PERGAMON

Phytochemistry 51 (1999) 1133–1137

PHYTOCHEMISTRY

Flavonoids from blue flowers of *Nymphaèa caerulea*

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Received 9 October 1998; received in revised form 4 December 1998; accepted 10 December 1998

Abstract

Seven flavonols including the novel 3-(2″-acetylrhamnosides) of myricetin and quercetin (**2** and **6**), the rare kaempferol 3-(2″-acetylrhamnoside) and quercetin 3-(3″-acetylrhamnoside), in addition to the 3-rhamnosides of kaempferol, quercetin and were isolated from blue flowers of the African water lily *Nymphaèa caerulea* (=*Nymphaèa capensis*). Their structures were elucidated by a combination of chromatography and homo- and heteronuclear two-dimensional NMR techniques and electrospray MS for compound **2**.

Keywords: Water lily; *Nymphaèa caerulea*; Nymphaèaceae; Flavonol 3-rhamnosides; Myricetin 3-(2″-acetylrhamnoside); Quercetin 3-(2″-acetylrhamnoside); Kaempferol 3-(2″-acetylrhamnoside); Quercetin 3-(3″-acetylrhamnoside)

1. Introduction

Anthocyanin-based flower colours are in general influenced by several factors, including copigmentation with other flavonoids (Brouillard & Dangles, 1994). The water lilies (Nymphaèaceae) show a wide range of flower colours, embracing white, yellow, red and blue nuances (Beckett, 1984). Recently, myricetin 3-*O*-(α-rhamnopyranosyl(1 → 6)β-galactopyranoside) was isolated from the leaves and the 3′-xylosides of kaempferol, quercetin and quercetin 3-methyl ether from the red flowers of *Nymphaèa* × *marliacea* (Fossen, Frøystein, & Andersen, 1998a). The anthocyanins cyanidin 3-(2″-galloyl-6″-acetylgalactoside), and the 3-(2″-galloyl-6″-acetylgalactoside), 3-(6″-acetylgalactoside), 3-(2″-galloylgalactoside) and 3-galactoside of delphinidin have been found in different proportions in both flowers and leaves of *N.* × *marliacea* cultivars with red, pink and white flowers (Fossen, Larsen, & Andersen, 1998b), and the 3-(2″-galloylgalactosides) of delphinidin and cyanidin in leaves of two *Victoria* species (Strack, Wray, Metzger, & Grosse, 1992). From the blue flowers of the African water lily *N. caerulea* the unusual anthocyanins, 3′-(2″-galloylgalactoside) and 3′-(2″-galloyl-6″-acetylgalactoside) of delphinidin, have recently been identified (Fossen & Andersen, 1998). In this paper we report on the isolation and structure identification of seven flavonol 3-rhamnosides from the blue flowers of this latter species.

2. Results and discussion

The methanolic extract of *N. caerulea* petals was purified by partition against ethyl acetate followed by purification with column chromatography (Amberlite XAD-7 and Sephadex LH-20) and finally preparative HPLC. The pure flavonoids were checked for homogeneity by analytical HPLC. The compounds **1**, **3** and **4**, were identified as the 3-*O*-α-rhamnopyranosides of myricetin, quercetin and kaempferol, respectively. The ^{1}H and ^{13}C resonances for these flavonols were assigned by a combination of 1D and 2D ^{1}H and ^{13}C NMR spectroscopic techniques (Tables 2–3).

The aromatic region of the ^{1}H NMR spectrum of **6**

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0031-9422/99/$ - see front matter
PII: S0031-9422(99)00049-7

Table 1
Chromatographic (TLC, HPLC) and UV data for flavonols isolated from *Nymphaéa caerulea*; myricetin 3-rhamnoside (**1**), myricetin 3-(2″-acetylrhamnoside) (**2**), quercetin-3-rhamnoside (**3**), kaempferol 3-rhamnoside (**4**), quercetin 3-(3″-acetylrhamnoside) (**5**), quercetin 3-(2″-acetylrhamnoside) (**6**) and kaempferol 3-(2″-acetylrhamnoside) (**7**). nd means not determined

Compound	TLC (R_f)		HPLC t_R (min)	UV (methanol, nm)	
	AW	BA		Band I	Band II
1	0.43	0.65	15.74	353	255
2	0.58	0.82	19.11	nd	nd
3	0.51	0.74	18.81	348	254
4	0.46	0.81	20.68	343	262
5	0.51	0.88	20.25	nd	nd
6	0.63	0.85	21.35	347	256
7	0.55	0.93	23.55	324	263

showed an ABX system at δ 7.41 (H-6′), δ 7.43 (H-2′) and δ 7.01 (H-5′), and a 2H AX system at δ 6.47 (H-8) and δ 6.30 (H-6), respectively, in accordance with a quercetin-derivative. The crosspeaks at δ 7.41/158.6 (H-6′/C-2), δ 7.44/158.6 (H-2′/C-2), δ 6.47/158.0 (H-8/C-9) and δ 6.30/162.6 (H-6/C-5) in the HMBC spectrum were particular useful for assignment of the quaternary carbons of the aglycone. 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 δ 1.06 (J = 6.2 Hz) integrating for three protons was in accord with rhamnose H-6″ protons. The corresponding ^{13}C resonances for the sugar unit were thereafter assigned by the one-bond heteronuclear (HSQC) experiment Table 3. The relative large anomeric $^{1}J_{CH}$ at 179 Hz (Tvaroska & Taravel, 1995) showed that the anomer has an equatorially disposed hydrogen at C-1. Thus, based on ^{13}C and ^{1}H NMR shift values and coupling constants (Tables 1–3) the sugar unit was identified as α-rhamnopyranosyl. The crosspeak in the 2D long-range HMBC experiment at δ 5.49/135.1 between the anomeric rhamnosyl proton and C-3 of the aglycone showed that the sugar moiety was linked to the aglycone 3-position. The acyl moiety was identified as acetic acid by the 3H singlet at δ 2.17 (H-2‴) in the ^{1}H spectrum of **6** and the two carbon resonances at δ 171.91 (C-1‴) and δ 20.82 (C-2‴) in the SEFT spectrum. The crosspeaks at δ 2.17/171.5 (H-2‴/C-1‴) and δ 2.17/20.3 (H-2‴/C-2‴) in the HMBC spectrum confirmed the acetyl group. The linkage point between the sugar unit and the acetyl group was indicated to be at the 2″-hydroxyl by the downfield shifts of H-2″ (1.2 ppm) and C-2″ (1.5 ppm), and the upfield shifts of C-1″ (3.2 ppm) and C-3″ (1.7 ppm) compared to the analogous resonances of quercetin 3-rhamnoside, **3**. The crosspeak in the 2D long-range HMBC experiment at

Table 2
^{1}H NMR spectral data for myricetin 3-rhamnoside (**1**), myricetin 3-(2″-acetylrhamnoside) (**2**), quercetin-3-rhamnoside (**3**), kaempferol 3-rhamnoside (**4**), quercetin 3-(3″-acetylrhamnoside) (**5**), quercetin 3-(2″-acetylrhamnoside) (**6**) and kaempferol 3-(2″-acetylrhamnoside) (**7**) in CD_3OD at 25°C. un = unresolved

	1 (δ (ppm) J (Hz))	**2** (δ (ppm) J (Hz))	**3** (δ (ppm) J (Hz))	**4** (δ (ppm) J (Hz))	**5** (δ (ppm) J (Hz))	**6** (δ (ppm) J (Hz))	**7** (δ (ppm) J (Hz))
Aglycone							
6	6.33 d 1.9	6.32 d[a]	6.32 d 2.0	6.30 d 1.9	6.30 d un	6.30 d 2.1	6.28 d 2.0
8	6.49 d 1.9	6.48 d 2.0	6.49 d 2.0	6.48 d 1.9	6.47 d un	6.47 d 2.1	6.45 d 1.9
2′	7.07 s	7.08 s	7.46 d 2.3	7.86 d 8.6	7.43 d 2.2	7.43 d 2.1	7.87 d 8.8
3′				7.03 d 8.7			7.03 d 8.8
5′			7.03 d 8.2	7.03 d 8.7	7.00 d 8.1	7.01 d 8.2	7.03 d 8.8
6′	7.07 s	7.08 s	7.43 dd 8.2, 2.3	7.86 d 8.6	7.45 dd 8.1, 2.3	7.41 dd 8.2, 2.1	7.87 d 8.8
3-O-α-rhamnopyranoside							
1″	5.44 d 1.5	5.53 d 1.6	5.48 d 1.6	5.47 d 1.6	5.45 d 1.6	5.49 d 1.5	5.49 d 1.5
2″	4.35 dd 1.5, 3.3	5.58 dd 1.6, 3.3	4.34 dd 1.6, 3.0	4.31 dd 1.6, 3.3	4.47 dd 1.6, 3.1	5.54 dd 1.5, 3.2	5.53 dd 1.5, 3.4
3″	3.92 dd 3.3, 9.5	4.07 dd 3.3, 9.5	3.87 dd 3.2, 9.5	3.80 dd 3.4, 9.0	5.10 dd 3.1, 9.3	4.00 dd 3.2, 9.7	3.97 dd 3.4, 9.5
4″	3.47 t 9.5	[a]	3.46 t 9.5	3.42 m	[a]	3.00 m	3.37 m
5″	3.64 dd 9.5, 6.2	3.60 dd 9.5, 6.3	3.55 dd 9.5, 6.2	3.40 m	3.63 dd 9.1, 5.5	3.52 m	3.48 dd 9.6, 6.2
6″	1.08 d 6.2	1.11 d 6.1	1.07 d 6.2	1.01 d 5.6	1.06 d 5.8	1.06 d 6.2	1.05 d 6.3
2″-acetyl							
2‴		2.02 s				2.17 s	2.16 s
3″-acetyl							
2‴					2.23 s		

[a] Not determined.

Table 3
^{13}C NMR spectral data[a] for myricetin 3-rhamnoside (**1**), myricetin 3-(2″-acetylrhamnoside) (**2**), quercetin-3-rhamnoside (**3**), kaempferol 3-rhamnoside (**4**), quercetin 3-(3″-acetylrhamnoside) (**5**) and quercetin 3-(2″-acetylrhamnoside) (**6**) in CD_3OD at 25°C

	1 (δ (ppm))	**2** (δ (ppm))	**3** (δ (ppm))	**4** (δ (ppm))	**5** (δ (ppm))	**6** (δ (ppm))
Aglycone						
2	158.51	158.51	158.56	159.32	159.35	159.27
3	136.34	135.60	136.28	136.23	[b]	135.56
4	179.72	179.43	179.69	179.66	[b]	179.43
5	163.21	163.25	163.25	163.26	[b]	163.24
6	99.84	99.77	99.84	99.84	[b]	99.84
7	165.88	165.89	165.89	165.93	[b]	165.90
8	94.74	94.74	94.74	94.75	[b]	94.71
9	159.51	159.44	159.35	158.59	[b]	158.52
10	105.92	105.85	105.94	105.94	[b]	105.84
1′	121.99	121.77	123.01	122.64	123.12	122.77
2′	109.64	109.55	116.97	131.91	116.84	116.83
3′	146.86	146.95	149.83	116.54	149.89	149.90
4′	137.90	137.99	146.45	161.62	146.66	146.52
5′	146.86	146.95	116.40	116.54	[b]	116.47
6′	109.64	109.55	122.90	131.91	[b]	122.81
3-O-α-rhamnopyranoside						
1″	103.64	100.38	103.58	103.52	103.27	100.36
2″	71.90	73.43	71.94	71.93	69.71	73.39
3″	72.15	70.53	72.15	72.13	75.36	70.49
4″	73.37	73.63	73.30	73.19	73.27	73.50
5″	72.07	[b]	72.07	72.05	70.48	72.11
6″	17.67	17.74	17.69	17.66	17.71	17.70
X″-acetyl						
1‴		172.00			172.78	171.91
2‴		20.86			[b]	20.82

[a] The shift values are determined from the SEFT spectrum.
[b] Not determined.

δ 5.54/171.5 between H-2″ and C-1‴ confirmed this linkage point. Thus, the identity of **6** was found to be the novel flavonol quercetin 3-*O*-(2″-*O*-acetyl-α-rhamnopyranoside).

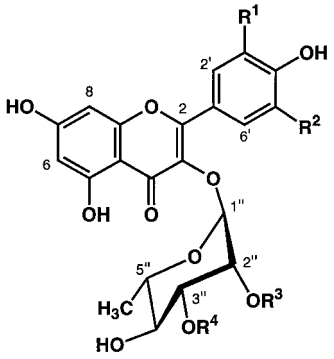
The aromatic region of the ^{1}H NMR spectrum of **2** showed a 2H singlet at δ 7.08 (H-2′/6′) and an AX system at δ 6.48 (H-8) and 6.32 ppm (H-6), respectively, in accordance with myricetin. The sugar region of the ^{1}H spectrum of **2** was similar to the corresponding sugar region of **6**, in agreement with α-rhamnosyl acylated with acetyl at the 2″-position. The proton and carbon resonances of **2** were assigned by means of the 1D ^{13}C SEFT spectrum and the 2D HMBC and HSQC spectra. A molecular related ion of *m*/*z* 507 confirmed the identity of **2** to be the novel compound myricetin 3-*O*-(2″-*O*-acetyl-α-rhamnopyranoside). The compound, myricetin 3-*O*-α-L-(acetyl)-rhamnopyranoside, without determination of the acetyl moiety has previously been identified in *Betula pubescens* leaves (Ossipov et al., 1995).

The aromatic region of the ^{1}H NMR spectrum of **5** was in accordance with quercetin Table 2. All the sugar proton resonances were assigned by comparison of similar spectra of **3** and **6** and determination of coupling constants in the ^{1}H spectrum Table 2. The corresponding ^{13}C resonances for the sugar unit were thereafter assigned by the one-bond heteronuclear (HSQC) experiment Table 3, and the sugar unit was identified as α-rhamnosyl. The downfield shifts of H-3″ (1.3 ppm) and C-3″ (3.2 ppm) in the ^{1}H and the ^{13}C SEFT spectra, respectively, and the upfield shift of C-2″ (2.2 ppm) (Tables 2 and 3) showed the presence of acylation at the 3″-hydroxyl. Thus, the identity of **5** was determined to be quercetin 3-*O*-(3″-*O*-acetyl-α-rhamnopyranoside). This pigment has previously been isolated from *Pteris grandifolia* (Pteridaceae) (Tanaka, Murakami, Saiki, Chen, & Gomez, 1978).

The aromatic region of the ^{1}H NMR spectrum of **7** showed an AA′XX′ system at δ 7.87 (H-2′/6′) and δ 7.03 (H-3′/5′) and an AX system at δ 6.45 (H-8) and δ 6.28 (H-6), respectively, in accordance with a kaempferol-derivative. The sugar region of the ^{1}H spectrum of pigment **7** was similar to the corresponding sugar region of pigment **6**, and thus showed one unit of α-

rhamnose in addition to an acetyl unit. 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 notable downfield shift of the H-2″ signal (1.2 ppm) compared to the corresponding signal of **4**, confirmed the linkage between the acetyl group and the rhamnose 2″-hydroxyl. Thus, the identity of **7** was found to be kaempferol 3-*O*-(2″-*O*-acetyl-α-rhamnopyranoside). This pigment has previously been isolated from the rhizomes of *Zingiber zerumbet* (Zingiberaceae) (Masuda, Jitoe, Kato, & Nakatani, 1991).

It is interesting to note that all the isolated flavonols, **1**–**7**, from the blue flowers of *N. caerulea* have a rhamnosyl moiety in the aglycone 3-position, while all the flavonols which were isolated from the red petals of *N.* × *marliacea* were flavonol 3′-xylosides (Fossen et al., 1998a). In contrast to this, red flowers of this latter species have anthocyanins with galactosyl moieties in the 3-position (Fossen et al., 1998b), while the blue flowers of *N. caerulea* contain analogous anthocyanins with galactosyl moieties in the 3′-position.



	R^1	R^2	R^3	R^4
1	OH	OH	H	H
2	OH	OH	CH_3CO	H
3	OH	H	H	H
4	H	H	H	H
5	OH	H	H	CH_3CO
6	OH	H	CH_3CO	H
7	H	H	CH_3CO	H

3. Experimental

3.1. Plant material

Flowers (500 g) of the African water lily *N. caerulea* (= *N. capensis*) were collected in September 1997 from Wamiko water pond, west of Kampala, Uganda. A voucher specimen has been deposited at Makerere University herbarium, Uganda.

3.2. Isolation of pigments

The flowers were cut into pieces and extracted with 5% acetic acid in MeOH at 5°C. The filtered extract was concd under red. pres., purified by partition against ethyl acetate and applied to an Amberlite XAD-7 column (Andersen, 1988). The flavonoids in this purified extract were separated by Sephadex LH-20 column chromatography and prep. HPLC according to previously published procedure (Fossen et al., 1998a).

3.3. High-performance liquid chromatography

The analytical HPLC system (Hewlett Packard, Model 1050) was equipped with a diode-array detector, a μl loop and a 200 × 4.6 mm ODS Hypersil column, 5 mm. For the preparative separations a Gilson 305/306 pump system was employed together with a Hewlett Packard 1040A UV detector and an 250 × 10 mm Econosil C18 column, 10 μm. Both HPLC systems were operated at room temperature using the same solvents: HCO_2H–H_2O (1:9) (A) and HCO_2H–H_2O–MeOH (1:4:5) (B). The elution profile for analytical HPLC consisted of isocratic elution (90% A, 10% B) for 4 min, linear gradient from 10 to 100% B during the next 17 min, isocratic elution (100% B) for 4 min followed by linear gradient from 100 to 10% B for 1 min. The flow rate was 1.2 ml min^{-1}, and aliquots of 15 μl were injected. The elution profile for preparative separations consisted of isocratic elution (90% A, 10% B) for 4 min, linear gradient from 10% to 100% B during the next 17 min, isocratic elution (100% B) for 12 min, Followed by linear gradient from 100% to 10% B for 1 min. The flow rate was 4.0 ml min^{-1}.

3.4. Spectroscopy

UV absorption spectra were recorded in MeOH. Spectral measurements were made over the wavelength range 240–600 nm in steps of 2 nm. The NMR experiments (DQF–COSY, HMBC, HSQC, 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 3.4 from TMS, respectively). See Fossen et al. (1998a) for more experimental details. The mass spectrum was 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 a methanol–water (50:50) mixture containing 0.1% formic acid. The carrier was pumped into the source at a flow rate of 100 ml min^{-1}. The samples were dissolved in 3% formic acid (in methanol) prior to analysis.

Acknowledgements

T. F. gratefully acknowledges the Norwegian Research Council for a fellowship. The authors acknowledge the Norwegian Universities' Committee for Development, Research and Education (NUFU) for financial support.

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