Phytochemistry, 1978, Vol. 17, pp. 523–526 Pergamon Press Printed in England

MASS SPECTROMETRY OF SILYLATED FLAVONE AND FLAVANONE GLYCOSIDES*

HELMUT SCHELS†, H. DIETMAR ZINSMEISTER† and KARL PFLEGER‡

†Fachbereich 16-Botanik, Universität des Saarlandes, 6600 Saarbrücken, GFR; ‡Fachbereich 3-Pharmakologie und Toxikologie, Universität des Saarlandes, 6650 Homburg, GFR

(*Revised received* 14 *September* 1977)

Key Word Index—MS fragmentation; flavone-*O*-glycosides; flavone-*C*-glycoside; flavone-*C*/*O*-diglycosides; flavanone-*O*-glycosides; TMSi-derivatives; trimethylsilylation.

Abstract—The electron impact mass spectra of 14 trimethylsilylated flavone and flavanone mono- and diglycosides are reported for the first time. All spectra show well defined molecular ion peaks and those of *O*-glycosides additionally give evidence of the aglycone and the sugar(s), the sugar attachment, the sugar sequence and the interglycosidic linkage (of flavone and flavanone biosides).

INTRODUCTION

In an earlier paper [1], the mass spectrometric investigation of flavonol-*O*-glycosides was reported. There have been shown some substantial advantages of silylation in comparison to methylation. Therefore this new method was also applied to other classes of flavonoid glycosides.

RESULTS AND DISCUSSION

The 14 flavonoid glycosides investigated are listed in Table 1. The characteristic fragmentation patterns, the 'nomenclature', used for the different fragments and the mass spectra representing each group of glycosides are shown in Figs 1–3. The major fragments of all investigated silylated flavonoids, along with their intensities are given in Table 2.

Molecular ion peaks

As shown already for trimethylsilylated flavonol-*O*-glycosides [1], all flavone and flavanone glycosides possess M^+ peaks with a measurable intensity (at least 2%). In addition, also a more prominent $M^+ - 15$ peak (up to 52%) appeared. According to the hydrolytic behaviour in acidic solution [2], the intensity of M^+ and $M^+ - 15$ peaks respectively were influenced by the molecular structure (position of the sugar attachment, kind of sugar, type of glycosidation). Thus luteolin-5-*O*-glucoside showed the lowest $M^+ - 15$ peak, followed by 7-*O*-biosides and 7-*O*-monoglycosides. Among those with a most intense $M^+ - 15$ peak (relative intensity 52%) was scutellarein-7-*O*-glucuronide, which is known to be very resistant to acidic hydrolysis. The only flavone-*C*-monoglycoside (vitexin) studied showed the same phenomenon. A striking difference in MS with methylated derivatives is that flavanones showed no ring fission of the heterocycle [3, 4]. Scutellarein-7-*O*-glucuronide differs clearly from all other glycosides investigated presenting $M^+ - 72$ and $(M^+ - 15) - 72$ peaks which seems to be characteristic for glucuronides, since they occur also with flavonol-*O*-glucuronides [5]. This seems to be analogous to the splitting off of MeOH, which has been observed for methylated derivatives of glucuronides [6].

Table 1. Flavonoid glycosides investigated

No.	Compound
Flavone-5-*O*-monoglycoside	
1	luteolin-5-*O*-glucoside*
Flavone-7-*O*-mono- and biosides	
2	apigenin-7-*O*-glucoside*
3	scutellarein-7-*O*-glucuronide (scutellarein)*
4	acacetin-7-*O*-rutinoside (acaciin)*
5	pectolinaringenin-7-*O*-rutinoside (pectolinarin)*
6	apigenin-7-*O*-apioglucoside (apiin)*
7	apigenin-7-*O*-neohesperidoside (rhoifolin)*
8	diosmetin-7-*O*-rutinoside (diosmin)*
Flavone-*C*-(*O*)-mono- and diglycosides	
9	apigenin-8-*C*-glucoside (vitexin)*
10	vitexin-4′-*O*-xyloside†
11	vitexin-4′-*O*-rhamnoside*
Flavanone-7-*O*-mono- and biosides	
12	naringenin-7-*O*-glucoside‡
13	naringenin-7-*O*-neohesperidoside (naringin)*
14	hesperetin-7-*O*-rutinoside (hesperidin)*

*C. Roth, Karlsruhe, GFR
†Isolated from *Vitex lucens*.
‡Synthesized.

Aglycones

The mass spectra of the flavone and flavanone-*O*-glycosides reveal that the fission of aglycone sugar linkage involves hydrogen and/or trimethylsilyl transfer leading to the following 'aglycone-fragments': A + R, (A + R) − 15, A + H, (A + H) − 15. In the case of flavone-*C*-glycosides and flavone-*C*/*O*-glycosides respectively, the sugar in the C-8 position is not split off as such, as with *O*-linked sugars, but there occurs

*Part 2 in the series 'Mass spectrometry of silylated flavonoid glycosides'. For Part 1 see ref. 1.

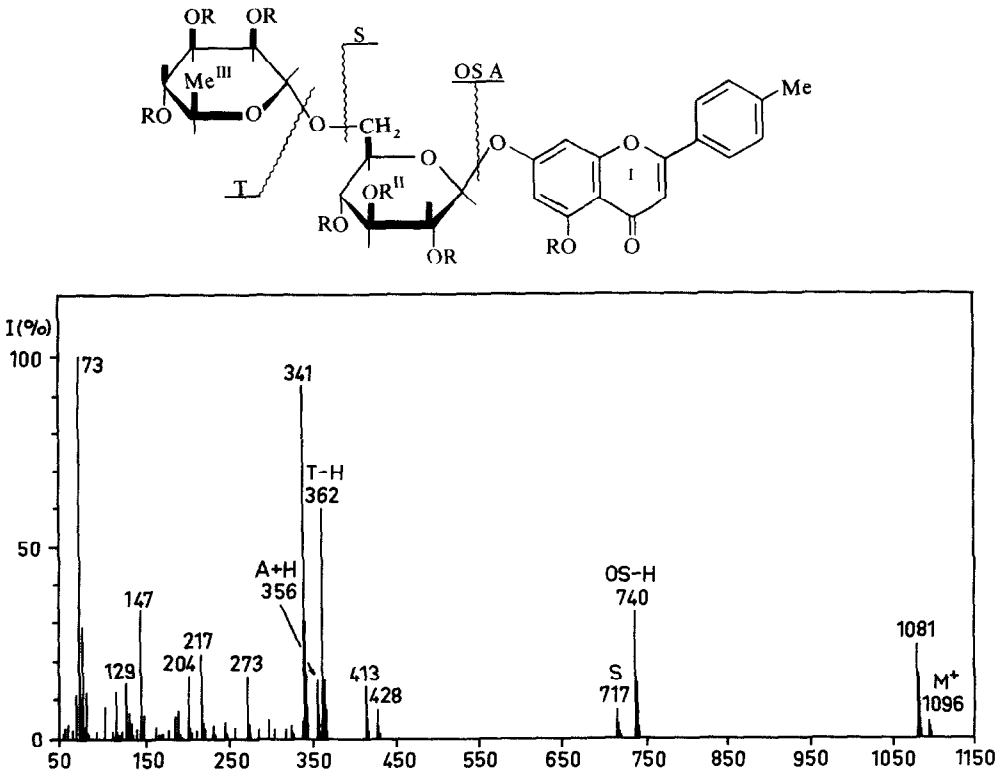

Fig. 1. Scheme of the MS-fragmentation and mass spectrum of a silylated flavone-7-*O*-glycoside (acaciin). S = I + II, OS = II + III, R = $Si(Me)_3$, I = relative intensity (%).

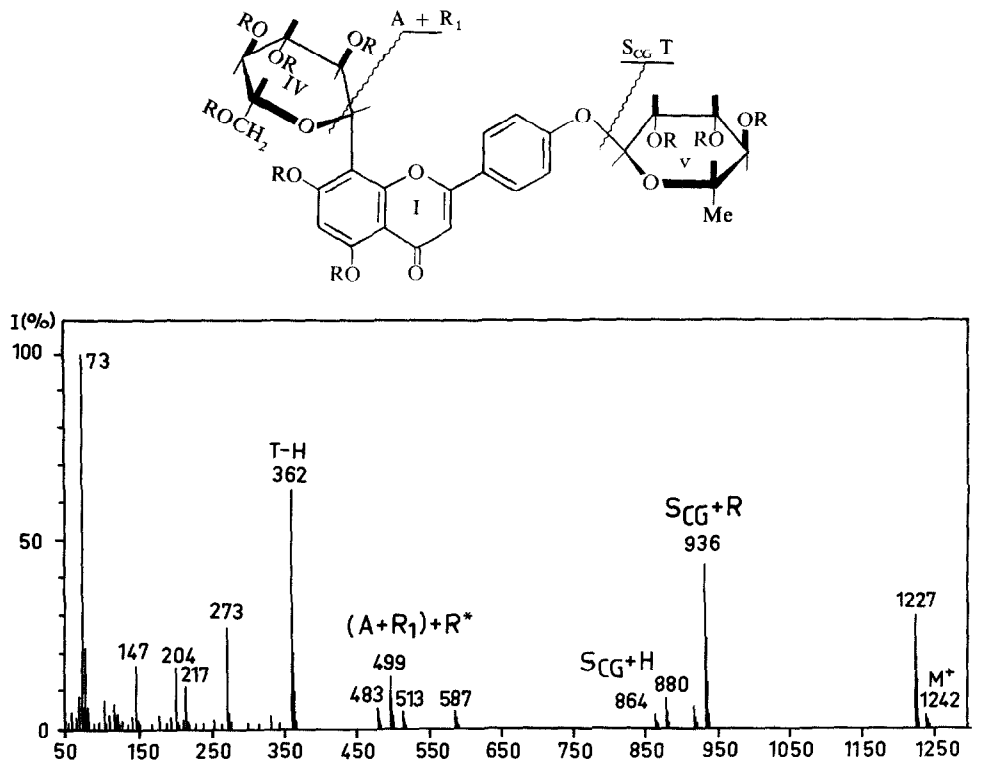

Fig. 2. Scheme of the MS-fragmentation and mass spectrum of a silylated flavone-8-*C*-4′-*O*-glycoside (vitexin-4′-*O*-rhamnoside). S_{CG} = I + IV, R = $Si(Me)_3$, R_1 = $-CH_2$, I = relative intensity (%).

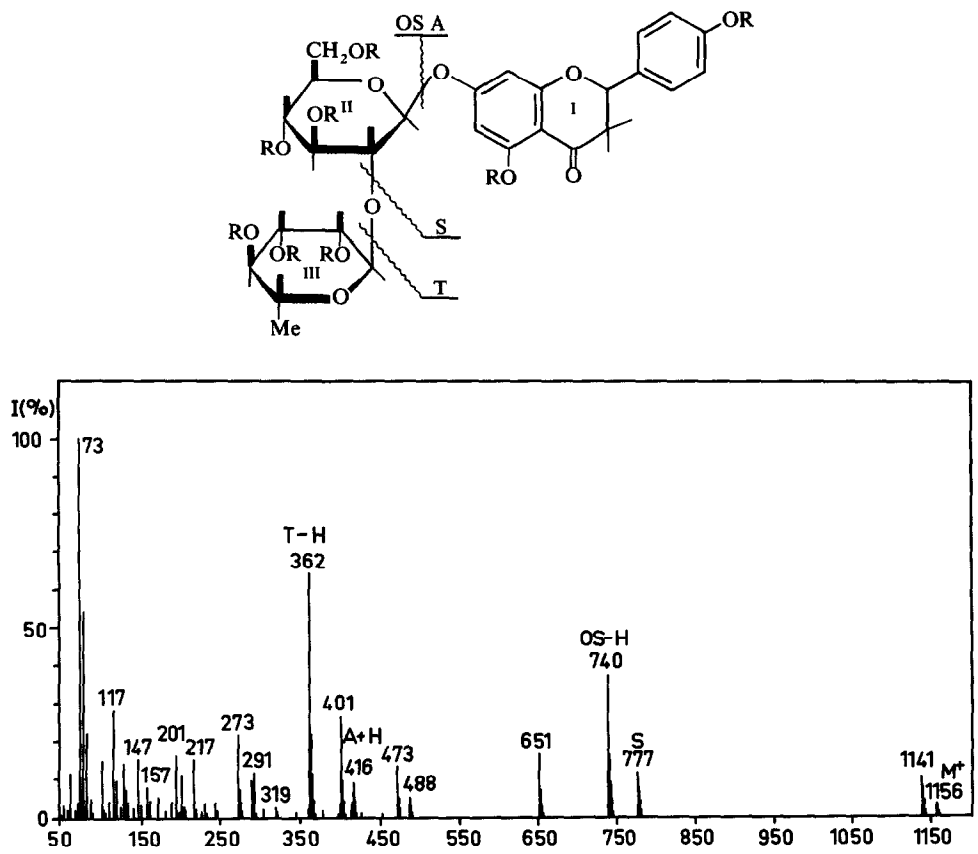

Fig. 3. Scheme of the MS-fragmentation and mass spectrum of a silylated flavanone-7-*O*-glycoside (naringin). S = 1 + II, OS = II + III, R = $Si(Me)_3$, I = relative intensity (%).

ring fission of the glucose molecule and a CH_2-residue (R_1) remains (Fig 2); this is typical for permethylated derivatives too [7]. This leads for silylated vitexin to the fragment A + R_1 at *m/e* 499. Vitexin-4'-*O*-xyloside and vitexin-4'-*O*-rhamnoside showed the fragments (A + R_1) + R also at *m/e* 499 produced by splitting off the sugar at 4'-position and transfer of R to the aglycone. The most prominent aglycone fragment of flavone- and flavanone-7-*O*-glycosides (Figs 1 and 3) is as that for flavonol-7-*O*-glycosides [1]: (A + H) − 15. These aglycone fragments of flavone glycosides differ strikingly from those of flavanone-glycosides in having much higher relative intensities. In contrast, the only flavone-5-*O*-monoside investigated possesses as the most prominent

Table 2. MS data of silylated flavone- and flavanone-glycosides

	Flavone 5-*O*-mono, 7-*O*-mono- and biosides								Flavone-C-(*O*)-mono- and diglycosides			Flavanone-7-*O*-mono- and biosides		
Compounds	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Fragments														
M^+	952 (2)	864 (2)	966 (4)	1096 (4)	1126 (3)	1140 (2)	1154 (3)	1184 (3)	936 (10)	1228 (3)	1242 (3)	866 (3)	1156 (3)	1186 (2)
$M^+ - 15$	937 (5)	849 (32)	951 (52)	1081 (25)	1111 (17)	1125 (10)	1139 (14)	1169 (24)	921 (52)	1213 (29)	1227 (29)	851 (27)	1141 (10)	1171 (10)
S_{CG} + R	—	—	—	—	—	—	—	—	—	936 (35)	936 (43)	—	—	—
S/S_{CG} + H*	—	—	—	717 (7)	747 (8)	775 (4)	775 (6)	805 (6)	—	864 (3)*	864 (3)*	—	777 (11)	807 (6)
OS-H	—	—	—	740 (33)	740 (32)	726 (5)	740 (18)	740 (31)	—	—	—	—	740 (37)	740 (21)
OS-ROH	—	—	—	651 (0)	651 (0)	637 (22)	651 (29)	651 (0)	—	—	—	—	651 (17)	651 (0)
(A + R) − 15	559 (27)	471 (13)	559 (10)	413 (13)	443 (19)	471 (17)	471 (28)	501 (8)	—	—	—	473 (16)	473 (13)	503 (23)
A + R_1/(A + R_1) + R*	—	—	—	—	—	—	—	—	499 (26)	499 (11)*	499 (13)*	—	—	—
A + H	502 (100)	414 (15)	502 (15)	356 (15)	386 (16)	414 (7)	414 (13)	444 (18)	—	—	—	416 (16)	416 (9)	446 (3)
(A + H) − 15	487 (15)	399 (68)	487 (91)	341 (93)	371 (87)	399 (32)	399 (50)	429 (91)	—	—	—	401 (29)	401 (26)	431 (28)
T-H	450 (54)	450 (35)	464 (43)	362 (60)	362 (48)	348 (13)	362 (72)	362 (61)	—	348 (61)	362 (64)	450 (61)	362 (64)	362 (59)
T-ROH	361 (33)	361 (54)	375 (29)	273 (16)	273 (18)	259 (64)	273 (11)	273 (12)	—	259 (32)	273 (27)	361 (35)	273 (21)	273 (14)
Ion *m/e* 217	(16)	(29)	(21)	(22)	(30)	(30)	(27)	(16)	(10)	(24)	(11)	(19)	(15)	(37)
Ion *m/e* 204	(4)	(5)	(7)	(15)	(16)	(5)	(14)	(21)	(30)	(9)	(15)	(11)	(10)	(43)
Ion *m/e* 147	(16)	(39)	(30)	(34)	(17)	(42)	(40)	(25)	(47)	(33)	(15)	(15)	(15)	(29)
Ion *m/e* 73	(86)	(100)	(100)	(100)	(100)	(100)	(100)	(100)	(100)	(100)	(100)	(100)	(100)	(100)

Relative intensity (%) in parenthesis.
S_{CG} = aglycone plus C-bonded glucose at C-8.

aglycone fragment A + H, like flavonol-3-*O*-glycosides [1].

Sugars, sugar sequence, sugar attachment and interglycosidic linkage

The formation of sugar fragments is the same as reported for flavonol-*O*-glycosides [1]. This is also so for the two sugars apiose and xylose with mass 348 (T − H; see Fig. 2). Exceptionally, there is not a T-series sugar fragment for C − 8 bonded glucose of *C*-glycosides (compounds **9–11**). Compounds **10** and **11** show prominent S_{CG} + R-peaks at *m/e* 936 and minor peaks: S_{CG} + H at *m/e* 864. This is due to splitting off the *O*-bonded sugar at C − 4′ and the simultaneous trimethylsilyl- and hydrogen transfer, whereas the C − 8 bonded sugar remains attached to the aglycone (Fig. 2). It is not possible to differentiate between pyranose and furanose forms of sugars in glycosides as can be done for free sugars [8], but this is not a specific disadvantage of persilylation [10]. As with the flavonol-*O*-glycosides, the sugar sequence in flavone and flavanone biosides can be established by the fragments of the T and S series [1]. As stated above, the sugar attachment of C − 5 and C − 7 substituted flavones can be distinguished by their typical aglycone fragments. The position of the interglycosidic linkage can be determined as described earlier [1].

Mass spectrometric studies on flavonoid glycosides [1, and this paper] have some substantial advantages for *O*-glycosides, using trimethylsilylated derivatives instead of permethylated or perdeuteromethylated products. Thus in the case of persilylation, complete derivatization takes place resulting in one silylation product, whereas with permethylation one may obtain up to a dozen methylation products due to incomplete or excessive methylation, which have to be further purified, before analysis [7]. Thus silylation provides a more rapid technique for all *O*-glycosides (42) of various flavonoid types. The determination of the molecular mass by the silylation method is very easy, since there are always distinct M^+ and corresponding $M^+ - 15$ peaks, which is not always true for methylated flavonoid *O*-glycosides. A further advantage of silylation is shown with alkali labile compounds (e.g. flavanones) which may be degraded by methylation (ring fission) under alkaline conditions. This method is however not as satisfactory for *C*-glycosides, which are better analyzed by using permethylated derivatives [7]; it is, however, a good tool for quick determination of the molecular mass for an unknown *C*-glycoside.

EXPERIMENTAL

Trimethylsilylation and MS was as described earlier [1, 9].

Acknowledgements—The support of Deutsche Forschungsgemeinschaft and the Bundesministerium für Technologie und Forschung to K.P. is gratefully acknowledged. We thank H. Wagner, Munich, GFR, for a purified extract of *Vitex lucens*.

REFERENCES

1. Schels, H., Zinsmeister, H D. and Pfleger, K. (1977) *Phytochemistry* **16**, 1019.
2. Harborne, J. B. (1965) *Phytochemistry* **4**, 107
3 Schmid, R. D. (1972) *Tetrahedron* **29**, 3259.
4. Schmid, R. D., Mues, R., McReynolds, J. H., Vander Velde, G., Nakatani, N., Rodriguez, E. and Mabry, T. J. (1973) *Phytochemsitry* **12**, 2765.
5. Schels, H., Zinsmeister, H D. and Pfleger, K. unpublished.
6. Wagner, H. and Seligmann, O. (1973) *Tetrahedron* **29**, 3029.
7. Bouillant, M.-L., Favre-Bonvin, J. and Chopin, J. (1975) *Phytochemistry* **14**, 2267.
8. De Jongh, D. C., Radford, T., Hribar, J. D., Hanessian, S., Bieber, M., Dawson, G. and Sweeley, C. C. (1969) *J Am. Chem. Soc.* **91**, 1728.
9. Schels, H. and Pfleger, K. (1978) Varian Application. Note 24.
10. Schmid, R. D, Varenne, P and Paris, R. (1972) *Tetrahedron* **28**, 5037.