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MYRICETIN 3-RHAMNOSYL(1 → 6)GALACTOSIDE FROM *NYMPHAÉA* X *MARLIACEA*

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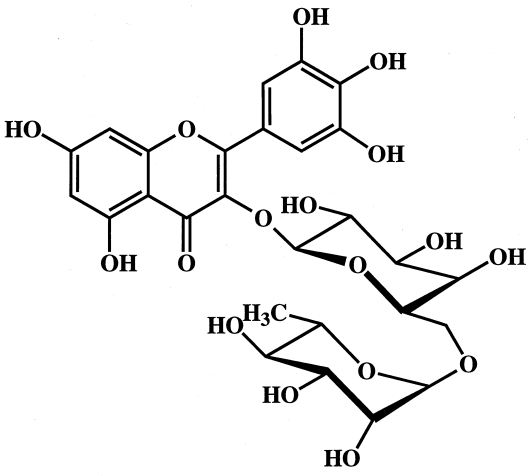
Key Word Index—*Nymphaéa* x *marliacea*; Nymphaéaceae; flavonoids; myricetin 3-rhamnosyl(1 → 6)galactoside; flavonol 3′-xylosides; MS; ^{13}C-NMR.

Abstract—The novel flavonol, myricetin 3-*O*-α-rhamnopyranosyl(1 → 6)β-galactopyranoside was isolated from leaves of the water lily *Nymphaéa* x *marliacea* (white petals), while four flavonols, quercetin and the 3′-xylosides of myricetin, quercetin and quercetin 3-methyl ether were isolated from red petals of the variety *Escarboucle*. Their structures were elucidated by homo- and heteronuclear two-dimensional NMR techniques, other spectroscopic techniques and chromatography. This is the first report of flavonol 3′-xylosides as flower pigments.

INTRODUCTION

The water lilies (Nymphaéaceae) are becoming increasingly popular as ornamental plants. In addition to decorative leaves these species and their hybrids show a wide range of flower colours. Recent studies on the genera *Victoria* and *Nymphaéa* have shown the presence of some unusual anthocyanins [1–3]. Here, we describe the isolation and structural elucidation of a new flavonol from the leaves of *Nymphaéa* x *marliacea* and three flavonol 3′-xylosides from the red flowers of *N.* x *marliacea* var. *Escarboucle*.



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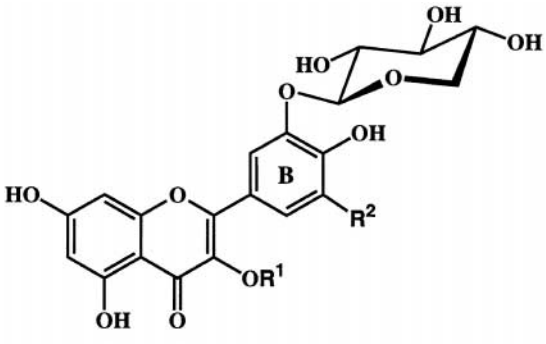
RESULTS AND DISCUSSION

The methanolic extracts of the water lily leaves (*Nymphaéa* x *marliacea* with white petals) and red petals (var. *Escarboucle*) were purified by partition against organic solvents followed by Amberlite XAD-7 column chromatography. Compound **1** was isolated from the purified leaf extract by Sephadex LH-20 gel filtration followed by preparative HPLC, while compounds **2**–**5** were isolated from the puri-



	R^1	R^2
3:	H	OH
4:	H	H
5:	Me	H

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Table 1. Retention times (HPLC and TLC) and UV absorption spectra for flavonols from *Nymphaéa* x *marliacea*; myricetin-3-rhamnosyl(1 → 6) galactoside (**1**), myricetin 3′-xyloside (**3**), quercetin 3′-xyloside (**4**) and quercetin 3-methyl ether 3′-xyloside (**5**)

Compound	HPLC, R_t(min)	TLC (hR_F)		UV					
		AW	BAW	MeOH	NaOMe	$AlCl_3$	$AlCl_3$–HCl	NaOAc	NaOAc–H_3BO_3
1	13.99	69	55	366, 303, 260	410, 324, 268	430, 308 sh, 272	410, 369 sh, 307, 274	411, 326, 272	389, 302, 260
3	17.22	8	45	365, 296 sh, 265 sh, 255	404, 328, 274	450, 305 sh, 273	421, 271	397, 323, 273	379, 302, 261
4	19.93	13	67	367, 266 sh, 252	412, 323, 276	424, 354, 302 sh, 266	423, 353, 295 sh, 267	396, 317, 273	368, 267, 253
5	20.66	21	60	354, 306 sh, 267	402, 327, 273	410, 353, 303, 273	406, 349, 299 sh, 273	399, 326, 272	353, 309 sh, 266

fied petal extract by preparative HPLC. The pure flavonoids were checked for homogeneity by analytical HPLC. The retention times (HPLC and TLC) and UV absorption spectra of **1**, **3**–**5** are found in Table 1.

The aromatic region of the ^{1}H NMR spectrum of **1** showed a singlet at 7.50 ppm integrating for two protons (H-2′/6′) and an AX system at 6.48 ppm (H-8) and 6.29 ppm (H-6), respectively, in accordance with a myricetin derivative (Table 2). The two anomeric signals at 5.18 ppm (*d*, J = 8.0 Hz) and 4.63 ppm (*d*, J = 1.5 Hz), respectively, indicated the presence of two linked sugar units with β- and α-configurations, respectively. All the sugar proton resonances were assigned by the DQF-COSY experiment, using the anomeric protons and the sugar H-6 protons as entry points (Table 2). The doublet at 1.28 ppm (J = 6.2 Hz) integrating for three protons was of particular value for identification of the rhamnose H-6 protons. The corresponding ^{13}C resonances for the sugars were thereafter assigned by the one-bond heteronuclear (HSC) experiment (Table 3). Thus, based on ^{13}C and ^{1}H NMR shift values and coupling constants (Tables 2 and 3) the sugar units were identified as β-galactosyl and α-rhamnosyl, respectively. The crosspeak at 5.2/136.1 ppm in the 2D long-range HMBC experiment between the anomeric galactosyl proton and C-3 showed that the sugar moiety was linked to the 3-position of the aglycone. The linkage point between the two sugar units was indicated to be at the galactosyl 6″-hydroxyl by the downfield shift of C-6″ (67.34 ppm) in the SEFT spectrum (cf. Ref. [2]). The crosspeaks at 4.63/67.3, 3.84/101.8 and 3.52/101.8 ppm between H-1‴/C-6″, H-6A″/C-1‴ and H-6B″/C-1‴, respectively, in the 2D long-range HMBC experiment confirmed this linkage point. A molecular ion [M^+] of 627.2 amu confirmed the

Table 2. ^{1}H NMR spectral data[a] for myricetin 3-rhamnosyl(1 → 6)galactoside (**1**), quercetin (**2**), myricetin 3′-xyloside, (**3**), quercetin 3′-xyloside (**4**) and quercetin 3-methyl ether 3′-xyloside (**5**) in CD_3OD at 25°C

Position	**1**		**3**		**4**		**5**		**2**	
	δH (ppm)	*J* (Hz)	δH (ppm)	*J* (Hz)	δH (ppm)	*J* (Hz)	δH (ppm)	*J* (Hz)	δH (ppm)	*J* (Hz)
6	6.29	*d*, 1.9	6.27	*d*[b]	6.27	*d*[b]	6.28	*d*, 1.9	6.28	*d*, 1.9
8	6.48	*d*, 1.9	6.46	*d*[b]	6.47	*d*[b]	6.47	*d*, 1.9	6.49	*d*[b]
2′	7.50	*s*	7.76	*d*, 1.2	8.17	*s*	8.08	*d*, 2.1	8.19	*m*
5′					7.06	*d*, 8.1	6.97	*d*, 8.9	7.00	*d*, 8.6
6′	7.50	*s*	7.60	*d*[b]	7.91	*d*, 8.0	7.71	*dd*, 8.7, 2.1	8.18	*m*
3-OCH_3							3.90	*s*		
1″	5.18	*d*, 8.0	4.90	*d*, 7.6	4.91	[c]	4.90	*d*, 7.6		
2″	3.93	*dd*, 8.0, 9.5	3.63	*dd*, 7.6, 9.0	3.63	*dd*, 8.1, 8.7	3.63	*dd*, 7.8, 8.8		
3″	3.69	*m*	3.56	*t*, 8.9	3.55	*t*, 8.9	3.55	*t*, 8.9		
4″	3.92	*d*, 3.0	3.71	*m*	3.71	*m*	3.71	*m*		
5A″	3.76	*m*, 6.0, 6.5	4.10	*dd*, 11.4, 5.3	4.11	*dd*, 11.3, 5.2	4.06	*dd*, 11.5, 5.4		
5B″			3.48	*dd*, 11.1, 10.5	3.48	*dd*, 11.0, 10.8	3.48	*dd*, 11.2, 10.3		
6A″	3.85	*dd*, 10.1, 5.9								
6B″	3.53	*dd*, 10.2, 6.7								
1‴	4.63	*d*, 1.5								
2‴	3.67	*m*								
3‴	3.60	*dd*, 9.5, 3.2								
4‴	3.38	*t*, 9.5								
5‴	3.63	*dd*, 9.5, 6.2								
6‴	1.28	*d*, 6.2								

[a]The coupling constants are based on direct readings from the resonances in the ^{1}H NMR spectra.
[b]Unresolved.
[c]Hidden under solvent peak.

Table 3. ^{13}C NMR spectral data for myricetin 3-rhamnosyl(1 → 6)galactoside (**1**), myricetin 3′-xyloside (**3**), quercetin 3′-xyloside (**4**) and quercetin 3-methyl ether 3′-xyloside (**5**) in CD_3OD at 25°C

Position	**1**		**3**		**4**		**5**	
	δC (ppm)	SEFT[a]	δC (ppm)	SEFT[a]	δC (ppm)	SEFT[a]	δC (ppm)	SEFT[a]
2	158.36	↑	147.23[b]	↑	147.17[b]	↑	157.13	↑
3	136.08	↑	137.60	↑	137.47	↑	139.68	↑
4	179.33	↑	177.33	↑	177.36	↑	179.99	↑
5	162.90	↑	162.51	↑	162.53	↑	163.11	↑
6	99.93	↓	99.28	↓	99.30	↓	99.82	↓
7	166.10	↑	165.64	↑	165.65	↑	165.98	↑
8	94.76	↓	94.37	↓	94.42	↓	94.73	↓
9	158.79	↑	158.14	↑	158.17	↑	158.36	↑
10	105.52	↑	104.52	↑	104.55	↑	105.91	↑
1′	121.61	↑	123.28	↑	124.31	↑	123.06	↑
2′	110.12	↓	110.36	↓	118.54	↓	119.06	↓
3′	146.31	↑	147.01[b]	↑	146.32[b]	↑	146.41	↑
4′	138.22	↑	138.85	↑	150.49	↑	151.66	↑
5′	146.31	↑	146.88[b]	↑	117.19	↓	117.43	↓
6′	110.12	↓	111.50	↓	124.92	↓	125.60	↓
3-methoxy							60.59	↓
1″	106.17	↓	104.95	↓	104.86	↓	104.78	↓
2″	73.24	↓	74.65	↓	74.63	↓	74.60	↓
3″	72.03	↓	77.41	↓	77.39	↓	77.29	↓
4″	70.20	↓	71.04	↓	71.03	↓	70.98	↓
5″	75.24	↓	67.10	↑	67.12	↑	67.12	↑
6″	67.34	↑						
1‴	101.95	↓						
2‴	75.09	↓						
3‴	72.26	↓						
4‴	73.87	↓						
5‴	69.71	↓						
6‴	17.96	↓						

[a]SEFT = coupling modulated spin echo NMR experiment: Cq and CH_2, ↑; CH and CH_3 ↓.
[b]Assignments with the same superscript may be reversed.

identity of **1** to be the novel compound myricetin 3-*O*-α-rhamnopyranosyl(1 → 6)β-galactopyranoside.

Compound **2** cochromatographed (HPLC) with and showed similar chemical shifts and coupling constants (Table 2) to quercetin. The sugar regions in the SEFT spectra of **3**–**5** (67–78 ppm) showed four resonances in addition to the anomeric signals (Table 3). The corresponding sugar protons were then assigned from the HSC experiments (Table 2). The shift values, coupling constants and integration data showed the glycosyl moieties of **3**–**5** to be one β-xylopyranosyl unit in each of the three compounds [4].

The aromatic region of the ^1H NMR spectrum of **3** showed an AX system at 7.76 ppm (H-2′) and 7.60 ppm (H-6′) and an AX system at 6.46 ppm (H-8) and 6.27 ppm (H-6), respectively, in accordance with a myricetin derivative with an asymmetrical *B*-ring due to sugar substitution of the 3′-hydroxyl. The aromatic region of the ^1H NMR spectrum of **4** showed an AMX system at 7.91 ppm (H-6′), 8.17 ppm (H-2′) and 7.06 ppm (H-5′) and an AX system at 6.47 ppm (H-8) and 6.27 ppm (H-6), respectively, in accordance with a quercetin-derivative. The strong crosspeak in the ROESY experiment at 4.9/8.2 ppm between the anomeric proton and H-2′, in addition to absence of other crosspeaks between the anomeric proton and other aromatic protons revealed the connection point between the xylose and the aglycone at the 3′-hydroxyl. The aromatic region of the ^1H NMR spectrum of **5** was similar to the same region of **4** (Table 2). The presence of a methoxyl singlet at 3.90 ppm indicated **5** to be a methyl ether of quercetin. The crosspeak at 3.9/139.5 ppm between the methoxyl protons and C-3 in the 2D long-range HMBC experiment confirmed the identity of the aglycone as quercetin-3-methyl ether. In the same spectrum a crosspeak at 4.9/146.1 ppm between H-1″ and C-3′ showed the connection point between the sugar and the aglycone. As for compound **4**, the strong crosspeak at 4.9/8.1 ppm between the anomeric proton and H-2′ in the ROESY spectrum confirmed this linkage. Thus, **3**–**5** were identified as the 3′-β-xylopyranosides of myricetin, quercetin and quercetin 3-methyl ether, respectively. This is the first report of flavonol 3′-xylosides as flower pigments, and these compounds may have chemotaxonomic potential in the genus *Nymphaeae*.

EXPERIMENTAL

Plant material

Flowers of *Nymphaéa* x *marliacea* var. *Escarboucle* and leaves of *N.* × *marliacea* (white flowered cultivar), respectively, were collected during June–September 1996 in the Botanical

Garden of the University of Bergen (BG) and frozen (−20°C). Samples of the cultivar with red flowers, which belongs to var. *Escarboucle*, were also collected at ARBOHA, Milde, Bergen. Voucher specimens have been deposited in BG.

Isolation of pigments

The samples were extracted with 0.5 M citric acid in MeOH at 5°C. The filtered extract of the red flowers of var. *Escarboucle* was concd under red. pres. and purified by partition against hexane. The flavonoids were extracted from the concd aqueous extract with EtOAc and applied to an Amberlite XAD-7 column [5]. The flavonoids in this purified extract were separated by prep. HPLC. The concd methanolic leaf extract of the white flower variety was purified by partition against both hexane and EtOAc and applied to an Amberlite XAD-7 column. The flavonoids in this purified extract were separated by Sephadex LH-20 CC [2].

High-performance liquid chromatography

The analytical HPLC system (Hewlett Packard, Model 1050) was equipped with a diode-array detector, a 20 μl loop and an 200×4.6 mm ODS Hypersil column, 5 μm. For the preparative separations a Gilson 305/306 pump system was employed together with a Hewlett Packard 1040A UV detector and a 250×10 mm Econosil C18 column, 10 μm. Both HPLC systems were operated at room temp. using the same solvents: HCOOH–H_2O (1:9) (A) and HCOOH–H_2O–MeOH (1:4:5) (B). The elution profile for analytical HPLC consisted of isocratic elution (90% A, 10% B) for 4 min followed by a linear gradient from 10 to 100% B for 17 min and then isocratic elution (100% B) for 4 min followed by linear gradient from 100 to 10% B for 1 min. The flow rate was 1.0 ml min^{-1} and aliquots of 15 μl were injected. The elution profile for preparative separations consisted of an isocratic elution (90% A, 10% B) for 4 min, a linear gradient from 10 to 100% B for 17 min, an isocratic elution for 12 min, followed by linear gradient from 100 to 10% B for 1 min. The flow rate was 4.0 ml min^{-1}.

Thin-layer chromatography

Analyt. TLC was carried out on microcrystalline cellulose (F1440, Schleicher and Schull) with the solvents AW (15% HOAc) and BAW (*n*-BuOH–HOAc–H_2O, 4:1:5, upper phase).

Spectroscopy

Shift reagents for UV spectroscopy were prepared and used according to Markham [6]. The NMR experiments (DQF-COSY, HMBC, HSC, SEFT) were obtained at 600.13 and 150.92 MHz for ^1H and ^{13}C, respectively, on a Bruker DRX-600 instrument at 25°C. The deuteriomethyl ^{13}C signal and the residual ^1H signal of the solvent (CD_3OD) were used as secondary references (δ 49.0 and δ 3.4 from TMS, respectively) (see Ref. [2] for more experimental details). MS were obtained on a Quattro II MS/MS (Micromass, UK) by flow injection into the electrospray source. The instrument was operated in the positive ion mode and the mobile phase carrier was an MeOH–H_2O (1:1) mixture containing 0.1% HCOOH. The carrier was pumped into the source at a flow rate of 100 μl min^{-1}. The samples were dissolved in 3% HCOOH (in MeOH) prior to analysis.

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