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A new gallic acid fructose ester from *Saxifraga stellaris*

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Abstract

Seven compounds have been isolated from methanol extracts of whole *Saxifraga stellaris* (Saxifragaceae) plants. They were identified as the new compound, 6-*O*-galloyl fructose, together with 3-*O*-[2-*O*-(β-D-xylopyranosyl)-β-D-galactopyranosyl]-kaempferol, 3-*O*-[2-*O*-(β-D-xylopyranosyl)-β-D-galactopyranosyl]-quercetin, trifolin, hyperin, resveratrol-3-*O*-glucoside, triandrin, by chemical and spectroscopic methods. Their free radical scavenging properties are also described. © 1998 Published by Elsevier Science Ltd. All rights reserved.

Keywords: Saxifraga stellaris; Saxifragaceae; Flavonoid glycosides; Stilbene glycoside; 6-*O*-galloylfructose; Triandrin; Free radical scavengers

1. Introduction

The genus *Saxifraga* (Saxifragaceae) is found in the mountain regions of the northern hemisphere. *Saxifraga stellaris* L., one of the 26 species of *Saxifraga* from Switzerland, is a small thick-leaved plant that can be found around the Polar circle and in all the mountain regions of Europe (Hess & Hirzel, 1977). Although flavonoids have been described from *Saxifraga* (Jay & Lebreton, 1965; Pawlowska, 1976; Miller & Bohm, 1980; Bohm, Bhat, & Miller, 1984), little is known about the constituents of the genus. As no phytochemical study on *S. stellaris* has yet been undertaken, it was decided to investigate this plant.

2. Results and discussion

Dried whole plants of *S. stellaris* were extracted at room temperature with solvents of increasing polarity (dichloromethane and methanol). The methanol extract was fractionated by a combination of gel filtration on Sephadex LH-20, silica gel column chromatography and low pressure LC on an octadecylsilyl phase to afford compounds **1**–**7** (see Section 3).

The molecular formula $C_{13}H_{16}O_{10}$ of compound **1** was established on the basis of ^{1}H and ^{13}C NMR spectra (multiplicities of the carbons established by DEPT experiments) and thermospray (TSP) — MS (*m*/*z* 332 $[M]^{+}$). The EI-MS gave a fragment ion at *m*/*z* 170 $[M\text{-}162]^{+\cdot}$, corresponding to the loss of a hexosyl moiety. The ^{1}H NMR spectrum showed signals at δ 4.36 (1H, dd, $J = 3$ Hz, 11 Hz), 4.12 (1H, d, $J = 11$ Hz), 3.89 (2H, m), 3.78 (1H, m), 3.31 (2H, m) which confirmed the presence of a sugar moiety. One additional signal at δ 6.98 (2H, d, $J = 6$ Hz), integrating for two protons, was also seen in the low field region, suggesting the presence of a phenolic derivative. The coupling constant of 6 Hz indicated a *meta* substitution of the two protons. The ^{13}C NMR spectrum exhibited typical signals for a galloyl moiety at δ 165.7 (C-1), 145.4 (C-4 and C-6), 138.3 (C-5), 119.3 (C-2), 108.7 (C-3), 108.6 (C-7). In addition, twelve signals for fructofuranose, 104.4 and 102.4 (C-2′), 82.7 and 78.5 (C-5′), 77.9 and 75.7 (C-3′), 77.0 and 75.1 (C-4′), 66.2 and 65.0 (C-6′), 63.4 and 62.8 (C-1′), were shown, suggesting that **1** exists in solution as an equilibrium mixture of β-furanose and α-furanose forms (Yoshiki, Gen-Ichiro, & Itsuo, 1984). The signals were attributed by gradient HSQC and gradient HMBC.

The attachment of the galloyl moiety at position C-6′ of the fructose moiety was proved by additional NMR experiments: a correlation between H-6′ and H-5′ was seen in the ^{1}H–^{1}H COSY spectrum, confirmed by observation of a NOE on H-5′ after irradiation of H-6′. Correlation of H-6′ with C-1 was established by

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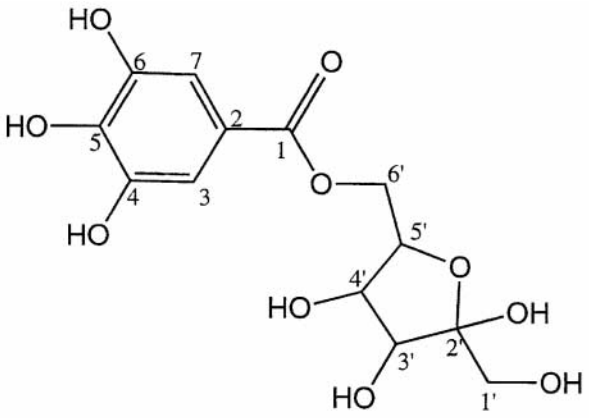

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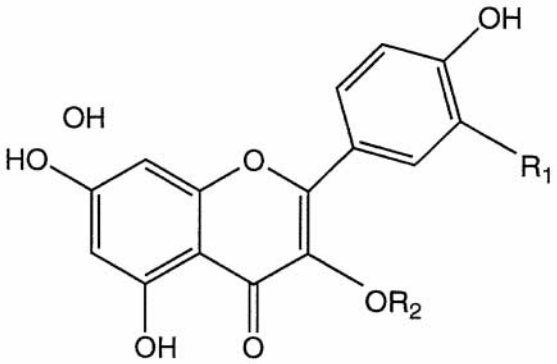
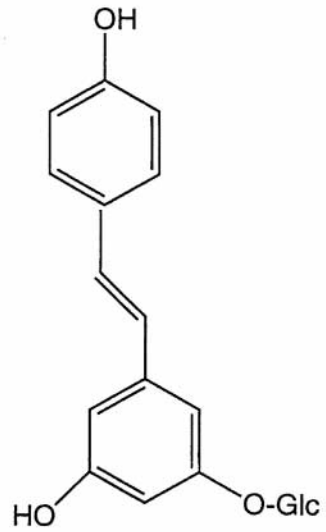


	R_1	R_2
2	-H	-Gal^2-Xyl
3	-OH	-Gal^2-Xyl
4	-H	-Gal
5	-OH	-Gal



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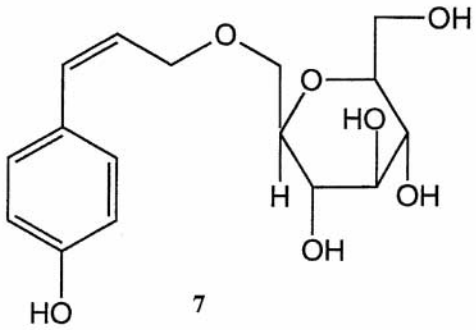

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^1H–^{13}C gradient HMBC long range NMR measurements (Fig. 1). Since the galloyl group is attached at position C-6 of the fructose, the pyranose form of the sugar can be excluded (Yoshiki et al., 1984). The presence of fructose was confirmed by TLC comparison with an authentic sample after basic hydrolysis. Thus, compound **1** is 6-*O*-galloylfructose, a new natural product for which we propose the name stellarin.

Compounds **2**–**7** were identified by comparison of their spectral data (UV, D/CI-MS, ^1H and ^{13}C NMR) with literature values as 3-*O*-[2-*O*-(β-D-xylopyranosyl)-β-D-galactopyranosyl]-kaempferol (**2**) (Lone, Jens, &

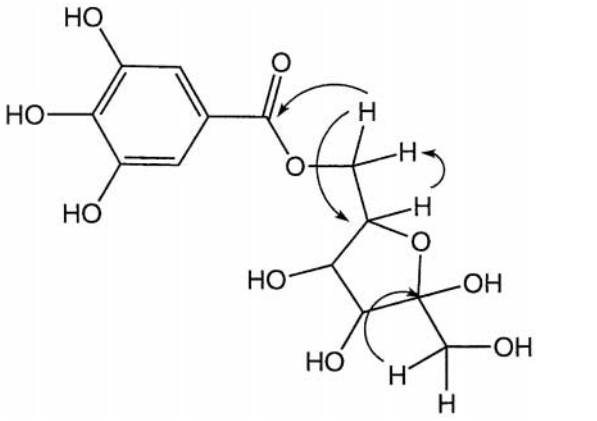

Fig. 1. Selected ^{1}H–^{13}C long range correlations of compound **1** by gradient HMBC and COSY ^{1}H–^{1}H.

Hilmer, 1982) 3-*O*-[2-*O*-(β-D-xylopyranosyl)-*β*-D-galactopyranosyl]-quercetin (**3**) (Lone et al., 1982), trifolin (**4**) (Markham & Ternai, 1976), hyperin (**5**) (Markham & Ternai, 1976), resveratrol-3-*O*-glucoside (**6**) (Fujinori, Satoshi, & Junya, 1992) and triandrin (**7**) (Lovina, Siegfried, & Hutchings, 1992).

Radical-scavenging properties of compounds **1**–**7** were evaluated against the DPPH (2,2-diphenyl-1-picrylhydrazyl) radical (Lamaison, Petitjean-Freyet, & Carnat, 1991; Bors, Saran, & Elstner, 1992; Choi, Lee, & Kang, 1994). By using DPPH as a TLC spray reagent (Cuendet, Hostettmann, & Potterat, 1997), **1**–**7** (10 μg) appeared as yellow spots against a purple background. Compounds **1**–**7** were also tested against DPPH in a spectrophotometric assay. Quercetin and BHT (2,6-di(*tert*-butyl)-4-methylphenol) were used as reference compounds. The activities of compounds **1**, **3** and **5** were comparable with quercetin and were higher than that of BHT (Table 1). Compounds **2** and **4**, lacking the catechol moiety, were less active. This is in agreement with structure–activity relationships previously established for flavonoids which show the importance of the catechol moiety for radical–scavenging activity (Bors, Heller, Michel, & Saran, 1990).

Table 1
Scavenging activity of compounds **1**–**7** in a DPPH assay

Compound	Concentration [μM]					
	80.0	40.0	20.0	10.0	5.0	2.5
1	95.0*	94.8	62.8	38.6	15.5	15.2
2	19.1	13.3	11.0	8.5	8.4	9.4
3	90.9	92.9	68.8	37.6	22.1	14.7
4	23.3	16.5	12.7	10.3	10.2	10.2
5	94.9	94.5	90.0	55.3	31.1	20.9
6	22.7	17.3	10.9	8.2	7.2	7.6
7	16.7	13.9	10.4	8.6	8.0	8.6
BHT	64.8	43.6	28.1	19.6	15.2	12.7
Quercetin	95.4	95.0	64.9	41.4	23.0	14.3

*Absorbance of test solution in % measured after 30 min at 517 nm.

3. Experimental

3.1. General

TLC: Silica gel $60F_{254}$ A1 sheets (Merck), detection with Godin reagent (Godin, 1954). $[\alpha]_D$: Perkin-Elmer-241 polarimeter. UV: Perkin Elmer Lambda 20 spectrophotometer and UV spectra recorded in MeOH. For open CC, silica gel 60 (63–200 μm) and for Lobar LPLC, RP-18 (15–40 μm) were used. Analytical HPLC was performed on a HP-1090 instrument equipped with a photodiode array detector. Frs were analyzed on Nova-pak C18 columns, (4 μm, 150 × 3.9 mm, i.d., Waters) with a gradient of 5–30% MeOH in water over 20 min, 30–35% over 20 min and 35–100% over 10 min, at a flow rate of 1 ml min^{-1}. EI-MS and D/CI-MS: Finnigan-MAT TSQ-700 triple stage quadrupole instrument (NH_3, positive ion mode). ^{1}H and ^{13}C-NMR spectra were measured on a Varian Innova-500: samples were dissolved in DMSO-d_6 and TMS was used as int. standard. Complete attribution was performed on the basis of 2D-experiments (COSY, gradient HMBC, gradient HSQC).

3.2. Plant material and samples

Saxifraga stellaris was collected near the Grand St. Bernard Pass (VS, Switzerland) in August 1996 and identified by E. Anchisi, Jardin Botanique de Champex (VS, Switzerland). A voucher specimen (No. 96133) has been deposited at the Institute of Pharmacognosy and Phytochemistry, University of Lausanne, Switzerland.

3.3. Extraction and isolation

The dried whole plant of *S. stellaris* (250 g) was extracted at room temp. successively with CH_2Cl_2 (3 × 2 l) and MeOH (3 × 2 l). A portion (8 g) of the methanol extract (18 g) was separated on a silica gel open column (70 × 4 cm) with a step-gradient of MeOH–EtOAc 1:4 (2 l), 1:2 (900 ml) and finally MeOH to give 6 frs (A–F). Fraction C (1.1 g) was suspended in 200 ml of water and extracted 3 times with 200 ml of *n*-BuOH. The *n*-BuOH fr (700 mg) was then fractionated on Sephadex LH-20 ($CHCl_3$–MeOH 1:1) to give 4 frs (C1–C4). Fraction C2 was separated by Lobar RP-18 (MeOH–H_2O 4:6) to give compounds **2** (15 mg) and **4** (10 mg). Fraction C3 was separated by Lobar RP-18 with a step-gradient of (MeOH–H_2O 3:7, 4:6) to give compound **5** (76 mg) and 3 frs (C31–C33). Fraction C31 was separated by Lobar RP-18 (MeOH–

H_2O 5:95) to give compound **1** (15 mg). Fraction C32 was separated by Lobar RP-18 (MeOH–H_2O 35:65) to give compound **6** (43 mg).

The other portion (10 g) of the methanol extract was suspended in 1 l of water and extracted 3 times with 1 l of *n*-BuOH. The *n*-BuOH fr (3 g) was then fractionated on a silica gel column (60 × 4 cm) with a step-gradient of $CHCl_3$–MeOH–H_2O (50:5:1, 50:10:1, 65:35:5) and finally MeOH to give 5 frs (G–K). Fraction G was filtered on Sephadex LH-20 (MeOH) to give 6 frs (G1–G6). Fraction G1 was separated by Lobar RP-18 with a step-gradient of MeOH–H_2O (8:2, 6:2, 1:1, 1:3), MeOH and finally EtOAc, to give compound **7** (4 mg). Fraction H (287 mg) was separated by Lobar RP-18 with a step-gradient of MeOH–H_2O (8:2, 6:4, 4:6), to give compound **3** (48 mg).

*3.4. 6-O-Galloylfructose (**1**)*

White glass. TLC: ($CHCl_3$–MeOH–H_2O, 65:35:5) R_f 0.52. UV λ_{max}^{MeOH} nm (log ϵ): 276 (5.28), 218 (5.67); $[\alpha]_D^{21} = +30.8^\circ$ (MeOH; c 0.1); ^{1}H NMR: δ 6.98 (d, $J = 6$ Hz, H-3, H-7), 4.36 (1H, dd, $J = 3$ Hz, 11 Hz, H-6′), 4.12 (1H, d, $J = 11$ Hz, H-6′), 3.89 (2H, m, H-3′, H-4′), 3.78 (1H, m, H-5′), 3.31 (2H, m, H-1′); ^{13}C NMR: δ 165.7 (C-1), 145.4 (C-4 and C-6), 138.3 (C-5), 119.3 (C-2), 108.7 (C-3), 108.6 (C-7), 104.4 and 102.4 (C-2′), 82.7 and 78.5 (C-5′), 77.9 and 75.7 (C-3′), 77.0 and 75.1 (C-4′), 66.2 and 65.0 (C-6′), 63.4 and 62.8 (C-1′).

Hydrolysis of compound **1**: 2 mg of **1** were treated with 1 ml 0.5 N KOH at room temperature for 1 min. The identity of the sugar was confirmed by TLC analysis (EtOAc–MeOH–H_2O–AcOH, 65:15:15:20) of the reaction mixture with a genuine sample of fructose.

3.5. Reduction of 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical

5 μl of a soln. containing the compound to be tested were added to 225 μl of MeOH and 50 μl of a 0.004% MeOH soln. of DPPH. Absorbance at 517 nm was determined after 30 min and the percent of activity was calculated.

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