Phytochemistry, Vol 23, No 11, pp 2653–2657, 1984
Printed in Great Britain

0031–9422/84 $3 00 + 0 00

STRUCTURAL DETERMINATION OF 6-*C*-DIGLYCOSYL-8-*C*-GLYCOSYLFLAVONES AND 6-*C*-GLYCOSYL-8-*C*-DIGLYCOSYLFLAVONES BY MASS SPECTROMETRY OF THEIR PERMETHYL ETHERS

M L BOUILLANT, F FERRERES DE ARCE*, J FAVRE-BONVIN, J CHOPIN, A ZOLL† and G MATHIEU†

Laboratoires de Chimie Biologique et de Phytochimie, Université Claude Bernard (Lyon I), 69622 Villeurbanne Cedex, France, †Laboratoire de Pharmacognosie, Faculté de Pharmacie, Université de Dijon, 21033 Dijon Cedex, France

(*Received* 27 *February* 1984)

Key Word Index—*Spergularia rubra*, *Stellaria holostea*, Caryophyllaceae, *C*-glycosylflavones, vicenin-2 X‴-*O*-glucoside diferuloylester, vicenin-2 6″-*O*-glucoside, schaftoside 6″-*O*-glucoside, 6-*C*-cellobiosyl-8-*C*-glucosylacacetin, permethyl ethers, EIMS

Abstract—Permethylated 6-*C*-diglycosyl-8-*C*-glycosylflavones and 6-*C*-glycosyl-8-*C*-diglycosylflavones gave well defined EIMS including the molecular peak and a fragmentation pattern characteristic of the 6-*C*-glycosyl residue X‴-*O*-glycosides (8-*C*-disaccharides) are thus easily distinguished from X″-*O*-glycosides (6-*C*-disaccharides) and, in the latter, the position of the *O*-glycosidic bond should be deduced from the MS, after acid hydrolysis Three new *C*-glycosylflavones have been characterized in this way from *Spergularia rubra* and *Stellaria holostea*

INTRODUCTION

In the first paper of this series [1], mass spectrometry of permethylated 5,7-dihydroxy-6,8-di-*C*-glycosylflavones has been shown to be extremely useful in the structural study of these natural compounds, because the observed fragmentation pattern, governed by the sugar in position 6, allows identification of the latter as an hexose, pentose or deoxyhexose In the following papers [2–6], the work was extended to *O*-glycosyl-6-*C*-glucosylflavones It has been shown that the determination of an *O*-glycosidic bond position in 6-*C*-glycosylglucosylflavones is possible from the MS of their permethyl ethers and of the hydrolysis products of the latter In the present paper, we now show that permethylated X″-(substitution on the sugar in position 6) or X‴-(substitution on the sugar in position 8) *O*-glycosyl-6,8-di-*C*-glycosylflavones give well-defined EIMS including the molecular peak The MS of X‴-*O*-glycosides are easily distinguished from those of X″-*O*-glycosides In the latter, the position of the *O*-glycosidic bond may be deduced from the fragmentation pattern of the permethyl ethers and of their hydrolysis products In this way, a diferuloyl ester of a 6-*C*-glucosyl-8-*C*-glucosylglucosylapigenin (vicenin-2 X‴-*O*-glucoside) from *Spergularia rubra*, 6-*C*-(6-*O*-glucosylglucosyl)-8-*C*-glucosylapigenin (vicenin-2 6″-*O*-glucoside) and 6-*C*-(6-*O*-glucosylglucosyl)-8-*C*-arabinosylapigenin (schaftoside 6″-*O*-glucoside) from *Stellaria holostea* have been characterized

RESULTS AND DISCUSSION

The existence of β-*O*-glucosides of 6,8-di-*C*-glycosylflavones in which the *O*-glucosyl residue is attached to one of the *C*-glycosyl residues has been reported by Ockendon *et al* [7] in *Psoralea* on the basis of UV data and β-glucosidase hydrolysis, but nothing more was known about the position of the *O*-glucosyl residue During a study of the butanol-soluble fraction of ethanolic extracts from aerial parts of the Caryophyllaceae *Spergularia rubra* and *Stellaria holostea*, three compounds were isolated, which all gave glucose accompanied by vicenin-2 (6,8-di-*C*-β-D-glucopyranosylapigenin) or schaftoside (6-*C*-β-D-glucopyranosyl-8-C-α-L-arabinopyranosylapigenin) on acid hydrolysis From the UV data, glucose is bound to the *C*-glycosyl residues of vicenin-2 or schaftoside in these compounds

Compound **1**, isolated from *Spergularia rubra*, gave an apigenin-like UV spectrum with a stronger band I λ_{max} 272 nm (0 55) and 328 nm (0 82) However diagnostic shifts with $AlCl_3$, $AlCl_3$ + HCl, NaOAc and NaOMe showed free 5, 7 and 4′ hydroxyl groups [8] Alkaline hydrolysis led to ferulic acid and a new compound **1′** which gave the same UV spectrum and diagnostic shifts as apigenin Acid hydrolysis of **1′** led to vicenin-2 and glucose From the UV data, ferulic acid and glucose are not bound to phenolic hydroxyl groups When **1** was permethylated, the EIMS of the main band isolated by TLC of the permethylation mixture showed a fragmentation pattern characteristic of a PM 6-*C*-hexosyl-8-*C*-glycosylflavone $[M]^+$, $[M-15]^+$, $[M-31]^+$, $[M-47]^+$, $[M-63]^+$, $[M-103]^+$, $[M-163]^+$, $[M-175]^+$, $[M-189]^+$, $[M-205]^+$ [1], with the usual metastable ion corresponding to the transition M → M − 31 [2, 6] However the corresponding molecular peak was found at *m/z* 924, i e 28 m u less than the expected value for a PM O-hexosylvicenin-2 This difference can be explained by the presence of two feruloyl groups in **1** (in agreement with the higher absorption at 328 nm than at 272 nm) because they can be lost after permethylation by transesterification or saponification during the processing of the reaction mixture, leaving two free hydroxyl groups in the reaction product If so, these hydroxyl groups

*Present address Centro de Edafologia y Biologia Aplicada del Segura, C S I C, Murcia, Spain

cannot belong to the 6-*C*-glycosyl residue since the latter must be a fully methylated hexosyl residue from the observed fragmentation pattern Therefore, they belong to the 8-*C*-glycosyl residue which must be a 8-*C*-glucosylglucosyl one since acid hydrolysis of **1'** gives glucose and vicenin-2

When the above permethyl derivative of **1** was hydrolysed with HCl, a new product could be separated on TLC, the EIMS of which again showed the characteristic fragmentation pattern of a PM 6-*C*-hexosyl-8-*C*-glycosylflavone, with a molecular peak at *m/z* 734, i e 14 m u less than PM vicenin-2, in agreement with the presence of only one free hydroxyl group on the 8-*C*-glucosyl residue resulting from the hydrolysis of the *O*-glucosidic bond In order to verify that the presence of a free hydroxyl group on the 8-*C*-glycosyl residue does not affect the fragmentation pattern, a standard compound was prepared by tritylation of vicenin-1 (6-*C*-*β*-D-xylopyranosyl-8-*C*-*β*-D-glucopyranosylapigenin, **5**), tritylation taking place at the 6-CH_2OH of the 8-*C*-glucosyl residue The resulting 6'''-*O*-tritylvicenin-1 (**6**) was permethylated and the permethyl derivative was detritylated with acid, leading to the expected 6'''-desmethyl PM vicenin-1 (**7**) bearing a free hydroxyl group on the 8-*C*-glucosyl residue as shown by the molecular peak at *m/z* 690 found in its MS The observed fragmentation pattern of the fully methylated 6-*C*-xylosyl residue was the same as in the MS of PM vicenin-1 [1], demonstrating its independence from the presence of the free 6'''-hydroxyl group

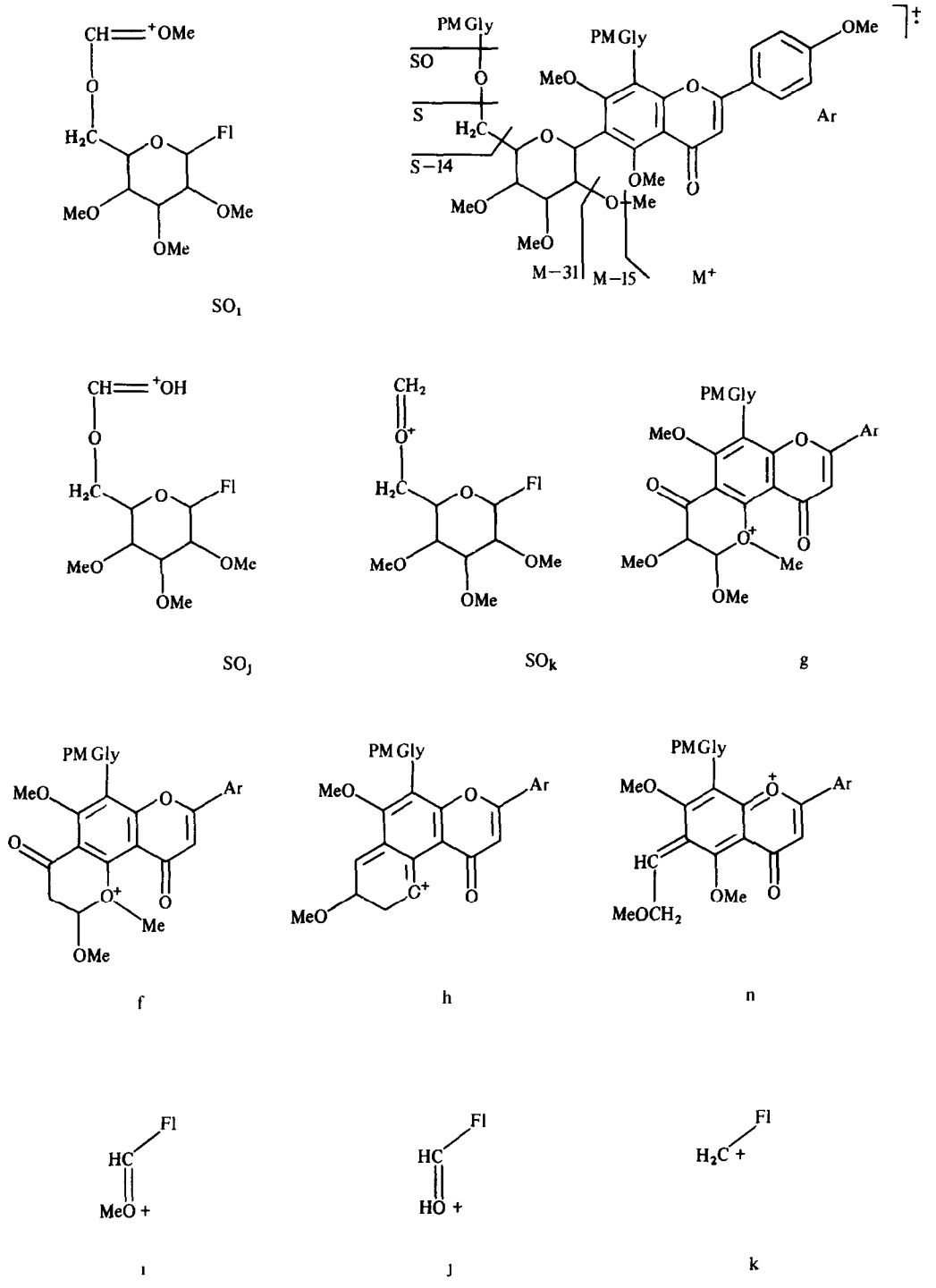
It can thus be concluded that, in **1**, ferulic acid was attached to two hydroxyl groups of the *O*-glucosyl residue linked to the 8-*C*-glucosyl moiety of vicenin-2 However, the fragmentation pattern being that of the PM-6-*C*-glucosyl moiety, the positions of the *O*-feruloyl groups and of the *O*-glucosidic bond cannot be deduced from the MS of PM derivatives and the proposed structure for **1** is thus X'''-*O*-(x,y-di-*O*-feruloylglucosyl)-vicenin-2 or apigenin-6-*C*-glucoside-8-*C*-(x,y-di-*O*-feruloylglucosyl)glucoside

The above results show that PM X'''-*O*-glycosyl-6,8-di-*C*-glycosylflavones (PM flavones 6-*C*-glycosides-8-*C*-glycosylglycosides) and their hydrolysis products give well-defined EIMS including the molecular peak and the same fragmentation pattern as that of the corresponding PM-6,8-di-*C*-glycosylflavones

From *Stellaria holostea*, two compounds, **2** and **3**, were isolated which showed striking similarities with **1** from *Spergularia rubra* in their UV spectra and hydrolysis products, since both compounds gave glucose on acid hydrolysis besides vicenin-2 from **2** and schaftoside from **3** When **2** and **3** were permethylated, the EIMS of their PM derivatives showed the same fragmentation pattern, characteristic of a PM 6''-*O*-glycosyl-6-*C*-glycosylflavone $[M]^+$, $[M-15]^+$, $[M-31]^+$, $[SO]^+_i$, $[SO]^+_j$, $[SO]^+_k$, $[SO]^+$, $[SO-2]^+$, $[S+2]^+$, $[S]^+ < [S-14]^+$, $[S-32]^+$, $[S-14-32]^+$, $[g]^+$, $[f]^+$, $[n]^+$, $[h]^+$, $[i]^+$, $[j]^+$, $[k]^+$ (see ref [2] and Scheme 1) The only differences were the higher importance of the ions $[M]^+$, $[M-15]^+$, $[M-31]^+$ (a common feature of the MS of PM 6,8-di-*C*-glycosylflavones when compared to those of PM-6-*C*-glycosylflavones [1]) and the presence of an as yet unexplained important ion $[M-158]^+$ The molecular peaks found at *m/z* 952 for PM **2** and 908 for PM **3** corresponded to a PM *O*-hexosylvicenin-2 and a PM *O*-hexosylschaftoside, respectively In order to confirm the 6''-position of the *O*-glucosyl residue, PM **2** and PM **3** were hydrolysed with acid and the new products separated on TLC Their MS again showed the same fragmentation pattern, characteristic of 6''-desmethyl PM 6-*C*-glucosylapigenin $[M]^+$, $[M-15]^+$, $[M-31]^+$, $[M-89]^+$, $[M-149]^+$, $[M-161]^+$, $[M-175]^+$, $[M-191]^+$ with the relative importances $[M-31]^+ > [M]^+$, $[M-161]^+ > [M-175]^+$ and $[M-89]^+ \gg [M-103]^+$ [4] Again the only differences were the higher importance of the ions $[M]^+$, $[M-15]^+$, $[M-31]^+$ (in agreement with a PM 6,8-di-*C*-glycosylflavone) and of the ion h $[M-149]^+$ The molecular peaks were found at *m/z* 734 and 690, i e 14 m u less than PM vicenin-2 and PM schaftoside, respectively, in agreement with the presence of only one free hydroxyl group on the 6-*C*-glucosyl residue resulting from the hydrolysis of the *O*-glucosidic bond A direct confirmation of the 6''-position of this free hydroxyl group in the product derived from PM **3** could be obtained by tritylation of schaftoside **8** on the 6-CH_2OH of the 6-*C*-glucosyl moiety, permethylation of the resulting 6''-tritylschaftoside (**9**) and detritylation of the latter with acid The resulting 6''-desmethyl PM schaftoside (**10**) showed the same MS as the acid hydrolysis product derived from PM **3** and could not be separated from it on TLC It follows that **3** is 6''-*O*-glucosylschaftoside (apigenin 6-*C*-(6-*O*-glucosylglucoside)-8-*C*-arabinoside) and **2** is 6''-*O*-glucosylvicenin-2 (apigenin 6-*C*-(6-*O*-glucosylglucoside)-8-*C*-glucoside)

The above MS data suggest that the EIMS of PM 6-*C*-glycosylglycosyl-8-*C*-glycosylflavones show the same fragmentation pattern as the MS of the corresponding PM 6-*C*-glycosylglycosylflavones In order to verify this assumption, a standard compound was synthesized from natural cytisoside (8-*C*-glucosylacacetin) by 6-*C*-glycosylation [9] with the easily accessible acetobromocellobiose The obtained 6-*C*-cellobiosyl-8-*C*-glucosylacacetin (acacetin 6-*C*-(4-*O*-glucosylglucoside)-8-*C*-glucoside, **4**) was permethylated As expected from the above data, the EIMS of its PM derivative showed the molecular peak at *m/z* 952 and the same fragmentation pattern as the MS of PM 6-*C*-cellobiosylacacetin $[M]^+$, $[M-15]^+$, $[M-31]^+$, $[SO_n+1]^+$, $[SO_i]^+$, $[SO_j]^+$, $[SO]^+$, $[SO-2]^+$, $[S]^+ > [S-14]^+$, $[S-32]^+$, $[g]^+$, $[f]^+$, $[n]^+$, $[h]^+$, $[i]^+$, $[j]^+$, $[k]^+$ [2], with a higher importance of $[M]^+$, $[M-15]^+$, $[M-31]^+$ and $[h]^+$ No important peak was found at $[M-158]$ From the MS comparison of PM **4** and PM **2**, it can be concluded that the differences previously observed [2] between the EIMS of PM 4''-*O*-glycosyl-6-*C*-glucosylflavones and PM 6''-*O*-glycosyl-6-*C*-glucosylflavones are not affected by the addition of a 8-*C*-glycosyl residue Since it has been previously shown [2–4] that the determination of *O*-glycosidic bond position in 6-*C*-glycosylglycosylflavones is possible from the MS of their PM ethers and of the hydrolysis products of the latter, we can now extend this possibility to 6-*C*-glycosylglycosyl-8-*C*-glycosylflavones with a reasonable certainty and propose the following conclusions

PM 6,8-*C*-glycosylflavones *O*-glycosides in which the *O*-sugar is linked to one *C*-sugar give well-defined EIMS including the molecular peak and a fragmentation pattern characteristic of the 6-*C*-glycosyl residue, thus allowing to distinguish easily 6-*C*-monosaccharidic residues (as found in X'''-*O*-glycosides, i e 6-*C*-glycosides-8-*C*-glycosylglycosides) from 6-*C*-disaccharidic residues (as found in X''-*O*-glycosides, i e 6-*C*-glycosylglycosides-8-*C*-glycosides)



Scheme 1 Fragmentation pattern of PM 6-*C*-(6-*O*-glycosylglucosyl)-8-*C*-glycosylapigenins Fl = permethyl 8-*C*-glycosylflavone residue

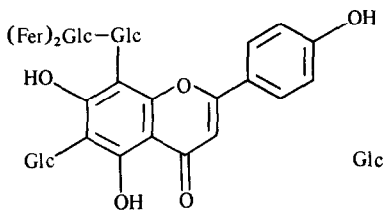
Moreover, in the latter case, the position of the *O*-glycosidic bond should be deduced from the fragmentation patterns of the PM ether and its acid hydrolysis product, as previously shown with PM acacetin 6-*C*-glycosylglycosides, because these fragmentation patterns have been shown to be independent from the presence of a 8-*C*-glycosyl residue for 6″- and 4″-*O*-glycosides and the same behaviour is expected for 2″- and 3″-*O*-glycosides

EXPERIMENTAL

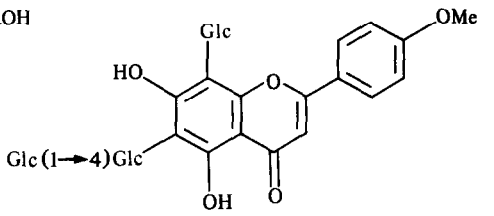
EIMS were recorded on an AEIMS 902 spectrograph Perméthylation, tritylation and acid hydrolysis of permethyl and trityl derivatives were carried out as previously described [6]

Plant material and isolation Spergularia rubra see ref [5], *Stellaria holostea* see ref [10]

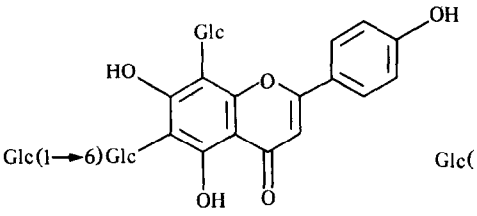
Apigenin-6-C-glucoside-8-C-(x,y-di-O-feruloylglucosyl)gluco-



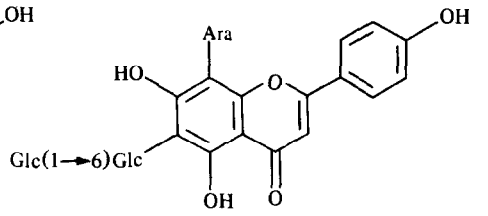
1



4

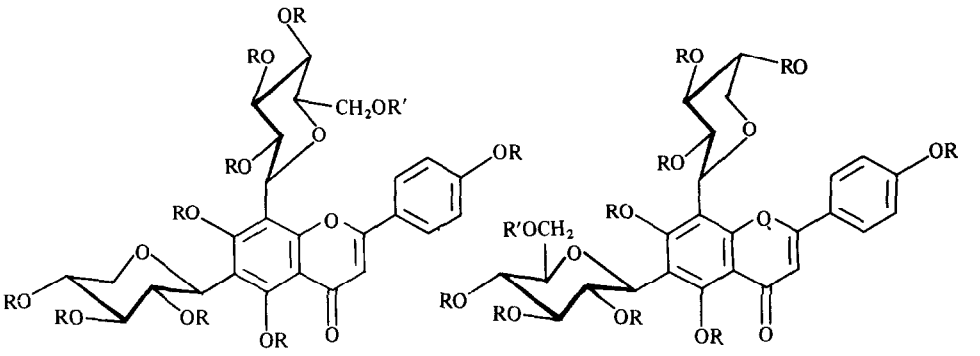


2



3

Fer = feruloyl, Glc = glucosyl, Ara = α–L– arabinopyranosyl



5 R = R' = H
6 R = H, R' = C(C$_6$H$_5$)$_3$
7 R = Me, R' = H

8 R = R' = H
9 R = H, R' = C(C$_6$H$_5$)$_3$
10 R = Me, R' = H

side (**1**) Colour UV dark purple, +AlCl$_3$ yellow, +basic lead acetate orange–red TLC (cellulose) R_f 0·66 (vicenin-2 0·40) (15% HOAc), 0·63 (vicenin-2 0·37) (BAW) UV λ_{max}^{MeOH} nm 272, 300 sh, 328, +NaOMe 280, 336 sh, 386, +AlCl$_3$ and AlCl$_3$ +HCl 278, 300, 334, 380 sh, +NaOAc 281, 317 sh, 332, 387 Alkaline hydrolysis (4 N NaOH + MeOH (1 1), N$_2$, 12 hr, room temp) gave ferulic acid (UV, TLC) and **1'** (UV dark purple, +AlCl$_3$ yellow, +basic lead acetate yellow, same UV spectrum and diagnostic shifts as apigenin) Acid hydrolysis (2 N HCl + MeOH (1 1), 2 hr, 100°) of **1'** gave vicenin-2 (UV, TLC) and glucose (TLC)

Permethyl derivative of **1** EIMS (70 eV) m/z > 700 (rel int) 924 [M]$^+$ (29), 909 [M − 15]$^+$ (29), 893 [M − 31]$^+$ (100), 879 [M − 45]$^+$ (6), 877 [M − 47]$^+$ (9), 863 [M − 61]$^+$ (6), 861 [M − 63]$^+$ (4), 821 [M − 103]$^+$ (12), 791 [M − 133]$^+$ (4), 763 [M − 161]$^+$ (10), 761 [M − 163]$^+$ (27), 749 [M − 175]$^+$ (29), 735 [M − 189]$^+$ (6), 719 [M − 205]$^+$ (9), metastable ion 863 (M → M − 31) Acid hydrolysis product EIMS (70 eV) m/z > 500 (rel int) 734 [M]$^+$ (32), 719 [M − 15]$^+$ (28), 703 [M − 31]$^+$ (100), 689 [M − 45]$^+$ (14), 687 [M − 47]$^+$ (11), 671 [M − 63]$^+$ (7), 631 [M − 103]$^+$ (14), 601 [M − 133]$^+$ (5), 573 [M − 161]$^+$ (11), 571 [M − 163]$^+$ (32), 559 [M − 175]$^+$ (37), 545 [M − 189]$^+$ (15), 529 [M − 205]$^+$ (8), métastable ion 673 (M → M − 31) TLC (silica gel) R_f 0·52, PM 1 0·70 (CHCl$_3$–EtOAc–Me$_2$CO, 5 1 4)

6'''-Desmethyl PM vicenin-1 (**7**) EIMS (70 eV) m/z > 450 (rel int) 690 [M]$^+$ (7), 675 [M − 15]$^+$ (22), 659 [M − 31]$^+$ (100), 643 [M − 47]$^+$ (12), 631 [M − 59]$^+$ (7), 629 [M − 61]$^+$ (10), 627 [M − 63]$^+$ (7), 601 [M − 89]$^+$ (4), 571 [M − 119]$^+$ (22), 559 [M − 131]$^+$ (20), 545 [M − 145]$^+$ (12), 529 [M − 161]$^+$ (13), 515 [M − 175]$^+$ (7)

Apigenin 6-C-(6-O-*glucosylglucoside*)-8-C-*glucoside* (**2**) Colour UV dark purple, +AlCl$_3$ yellow, +basic lead acetate yellow TLC (cellulose) R_f 0·50 (vicenin-2 0·40) (15% HOAc), 0·26 (vicenin-2 0·37) (BAW) UV λ_{max}^{MeOH} nm 272, 330, +NaOMe 282, 334, 400, +AlCl$_3$ and AlCl$_3$ +HCl 280, 305, 350, 380, +NaOAc 282, 336, 392 Acid hydrolysis gave vicenin-2 (UV, TLC) and glucose (TLC)

Permethyl derivative of **2** EIMS (70 eV) m/z > 500 (rel int)

952 $[M]^+$ (70), 937 $[M-15]^+$ (36), 921 $[M-31]^+$ (100), 794 (93), 777 $[M-175, SO_i]^+$ (46), 763 $[M-189, SO_j]^+$ (30), 747 $[M-205, SO_k]^+$ (26), 733 $[M-219, SO]^+$ (56), 731 $[SO-2]^+$ (43), 719 $[S+2]^+$ (30), 717 $[M-235, S]^+$ (26), 703 $[S-14]^+$ (50), 685 $[S-32]^+$ (20), 671 $[S-14-32]^+$ (23), 645 $[g]^+$ (33), 615 $[f]^+$ (20), 587 $[n]^+$ (76), 585 $[h]^+$ (80), 573 $[i]^+$ (86), 559 $[j]^+$ (33), 543 $[k]^+$ (23), metastable ion 891 ($M \rightarrow M-31$) Acid hydrolysis product EIMS (70 eV) $m/z > 500$ (rel int) 734 $[M]^+$ (16), 719 $[M-15]^+$ (34), 703 $[M-31]^+$ (100), 687 $[M-47]^+$ (4), 671 $[M-63]^+$ (6), 645 $[M-89]^+$ (14), 615 $[M-119]^+$ (4), 601 $[M-133]^+$ (8), 587 $[M-147]^+$ (8), 585 $[M-149]^+$ (32), 573 $[M-161]^+$ (33), 559 $[M-175]^+$ (14), 543 $[M-191]^+$ (9), metastable ion 673 ($M \rightarrow M-31$) TLC (silica gel) 0 40, PM 2 0 57 ($CHCl_3$–EtOAc– Me_2CO, 5 1 4)

Apigenin 6-C-(6-O-glucosylglucoside)-8-C-arabinoside (**3**). Colour UV dark purple, $+AlCl_3$ yellow, + basic lead acetate yellow TLC (cellulose) R_f 0 54 (vicenin-2 0 40) (15% HOAc), 0 33 (vicenin-2 0 37) (BAW) UV λ_{max}^{MeOH} nm 272, 330, + NaOMe 282, 334, 400; $+AlCl_3$ and $AlCl_3$ + HCl 280, 305, 350, 380, + NaOAc 282, 338, 392 Acid hydrolysis gave schaftoside (UV, TLC) and glucose (TLC)

Permethyl derivative of **3** EIMS (70 eV) $m/z > 500$ (rel int) 908 $[M]^+$ (46), 893 $[M-15]^+$ (36), 877 $[M-31]^+$ (100), 733 $[M-175, SO_i]^+$ (50), 719 $[M-189, SO_j]^+$ (30), 703 $[M-205, SO_k]^+$ (20), 689 $[M-219, SO]^+$ (53), 687 $[SO-2]^+$ (36), 675 $[S+2]^+$ (42), 673 $[M-235, S]^+$ (30), 659 $[S-14]^+$ (73), 641 $[S-32]^+$ (22), 627 $[S-14-32]^+$ (30), 601 $[g]^+$ (45), 543 $[n]^+$ (74), 541 $[h]^+$ (82), 529 $[i]^+$ (110), 515 $[j]^+$ (68), 499 $[k]^+$ (30), metastable ion 847 ($M \rightarrow M-31$) Acid hydrolysis product EIMS (70 eV) $m/z > 450$ (rel int) 690 $[M]^+$ (21), 675 $[M-15]^+$ (38), 659 $[M-31]^+$ (100), 643 $[M-47]^+$ (6), 627 $[M-63]^+$ (8), 601 $[M-89]^+$ (18), 681 (11), 571 $[M-119]^+$ (9), 559 $[M-131]^+$ (23), 541 $[M-149]^+$ (40), 529 $[M-161]$ (32), 515 $[M-175]^+$ (21), 499 $[M-191]^+$ (9), metastable ion 629 ($M \rightarrow M-31$) TLC silica gel R_f 0 07 ($CHCl_3$–EtOAc–Me_2CO, 5 4 1), 0 15 ($CHCl_3$–Me_2CO, 4 1), 0 36, PM 3 0 55 ($CHCl_3$–EtOAc–Me_2CO, 5 1 4)

6″-Desmethyl PM schaftoside (**10**) EIMS (70 eV) $m/z > 450$ (rel int) 690 $[M]^+$ (19), 675 $[M-15]^+$ (31), 659 $[M-31]^+$ (100), 643 $[M-47]^+$ (5), 627 $[M-63]^+$ (7), 601 $[M-89]^+$ (13), 585 (5), 571 $[M-119]^+$ (7), 559 $[M-131]^+$ (5), 541 $[M-149]^+$ (25), 529 $[M-161]^+$ (31), 515 $[M-175]^+$ (13), 499 $[M-191]^+$ (9), metastable ion 629 ($M \rightarrow M-31$) TLC (silica gel) R_f 0 07 ($CHCl_3$–EtOAc–Me_2CO, 5 4 1), 0 15 ($CHCl_3$–Me_2CO, 4 1), 0 36 ($CHCl_3$–EtOAc–Me_2CO, 5 1 4)

C-Cellobiosylation of cytisoside Acetobromocellobiose (7 5 g) in $CHCl_3$ (40 ml) was added to a stirred soln of cytisoside (539 mg) and LiOMe (184 mg Li) in MeOH (40 ml) and the mixture was left overnight at room temp After evapn under red pres, the residue was extracted with H_2O (70 ml) and the aq extract chromatographed on a polyamide column (2 × 75 cm) Elution with H_2O was followed by TLC (silica gel) in EtOAc–pyridine–H_2O–MeOH (16 4 2 1) and spraying with bis-diazotized benzidine and Na_2CO_3 6-C-*Cellobiosylcytisoside* (**4**), dark purple under UV, colourless with bis-diazotized benzidine, showed R_f 0 33 (6-*C*-glucosylcytisoside 0 50, colourless, cytisoside 0 80, brown–red) and UV λ_{max}^{MeOH} nm 272, 325, + NaOAc 282, 300 sh, 370 *Permethyl ether* EIMS (70 eV) $m/z > 500$ (rel int) 952 $[M]^+$ (21), 937 $[M-15]^+$ (33), 921 $[M-31]^+$ (77), 905 $[M-47]^+$ (10), 792 $[M-160, SO_n+1]^+$ (11), 777 $[M-175, SO_i]^+$ (9), 763 $[M-189, SO_j]^+$ (5), 747 $[M-205, SO_k]^+$ (7), 733 $[M-219, SO]^+$ (14), 731 $[SO-2]^+$ (16), 717 $[M-235, S]^+$ (28), 703 $[S-14]^+$ (17), 685 $[S-32]^+$ (17), 645 $[g]^+$ (12), 615 $[f]^+$ (8), 587 $[n]^+$ (30), 585 $[h]^+$ (42), 573 $[i]^+$ (100), 559 $[j]^+$ (31), 543 $[k]^+$ (11), metastable ion 891 ($M \rightarrow M-31$)

REFERENCES

1 Bouillant, M L, Favre-Bonvin, J and Chopin, J (1975) *Phytochemistry* **14**, 2267

2 Bouillant, M L, Besset, A, Favre-Bonvin, J and Chopin, J (1978) *Phytochemistry* **17**, 527

3 Besson, E, Besset, A, Bouillant, M L, Chopin, J, Van Brederode, J and Van Nigtevecht, G (1979) *Phytochemistry* **18**, 657

4 Bouillant, M L, Besset, A, Favre-Bonvin, J and Chopin, J (1979) *Phytochemistry* **18**, 690

5 Bouillant, M L, Ferreres de Arce, F, Favre-Bonvin, J, Chopin, J, Zoll, A and Mathieu, G (1979) *Phytochemistry* **18**, 1043

6 Bouillant, M L, Besset, A, Favre-Bonvin, J and Chopin, J (1980) *Phytochemistry* **19**, 1755

7 Ockendon, D J, Alston, R E and Naifeh, K (1966) *Phytochemistry* **5**, 601

8 Mabry, T J, Markham, K R and Thomas, M B (1970) *The Systematic Identification of Flavonoids* Springer, Berlin

9 Chopin, J (1971) in *Pharmacognosy and Phytochemistry* (Wagner, H and Horhammer, L, eds) p 111 Springer, Berlin

10 Zoll, A and Nouvel, G (1974) *Plant Méd Phytothér* **8**, 134