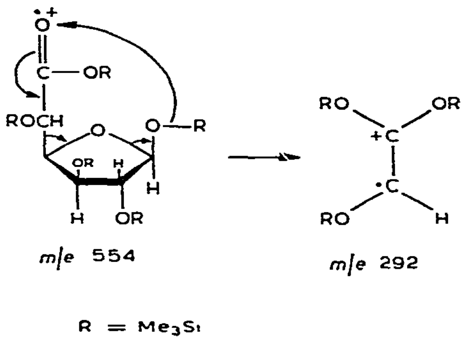
TABLE II

COMPARISON OF ION ABUNDANCE[a] FOR *O*-TRIMETHYLSILYL DERIVATIVES OF ISOMERIC HEXURONIC ACIDS

m/e	D-*Glucuronic acid*		D-*Galacturonic acid*		D-*Mannuronic acid*	L-*Iduronic acid*
	α-p[b]	β-p	α-p	β-p	β p	α,β-p
539	0.17	0.11	0.37	0.40	0.43	0.07
464	0.05	0.05	0.04	0.03	0.09	0.17
449	0.96	0.95	0.44	0.54	0.74	0.38
437	—	0.01	—	0.01	0.01	—
421	0.14	0.15	0.18	0.18	0.17	0.10
408	0.03	0.11	0.03	0.03	0.04	0.04
392	0.02	0.07	—	0.06	—	0.02
347	0.12	0.12	0.13	0.13	0.16	0.18
325	0.38	0.41	0.38	0.39	0.54	0.38
333	0.37	0.74	0.29	0.43	0.86	0.42
319	0.19	0.16	0.34	0.16	0.21	0.30
305	4.61	4.11	3.08	2.78	5.36	1.77
292	4.51	4.16	3.73	3.67	4.92	1.23
291	0.36	0.39	0.28	0.27	0.37	0.30
245	0.49	0.37	0.47	0.36	--	0.33
243	0.15	0.16	0.16	0.21	—	0.21
233	1.59	1.53	0.89	0.98	1.69	0.21
232	0.36	0.35	0.27	0.25	0.39	0.11
231	0.14	0.17	0.15	0.15	0.12	0.22
217	16.60	17.60	13.20	11.70	17.60	7.53
204	11.20	10.60	14.20	13.90	12.20	12.60
191	4.51	4.10	2.67	2.62	3.94	1.42
189	0.59	0.67	0.59	0.59	0.67	1.05

[a] Abundance of each ion measured relative to the total ion abundance in each spectrum [b] *p* = pyranose

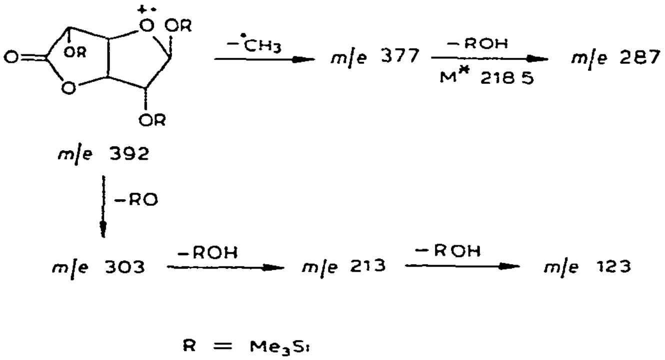
The formation of the ion *m/e* 292 can only have taken place by a McLafferty-type rearrangement of a trimethylsilyl group to the carbonyl group of the carboxyl group through a seven- or eight-membered ring intermediate, the latter being sterically more favourable (Scheme 10) Its formation demonstrates that it is characteristic of the carboxyl group The production of this peak was noted at similar intensities (when measured as a percentage of the total intensity) for each hexuronic acid, with the exception of L-iduronic acid (Table II) This situation arises because, in all the other samples studied, the carboxyl group would have been mainly in the equatorial position (in the pyranose form), whereas it would have been axially orientated in L-iduronic acid.



Scheme 10 The formation of the fragment *m/e* 292 in furanoid structures

β-D-*Glucofuranurono-6,3-lactone* (*Table III*)

In all cases studied, a molecular ion (*m/e* 392) was found together with the M — 15 ion (*m/e* 377) of greater, relative intensity The normal loss of trimethylsilanyl radicals (—89) and trimethylsilanol units produces a series of fragments of little structural importance (Scheme 11)



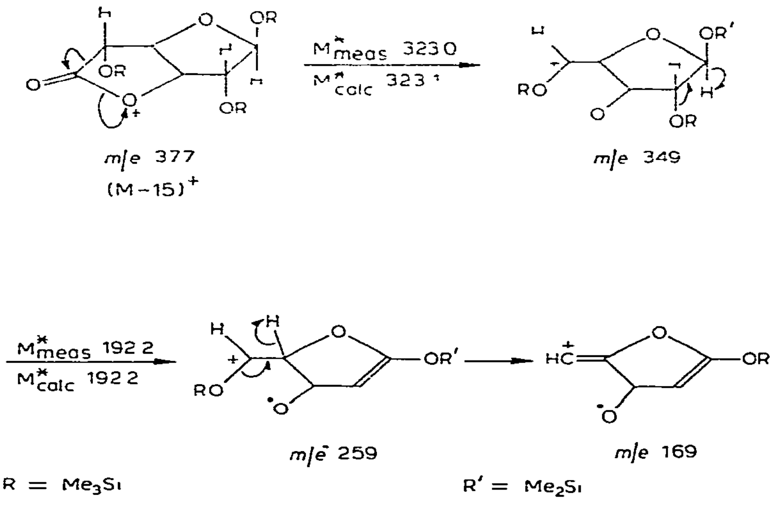
Scheme 11 Non-specific fragmentation of the molecular ion for the lactone structures

Loss of the C-6 unit as carbon monoxide from the M — 15 ion gives rise to the series of ions having *m/e* 349, 259, and 169 (Scheme 12) The fragment *m/e* 348 also arises from the loss of C-6, as carbon dioxide from the molecular ion This odd-electron ion fragments to produce the two major ions of the spectra, namely, *m/e* 230 and 217 Because of the structure of the lactone, the origin of the *m/e* 217 fragment is probably in the C-1–C-3 structure, although its production is not as simple as with the uronic acid derivatives (Scheme 13)

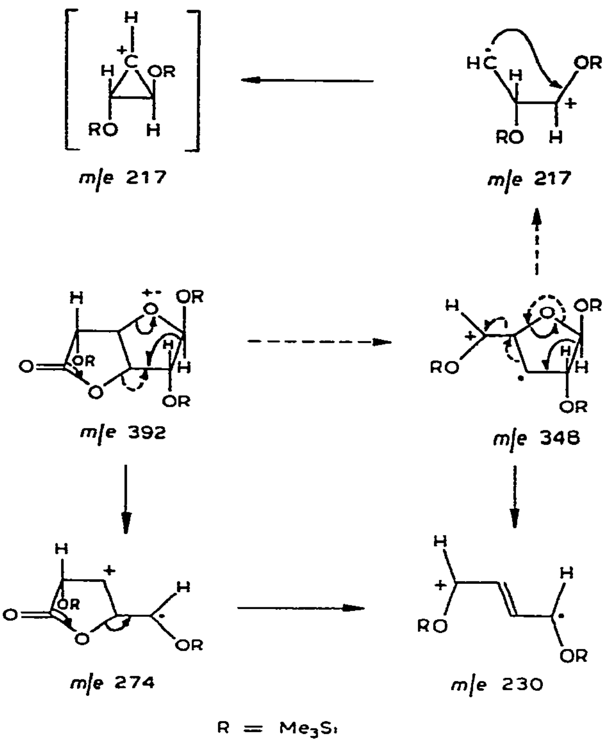
TABLE III

MASS-SPECTRAL DATA FOR THE *O*-TRIMETHYLSILYL DERIVATIVE OF β-D-GLUCOFURANURONO-6,3-LACTONE

m/e	*Relative abundance* (%)	m/e	*Relative abundance* (%)
392	0 4	204	2 05
377	9 1	197	1 4
359	0 4	191	2 1
349	0 4	189	4 3
347	2 6	177	0 6
333	0 7	169	2.2
331	0 3	157	1 5
319	2 4	156	1 0
305	0 2	149	3 4
303	0 2	147	17 8
302	0 2	145	3 1
291	1 2	143	2 0
287	5 7	133	5 1
285	0 4	131	2 6
259	4 4	129	6 5
258	1 0	123	0 7
245	8 2	117	2 5
243	5 3	113	2 2
230	84 0	103	5 6
217	14 1	95	0 3
215	1 7	75	13 3
213	3 7	73	100

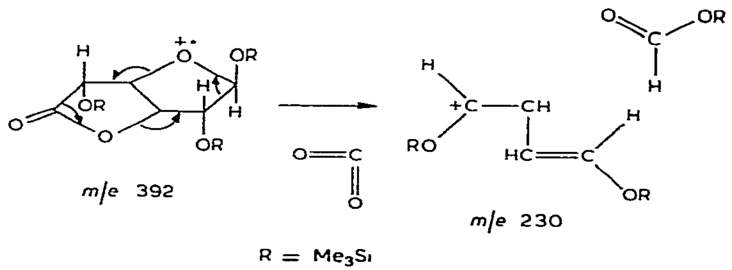


Scheme 12 The formation of the fragment *m/e* 349



Scheme 13 The formation of the fragment *m/e* 230

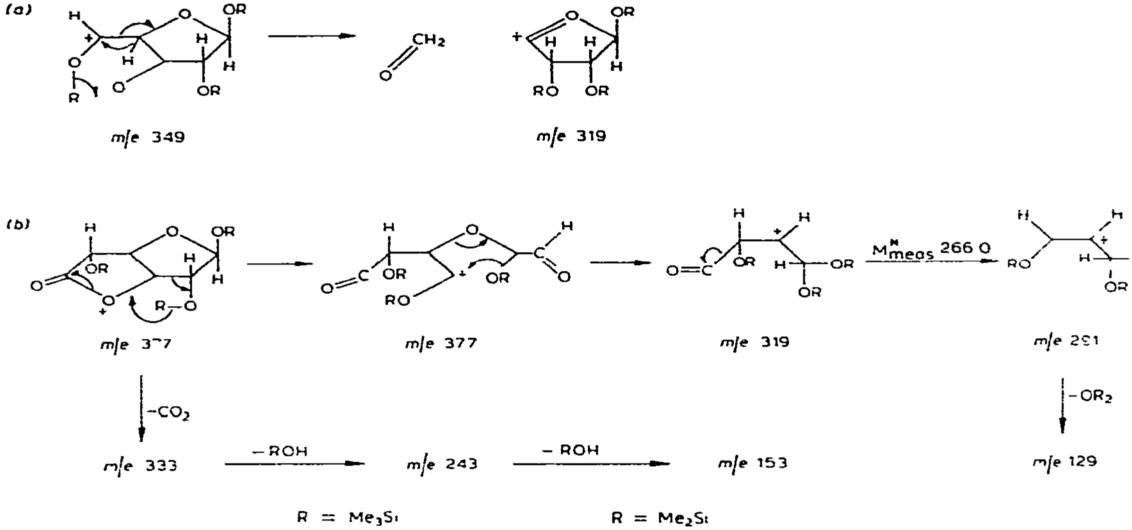
The major ion of the spectra, following the *m/e* 73 ion, is the odd-electron ion having *m/e* 230. A metastable-ion peak at *m/e* 134.8 suggests that its origin lies with the molecular ion. The fragmentation of the molecular ion, *via* a circulation of electrons round the double-ring system, produces two stable, neutral molecules as by-products which may promote this mode of breakdown (Scheme 14). Formation of this ion can also take place through a two-stage fragmentation from the molecular ion, through the fragments having *m/e* 348 and 274 (Scheme 13). Both these ions are found in the mass spectra, and their fragmentation may help explain the great abundance of the



Scheme 14 The formation of the fragment *m/e* 230

m/e 230 ion Its formation, in all of the hexuronolactone spectra studied, is characteristic of these structures Fragmentation of this ion leads to the formation of the ions *m/e* 215 (loss of methyl radical), 157 (loss of trimethylsilyl radical), and 73 (trimethylsilyl radical)

The loss of the C-6 unit as carbon dioxide was the basis of the production of several ions, including *m/e* 333 from (M−15), and *m/e* 243 from (M−15−90) (Scheme 15)



Scheme 15 The formation of the fragment *m/e* 319

The formation of the ion *m/e* 319 was thought to arise from (M−15), through loss of the C-6 unit to give *m/e* 349, followed by a rearrangement of a trimethylsilyl group with the loss of a formaldehyde unit Subsequent loss of carbon monoxide leads to the formation of the ion *m/e* 291 (Scheme 15) The other major peak normally found in the mass spectra of trimethylsilyl derivatives of monosaccharides, namely, the *m/e* 305 fragment, was absent in the spectra of the hexuronolactones

Many of the breakdown pathways are similar to those found by Kováčič and Bauer[14] in their studies of the mass spectra of free D-glucuronolactone Rupture of the two-ring system occurs initially in a similar manner, although the trimethylsilyl derivative undergoes many further characteristic fragmentations

EXPERIMENTAL

Materials — D-Glucurono-6,3-lactone, D-glucuronic acid, D-galacturonic acid, and D-mannurono-6,3-lactone were commercial samples L-Idurono-6,3-lactone was obtained from the 1,2-*O*-isopropylidene derivative (1 mg, kindly provided by

Professor D Horton, Ohio State University, U S A) by hydrolysis with M hydrochloric acid (1 ml) at 100° for 3 h, removal of hydrogen chloride by evaporation *in vacuo* with aqueous sodium hydroxide in the receiver, and evaporation to dryness Sodium L-iduronate was prepared by addition of an equivalent amount of M sodium carbonate to an aqueous solution of L-idurono-6,3-lactone (1 mg/ml) Hexamethyldisilazane and chlorotrimethylsilane, silylation grade, were obtained from Pierce and Warriner (UK) Ltd All solvents were dried and redistilled

Preparation of samples for mass spectrometry — The trimethylsilyl derivatives of the hexuronic acids and their lactones were prepared by the method previously described[8] Hexamethyldisilazane (100 μl) and chlorotrimethylsilane (50 μl) were mixed and a solution of the hexuronic acid in methyl sulphoxide (500 μl) was added The resulting, upper layer was separated, and the remaining silylating reagents and hexamethyldisiloxane were removed by evaporation *in vacuo* at 50–60° The resulting oil was dissolved in hexane and stored ready for analysis

Mass spectrometry was performed with a combined gas-chromatograph (Perkin-Elmer Model 881)-mass spectrometer (AEI MS 902) G l c runs were made with a glass, coiled column (304 cm × 5 mm) of liquid phase OV-1 on Chromosorb oven temperature, 175° carrier gas flow-rate, 40 ml/min All spectra were recorded at 70 eV, with a source temperature of 220° An enhanced metastable-ion spectrum was obtained from the same instrument

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