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FLAVONOL GLYCOSIDES FROM *SEDUM TELEPHIUM* SUBSPECIES *MAXIMUM* LEAVES

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Key Word Index—*Sedum telephium* ssp. *maximum*; Crassulaceae; flavonols; kaempferol 3-neohesperidoside-7-rhamnoside; quercetin 3-neohesperidoside-7-rhamnoside; NOE.

Abstract—Two new flavonol glycosides, kaempferol 3-*O*-β-neohesperidoside-7-*O*-α-rhamnoside and quercetin 3-*O*-β-neohesperidoside-7-*O*-α-rhamnoside were identified in the fresh leaves of *Sedum telephium* ssp. *maximum*. The known compounds, quercetin, kaempferol and their 3-glucosides, 7-rhamnosides and 3,7-dirhamnosides were also identified. All compounds were characterized by means of chemical and spectroscopic methods. NOE experiments were performed to detect the glycosidic bond at the 7-hydroxyl of the aglycone molecules.

INTRODUCTION

The genus *Sedum* (Crassulaceae) is found mainly in various East-European regions and a large number of *Sedum* species are used pharmaceutically. The drug mostly derives from the aerial parts of the plants. The fresh leaves and their juice serve for both topical applications and internal use [1].

Sedum telephium L., in particular, was widely known in antiquity for its local anti-inflammatory activity; it is presently found all over Europe, the leaves being used for wound healing and for the treatment of various local inflammatory processes [1]. *Sedum telephium* is also quoted in popular Tuscan medical texts where it is called 'Erba di San Giovanni'.

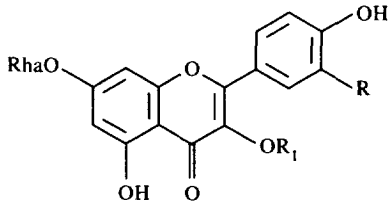
The anti-inflammatory, keratolytic and analgesic activity of *S. telephium* L. ssp. *maximum* Schinz & Thell. leaves has been confirmed by numerous experiments carried out at the Emergency Unit of the Torre Galli Hospital, Florence in the treatment of various local inflammatory conditions [2].

In a more recent investigation of this plant, polysaccharides isolated and identified from leaf tissue have been shown to have anti-inflammatory potential [3].

The aim of the present work was to investigate the flavonoid constituents of *S. telephium* ssp. *maximum* leaves as these compounds have been suggested to contribute to its anti-inflammatory activity.

RESULTS AND DISCUSSION

According to the literature [4–7], the genus *Sedum* appears to be rich in flavonoids, and our investigation on the leaves of *S. telephium* ssp. *maximum* confirmed these findings. Two tri-glycosylated flavonols, kaempferol 3-*O*-α-L-rhamnopyranosyl (1 → 2)-β-D-glucopyranoside-7-*O*-α-L-rhamnopyranoside (**1**) and quercetin 3-*O*-α-L-rhamnopyranosyl (1 → 2)-β-D-glucopyranoside-7-*O*-α-L-rhamnopyranoside (**2**), together with quercetin 3,7-*O*-α-L-dirhamnopyranoside (**6**) and the previously reported kaempferol 3-*O*-β-D-glucopyranoside-7-*O*-α-L-rhamnopyranoside (**3**), quercetin 3-*O*-β-D-glucopyranoside-7-*O*-α-L-rhamnopyranoside (**4**), and kaempferol 3,7-*O*-α-L-dirhamnopyranoside (**5**) [8] were isolated and identified. For each compound, the ^{1}H NMR spectrum in MeOH-d_4 was recorded. This is the first report on the occurrence of tri-glycosylated flavonols in this plant, and to our knowledge also of products **1** and **2**. No myricetin glycosides were found, although they were previously reported from *S. telephium* ssp. *maximum* [8]. In the present study kaempferol derivatives were found to be more abundant



	R	R^1
1	H	Glc(2-1)Rha
2	OH	Glc(2-1)Rha
3	H	Glc
4	OH	Glc
5	H	Rha
6	OH	Rha

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than quercetin glycosides with kaempferol 3,7-dirhamnoside (**5**) as the main flavonol glycoside in the leaf tissue.

Glycoside **1** gave kaempferol, glucose and rhamnose after acid hydrolysis; the MS spectrum in FAB showed $[MH]^+$ of 741 with other typical fragments due to the loss of glucose and rhamnose. The UV spectra showed the presence of a kaempferol derivative with free hydroxyl groups at 5 and 4'. The ^1H NMR spectrum indicated the sugar configuration and the sequence of the glucose and rhamnose units to the aglycone. The four doublets between δ6.45–8.08 are in agreement with the signals of kaempferol. Three signals deriving from anomeric protons were observed: the doublet at δ5.77 (diaxial coupling $J = 7.0$ Hz) was assigned to the anomeric proton of D-glucose β-linked to the 3-hydroxyl of kaempferol. The other two signals at δ5.25 and 5.58 (diequatorial coupling $J = 2.0$ Hz) corresponded to the anomeric protons of the L-rhamnopyranose moieties. The signal at δ5.58 was assigned to the L-rhamnose α-linked to the 7-hydroxyl of kaempferol. In fact, when this signal was presaturated an intense NOE enhancement of the doublets relative to protons H-6 and H-8 was observed. The signal at δ5.25 corresponded to a rhamnose unit α-linked to the glucose. The chemical shift of this anomeric proton was further downfield than the same signal of the interglycosidic bond (6-1) of the rutin (+0.91). Also, the glucose anomeric proton at δ5.77, was further downfield (+0.44) than the same signal of the other products **3** and **4**, in which the glucose unit was present without interglycosidic bonds. In the light of these data, the signal at δ5.25 was attributed to a rhamnose unit which was α-linked to the 2''-hydroxyl of glucose. The doublet at δ1.25 was assigned to the methyl group of rhamnose α-linked to the 7-hydroxyl according to the ^1H NMR spectra of **3** and **6**. Therefore, the other signal, at δ0.95, was attributed to a rhamnose unit α-linked to the 2''-hydroxyl of glucose.

In the ^{13}C NMR spectrum of **1** the presence of signals at δ161.6 and 133.0 agreed with the glycosylation at C-7 and C-3, respectively [9]. Three anomeric carbon signals were observed at δ100.6, 98.4 and 98.3; the remaining sugar signals were characteristic of the pyranose form [10], and appeared in the range δ77.6–17.2. The signal for C-2 of the glucose, which was β-linked to the 3-hydroxyl, was at δ77.5. We observed a downfield shift (+3.3) between **1** and the value of the same signal for the kaempferol glycosides, in which an unsubstituted glucose unit is β-linked at the 3-hydroxyl [9]. This deshielding is due to the interglycosidic bond between C-2'' and C-1'''. Therefore, the glycosidic fragment, which is β-linked to the 3-hydroxyl, appeared to be neohesperidose [11].

On the basis of these data, **1** was identified as kaempferol 3-*O*-α-L-rhamnopyranosyl (1→2)-β-D-glucopyranoside-7-*O*-α-L-rhamnopyranoside.

Glycoside **2** gave quercetin, glucose and rhamnose after acid hydrolysis. The MS spectrum in positive FAB showed $[MH]^+$ 757, and the UV spectra suggested the presence of quercetin with free 3'-, 4'- and 5-hydroxyls. The signals of the ^1H NMR spectrum, between δ7.65 and 6.45, showed a typical quercetin pattern. Also, three signals derived from anomeric protons were observed. These signals were at exactly the same chemical shifts as **1**. Moreover, two doublets at δ1.25 and 0.97 with coupling constants of 3.6 Hz confirmed the presence of two rhamnose units bound in the same position as in **1**. The other signals of the ^1H NMR spectrum (range δ3.0–4.2) appeared to overlap with the same signals as **1**. Thus, **2** was identified as quercetin 3-*O*-α-L-rhamnopyranosyl (1→2)-β-D-glucopyranoside-7-*O*-α-L-rhamnopyranoside.

The UV data of **3**–**6**, both in methanol and with standard shift reagents, were in agreement with glycosylation at the 3-hydroxyl and 7-hydroxyl of the aglycone molecules. After enzymic hydrolysis with α-rhamnosidase [12], the following compounds were obtained: kaempferol 3-glucoside from **3**, quercetin 3-glucoside from **4**, kaempferol from **5** and quercetin from **6**. These products of enzyme hydrolysis were identified by co-chromatography with authentic samples.

The ^1H NMR NOE experiments appeared to be an efficient tool for establishing the presence of the sugar bond at the 7-hydroxyl of the kaempferol and quercetin molecules. The NOE experiments were carried out by presaturating the signals of the rhamnose anomeric protons. Significant nuclear Overhauser enhancements were observed for each glycoside, between the anomeric protons and the H-6 and H-8 protons of the aglycone, with signal increases between 10 and 17%.

EXPERIMENTAL

Plant material. Leaves of *S. telephium* ssp. *maximum* were obtained from plants grown in an experimental field in a hilly area near Florence; they were collected and immediately frozen in August 1992. For this study they were carefully dried in an airy oven at 38°.

Instruments. The ^1H NMR spectra were performed on a Varian Gemini 200 at 200 MHz. The NOE experiments were performed using a Varian NOE diff. program version 6.3 A. ^{13}C NMR spectra were recorded with a Varian Gemini 300. MS-FAB spectra were recorded with a Kratos-MS 50. Analyt. HPLC was performed with a Hewlett Packard chromatograph equipped with a diode array detector.

Extraction. Dried ground leaves were first extracted with petrol, then with 70% aq. MeOH in a blender at room temp. The concd MeOH extract was successfully partitioned between H_2O and Et_2O, H_2O and EtOAc. The EtOAc extract and the final aq. fr. were used to isolate the 6 flavonol glycosides.

Isolation of the flavonoids. The EtOAc extract was chromatographed on a Kielsegel 60 (Merck), 230–400 mesh column (i.d. = 3.2 mm, l = 32 mm), using $CHCl_3$–MeOH–H_2O 7:3:0.5 as eluent. The aq. fr. was chromatographed on a dry Kielsegel 60 A column (ICN-Biomedicals, Gmbh, Eschwege) using EtOAc–MeOH–H_2O–HCO_2H 77:13:10:4 as eluent. The frs obtained were checked by HPTLC using silica gel plates and the following eluents: $CHCl_3$–MeOH–H_2O 7:3:0.5 (eluent A); EtOAc–MeOH–H_2O–HCO_2H 77:13:10:4 (eluent B) and EtOAc–HCO_2H–HOAc–H_2O 100:11:11:27 (eluent C). The glycosides were isolated from the frs by semi-

prep. HPLC (column Hibar LiChrosorb C18, 7 μ, i.d. 10 mm, length 250 mm; UV detector 280 nm; flow 6 ml min^{-1}; solvent A: H_2O pH 3.6 with HOAc and solvent B: acetonitrile; various linear solvent gradients were used from 100% A to 75% A and 25% B).

To verify purity, each compound was dissolved in acetonitrile–H_2O (pH 2.8 with HCl) 2:8 and analysed with a 4-ramp linearly programmed solvent gradient. Initial conditions: 100% A; final conditions 75% A (column Hibar LiChrosorb C 18, 5 μ, i.d. 4.6 mm, length 125 mm; flow 1.5 ml min^{-1}; solvent A: H_2O pH 3.6 with HOAc, solvent B: acetonitrile and solvent C: MeOH).

Flavonoid identification. Enzymic hydrolyses were carried out in acetate buffers with β-glucosidase at pH 4.5 or with Sigma hesperidinase, as a source of α-rhamnosidase, at pH 3.8. Acid hydrolyses were conducted with 1 M HCl at 100° for 1 hr. The sugars were identified by GLC as trimethylsilyl-derivatives (TRI-SIL^R-Z reagent—Pierce) in comparison with standards. The standards of aglycones and glycosides for HPTLC control were purchased from Extrasynthèse (BP-62-ZI Lyon Nord, Genay France).

Kaempferol 3-O-α-L-*rhamnopyranosyl* (1 → 2)-β-D-*glucopyranoside*-7-O-α-L-*rhamnopyranoside* (**1**). HPTLC R_f: eluents A = 0.16, B = 0.17, C = 0.2; UV λ_{max}^{MeOH} nm: 228 (sh), 265, 316 (sh), 346; + NaOMe: 241, 271, 346 (sh), 380; + NaOAc: 265, 370; + $AlCl_3$: 234 (sh), 273, 300 (sh), 350, 395; + $AlCl_3$–HCl: 234 (sh), 273, 300 (sh), 344, 395. FAB-MS (thioglycerol): 741 $[MH]^+$, 595 $[MH - rha]^+$, 579 $[MH - glu]^+$, 286. ^1H NMR (MeOH-d_4): δ8.08 (*d*, *J* = 8.4 Hz, H-2′ and 6′), 6.91 (*d*, *J* = 8.4 Hz, H-2′ and 5′), 6.75 (*dm*, *J* = 2.0 Hz, H-6), 6.45 (*d*, *J* = 2.0 Hz, H-8), 5.77 (*d*, *J* = 7.0 Hz, H-1″), 5.58 (*d*, *J* = 2.0 Hz, H-1‴), 5.25 (*d*, *J* = 2.0 Hz, H-1 R7), 4.0–3.55 (*m*, sugar protons), 1.25 (*d*, *J* = 5.6 Hz, H-6 R7), 0.95 (*d*, *J* = 5.6 Hz, H-6‴). ^{13}C NMR (DMSO-d_6, TMS as int. standard): δ177.5 (C-4), 161.6 (C-7), 160.8 (C-5), 160.1 (C-4′), 156.7 (C-9), 155.9 (C-2), 133.0 (C-3), 130.9 (C-2′ and C-6′), 120.7 (C-1′), 115.1 (C-3′ and C-5′), 105.6 (C-10), 100.6 (C-1‴), 99.4 (C-6), 98.4 (C-1″), 98.3 (C-1 R7), 94.5 (C-8), 77.6 (C-2″), 77.5 (C-3‴), 77.2 (C-5″), 71.8 (C-4 R7), 71.6 (C-4‴), 70.6 (C-3 R7), 70.5 (C-3‴), 70.2 (C-2‴), 70.2 (C-2 R7), 70.1 (C-4″), 69.8 (C-5 R7), 68.3 (C-5‴), 60.8 (C-6″), 17.9 (C-6‴), 17.2 (C-6 R7).

Quercetin 3-O-α-L-*rhamnopyranosyl* (1→2)-β-D-*glucopyranoside*-7-O-α-L-*rhamnopyranoside* (**2**). HPTLC R_f: eluents A = 0.1, B = 0.14, C = 0.2; UV λ_{max}^{MeOH} nm: 257, 360; + NaOMe: 268, 330 (sh), 386; + NaOAc: 260, 330 (sh), 383; + $AlCl_3$: 273, 300 (sh), 436; + $AlCl_3$–HCl: 270, 296 (sh), 358 (sh), 402. FAB-MS (DMSO + KI): 795 $[MK]^+$, 757 $[MH]^+$, 611 $[MH - rha]^+$, 595 $[MH - glu]^+$. ^1H NMR (MeOH-d_4): δ7.65 (*m*, H-2′ and 6′), 6.87 (*d*, *J* = 9.0 Hz, H-5), 6.74 (*d*, *J* = 2.0 Hz, H-2 and 8), 6.45 (*d*, *J* = 2.0 Hz, H-6), 5.77 (*d*, *J* = 7.3 Hz, H-1″), 5.56 (*d*, *J* = 1.5 Hz, H-1 R7), 5.23 (*d*, *J* = 1.5 Hz, H-1‴), 4.01–3.47 (*m*, sugar protons), 1.25 (*d*, *J* = 5.8 Hz, H-6 R7), 0.97 (*d*, *J* = 6.2 Hz, H-6‴).

Kaempferol 3-O-β-D-*glucopyranoside*-7-O-α-L-*rhamnopyranoside* (**3**). HPTLC R_f: eluents A = 0.37, B = 0.36, C = 0.38; ^1H NMR (MeOH-d_4): δ8.09 (*d*, *J* = 9.0 Hz, H-2′ and H-6′), 6.9 (*d*, *J* = 9.0 Hz, H-3′ and 5′), 6.77 (*d*, *J* = 2.0 Hz, H-8), 6.47 (*d*, *J* = 2.0 Hz, H-6), 5.58 (*d*, *J* = 1.3 Hz, H-1‴), 5.34 (*d*, *J* = 6.4 Hz, H-1″), 4.03–3.44 (*m*, sugar protons), 1.25 (*d*, *J* = 6.2 Hz, H-6‴).

Quercetin 3-O-β-D-*glucopyranoside*-7-O-α-L-*rhamnopyranoside* (**4**). HPTLC R_f: eluents A = 0.25, B = 0.3, C = 0.32; ^1H NMR (MeOH-d_4): δ7.73 (*d*, *J* = 2.2 Hz, H-2′), 7.63 (*d*, *J* = 8.5 Hz, H-6′), 6.88 (*d*, *J* = 8.5 Hz, H-5′), 6.76 (*d*, *J* = 1.6 Hz, H-8), 6.48 (*d*, *J* = 1.6 Hz, H-6), 5.57 (*d*, *J* = 1.2 Hz, H-1 R7), 5.33 (*d*, *J* = 7.3 Hz, H-1″), 4.04–3.39 (*m*, sugar protons), 1.26 (*d*, *J* = 5.8 Hz, H-6 R7).

Kaempferol 3,7-O-α-L-*dirhamnopyranoside* (**5**). HPTLC R_f: eluents A = 0.43, B = 0.47, C = 0.54; ^1H NMR (MeOH-d_4): δ7.81 (*d*, *J* = 8.8 Hz, H-2′ and 6′), 6.95 (*d*, *J* = 8.6 Hz, H-3′ and 5′), 6.75 (*d*, *J* = 2.0 Hz, H-8), 6.48 (*d*, *J* = 2.0 Hz, H-6), 5.57 (*d*, *J* = 1.7 Hz, H-1‴), 5.41 (*d*, *J* = 1.7 Hz, H-1″), 4.23 (*d*, *J* = 3.3 Hz, H-2″), 4.02 (*d*, *J* = 3.3 Hz, H-2‴), 4.1–3.4 (*m*, sugar protons), 1.27 (*d*, *J* = 5.6 Hz, H-6‴), 0.94 (*d*, *J* = 5.6 Hz, H-6″).

Quercetin 3,7-O-α-L-*dirhamnopyranoside* (**6**). HPTLC R_f: eluents A = 0.32, B = 0.41, C = 0.5; ^1H NMR (MeOH-d_4): δ7.38 (*s*, H-2′), 7.35 (partially superimposed with H-2′), (*d*, *J* = 7.7 Hz, H-6′), 6.93 (*d*, *J* = 7.7 Hz, H-5′), 6.74 (*d*, *J* = 1.8 Hz, H-8), 6.49 (*d*, *J* = 1.8 Hz, H-6), 5.56 (*d*, *J* = 1.5 Hz, H-1 R7), 5.38 (*d*, *J* = 1.5 Hz, H-1‴), 4.23–3.38 (*m*, sugar protons), 1.27 (*d*, *J* = 5.8 Hz, H-6 R7), 0.96 (*d*, *J* = 5.8 Hz, H-6″). (R7 = rhamnose moiety attached at C-7).

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REFERENCES

1. Gnedkov, P. A. (1977) *Rastit. Resur.* **13**, 544.
2. Balatri, S. (1981) in *L'uso del Sedum telephium L. nella pratica chirurgica minore*, XLII, 3. Boll. Soc. Tosco-Umbra Chir.
3. Sendl, A., Mulinacci, N., Vincieri, F. F. and Wagner, H. (1993) *Phytochemistry* **34**, 1357.
4. Wolbis, M. (1989) *Phytochemistry* **28**, 2187.
5. Khetwal, K. S., Manral, K. and Verma, D. L. (1988) *Planta Med.* **54**, 89.
6. Gerard, G., Nieman, J., Visser-Simons, J. M. J. and Henk't Hart (1976) *Planta Med.* **30**, 384.
7. Denton, M. F. and Kerwin, J. L. (1980) *Can. J. Botany* **58**, 902.
8. Wolbis, M. (1987) *Acta Pol. Pharm.* **44**, 567.
9. Pawan, K., Agrawal and Mahesh, C. B. (1989) in *Carbon-13 NMR of Flavonoids* (Agrawal, P. K., ed.), pp. 334–347. Elsevier Science, Amsterdam.
10. Pomiiio, A. B. and Zallocchi, E. M. (1989) *J. Nat. Prod.* **52**, 511.
11. Markam, K. R., Ternai, B., Stanley, R., Geiger, H. and Mabry, T. J. (1978) *Tetrahedron* **34**, 1389.
12. Harborne, J. B. and Boardley, M. (1983) *Phytochemistry* **22**, 622.