Phytochemistry, Vol. 28, No. 5, pp. 1513–1516, 1989.
Printed in Great Britain.

0031 9422/89 $3.00 + 0.00

TWO ISORHAMNETIN TRIGLYCOSIDES FROM *ANTHYLLIS SERICEA*

J. ALBERTO MARCO,* J. ADELL, O. BARBERA,† D. STRACK‡ and V. WRAY§

Departamento de Quimica Orgánica, Facultad de Quimicas, Universidad de Valencia, E-46100 Burjasot, Valencia, Spain; †Departamento de Didáctica de las Ciencias Experiments, E.U.P.E.G.B., Universidad de Valencia, E-46006 Valencia, Spain; ‡Institut für Pharmazeutische Biologie der Technischen Universität Braunschweig, Mendelssohnstr. 1, D-3300 Braunschweig, F.R.G.; §Gesellschaft für Biotechnologische Forschung, Mascheroder Weg 1, D-3300 Braunschweig, F.R.G.

(*Received* 24 *June* 1988)

Key Word Index—*Anthyllis sericea*; Leguminosae; flavonol glycosides; isorhamnetin 3-*O*-(2-*O*-β-glucopyranosyl-6-*O*-α-rhamnopyranosyl)-β-galactopyranoside; isorhamnetin 3-*O*-(2-*O*-β-glucopyranosyl)-β-galactopyranoside-7-*O*-β-glucopyranoside; ^{1}H NMR; ^{13}C NMR.

Abstract—A fraction of a methanolic extract of aerial parts of *Anthyllis sericea* yielded vicenin 2 and the two new compounds isorhamnetin 3-*O*-(2-*O*-β-glucopyranosyl-6-*O*-α-rhamnopyranosyl)-β-galactopyranoside and isorhamnetin 3-*O*-(2-*O*-β-glucopyranosyl)-β-galactopyranoside-7-*O*-β-glucopyranoside.

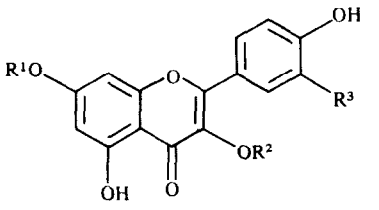
INTRODUCTION

We have recently reported [1] on the isolation of several flavonoid glycosides from a fraction of a methanolic extract of *Anthyllis sericea* Lag. non Willd. (syn. *A. henoniana* Cosson ex Batt.) (Leguminosae). Among these compounds, the diglycoside isorhamnetin 3-*O*-(2-*O*-β-D-glucopyranosyl)-β-D-galactopyranoside was described for the first time as a natural product. We now wish to communicate the results of our study on another fraction of the mentioned extract, thus completing the chemical investigation of the species. This has allowed the isolation and characterization of the two new isorhamnetin triglycosides **1** and **2** and of the di-*C*-glycosylflavone vicenin 2.

RESULTS AND DISCUSSION

Extraction of aerial parts of *A. sericea* and subsequent chromatography yielded compounds **1** and **2**, which were isolated in small amounts as mixtures of isorhamnetin/kaempferol glycosides (NMR analysis). We had already found such aglycone pairs as mono- and diglycosides with various sugar residues [1]. The separation of both components, which had been very lengthy and difficult in the case of the diglycosides, proved completely impossible by traditional methods of TLC and column chromatography at normal pressure for the triglycosides. Recourse had to be made to HPLC, which permitted the separation and purification of the isorhamnetin derivatives **1** and **2** (see Experimental). The presumed kaempferol glycosides **3** and **4** were present in too small amounts to allow their characterization.

Compound **1** was a yellow material with the chromatographic mobility of a triglycoside [2]. Acid hydrolysis yielded isorhamnetin, glucose, galactose and rhamnose. The negative ion FAB mass spectrum showed a $[M-H]^-$ peak at *m/z* 785, in accord with the molecular formula $C_{34}H_{42}O_{21}$. A 200 MHz ^{1}H NMR spectrum in



	R^1	R^2	R^3
1	H	βGal (2→1β)Glc / (6→1α)Rha	OMe
2	βGlc	βGal (2→1β)Glc	OMe
3	H	βGal (2→1β)Glc / (6→1α)Rha	H
4	βGlc	βGal (2→1β)Glc	H

DMSO-d_6 (Table 1) showed the expected aromatic pattern as well as three doublets at δ5.64 (J = 7.6 Hz), 4.58 (J = 7.4 Hz) and 4.37 (J = 1.3 Hz), which were attributed to anomeric protons. The last signal was clearly originated in the rhamnose residue, its high field position pointing to a sugar–sugar linkage. Since we had already found an isorhamnetin 2-glucogalactoside and an isorhamnetin robinobioside (6-rhamnogalactoside) in the same species [1], we supposed **1** to have the sugar pattern resulting from the superposition of both carbohydrate moieties, i.e. 2-glucosyl-6-rhamnosylgalactose. The location of sugar residues at the other ring phenolic positions (C-5,7,4′) could be excluded by the position of the NMR signals of H-6, H-8 and H-5′, which were almost coincident with those of isorhamnetin 3-galactoside [3].

In order to confirm this conclusion, we undertook a careful study of the sugar part of **1** by two-dimensional ^{1}H shift correlation (COSY) at 300 and 400 MHz, the higher resolution aimed at determining as far as possible the coupling constants in the hexose rings. The results of the study are given in Table 2. Starting from the doublet

*Author to whom correspondence should be addressed.

Table 1. ^{1}H NMR spectra of compounds **1** and **2**

Compound	Aromatic protons					Anomeric protons†			OMe	Me_{rha}
	H-6	H-8	H-2′	H-5′	H-6′	H-1″	H-1‴	H-1⁗		
1*	6.19 *d*	6.43 *d*	7.87 *d*	6.90 *d*	7.60 *dd*	5.64 *d*	4.58 *d*	4.37 *d*	3.86 *s*	1.04 *d*
	(1.9)	(1.9)	(1.9)	(8.5)	(8.5; 1.9)	(7.6)	(7.4)	(1.3)		(6.1)
1‡	6.25 *d*	6.45 *d*	7.98 *d*	6.96 *d*	7.68 *dd*	5.49 *d*	4.82 *d*	4.54 *d*	4.02 *s*	1.21 *d*
	(1.7)	(1.7)	(1.8)	(8.4)	(8.4; 1.8)	(7.7)	(7.5)	(1.3)		(6.2)
2*	6.42 *d*	6.83 *d*	7.90 *d*	6.90 *d*	7.66 *dd*	5.72 *d*	4.62 *d*	5.15 *d*	3.88 *s*	
	(1.9)	(1.9)	(1.8)	(8.5)	(8.5; 1.8)	(7.6)	(7.3)	(7.4)		
2‡	6.53 *d*	6.84 *d*	8.00 *d*	6.96 *d*	7.71 *dd*	5.63 *d*	4.81 *d*	5.11 *d*	4.02 *s*	
	(1.8)	(1.8)	(2.1)	(8.5)	(8.5; 2.1)	(7.7)	(7.4)	(7.7)		

*At 200.13 MHz in DMSO-d_6 (30°); δ values are followed by multiplicity and below, in parentheses, coupling constants in Hz. Only aromatic, anomeric, methyl and methoxyl signals are given.

†″indicates the galactose, ‴ denotes the glucose bound to C-2″ and ⁗ means the third sugar residue (glucose or rhamnose), respectively.

‡At 300 MHz in CD_3OD (25°).

Table 2. ^{1}H NMR of **1** and **2** (sugar part)*

H	1	2
1″	5.49 *d*	5.63 *d*
	(7.7)	(7.7)
2″	4.13 *dd*	4.12 *dd*
	(9.3; 7.7)	(9.5; 7.7)
3″	3.79 *dd*	3.81 *dd*
	(9.3; 3.5)	(9.5; 3.4)
4″	3.86 *dd*	3.91 *dd*
	(3.5; <1)	(3.4; <1)
5″	3.70–3.50	3.70–3.50
$6''_{A+B}$	overlapped *m*	overlapped *m*
1‴	4.82 *d*	4.81 *d*
	(7.5)	(7.4)
2‴	3.42	3.40
	overlapped *m*	overlapped *m*
1⁗	4.54 *d*	5.11 *d*
	(1.3)	(7.7)
$6''''_{A+B}$	1.21 *d* (3H)	3.95 *dd* (1H)
	(6.1)	(12.0; 2.2)
		3.80–3.70 (1H)
		overlapped *m*

*At 400 MHz in CD_3OD (25°). See footnotes * and † to Table 1.

at δ5.49 (in CD_3OD), undoubtedly originated in the anomeric hydrogen of the aglycone-sugar linkage (H-1″), we could pursue the proton chain up to H-4″. The small value of the coupling constants $J_{3'',4''}$ and $J_{4'',5''}$ evidenced the equatorial nature of H-4″ [1], thus showing this sugar residue to be galactose. The rather low field position of H-2″ (Table 2) pointed to a second sugar moiety being bonded through this hydroxyl group. That this sugar residue corresponded to glucose was strongly supported by the observation of a NOE effect at H-2″ after saturation of the doublet at δ4.82 (H-1‴), the signal necessarily arising in the glucose anomeric proton (axial–axial coupling J = 7.5 Hz). Furthermore, the position of the signal of H-4″ at relatively high field suggested that the hydroxyl at C-4″ was not glycosylated.

The ^{13}C NMR spectrum was in a very good accord with the proposed structure [4, 5]. The signals at δ79.54 and 64.91 were assigned to C-2″ and C-6″ and appeared thus at the expected positions if one assumed the mentioned galactose (2→1) glucose and galactose (6→1) rhamnose linkages [1, 4, 6–10]. For instance, the trisaccharide part of **1** is not common. Although derivatives of kaempferol [11] and isorhamnetin [12, 13] have been described with a triglycoside residue at C-3 consisting of galactose, glucose and rhamnose, the exact nature of the triose part was not ascertained in these cases.

Compound **2** had a similar colour but a somewhat lower chromatographic mobility than **1**. Acid hydrolysis yielded isorhamnetin, glucose and galactose. The true molecular size followed again from a negative ion FAB mass spectrum, which showed peaks at m/z 801 and 639. This agreed with a molecular formula $C_{34}H_{42}O_{22}$ (M_r 802) and thus characterized **2** also as a triglycoside. The aromatic part in the ^{1}H NMR spectrum of **2** (200 MHz, DMSO-d_6) showed the same pattern as that of **1** but with marked downfield shifts in the signals of H-6 and H-8 (Table 1). This suggested one glycosyl residue being bound to C-7. Moreover, the appearance of two anomeric signals above δ5 pointed to the presence of two aglycone-sugar linkages, the other anomeric signal being located at δ4.62, more typical of a sugar–sugar linkage. All three anomeric signals had coupling constants of *ca* 7.5 Hz, an usual value for β-glucopyranose and galactopyranose rings [1, 3]. These data led us to conclude that **2** was an isorhamnetin bearing sugar residues at C-3 and C-7. Hence, the structure of isorhamnetin 3-*O*-(2-*O*-β-D-glucopyranosyl)-β-D-galactopyranoside-7-*O*-β-D-glucopyranoside seemed us to be the most probable one.

Higher field NMR (400 MHz, CD_3OD) and two-dimensional COSY spectra were again utilized to confirm this structural assignment. As in **1**, the hydrogen chain of the first hexose moiety (starting from the doublet at δ5.63) could be traced back up to H-4″, thus showing that galactose was bound to C-3 (see small coupling constants $J_{3'',4''}$ and $J_{4'',5''}$ in Table 2). A glucose residue had to be connected through C-2″ in order to explain the rather low

Table 3. ^{13}C NMR spectra of compounds **1** and **2***

	Aromatic part			Sugar part†	
C	1	2	C	1	2
2	156.34[a]	156.27[a]	1″	98.80	98.45
3	132.78	133.18	2″	79.54	79.84
4	177.30	177.52	3″	73.11[c]	73.06[c]
5	161.17	160.79	4″	67.74	67.66
6	98.80	99.24[b]	5″	73.53[c]	75.86[d]
7	164.51	162.78	6″	64.91	60.01
8	93.79	94.55	1‴	103.77[b]	103.68
9	156.06[a]	155.81[a]	2‴	74.18	74.22
10	103.53[b]	105.33	3‴	76.83[d]	76.76[d]
1′	121.10	120.91	4‴	69.84	69.80[e]
2′	113.19	113.23	5‴	76.55[d]	76.53[d]
3′	147.04	147.08	6‴	60.06	60.80
4′	149.53	149.69	1⁗	99.99	99.87[b]
5′	115.24	115.25	2⁗	70.61[e]	73.28[c]
6′	122.55	122.69	3⁗	70.39[e]	76.44[d]
			4⁗	71.91	69.62[e]
OMe	55.92	55.94	5⁗	68.25	77.23[d]
			6⁗	17.88	60.62

*At 50.32 MHz in DMSO-d_6 (30°). The signals with the same superscript ([a, b, c, ...]) may be interchanged within the corresponding spectrum.

†See corresponding footnote to Table 1.

field position of the signal of H-2″ (*vide supra*). The third sugar residue (glucose) was thus located at C-7. As a further support of this, NOE effects were detected at H-2″ while saturating the doublet at δ4.81 (H-1‴) and at H-6/H-8 by saturation of the signal at δ5.11 (H-1⁗). As in the case of **1**, the ^{13}C NMR spectrum of **2** showed signals in the expected positions for the proposed structure [1, 4–10].

The limited chemical data available on the genus *Anthyllis*, which is included into the tribe Loteae, do not permit definitive conclusions about the taxonomic significance of the compounds isolated from *A. sericea*. Jay *et al.* [14] pointed out that lack of 5-oxygenation and the presence of 8-oxygenation, 7-*O*-methylation, B-ring trioxygenation and *C*-glycosylation are frequent structural features of flavonoids isolated from the tribe Loteae. Within this tribe, woody species of the genus *Anthyllis* (*A. barba-jovis*, *A. hermanniae*, *A. montana*, etc.) usually yielded flavonoids with the mentioned structural features but without 7-*O*-methylation. Herbaceous species however (e.g. *A. vulneraria*), contain mainly flavonoids without B-ring trisubstitution and 8-oxygenation but with 7-*O*-methylation. We had already observed this pattern in the flavonoids isolated from the herbaceous *A. onobrychioides* [3]. *A. sericea*, a woody species, seems to conform to the predicted behaviour in that it only shows flavonoids without 7-*O*-methyl substitution. On the other hand, although neither 5-deoxyflavonoids nor 8-oxygenated flavonoids were found in this species, we report for the first time in the genus the presence of *C*-glycosylflavones.

EXPERIMENTAL

NMR spectra were measured at 200, 300 and 400 MHz. The solvent signals were taken as the ref.

Extraction and chromatography. The plant material has been already described [1]. A *n*-BuOH extract, obtained as reported there, was concd *in vavuo* and chromatographed on a polyamide column with H_2O. After TLC analysis (spraying with Neu reagent), two flavonoid-containing fractions (B-1 and B-2) were collected. Fraction B-1 was then re-chromatographed on polyamide with H_2O: the flavonoid material was subsequently purified by paper chromatography (TBA) and CC on Sephadex LH-20 (20% aq. MeOH), affording the isolation of **1** as a mixture (15 mg) with the corresponding kaempferol glycoside. Fraction B-2 gave three fractions (B-21 to B-23) after column chromatography on polyamide (elution with H_2O). The more polar fraction (B-21) was percolated through Sephadex LH-20 (MeOH), giving **2** as a mixture (10 mg) with the corresponding kaempferol glycoside. B-22 was shown to be a mixture of the products of fraction B-21 and vicenin 2. Separation took place by CC on Sephadex LH-20 (elution with 10% aq. MeOH), allowing the isolation of 8 mg of pure vicenin 2. The third fraction (B-23) contained 25 mg of the already described isorhamnetin 3-*O*-glucogalactoside [1].

Acid hydrolyses were performed on the two mentioned isorhamnetin/kaempferol mixtures under the standard conditions [3]. This gave in each case the two aglycones isorhamnetin and kaempferol, identified by comparison with authentic samples, and the corresponding sugar mixture, which was analysed by GC of the silylated derivative. The ^{13}C NMR spectra were also measured on the isorhamnetin/kaempferol mixtures. The homogeneity of the sugar part of the spectrum in both cases pointed to the identity of this part for both isorhamnetin and kaempferol derivatives.

Final purification of **1** and **2** took place by HPLC (Waters/Millipore GmbH, system 600) under the following conditions: prepacked Multosorb-C_{18} column (10 μm, 250 × 20 mm i.d.) from CS-Chromatographie Service Eschweiler, FRG, UV detection at 360 nm. Elution system: linear gradient within 100 min from solvent A (0.5% HCOOH in H_2O) to 50% solvent B (H_2O–MeOH–MeCN 1:1:1) in A, flow rate of 30 ml/min. R_ts: **1**, 73 min; **2**, 49 min. *Ca.* 1.5 mg were isolated of each compound for ^{1}H NMR and mass spectral purposes.

Acknowledgements—O.B. thanks the Conselleria de Cultura, Educació i Ciencia de la Generalitat Valenciana for a grant. Support by the Deutsche Forschungsgemeinschaft to D.S. is gratefully acknowledged.

REFERENCES

1. Adell, J., Barberá, O. and Marco, J. A. (1988) *Phytochemistry* **27**, 2967.
2. Harborne, J. B. (1967) *Comparative Biochemistry of the Flavonoids*, Chap. 2. Academic Press, London.
3. Barberá, O., Sanz, J. F., Sanchez Parareda, J. and Marco, J. A. (1986) *Phytochemistry* **25**, 2361.
4. Markham, K. R., Ternai, B., Stanley, R., Geiger, H. and Mabry, T. J. (1978) *Tetrahedron* **34**, 1389.
5. Agrawal, P. K. and Rastogi, R. P. (1981) *Heterocycles* **16**, 2181.
6. Andersen, W. K., Omar, A. A. and Christensen, S. B. (1987) *Phytochemistry* **26**, 291.
7. Beier, R. C., Mundy, B. P. and Strobel, G. A. (1980) *Can. J. Chem.* **58**, 2800.
8. Bock, K. and Pedersen, C. (1983) *Adv. Carbohydr. Chem. Biochem.* **41**, 27.
9. Bock, K., Pedersen, C. and Pedersen, H. (1984) *Adv. Car-*

bohydr. Chem. Biochem. **42**, 193.
10. Dutton, G. G. S., Merrifield, E. H., Lafitte, C., Pratviel-Sosa, F. and Wylde, R. (1982) *Org. Magn. Reson.* **20**, 154.
11. El-Sherbeiny, A. A., El-Ansari, M. A., Nawwar, M. A. M. and El-Sayed, N. H. A. (1977) *Planta Med.* **32**, 165.
12. Tappi, G. and Menziani, E. (1955) *Gazz. Chim. Ital.* **85**, 694.
13. Wagner, H., Iyengar, M. A., Seligmann, O., Hörhammer, L. and Herz, W. (1972) *Phytochemistry* **11**, 2630.
14. Jay, M., Voirin, B., Hasan, A., Gonnet, J. F. and Viricel, M. R. (1980) *Biochem. Syst. Ecol.* **8**, 127.