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# STRUCTURE OF A 6,8-DI-*C*-PENTOSYLAPIGENIN FROM *MOLLUGO PENTAPHYLLA*

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**Key Word Index**—*Mollugo pentaphylla*; Aizoaceae; *C*-glycosylflavones; 6-*C*-β-D-xylopyranosyl-8-*C*-α-L-arabinopyranosylapigenin.

**Abstract**—One of the di-*C*-pentosylflavones isolated from *Mollugo pentaphylla* was identified as 6-*C*-β-D-xylopyranosyl-8-*C*-α-L-arabinopyranosylapigenin in spite of the 6-*C*-arabinosyl structure suggested by the MS of its permethyl derivative.

## INTRODUCTION

It has been shown earlier [1] that the permethyl (PM) derivatives of three compounds isolated from *Mollugo pentaphylla* all gave MS of PM 6,8-di-*C*-pentosylapigenins in which the relative intensities of $[M-119]^+$, $[M-131]^+$ and $[M-145]^+$ ions were in the order $[M-131]^+ > [M-119]^+ > [M-145]^+$. Such an order has always been observed in the MS of PM 6-*C*-arabinosyl-8-*C*-hexosylflavones when the sugar attached on C-8 was glucose [2] or galactose [3]. It was therefore postulated as characteristic of a 6-*C*-arabinosyl residue when found in the MS of the PM derivatives of several natural 6,8-di-*C*-pentosylflavones [2, 4–8]. This hypothesis proved to be valid for 6,8-di-*C*-arabinosylflavones, as shown by synthetic 6,8-di-*C*-α-L-arabinopyranosylacacetin [9] and natural apigenin and tricin 6,8-di-*C*-arabinopyranosides [10]. However, we have now shown it is not valid for *C*-arabinosyl-*C*-xylosylflavones since we report one of the 6,8-di-*C*-pentosylapigenins from *Mollugo pentaphylla* to be 6-*C*-β-D-xylopyranosyl-8-*C*-α-L-arabinopyranosylapigenin.

## RESULTS AND DISCUSSION

When the ethyl acetate-soluble fraction of the ethanol extract from the aerial parts of the plant was chromatographed on a polyamide column, elution with water led first to a mixture of compounds appearing on TLC as brown–red spots after spraying with bis-diazotized benzidine, then to compound **1** [1] giving a yellow spot with the same reagent and migrating between natural 6,8-di-*C*-α-L-arabinopyranosylapigenin and synthetic 6,8-di-*C*-β-D-xylopyranosylapigenin [11]. Purification of **1** was achieved by PPC in 15% acetic acid and crystallization from aqueous methanol. **1** showed the UV spectrum and diagnostic shifts [12] of apigenin with free 5, 7 and 4′-hydroxyl groups and was not chromatographically changed after alkaline or acid hydrolysis. The PM derivative gave the MS of a PM 6,8-di-*C*-pentosylapigenin in which $[M-131]^+ > [M-119]^+ > [M-145]^+$ and migrated on TLC between PM 6,8-di-*C*-α-L-arabinopyranosylapigenin and PM 6,8-di-*C*-β-D-xyopyranosylapigenin. The $^1$H NMR spectrum of the perdeuteriomethylated (PDM) derivative confirmed the 6,8-disubstituted apigenin structure from the absence of H-6 and H-8 signals and the presence of two doublets (H-2′, H-6′ and H-3′, H-5′) and of one singlet (H-3) in the aromatic region. As already observed in the spectra of other PDM di-*C*-glycosylflavones [3], the two anomeric protons appeared as four doublets with a coupling constant (*ca* 10 Hz) characteristic of a *trans* diaxial relationship in a pyranose ring. Moreover the nature of the sugar residues could be deduced from comparison (see Fig. 1) with the $^1$H NMR spectra of PDM molludistin (8-*C*-α-L-arabinopyranosyl-7-*O*-methylapigenin) [13], PDM vitexin (8-*C*-β-D-glucopyranosylapigenin) and PDM vicenin 1 (6-*C*-β-D-xylopyranosyl-8-*C*-β-D-glucopyranosylapigenin).

The characteristic signals of H-2″, H-3″, H-4″ and H-5″ of PDM molludistin were found as the same δ values in the spectrum of PDM **1** in which they are flanked by two multiplets (2H each) superimposable with the corresponding signals present at the same δ values in the spectrum of PDM vicenin 1. The latter could easily be attributed to the xylose residue since they are external to the glucose signals present at the same δ values in the spectrum of PDM vitexin.

On the other hand, the signals of H-2″, H-3″, H-4″ and H-5″ of PDM 6-*C*-α- and β-L-arabinofuranosyl and 6-*C*-β-L-arabinopyranosylacacetins [14] exhibited δ values quite different from those observed in the spectrum of PDM **1**, thus excluding the possibility of 6,8-di-*C*-arabinosylapigenin structures with different arabinosyl residues such as those observed when 6,8-di-*C*-α-L-arabinopyranosylapigenin is heated with acid [6]. The coincidences between the $^1$H NMR spectra of PDM molludistin, PDM vicenin and PDM **1** strongly suggested the attachment of xylose on C-6 and of arabinose on C-8 and this also agreed with the CD

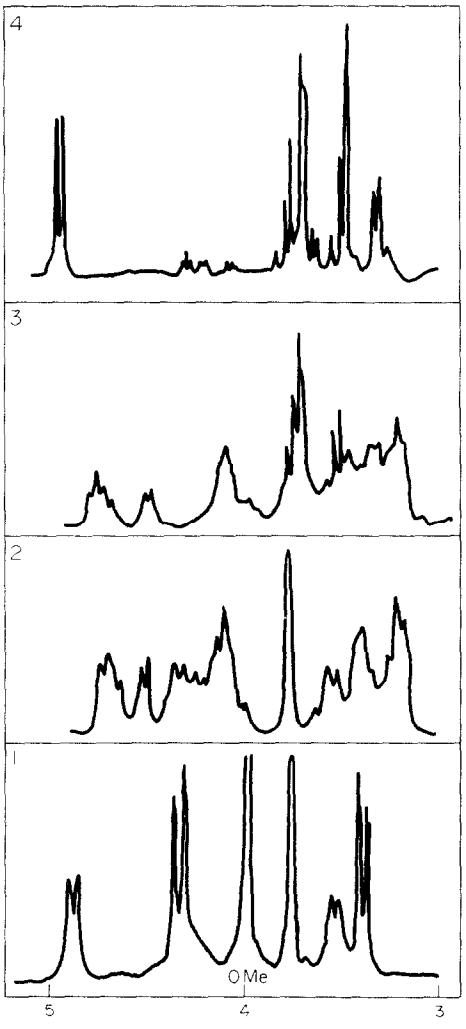


Fig. 1. $^1$H NMR spectra (sugar protons) of the perdeuteriomethyl derivatives of molludistin (1), compound 1 (2), vicenin-1 (3) and vitexin (4).

spectrum of 1 being similar to that of schaftoside (6-*C*-*β*-D-glucopyranosyl-8-*C*-L-arabinopyranosylapigenin) [15]. This was definitely proved by the MS of the permethyl derivative of the isopropylidene ketal, which was obtained from 1 by prolonged stirring with acetone and paratoluenesulfonic acid. The MS was in agreement with a PM monoisopropylidene derivative and the fragmentation pattern remained the same as that of PM 1, showing that the 6-*C*-glycosyl residue (which governs the fragmentation pattern of PM 6,8-di-*C*-glycosylflavones [2]) does not bear the isopropylidene group. Therefore the xylopyranosyl residue, unable to give an isopropylidene derivative, is attached on C-6 and the final structure of 1 is 6-*C*-*β*-D-xylopyranosyl-8-*C*-*α*-L-arabinopyranosylapigenin.

To our knowledge, 1 is the first 6,8-di-*C*-pentosylflavone containing both arabinose and xylose to be characterized. However this type of compound may be widely spread in the plant kindgom, as indicated by the identification of 1 with the 6,8-di-*C*-pentosylapigenin isolated from *Lespedeza cuneata* (Leguminosae) [16] (identity of the $^1$H NMR spectra of the PDM derivatives) and from *Cerastium arvense* (Caryophyllaceae) [17] (identity of the MS and TLC of the PM derivatives). When 1 was heated with acid and the resulting mixture permethylated, TLC of the latter led, besides PM 1, to another PM derivative which showed the MS of a PM 6,8-di-*C*-pentosylapigenin (again with $[M-131]^+ > [M-119]^+ > [M-145]^+$ but with a larger difference between $[M-131]^+$ and $[M-119]^+$) and co-chromatographed with the PM derivative of 2 previously isolated with 1 from *Mollugo pentaphylla* [1]. Thus 2 is probably the Wessely–Moser isomer of 1, i.e. 6-*C*-arabinosyl-8-*C*-xylosylapigenin.

It can be concluded that the relative intensities of $[M-119]^+$, $[M-131]^+$ and $[M-145]^+$ ions in the MS of PM 6,8di-*C*-pentosylflavones cannot be used in the case of *C*-arabinosyl-*C*-xylosylflavones for identifying the sugar residue attached on C-6.

## EXPERIMENTAL

$^1$H NMR spectra were recorded on a CAMECA (250 MHz) instrument and MS on an AEI MS 902 (70 eV). For permethylation and purification of PM derivatives see ref. [2].

*Plant material and extraction.* See ref. [1].

*Isolation.* The EtOAc-soluble fraction (2.2 g) was adsorbed on polyamide and applied in $H_2O$ to a polyamide column (3.5 × 36 cm). Elution was carried out with $H_2O$ (7 l.) followed by 50% MeOH (2 l.) and MeOH (5 l.). TLC (Si gel) in EtOAc–$C_5H_5N$–$H_2O$–MeOH (16:4:2:1) of each 10th fraction (7 ml) showed 1 present in fractions 490–1150. Pure samples were obtained by PPC on Whatman 3 MM paper in 15% HOAc and crystallization from aq. MeOH (35 mg).

6-*C*-*β*-D-*xylopyranosyl*-8-*C*-*α*-L-*arabinopyranosylapigenin* (1). Mp 220–230° (dec) UV $\lambda_{max}^{MeOH}$ nm: 271, 330; +NaOAc 277, 350; +NaOAc + $H_3BO_3$ 273, 330; + $AlCl_3$ 279, 305, 348, 381; + $AlCl_3$ + HCl 280, 304, 344, 381; +NaOH (0.1 N) 281, 332, 397. CD $[\theta]_{250}-9870$, $[\theta]_{302}+11100$ (MeOH). TLC Si gel $R_f$ 0.50 (EtOAc–$C_5H_5N$–$H_2O$–MeOH 16:4:2:1), cellulose 0.33 (15% HOAc); 6,8-di-*C*-*α*-L-arabinopyranosylapigenin 0.47, 0.31; 6,8-di-*C*-*β*-D-xylopyranosylapigenin 0.54, 0.35. Permethyl ether: EIMS 70 eV, *m/z* > 400 (rel. int.) 660 $[M]^+$ (17), 645 $[M-15]^+$ (24), 629 $[M-31]^+$ (100), 541 $[M-119]^+$ (20), 529 $[M-131]^+$ (21), 515 $[M-145]^+$ (12); TLC Si gel $R_f$ 0.21 ($CHCl_3$–EtOAc–$Me_2CO$, 5:4:1), PM 6,8-di-*C*-*α*-L-arabinopyranosylapigenin 0.11, PM 6,8-di-*C*-*β*-D-xylopyranosylapigenin 0.30. Perdeuteriomethyl ether: $^1$H NMR (250 MHz, $CDCl_3$) *δ* 8.06 (2H, *d*, *J* = 9 Hz, H-2′, H-6′), 7.01 (2H, *d*, *J* = 9 Hz, H-3′, H-5′), 6.64 (1H, *s*, H-3), 4.71 (*d*, *J* = 10 Hz), 4.65 (*d*, *J* = 12 Hz), 4.51 (*d*, *J* = 10 Hz) (H-1″, H-1‴), 4.30 (2H, *m*, *J* = 12 Hz, *W* = 56 Hz, H-5″$_{eq}$ Ara, H-2″ Ara), 4.11 (2H, *m*, *W* = 56 Hz, 2H-xyl), 3.77 (1H, *br s*, $W_{1/2}$ = 8 Hz, H-4″ Ara), 3.55 (1H, *br d*, *J* = 12 Hz, H-5″$_{ax}$ Ara), 3.39 (2H, *m*, $W_{1/2}$ = 30 Hz, H-3″ Ara, 1H-Xyl), 3.21 (2H, *m*, $W_{1/2}$ = 26 Hz, 2H-xyl). PDM molludistin: 4.88 (1H, *d*, *J* = 9.5 Hz, H-1″), 4.32 (1H, *dd*, $J_{gem}$ = 13 Hz, $J_{4',5'}$ = 1.9 Hz, H-5″$_{eq}$), 4.30 (1H, *br m*, H-2″), 3.98 (3H, *s*, OMe), 3.75 (1H, *br s*, $W_{1/2}$ = 8 Hz, H-4″), 3.52 (1H, *br d*, $J_{gem}$ = 13 Hz, H-5″$_{ax}$), 3.39 (1H, *dd*, $J_{2',3'}$ = 9.5 Hz, $J_{3',4'}$ = 3.5 Hz, H-3″). PDM vitexin: 4.94 (1H, *d*, *J* = 10 Hz, H-1″), 3.70 (3H, *m*, *W* = 50 Hz), 3.48 (2H, *m*, *W* = 25 Hz), 3.30 (1H, *m*, *W* = 25 Hz). PDM vicenin-1: 4.80 (*d*, *J* = 10 Hz), 4.76 (*d*, *J* = 10 Hz), 4.71

(*d*, $J = 10$ Hz), 4.50 (*d*, $J = 10$ Hz) (H-1″, H-1‴), 4.12 (2H, *m*, $W = 56$ Hz, 2H-xyl), 3.73 (3H, *m*, $W = 30$ Hz, 3H-glc), 3.48 (2H, *m*, $W = 25$ Hz, 2H-glc), 3.37 (2H, *m*, $W = 40$ Hz, 1H-glc, 1H-xyl), 3.23 (2H, *m*, $W_{1/2} = 25$ Hz, 2H-xyl).

*Permethylisopropylidene ketal.* No reaction being observed by TLC after prolonged stirring in $Me_2CO$ with dry $CuSO_4$, **1** (2.5 mg) was stirred at room temp. in $Me_2CO$ (2 ml) with paratoluenesulfonic acid (1.3 mg) for 5 days. The reaction mixture was evaporated to dryness after neutralization with $NH_4OH$ and permethylated. TLC (Si gel) in $CHCl_3$–EtOAc–$Me_2CO$ (5:4:1) of the crude product showed two main bands $R_f$ 0.25 (co-chromatography with PM **1**) and 0.36. The latter gave the MS of a PM monoisopropylidene derivative of **1**: EIMS 70 eV, $m/z > 400$ (rel. int.) 672 $[M]^+$ (14), 657 $[M-15]^+$ (25), 641 $[M-31]^+$ (100), 625 $[M-47]^+$ (14), 613 $[M-59]^+$ (8), 611 $[M-61]^+$ (11), 609 $[M-63]^+$ (11), 553 $[M-119]^+$ (28), 541 $[M-131]^+$ (29), 527 $[M-145]^+$ (19).

*Acid isomerization.* **1** (2 mg) was heated with MeOH–4 N HCl (1:1) (2 ml) at 100° in a sealed tube for 7 hr. The reaction mixture was evaporated to dryness after neutralization with NaOH and permethylated. TLC (Si gel) in $CHCl_3$–EtOAc–$Me_2CO$ (5:4:1) showed two main bands $R_f$ 0.25 (co-chromatography with PM **1**) and 0.29. The latter gave the MS of a PM 6, 8 - di - *C* - pentosylapigenin: EIMS 70 eV, $m/z > 400$ (rel. int.) 660 $[M]^+$ (14), 645 $[M-15]^+$ (33), 629 $[M-31]^+$ (100), 613 $[M-47]^+$ (18), 599 $[M-61]^+$ (14), 541 $[M-119]^+$ (31), 529 $[M-131]^+$ (43), 515 $[M-145]^+$ (17), and co-chromatographed with the PM derivative of **2** previously isolated from the same source [1].

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## NOTE ADDED IN PROOF

The $^{13}C$ NMR spectrum of **1** supports the proposed structure [Markham, K. R., personal communication].