Phytochemistry, 1977, Vol. 16, pp. 1019–1023. Pergamon Press. Printed in England.

MASS SPECTROMETRY OF SILYLATED FLAVONOL *O*-GLYCOSIDES*

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(*Received* 23 *November* 1976)

Key Word Index—MS fragmentation; flavonol-*O*-glycosides; mono-, di-, triglycosides; TMSi- derivatives; trimethylsilylation.

Abstract—The electron impact mass spectra of 19 trimethylsilylated flavonol mono-, di- and -triglycosides are reported for the first time. All spectra show well defined molecular ion peaks and additionally give evidence of the aglycone and the sugar(s), the sugar attachment, the sugar sequence and the interglycosidic linkage in the case of flavonol biosides.

INTRODUCTION

For the mass spectrometric investigation of flavonoid *O*-glycosides, methylated or perdeuteromethylated derivatives are usually used [1–3]. The disadvantage of this is, however, either incomplete or excessive methylation and the methylation products need further purification. Thus, an easier method was sought. Since 1957 TMSi-derivatives [4] have been widely used in mass spectrometry. For flavonoids, TMSi-derivatives have already been used for GC–MS of aglycones [5] and for establishing the molecular mass of a flavonoid-*C*-glycoside (Saponaretin) [6]. This paper reports the mass spectrometric analysis of flavonol *O*-glycosides using trimethylsilylated derivatives. The problem of exact mass measurement for the flavonoid di- and -triglycosides which possess as silylated products molecular weights of over 1000 has been overcome using a new calibration method [7].

RESULTS AND DISCUSSION

The 19 flavonol *O*-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

In all mass spectra of trimethylsilylated flavonol *O*-glycosides the M^+ peak could be detected and showed always a relative high intensity, mainly in comparison with methylated 3-*O*-glycosides [1–3], of at least 1% (the base peak = 100% relative intensity is always represented by the most intense peak of a MS). In addition, a more prominent $M^+ - 15$ peak of upto 44% relative intensity appeared. As a characteristic feature, the M^+ and $M^+ - 15$ peaks are the first of a group of bands due to the isotropic pattern of silicon. These occurred for all other fragments of the mass spectra.

Table 1. Flavonol glycosides investigated

No.	Compound
3-*O*-mono- and diglycosides	
1	kaempferol-3-*O*-glucuronide*
2	kaempferol-3-*O*-glucuronic acid methyl ester†
3	quercetin-3-*O*-rhamnoside (quercitrin)‡
4	quercetin-3-*O*-galactoside (hyperoside)‡
5	quercetin-3-*O*-glucoside (isoquercitrin)*
6	quercetin-3-*O*-glucuronide (quercituron)*
7	myricetin-3-*O*-rhamnoside (myricitrin)‡
8	kaempferol-3-*O*-rutinoside (nicotiflorin)*
9	kaempferol-3-*O*-sophoroside§
10	quercetin-3-*O*-rutinoside (rutin)*
3,7-*O*-di- and triglycosides	
11	kaempferol-3,7-*O*-dirhamnoside (kaempferitrin)§
12	kaempferol-3,7-*O*-diglucoside (paeoniside)*
13	kaempferol-3-*O*-rhamnoside-7-*O*-glucoside*
14	kaempferol-3-*O*-galactoside-7-*O*-rhamnoside†
15	quercetin-3,7-*O*-diglucoside‖
16	kaempferol-3-*O*-robinobioside-7-*O*-rhamnoside (robinin)‡
7-*O*-mono- and -diglycosides	
17	kaempferol-7-*O*-rhamnoside*
18	kaempferol-7-*O*-glucoside (populnin)*
19	kaempferol-7-*O*-neohesperidoside‖

* Isolated from *Phaseolus vulgaris*
† Synthesized
‡ From C. Roth, Karlsruhe, GFR.
§ From Prof. Dr. K. Egger.
‖ From Prof Dr. T. Mabry.

Aglycones

The mass spectra reveal that the fission of aglycone-sugar linkage involves hydrogen and/or trimethylsilyl transfer leading to the following 'aglycone-fragments': A + 2R, A + H + R, A + R, A + 2H, A + H

* Part 1 in the series 'Mass Spectrometry of silylated flavonoid glycosides'.

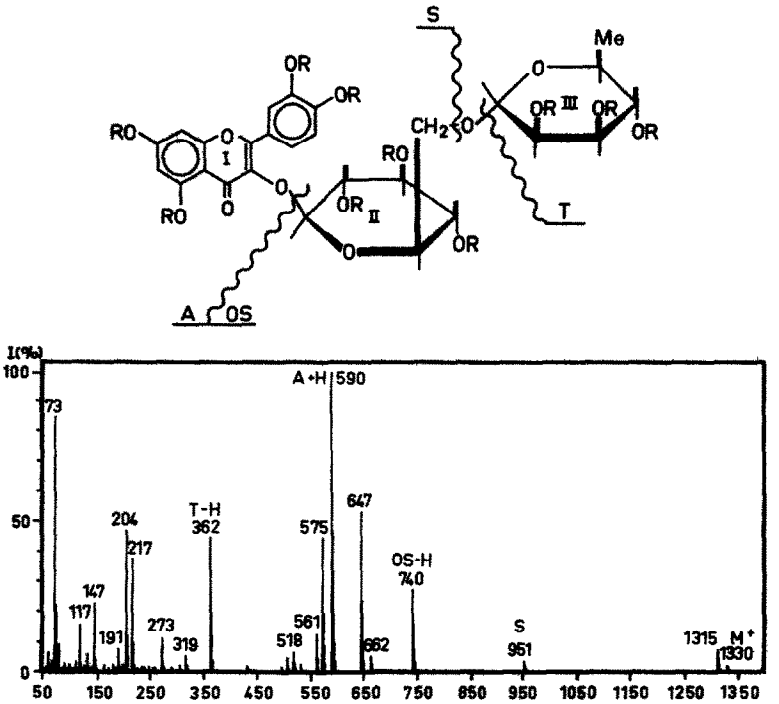

Fig. 1. Scheme of the MS-fragmentation and MS of a silylated flavonol-3-*O*-glycoside (rutin) S = I + II, OS = II + III, R = $Si(Me)_3$, I = relative intensity (%).

and the corresponding masses minus 15. The 3-*O*-mono- and biosides show a very intense A + H peak which frequently represents the base peak. The 7-*O*-glycosides differ typically from 3-*O*-glycosides by showing an (A + H)-15 peak of nearly 100% relative intensity. The most intensive 'aglycone-fragment' of the 3,7-*O*-di- and -triglycosides is (A + H + R)-15. Subsequent fragmentation of the aglycone, e.g. by retro-Diels-Alder cleavage, gives no further informations on the substitution pattern of the aglycone [1].

Sugars, sugar sequence and sugar attachment

The formation of sugar fragments is always combined with the loss of hydrogen. These fragments possess a fairly high intensity upto 81% and, dependent on the molecular constitution, they are as follows: T-H (compounds 1–10, 17–19); T_1-H and T_2-H (compounds 11–16); OS-H (compounds 8–10, 16, 19). The typical masses to be found for the different sugars are 828 (sophorose), 740 (rutinose, neohesperidose, robinobiose), 464 (glucuronic acid), 406 (glucuronic acid methyl ester), 450 (glucose, galactose), 362 (rhamnose).

Mono- and biosides of flavonols are not only distinguished by molecular mass but also by the appearance of an additional OS-H fragment for biosides. Further sugar fragments are formed by the loss of trimethylsilanol (ROH): T-ROH (compounds 1–10, 17–19);

Table 2. MS data of

Compound	3-*O*-mono-and biosides							
Fragments	1	2	3	4	5	6	7	8
M^+	966(1)	908(2)	952(5)	1040(2)	1040(2)	1054(4)	1040(2)	1242(1)
M^+-15	951(11)	893(20)	937(17)	1025(7)	1025(9)	1039(21)	1025(9)	1227(5)
S/S_3*	—	—	—	—	—	—	—	863(5)
(S_1 + R)-15	—	—	—	—	—	—	—	—
S_1 + H	—	—	—	—	—	—	—	—
(S_2 + R)-15	—	—	—	—	—	—	—	—
S_2 + H	—	—	—	—	—	—	—	—
OS-H	—	—	—	—	—	—	—	740(29)
OS-ROH	—	—	—	—	—	—	—	651(0)
(A + 2R)-15	—	—	—	—	—	—	—	—
(A + R)-15/(A + R + H)-15*	559(55)	559(41)	647(69)	647(95)	647(79)	647(60)	735(39)	559(46)
A + H/A + 2H*	502(100)	502(100)	590(100)	590(100)	590(96)	590(100)	678(95)	502(100)
(A + H)-15/(A + 2H)-15*	487(86)	487(76)	575(70)	575(92)	575(50)	575(73)	663(38)	487(44)
T-H/T_1-H*	464(49)	406(51)	362(74)	450(40)	450(44)	464(47)	362(44)	362(43)
T-ROH/T_1-ROH*	375(61)	317(25)	273(17)	361(55)	361(16)	375(66)	273(8)	273(14)
T_2-H	—	—	—	—	—	—	—	—
T_2-ROH	—	—	—	—	—	—	—	—
mass 217	(54)	(37)	(49)	(74)	(44)	(47)	(28)	(34)
mass 204	(20)	(13)	(14)	(14)	(11)	(18)	(11)	(27)
mass 147	(66)	(63)	(51)	(56)	(41)	(58)	(34)	(23)
mass 73	(89)	(88)	(71)	(95)	(100)	(82)	(100)	(92)

? = intensity could not be measured by peak over-lapping; relative intensity (%) in parenthesis

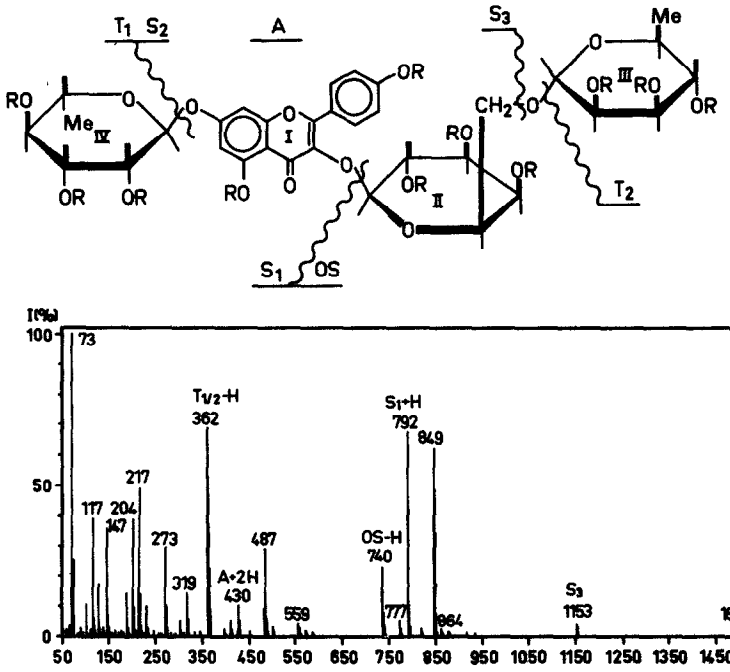

Fig. 2. Scheme of the MS-fragmentation and MS of a silylated flavonol-3,7-*O*-glycoside (robinin) S_1 = I + IV, S_2 = I + II + III, S_3 = I + II + IV, OS = II + III, R = Si(Me)$_3$, I = relative intensity (%).

T_1-ROH and T_2-ROH (compounds 11–16) and OS-ROH (compounds 9 and 19).

Additionally, other fragments [8] of these sugars are distributed over the lower mass range. In all flavonoid-*O*-glycoside spectra, the fragments 217, 204, 147, 117 and 73 occur with intense signals, the latter often representing the base peak. It is not possible from these data to distinguish between the isomeric sugars glucose and galactose or rutinose and robinobiose respectively. In the case of flavonol biosides, it is possible to deduce the sugar sequence because the terminal sugar always produces a very intense T-fragment (relative intensity >30%) accompanied with a high T-ROH or T_1-ROH and/or T_2-ROH signal. This can be demonstrated in compounds 8, 10, 16, 19 where rhamnose as the terminal sugar produces the T-H ion at *m/e* 362. On splitting off the terminal sugar, a charge may also remain on the rest of the molecule. Then, fission occurs—in contrast to formation of T-fragments—between the ethereal oxygen and the carbon of the aglycone-linked sugar. This cleavage results in the fragments S(compounds 8–10, 19) and S_3 (compound 16), which also appear in the MS of methylated and perdeuteromethylated flavonoid-*O*-glycosides [1]. These spectra did not show

silylated flavonol-glycosides

7-*O*-mono- and biosides					3,7-di-and triglycosides					
9	10	17	18	19	11	12	13	14	15	16
1330(1)	1330(1)	864(4)	952(8)	1242(2)	1154(1)	1330(1)	1242(3)	1242(1)	1418(1)	1532(1)
1315(4)	1315(7)	849(44)	937(42)	1227(13)	1139(10)	1315(5)	1227(9)	1227(6)	1403(5)	1517(4)
863(6)	951(3)	—	—	863(7)	—	—	—	—	—	1153(4)*
—	—	—	—	—	849(74)	937(26)	937(88)	849(100)	1025(42)	849(62)
—	—	—	—	—	792(89)	880(54)	880(34)	792(49)	968(68)	792(67)
—	—	—	—	—	849(74)	937(26)	849(7)	937(19)	1025(42)	1227(0)
—	—	—	—	—	792(89)	880(54)	792(19)	880(8)	968(68)	1170(0)
828(13)	740(27)	—	—	740(27)	—	—	—	—	—	740(23)
739(23)	651(0)	—	—	651(19)	—	—	—	—	—	651(0)
—	—	—	—	—	559(32)	559(14)	559(5)	559(46)	647(2)	559(4)
559(50)	647(52)	559(21)	559(31)	559(13)	487(69)*	487(65)*	487(15)*	487(58)*	575(50)*	487(29)*
502(55)	590(100)	502(5)	502(14)	502(41)	430(9)*	430(45)*	430(9)*	430(23)*	518(66)*	430(10)*
487(31)	575(44)	487(84)	487(94)	487(97)	415(7)*	415(25)*	415(3)*	415(14)*	503(33)*	415(4)*
450(38)	362(44)	362(55)	450(56)	362(34)	362(48)*	450(81)*	450(41)*	362( )	450(46)*	362(69)*
361(59)	273(11)	273(14)	361(46)	273(15)	273(21)*	361(44)*	361(52)*	273(7)*	361(42)*	273(30)*
—	—	—	—	—	362(48)	450(81)	362(81)	450(38)	450(46)	362(69)
—	—	—	—	—	273(21)	361(44)	273(26)	361(39)	361(42)	273(30)
(39)	(37)	(34)	(53)	(23)	(45)	(85)	(41)	(39)	(60)	(49)
(25)	(46)	(10)	(22)	(12)	(16)	(21)	(18)	(11)	(20)	(39)
(24)	(22)	(44)	(40)	(28)	(15)	(73)	(22)	(25)	(39)	(35)
(100)	(85)	(100)	(100)	(100)	(100)	(100)	(100)	(80)	(100)	(100)

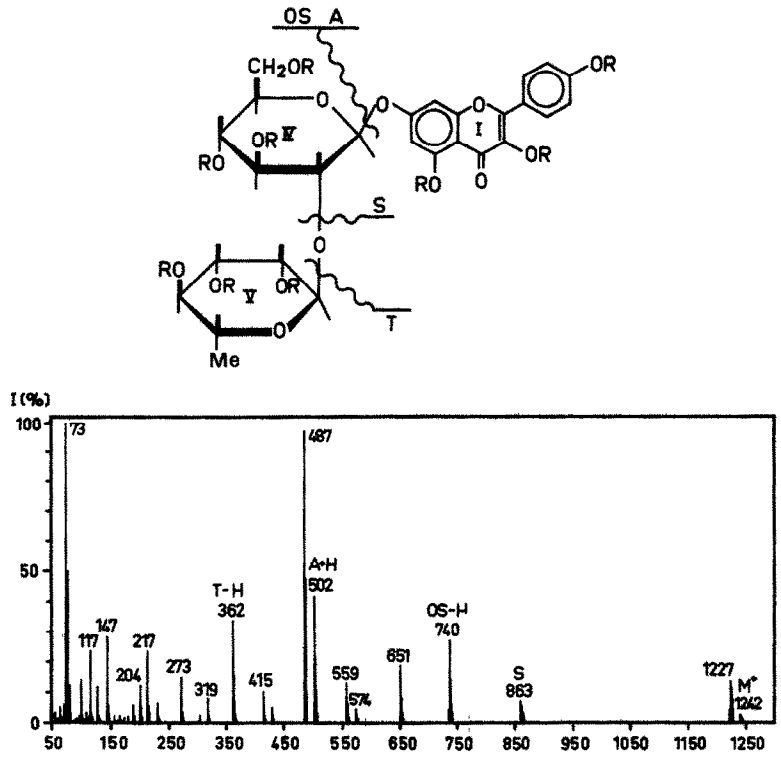

Fig. 3. Scheme of the MS-fragmentation and MS of a silylated flavonol-7-*O*-glycoside (kaempferol-7-*O*-neohesperidoside) S = I + IV, OS = IV + V, R = $Si(Me)_3$, I = relative intensity (%).

fragments S-A (compounds 8–10, 19) and S_3-S_1 (compound 16) due to the residue of the aglycone-linked sugar. In the case of 3,7-*O*-glycosides, the sugars are split off both in the 3-position (resulting in fragments S_1) and the 7-position (resulting in fragments S_2). These fragments are always formed with hydrogen transfer (e.g. $S_1 + H$) and accompanied by fragments formed with trimethylsilyl transfer (e.g. $S_1 + R$) and the corresponding masses minus 15 (e.g. $(S_1 + H)$-15, $(S_1 + R)$-15). From the relative intensity of the ions S_1 and S_2 respectively, it is possible to deduce the specific attachment of the sugars at the C_3- and C_7-positions of flavonol-3,7-*O*-di and triglycosides if there are two sugars of different masses. As with acidic hydrolysis, the cleavage of the sugar at 3-position is preferred to that at the 7-position. Therefore in MS of silylated flavonol glycosides there are more intense signals of the fragments $S_1 + R$, $(S_1 + R)$-15, $S_1 + H$ (resulting from aglycone plus sugar at C_7) than $S_2 + R$, $(S_2 + R)$-15, $S_2 + H$ (resulting from aglycone plus sugar at C_3; compare table 2). A similar behaviour is reported for methylated products [3]. Thus it is easy to distinguish between kaempferol-3-*O*-rhamnoside-7-*O*-glucoside (compound 13) and kaempferol-3-*O*-galactoside-7-*O*-rhamnoside (compound 14). The type of glycosidation (3-*O*-, 7-*O*- or 3,7-*O*-glycosides) is also indicated by different aglycone fragments as already mentioned above.

Interglycosidic linkage

The position of the interglycosidic linkage can easily be detected in the case of 1 → 2- and 1 → 6-linked biosides respectively. The 1 → 6-linked sugars, e.g. rutinose and robinobiose (compounds 8, 10, and 16) show only one intense peak due to the disaccharide moiety OS-H. In contrast the 1 → 2-linked biosides, e.g. neohesperidose (compound 19) and sophorose (compound 9) possess, in addition to the OS-H fragments, peaks OS-ROH at *m/e* 651 and 739 respectively. Rutinose and neohesperidose cannot however be differentiated by the typical fragment for silylated 1 → 6-bonded carbohydrates at *m/e* 495 [9, 10], since this fragment did not appear in our spectra.

ADDENDUM

After completing this manuscript, some more flavonol-*O*-glycosides for mass spectrometry became available. Azaleatin-3-*O*-rhamnoside (azalein), jaceidin-7-*O*-glucoside (jacein), quercetin-4'-*O*-glucoside (spiraeoside), kaempferol-3-*O*-xylogalactoside, isorhamnetin-3,7-*O*-diglucoside, myricetin-3-*O*-rutinoside, provided by E. Wollenweber, and kaempferol-3-*O*-(*p*-coumaryl)-glucoside (tiliroside) provided by Fa-ching Chen. The 3-*O*- and 3,7-*O*-flavonol glycosides showed corresponding mass spectra according to the reported results, while the only 4'-*O*-flavonol glycoside (spiraeoside) could be distinguished by mass spectrometry from the others.

EXPERIMENTAL

Trimethylsilylation. To 1 mg of each flavonol glycoside, dissolved in 0.5 ml Py. (dried over KOH), 0.2 ml *N, O-bis*-trimethylsilylacetamide [11] (E. Merck, Darmstadt, GFR) were added. The mixture was kept for 6 hr at room temp. for silylation. 1–2 μl of this solution were used for the MS.

MS. All spectra were recorded on a Varian mass spectrometer data system MAT 311 A-100 MS with a direct inlet system under the following conditions: ionisation energy 90 eV, acceleration voltage 1.8 kV, emission current 2 mA, ion source temperature 150°, scan rate 12.6 sec/dec, resolution 2000 at 5% valley, sample frequency 3 kHz. The calibration of the mass spectrometer data system was achieved as described earlier [7].

Acknowledgements—We are grateful to T. J. Mabry, Austin, Texas (USA), K. Egger, Heidelberg (GFR), E. Wollenweber, Darmstadt (GFR) and Fa-ching-Chen, Taipei (ROC) for flavonoid samples.

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