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C-GALACTOSYLFLAVONES FROM *POLYGONATUM MULTIFLORUM*

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Key Word Index—*Polygonatum multiflorum*; Liliaceae; *C*-glycosylflavones; 8-*C*-galactosylapigenin; 6-*C*-galactosyl 8-*C*-arabinosylapigenin; ^{13}C-NMR spectra; isopropylidene derivatives.

Abstract—8-*C*-Galactosylapigenin and 6-*C*-galactosyl-8-*C*-arabinosylapigenin were isolated from the leaves of *Polygonatum multiflorum* (L.) All. Structural assignments for the latter compound were made on the basis of mass, CD and ^{13}C-NMR spectra.

INTRODUCTION

In a previous paper [1], three flavonoids (P, R and S) isolated from the fresh leaves of *Polygonatum multiflorum* were considered as *C*-glycosylapigenin derivatives on the basis of chemical, UV and ^{1}H-NMR spectral properties.

MS studies led us to revise the previously suggested structures and we now describe the identification of compound P as 8-*C*-galactosylapigenin and the characterization of compound S as 6-*C*-galactosyl-8-*C*-arabinosylapigenin.

RESULTS AND DISCUSSION

Compound P showed the same UV spectrum and diagnostic shifts [2] as apigenin and the chromatographic properties of a glycoside, but gave no sugar on acid hydrolysis. An 8-*C*-hexosylapigenin structure resulted from the NMR spectrum of the TMS derivative, H-6 appearing as a singlet (δ 6.12), 7 to 9 protons being found in the region δ 3.0–5.0 and one TMS signal (2″-OTMS) being shifted to higher field (δ −0.27), the other TMS signals clearly divided into three aromatic and three aliphatic TMS ethers. MS of permethylated compound P confirmed this assignment by showing the expected molecular peak M^+ 530 and the same fragmentation pattern as PM8-*C*-glucosylapigenin [3]. However, co-chromatography of permethylated compound P and PM 8-*C*-glucosylapigenin showed them to be different. Finally, compound P was identified with synthetic 8-*C*-galactosylapigenin [4] by co-chromatography. Following the recent discovery [5] of 8-*C*-galactosylapigenin from *Briza media* [6], this identification of compound P represents only the second report of a mono-*C*-galactosylflavone in plants, again in the monocotyledons.

Compound S, like P, showed the same UV spectrum and diagnostic shifts as apigenin, but the chromatographic properties suggested a more hydrophilic structure. Again no sugar was found after acid hydrolysis. A 6,8-di-*C*-glycosylapigenin structure assignment resulted from the NMR spectrum of the TMS derivative: absence of H-6 and H-8, presence of 16–19 protons in the region δ 3.0–5.0, of three aromatic TMS ethers and of two-TMS signals (2″- and 2‴-OTMS) shifted to higher field (δ −0.18 and −0.33). Excess sugar protons probably came from an impurity since, in agreement with the absence of sugar after acid hydrolysis, permethylated compound S exhibited a typical mass spectrum of PM 6,8-di-*C*-glycosylflavone. The molecular peak M^+ 704 agreed with a PM *C*-pentosyl-*C*-hexosylapigenin structure and the relative importance of M-175 and M-131 peaks with a PM 6-*C*-hexosyl-8-*C*-pentosyl apigenin structure [3]. Two compounds of this type have been described, 6-*C*-glucosyl-8-*C*-xylosylapigenin (vicenin-3) [7] and 6-*C*-glucosyl-8-*C*-arabinosylapigenin (schaftoside) [8], both of which proved to be different from compound S by co-chromatography of free and permethylated substances.

Unfortunately, no sugar could be found in the $FeCl_3$ oxidation products of compound S whereas glucose and arabinose were characterized in a parallel experiment with schaftoside [8]. A first indication about the possible nature of the *C*-pentosyl residue in compound S was afforded by the presence of a secondary constituent (PMS′) in the crude permethylation mixture. The MS of this product showed it to be a PM 6-*C*-pentosyl-8-*C*-hexosylapigenin in which the relative abundance of the peaks M-131 > M-119 > M-145 favoured a 6-*C*-arabinosyl structure [3]. As the natural co-occurrence of Wessely–Moser isomers has been frequently mentioned [9], the secondary compound S′ could be the Wessely–Moser isomer of compound S, suggesting the presence of a *C*-arabinosyl residue in the latter. To substantiate this assumption, pure compound S was isomerized by heating with acid and the crude mixture permethylated. Indeed, TLC of the permethylation products afforded a small proportion of a constituent showing the same chromatographic behaviour and MS characteristics as PMS′.

With regard to the *C*-hexosyl residue in compound S, it could be a *C*-galactosyl moiety, in view of the co-occurrence of S with P in the same plant. Furthermore, compound S and schaftoside were not separated by PC in the usual solvent systems BAW (4:1:5) and 15% HOAc, but schaftoside showed a higher R_f than S on silica gel TLC in a solvent system in which 6-*C*-glucosylapigenin

migrated faster than 6-*C*-galactosylapigenin [9]. A similar behaviour was observed with the permethyl derivatives. Also, S showed higher positive values of $[\alpha]_D$ and molecular ellipticities in the region 250–275 nm that schaftoside. Methyl D-galactopyranosides exhibit a higher M_D than the corresponding methyl D-glucopyranosides [10] and 6-*C*-β-D-galactopyranosylapigenin a higher molecular ellipticity (θ_{270} + 14300) than 6-*C*-β-D-glucopyranosylapigenin (θ_{265} + 8730) [11].

Further evidence was obtained in the course of preparing the isopropylidene derivatives on a microscale and then characterizing them by MS after permethylation. Indeed, schaftoside thus led to a compound (PMIPSch) showing the mass spectrum expected for a permethyl mono-isopropylidene schaftoside (M^+ 716) bearing the isopropylidene group on the 8-*C*-arabinosyl residue since the fragmentation pattern, governed by the 6-*C*-glycosyl residue, remained the same as that of permethyl schaftoside [3]. On the other hand, three isopropylidene derivatives were obtained from compound S. After permethylation, two of them (PMIPS1 and PMIPS2) showed the MS expected for permethyl mono-isopropylidene derivatives (M^+ 716) and the third (PMIPS3) the MS of a permethyl diisopropylidene derivative (M^+ 728). These results were in agreement with the ability of both the *C*-D-galactopyranosyl and *C*-L-arabinopyranosyl residues to give 3,4-*O*-isopropylidene-ketals and with the presence of the M-15 and M-31 peaks (originating from the 2-methoxyl group of the permethyl 6-*C*-glycosyl residue [12]) in the MS of all these derivatives.

Since the MS of PMIPS1 and PMIPSch showed the same relative importance of the characteristic peaks M-175 and M-31, it seemed evident that the isopropylidene group is on the 8-*C*-arabinosyl residue in PMIPS1 especially since the M-175 peak was considerably lowered in the MS of both PMIPS2 and PMIPS3. Moreover the M-119, M-131 and M-145 peaks, which characterize the fragmentation of a permethylated *C*-pentosyl residue [3], were present in the MS of PMIPS2 (in agreement with the presence of the isopropylidene group on the 6-*C*-galactosyl-residue), but were absent in the MS of PMIPS1 and negligible in the MS of PMIPS3.

Finally, further evidence came from the comparison of ^{13}C-NMR spectra of iso-orientin (6-*C*-β-D-glucopyranosylluteolin [13], schaftoside, compound S, molludistin (8-*C*-α-L-arabinopyranosyl-7-*O*-methylapigenin) [14] and 8-*C*-β-D-galactopyranosylapigenin 6″-acetate [15]. In all these spectra, the sugar signals could be clearly distinguished. From the spectrum of iso-orientin, the 6-*C*-glucosyl signals could be tentatively assigned in the spectrum of schaftoside and the 8-*C*-arabinosyl signals deduced by difference. Subtraction of the latter in the spectrum of compound S left the 6-*C*-galactosyl signals. The results obtained were in good agreement with the observed 8-*C*-arabinosyl signals in the spectrum of molludistin and with the 8-*C*-galactosyl signals tentatively deduced from the spectrum of 8-*C*-β-D-galactopyranosylapigenin 6″-acetate [15]. Thus, the 6-*C*-β-D-galactopyranosyl-8-*C*-α-L-arabinopyranosylapigenin structure can be assigned to compound S, the first natural galactose-containing 6,8-di-*C*-glycosylflavone to be reported.

EXPERIMENTAL

PMR spectra were recorded on a Varian A-60 instrument with TMS as int. stand., ^{13}C-NMR spectra on a Varian FT XL-100 (25,2 MHz) at ord. temp. in d_6-DMSO, MS on an AEI MS 902 (70 eV).

Plant. Polygonatum multiflorum L., Liliaceae, collected in Poznan district. The reference samples (No. 037285–037287) are deposited in the Department of Pharm. Botany, Institute of Biology and Pharmacy, Medical Academy, Poznan, Poland.

Isolation. Fresh leaves (5 kg) were extracted under reflux with MeOH. After concn of the MeOH extract under red. press., the residue (150 g) was macerated with hot H_2O and the H_2O phase extracted sequentially with $CHCl_3$ (1.4 g), Et_2O (0.1 g), EtOAc (5.2 g) and EtOAc–MeOH (9:1) (7 g). Compound S (206 mg) was obtained from the remaining H_2O phase after 2 months standing at 4°. Compound P (11 mg) was purified by chromatography of the EtOAc fraction (3 g) on polyamide with elution by MeOH in 50% EtOH. Compound S appeared in the first, compound R in the intermediate and compound P in the last fractions.

Compound P. Yellow needles (50% MeOH) mp 202–204°; UV λ_{max}^{MeOH} nm 270, 333; + $AlCl_3$ 278, 306, 352, 386; + $AlCl_3$ + HCl 278, 303, 345, 382; + NaOAc 280, 300, 382; + NaOAc + H_3BO_3 273, 333; + NaOMe 280, 330, 395; IR (KBr) ν_{CO} 1650 cm^{-1}; NMR (TMS ether in CCl_4) $\delta_{TMS}^{10^{-6}}$: 7.72 (2H, *d*, *J* = 8,5 Hz) H-2′, 6′; 6,81 (2H, *d*, *J* = 8.5 Hz) H-3′, 5′; 6,27 (1H, *s*) H-3; 6, 14 (1H, *s*) H-6; 4, 85 (1H, *d*, *J* = 8 Hz) H-1″; 5.0–3.0 (7–9 H, *m*) sugar protons; 0.36 and 0.28 (*s*) arom. OTMS; 0.18 and 0.08 (*s*) aliph. OTMS; −0.27 (*s*) 2″-OTMS; PC (Whatman N°1) R_f 0.45 (BAW 4:1:5), 0.25 (15% HOAc); TLC (activated Si gel) R_f 0.60 (EtOAc–Py–H_2O–MeOH, 16:4:2:1) (8-*C*-β-D-galactopyranosylapigenin: 0.60; 8-*C*-β-D-glucopyranosylapigenin: 0.73). Permethylation [3] of compound P and TLC of the mixture on Si gel in $CHCl_3$–EtOAc–Me_2CO (5:4:1) led to two main bands R_f 0.11 (PM 8-*C*-β-D-glucopyranosylapigenin: 0.16) and 0.56. The former showed the MS of a PM 8-*C*-hexosylapigenin: (*m/e*) 530 (M^+, 100%), 369 (M-161, 11%), 355 (M-175, 100%), 341 (M-189, 39%), 325 (M-205, 23%), 311 (M-219, 9%); the latter showed the MS of a *C*-methyl PM 8-*C*-hexosylapigenin (same peaks 14 mu higher), a by-product of permethylation commonly observed from 8-*C*-glycosyl flavones. TLC (Si gel) in $CHCl_3$–EtOAc–Me_2CO (5:1:4), PM compound P: R_f 0.31; PM 8-*C*-β-D-glucopyranosylapigenin: 0.37.

Compound S. Yellow needles (H_2O) mp 219–222°; $[\alpha]_D$ + 109°, schaftoside: +95° (DMSO–H_2O, 1:4, *c* = 20 g/l); CD(MeOH) $[\theta]_{271}$ +5140, $[\theta]_{250}$ −4930, schaftoside: $[\theta]_{275}$ +2540, $[\theta]_{250}$ −16900; UV λ_{max}^{MeOH} nm 271, 332; + $AlCl_3$ 280, 306, 355, 386 *sh*; + $AlCl_3$ + HCl 279, 302, 346, 379 *sh*; + NaOAc 280, 332, 396; + NaOMe 281, 331, 399; IR (KBr) ν_{CO} 1640 cm^{-1}; ^{1}H-NMR (TMS ether in CCl_4) $\delta_{TMS}^{10^{-6}}$: 7.85 (2H, *d*, *J* = 8.5 Hz) H-2′, 6′; 6.78 (2H, *d*, *J* = 8.5 Hz) H-3′, 5′; 6.26 (1H, *s*) H-3; 5.0–3.0 (16–19H, *m*) sugar protons; 0.40 and 0.28 (*s*) arom. OTMS; 0.18 and 0.08 (*s*) aliph. OTMS; −0.18 (*s*) 2″-OTMS; −0.33 (*s*) 2‴-OTMS; PC (Whatman N°1) R_f 0.18 (BAW 4:1:5), 0.44 (15% HOAc); TLC (activated Si gel) R_f 0.04 (EtOAc–Py–H_2O–MeOH, 80:20:10:5), schaftoside: 0.14. Permethylation [3] of compound S and TLC of the mixture on Si gel in $CHCl_3$–EtOAc–Me_2CO (5:4:1) led to one main band (R_f 0.14, PM schaftoside: 0.20, PM vicenin-3: 0.28) and a narrow fringe (R_f 0.10, PM isochaftoside: 0.17). The former (PMS) showed the MS of a PM 6-*C*-hexosyl-8-*C*-pentosylapigenin: (*m/e*) 704 (M^+, 22%), 689 (M-15, 39%), 673 (M-31, 100%), 573 (M-131, 12%), 541 (M-163, 42%), 529 (M-175, 59%). 515 (M-189, 18%), the latter (PMS′) the MS of a PM 6-*C*-arabinosyl-8-*C*-hexosylapigenin: (*m/e*) 704 (M^+, 19%), 689 (M-15, 32%), 673 (M-31, 100%), 585 (M-119, 14%), 573 (M-131, 24%), 559 (M-145, 13%), 541 (M-163, 26%), 529 (M-175, 32%), 515 (M-189, 19%). ^{13}C-NMR (d_6-DMSO): [illegible] schaftoside 182.0 (C-4), 163.7 (C-2), 161 (C-4′), 160.9 (C-5?), 158.0 (C-7?), 129.0 (C-9?), 128.9 (C-2′, 6′), 120.9 (C-1′), 115.7 (C-3′, 5′), 108.1 (C-6), 104.2 (C-8), 103.2 (C-10), 102.2 (C-3), sugar C (see Table 1); compound S: 182.0 (C-4), 163.9 (C-2), 160.9 (C-4′, C-5?), 158.2 (C-7?), 155.0 (C-9?), 129.3 (C-2′, 6′), 120.8 (C-1′), 115.7 (C-3′, 5′), 108.1 (C-6), 104.6 (C-8), 103.3 (C-10), 101.9 (C-3), sugar C (see Table 1).

Acid isomerization. Compound S (3 mg) was heated with MeOH–4N HCl (1:1) (2 ml) at 100° for 6 hr. After evaporation and permethylation, TLC of the mixture on Si gel in $CHCl_3$–

Table 1. ^{13}C NMR spectra of glycosylflavones

	δ_{TMS} of *C*-glycosyl C atoms												
Schaftoside	81.1	78.3	—	74.5	73.9	73.25	71.0	70.7	69.9	—	—	68.6	60.5
Isoorientin	81.35	78.88	—	—	—	73.10	—	70.36	—	—	—	—	61.47
Molludistin	—	—	—	74.88	74.05	—	71.10	—	68.93	—	—	67.86	—
Compound S	79.0	74.8	74.5	74.4	73.5	—	71.0	—	69.4	68.9	68.3	68.2	60.7
8-Galactosylapigenin	79.0	75.0	73.8	—	—	—	—	—	—	69.4	68.2	—	61.5

EtOAc–Me_2CO (5:1:4) led to one main band and a lower narrow band which was eluted. Cochromatography with PMS' in the same solvent showed no difference and the MS was that of a PM 6-*C*-arabinosyl-8-*C*-hexosylapigenin: (*m/e*) 704 (M^+, 22%), 689 (M-15, 30%), 673 (M-31, 93%), 585 (M-119, 26%), 573 (M-131, 30%), 559 (M-145, 18%), 529 (M-175, 30%).

Isopropylidene (IP) derivatives were prepared from compound S and schaftoside (2 mg) with 2,2-dimethoxypropane and 6N HCl-dioxane in anhydrous DMF according to [16] and their presence controlled by TLC of the $CHCl_3$ extract on Si gel in EtOAc–Py–H_2O–MeOH (80:12:10:5), the IP derivatives migrating faster than the starting product. After evaporation of the $CHCl_3$ extract and permethylation, TLC of the mixture on Si gel H in $CHCl_3$–EtOAc–Me_2CO (5:1:4) showed two bands from schaftoside, one (R_f 0.60) identical with PM schaftoside, the other (R_f 0.64) (IPSch) giving the MS of a PM mono-IP derivative: (*m/e*) 716 (M^+, 17%), 701 (M-15, 32%), 685 (M-31, 100%), 613 (M-103, 14%), 553 (M-163, 36%), 541 (M-175, 57%), 527 (M-189, 15%). From compound S, four bands were obtained, R_f 0.52, 0.56, 0.61 and 0.66, the first being identical with PMS; the second band (PMIPSI) gave the MS of a PM mono-IP derivative: (*m/e*) 716 (M^+, 27%), 701 (M-15, 30%), 685 (M-31, 75%), 577 (M-139, 25%), 553 (M-163, 30%), 541 (M-175, 45%), 527 (M-189, 10%), 368 (100%), the third band (PMIPS2) also gave the MS of a PM mono-IP derivative: (*m/e*) 716 (M^+, 30%), 701 (M-15, 49%), 685 (M-31, 100%), 641 (M-75, 6%), 627 (M-89, 5%), 609 (M-107, 6%), 597 (M-119, 6%), 585 (M-131, 16%), 571 (M-145, 5%), 569 (M-147, 13%), 553 (M-163, 6%), 541 (M-175, 22%), 527 (M-189, 6%), 515 (M-201, 16%), 497 (M-219, 13%), the last band (PMIPS3) gave the MS of a PM di-IP derivative: (*m/e*) 728 (M^+, 25%), 713 (M-15, 41%), 697 (M-31, 100%), 653 (M-75, 8%), 639 (M-89, 14%), 623 (M-105, 8%), 611 (M-117, 10%), 593 (M-135, 20%), 581 (M-147, 23%), 565 (M-163, 7%), 553 (M-175, 26%), 539 (M-189, 11%), 527 (M-201, 29%), 509 (M-219, 19%).

$FeCl_3$ oxidation [17] of compound S (25 mg) and schaftoside (25 mg) by heating 6 hr at 100° with $FeCl_3$ (240 mg) in H_2O (1 ml). Deionisation with Amberlite IR 120 and IR 45. TLC on HNa_2PO_4-impregnated Si gel in Me_2CO–H_2O (9:1). Arabinose and glucose spots from schaftoside; no spot from compound S.

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