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MASS SPECTROMETRY OF TRIMETHYLSILYL DERIVATIVES OF GIBBERELLIN GLUCOSIDES AND GLUCOSYL ESTERS

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Abstract—The mass spectra of trimethylsilyl ethers of six gibberellin-β-D-glucopyranosyl ethers and five gibberellin-β-D-glucopyranosyl esters are discussed. The fragmentation patterns are shown to be affected by the structural variations of the aglycones.

INTRODUCTION

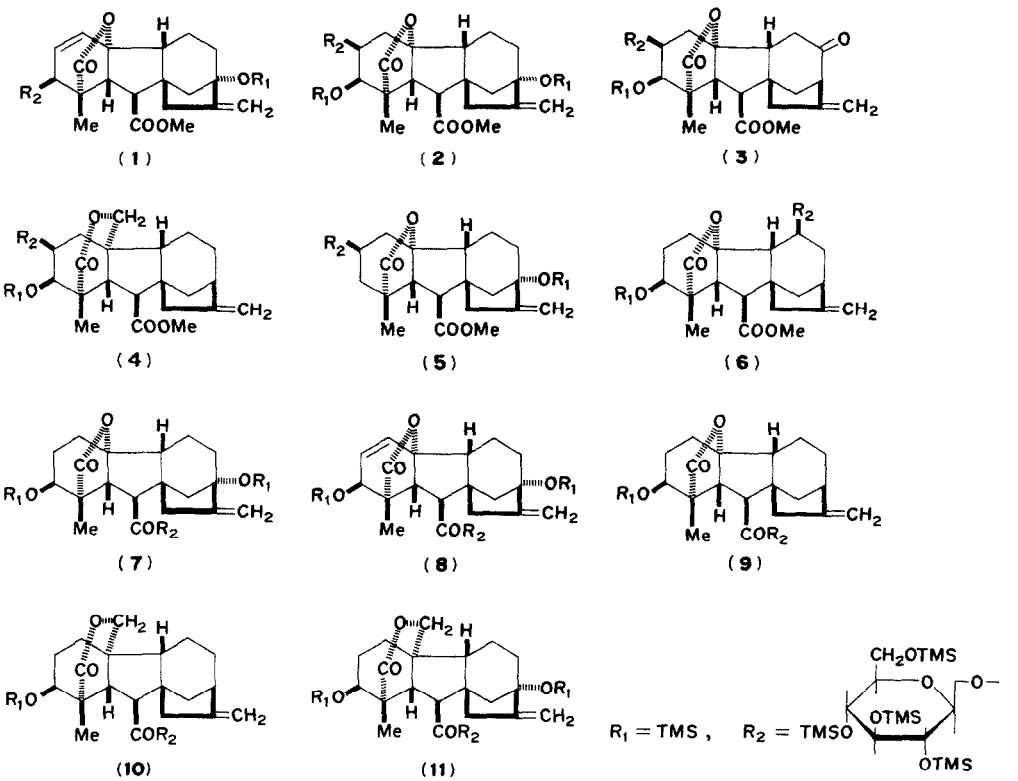
In recent years several conjugates of gibberellins, (*viz.* gibberellin-β-D-glucopyranosyl ethers and gibberellin-β-D-glucopyranosyl esters) have been isolated from various plant sources [1–6]. The MS fragmentation patterns of many sugars and glycosides have been already studied [7–12]. However, most of the compounds so far studied have had rather simple structures. In this paper, we outline the MS of the trimethylsilyl (TMS) derivatives of eleven gibberellin-β-D-glucopyranosides [13]. They show the fragmentation patterns which differ from those reported for other glycosides and were shown to be affected by the structural variations of the aglycones.

RESULTS AND DISCUSSION

The mass spectra of trimethylsilyl ethers of gibberellin-β-D-glucopyranoside methyl esters. The MS of TMS ethers of methyl esters of 3-*O*-β-D-glucopyranosylgibberellin A_3 (**1**), 2-*O*-β-D-glucopyranosylgibberellin A_8 (**2**), 2-*O*-β-D-glucopyranosylgibberellin A_{26} (**3**), 2-*O*-β-D-glucopyranosylgibberellin A_{27} (**4**), 2-*O*-β-D-glucopyranosylgibberellin A_{29} (**5**) and 11-*O*-β-D-glucopyranosylgibberellin A_{35} (**6**) were determined. The mass spectra of **1** is shown in Fig. 1. In these six compounds, the ions derived from glucosyl residue are much more abundant than those retained fragments of the gibberellin. However, the latter are much more informative and the peaks from them are summarized in Table 1 in which the most intense peak is represented as the base peak (100% intensity).

Molecular ion peaks were observed in all spectra. The fragmentation associated with the breakdown of the TMS group gives rise to the ions at M-15 (Me·), M-58 (Me–Si–Me), M-72 ($Me_2SI{=}CH_2$), M-87 (Me· + $Me_2Si{=}CH_2$) and M-105 (Me· + Me_3SiOH). The ion at M-103 arises from loss of CH_2OSiMe_3 group from the glucosyl moiety. This group of ions, including the molecular ion, is more abundant in compounds with a 13-OTMS group (compounds **1**, **2**, **5**) than in those without this group. This is in agreement with the observation that gibberellin methyl esters with a 13-OTMS group give more intense molecular ion peaks than those without such group [15].

Decomposition of the glucopyranosyl moiety gives rise to ions which retain the gibberellin skeleton with or without a certain fragment from the sugar moiety. These ions appear at M-335, M-336,



M-349, M-421, M-422, M-450, M-451, M-467 and M-468.

The ion at M-336, as well as that at M-349, has often been observed in the MS of TMS ethers of glucosides reported so far [7, 8]. This ion, which arizes from cleavage of both the C-2′-C-3′ and the C-1′-ether oxygen bond of glucose, is observed in **1**, **5** and **6** (Table 1). The structure can be shown as **a** (Table 2) where the aglycone is represented as "ROH". The ion at M-335 is possibly formed by rearrangement of a hydrogen atom to the ion **a** and is represented as **b**. It is noteworthy that this ion occurs in **2**, **3** and **4** which contain in commonly both a 2-*O*-glucopyranosyl group and a 3-OTMS group.

The ion at M-349, which was abundantly observed in every compound, is considered to retain C-1′ to which OTMS group rearranged, and to have the structure **c** (Table 2). The ion at M-421 (**c**-72) is observed in **1** (7%), **2** (91%), **3** (100%), **4**

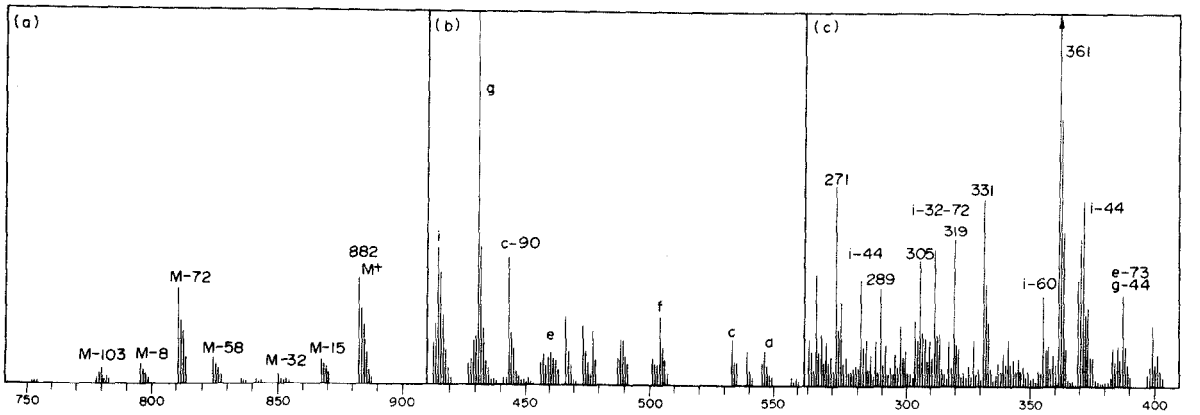


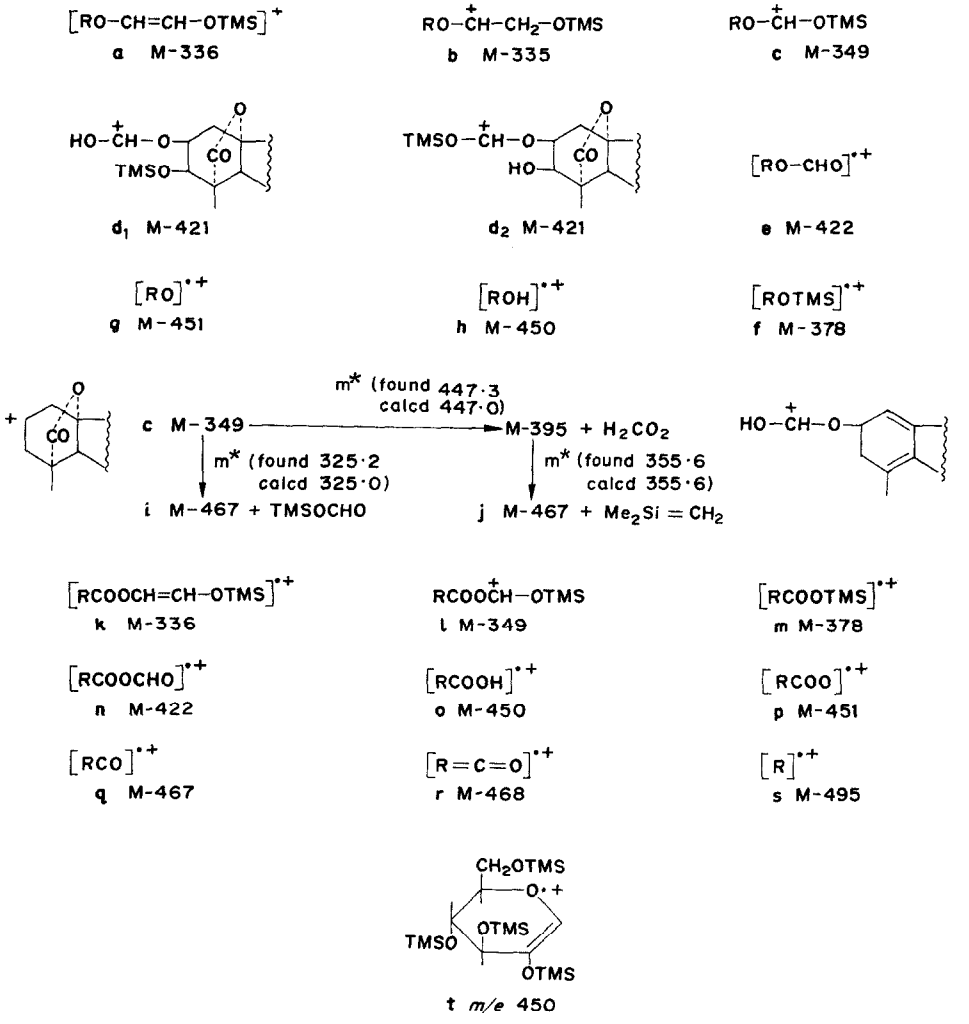
Fig. 1. Mass spectrum of 3-*O*-β-D-glucopyranosyl gibberellin A_3.

Table 1. Relative-intensity data of fragment ions formed in the mass spectra of TMS ethers of gibberellin-β-D-glucopyranosyl ether methyl esters

Mass lost	Compound (MW) 1 (882)	2 (972)	3 (898)	4 (898)	5 (884)	6 (884)
0 (M^+)	28	4	t	1	18	t
15	6	2	t	1	9	1
31		t†			1	1
32	3		t	1		
41	1	t			3	
44					3	
58	6	2			3	
59					2	
72	25	23			6	
87	5	7	1	3	3	1
103	4	1	4	3		1
105	2	1	1	1	7	1
335		1	12	8		5
336	8				8	5
349	11	57	29	52	93	100
378	18	t	t	10	t	43
381	6	22			38	3
393	12	18	18	10		9
394	12	16	7	6		10
395	7	13	7	2	55	19
408		27	18			
409	15	18	4	2	11	
421	7	91	100	100	13	6
422	8	28			30	
425	8	17	12			4
427					16	7
437				3	14	7
439	34	35	19		26	21
450		16	11	16		2
451	100		6	3	13	3
453	10	25				5
455					17	6
467	36	50	26	27	100	74
468	18	9		5	13	16
481	8	17				
483	16		7	7	15	
485	4	23	10	3	12	35
494		41				
495	24			5	11	
497	10			5		
499	10	19	7	4	35	13
511	50	100	48	69	68	8
512	40	10	6	2	16	12
513	28	15	8	2	25	25
523	10	25			25*	90*
524				17		
525	12	14	8	7	10	
527	24	15	10	6	29	10
529			11		7	5
531	12					
539	8	42	12	54	20	7
543	8	15	6	5		7
545				3		12
555	12	25	6	5	10	
557	6	26	12	10	41	27
571	38	34	23	19	30	8
583		20			6	
585	16	18	19	14	16	7
599		30	17	27	16	13
601	28	59	32		27	65
615		24		57	16	

* These ions contain the ion (*m/e* 361) arising from glucosyl group.
† t: tracc.

Table 2. Ions formed from the MS fragmentation of gibberellin *O*-glucosides and glucose esters



(100%), **5** (13%) and **6** (6%). This ion is very abundant in compounds **2**, **3** and **4** and is possibly represented by $\mathbf{d}_1$ or $\mathbf{d}_2$ (Table 2); the vicinal glycol system in ring A seems to stabilize the positive charge and perhaps explains the fact that the ion is of low intensity in compounds **1**, **5** and **6** which do not contain this grouping.

In addition to M-421, an ion at M-422 (**c**-73) is observed in **1** (8%), **2** (28%) and **5** (30%), its structure being represented as **e** which retains C-1′ as a formyloxy group. It is also possible that this ion is formed from the ion at M-378 with loss of CO_2. The latter ion (M-378) may arise from cleavage of the glucosidic linkage and rearrangement of O-TMS (or TMS) group. The structure **f** may correspond to TMS ether of aglycone. This ion is observed in **1** (18%), **4** (10%) and **6** (43%) but is not clearly seen in the spectra of the other compounds.

The ion at M-451, arising from elimination of glucopyranosyl group has the structure **g**, being observed in **1** (100%) and **5** (13%). The rearrangement of a hydrogen atom to the ion **g** gives rise to the ion **h** at M-450 which is observed in **2** (16%), **3** (11%), **4** (16%) and **6** (2%). The abundant ion at M-467 is found to be a mixture of the ion **i** and **j** based on calculation of the metastable ions in **5**. The ions **i** and **j** arise from the ion **c** (M-378) with loss of TMS formate and of H_2CO_2 and $Me_2Si{=}CH_2$ respectively (Table 2). Since the TMS ether of methyl gibberellenate glucopyranosyl eth-

er [17] also gives rise to an abundant ion at M-467 and in this process only the elimination of TMS-OCHO is possible, it seems likely that this type of ion is also rather predominant in other glucosides.

The fragments described above subsequently decompose with loss of mass units 15 (Me·), 18 (H_2O), 31:32 (MeO–MeOH), 44:46 (CO_2–H_2CO_2), 59:60 (MeOCO·–MeOCHO), 72:73 ($Me_2Si{=}CH_2$–Me_3Si·), 90 (TMSOH) and their combination to give rise to the following ions: M-393 (**c**-44 or **f**-15), M-395 (**c**-46 or **b**-60), M-408 (**c**-59 or **b**-73), M-409 (**c**-60, **f**-31 or **a**-73), M-439 (**c**-90), M-483 (**g**-32), M-485 (**c**-46-90), M-495 (**g**-44 or **e**-73), M-499 (**i,j**-32), M-511 (**i**-44 or **g**-60), M-512 (**h**-18-44), M-513 (**i,j**-46), M-523 (**h**-73 or **g**-72), M-525 (**d**-44-60), M-527 (**i,j**-60), M-539 (**i,j**-72), M-557 (**i,j**-90), M-571 (**i,j**-32-72), M-585 (**i,j**-46-72), M-599 (**i,j**-60-72) and M-601 (**i,j**-18-44-72).

It should be noted that among these ions, the one at M-511 is very abundant in compounds containing glucosyl moiety in the ring A, i.e. **1** (50%), **2** (100%), **3** (48%), **4** (69%) and **5** (68%), whereas it is very low (8%) in **6** which has the glucopyranosyl moiety in ring C (Tables 1 and 2). This ion (M-511) may be derived from the ion **i** by loss of CO_2, and the elimination of CO_2 seems to be enhanced by the positive charge of ring A which is in turn stabilized by the resulting double bond.

The fragments due to the decomposition of glucopyranosyl group appear in every compound at *m/e* 361, 332, 331, 319, 305, 289, 271, 217, 204, 191 and so on. Since their origin and structures have been adequately discussed previously [7], they are not referred to here.

The mass spectra of trimethylsilyl ethers of gibberellin-β-D-glucopyranosyl esters. Mass spectra of TMS ethers of gibberellin A_1-β-D-glucopyranosyl ester (**7**), gibberellin A_3-β-D-glucopyranosyl ester (**8**), gibberellin A_4-β-D-glucopyranosyl ester (**9**), gibberellin A_{37}-β-D-glucopyranosyl ester (**10**), gibberellin A_{38}-β-D-glucopyranosyl ester (**11**) were next examined. The spectra of these compounds are summarized in Table 3 in the same way as before.

Molecular ion peaks are observed in all compounds. The decomposition of TMS group gives rise to ions at M-15, M-72, M-88, M-90, M-103, M-104 and M-144:145. But these ions are weak in every compound and of little practical value. The ions retaining gibberellin moiety, with or without a certain fragment from the sugar, are much more abundant and informative. Their structures were deduced in the same way as for the case of the glucopyranosides: here the aglycone is represented as "RCOOH".

Table 3. Relative-intensity data of fragment ions formed in the mass spectra of TMS ethers of gibberellin-β-D-glucopyranosyl esters

Mass lost	Compound (MW) **7** (942)	**8** (940)	**9** (854)	**10** (868)	**11** (956)
0 (M^+)	11	9	3	1	2
15	3	4	2	6	2
72	9	13	2		2
87	5	8	4	2	1
90	3	5	4	1	
103	1			2	
105	1	2	7	4	2
144	1	2		8	6
145		3	1	5	5
336	10	25	8	1	4
349			3	2	3
378	100	100	100	100	100
393	8	14	22	7	14
395	10	10	42	7	11
422	7	8	15	19	14
423	6	9	24		
450	50	90	8	10	33
451	13	21	5	3	8
465	19	27	12	5	34
466	28	27	26	9	62
467	20	25	72	28	34
468	11	15	45	6	
479		7			6
483	4	6		1	
485	4		14		
495	33	60	30	8	32
513	7	17	26	2	3
527	9	8	3		
539	5	27	14	5	20
540	3	8	18	8	18
557	5	18	8		3
567	3	6	10		8
585	8	25	44	17	35

Two ion peaks at M-335 (**b**) and M-421 (**d**) which appear in the glucopyranosyl ethers are scarcely observed in the esters, whereas those at M-336 (**k**) and M-422 (**n**) appear with moderate intensities in all cases. The intensity of the ion at M-349 (**l**) is very low in **9** (3%), **10** (2%) and **11** (3%), and not observed in **7** and **8**, (Table 3) although it is very abundant in all the glucopyranosyl ethers (see Table 1). On the other hand, the ion at M-378 (**m**) is the most abundant (100%) in every ester, while it is rather low in TMS ethers of glucopyranosides.

Its structure corresponds to the TMS ester of the aglycone. This characteristic fragment is useful therefore, not only to differentiate glucose esters from *O*-glucosides but also to determine the molecular weight of the aglycone.

The abundant ion at M-450 (**o**) is due to the carboxylic acid which arises from elimination of the glucopyranosyl group and rearrangement of hydrogen while the ion at M-451 is due to the carboxylate ion (**p**).

The ions at M-467 (**q**)/468 (**r**) correspond to the ions at M-31:32, while the abundant ion at M-495 (**s**) corresponds to that at M-59 observed in the MS of gibberellin methyl esters and their TMS ethers.

It should be noted that the features listed above are more or less common to all the glucopyranosyl esters examined, while in the case of *O*-glucopyranosides, some variations of fragmentation patterns were observed. This may be due to the fact that in the esters the glucopyranosyl group is attached exclusively to the 6-carboxyl group whereas in *O*-glucosides the position of the glucopyranosyl group is variable.

Other abundant ions arising from the esters are derived by loss of CO_2, H_2CO_2, $Me_3Si\cdot$ and $Me_2Si{=}CH_2$ from the ions **l**-**s**; they are observed at M-393 (**l**-44 or **m**-15), M-395 (**l**-46), M-423 (**k**-15-72), M-465 (**o**-15), M-466 (**n**-44), M-513 (**q**-46), M-539 (**q**-72 or **s**-44), M-540 (**r**-72), M-557 (**q**-90), M-567 (**s**-72), M-585 (**s**-90, **q**-46-72 or **q**-28-90).

The fragment ions associated with the glucosyl group are observed at *m/e* 450 (**t**), 435 (450–15), 378 (450–72), 361, 332, 331, 319, 305, 271, 217, 204, 191 and so on in each compound. The first three ions are not observed in glucopyranosyl ethers, and are thus characteristic of glucopyranosyl esters.

EXPERIMENTAL

The mass spectra were determined using the direct inlet system of an Hitachi RMU-6L mass spectrometer at an ionizing potential of 70 eV and a sample vapourizing temp of 140–170°.

Natural gibberellin-β-D-glucopyranosyl ethers (glucosides) were used: the glucosides of gibberellin A_3, A_8, A_{26}, A_{27} and A_{29} were isolated from *Pharbitis nil* [1] and that of gibberellin A_{35} from *Cytisus scoparius* [2]. They were methylated with CH_2N_2 before trimethylsilylation. β-D-Glucopyranosyl esters of gibberellin A_1, A_3, A_4 and A_{37} were synthesized as reported by Hiraga *et al.* [6] and the ester of gibberellin A_{38} was one isolated from *Phaseolus vulgaris* [5]. These glucose esters were trimethylsilylated directly in a sample probe filled with quartz fibre using *N,O-bis*(trimethylsilyl)acetamide, TMCS and C_5H_5N in the usual manner. After 2 min reaction excess reagent in the probe was evaporated for 20 min at 25° by diffusion pump and the probe inserted into the sample chamber.

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