*Phytochemistry*, 1978, Vol 17, pp 527–533. Pergamon Press Printed in England

# STRUCTURAL DETERMINATION OF *C*-GLYCOSYLFLAVONES BY MASS SPECTROMETRY OF THEIR PERMETHYL ETHERS: *O*-GLYCOSYL-6-*C*-GLYCOSYLFLAVONES

Marie-Louise Bouillant*, André Besset*, Jean Favre-Bonvin† and Jean Chopin*
Laboratoires de Chimie biologique* et de Phytochimie†, Université Claude Bernard, Lyon I, 69621 Villeurbanne, France

(*Received* 15 *July* 1977)

**Key Word Index**—MS; MS fragmentation; *C*-glycosylflavones; *O*-glycosyl-6-*C*-glycosylflavones; 6-*C*-diholosylflavones; 6-*C*-neohesperidosylacacetin; 6-*C*-cellobiosylacacetin; 6-*C*-rutinosylacacetin; permethylation.

**Abstract**—Permethylated *O*-glycosyl-*C*-glycosylflavones give well defined MS including an important molecular peak. Permethyl 6-*C*-glycosylflavones *O*-glycosylated on a phenolic hydroxyl group are easily distinguished from the isomeric permethyl 6-*C*-diholosylflavones. In both types, the position of the *O*-glycosidic bond can be deduced from the MS, eventually after acid hydrolysis. 2″-*O*-glycosyl-6-*C*-glycosylflavones can be differentiated from their 8-*C* isomers.

## INTRODUCTION

In the first paper of this series [1], mass spectrometry has been shown to be useful in the structural study of 6-*C*, 8-*C* and 6,8-di-*C*-glycosylflavones. *C*-glycosylflavones often occur in Nature as *O*-glycosides and most of these are *O*-monosides which can be divided in two types: *x* (or -*x*′) -*O*-glycosyl *C*-glycosylflavones, in which the *O*-glycosyl moiety is bound to a phenolic hydroxyl group of the flavone; -*x*″-*O*-glycosyl *C*-glycosylflavones or *C*-diholosylflavones, in which the *O*-glycosyl moiety is bound to an alcoholic hydroxyl group of the *C*-glycosyl residue.

In both types, the *O*-glycosyl group and the *C*-glycosylflavone can be easily identified after acid hydrolysis. In the first type, the *O*-glycosyl position can be deduced from the comparison of UV spectral shifts [2] before and after hydrolysis, as in the case of flavone *O*-glycosides. In the second type, however, the UV spectral shifts remain unchanged after hydrolysis and location of the *O*-glycosidic bond is much more difficult than in the case of flavone *O*-diholosides, since the *C*-glycosyl moiety cannot be detached from the flavone by acid hydrolysis after permethylation.

Indeed, only a few *C*-diholosylflavone structures have been elucidated until now and all but two are *O*-2″-glycosides, the structures of which have been solved by using the high field shift of the equatorial 2″-OAc or 2″-OMe signal in the NMR spectrum of 6- or 8-*C*-glucosyl flavones acetates or methyl ethers [3]. The only other examples are 4″-*O*-rhamnosylvitexin from *Crataegus curvisepala* [4], and 6″-*O*-arabinosyl isoorientin from *Swertia perennis* [5]. The structure of the former was deduced from identification of tartaric acid as a product of periodate oxidation followed by nitric acid oxidation; however, this may be questioned since *O*-rhamnosyl vitexins from other *Crataegus* species have been shown to be 2″-*O*-rhamnosylvitexin [6, 7]. The structure of the latter was inferred from comparison of its $^{13}$C NMR spectrum with that of isoorientin.

In previous preliminary papers [8, 9], we showed that mass spectrometry of permethyl ethers allowed one to differentiate *x* (or *x*′)-*O*-glycosyl 6-*C*-glycosylflavones from 6-*C*-diholosylflavones. Following the synthesis of some 6-*C*-diholosylflavones by *C*-diholosylation of 5,7-dihydroxyflavones [10, 11], we can now report on the separation of 2″-, 4″- and 6″-*O*-glycosyl 6-*C*-glycosylflavones from each other by mass spectrometry of their permethyl ethers.

## RESULTS AND DISCUSSION

The compounds studied are listed in Table 1. Apart from **9** and **20** which are derived respectively from 6-*C*-

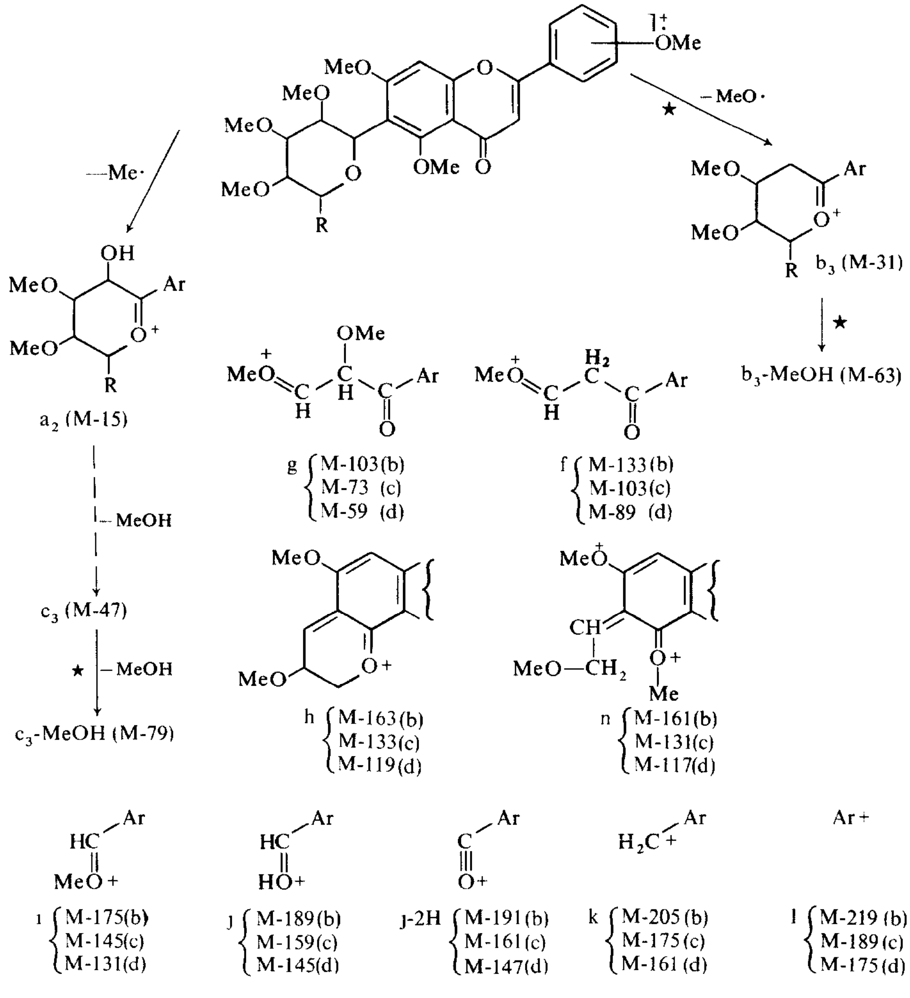
Table 1. *C*-Glycosyl flavones studied

| | Compound* |
|---|---|
| **1** | PM 7-*O*-glucosylisovitexin (PM saponarin) |
| **2** | PM 7-*O*-glucosylisoorientin (PM lutonarin) |
| **3** | PM 4′-*O*-glucosylisovitexin |
| **4** | PM 4′-*O*-glucosylisoorientin |
| **5** | PM 3′-*O*-glucosylisoorientin |
| **6** | PM 2″-*O*-glucosylisovitexin |
| **7** | PM 2″-*O*-glucosylisoorientin |
| **8** | PM 2″-*O*-rhamnosylisovitexin |
| **9** | PM 2″-*O*-rhamnosyl 6-*C*-xylosylluteolin |
| **10** | PM 4″-*O*-glucosylisovitexin |
| **11** | PM 4″-*O*-glucosylisoorientin |
| **12** | PM 6″-*O*-rhamnosylisovitexin |
| **13** | PM 6″-*O*-arabinosylisoorientin |
| **14** | 2″, 3″, 4′, 4″, 5, 6″-hexa-*O*-methyl isovitexin (7-OH free) |
| **15** | 3″, 4′, 4″, 5, 6″, 7-hexa-*O*-methylisovitexin (2″-OH free) |
| **16** | 2″, 3″, 4′, 5, 6″, 7-hexa-*O*-methylisovitexin (4″-OH free) |
| **17** | 2″, 3′, 3″, 4′. 5, 6″, 7-hepta-*O*-methylisoorientin (4″-OH free) |
| **18** | 2″, 3″, 4′, 4″, 5, 7-hexa-*O*-methylisovitexin (6″-OH free) |
| **19** | 2″, 3′, 3″, 4′, 4″, 5, 7-hepta-*O*-methylisoorientin (6″-OH free) |
| **20** | PM 2″-*O*-xylosylvitexin |

* All sugars are in the pyranose form. D-glucose, D-galactose, D-xylose are β-linked, L-rhamnose and L-arabinose are α-linked.

xylosylluteolin and 8-*C*-glucosylapigenin (vitexin), all the others are 6-*C*-glucosylapigenin (isovitexin) or 6-*C*-glucosylluteolin (isoorientin) derivatives. In addition to PM 7-*O*-glucosylisovitexin **1** and PM *x*″-O-glycosylisovitexins and isoorientins **6–8** and **10–13**, we also examined their acid hydrolysis products **14–19** which can be used as standards independent of the *O*-glycosyl moiety.

Scheme 1 shows the fragmentation pattern of PM 6-*C*-glycopyranosyl flavones, based on the study of various deuteromethyl ethers [11, 12]. The nomenclature used in [1] is extended below to the fragments derived from PM *O*-glycosyl-6-*C*-glycosylflavones.



Scheme 1. Fragmentation pattern of PM 6-*C*-glycosylflavones: (a) Ar = PM flavone radical; losses for (b) a PM *C*-hexosylflavone (R = $CH_2OMe$) (c) a PM *C*-deoxyhexosylflavone (R = Me); (d) a PM *C*-pentosylflavone (R = H); ★ metastable transition.

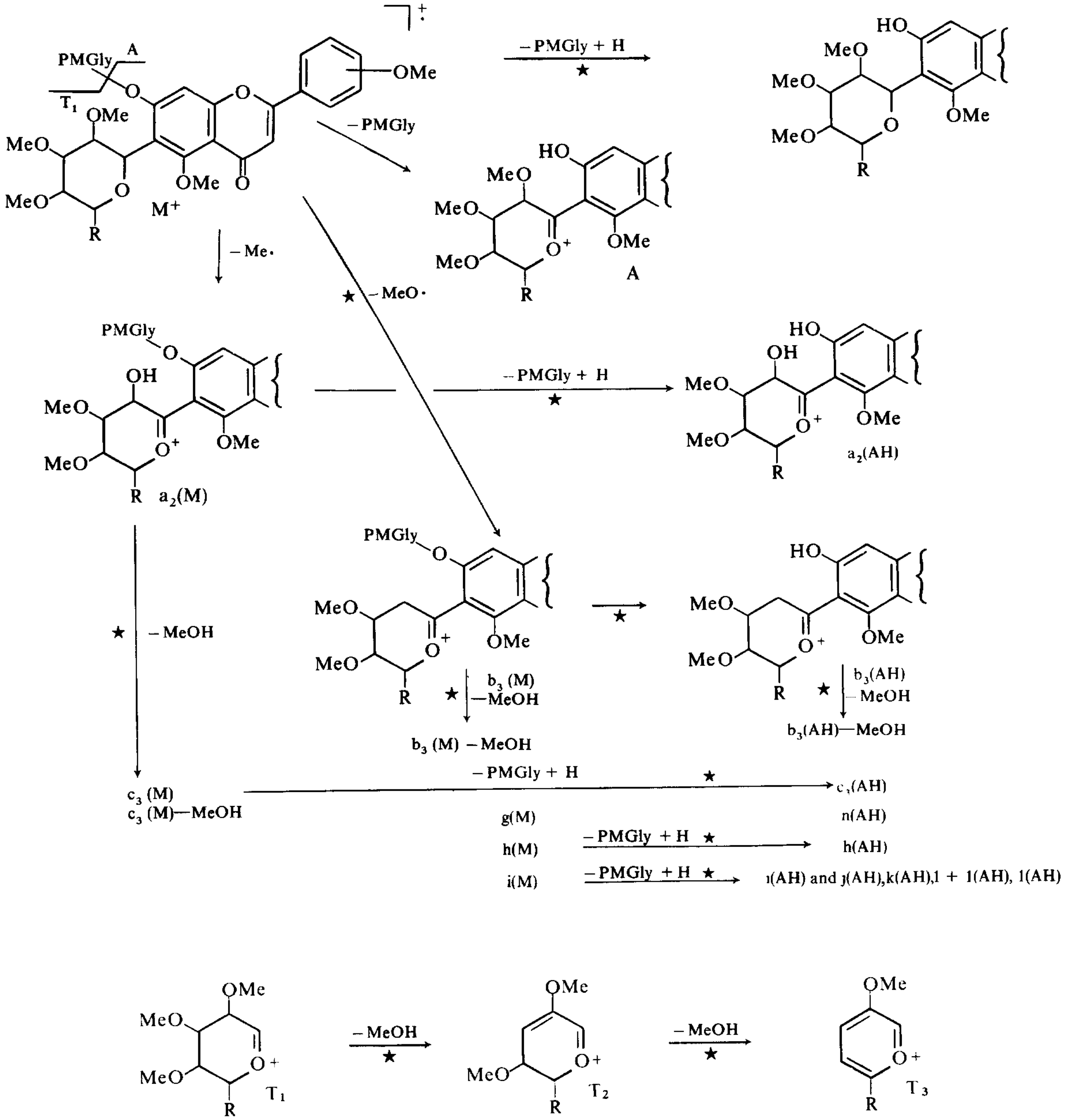
*MS of PM* x (*or* x′)-O-*glycosyl* 6-C-*glycosylflavones*

Five compounds have been permethylated: natural saponarin (7-*O*-glucosylisovitexin) from *Saponaria officinalis* [13], synthetic lutonarin (7-*O*-glucosylisoorientin) [14], natural 4′-*O*-glucosylisovitexin and 4′-*O*-glucosylisoorientin from *Gentiana lutea* [15] and 3′-*O*-glucosylisoorientin from *Gentiana nivalis* [16], leading to the permethyl ethers **1** to **5** respectively. The main fragments observed in their MS are listed in Table 2.

*PM* 7-O-*glycosyl*-6-C-*glycosylflavones*. The fragmentation pattern, deduced from the MS of **1** and **2**, is shown in Scheme 2. These MS are characterized by two homologous series of peaks, both corresponding to the fragmentation of PM 6-*C*-glycosylflavones (Scheme 1), the first series related to the molecular peak, the second to the aglycone peak AH. *Consequently, the difference M–A gives the nature* (*hexose, pentose or deoxyhexose*) *of the* 7-O-*bound sugar.*

Two peaks exhibit a much higher intensity than the corresponding ones in the MS of PM 6-*C*-glycosylflavones: $c_3$ (AH) and $b_3$-MeOH (AH), the latter being derived from $b_3$ (AH) by loss of MeOH as shown by a metastable ion. Stabilization of these two ions by the absence of 7-*O*-substitution is confirmed by the MS of **14**, the acid hydrolysis product of **1** (see Table 4).

As in the MS of PM 6-*C*-glycosylflavones, *the ion i* (*AH*) *allows identification of the* C-*bound sugar* by



Scheme 2. Fragmentation pattern of PM 7-*O*-glycosyl-6-*C*-glycosylflavones: ★ metastable transition.

comparison with A: i (AH) = AH-175 for an hexose, AH-131 for a pentose, AH-145 for a deoxyhexose; *the flavone residue can be deduced from i (AH)*: 341 for a *C*-glycosylapigenin and 371 for a *C*-glycosylluteolin derivative, rather than from the weaker ion l (AH) of the flavone residue itself. Comparison of the MS of **1** and **2** shows that the B-ring does not interfere with the fragmentation pattern. The situation is not the same when *O*-glycosylation takes place on the B-ring.

*PM x'-O-glycosyl-6-C-glycosylflavones* (**3–5**). As can be seen in Table 2, the MS of the isovitexin derivative **3** is quite different from those of the isoorientin derivatives **4** and **5** which cannot be differentiated from each other. A common feature however characterizes the three compounds as 6-*C*-glucosylflavone derivatives: the presence of M, M-15, M-31 and M-175 peaks. Their intensities are about the same in **3** as in **1** and **2**, but considerably lowered in **4** and **5**. This striking difference in the stability of the molecular ions results from the presence of a methoxyl group in the *o*-position to the *O*-glycosidic bond in the B-ring. A clear distinction can be made between the MS of **1** and **2** on the one hand and those of **3**, **4** and **5** on the other hand, by the transfer of one hydrogen atom from the *O*-glycosyl residue to the oxygen atom on rupture of the *O*-glycosidic bond, giving rise to the ion AH, the parent peak in the MS of **4** and **5**.

This transfer is always observed with PM flavone *O*-glycosides [17] and most likely takes place with **1** and

Table 2. MS data for PM *x* (or *x*′)-*O*-glucosyl 6-*C*-glucosylflavones **1–5**: *m/e* values (relative intensities %) of the main fragments (⩾5 %)

| Compound | | 1 | 2 | 3 | 4 | 5 |
|---|---|---|---|---|---|---|
| $M^+$ | | 734(43) | 764(37) | 734(37) | 764(8) | 764(9) |
| $a_2$(M) | M-15 | 719(36) | 749(28) | 719/30) | 749(3) | 749(6) |
| $b_3$(M) | M-31 | 703(100) | 733(76) | 703(100) | 733(7) | 733(17) |
| $c_1$(M) | M-45 | 689(5) | — | 689(7) | — | — |
| $c_3$(M) | M-47 | — | — | 687(17) | — | — |
| $b_3$-MeOH(M) | M-63 | 671(7) | 701(8) | — | — | — |
| g(M) | M-103 | 631(5) | — | 631(11) | — | — |
| g-2(M) | M-105 | 629(6) | — | — | — | — |
| h(M) | M-163 | 571(18) | 601(20) | 571(12) | — | — |
| i(M) | M-175 | 559(25) | 589(25) | 559(44) | — | 589(8) |
| k + 1(M) | M-204 | 530(5) | — | — | — | 560(11) |
| AH | M-218 | 516(11) | 546(12) | 516(35) | 546(100) | 546(100) |
| A = 1 (M) | M-219 | 515(36) | 545(32) | 515(5) | — | — |
| $a_2$(AH) | AH-15 | 501(43) | 531(40) | 501(5) | — | — |
| A-O | A-16 | 499(16) | 529(17) | — | — | — |
| $b_3$(AH) | AH-31 | 485(100) | 515(100) | 485(10) | — | 515(6) |
| $c_1$(AH) | AH-45 | 471(15) | 501(20) | — | — | |
| $c_3$(AH) | AH-47 | 469(21) | 499(24) | — | — | — |
| $b_3$-MeOH(AH) | AH-63 | 453(75) | 483(80) | — | — | — |
| g(AH) | AH-103 | — | 443(8) | — | — | — |
| g-2(AH) | AH-105 | 411(11) | 441(12) | — | — | — |
| n(AH) | AH-161 | 355(32) | 385(36) | 355(17) | 385(12) | 385(17) |
| h + 1(AH) | AH-162 | — | — | 354(30) | 384(30) | 384(50) |
| h(AH) | AH-163 | 353(32) | 383(40) | 353(8) | 383(5) | — |
| i(AH) | AH-175 | 341(61) | 371(64) | 341(19) | 371(9) | 371(16) |
| j(AH) | AH-189 | 327(18) | 357(20) | 327(12) | — | 357(9) |
| j-2(AH) | AH-191 | 325(18) | 355(20) | 325(14) | 355(7) | 355(9) |
| k(AH) | AH-205 | 311(14) | 341(24) | 311(10) | 341(5) | 341(8) |
| 1 + 1(AH) | AH-218 | 298(7) | 328(16) | 298(5) | — | 328(7) |
| 1(AH) | AH-219 | 297(8) | 327(12) | 297(7) | — | 327(15) |

**2** also. In that case however, the hydrogen atom can be transferred from the vicinal *C*-glycosyl instead of the *O*-glycosyl residue, thus leading to the ion A. Other characteristic features of the MS of **3**, **4** and **5** are the almost complete disappearance of the ions $a_2$ (AH), $b_3$ (AH), $c_3$ (AH) and $b_3$-MeOH (AH), the lowered abundance of the ions n (AH), h (AH) and i (AH), and the appearance of an important peak h + 1 (AH) derived from AH (metastable ion).

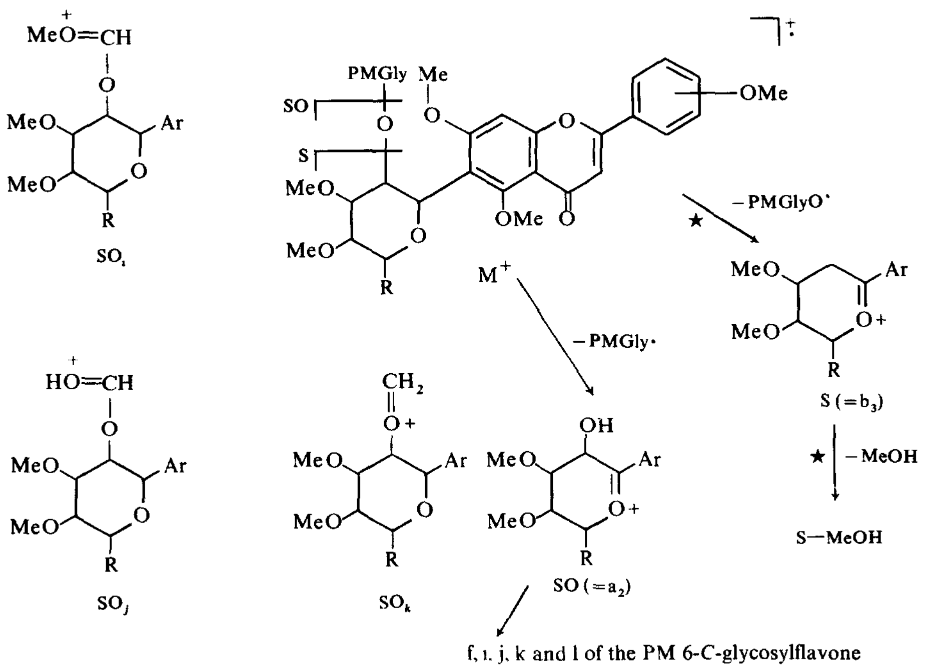
### *MS of PM 6-C-diholosylflavones*

Eight permethyl ethers of this type have been prepared: PM 2″-*O*-glucosylisovitexin **6** and PM 2″-*O*-glucosylisoorientin **7** from melosides A and L of *Cucumis melo* [9]; PM 2″-*O*-rhamnosylisovitexin **8** from synthetic 6-*C*-neohesperidosylacetin [10, 11]; PM 2″-*O*-rhamnosyl-6-*C*-xylosylluteolin **9** from the natural product of *Phlox drummondii* [18]; PM 4″-*O*-glucosylisovitexin **10** from synthetic 6-*C*-cellobiosylacacetin [11]; PM 4″-*O*-glucosylisoorientin **11** from synthetic 6-*C*-cellobiosyl-3′,4′-di-*O*-methylluteolin [11]; PM 6″-*O*-rhamnosylisovitexin **12** from synthetic 6-*C*-rutinosylacacetin [11] and PM 6″-*O*-arabinosylisoorientin **13** from the natural product of *Swertia perennis* [5].

The main fragments observed in their MS are listed in Table 3.

*PM 2″-O-glycosyl-6-C-glycosylflavones* (**6**–**9**). Their fragmentation pattern is shown in Scheme 3. The characteristic feature of their MS compared with those of PM 6-*C*-glycosylflavones, is the absence of the M-15 and M-31 peaks, replaced by the ions SO (40–60 %) and S (100 %), derived from the elimination of the permethylated 2″-*O*-glycosyl residue without and with the oxygen atom of the glycosidic bond respectively. Between the rather weak molecular peak (5–13 %) and the ion SO are found some ions $SO_i$, $SO_j$, $SO_k$, in which fragments of the *O*-glycosyl residue remain bound to SO (see the fragments i, j and k of Scheme 1). At lower *m/e* values, the most important peak corresponds to the ion j (Ar—CH=$\overset{+}{\mathrm{O}}$H) of the corresponding PM 6-*C*-glycosylflavone MS (see Scheme 1) instead of the ion i (Ar—CH=$\overset{+}{\mathrm{O}}$Me), base peak in the MS of PM 6-*C*-glycosylflavones, in which the C-1″ methoxyl group is transferred from C-3″ [11]. Fragmentation of j follows the usual path until the ion 1 corresponds to the permethylflavone residue. Comparison of the MS of **6**, **7**, **8** and **9** shows that the main features of the fragmentation pattern are not affected by the B-ring of the flavone, or by the nature of the *O*-glycosyl and *C*-glycosyl residues.

*PM 4″-O-glycosyl 6-C-glycosylflavones* (**10**, **11**). Their identification as 6-*C*-glycosylflavone derivatives immediately results from the presence of important M, M-15 and M-31 peaks. The base peak corresponds to the ion i (Ar—CH=$\overset{+}{\mathrm{O}}$Me) of the MS of the corresponding PM 6-*C*-glycosylflavone. Identification of the *O*-glycosyl residue follows from the peak S (18–25 %) being considerably lower than in the MS of PM 2″-*O*-glycosides. Excepted for $SO_{n+1}$, the ions SO and $SO_{i-k}$ are weak or very weak. At lower *m/e* values, the ion n of the corresponding PM 6-*C*-glycosylflavone is found here in higher abundance (24–36 %) and the ion n + 2 is notable



Schem 3. Fragmentation pattern of PM 2″-O-glycosyl 6-C- glycosylflavones: ★ metastable transition.

(19–21 %). Fragmentation of i follows the usual pathway until the ion l (permethylflavone residue). These MS are thus quite different from those of PM 2″-O-glycosides as well as those of x (or x′)-O-glycosides.

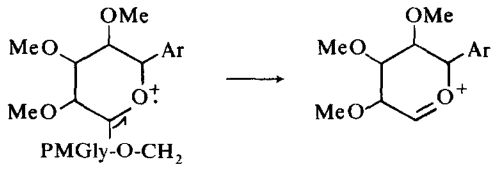
*PM 6″-O-glycosyl-6-C-glycosylflavones* (**12–13**). Their fragmentation pattern is very similar to that of 4″-O-glycosides. However, a significant difference is the presence, in the MS of 6″-O-glycosides, of a peak S-14 (*ca* 20 %) corresponding to the elimination of the C-5″ PM glycosyloxymethyl side chain (see Scheme 4). Another, less significant, difference is the greater abundance of the ions $SO_{i-k}$.

*PM 2″-O-glycosyl-8-C-glycosylflavones* (**20**). As seen above, all the MS of PM 6-C-glycosylflavone O-glycosides exhibit M, M-15 and M-31 peaks, excepted for 2″-O-glycosides. Since the M-15 and M-31 peaks are absent in the MS of PM 8-C- glycosylflavones, it was necessary to compare the MS of a PM 2″-O-glycosyl 8-C-glycosylflavone with those of PM 2″-O-glycosyl 6-C-glycosylflavones. The main peaks of the MS of PM 2″-O-xylosylvitexin **20**, prepared from the natural product of *Vitex lucens* [3], are listed in Table 3. The striking difference concerns the ion S, which is very weak here instead of being the base peak in the MS of PM 2″-O-glycosyl-6-C-glycosylflavones. Indeed, the MS of **20** is characterized by a stronger molecular peak (37 %), giving the ion SO (63 %) by loss of the PM O-glycosyl residue, the base peak j ($Ar{-}CH{=}\overset{+}{O}H$) being directly formed from SO as shown by metastable ions. Therefore, 2″-O-glycosyl 6-C-glycosylflavones can be as well distinguished from their Wessely–Moser isomers as they can be from their 4″- and 6″-isomers.

### *MS of hydrolysis products of PM O-glycosyl-6-C-glucosylflavones* (14–19)

Looking for a better distinction between 4″- and 6″-O-glycosides of 6-C-glycosylflavones led us to study the MS of the acid hydrolysis products of the PM O-glycosyl-6-C-glucosylflavones. 2″, 3″, 4′, 4″, 5, 6″-hexamethylisovitexin **14** (7-OH free) was prepared from **1**; 3″, 4′, 4″, 5, 6″, 7-hexamethylisovitexin **15** (2″-OH free) from **8**; 2″, 3″, 4′, 5, 6″, 7-hexamethylisovitexin **16** and 2″, 3′, 3″, 4′, 5, 6″, 7-heptamethylisoorientin **17** (4″-OH free) from **10** and **11**; 2″, 3″, 4′, 4″, 5, 7-hexamethylisovitexin **18** and 2″, 3′, 3″, 4′, 4″, 5, 7-heptamethylisoorientin **19** (6″-OH free) from **12** and **13**.

The main fragments observed in their MS are listed in Table 4. As expected, the MS of **14** (7-OH free) is quite different from the others and from the MS of PM isovitexin due to the appearance of strong peaks at M-32, M-32-15, M-32-31, M-32-103 (base peak), M-32-133 beside the classical fragments M, M-15, M-31, M-103, M-133, M-175 (33 % only). This strongly suggests elimination of methanol from the starting product, paralleling loss of water from free C-glycosylflavones [19]. Comparison of the MS of the hydrolysis products



Scheme 4. S-14 ion in the fragmentation pattern of 6″-O- glycosyl-6-C-glycosyl flavones.

Table 3. MS data for PM 6-*C*-diholosylflavones **6–13** and PM 8-*C*-diholosylflavone **20**: *m/e* values (relative intensities %) of the main fragments (≥5%)

| Compound | **6** | **7** | **8** | **9** | **10** | **11** | **12** | **13** | **20** |
|---|---|---|---|---|---|---|---|---|---|
| $M^+$ | 734(4) | 764(5) | 704(13) | 690(8) | 734(22) | 764(66) | 704(19) | 720(17) | 690(37) |
| M-15 | — | — | — | — | 719(6) | 749(15) | 689(10) | 705(7) | — |
| M-31 | — | — | — | — | 703(18) | 733(42) | 673(22) | 689(23) | — |
| $SO_{n+1}$ | — | — | — | — | 574(14) | 604(19) | — | — | — |
| $SO_i$ | 559(6) | — | 559(6) | 545(9) | — | — | 559(15) | 589(21) | — |
| $SO_j$ | 545(7) | — | 545(13) | 531(17) | — | — | 545(12) | 575(7) | 545(8) |
| $SO_k$ | 529(8) | — | — | 515(25) | — | — | 529(8) | 559(5) | — |
| SO | 515(42) | 545(44) | 515(59) | 501(60) | — | 545(6) | 515(13) | 545(15) | 515(63) |
| SO-2 | 513(9) | 543(6) | — | 499(23) | — | 543(11) | 513(23) | 543(16) | — |
| S+ 2 | — | — | — | — | — | — | 501(10) | 531(13) | — |
| S | 499(100) | 529(100) | 499(100) | 485(100) | 499(18) | 529(25) | 499(14) | 529(16) | 499(8) |
| S-14 | — | — | — | — | — | — | 485(21) | 515(22) | 485(6) |
| S-32 | 467(6) | 497(7) | 467(6) | 453(7) | 467(8) | 497(11) | 467(8) | 497(9) | 467(10) |
| S-14-32 | — | — | — | — | — | 483(7) | 453(7) | 483(9) | — |
| g | — | — | — | — | 427(5) | — | 427(13) | 457(10) | — |
| g-2 | — | — | — | — | 425(5) | — | 425(5) | 455(14) | — |
| f | 397(7) | — | 397(7) | — | — | — | 397(11) | 427(7) | 397(5) |
| n + 2 | — | — | — | — | 371(19) | 401(21) | 371(15) | 401(6) | — |
| n | — | — | — | — | 369(24) | 399(36) | 369(32) | 399(50) | — |
| h | — | — | — | — | 367(8) | 397(13) | 367(15) | 397(15) | — |
| i | 355(10) | 385(7) | 355(7) | 385(20) | 355(100) | 385(100) | 355(100) | 385(100) | 355(13) |
| j | 341(42) | 371(34) | 341(61) | 371(88) | 341(25) | 371(27) | 341(26) | 371(23) | 341(100) |
| j-2H | 339(8) | 369(5) | 339(7) | 369(8) | 339(8) | 369(13) | 339(11) | 369(14) | 339(9) |
| k | 325(14) | 355(9) | 325(13) | 355(12) | 325(10) | 355(13) | 325(15) | 355(25) | 325(47) |
| k-2 | 323(6) | — | 323(8) | 353(8) | 323(8) | 353(11) | 323(15) | 353(14) | — |
| l + 1 | — | — | 312(8) | 342(8) | — | 342(11) | 312(7) | 342(6) | 312(22) |
| l | 311(8) | 341(5) | 311(8) | 341(8) | 311(7) | 341(13) | 311(13) | 341(16) | 311(13) |
| l-2 | — | — | — | — | — | — | 309(5) | 339(11) | — |

Table 4. MS data for the hydrolysis products of some PM C-glucosylflavones **14–19**: *m/e* values (relative intensities %) of the main fragments (≥5%)

| Compound | **14** | **15** | **16** | **17** | **18** | **19** |
|---|---|---|---|---|---|---|
| $M^+$ | 516(8) | 516(69) | 516(37) | 546(26) | 516(31) | 546(41) |
| M-15 | — | 501(6) | 501(24) | 531(27) | 501(26) | 531(32) |
| M-17 | — | 499(13) | — | — | — | — |
| M-31 | 485(75) | 485(15) | 485(100) | 515(100) | 485(97) | 515(100) |
| M-32 | 484(57) | — | — | — | — | — |
| M-45 | — | 471(38) | 471(8) | 501(7) | — | 501(5) |
| M-47 | 469(71) | 469(19) | — | 499(6) | 469(6) | 499(6) |
| M-63 | 453(57) | 453(7) | — | 483(5) | 453(7) | 483(7) |
| M-79 | 437(13) | — | — | — | — | — |
| M-89 | — | — | 427(9)g | 457(9)g | 427(22)g | 457(23)g |
| M-95 | 421(11) | — | — | — | — | — |
| M-103 | 413(8)g | 413(36) | — | — | 413(7) | — |
| M-105 | — | 411(19) | — | — | 411(6) | — |
| M-119 | — | 397(6) | 397(8)f | 427(8)f | 397(12)f | 427(9)f |
| M-121 | — | — | 395(9) | 425(11) | — | — |
| M-131 | — | 385(5) | 385(11) | — | 385(23) | — |
| M-133 | 383(15)f | 383(9) | — | — | 383(7) | 413(9) |
| M-135 | 381(100) | — | — | — | — | — |
| M-149 | 367(17) | 367(6) | 367(18)h | 397(24)h | 367(22)h | 397(23)h |
| M-161 | 355(14) | 355(23)i | 355(41)i | 385(47)i | 355(100)i | 385(100)i |
| M-163 | 353(17) | 353(11) | 353(6) | 383(7) | 353(12) | — |
| M-165 | 351(40) | 351(6) | — | — | — | — |
| M-175 | 341(33)i | 341(100)j | 341(63)j | 371(86)j | 341(48)j | 371(41)j |
| M-177 | 339(18) | 339(11) | 339(7) | — | 339(16) | 369(16) |
| M-189 | 327(11)j | 327(30) | 327(9) | 357(21) | 327(6) | — |
| M-191 | 325(16) | 325(27)k | 325(12)k | 355(19)k | 325(21)k | 355(23)k |
| M-193 | 323(12) | 323(15) | 323(11) | 353(14) | 323(14) | 353(16) |
| M-205 | 311(18)k | 311(27)l | 311(11)l | 341(20)l | 311(17)l | 341(18)l |
| M-207 | 309(19) | — | — | — | — | — |
| M-218 | 298(9) | — | — | — | — | — |
| M-219 | 297(12) | 297(8) | 297(6) | 327(9) | 297(7) | 327(5) |

derived from PM 6-*C*-diholosylflavones led to the desired results, since a new and better criterion was found to differentiate 4″- from 6″-*O*-glycosides. Here again, the MS of **15** (2″-OH free) is different from the others. The M-15 and M-31 peaks are very weak, but their presence shows some participation of methoxyl groups in positions other than 2″. The unusual ion M-17 results from the elimination of 2″-OH. The most important peaks are given by the ions $M^+$, M-45, M-103 and j (base peak). As for the *O*-glycosides themselves, the ion i is much weaker than j. In the MS of **16**, **17** (4″-OH free) and **18**, **19** (6″-OH free), the M, M-15 and M-31 peaks are again as intense as they are in the MS of PM isovitexin and M-31 is even higher than in the latter, being the base peak in most cases. The most significant differences between **16** or **17** and **18** or **19** are in the relative abundances of the ions i ($\text{Ar—CH}=\overset{+}{\text{O}}\text{Me}$, M-161) and j ($\text{Ar—CH}=\overset{+}{\text{O}}\text{H}$, M-175). In the MS of **16** and **17**, j (about 60%) > i (about 40%) whereas i (base peak) > j (<50%) in the MS of **18** and **19**, which, in this respect, do not differ from those of PM-6-*C*-glycosylflavones.

It can be concluded that permethylation and mass spectrometry afford a simple way for structural determination of *O*-glycosyl 6-*C*-glycosylflavones on a microscale, since 7-, 4′- (or 3′-) and *x*″-*O*-glycosides can be distinguished from each other. 2″-*O*-glycosides can be identified at first glance from the absence of M-15 and M-31 peaks (respectively replaced by M-permethylglycosyl and M-permethylglycosyloxy) in the MS of their permethyl ethers. In all others, the 6-*C*-glycosylation immediately appears from the presence of M-15 and M-31 peaks. 7-*O*-glycosides are characterized by a parallel fragmentation of the aglycone, absent in the other MS, and 6″-*O*-glycosides by elimination of the 5″ permethylglycosyloxymethyl side chain. Further differentiation of PM 4″- and 6″-*O*-glycosides can be obtained from the MS of their acid hydrolysis products.

## EXPERIMENTAL

Permethylation was carried out using the method previously described in [1]. Acid hydrolyses were carried out, in sealed tubes, with 4N HCl–MeOH (1:1) at 120° for 1–2 hr. Pure PM *O*-glycosyl *C*-glycosyl flavones and their hydrolysis products can be obtained by TLC (Si gel) using $CHCl_3$–EtOAc–$Me_2CO$ (5:1:4) and (5:4:1) respectively. MS were recorded on an AEI MS 902 Spectrograph, to 70 eV. Temps (sample and source in the same order) varied between 150 and 190°.

*Acknowledgements*—This work was supported by CNRS (AI 031196). MS spectra were registered in the Centre de Spectrométrie de masse de Lyon, Université Lyon I, France. The authors thank Prof. A. Jacot-Guillarmod and Dr. K. Hostettmann (Institut de Chimie of the Neuchâtel University, Switzerland), Dr. R. M. Horowitz (Fruit and Vegetable Chemistry Laboratory, Pasadena, USA), Prof. W. Rahman (Department of Chemistry, Aligarh Muslim University, Aligarh, India) and Dr. B. Monties (INRA, Centre de Grignon, France) for samples of natural compounds.

## REFERENCES

1. Bouillant, M. L., Favre-Bonvin, J. and Chopin, J. (1975) *Phytochemistry* **14**, 2267.
2. Mabry, T. J., Markham, K. R. and Thomas, M. B. (1970) *The Systematic Identification of Flavonoids.* Springer-Verlag, Berlin.
3. Horowitz, R. M. and Gentili, B. (1966) *Chem. Ind.* (*Lond.*) 625.
4. Batyuk, V. S., Chernobrovaya, N. V. and Prokopenko, A. P. (1966) *Khim. Prir. Soedin.* **2**, 90.
5. Hostettmann, K. and Jacot-Guillarmod, A. (1976) *Helv. Chim. Acta* **59**, 1584.
6. Nikolov, N. (1971) *Farmatsiya* (*Sofia*) **21**, 42; Nikolov, N., Horowitz, R. M. and Gentili, B. (1976) *Intl. Congress for Research on Medicinal Plants.* Section A, Munich.
7. Chopin, J., Dellamonica, G., Bouillant, M. L., Besset, A., Popovici, G. and Weissenbock, G. (1977) *Phytochemistry* **16**, 2041.
8. Bouillant, M. L., Favre-Bonvin, J. and Chopin, J. (1975) *Convegno internazionale sui polifenoli,* Gargnano sul Garda, p. 232.
9. Monties, B., Bouillant, M. L. and Chopin, J. (1976) *Phytochemistry* **15**, 1053.
10. Chopin, J., Besset, A. and Bouillant, M. L. (1977) *Compt. Rend.* **284**, 1007.
11. Besset, A. (1977) Thèse de Doctorat de 3è cycle, Lyon, n° 659.
12. Bouillant, M. L. (1976) Thèse de Doctorat d'Etat, Lyon, n° 7652.
13. Barger, G. (1906). *J. Chem. Soc.* **89**, 1210.
14. Planche, G., Bouillant, M. L. and Chopin, J. unpublished results.
15. Hostettmann, K., Bellmann, G., Tabachi, R. and Jacot-Guillarmod, A. (1973) *Helv. Chim. Acta* **56**, 3050.
16. Hostettmann, K. and Jacot-Guillarmod, A. (1974) *Helv. Chim. Acta* **57**, 204.
17. Mabry, T. J. (1975) *Mass Spectrometry of Flavonoids,* in *The Flavonoids* p. 122. Chapman & Hall, London.
18. Mabry, T. J., Yoshioka, H., Sutherland, S., Woodland, S., Rahman, W., Ilyas, M., Usmani, J. N., Hameed, N., Chopin, J. and Bouillant, M. L. (1971) *Phytochemistry* **10**, 677.
19. Prox, A. (1968) *Tetrahedron* **24**, 3697.