PERGAMON

Phytochemistry 60 (2002) 835–838

PHYTOCHEMISTRY

www.elsevier.com/locate/phytochem

Kaempferol triosides from *Silphium perfoliatum*

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Received 20 August 2001; received in revised form 12 March 2002

Abstract

Two apiose-containing kaempferol triosides, together with nine known flavonoids were isolated from the leaves of *Silphium perfoliatum* L. Their structures were elucidated by acid hydrolysis and spectroscopic methods including UV, LSI MS, FAB MS, CI MS, ^{1}H, ^{13}C and 2D-NMR, DEPT, HMQC and HMBC experiments. The two new compounds were identified as kaempferol 3-*O*-β-D-apiofuranoside 7-*O*-α-L-rhamnosyl-(1‴→6‴)-*O*-β-D-galactopyranoside and kaempferol 3-*O*-β-D-apiofuranoside 7-*O*-α-L-rhamnosyl-(1‴→ 6‴)-*O*-β-D (2‴-*O*-*E*-caffeoylgalactopyranoside). © 2002 Elsevier Science Ltd. All rights reserved.

Keywords: *Silphium perfoliatum* L.; *Asteraceae*; Kaempferol triosides; Kaempferol caffeoyltrioside; Apiose-containing kaempferol triosides

1. Introduction

Silphium perfoliatum L. tribe *Heliantheae* (*Asteraceae*), also known as cup-plant or Indian-cup, is a perennial herb native to North America (Britton and Brown, 1958). Earlier chemical screening of *S. perfoliatum* detected mono-, di-, tri- and sesquiterpenoids (Bohlmann and Jakupovic, 1979, 1980; Davidyants and Abubakirov, 1992; Wolski et al., 2000), carbohydrates, simple flavonoids and ascorbic acid (Davidyants and Abubakirov, 1992), coumarins (Kowalski et al., 1998), protein complexes (Glaubertiene and Marciulionis, 1980) and phenolic acids (Wojcińska and Drost-Karbowska, 1998). The herb is used as a tonic, diaphoretic and diuretic (Heilpflanzen, 1996–1999), whereas roots are used as poultice for bleeding wounds, in backache and haemorrhagic (Hocking, 1997). Terpenoids from the leaves were found to have hypocholesterolemic and hypotriglyceridemic effects (Syrov et al., 1992).

We report here the isolation and identification of two new compounds, kaempferol 3-*O*-β-D-apiofuranoside 7-*O*-α-L-rhamnosyl-(1‴→6‴)-*O*-β-D-galactopyranoside **1** and its caffeoyl ester **2**, both of which contain apiose, together with nine other known flavonoids.

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2. Results and discussion

The known compounds, kaempferol and its 3-*O*-glucoside, 3-*O*-galactoside, 3-*O*-rutinoside and 3-*O*-robinobioside, quercetin and its 3-*O*-galactoside, 3-*O*-glucoside and 3-*O*-rutinoside, were all identified by comparison of their ^{1}H, ^{13}C NMR spectra with literature data (Agrawal and Bansal, 1989; Markham et al., 1978). Acid hydrolysis of both **1** and **2** afforded kaempferol, galactose, rhamnose and apiose; in addition caffeic acid was detected in the hydrolysate of **2**. The UV spectra in methanol and with diagnostic reagents of both **1** and **2** were in accord with 3,7-di-*O*-substituted flavonoids (Mabry et al., 1970); for **2** an additional shoulder was observed at 300 nm due to a caffeoyl moiety.

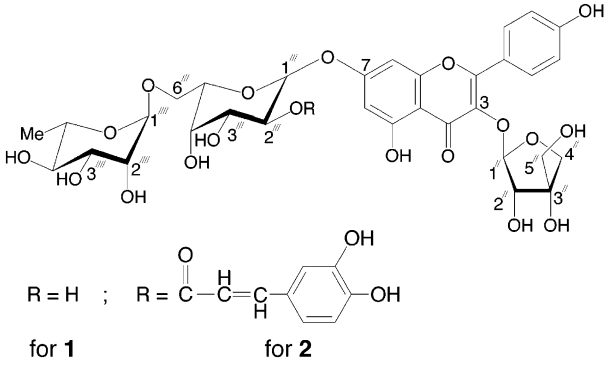
^{1}H NMR for both **1** and **2** exhibited A and B ring signals typical of kaempferol 3,7-di-*O*-substituted glycosides (Mabry et al., 1970) and signals for three anomeric protons of three sugar moieties were detected for both compounds: one signal for a 3-*O*-apiofuranosyl anomeric proton at δ 5.63 for **1** as *d*, $J = 3.16$ Hz and at δ 5.62 *d*, $J = 3.65$ Hz for **2**; the apiose $J_{1,2}$ ~3–4 Hz confirms the more stable β-D-*erythro*furanoside form of apiose (Angyal and Pickles, 1972; Angyal et al., 1977; Ranganathan et al., 1980; Ingham et al., 1986; Silva et al., 2000). The *erythro* relationship was also supported by the chemical shift difference of 2.64 ppm observed

PII: S0031-9422(02)00133-4

for C-2″ and C-3″ in both **1** and **2** (Bashir et al., 1990). A second signal for an anomeric proton of a 7-*O*-galactosyl was detected at δ 5.32 *d*, $J=7.75$ Hz for **1** and at δ 5.6 *d*, $J=7.98$ Hz for **2**, and a third upfield anomeric proton signal typical for a *O*-α-L-terminal rhamnosyl unit linked to galactose was detected at δ 4.39 Hz *d*, $J=1.26$ Hz for **1** and at **δ** 4.37 *d*, $J=1.21$ Hz for **2**; in addition, **2** exhibited two *trans* olefinic proton signals at δ 7.5 *d*, $J=15.77$ Hz and at δ 6.26 *d*, $J=15.92$ Hz, as well as a *d* at δ 6.76 $J=8.12$ Hz for caffeic H-5, a *dd* at δ 6.98 $J=1.93$, 8.25 for caffeic H-6 and at δ 7.04 *d*, $J=1.94$ for H-2, all indicative of a caffeoyl moiety in **2** (Kraut et al., 1993).

^{13}C DEPT experiments showed three methylene, one methyl, 18 methine and ten quaternary carbons for **1** and three methylene, one methyl, 23 methine and 14 quaternary carbons for **2**, which together with FAB negative at *m*/*z* 725 $[M-H]^-$ for **1** and 887 $[M-H]^-$ for **2** established a molecular ion formula of $C_{32}H_{38}O_{19}$ for **1** and $C_{41}H_{44}O_{22}$ for **2**. The first loss of an apiose unit giving *m*/*z* 593 [M-l-132]$^-$ for **1** supports the presence of apiose not linked to any other sugar moiety. The rhamnosyl terminal sugar was shown to be linked to galactose through a 1‴→6‴ bond for both **1** and **2** on the basis of the galactosyl C-6‴ downfield shift of $\sim+5.0$ ppm at δ 65.32 ppm for **1** and at **δ** 65.30 ppm for **2**. In addition a consequent upfield shift of rhamnosyl C-2‴ of −0.63 ppm (Markham et al., 1978) together with the HMBC correlation of the rhamnosyl H-l‴ to the galactosyl C-6‴ confirm the 1‴→ 6‴ linkage between these two sugar moieties. The caffeoyl moiety in **2** was assigned to the galactosyl C-2‴ based on the observed downfield shift of galactosyl H-l‴ of +0.29 and H-2‴ of +1.75 ppm and the downfield shifts of galactosyl C-2‴ of +2 ppm with consequent upfield shift of both galactosyl C-1‴ and C-3‴ of −2.7 and −2.06 ppm, respectively. The correlation between caffeoyl C=0 and both of galactosyl H-l‴ and galactosyl H-2‴ by HMBC confirms the previous assignment.

The data indicate that **1** is kaempferol 3-*O*-β-D-apiofuranoside 7-*O*-α-L-rhamnosyl-(1‴→ 6‴)-*O*-β-D-galactopyranoside and **2** is kaempferol 3-*O*-β-D-apiofuranoside 7-*O*-α-L-rhamnosyl-(1‴→ 6‴)-*O*-β-D-(2‴-*O*-*E*-caffeoyl-galactopyranoside).



3. Experimental

3.1. Spectral data

^{1}H and ^{13}C NMR spectra were recorded on a 500 MHz Bruker AMX and a Varian Unity Inova with TMS as an internal standard and DMSO-d_6 as solvent. FAB and CI mass spectra were obtained using a Finnigan MAT TSQ 700 spectrometer, LSI MS were recorded with AMD 604 Intectra GmbH Mass spectrometer, while UV in MeOH were recorded using a Lambda 20 Perkin Elmer spectrophotometer.

3.2. Plant material

Aerial parts of *S. perfoliatum* were collected in the summer of 1999 from the Medicinal Plantń Garden, K. Marcinkowski Univeristy of Medical Sciences, Poznań, Poland. The plant was identified by Professor K. Latowski, and a voucher specimen has been deposited in the Department of Pharmacognosy, K. Marcinkowski Univeristy of Medical Sciences.

3.3. Extraction and isolation of flavonoids

The dried aerial parts of *S. perfoliatum* (945 g) were extracted with petroleum ether (40–60 °C) for 2 h to remove fats. The residue remaining was extracted with MeOH (5×5 l each), and the MeOH extract was evaporated under reduced pressure affording a dry extract 255 g). The latter residue was extracted with CCl_4, $CHCl_3$ and EtOAc yielding 14.5, 8.9 and 44.4 g, respectively and a gummy water residue. The EtOAc extract (44.4 g) was subjected to a Sephadex LH-20 column using MeOH–H_2O (1:1), yielding fractions containing the aglycones, monoglycosides and the new acylated trioside **2** (4.5 mg). The gummy H_2O residue was subjectec to polyamide (Roth GmbH) using H_2O as eluant, then with increasing amounts of MeOH. Fractions obtained from the polyamide column were then applies to Sephadex LH-20 to give pure samples of the flavonoid diglycosides and the new apiose trioside **1** (5.6 mg).

3.4. Hydrolysis experiments

Acid hydrolysis was performed using 1% aq. HC1 at 100 °C for 90 min. For aglycone detection, the final aqueous acidic mixture was extracted with EtOAc, then the aqueous layer was neutralized for determination of the released sugar moieties using silica gel plates with *n*-PrOH:EtOAc:H_2O (7:2:1), giving R_f values × 100 of 0.31, 0.63 and 0.76 for galactose, apiose and rhamnose, respectively. Aniline phthalate was employed as a spray for color detection of the sugars.

3.5. Kaempferol 3-O-β-D-apiofuranoside 7-O-α-L-rhamnosyl (1‴→6″)-O-β-D-galactopyranoside (1)

Yellow amorphous powder (5.6 mg), soluble in H_2O and 70% aq. EtOH. R_f (silica gel) × 100, 0.56 in BAW and 0.46 in H_2O; it gave a brown color which changed to yellow with ammonia vapor under UV light; yellow color with Naturstoff reagenz A under UV light; UV λ max MeOH: 244.5 *sh*, 266.6, 291.7 *sh*, 319.5, 350; +NaOMe 267.3, 401.9; +NaOAc 266.2, 362.2, 402.8 *sh*; NaOAc + H_3BO_3 266.3, 294.4 *sh*, 352.8; +A1C13 254.1 *sh*, 274.35, 301.56, 352.9, 399; +HC1 274.1, 301.0, 348.7, 397.9. FAB-MS (neg.): *m/z* 725 $[M-l]^-$, *m/z* 593 [M-l-apio]$^-$, *m/z* 579 [M-l-rham]$^-$; FAB-MS (pos.): *m/z* 727 $[M+l]^+$, *m/z* 581 $[M+l\text{-rham}]^+$; CI-MS (neg.): *m/z* 593 [M-l-apio]$^-$, *m/z* 417 [M-1-rham-galc]$^-$, *m/z* 285 [M-1-rham-galc-apio]$^-$; CI-MS (pos.): *m/z* 595 $[M+l\text{-apio}]^+$, *m/z* 418 $[M+l\text{-rham-galc}]^+$, *m/z* 287 [aglycone+I]$^+$; LSI MS: *m/z* 726.8 $[M+l]^+$and *m/z* 580.7 $[M+l\text{-rham}]^+$, *m/z* 418.9 [aglycone + 1 + apio]$^+$, *m/z* 287 [aglycone + 1]$^+$. ^{1}H NMR of **1** (DMSO-d_6): δ 8.09 (2H, *d*, *J* = 8.79 Hz, H-2′, 6′), 6.84 (2H, *d*, *J* = 8.79 Hz, H-3′, 5′), 6.72 (1H, *d*, *J* ~1.00 Hz, H-8), 6.38 (1H, *d*, *J* ~1.00 Hz, H-6), 5.63 (1H, *d*, *J* = 3.16 Hz, apio H-l″), 5.32 (1H, *d*, *J* = 7.75 Hz, galc-H-l‴), 4.39 (1H, *d*, *J* = 1.26 Hz, rham H-l⁗), 4.06 (1H, *d*, *J* = 9.48 Hz, apio- H-4″a), 3.76 (1H, *d*, *J* = 9.27 Hz, apio H-4″b), 4.18 (1H, *d*, *J* = 3.42 Hz, apio H-2″), 3.25–3.65 (10 H, *m*, galc H-2‴,3‴,4‴,5‴,6‴-apio H-5″ a b, rham H-2⁗,3⁗,5⁗), 3.1 (1H, *t*, *J* = 9.32, 9.48 Hz, rham H-4⁗), 1.05 (3H, *d*, *J* = 6.18 Hz, rham 6 CH_3). ^{13}C NMR: δ 177.00 (C-4), 162.38 (C-7), 160.85 (C-5), 160.21 (C-4), 157.03 (C-2), 155.90 (C-9), 133.37 (C-3), 131.00 (C-2′, 6′), 121.00 (C-1′), 115.20 (C-3′,5′) 107.04 (C-l″), 106.00 (C-10), 101.93 (C-1‴), 99.99 (C-1⁗), 99.18 (C-6), 94.25 (C-8), 78.58 (C-3″), 75.94 (C-2″), 74.51 (C-4″), 73.53 (C-5‴), 72.86 (C-3‴), 71.81 (C-4⁗), 70.99 (C-2⁗). 70.49 (C-3⁗), 70.28 (C-2‴) 68.15 (C-5⁗), 67.93 (C-4‴), 65.32 (C-6‴), 61.85 (C-5″), 17.80 (C-6⁗).

3.6. Kaempferol 3-O-β-D-apiofuranoside 7-O-α-L-rhamnosyl-(1⁗→6‴)-O- β-D (2‴-O-E-caffeoylgalactopyranoside) (2)

Yellow amorphous powder (4.5 mg), soluble in H_2O and 70% EtOH. R_f × 100: 0.74 in BAW and 0.24 in H_2O; it gave a brown color which changed to yellow with ammonia vapor under UV light; yellow color with Naturstoffreagenz A under UV light and green color with $FeCl_3$; UV λ max MeOH: 256.0 *sh*, 267.2, 300 *sh*. 332.7; +NaOMe 269.1, 384.8; +NaOAc 259.0 *sh*, 267.3, 296.4, 333.9; NaOAc + H_3BO_3 265.3. 296.5, 347.9; +A1C13 270.9, 302.7, 358.5, 409.0 *sh*; +HC1 277.2, 299.33, 337.1, 397.0. FAB-MS (neg.): *m/z* 887 $[M-l]^-$, *m/z* 755 [M-l-apio]$^-$, *m/z* 447 [M-1-apio-rham-caff]$^-$, *m/z* 417 [M-1-rham-caff-gal]$^-$; FAB-MS (pos.): *m/z* 743 $[M+1\text{-rham}]^+$, *m/z* 449 [M + 1-apio-rham-caff]+, *m/z* 419 [M + 1-rham-caff-gal]+ CI-MS (neg.): *m/z* 887 [M-l]$^-$, *m/z* 611 [M-1-apio-rham]$^-$; CI-MS (pos.): *m/z* 449 [M + l-rham-caff-apio]+, *m/z* 419 [M + l-rham-caff-gal]$^+$, *m/z* 287 [aglycone + H]$^+$ ^{1}H NMR of **I** (DMSO-d_6): δ 8.08 (2H, *d*, *J* = 8.96 Hz, H-2′, 6′), 7.5 (1H, *d*, *J* = 15.77 Hz, caff- H-7), 7.04 (1H, *d*, *J* = 1.94 Hz, caff-H-2), 6.98 (1H, *dd*, *J* = 1.93, 8.25 caff-H-6), 6.87 (2H, *d*, *J* = 8.93 Hz, H-3′, 5′), 6.76 (1H, *d*, *J* = 8.12 Hz caff-H-5), 6.71 (1H, *d*, *J* = 2.83 Hz, H-8). 6.36 (1H, *d*, *J* = 2.52 Hz, H-6), 6.26 (1H, *d*, *J* = 15.92 Hz, caff-H-8), 5.62 1H, *d*, *J* = 3.53 Hz, apio- H-1″), 5.6 (1H, *d*, *J* = 7.98 Hz, galc- H-l‴), 5.2 (1H, *dd*, *J* = 8.01; 9.2 Hz, galc H-2‴), 4.37 (1H, *d*, *J* = 1.21 Hz, rham-H-1⁗) 4.17 (1H, *d*, *J* = 3.36 Hz, apio- H2″), 4.05 (1H, *d*, *J* = 9.51 Hz,apio-H-4″a), 3.76 (1H, *d*, *J* = 9.44 Hz, apio- 4″b), 3.35–3.75 (2H, *m*, apio- H-5″ a,b), 3.1–3.65 (*m*, other sugar protons), 1.06 (3H, *d*, *J* = 6.17 Hz, rham-6CH_3). ^{13}C NMR: δ 177.32 (C-4), 165.98 (C-9 caff) 162.47 (C-7), 160.85 (C-5), 160.21 (C-4′), 157.06(C-2), 155.94 (C-9), 148.35 (C-4 caff),145.58 (C-3 caff), 145.13 (C-7 caff), 132.97 (C-3), 131.06 (C-2′, 6′), 125.56 (C-l caff), 121.22 (C-l′), 120.54 (C-6 caff), 115.79 (C-5 caff), 115.14 (C-3′, 5′), 114.71 (C-2 caff), 114.31 (C-8 caff) 107.12 (C-1″), 105.44 (C-10), 100.15 (C-1⁗), 99.23 (C-1‴), 99.08 (C-6), 94.42 (C-8), 78.67 (C-3″), 76.05 (C-2″), 74.60 (C-4″), 73.69 (C-5‴),72.29 (C-2‴), 71.86 (C-4⁗), 70.80 (C-3‴), 70.58 (C-3⁗), 70.36 (C-2⁗), 69.76 (C-4‴), 68.29 (C-5⁗), 65.30 (C-6‴), 61.95 (C-5″), 17.88 (CH_3 rham).

Acknowledgements

The research was supported at The University of Texas at Austin by a grant from the Robert A. Welch Foundation (F-130) to T. J. Mabry and by the State Polish Committee for Scientific Research to M.W. grant 4P05f 00815.

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