Positive and Negative Ion Mass Spectra of Flavonoid Glycosides by Fast Atom Bombardment

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(Received May 18, 1987)

Flavonoid glycosides (22 compounds) were measured by fast atom bombardment, and the positive and negative ion mass spectra were compared. The mass spectra showed clear molecular ion species. Fast atom bombardment mass spectrometry provided useful information for the structural elucidation of flavonoid glycosides.

Positive ion spectra in particular were useful for flavonol 3,7-*O*-diglycosides, while the negative ion spectra were useful for flavonoid glycoside methyl ether.

1. Introduction

In previous papers, we have described the structure elucidation of flavonoid glycosides¹⁾ by electron ionization(EI) mass spectrometry and the scope and limitation of a new technique of fast atom bombardment (FAB) mass spectrometry for the characterization of chlorogenic acids.²⁾

This paper describes positive and negative ion FAB mass spectra for the structural elucidation of flavonoid glycosides, such as flavonoid monoglycosides, flavonol 3,7-*O*-diglycosides, flavonoid *O*-diglycosides, and flavonoid *O*-triglycosides (Table I).³⁾

2. Experimental

Mass spectrometry——The FAB spectra were recorded using a mass spectrometer designed according to the specifications of Dr.R. Rhyage and Dr.Margith Jansson from the Laboratory of Mass Spectrometry at the Karolinska Institute in Stockholm, with Shimadzu GC/MS DF-6020. The collision gas used was Ar or Xe (ion gun condition : 7 kV or 6 kV) and a matrix used was glycerol or thioglycerol.

Materials(Table I)——Compound 1, 3, 4, 5, 6, 7, 8, 12, 13, 14, 15, 16, 20, 21, and 22 were isolated from the leaves of *Trachlospermum asiaticum* var. *intermedium*,^{8,9)} *Cerbera manghas*,^{10,11)} *Apocynum venetum* var. *basikurumon*,¹²⁾ *Ochirosia nakaiana*,¹³⁾ *Wagneriopteris formosa*¹⁴⁾ and *Vinca*

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major.¹⁵⁾ Compounds 2, 9, 10, 11, 17, and 19 were supplied by Prof. J.Sakakibara, Dr.M. Ogawa, Dr.Y.Tokuoka and Prof. N.Morita. Samples were prepared by dissolving the flavonoid glycosides(200-300 μ g) in CH₃OH and then mixing the solutions with glycerol or thioglycerol (1 μ l) on the target. Since stable ion currents were obtained for several minutes, in a number of cases, both positive and negative ion mass spectra were recorded for each sample. The mass spectrometer was scanned over a mass range of 5-800(m/z). Background peaks due to glycerol ions were subtracted from measured spectra.

3. Results and Discussion

Positive ion FAB spectra of mono-glycosides, compounds 1-11, showed significant peaks of protonated molecule $[M+H]^+$ as shown in Table II. The $[M+H]^+$ ion peaks were observed at m/z 433 for the compound 1; m/z 447 for 2; m/z 449 for 3, 4, 5 and 8; m/z 465 for 6, 7 and 9; m/z 547 for 10(quercetin 7-*O*-(6''-*O*-tigloyl)glucoside); and m/z 467 for 11, respectively. In the EI spectra of compounds 1-11, in contrast, molecular ions were not observed, because they are thermolabile and involatile. In the positive ion spectra, the relative intensities of $[M+$

Table I. Flavonoid *O*-glycosides Examined

No.	Compounds
Monoglycosides	
1.	apigenin 7- <i>O</i> -glucoside(cosmosiin)
2.	4- <i>O</i> -methylapigenin 7- <i>O</i> -glucoside(tilianin)
3.	luteolin 7- <i>O</i> -glucoside
4.	luteolin 4'- <i>O</i> -glucoside
5.	kaempferol 3- <i>O</i> -glucoside(astragalin)
6.	quercetin 3- <i>O</i> -glucoside(isoquercitrin)
7.	quercetin 3- <i>O</i> -galactoside(hyperoside)
8.	quercetin 3- <i>O</i> -rhamnoside(quercitrin)
9.	myricetin 3- <i>O</i> -rhamnoside(myricitrin)
10.	quercetin 7- <i>O</i> -(6''- <i>O</i> -tigloyl)glucoside(eudiposide)
11.	hesperetin 7- <i>O</i> -glucoside
Diglycosides	
12.	apigenin 7- <i>O</i> -neohesperidoside(rhoifolin)
13.	apigenin 7- <i>O</i> -gentiobioside
14.	luteolin 7- <i>O</i> -gentiobioside
15.	kaempferol 3- <i>O</i> -hesperidoside(nicotiflorin)
16.	quercetin 3- <i>O</i> -hesperidoside(rutin)
17.	quercetin 3- <i>O</i> -glucoside 7- <i>O</i> -rhamnoside(antoside)
18.	kaempferol 3- <i>O</i> -arabinoside 7- <i>O</i> -rhamnoside
19.	4'- <i>O</i> -methyl-8- γ , γ -dimethylallylkaempferol 3- <i>O</i> -rhamnoside-7- <i>O</i> -glucoside
Triglycosides	
20.	kaempferol 3- <i>O</i> -(2 ^c - <i>O</i> -rhamnosyl)hesperidoside(clitorin)
21.	kaempferol 3- <i>O</i> -robinobioside 7- <i>O</i> -rhamnoside(robinin)
22.	kaempferol 3- <i>O</i> -robinobioside 7- <i>O</i> -glucoside

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Table II. Main Positive Ions in FAB Mass Spectra of Flavonoid Glycosides. A : Aglycone.

Monoglycosides											
Compounds No. <i>m/z</i> (%)	1	2	3	4	5	6	7	8	9	10	11
[M+H] ⁺	433 (45)	447 (47)	449 (52)	449 (51)	449 (35)	465 (7)	465 (40)	449 (36)	465 (27)	547 (3)	467 (31)
[M] ⁻	432 (10)	446 (21)	448 (19)	448 (12)	448 (3)	464 (2)	464 (3)	448 (22)	464 (5)	546 (1)	466 (7)
[M+H-O] ⁻	417 (5)	431 (3)	433 (5)	433 (12)	433 (2)	449 (1)	449 (11)	433 (16)	449 (9)	531 (---)	451 (9)
[A+H] ⁻	271 (100)	285 (100)	287 (100)	287 (100)	287 (100)	303 (100)	303 (100)	303 (100)	319 (100)	303 (100)	303 (100)
[A+H-O]	255 (58)	269 (7)	271 (20)	271 (82)	271 (11)	287 (29)	287 (55)	287 (47)	303 (21)	287 (13)	287 (45)

Table III. Main Positive Ions in FAB Mass Spectra of Flavonoid Glycosides.

A : Aglycone, S_t : Terminal sugar, S_a : Sugar of C-3, S_b : Sugar of C-7.

Disaccharides									Triglycosides		
Compounds No. <i>m/z</i> (%)	12	13	14	15	16	17	18	19	20	21	22
[M+H] ⁺	579 (9)	595 (6)	611 (16)	595 (10)	611 (8)	611 (2)	565 (16)	677 (3)	741 (19)	741 (10)	757 (4)
[M] ⁻	578 (4)	594 (3)	610 (7)	594 (6)	610 (6)	610 (-)	564 (-)	676 (-)	740 (10)	740 (5)	756 (1)
[M+H-O] ⁻	563 (1)	579 (3)	595 (7)	579 (1)	595 (2)	595 (-)	549 (-)	---	725 (3)	---	
[M+H-S _t] ⁺	433 (2)	433 (6)	449 (13)	449 (7)	465 (6)				595 (14)		
[M+H-S _t -O] ⁺									579 (5)		
									449 (15)		
									433 (6)		
[M+H-S _a] ⁺						449 (6)	433 (22)	531 (10)		433 (22)	449 (16)
[M+H-S _b] ⁺						465 (2)	419 (4)	515 (2)		595 (7)	611 (3)
[A+H] ⁺	271 (100)	271 (100)	287 (100)	287 (100)	303 (100)	303 (100)	287 (100)	369 (100)	287 (100)	287 (100)	287 (100)
[A+H-O] ⁺	255 (21)	269 (57)	271 (68)	271 (19)	287 (36)	287 (47)	271 (15)	353 (26)	271 (33)	271 (33)	271 (20)

$\text{H}]^+$ peaks of all compounds were more than 20%, except for 6 and 10 (3.8% and 7.4%, respectively). Aglycone moieties, which are protonated aglycones $[\text{A}+\text{H}]^+$, were observed at m/z 271 for 1; m/z 285 for 2; m/z 287 for 3, 4 and 5; m/z 303 for 6, 7, 8, 10 and 11; and m/z 319 for 11, respectively, as the base peak. All these peaks were characteristic of monoglycosides. The positive ion spectra, however, did not show intense peaks indicating the presence of fragment ions formed by the cleavage of aglycone and sugar moieties. Fragment ions whose mass numbers were corresponding to the loss of an oxygen atom from $[\text{M}+\text{H}]^+$ ion were detected at m/z 417 for 1; m/z 431 for 2; m/z 433 for 3, 4, 5 and 8; m/z 449 for 6, 7 and 9; m/z 531 for 10, and m/z 451 for 11, respectively. The compound 15 was measured by high resolution mass spectrometry and such peak was assumed to be $[\text{M}+\text{H}-\text{O}]^+$, although the difference between the observed value and the calculated value was a little too large. (High Resolution Mass Calcd. for $\text{C}_{27}\text{H}_{31}\text{O}_{14}$: 579.1712. Found : 579.1886). Fragment peaks corresponding to the loss of an oxygen atom from $[\text{A}+\text{H}]^+$ ion were observed at m/z 255 for 1; m/z 269 for 2; m/z 271 for 3, 4 and 5; m/z 287 for 6, 7, 8 and 10; and m/z 303 for 9 and 11, respectively.

Positive ion spectra of diglycosides (Table III), compounds 12, 13, 14, 15, 16, 17, 18 and 19, showed protonated molecule $[\text{M}+\text{H}]^+$, as significant peaks at m/z 579 for 12; m/z 595 for 13 and 15; m/z 611 for 14, 16 and 17; and m/z 565 for 18, respectively. The $[\text{A}+\text{H}]^+$ ions were observed at m/z 271 for 12 and 13; m/z 287 for 14 and 15; m/z 303 for 16, respectively, as the base peak. Fragment ions due to the loss of terminal sugar moiety

from $[\text{M}+\text{H}]^+$ ion appeared as significant peaks at m/z 433 for 12 and 13; m/z 449 for 14 and 15; and m/z 465 for 16, respectively. In these cases, mass numbers of terminal sugars were determined by measuring the eliminated mass units. This calculation provides useful information for the structural elucidation of the compounds.

In particular, the positive ion spectra of flavonol 3,7-*O*-diglycosides, 17, 18 and 19 (Table III) exhibited fragment ions due to the loss of terminal sugar of glycosides, as significant peaks at m/z 499 and 465 for 17; m/z 433 and 419 for 18; and m/z 531 and 515 for 19, respectively. The relative peak intensities at m/z 449 of 17; m/z 433 of 18; and m/z 531 of 19, formed by the loss of sugar (S_a) at C-3, were higher than those of the fragment ions due to the loss of sugar (S_b) at C-7, which were m/z 465 for 17; m/z 419 for 18; and m/z 515 for 19, respectively, (Fig.1). The results indicate that the loss of sugar at C-3 may occur in preference compared to the loss at C-7. The C-3 position is assumed to be activated by the adjacent carbonyl group at C-4. Similar results were also observed on the acidic hydrolysis of flavonol 3,7-*O*-diglycosides, and in EI spectra of permethylated or pertrimethylsilylated flavonol 3,7-*O*-diglycosides.⁴⁻⁷⁾ The relative intensities of those ions suggest that there is the specific attachment of the sugar of flavonol 3,7-*O*-diglycosides, if the compound has two sugars of different masses in a molecule. The results are useful for structure determination of unmodified flavonol 3,7-*O*-diglycosides.

Positive ion spectra of flavonol 3-*O*-triglycosides and 3,7-*O*-triglycosides, compounds 20, 21 and 22 showed the protonated

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Fig. 1. Positive Ion Spectra of Flavonol 3, 7-*O*-glycosides.

(17 : antoside, 18 : kaempferol 3-*O*-arabinoside 7-*O*-rhamnoside, 19 : 4'-*O*-methyl-8- γ , γ -dimethylallylkaempferol 3-*O*-rhamnoside 7-*O*-glucoside)

molecule $[M+H]^+$ peaks at m/z 741 and 757 with the relative intensity of about 3%. This indicate that the FAB mass spectrometry is useful for the determination of molecular weight of thermolabile and involatile flavonoid oligosaccharides. Aglycone ions appeared as the base peak at m/z 287, and fragment ions due to the loss of sugar from the $[M+H]^+$ ions showed peaks at m/z 595 and 449 for **20**; m/z 595 and 433 for **21**; and m/z 611 and 449 for **22**, respectively. All these results were similar to those found in the case of flavonoid monoglycosides and diglycosides (compounds **1-19**). In particular, the relative intensities of m/z 433 of **21** and m/z 449 of **22** were higher than

those of m/z 595 of **21** and m/z 611 of **22**.

Negative ion FAB spectra of the compounds **1-11** (Table IV) showed significant peaks due to the elimination of a proton from the molecule. In all cases examined, we also observed $[A-H]^-$ ions as the base peak corresponding to the loss of a proton from aglycone moiety and the background ions of relative intensities lower than those of positive ions (Fig. 2). These features were characteristic for the negative ion FAB spectra. The spectra also indicate the presence of fragment ions due to the elimination of an oxygen atom from $[A-H]^-$ and $[M-H]^-$ ions, respectively. Those peaks were observed at m/z 253 for **1**; m/z 269 for **3**, **4** and **5**;

Table IV. Main Negative Ions in FAB Mass Spectra of Flavonoid Glycosides.

A : Aglycone, T : Tigloyl,

Monoglycosides											
Compounds No. $m/z(\%)$	1	2	3	4	5	6	7	8	9	10	11
$[M]^-$	432 (24)	446 (10)	448 (23)	448 (15)	448 (53)	464 (50)	464 (45)	448 (38)	464 (48)	546 (31)	466 (29)
$[M-H]^-$	431 (32)	445 (15)	447 (35)	447 (30)	447 (88)	463 (86)	463 (71)	447 (76)	463 (92)	545 (34)	465 (68)
$[M-H-CH_3]^-$		430 (14)									450 (11)
$[M-H-O]^-$	415 (5)	429 (5)	431 (11)	431 (6)	431 (6)	447 (16)	447 (16)	431 (10)	447 (14)	529 (3)	449 (20)
$[M-H-T]^-$										463 (2)	
$[M-H-T-O]^-$										447 (1)	
$[A-H]^-$	269 (100)	283 (100)	285 (100)	285 (100)	285 (100)	301 (100)	301 (100)	301 (100)	317 (100)	301 (100)	301 (100)
$[A-H-CH_3]^-$		268 (36)									286 (28)
$[A-H-O]^-$	253 (41)	267 (29)	269 (76)	269 (32)	269 (28)	285 (46)	285 (64)	285 (48)	301 (42)	285 (15)	285 (15)

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m/z 285 for 6, 7, 8, 10 and 11, respectively. It is a characteristic of the FAB mass spectra of these compounds, namely, $[M-H]^-$ and $[A-H]^-$ ions in the negative ion spectra, as well as $[M+H]^+$ and $[A+H]^+$ ions in the positive ion spectra showed the elimination of an oxygen atom. The negative ion spectra of 2 and 11 showed fragment ions due to the elimination of a methyl radical from the $[M-H]^-$ ion at m/z 430 for 2 and m/z 450 for 11 and, as well, the presence of fragment ions due to the elimination of a methyl radical from the $[A-H]^-$ ion at m/z 268 for 2 and m/z 286 for 11, respectively.

Negative ion spectra of compound 10 showed significant fragment peaks at m/z 463 and m/z 447, corresponding to the loss of the tigloyl (T_1) (Table IV) group from the molecular ion. Such fragment peaks were not observed in the positive ion spectra.

Negative ion spectra of compounds 12-22 (Table V) showed the $[M-H]^-$ and $[A-H]^-$ ions as intense or the base peaks. These results were similar to those seen in the positive ion spectra. However, the peaks at m/z 660 for 19; m/z 430 for 2; and m/z 452 for 11 were not appeared in the positive ion spectra. The results that the latter ions are

Table V. Main Negative Ions in FAB Mass Spectra of Flavonoid Glycosides.

A : Aglycone, S_t : Terminal sugar, S_s : Sugar of C-3, S_b : Sugar of C-7.

Compounds No. $m/z(\%)$	Diglycosides								Triglycosides		
	12	13	14	15	16	17	18	19	20	21	22
$[M]^-$	578 (20)	594 (13)	610 (14)	594 (8)	610 (30)	610 (16)	564 (8)	676 (2)	740 (6)	740 (15)	756 (3)
$[M-H]^-$	577 (23)	593 (17)	609 (24)	593 (11)	609 (34)	609 (23)	563 (13)	675 (4)	739 (7)	739 (16)	755 (5)
$[M-H-CH_3]^-$								660 (1)			
$[M-H-O]^-$	561 (2)	577 (7)	593 (5)	577 (1)	593 (10)	593 (9)			723 (3)		739 (-)
$[M-H-S_s]^-$	431 (8)	431 (6)	447 (17)	447 (5)	463 (43)				593 (6)		593 (15)
									447 (3)		577 (4)
$[M-H-S_a]^-$						447 (35)	431 (15)	529 (7)		431 (10)	447 (12)
$[M-H-S_b]^-$						463 (28)	417 (21)	513 (51)		593 (15)	609 (4)
$[A-H]^-$	269 (100)	269 (100)	285 (100)	285 (100)	301 (100)	301 (100)	285 (100)	367 (100)	285 (100)	285 (100)	285 (100)
$[A-H-CH_3]^-$								352 (15)			
$[A-H-O]^-$	253 (13)	253 (25)	269 (49)	269 (20)	285 (79)	285 (70)	269 (38)	351 (36)	269 (23)	269 (52)	269 (56)

Fig. 2. Negative Ion Spectra of Flavonol 3,7-*O*-glycosides.

(17 : antoside, 18 : kaempferol-3-*O*-arabinoside 7-*O*-rhamnoside, 19 : 4'-*O*-methyl 8- γ , γ -dimethylallylkaempferol 3-*O*-rhamnoside 7-*O*-glucoside)

observed only in the negative ion spectra are characteristic and useful for the structure elucidation of flavonoid glycosides methyl ethers.

In the negative ion spectra of 17, 18 and 19: the relative intensity of the peak at m/z 447 corresponding to the $[M-H-S_a]^-$ ion was higher than that of m/z 463 corresponding to the $[M-H-S_b]^-$ ion of 17, as well as in the positive ion spectra. In contrast, the $[M-H-S_a]^-$ ion peak at m/z 529 and 431 of 18 and 19 were less intense than the $[M-H-S_b]^-$ peak of 18 and 19 as shown in Fig. 2.

In the negative ion spectra of 20, 21 and 22, significant peaks due to the elimination of sugar moiety at C-3 or C-7 of aglycone from the $[M-H]^-$ ion were observed at m/z 593 and 447 for 20; m/z 593 and 431 for 21 and m/z 609 and 447 for 22 as well as those seen in the positive ion spectra.

4. Conclusion

In all cases studied, the quasi-molecular ions of flavonoid glycosides were observed in the FAB spectra. The negative ion spectra were useful to elucidate structures of flavonoid glycoside methyl ether and allyl ester. The positive ion spectra provided information about molecular weight and sugar sequence. In particular, judging from relative intensities of ions formed by the loss of sugars, the specific attachment of the sugar of flavonol 3,7-*O*-diglycosides was assumed to occur, if there are two sugars of different masses in the compound. Thus, the information obtained from both positive and negative ion spectra is considered to be important for structural elucidation of these compounds.

Acknowledgements

The authors are grateful to Professor Jinsaku Sakakibara and Dr. Munebara Ogawa of Nagoya City University for samples antoside and eudiposide, Dr. Yasuo Tokuoka of Tohoku University for a sample of Epimedeside A, Professor Naokata Morita of Toyama Medical and Pharmaceutical University for samples of tilianin and hesperetin 7-*O*-glucoside, and Professor Makoto Suzuki of Meijo University for helpful discussion.

References

- 1) A. Sakushima and S. Nishibe, *Phytochemistry* **27**, 915 (1988)
- 2) A. Sakushima, S. Hisada, S. Nishibe, and H. Brandenberger, *Phytochemistry*, **24**, 325 (1985).
- 3) This work was presented at the Japanese society for medical mass spectrometry (*Iyo Masu Kenkyukai Koenshu* **9**, 217 (1984)). Recently, F. W. Crow et al. has reported on the applicability of fast atom bombardment regarding structural information for some glycosides. F.W. Crow, K.B. Tomer, J.H. Looker and M.L. Gross *Anal. Biochem.* **155**, 286 (1986).
- 4) C.W. Glennie and G.J.B. Harborne, *Phytochemistry*, **10**, 1325 (1971).
- 5) J.B. Harborne, T.J. Mabry, and H. Mabry, "The Flavonoids" Chapman and Hall, London. (1975) p-387.
- 6) J.B. Harborne, T.J. Mabry, and H. Mabry, "The Flavonoids" Chapman and Hall, London. (1975) p-78.
- 7) H. Schels, H.D. Zinsmeister, and K. Pfeleger, *Phytochemistry*, **16**, 1019 (1977).
- 8) H. Wagner, and O. Seligmann, *Tetrahedron*, **29**, 3029 (1973).
- 9) I. Inagaki, S. Hisada, S. Nishibe, and A. Sakushima, *Yakugaku Zasshi*, **93**, 1231 (1973).
- 10) A. Sakushima, S. Hisada, S. Nishibe, and I. Inagaki, *Yakugaku Zasshi*, **93**, 1127 (1973).
- 11) A. Sakushima, S. Nishibe, S. Hisada, Y. Noro, and Y. Hisada, *Yakugaku Zasshi*, **96**, 1046 (1976).
- 12) A. Sakushima, S. Hisada, Y. Ogihara, and S. Nishibe, *Chem. Pharm. Bull.*, **28**, 1219 (1980).
- 13) A. Sakushima, S. Nishibe, and S. Hisada, *Ya-*

A. Sakushima, S. Nishibe, T. Takeda and Y. Ogihara

- kugaku Zasshi*, 98, 1395 (1978).
- 14) A. Sakushima, S. Hisada, S. Nishibe, and Y. Ogihara, *Shoyakugaku Zasshi*, 34, 92 (1980).
- 15) T. Murakami, T. Satake, C. Hirasawa, Y. Ikeno, Y. Saiki and C-H. Chen, *Yakugaku Zasshi*, 104, 142 (1984).
- 16) A. Sakushima and S. Nishibe, unpublished data.
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Flavonoid triglycosides
Fast atom bombardment mass spectrometry
Positive-ion
Negative-ion

Keywords

Flavonoid glycosides