Three New Flavonol Malonylrhamnosides from *Ribes alpinum*

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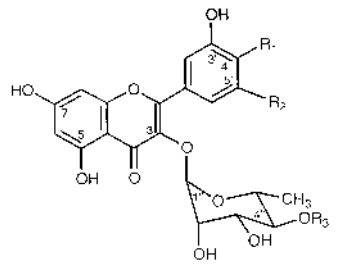
Three new flavonol malonylrhamnosides, 3-*O*-(4″-*O*-malonyl)-α-L-rhamnopyranosides of mearnsetin, myricetin and quercetin respectively, together with the corresponding mearnsitrin, myricitrin, quercitrin and the 4-*O*-methyl phloracetophenone 2-*O*-β-D-glucopyranoside, were isolated from the leaves of *Ribes alpinum* and fully characterized by spectrocopic methods including 2D NMR.

Key words *Ribes alpinum*; Grossulariaceae; flavonol malonylrhamnoside; mearnsitrin; myricitrin; quercitrin

Ribes alpinum L. (alpine currant) (Grossulariaceae), is a shrub widespread in montainous areas across western Europe. Unlike *R. nigrum* (black currant), *R. rubrum* (red currant) and *R. grossularia* (gooseberry), this species is not cultivated to-day, although its berries collected in the wild were traditionally used to prepare a cheap 'wine'. Renewed attention is currently being given to these species as part of the revival of 'natural' food sources and the search for their valorization as pharmaceuticals; here the use of leaf infusions in traditional medicine draws attention to their polyphenolic content, such molecules being the active components of many commercialized plant preparations. While hydrolysates from *R. nigrum* and other species are currently screened for their antioxydant flavonols, such work deals mainly with the edible berries.[1,2] As for the leaves, most knowledge dates from chemotaxonomic surveys of flavonol aglycones[3,4]; sampling investigated for native *O*-glycosides is very limited.[5—7] As part of a multidisciplinary analysis of regional wild and cultivated *Ribes* biodiversity, the flavonoid pattern of leaves from 120 individual plants, half representing *R. alpinum*, was assessed. HPLC coupled with PDA detection revealed that *R. alpinum* differed from the other species under study, in agreement with its botanical assignment to subgenus *Berisia* (Leclerc *et al*., to be published). We report here the isolation and structural elucidation of the six major flavonol glycosides characteristic of the leaves of *R. alpinum*, three of which were hitherto undescribed. A phloracetophenone glycoside was also isolated.



	R_1	R_2	R_3
1	OCH_3	OH	$COCH_2CO_2H$
2	OH	OH	$COCH_2CO_2H$
3	OH	H	$COCH_2CO_2H$
4	OCH_3	OH	H
5	OH	OH	H
6	OH	H	H

The ethanolic extract of dried leaves from *R. alpinum*, once defatted, was fractionated by a combination of gel filtration and chromatographic techniques including cellulose preparative TLC and diol-bonded silica CC to give **1**—**7**.

Analysis of the ^{1}H- and ^{13}C-NMR spectra of compound **1** (Table 1; all assignments based on HSQC-TOCSY and HMBC experiments) showed the presence of one aromatic and one aliphatic system as well as a sugar moiety. The ^{1}H-NMR resonances of two *meta* coupled doublets at δ 6.23 and 6.40 ppm (1H, J=1.9 Hz), correlated with the carbons at 99.7 and 94.7 ppm respectively in the HSQC spectrum, characterized the 6- and 8-protons of a flavonoid 5, 7 dihydroxy A-ring.[8] The doublet at δ 6.80 (2H, J=0.9 Hz) was attributed

Table 1. ^{1}H- and ^{13}C-NMR Data of **1** in DMSO-d_6 (^{13}C: 125 MHz, ^{1}H: 500 MHz, δ ppm, J Hz)

C-	^{13}C	^{1}H	HMBC
Mearnsetin			
2	158.3		
3	135.6		
4	178.4		
5	161.9		
6	99.7	6.23 (d, 1.9)	C-5, C-7, C-8, C-10
7	165.5		
8	94.7	6.40 (d, 1.9)	C-6, C-7, C-9, C-10
9	157.5		
10	104.9		
1′	125.6		
2′/6′	108.9	6.80 (d, 0.9)	C-2, C-1′, C-3′/5′, C-4′
3′	151.6		
4′	138.6		
5′	151.6		
4′-OCH_3	60.7	3.74 (s)	C-4′
3-*O*-Rhamnose			
1″	102.7	5.10 (d, 1)	C-3
2″	70.3	4.05 (br d)	C-3″, C-4″
3″	69.5	3.76 (dd,. 3.2, 9.7)	C-4″
4″	74.6	4.70 (dd, 9.7, 9.8)	C-3″, C-5″, C-6″, C-1‴
5″	68.5	3.30 (dd, 6.1, 9.8)	C-4″
6″	17.8	0.80 (d, 6.1)	C-4″, C-5″
4″-*O*-Malonic acid			
1‴	169.9		
2‴	45	2.93/3.08 (d, 14)	C-1‴, C-3‴
3‴	169.5		

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Table 2. ^{1}H- and ^{13}C-NMR Data of **2** and **3** in DMSO-d_6 (^{13}C: 125 MHz, ^{1}H: 500 MHz, δ ppm, J Hz)

	2				**3**		
C-	^{13}C	^{1}H	HMBC	C-	^{13}C	^{1}H	HMBC
Myricetin				Quercetin			
2	158.6			2	158.9		
3	135.2			3	135.7		
4	178.6			4	178.4		
5	162.2			5	162.5		
6	99.6	6.20 (d, 2.0)	C-5, C-7, C-8, C-10	6	99.6	6.21 (d, 1.9)	C-5, C-7, C-8, C-10
7	165.1			7	165.4		
8	94.5	6.40 (d, 2.0)	C-6, C-7, C-9, C-10	8	94.5	6.40 (d, 1.9)	C-6, C -7, C-9, C-10
9	157.3			9	157.4		
10	104.9			10	104.9		
1′	120.3			1′	121.2		
2′/6′	108.8	6.85 (d, 0.6)	C-2, C-1′, C-3′/5′, C-4′	2′	117.2	7.26 (d, 1.9)	C-2, C-1′, C-3′, C-4′
3′	147.0			3′	146.9		
4′	137.6			4′	150.0		
5′	147.0			5′	117.2	6.91 (d, 8.2)	C-1′, C-3′, C-4′, C-6′
				6′	121.9	7.20 (dd, 1.9, 8.2)	C-2, C-1′, C-4′, C-5′
3-*O*-Rhamnose							
1″	102.8	5.10 (d, 1)	C-3	1″	102.3	5.21 (d, 1)	C-3
2″	70.8	4.05 (br d)	C-3″, C-4″	2″	70.4	4.05 (m)	C-4″
3″	68.9	3.80 (dd, 3.1, 9.7)	C-4″, C-5″	3″	69.4	3.75 (dd, 3.5, 9.8)	C-4″
4″	75	4.75 (dd, 9.7, 9.9)	C-3″, C-5″, C-6″, C-1‴	4″	74.8	4.65 (dd, 9.8, 9.9)	C-3″, C-5″, C-6″, C-1‴
5″	68.7	3.65 (dd, 6.3, 9.9)	C-3″, C-4″	5″	68.5	3.29 (dd, 6.2, 9.9)	C-4″
6″	17.8	0.80 (d, 6.3)	C-4″, C-5″	6″	17.9	0.70 (d, 6.2)	C-4″, C-5″
4-*O*-Malonic acid							
1‴	167.8			1‴	169.3[a]		
2‴	43.1	3.33 (s)	C-1‴, C-3‴	2‴	45.8	3.04/3.13 (d, 14)	C-1‴, C-3‴
3‴	169.2			3‴	169.3[a]		

a) Broad signal, corresponding to 2 carbons according to the intensity of the correlation cross peaks $C_{169.3}/H_{3.04\ 3.13}$.

to the *meta*-related 2′- and 6′-protons of the 3′, 4′, 5′ tri-*O*-substituted B-ring. Additionally, the ^{1}H-NMR spectrum exhibited one singlet at δ 3.74 (3H) revealing a methoxy group correlated with the carbon resonance at δ 138.6 (C-4′) on the HMBC spectrum. Thus the aglycone of **1** was identified as 3,5,7,3′,5′-pentahydroxy-4′-methoxyflavone (mearnsetin). On the basis of the chemical shifts, multiplicity of the signals and values of the coupling constants, the sugar was identified as α-L-rhamnopyranosyl.[9] Its position was determined by the long-range correlation between C-3 (135.6) of the aglycone and its anomeric proton (δ 5.10, d, J=1 Hz) in HBMC experiments. The remaining signals of the 2D NMR spectra could be assigned to a malonyl residue.[10] The ^3J coupling between the downfield-shifted rhamnose H-4″ (δ 4.70) and the carbonyl ester carbon (δ 169.9) suggested the attachment of the malonyl at rhamnose C-4″ hydroxyl group.

Thus compound **1** was identified as mearnsetin 3-*O*-(4″-*O*-malonyl)-α-L-rhamnopyranoside. This identification was corroborated by ESIMS which exhibited a quasimolecular ion peak at *m/z* 563 $[M-H]^-$ and fragment ion peaks at *m/z* 477 $[M-H-86]^-$ and 331 $[M-H-86-146]^-$ indicating the successive elimination of one malonyl and one rhamnosyl moiety.

Similarly, NMR spectral data (Table 2) showed **2** and **3** to be 3-*O*-(4″-*O*-malonyl)-α-L-rhamnopyranosides of myricetin (3,5,7,3′,4′,5′-hexahydroxy flavone) and quercetin (3,5,7,3′,4′-pentahydroxy flavone) respectively.

Compounds **4**—**6** were proved to be identical with alkaline hydrolysis products from **1**—**3**, and thus were mearnsitrin, myricitrin and quercitrin respectively. While these flavonol rhamnosides are of common occurrence in the plant kingdom,[11] this is the first report of the malonyl esters **1**—**3**.

As for compound **7**, it was identified as 4-*O*-methyl phloracetophenone 2-*O*-β-D-glucopyranoside by comparison of its spectral data with the literature.[12]

Experimental

General Methods NMR experiments were performed on a Bruker DRX 500 spectrometer (500 MHz for ^{1}H and 125 MHz for ^{13}C spectra) with inverse and direct gradient probes. 2D matrices were recorded with very high resolution (2K) to resolve carbons separated by few Hz. Linear prediction was systematically applied before Fourier transform in F1. Negative ion ESIMS were measured on a Hewlett-Packard 1100 MSD system. UV spectra were recorded on a Kontron UVIKON 860 system. HPLC analyses were performed using a Kontron 322-360-MT450 system coupled to a photodiode array detector Waters 991 (chromatographic system: column symmetryshield RP 18 5 μm, 4.6×250 mm, Waters; 110 min gradient from 1.6—42% of acetonitrile in water in the presence of 2% HOAc, flux 0.65 ml min^{-1}, detection at 280 and 350 nm).

Plant Material Leaves of *R. alpinum* were collected in May 1999 on a dozen natural sites of Massif Central, France.

Extraction and Isolation of Compounds 1—7 Leaves (dried in the dark at room temp.) of about 60 individuals were extracted twice with 80% EtOH (40 ml g^{-1}) by boiling under reflux for 20 min. Combined extracts (after individual HPLC analysis, data not shown) were evaporated to dryness under reduced pressure then taken up in H_2O. The H_2O crude extract was defatted after 48 h at 4 °C. On a CC of Sephadex LH-20 (Pharmacia), H_2O eluted fraction A (mainly **7**), 50% MeOH fraction B (mainly **1**, **4**) and MeOH fraction C (mainly **2**, **3**, **5**, **6**). Rechromatography of fraction A on a Sephadex LH-20 column with MeOH yielded **7**. Malonyl esters of fractions B and C were separated from the corresponding heterosides by prep. TLC on microcrystalline cellulose (Macherey-Nagel) with H_2O and eluted with H_2O (MeOH for the heterosides); filtration on cellulose and purification on Sephadex LH 20 with MeOH yielded **1** and **4** from fraction B. As for fraction C, MPLC on a Büchi 460×15 mm column over Lichroprep Diol (40—

63 μm, Merck) with $CHCl_3$–iso-PrOH 85 : 15 separated **2** and **3**, and prep. TLC on polyamide 6 (Macherey-Nagel) with toluene–MeOH–MeCOEt 4 : 3 : 3 (twice) separated **5** and **6**.

Alkaline Hydrolysis of 1—3 As described in ref. 10.

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