Phytochemistry, Vol. 27, No. 12, pp. 3941–3943, 1988.
Printed in Great Britain.

0031–9422/88 $3.00+0.00

FLAVONOL GLYCOSIDES FROM *SEDUM ACRE*

MARIA WOLBIŚ and MARIA KRÓLIKOWSKA

Department of Pharmacognosy, Institute of Technology and Chemistry of Drugs, Medical Academy of Łódź, 90–151 Łódź, Poland

Received in revised form 25 *April* 1988)

Key Word Index—*Sedum acre*; Crassulaceae; flavonols; quercetin; isorhamnetin; limocitrin 3-,7- and 3,7-glucosides; isorhamnetin 3-*O*-(2″-*O*-acetyl)-β-D-glucopyranoside.

Abstract—Three new flavonol glycosides, isorhamnetin 3-(2″-acetyl) glucoside, limocitrin 7-glucoside, and limocitrin 3,7-diglucoside, were isolated from the aerial parts of *Sedum acre*. The known compounds quercetin, isorhamnetin and their 3- and 3,7-di-glucosides, isorhamnetin7-glucoside and limocitrin and its 3-glucoside were also identified. The structure of the compounds was determined by means of spectroscopic and chemical methods.

INTRODUCTION

Sedum acre L. is the most common *Sedum* species in Poland. The aerial parts of the plant are used in folk medicine. The biological activity of the chemically investigated representatives of this genus is ascribed to alkaloids, organic acids, and polyphenolics including flavonoids [1, 2]. Previous flavonoid investigations of *S. acre* have shown the presence of rutin [3], various incompletely determined flavones and flavanones [4, 5], as well as isorhamnetin 3- and 7-glucosides, limocitrin 3-glucoside, isorhamnetin 3,7-di- or triglucoside and a possible isorhamnetin glucoester [6, 7]. In *S. acre* L. var. *sexangulare*, treated in the latest floristic literature as an independent species *S. sexangulare* L. [8, 9], 8-methoxykaempferol and its 3-rhamnoglucoside-7-rhamnoside have been reported [10, 11].

In the present investigation a further eight flavonoids have been characterized, including three described for the first time.

RESULTS AND DISCUSSION

In the aerial parts of *S. acre* 14 flavonoids were detected, of which 12 were separated and identified. Of the previously reported compound the presence of isorhamnetin 3- and 7-glucosides and limocitrin 3-glucoside was confirmed. Isorhamnetin 3,7-di-*O*-β-D-glucopyranoside has been fully characterized by other workers [6, 7]. However, the presence of rutin, flavanones and 8-methoxykaempferol and its glycoside, reported in the literature, could not be detected [3–5, 10, 11]. The remaining flavonoids have been isolated from the taxon for the first time. On the basis of chemical and spectroscopic investigations (UV, ^{1}H NMR) compound **1** was identified as isorhamnetin, **2** as quercetin, **3** limocitrin, **7** as quercetin 3-glucoside, **12** as quercetin 3,7-di-glucoside. The physicochemical properties of the isolated compounds are in agreement with those given in the literature, with the exception of those given for isorhamnetin 7-glucoside (its melting point and that of its heptaacetate and its UV spectrum) [7, 12].

A further three flavonoids (**5**, **9**, **10**) were isolated for the first time in nature. Compound **5** gave isorhamnetin and D-glucose on and hydrolysis, and during mild base hydrolysis gave isorhamnetin 3-glucoside. The UV and ^{1}H NMR spectra of the heptaacetate are identical with those obtained for isorhamnetin 3-glucoside. FDMS of **5** showed a quasimolecular ion at *m/z* 521 $[M+H]^+$ corresponding to the molecular formula $C_{24}H_{24}O_{13}$. In the IR spectrum, apart for the characteristic flavonoids signal the ester group signal was observed at 1730 cm^{-1}. The presence of a C=O group at δ169.4 ppm and Me at 20.9 ppm in the ^{13}C NMR spectrum, and a three-proton singlet at 2.14 ppm in ^{1}H NMR TMS ether spectrum can be ascribed to an acetyl function. From a comparison of the ^{1}H NMR spectra of the TMS ether of **5** and its reconstruction product it can be seen that the acetyl function is attached to the C-2″ of the β-D-glucopyranose (H-2″ signal, triplet of $J=8$ Hz, shifted downfield by *ca* 1.49 ppm, H-1″ signal, doublet of $J=8$ Hz shifted in the opposite direction by 0.33 ppm) [13, 14]. Specific rotation of **5**, −96.3°, is characteristic of glucosides acylated at C-2 of glucose [14, 15]. The acetylation position has been confirmed with ^{13}C NMR analysis (Table 1). The large difference in the position of signals of C-1″ and C-3″ in **5**, as compared with the respective signals in **4** which are shifted upfield by 3.0 ppm and 2.8 ppm, respectively, together with the unchanged position of the C-2″ signal and the agreement of the shifts of the remaining carbons with literature values [16] allowed identification of **5** as isorhamnetin 3-*O*-(2″*O*-acetyl)-β-D-glucopyranoside. The isomeric isorhamnetin 3-*O*-(6″-*O*-acetyl)-β-D-glucopyranoside, has been found in *Salix viminalis*, *Pinus contorta* and *Pinus silvestris* [17–19].

Compounds **9** and **10** on acid hydrolysis gave D-glucose and an aglycone, which on the basis of its melting point, acetate and UV and ^{1}H NMR spectra was characterized as limocitrin despite some differences from literature data (tetraacetate; mp [6, 7] 176–177°, [20] 155–156°). The glycosylation position, for **9** at C-7 and for **10** at C-3 and C-7, as well as the configuration of the glycosidic binding and the cyclic form of the sugar was determined by means of UV and ^{1}H NMR spectroscopic analysis of the TMS ethers of the compounds and their acetates [21]. FDMS showed quasimolecular ions, $[M+H]^+$ at *m/z* 509 and 671, corresponding to the molecu-

Table 1. ^{13}C NMR spectral data of compounds **4** and **5**

C	4	5
2	156.4	156.3
3	133.3	132.7
4	177.5	177.1
5	161.3	161.1
6	98.6	98.7
7	164.2	164.1
8	93.7	93.7
9	156.4	156.3
10	104.2	104.0
1′	121.3	120.8
2′	113.9	113.4
3′	149.5	149.5
4′	147.1	146.9
5′	115.3	115.2
6′	122.1	122.1
OMe	55.8	55.7
1″	101.4	98.4
2″	74.3	74.3
3″	76.7	73.9
4″	70.1	69.9
5″	77.4	77.5
6″	60.8	60.5
		169.4
OAc		20.9

300 MHz, DMSO-d_6, δ values in ppm from TMS

lar formulas of **9** $C_{23}H_{24}O_{13}$, and **10** $C_{29}H_{34}O_{18}$. From these results **9** is identified as limocitrin 7-*O*-β-D-glucopyranoside, and **10** as limocitrin 3,7-di-*O*-β-D-glucopyranoside.

Because of infraspecific cytological and morphological variations within the species *Sedum acre* [22], additional flavonoid investigations of plant material from various regions of Poland (Ciechocinek, Czestochowa, Gadańsk, Nowy Sacz, Wroclaw) were carried out. However, no qualitative flavonoid differences were found in the complex using chromatographic methods. Isorhamnetin and its glucosides were the major constituents in all the samples analysed.

EXPERIMENTAL

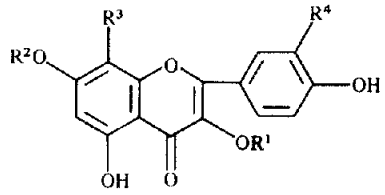
Plant material. Aerial parts of *S. acre* were collected from a natural population in Ozorków, region Łódź, Poland, at the time of flowering in June 1985. Taxonomic identification was performed by comparing the morphological features with those given for this taxon in refs [8, 9, 22]. A voucher specimen is deposited in the Herbarium of the Department of Pharmacognosy Medical Academy of Łódź, Poland.

General procedure. Mp uncorr.; FDMS-Varian MAT 711; ^{13}C NMR-Bruker MSL NMR; ^1H NMR 360/60 MHz, TMS as int. standard/. PC–Whatman No. 1 paper, solvent systems: (1) *n*-BuOH HOAc–H_2O (4:1:5), (2) PhOH–H_2O (3:1), (3) 15% HOAc, (4) conc. HCl–HOAc–H_2O (3:30:10); TLC-silica gel 60F 254 solvent system EtOH–25% NH_3 H_2O (20:1:4).

Flavonoids were localized in UV light. UV spectra and TMS ethers were obtained according to ref. [21]. Acid hydrolysis was conducted in: 2.5% H_2SO_4 for compounds **4**–**7**; 10% H_2SO_4 **8**–**12**; partial hydrolysis of **10**, **12** with 2.5% H_2SO_4. Acetylation was by standard procedures: glycosides–$Ac_2O+C_5H_5N$, aglycones–Ac_2O + NaOAc.

Extraction and isolation. Dried aerial parts of the plant (stalks, leaves and flowers) (2 kg), was submitted to initial extraction with petrol, $CHCl_3$ (Soxhlet), and then to exhaustive extraction with boiling MeOH (15 l × 4). 320 g of MeOH extract were dissolved in 2 l H_2O and subsequently extracted with: Et_2O (0.2 l × 30, E I 6.4 g), Et_2O–EtOAc (1:1, 0.2 l × 20, E II 11.2 g), EtOAc (0.4 l × 35, E III 21.1 g), EtOAc–MeOH (9:1, 0.4 l × 60, E IV 33.8 g). The ppts from the H_2O layer during extraction with EtOAc and EtOAc–MeOH (O I 3.1 g) were filtered off. After crystallization of E I with aq. MeOH compound **1** was obtained (0.5 g), and from E III **4** (6.2 g). Mother liquors from **1** and E II after combination with those from **4** and E IV were separated independently on MN-Polyamide SC6 columns (eluent H_2O–MeOH). From the 60–90% aq. MeOH fraction or E I additional amounts of **1** (0.3 g), **2** (90 mg), and **3** (0.2 g) were obtained. After the separation of E II from E III mother liquors the 40–60% aq. MeOH fraction gave an additional amount of **4** (3.2 g), and from the 65–90% aq. MeOH fraction **5** (2.1 g). Mother liquors from **4** and **5** and fractions eluted with 10–40% aq. MeOH from E IV were separated independently on MN-Polyamide SC6 columns (eluent C_6H_6–MeOH). After the separation of E III mother liquors the following compounds were obtained: **6** (0.2 g), **7** (0.1 g), **8** (0.3 g), **9** (80 mg). After the separation of fraction E IV the following compounds were obtained: **10** (0.3 g), **11** (0.8 g), **12** (60 mg). An additional amount of **11** (1.2 g) was obtained by multiple cryst. with aq. MeOH O I.

Isorhamnetin 3-O-(2″-O-acetyl)-β-D-glucopyranoside (**5**). Light-yellow needles, mp 156–158° (aq. MeOH); heptaacetate, needles, mp 160–161° (EtOH). PC R_f s: (1) 0.80 (2) 0.89, (3) 0.63. $[\alpha]_D^{20}$ −96.3° (MeOH; *c* 1.5). FDMS *m/z* 521/100%, $[M+H]^+$). IR ν_{max}^{KBr} cm^{-1}: 1658 C=O, γ-pyrone, 1730 (C=O, ester). UV-λ_{max} nm: MeOH, 253, 268 sh, 302 sh, 355; NaOMe, 268, 331, 414; $AlCl_3$, 267, 300 sh, 368 sh, 398; $AlCl_3$/HCl, 269, 300 sh, 360 sh, 398; NaOAc, 268, 316 sh, 372; NaOAc/H_3BO_3, 253, 268 sh, 300 sh, 356. ^1H NMR TMS ether (δ, ppm, CCl_4): 2.13 (*s*, OAc), 2.90–3.23 (*m*, H-5″), 3.36–3.86 (*m*, H-3″, 4″ and 2H-6″), 3.96 (*s*, OMe), 4.83 (*tr*, *J* = 8 Hz, H-2″), 5.50 (*d*, *J* = 8 Hz, H-1″), 6.10 (*d*, *J* = 2 Hz, H-6), 6.43 (*d*, *J* = 2 Hz, H-8), 6.80 (*d*, *J* = 8 Hz, H-5′), 7.40



1 R^1 = R^2 = R^3 = H, R^4 = OMe
2 R^1 = R^2 = R^3 = H, R^4 = OH
3 R^1 = R^2 = H, R^3 = R^4 = OMe
4 R^1 = Glc, R^2 = R^3 = H, R^4 = OMe
5 R^1 = (2-OAc)Glc, R^2 = R^3 = H, R^4 = OMe
6 R^1 = Glc, R^2 = H, R^3 = R^4 = OMe
7 R^1 = Glc, R^2 = R^3 = H, R^4 = OH
8 R^1 = R^3 = H, R^2 = Glc, R^4 = OMe
9 R^1 = H, R^2 = Glc, R^3 = R^4 = OMe
10 R^1 = R^2 = Glc, R^3 = R^4 = OMe
11 R^1 = R^2 = Glc, R^3 = H, R^4 = OMe
12 R^1 = R^2 = Glc, R^3 = H, R^4 = OH

(*dd*, $J=2$ and 8 Hz, H-6'), 7.76 (*d*, $J=2$ Hz, H-2'). ^{1}H NMR heptaacetate (δ, ppm, $CDCl_3$): 1.90 (*s*, OAc), 2.00 (s, 2 × OAc), 2.10 (*s*, OAc), 2.33 (*s*, 2 × OAc), 2.43 (*s*, OAc), 3.40–3.76 (*m*, H-5''), 3.80–4.10 (*m*, 2H-6''), 3.93 (*s*, OMe), 4.73–5.33 (*m*, H-2'', 3'' and 4''), 5.50 (*d*, $J=8$ Hz, H-1''), 6.76 (*d*, $J=2$ Hz, H-6), 7.06 (*d*, $J=8$ Hz, H-5'), 7.23 (*d*, $J=2$ Hz, H-8), 7.40–7.73 (*m*, H-2' and 6').

Acid hydrolysis products: isorhamnetin mp 310–312° (tetraacetate, mp 210–212°)/UV and ^{1}H NMR spectrum of the TMS ether identical with ref. [21]; D-glucose, R_fs: PC (1) 0.16, TLC 0.41. Base hydrolysis. **5** (50 mg) in 0.5% KOH (5 ml) was left for 20 min at room temp. The soln was acidified (5% HCl), isorhamnetin 3-glucoside was filtered and cryst. with aq. MeOH (mp, ^{1}H NMR spectrum of the TMS ether identical with that of **4**).

Limocitrin 7-O-β-D-*glucopyranoside* (**9**). Light-yellow needles, mp 265–268° (aq. MeOH); heptaacetate, amorphous compound, mp 111–113° (MeOH). PC R_fs: (1) 0.40 (2) 0.80, (3) 0.11. FDMS *m/z* 509 (100%, $[M+H]^+$), 346 (23%, A). UV λ_{max} nm: MeOH, 258, 272 sh, 333 sh, 384; NaOMe, 254 sh, 270, 450; $AlCl_3$, 269, 305 sh, 372 sh, 442: $AlCl_3$/HCl, 269, 305 sh, 368 sh, 440; NaOAc, 258, 272 sh, 335 sh, 390, 434 sh; NaOAc/H_3BO_3, 257, 273 sh, 335 sh, 384. ^{1}H NMR of the TMS ether (δ, ppm, CCl_4): 3.10–4.00 (m, H-2'', 3'', 4'', 5'' and 2H-6''), 3.90 (*s*, 2 × OMe), 4.86–5.06 (*m*, H-1''), 6.26 (*s*, H-6), 6.83 (*d*, $J=8$ Hz, H-5'), 7.60–7.83 (*m*, H-2' and 6'). ^{1}H NMR heptaacetate (δ, ppm, $CDCl_3$): 1.96 (*s*, OAc), 2.00 (*s*, OAc), 2.03 (*s*, OAc), 2.10 (*s*, OAc), 2.23 (*s*, OAc), 2.26 (*s*, OAc), 2.36 (*s*, OAc), 3.46–4.30 (*m*, H-5'' and 2H-6''), 3.93 (*s*, OMe), 3.96 (*s*, OMe), 4.86–5.26 (*m*, H-1'',2'',3'' and 4''), 6.73 (*s*, H-6), 7.10 (*d*, $J=8$ Hz, H-5'), 7.53–7.76 (*m*, H-2' and 6').

Acid hydrolysis products: limocitrin, mp 275–278° (tetraacetate, mp 174–176°) and D-glucose.

Limocitrin 3,7-di-O-β-D-*glucopyranoside* (**10**). Yellow needles, mp 262–264° (aq. MeOH); decaacetate, needles, mp 124–125° ($CHCl_3$–MeOH 1:1). PC R_fs: (1) 0.29 (2) 0.72, (3) 0.68. FDMS *m/z* 671 (100%, $[M+H]^+$), 509 (18%, $[M+H]^+$-glc). UV λ_{max} nm: MeOH, 256, 273, 362; NaOMe, 266, 305 sh, 415; $AlCl_3$, 243 sh, 274, 308 sh, 368, 415 sh; $AlCl_3$/HCl, 241 sh, 274, 306 sh, 364, 418; NaOAc, 257, 272, 370, 425 sh; NaOAc/H_3BO_3, 255, 273, 365. ^{1}H NMR TMS ether (δ, ppm, CCl_4): 2.96–4.03 (m, 12 sugar protons), 3.90 (*s*, 2 × OMe), 4.83–5.06 (*m*, H-1'''), 5.70–5.90 (*m*, H-1''), 6.30 (*s*, H-6), 6.86 (*d*, $J=8$ Hz, H-5'), 7.53 (*dd*, $J=2$ and 8 Hz, H-6'), 7.80 (*d*, $J=2$ Hz, H-2'). ^{1}H NMR decaacetate (δ, ppm, $CDCl_3$): 1.90 (*s*, OAc), 2.00 (*s*, 2 × OAc), 2.06 (*s*, 5 × OAc), 2.33 (*s*, OAc), 2.43 (*s*, OAc), 3.43–4.33 (*m*, H-5'', 2H-6'', H-5''' and 2H-6'''), 3.90 (*s*, OMe), 3.96 (*s*, OMe), 4.83–5.63 (*m*, H-1'', 2'',3'',4'' and H-1''',2''',3''',4'''), 6.76 (*s*, H-6), 7.10 (*d*, $J=8$ Hz, H-5'), 7.53–7.80 (*m*, H-2' and 6').

Acid hydrolysis products: limocitrin, mp 278–280° (tetraacetate, mp 176–177°), D-glucose and limocitrin 7-glucoside, mp 268–270° (UV spectrum identical with **9**).

REFERENCES

1. Shniyakina, G. P. and Krasnov, E. A. (1974) *Rastit. Resur.* **10**, 130.
2. Gnedkov, P. A. and Schroeter, A. I. (1977) *Rastit. Resur.* **13**, 554.
3. Mylius, E. (1872) *Arch. Pharm.* **201**, 97.
4. Nordal, A. (1946) *Johan Grundt Tanum Forlag, Oslo*, 166. (Chem. Abstr. **44**, 3216i).
5. Frigot, P. (1961) Travaux, Laboratoires de Matière Mèdicale, Paris **45**, 7.
6. Królikowska, M. (1966) *Roczniki Chem.* **40**, 717.
7. Królikowska, M. (1972) *Roczniki Chem.* **46**, 969.
8. Webb, D. A. (1964) *Flora Europaea.* 1. University of Cambridge Press.
9. Garcke, A. (1972) *Illustrierte Flora Deutschland und angrenzende Gabiete.* Parey, Berlin.
10. Combier, H., Markham, K., Audier, H., Lebreton, Ph., Mabry, T. J. and Jay, M. (1968) *C. R. Acad. Sci., Ser. D* **266** 2495.
11. Mnajed, H., Raynaud, J. and Combier, H. (1972) *C. R. Acad. Sci., Ser. D* **274**, 445.
12. Singh, K. N. and Pandey, V. B. (1986) *Phytochemistry* **25**, 2683.
13. Dossaji, S. and Kubo, I. (1980) *Phytochemistry* **19**, 482.
14. Isobe, T., Ito, N. and Nods, Y. (1980) *Phytochemistry* **19**, 1877.
15. Pearl, I. and Darling, S. F. (1959) *J. Org. Chem.* **24**, 731.
16. Markham, K. R., Ternai, B., Stanley, R., Geiger, H. and Mabry, T. J. (1978) *Tetrahedron* **34**, 1389.
17. Karl, Ch., Pedersen, P. A. and Schwarz, Ch. (1977) *Phytochemistry* **16**, 1117.
18. Higuchi, R. and Donnelly, D. M. X. (1978) *Phytochemistry* **17**, 787.
19. Zapesochnaya, G. G., Ivanova, S. Z., Medvedeva, S. A. and Tyukavkina, N. A. (1978) *Khim. Prir. Soedin.* **14**, 193.
20. Horowitz, R. M. and Gentili, B. (1961) *J. Org. Chem.* **26**, 2899.
21. Mabry, T. J., Markham, K. R. and Thomas, M. B. (1970) *The Systematic Identification of Flavonoids.* Springer, Berlin.
22. Hart, H. (1971) *Acta Bot. Neerl.* **20**, 282.