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Malonylated flavonol glycosides from the petals of *Clitoria ternatea*

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Abstract

Three flavonol glycosides, kaempferol 3-*O*-(2″-*O*-α-rhamnosyl-6″-*O*-malonyl)-β-glucoside, quercetin 3-*O*-(2″-*O*-α-rhamnosyl-6″-*O*-malonyl)-β-glucoside, and myricetin 3-*O*-(2″,6″-di-*O*-α-rhamnosyl)-β-glucoside were isolated from the petals of *Clitoria ternatea* cv. Double Blue, together with eleven known flavonol glycosides. Their structures were identified using UV, MS, and NMR spectroscopy. They were characterized as kaempferol and quercetin 3-(2^G- rhamnosylrutinoside)s, kaempferol, quercetin, and myricetin 3-neohesperidosides, 3-rutinosides, and 3-glucosides in the same tissue. In addition, the presence of myricetin 3-*O*-(2″-*O*-α-rhamnosyl-6″-*O*-malonyl)-β-glucoside was inferred from LC/MS/MS data for crude petal extracts. The flavonol compounds identified in the petals of *C. ternatea* differed from those reported in previous studies.

Keywords: *Clitoria ternatea* L.; Phaseoleae; Butterfly pea; Kaempferol 3-*O*-(2″-*O*-α- rhamnosyl-6″-*O*-malonyl)-β-glucoside; Quercetin 3-*O*-(2″-*O*-α-rhamnosyl-6″-*O*- malonyl)-β-glucoside; Myricetin 3-*O*-(2″,6″-di-*O*-α-rhamnosyl)-β-glucoside

1. Introduction

In the past 30 years, several researchers have examined the flavonoid constitutents of *Clitoria ternatea*. In these studies, the structural determination of ternatins, a group of 15 polyacylated anthocyanins found in the petals, has helped characterize the flower anthocyanins in this species. The success of these studies has been due, largely, to the use of recently developed techniques in natural product chemistry, such as RP-HPLC, high-magnetic-field NMR, and MS.

Since most flavonoid studies of the petals of *C. ternatea* have concentrated on the anthocyanins, information on the flavonol constituents of petals is fragmentary. Ranaganayaki and Singh (1979) reported kaempferol and Saito et al. (1985) detected kaempferol 3-glucoside, kaempferol 3-robinobioside-7-rhamnoside, quercetin, and quercetin 3-glucoside. Neither paper explained the quantitative relationships between these compounds. Although HPLC and MS are important for quantitative plant analysis (Harborne, 1998) and are widely used, these methods have not been applied thoroughly with respect to the flavonols of this species.

In the present study the flavonol constituents of *C. ternatea* petals were analyzed using an isolation experiment and LC/MS/MS. Three new glycosides and some glycosides new to *C. ternatea* were identified, and the quantitative relationships of all the constituents were determined.

2. Results and discussion

Fourteen flavonol glycosides, including three new compounds (**1**, **2**, and **3**), were isolated from 1% TFA–50% aq. MeOH extracts of the petals. These were structurally identified by spectroscopic methods, including UV, MS, and NMR spectroscopy. Although DMSO-d_6 is typically used as solvent for NMR analysis of flavonoids, methanol-d_4 was used because it gives better signal dispersion, especially for the ^{1}H NMR spectra of glycosides (Pauli, 2000), which facilitated the analyses. The NMR spectral data measured in methanol-d_4 are tabulated in Tables 1 and 2 because there is little published NMR data on flavonol glycosides

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Table 1
^{1}H NMR[a] (400 MHz) spectroscopic data for the isolated compounds

	1	**1**[b]	**2**	**2**[b]	**3**	**4**	**5**	**6**
Aglycone								
H-6	6.18 (*d*, 2.1)	6.19 (*d*, 2.1)	6.19 (*d*, 2.0)	6.18 (*d*, 2.0)	6.18 (*d*, 2.2)	6.18 (*d*, 2.2)	6.18 (*d*, 2.1)	6.18 (*d*, 2.1)
H-8	6.38 (*d*, 2.1)	6.41 (*d*, 2.1)	6.38 (*d*, 2.0)	6.38 (*d*, 2.0)	6.36 (*d*, 2.2)	6.37 (*d*, 2.2)	6.37 (*d*, 2.1)	6.37 (*d*, 2.1)
H-2′	7.99 (*d*, 8.9)	7.94 (*d*, 8.9)	7.57 (*d*, 2.1)	7.47 (*d*, 2.3)	7.23 (*s*)	7.58 (*d*, 2.2)	8.01 (*d*, 8.9)	8.04 (*d*, 8.9)
H-3′	6.88 (*d*, 8.9)	6.87 (*d*, 8.9)					6.89 (*d*, 8.9)	6.88 (*d*, 8.9)
H-5′	6.88(*d*, 8.9)	6.87 (*d*, 8.9)	6.86 (*d*, 8.9)	6.82 (*d*, 8.4)		6.88 (*d*, 8.8)	6.89 (*d*, 8.9)	6.88 (*d*, 8.9)
H-6′	7.99 (*d*, 8.9)	7.94 (*d*, 8.9)	7.56 (*dd*, 2.1, 8.9)	7.51 (*dd*, 2.3, 8.4)	7.23 (*s*)	7.60 (*dd*, 2.2, 8.8)	8.01 (*d*, 8.9)	8.04 (*d*, 8.9)
3-Glucosyl								
H-1	5.59 (*d*, 7.4)	5.53 (*d*, 7.3)	5.58 (*d*, 7.6)	5.55 (*d*, 7.7)	5.55 (*d*, 7.8)	5.58 (*d*, 7.7)	5.59 (*d*, 7.6)	5.72 (*d*, 7.4)
H-2	3.60 (*dd*, 7.4, 9.1)	3.43 (*m*)[c]	3.65 (*dd*, 7.6, 9.1)	3.1∼3.5 (4H, *m*)[c]	3.69 (*dd*, 7.8, 9.0)	3.64 (*dd*, 7.7, 9.4)	3.59 (*dd*, 7.6, 8.9)	3.61 (*dd*, 7.4, 9.1)
H-3	3.54 (*t*, 9.1)	3.38 (*m*)[c]	3.55 (*t*, 9.1)		3.53 (*t*, 9.0)	3.53 (*t*, 9.4)	3.53 (*t*, 8.9)	3.55 (*t*, 9.1)
H-4	3.33 (*t*, 9.1)	3.13 (*m*)[c]	3.36 (*t*, 9.1)		3.33 (*t*, 9.0)	3.34 (*t*, 9.4)	3.23 (*t*, 8.9)	3.28 (*t*, 9.1)
H-5	3.39 (*ddd*, 2.1, 5.1, 9.1)	3.33 (*m*)[c]	3.39 (*ddd*, 1.2, 4.4, 9.1)		3.32 (*ddd*, 1.0, 5.9, 9.0)	3.33 (*ddd*, 1.5, 5.6, 9.4)	3.33 (*ddd*, 1.0, 6.1, 8.9)	3.22 (*ddd*, 2.2, 5.8, 9.1)
H-6a	4.27 (*dd*, 2.1, 11.9)	4.16 (*dd*, 2.0, 12.0)	4.29 (*dd*, 1.2, 11.7)	4.19 (*dd*, 2.3, 12.0)	3.81 (*dd*, 1.0, 11.7)	3.81 (*dd*, 1.5, 11.5)	3.81 (*dd*, 1.0, 12.5)	3.72 (*dd*, 2.2, 12.3)
H-6b	4.13 (*dd*, 5.9, 11.9)	3.96 (*dd*, 5.4, 12.0)	4.13 (*dd*, 4.4, 11.7)	3.96 (*dd*, 5.4, 12.0)	3.40 (*dd*, 5.9, 11.7)	3.39 (*dd*, 5.6, 11.5)	3.37 (*dd*, 6.1, 12.5)	3.50 (*dd*, 5.8, 12.3)
2″-Rhamnosyl								
H-1	5.22 (*d*, 1.4)	5.05 (*d*, 1.0)	5.22 (*d*, 1.5)	5.06 (*d*, 1.0)	5.21 (*d*, 1.6)	5.22 (*d*, 1.5)	5.22 (*d*, 1.5)	5.22 (*d*, 1.6)
H-2	3.99 (*dd*, 1.4, 3.3)	3.72 (*m*)[c]	4.00 (*dd*, 1.5, 3.2)	3.72 (*m*)[c]	4.01 (*dd*, 1.6, 3.4)	4.00 (*dd*, 1.5, 3.4)	3.99 (*dd*, 1.5, 3.4)	3.99 (*dd*, 1.6, 3.4)
H-3	3.79 (*dd*, 3.3, 9.6)	3.47 (*m*)[c]	3.79 (*dd*, 3.2, 9.5)	3.46 (*m*)[c]	3.80 (*dd*, 3.4, 9.6)	3.79 (*dd*, 3.4, 9.4)	3.78 (*dd*, 3.4, 9.7)	3.77 (*dd*, 3.4, 9.6)
H-4	3.34 (*t*, 9.6)	3.13 (*m*)[c]	3.35 (*t*, 9.5)	3.1∼3.2 (*m*)[c]	3.35 (*t*, 9.6)	3.26 (*t*, 9.4)	3.33 (*t*, 9.7)	3.33 (*t*, 9.6)
H-5	4.06 (*dq*, 6.2, 9.6)	3.73 (*m*)[c]	4.08 (*dq*, 6.2, 9.5)	3.73 (*m*)[c]	4.10 (*dq*, 6.3, 9.6)	4.07 (*dq*, 6.1, 9.4)	4.05 (*dq*, 6.1, 9.7)	4.02 (*dq*, 6.1, 9.6)
H-6 ($C\underline{H}_3$)	1.00 (*d*, 6.2)	0.79 (*d*, 6.1)	1.01 (*d*, 6.2)	0.78 (*d*, 6.2)	1.03 (*d*, 6.3)	1.00 (*d*, 6.1)	0.98 (*d*, 6.1)	0.95 (*d*, 6.1)
6″-Rhamnosyl								
H-1					4.50 (*d*, 1.6)	4.50 (*d*, 1.7)	4.49 (*d*, 1.5)	
H-2					3.58 (*dd*, 1.6, 3.4)	3.58 (*dd*, 1.7, 3.4)	3.57 (*dd*, 1.5, 3.4)	
H-3					3.49 (*dd*, 3.4, 9.6)	3.48 (*dd*, 3.4, 9.5)	3.47 (*dd*, 3.4, 9.5)	
H-4					3.23 (*t*, 9.6)	3.22 (*t*, 9.5)	3.23 (*t*, 9.5)	
H-5					3.42 (*dq*, 6.2, 9.6)	3.41 (*dq*, 6.3, 9.5)	3.41 (*dq*, 6.2, 9.5)	
H-6($C\underline{H}_3$)					1.07 (*d*, 6.2)	1.07 (*d*, 6.3)	1.07 (*d*, 6.2)	
6″-Malonyl								
$C\underline{H}_2$	Missing[d]	3.03 (*s*)	Missing[d]	3.16 (*s*)				
Hydroxyl group								
5-O$\underline{H}$		12.60 (*s*)		12.62 (*s*)				

(*continued on next page*)

Table 1 (*continued*)

	7	8	9	10	11	12	13	14[c]
Aglycone								
H-6	6.17 (*d*, 2.1)	6.17 (*d*, 2.0)	6.21 (*d*, 1.8)	6.21 (*d*, 2.0)	6.21 (*d*, 1.8)	6.20 (*d*, 2.0)	6.20 (*d*, 2.2)	6.21 (*d*, 2.0)
H-8	6.36 (*d*, 2.1)	6.35 (*d*, 2.0)	6.40 (*d*, 1.8)	6.40 (*d*, 2.0)	6.39 (*d*, 1.8)	6.39 (*d*, 2.0)	6.39 (*d*, 2.2)	6.39 (*d*, 2.0)
H-2′	7.61 (*d*, 2.2)	7.23 (*s*)	8.05 (*d*, 9.0)	7.66 (*d*, 2.1)	7.28 (*s*)	8.05 (*d*, 9.0)	7.70 (*d*, 2.2)	7.29 (*s*)
H-3′			6.89 (*d*, 9.0)			6.88 (*d*, 9.0)		
H-5′	6.86 (*d*, 8.3)		6.89 (*d*, 9.0)	6.87 (*d*, 8.5)		6.88 (*d*, 9.0)	6.86 (*d*, 8.3)	
H-6′	7.60 (*dd*, 2.2, 8.3)	7.23 (*s*)	8.05 (*d*, 9.0)	7.62 (*dd*, 2.1, 8.5)	7.28 (*s*)	8.05 (*d*, 9.0)	7.58 (*dd*, 2.2, 8.3)	7.29 (*s*)
3-Glucosyl								
H-1	5.73 (*d*, 7.6)	5.73 (*d*, 7.7)	5.12 (*d*, 7.3)	5.10 (*d*, 7.7)	5.07 (*d*, 7.8)	5.24 (*d*, 7.3)	5.23 (*d*, 7.6)	5.27 (*d*, 7.5)
H-2	3.65 (*dd*, 7.6, 9.4)	3.70 (*dd*, 7.7, 9.2)	3.43 (*dd*, 7.3, 8.8)	3.46 (*dd*, 7.7, 8.9)	3.49 (*dd*, 7.8, 8.6)	3.44 (*dd*, 7.3, 9.1)	3.47 (*dd*, 7.6, 9.0)	3.50 (*dd*, 7.5, 8.5)
H-3	3.55 (*t*, 9.4)	3.55 (*t*, 9.2)	3.40 (*t*, 8.8)	3.40 (*t*, 8.9)	3.40 (*t*, 8.6)	3.41 (*t*, 9.1)	3.42 (*t*, 9.0)	3.43 (*t*, 8.5)
H-4	3.33 (*t*, 9.4)	3.39 (*t*, 9.2)	3.24 (*t*, 8.8)	3.26 (*t*, 8.9)	3.26 (*t*, 8.6)	3.32 (*t*, 9.1)	3.34 (*t*, 9.0)	3.37 (*t*, 8.5)
H-5	3.22 (*ddd*, 2.3, 5.6, 9.4)	3.22 (*ddd*, 2.3, 5.2, 9.2)	3.32 (*ddd*, 1.0, 6.1, 8.8)	3.32 (*ddd*, 1.2, 6.1, 8.9)	3.31 (*ddd*, 1.2, 5.7, 8.6)	3.19 (*ddd*, 2.3, 5.4, 9.1)	3.21 (*ddd*, 2.4, 5.4, 9.0)	3.23 (*ddd*, 2.4, 5.4, 8.5)
H-6a	3.72 (*dd*, 2.3, 12.1)	3.73 (*dd*, 2.3, 12.0)	3.80 (*dd*, 1.0, 12.5)	3.80 (*dd*, 1.2, 11.0)	3.80 (*dd*, 1.2, 11.0)	3.68 (*dd*, 2.3, 11.9)	3.70 (*dd*, 2.4, 12.0)	3.72 (*dd*, 2.4, 12.0)
H-6b	3.54 (*dd*, 5.6, 12.1)	3.58 (*dd*, 5.2, 12.0)	3.37 (*dd*, 6.1, 12.5)	3.38 (*dd*, 6.1, 11.0)	3.39 (*dd*, 5.7, 11.0)	3.52 (*dd*, 5.4, 11.9)	3.56 (*dd*, 5.4, 12.0)	3.61 (*dd*, 5.4, 12.0)
2″-Rhamnosyl								
H-1	5.22 (*d*, 1.5)	5.21 (*d*, 1.4)						
H-2	3.99 (*dd*, 1.5, 3.4)	4.00 (*dd*, 1.4, 3.3)						
H-3	3.77 (*dd*, 3.4, 9.6)	3.77 (*dd*, 3.3, 9.6)						
H-4	3.33 (*t*, 9.6)	3.33 (*t*, 9.6)						
H-5	4.03 (*dq*, 5.9, 9.6)	4.04 (*dq*, 6.2, 9.6)						
H-6 ($C\underline{H}_3$)	0.97 (*d*, 5.9)	0.98 (*d*, 6.2)						
6″-Rhamnosyl								
H-1			4.51 (*d*, 1.5)	4.51 (*d*, 1.5)	4.51 (*d*, 1.5)			
H-2			3.62 (*dd*, 1.5, 3.2)	3.62 (*dd*, 1.5, 3.4)	3.62 (*dd*, 1.5, 3.2)			
H-3			3.51 (*dd*, 3.2, 9.5)	3.53 (*dd*, 3.4, 9.6)	3.54 (*dd*, 3.2, 9.5)			
H-4			3.27 (*t*, 9.5)	3.27 (*t*, 9.6)	3.27 (*t*, 9.5)			
H-5			3.44 (*dq*, 6.1, 9.5)	3.44 (*dq*, 6.2, 9.6)	3.43 (*dq*, 6.1, 9.5)			
H-6 ($C\underline{H}_3$)			1.11 (*d*, 6.1)	1.11 (*d*, 6.2)	1.11 (*d*, 6.1)			

[a] In methanol-d_4 unless otherwise stated; the chemical shifts (in ppm) are reported relative to the solvent signal (3.30 ppm).
[b] In DMSO-d_6; the chemical shifts (in ppm) are reported relative to the solvent signal (2.49 ppm).
[c] Chemical shifts based on 2D ^1H–^1H COSY data.
[d] The signals are missing because of overlap with the solvent signal.
[e] In 5% DMSO-d_6–methanol-d_4.

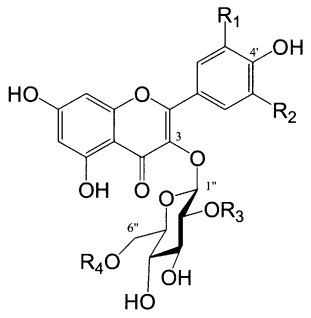
Table 2
^{13}C NMR[a] (100 MHz) spectroscopic data for the isolated compounds

	1	1[b]	2	2[b]	3	4	5	6	7	8	9	10	11	12	13	14[d]
Aglycone																
C-2	161.24	156.83	158.39	155.42	158.94	158.94	161.22	161.29	158.44	158.48	161.49	158.52	159.40	158.51	158.47	158.97
C-3	134.27	132.67	134.37	132.47	134.58	134.45	134.34	134.44	134.57	134.71	135.51	135.62	135.75	135.46	135.61	135.79
C-4	179.24	177.33	179.24	177.70	179.25	179.29	179.30	179.39	179.35	179.31	179.43	179.44	179.42	179.53	179.50	179.44
C-5	163.16	161.38	163.15	159.66	163.10	163.15	163.16	163.20	163.18	163.20	163.01	162.98	162.98	163.09	163.07	163.03
C-6	99.79	98.86	99.76	98.82	99.72	99.74	99.77	99.69	99.66	99.65	99.96	99.95	99.94	99.87	99.87	99.89
C-7	165.67	164.28	165.67	163.56	165.57	165.58	165.60	165.62	165.60	165.62	166.02	166.01	166.01	165.97	165.99	165.98
C-8	94.71	93.85	94.64	93.73	94.65	94.69	94.75	94.56	94.49	94.45	94.91	94.87	94.82	94.74	94.69	94.67
C-9	158.44	156.50	158.39	155.74	158.39	158.44	158.49	158.43	158.38	158.34	158.56	159.35	158.49	159.09	159.02	158.41
C-10	105.91	104.11	105.86	104.24	105.90	105.93	105.96	105.99	105.98	105.97	105.68	105.66	105.63	105.75	105.70	105.70
C-1′	123.18	120.99	123.30	121.72	122.53	123.45	123.17	123.14	123.22	122.43	122.76	123.15	122.13	122.80	123.18	121.97
C-2′	132.10	130.81	117.28	115.33	110.24	117.43	132.13	132.09	117.21	109.87	132.37	117.69	110.37	132.27	115.99	110.02
C-3′	116.08	115.22	145.94	145.27	146.35	145.91	116.14	116.09	146.00	146.46	116.13	145.84	146.35	116.07	149.84	146.47
C-4′	159.13	160.03	149.56	147.42	137.85	149.54	159.03	158.52	149.54	137.74	159.44	149.81	138.07	161.56	145.91	138.06
C-5′	116.08	115.22	115.95	116.17	146.35	116.06	116.14	116.09	115.98	146.46	116.13	116.06	146.35	116.07	117.54	146.47
C-6′	132.10	130.81	123.51	121.25	110.24	123.55	132.13	132.09	123.48	109.87	132.37	123.55	110.37	132.27	123.08	110.02
3-Glucosyl																
C-1	100.37	98.60	100.38	98.61	100.54	100.50	100.46	100.30	100.35	100.42	104.58	104.69	104.81	104.07	104.28	104.38
C-2	79.87	77.43	80.00	77.30	80.35	80.06	79.90	80.06	80.13	80.28	75.76	75.74	75.67	75.74	75.73	75.72
C-3	78.73	76.98	78.68	77.07	78.85	78.93	78.94	78.94	78.94	78.92	78.15	78.20	78.24	78.05	78.12	78.40
C-4	71.49	69.91	71.24	69.95	71.79	71.89	71.97	71.85	71.72	71.55	71.46	71.42	71.38	71.37	71.22	71.11
C-5	75.36	73.86	75.32	73.89	77.08	77.10	77.10	78.35	78.32	78.27	77.22	77.25	77.27	78.43	78.39	78.20
C-6	64.63	63.39	64.56	63.48	68.31	68.30	68.32	62.66	62.57	62.48	68.57	68.56	68.58	62.64	62.56	62.47
2″-Rhamnosyl																
C-1	102.62	100.79	102.67	100.72	102.74	102.64	102.59	102.60	102.64	102.72						
C-2	72.41	70.73	72.38	70.71	72.38	72.41	72.41	72.41	72.41	72.40						
C-3	72.35	70.73	72.31	70.75	72.29	72.31	72.33	72.31	72.31	72.30						
C-4	74.09	71.98	74.07	71.99	74.07	74.07	74.06	74.06	74.06	74.07						
C-5	69.93	68.48	69.98	68.43	70.03	69.96	69.91	69.91	69.96	70.03						
C-6	17.58	17.41	17.51	17.36	17.44	17.51	17.56	17.53	17.46	17.37						
6″-Rhamnosyl																
C-1					102.22	102.26	102.29				102.42	102.42	102.44			
C-2					72.17	72.15	72.12				72.09	72.12	72.13			
C-3					72.22	72.28	72.33				72.30	72.26	72.21			
C-4					73.92	73.88	73.83				73.89	73.94	73.98			
C-5					69.71	69.71	69.75				69.73	69.71	69.71			
C-6					17.79	17.81	17.84				17.91	17.87	17.84			
6″-Malonyl																
$\underline{C}H_2$	Missing[c]	41.31	Missing[c]	41.92												
R$\underline{C}$OOR′	168.23	166.60	168.30	166.55												
R$\underline{C}$OOH	169.96	167.80	170.58	167.81												

[a] In methanol-d_4, unless otherwise stated; the chemical shifts (in ppm) are reported relative to the solvent signal (49.0 ppm).
[b] In DMSO-d_6; the chemical shifts (in ppm) are reported relative to the solvent signal (39.70 ppm).
[c] The signals are missing because of overlap with the solvent signal.
[d] In 5% DMSO-d_6-methanol-d_4.

directly comparable to our results measured in methanol-d_4.

Eleven known compounds were also identified: quercetin 3-(2^G-rhamnosylrutinoside) (**4**), kaempferol 3-(2^G-rhamnosylrutinoside) (**5**), kaempferol 3-neohesperidoside (**6**), quercetin 3-neohesperidoside (**7**), myricetin 3-neohesperidoside (**8**) (Ismail and Alam, 2001), kaempferol 3-rutinoside (**9**), quercetin 3-rutinoside (**10**) (Kazuma et al., 2000), myricetin 3-rutinoside (**11**), kaempferol 3-glucoside (**12**) (Slimestad et al., 1995), quercetin 3-glucoside (**13**) (Slimestad et al., 1995), and myricetin 3-glucoside (**14**). In addition, the three new compounds were characterized as: kaempferol 3-*O*-(2″-*O*-α-rhamnosyl-6″-*O*-malonyl)-β-glucoside (**1**), quercetin 3-*O*-(2″-*O*-α-rhamnosyl-6″-*O*-malonyl)-β-glucoside (**2**), and myricetin 3-*O*-(2″,6″-di-*O*-α-rhamnosyl)-β-glucoside (**3**) (Fig. 1). The structural determination of the three new compounds (**1**, **2**, and **3**) is described below.



	R_1	R_2	R_3	R_4
1	H	H	rhamnosyl	malonyl
2	OH	H	rhamnosyl	malonyl
3	OH	OH	rhamnosyl	rhamnosyl
4	OH	H	rhamnosyl	rhamnosyl
5	H	H	rhamnosyl	rhamnosyl
6	H	H	rhamnosyl	H
7	OH	H	rhamnosyl	H
8	OH	OH	rhamnosyl	H
9	H	H	H	rhamnosyl
10	OH	H	H	rhamnosyl
11	OH	OH	H	rhamnosyl
12	H	H	H	H
13	OH	H	H	H
14	OH	OH	H	H

Fig. 1. Flavonol glycosides isolated from the petals of *Clitoria ternatea*. **1**, kaempferol 3-*O*-(2″-*O*-α-rhamnosyl-6″-*O*-malonyl)-β-glucoside; **2**, quercetin 3-*O*-(2″-*O*-α-rhamnosyl-6″-*O*-malonyl)-β-glucoside; **3**, myricetin 3-2^G-rhamnosylrutinoside; **4**, quercetin 3-2^G-rhamnosylrutinoside; **5**, kaempferol 3-2^G-rhamnosylrutinoside; **6**, kaempferol 3-neohesperidoside; **7**, quercetin 3-neohesperidoside; **8**, myricetin 3-neohesperidoside; **9**, kaempferol 3-rutinoside; **10**, quercetin 3-rutinoside; **11**, myricetin 3-rutinoside; **12**, kaempferol 3-glucoside; **13**, quercetin 3- glucoside; **14**, myricetin 3-glucoside.

Compounds **1** and **2** were obtained as light-yellow and dark-yellow amorphous powders, respectively. On acid hydrolysis of **1**, kaempferol, glucose, and rhamnose (glucose/rhamnose, 1/1) were identified by HPLC. From the acid hydrolysate of **2**, quercetin, glucose, and rhamnose (glucose/rhamnose, 1/1) were identified by HPLC. UV spectra of **1** and **2** in MeOH showed λ_{max} of 345 and 265 nm for **1**, and 357 and 257 nm for **2**. The shift in the λ_{max} on the addition of diagnostic reagents confirmed the presence of free hydroxyl groups (Markham, 1982), suggesting that **1** and **2** are each glycosylated at the 3-position of the aglycone.

HR-MALDI-TOF-MS of **1** and **2** showed molecular ions at *m/z* 703.1474 $[M+Na]^+$ and 719.1430 $[M+Na]^+$, which corresponded to the formulae $C_{30}H_{32}O_{18}Na$ (703.1486) and $C_{30}H_{32}O_{19}Na$ (719.1436), respectively. On ESI–MS of **1** and **2** in positive mode, peaks were observed at *m/z* 681 $[M+H]^+$ and *m/z* 697 $[M+H]^+$, respectively. The molecular ion of **1** was 16 mass units smaller than that of **2**, which corresponds to the difference in the number of hydroxyl groups on the B-ring of the flavonol aglycone. The molecular ion of **1** was 86 mass units larger than that of **6** or **9** (*m/z* 595 $[M+H]^+$), and that of **2** was also 86 mass units larger than that of **7** or **10** (*m/z* 611 $[M+H]^+$). The mass differences of 86 suggest that **1** and **2** each contain a malonyl group, which was also inferred from the MS/MS data. In the MS/MS of **1** (or **2**) (*m/z* 679 (or 695) $[M-H]^-$), the fragment ions found at *m/z* 635 (or 651) $[M-CO_2-H]^-$ and 593 (or 609) $[M-malonyl-H]^-$ are characteristic of a malonic acid ester (Stein and Zinsmeister, 1990).

The ^{1}H NMR spectrum in methanol-d_4 of **1** showed signals for kaempferol: δ 6.18 (*d*, *J* = 2.1 Hz, H-6), 6.38 (*d*, *J* = 2.1 Hz, H-8), 6.88 (*d*, *J* = 8.9 Hz, H-3′ and-5′), and 7.99 (*d*, *J* = 8.9, H-2′ and-6′) and anomeric protons at δ 5.59 (*d*, *J* = 7.4) and 5.22 (*d*, *J* = 1.4) characteristic of glucose and rhamnose, respectively (Table 1). The ^{1}H NMR of **2** in methanol-d_4 revealed the presence of quercetin: δ 6.19 (*d*, *J* = 2.0 Hz, H-6), 6.38 (*d*, *J* = 2.0 Hz, H-8), 7.57 (*d*, *J* = 2.1 Hz, H-2′), 6.86 (*d*, *J* = 8.9 Hz, H-5′), and 7.56 (*d*, *J* = 2.1 Hz, 8.9, H-6′) and similar anomeric protons to those in **1**. From the coupling constants of the anomeric protons on **1** and **2**, the configuration at C-1 of the glucose and rhamnose was determined to be the β- and α-configuration, respectively. In the differential NOE spectra of **1** and **2**, NOE was observed between the glucose H-2 and the rhamnose H-1: the intensity of the glucose H-2 signal increased on irradiation of the rhamnose H-1 signal (7.7 and 7.5% for **1** and **2**, respectively). These data suggest that the sugar moiety in **1** and **2** is a neohesperidose derivative. The hydroxymethyl proton signals (H-6″a and-6″b) of the glucose residue in **1** (δ 4.27 and 4.13, respectively) and **2** (δ 4.29 and 4.13, respectively) experienced ca. 0.6 ppm lowfield shifts due to malonylation, when compared to those in **6–8** or **12–14**. However, methylene protons of the malonyl group were not observed. All the other protons were assigned from the decoupling experiment and 2D ^{1}H-^{1}H COSY data.

The ^{13}C NMR spectra in methanol-d_4 of **1** and **2** were very similar, except for the signals corresponding to the flavonol aglycone (Table 2). Two characteristic carbonyl carbon signals of a malonyl group were observed at δ 168.23 and 169.96 for **1** and at δ 168.30 and 170.58 for **2**. However, no methylene carbon of a malonyl group was observed. All the other signals were assigned from HSQC and HMBC data. Comparison of the ^{13}C NMR spectroscopic data for **1** and **12** revealed that the glucose C-2″ and C-1″ experienced lowfield (Δ +4.2 ppm) and upfield (Δ −3.7 ppm) shifts due to the α- and β-effects of rhamnosylation, respectively (Agrawal and Bansal, 1989). Moreover, the glucose C-6″ and C-5″ were shifted lowfield (Δ +2.0 ppm) and upfield (Δ −3.0 ppm) due to the α- and β-effect of malonylation, respectively. The same phenomena were observed in the spectrum of **2** when compared to that of **13**.

The missing methylene proton and carbon signals in methanol-d_4 were independently confirmed by ^{1}H and ^{13}C NMR spectroscopy in DMSO-d_6 (Tables 1 and 2). In the spectra of **1**, signals at δ_H 3.03 (2H, *s*,–C$\underline{H}_2$–) and δ_C 41.31 (–$\underline{C}H_2$–) were observed in addition to ones at δ_C 166.60 (R$\underline{C}$OOR′) and 167.80 (R$\underline{C}$OOH). These signals were correlated in HSQC and HMBC. The methylene proton at δ_H 3.03 was correlated with the methylene carbon at δ_C 41.31 in HSQC, and with the carbonyl carbons at δ_C 166.6 and 167.8 in HMBC. Moreover, the glucose H-6″b (δ_H 3.96) was correlated with the ester carbonyl carbon (δ_C 166.6), and the rhamnose anomeric proton (δ_H 5.05) was correlated with the glucose C-2 (δ_C 79.87) in the HMBC spectra. These data proved the existence of a malonyl group at the C-6″ of glucose in the structure of **1**. In the spectra of **2**, signals at δ_H 3.16 (2H, *s*,–C$\underline{H}_2$–) and δ_C 41.92 (–$\underline{C}H_2$–) were observed in addition to those at δ_C 166.55 (R$\underline{C}$OOR') and 167.81 (R$\underline{C}$OOH). The existence of a malonic acid residue at the glucose C-6″ in the structure of **2** was inferred from the spectral similarity to **1**, although the HSQC and HMBC spectra of **2** gave insufficient information on the relation of these signals.

Furthermore, C-2,-3 and-4, signals in the ^{13}C NMR spectra in DMSO-d_6 of **1** and **2** experienced upfield (Δ ca. −9.3 ppm), lowfield (Δ ca. +3.0 ppm), and upfield (Δ ca. −1.7 ppm) shifts, respectively, as compared with the chemical shifts of the corresponding carbons in kaempferol and quercetin (Markham et al., 1978). These shifts showed that C-3 participated in *O*-glycosylation. Considering all the data, the structures of **1** and **2** were determined to be the 3-*O*-(2″-*O*-α-rhamnosyl-6″-*O*-malonyl)-β-glucosides of kaempferol and quercetin, respectively.

Compound **3** was obtained as a dark-yellow amorphous powder. On acid hydrolysis of **3**, myricetin, glucose, and rhamnose (glucose/rhamnose, 1/2) were identified by HPLC. The UV spectrum of **3** in MeOH showed λ_{max} of 356 and 256 nm. UV spectra analysis with diagnostic reagents indicated that the sugar residues are all attached to the 3-position of myricetin (Markham, 1982). HR-MALDI-TOF-MS of **3** showed a molecular ion at *m*/*z* 795.1951 $[M+Na]^+$, which corresponded to the formula $C_{33}H_{40}O_{21}Na$ (795.1960). The ESI–MS spectrum of **3** showed a molecular ion at *m*/*z* 773 $[M+H]^+$. On ESI–MS/MS of *m*/*z* 773, fragment ions at *m*/*z* 627 $[M-\text{rhamnosyl}+H]^+$, 481 $[M-(2\times\text{rhamnosyl})+H]^+$, and 319 $[\text{myricetin}+H]^+$ were observed.

The ^{1}H NMR spectrum of **3** was very similar to those of **4** and **5**, except for the signals derived from the flavonol aglycone (Table 1). Signals corresponding to a myricetin aglycone were observed at δ 7.23 (*s*, H-2′ and −5′), 6.18 (*d*, *J* = 2.2 Hz, H-6), and 6.36 (*d*, *J* = 2.2 Hz, H-8). The three anomeric protons at δ 5.55 (*d*, *J* = 7.8 Hz, H-1 of glucose), 5.21 (*d*, *J* = 1.6 Hz, H-1 of 2″-rhamnose), and 4.50 (*d*, *J* = 1.6 Hz, H-1 of 6″-rhamnose) were comparable to those of **4** and **5**. The configuration of these sugars at C-1 was determined as β for glucose and α for both rhamnoses from coupling constants. All the signals were assigned from the results of a decoupling experiment and 2D ^{1}H–^{1}H COSY data. In the differential NOE experiment, irradiation of the H-1 of 2″-rhamnose (δ 5.21) enhanced the H-2″ of glucose (δ 3.69) by 11%, and irradiation of the H-1 of 6″-rhamnose (δ 4.50) enhanced the H-6″a (δ 3.81) and H-6″b (δ 3.40) of glucose by 1.6 and 2.1%, respectively.

The ^{13}C NMR spectrum of **3** was similar to the spectra of **4** and **5** in the sugar region (Table 2). All the carbon signals were assigned from HSQC and HMBC data. When compared to the carbon signals of the glucosyl moiety in **14**, the glucose C-2″ and-1″ experienced lowfield (Δ +4.7 ppm) and upfield (Δ −3.9 ppm) shifts, respectively, due to the α- and β-effects of rhamnosylation at C-2. In addition, the C-6″ and-5″ experienced respective lowfield (Δ +5.6 ppm) and upfield (Δ −1.1 ppm) shifts due to the α- and β-effects of rhamnosylation at C-6.

In the HMBC spectrum, each rhamnose H-1 (δ_H 5.21 and 4.50) was correlated with the glucose C-2 at δ_C 80.35 and the glucose C-6 at δ_C 68.31, respectively. Finally, the glucose H-1 (δ_H 5.55) was correlated with the C-3 (δ_C 134.58) of the aglycone in the HMBC spectra. Considering these data, the structure of **3** was determined to be myricetin 3-*O*-(2″,6″-di-*O*-α-rhamnosyl)-β-glucoside (i.e. 3-2^G-rhamnosylrutinoside). Parker and Bohm (1975) previously reported myricetin 3-rhamnosylrutinoside but the bonding site of the rhamnose moiety was not determined.

This is the first report of myricetin glycosides from *C. ternatea*, although only as minor flavonoid components (1.9% of the total isolated flavonol weight, see Table 3). The majority were kaempferol glycosides (79.8%), followed by quercetin glycosides (18.3%). Kaempferol 3-neohesperidoside (**6**) was the major constituent (61.8%). Although kaempferol 3-robinobioside-7-rhamnoside was previously reported to be the major constituent in the flower (Saito et al., 1985), we could not detect it. In the LC/MS/MS analyses of the crude petal extract (see *Experimental*), each of the conspicuous HPLC peaks was identified as one of the 14 isolated flavonol glycosides or one of the known ternatins/anthocyanins (Table 3). The total area of the unidentified HPLC peaks was only 1.6% of the total peak area. Concentrating only on the flavonol glycosides, the relative area of an identified HPLC peak was proportional to the mol% ratio of the corresponding isolated compound. The only differences seen were for **1** and **2**, and these might have been due to demalonylation during purification. Our results show that the flavonol glycosides that we isolated constituted an absolute majority of the flavonol glycosides that accumulated in the

Table 3
Summary of LC/MS/MS and isolation experiment

Compounds	LC/MS/MS					Isolation	
	HPLC			MS and MS/MS (m/z)		Mol% ratio of isolated compounds	Wt. (mg) of isolated compounds
	t_R (min)	Peak area (%) at 350 nm		$[M+H]^+$	Fragment ion(s) of $[M+H]^+$		
		Total	Flavonols only				
1	30.1	5.2	5.7	681	535, 287	0.3	10.0
2	26.6	1.0	1.1	697	551, 303	0.1	4.0
3	18.2	0.3	0.4	773	627, 481, 319	0.4	15.8
4	20.9	3.5	3.9	757	611, 465, 303	3.5	132.9
5	23.7	22.1	24.5	741	595, 449, 287	13.3	489.6
6	26.1	42.5	46.9	595	449, 287	62.3	1899.6
7	23.1	3.5	3.9	611	465, 303	8.6	259.7
8	19.4	0.3	0.3	627	481, 319	0.5	16.3
9	31.1	0.6	0.7	595	449, 287	0.4	12.8
10	26.9	4.8	5.3	611	465, 303	4.3	129.8
11	22.7	1.0	1.1	627	481, 319	0.6	19.3
12	32.5	1.2	1.4	449	287	1.9	41.9
13	28.3	3.9	4.3	465	303	1.5	35.1
14	23.5	0.5	0.6	481	319	0.3	7.6
15[a]	22.5	n.d.[b]	n.d.[b]	713	567, 319	n.i.[c]	n.i.[c]
Ternatin A1	24.8	0.6		2107	1945, 1799, 1783, 1637, 1475, 1329, 1167		
Ternatin B1 and B2	30.4	3.6		1945, 1637	1475, 1329, 1167		
Ternatin D1	34.7	2.3		1783	1637, 1475		
Ternatin D2	32.6	1.4		1475	1329, 1167		
(other 11 peaks)		1.6					

[a] Myricetin 3-(2″-rhamnosyl-6″-malonyl)glucoside.
[b] Not detected.
[c] Not isolated.

petals. Therefore, for all practical purposes, kaempferol 3-robinobioside-7-rhamnoside is not present in the petals.

In addition, the existence of myricetin 3-(2″-rhamnosyl-6″-malonyl) glucoside was inferred from the LC/MS/MS experiment, although it was not observed as an HPLC peak. A mass chromatogram of m/z 713 ($[M+H]^+$) showed a single peak at t_R 22.5 (min), and the spontaneously obtained MS/MS spectrum of m/z 713 showed fragment ions at m/z 567 [M−rhamnosyl $+H]^+$ and 319 [M−rhamnosyl-malonylglucosyl $+H]^+$ (or [myricetin $+H]^+$). This fragment pattern corresponded to those of **1** and **2**.

Five flavonol glycoside sugars (2^G-rhamnosylrutinose, neohesperidose, rutinose, 2-rhamnosyl-6-malonylglucose, and glucose) occurred with each of the three flavonol aglycones. The 2-rhamnosyl-6-malonylglucose sugar moiety of **1** and **2** was first reported as in a flavonol glycoside, although it has been subsequently reported in a flavone glycoside (Stein and Zinsmeister, 1990).

Few malonylated flavonol glycosides have been reported in flowers or petals, although they have been reported in other parts of several species, including *Cicer arietinum* (aerial parts), *Pyrus communis* (leaves), and *Ranunculus fluitans* (leaves) (Williams and Harborne, 1994; Harborne and Williams, 1995, 1998, 2001). To our knowledge, only kaempferol 3-neohesperidoside-7-(6″-malonylglucoside) has been reported from flowers in *Crocus chrysanthus-biflorus* (Nørbæk et al., 1999).

3. Experimental

3.1. General

^1H and ^{13}C NMR spectra were recorded on a JEOL α-400 NMR spectrometer at 400 and 100 MHz, respectively, at 30 °C; the chemical shifts are reported in δ unit (ppm) values relative to the solvent (at δ_H 3.30, δ_C 49.0 for methanol-d_4, or δ_H 2.49, δ_C 39.7 for DMSO-d_6). 2-D NMR spectra were recorded with the JEOL 2-D programs (PDQF, HSQC, and HMBC). 1-D differential NOE spectra were recorded with the JEOL NOEDF program. The ^1H NMR spectrum of each compound was simulated using SwaN-MR 3.6.1 (Balacco, 1994) to check the signal assignments. UV/vis spectra were recorded with a Gene Spec III spectrometer (Naka Instruments). HR-MS measurements were carried out

on a PerSeptive Biosystems Voyager DE-STR. ESI–MS was measured on a PE Sciex API-300 or a Bruker Esquire3000 spectrometer. MPLC was performed using a LopODS (Nomura). Preparative HPLC was performed using a Develosil-10/20 column (20 i.d. × 250 mm, Nomura) on a Waters 616 LC system consisting of a 717 autosampler and a 486 UV/vis detector.

3.2. Plant material

Seeds of *C. ternatea* cv. Double Blue were purchased from Sakata Seed Ltd., and cultivated in a greenhouse. A voucher specimen (AGBC CLITE-DB01) is deposited in the laboratory of Aomori Green BioCenter. The petals of flowers that bloomed in the summer were harvested, dried in an oven at 50 °C (Terahara et al., 1990), and stored in a vacuum desiccator until use.

3.3. Extraction and isolation

The petals of *Clitoria ternatea* (106 g dry wt.) were homogenized and extracted with 50% MeOH–1% TFA (1 l × 3). Hydrophobic compounds were removed from the concentrated extract with $CHCl_3$ and EtOAc, successively (86.3 mg for $CHCl_3$ and 658.2 mg for EtOAc extract). The crude extract (83 g) was separated by MPLC (20–70% MeCN gradient/H_2O) to give two fractions (A: 20–70% MeCN fraction; 76 g, B: 70% MeCN fraction; 6.0 g). Fraction A contained almost all the anthocyanins and some sticky substances. Fraction B contained mainly flavonoid glycosides. Fraction B was further subjected to a Sephadex LH-20 column chromatography (5–70% MeCN gradient/H_2O) to give three fractions: Fractions C, 5–40% MeCN fraction, 1.6 g; D, 40–50% MeCN fraction, 1.5 g; and E, 50–70% MeCN fraction, 3.1 g. Fraction C contained anthocyanins. Preparative HPLC (5–40% MeCN–0.01M TFA gradient/0.01M TFA) of D gave five isolated compounds: **1** (10.0 mg), **2** (4.0 mg), **3** (15.8 mg), **4** (132.9 mg), and **5** (489.6 mg). Preparative HPLC (5–40% MeCN–0.01M TFA gradient/0.01M TFA) of E gave nine isolated compounds: **6** (1899.6 mg), **7** (259.7 mg), **8** (16.3 mg), **9** (12.8 mg), **10** (129.8 mg), **11** (19.3 mg), **12** (41.9 mg), **13** (35.1 mg), and **14** (7.6 mg).

3.4. Acid hydrolysis

Each isolated compound (100 μg) was dissolved in MeOH (5 μl) to which 2M HCl (5 μl) was added. The resulting solution was drawn into a 10-μl Ringcaps pipette (Hirschmann Laborgerate) and the sealed pipette heated on a block heater at 100 °C for 60 min. The solution in the pipette was diluted with H_2O (290 μl), passed through a Sep-Pak Vac 1-cc C18 Cartridge (Waters), and rinsed with H_2O (300 μl × 2). The water eluate (900 μl) was collected and submitted to HPLC analysis of the sugars. A further eluate with 40% MeCN–0.01M TFA (1 ml) was collected and submitted to HPLC analysis of the flavonoid aglycones. The HPLC conditions for the sugar analyses were: solvent, 0.3 M NaOH; column, Sucrebead I (2 i.d. × 250 mm, Shiseido); flow rate, 100 μl/min; temp., 20 °C. The HPLC conditions for the aglycone analyses were: solvent, 40% MeOH–0.01M TFA; column, Capcell Pak C_{18} UG 120 (3 i.d. × 250 mm, Shiseido); flow rate, 500 μl/min; temp., 50 °C.

3.5. LC/MS/MS experiment

A fresh petal was homogenized and extracted with aq. 50% MeOH containing 0.01M TFA (2 ml × 3). The crude extract was suspended in H_2O and passed through Sep-Pak C18 (Waters) and eluted with aq. 40% MeCN containing 0.01M TFA. The flavonoids in the eluate were analyzed with an LC/MS/MS system; the eluate from a Nanospace HPLC system (Shiseido) equipped with a Develosil C30-UG-5 (1.5 i.d. × 250 mm, Nomura) and a PDA detector was introduced into an Esquire3000 ion trap mass spectrometer (Bruker). MS data were collected in auto-MS/MS mode. The samples were eluted at 35 °C with a flow rate of 125 μl/min. The solvent system used was a linear gradient of 14% to 86% solvent B (40% MeCN–0.01M TFA) in solvent A (5% MeCN–0.01M TFA) over a period of 45 min. The results for flavonol glycosides are summarized in Table 3. The results for anthocyanins were as follows: Ternatin C5 (C5) (12.2 min, *m/z* 875), delphinidin 3-glucoside (16.3 min, *m/z* 465), C4 (16.7 min, *m/z* 1183), A3 (17.5 min, *m/z* 1491), B4 (22.5 min, *m/z* 1329), A2 and C3 (23.3 min, *m/z* 1021 and 1799, respectively), C2 (24.1 min, *m/z* 1491), A1 (24.8 min, *m/z* 2107), B3 (25.7 min, *m/z* 1637), D3 (26.7 min, *m/z* 1167), B1 and B2 (30.4 min, *m/z* 1637 and 1945, respectively), C1 (32.0 min, *m/z* 1329), D2 (32.6 min, *m/z* 1475), and D1 (34.7 min, *m/z* 1783).

3.6. Kaempferol 3-O-(2″-O-α-rhamnosyl-6″-O-malonyl)-β-glucoside (*1*)

Light-yellow amorphous powder. UV (MeOH) λ_{max} nm (log ϵ): 345 (4.19), 265 (4.24); NaOMe: 394, 324, 273 (intensity not decreased); $AlCl_3$: 394, 351, 302, 274; $AlCl_3$/HCl: 392, 346, 301, 273; NaOAc: 376, 307, 273; NaOAc/H_3BO_3: 351, 265. ^1H and ^{13}C NMR: see Tables 1 and 2. Differential NOE experiment data: enhancement of H-2 and-3 of glucose (7.7 and 3.4% increase, respectively) and H-2 of 2″-rhamnose (6.7%) on irradiation of H-1 of 2″-rhamnose. HR-MS: *m/z* 703.1474 $[M+Na]^+$, calc. for $C_{30}H_{32}O_{18}Na$, 703.1486. ESI-MS: *m/z* 681 $[M+H]^+$, 679 $[M-H]^-$. MS/MS of *m/z* 681: 535 $[M-\text{rhamnosyl}+H]^+$, 287 $[M-\text{rhamnosyl-malonylglucosyl}+H]^+$; MS/MS of *m/z* 679: 635 $[M-CO_2-H]^-$, 593 $[M-\text{malonyl}-H]^-$.

3.7. Quercetin 3-O-(2″-O-α-rhamnosyl-6″-O-malonyl)-β-glucoside (**2**)

Dark-yellow amorphous powder. UV (MeOH) λ_{max} nm (log ϵ): 357 (4.25), 257 (4.34); NaOMe: 399, 331, 270 (intensity not decreased); $AlCl_3$: 429, 272; $AlCl_3$/HCl: 399, 367, 270; NaOAc: 384, 324, 271; NaOAc/H_3BO_3: 374, 260. ^1H and ^{13}C NMR: see Tables 1 and 2. Differential NOE experiment data: enhancement of H-2 of glucose (7.5% increase) and H-2 of 2″-rhamnose (4.9%) on irradiation of H-1 of 2″-rhamnose. HR-MS: *m/z* 719.1430 $[M+Na]^+$, calc. for $C_{30}H_{32}O_{19}Na$, 719.1436. ESI-MS: *m/z* 697 $[M+H]^+$, 695 $[M-H]^-$. MS/MS of *m/z* 695: 551 $[M-\text{rhamnosyl}+H]^+$, 303 $[M-\text{rhamnosyl}-\text{malonylglucosyl}+H]^+$; MS/MS of *m/z* 695: 651 $[M-CO_2-H]^-$, 609 $[M-\text{malonyl}-H]^-$.

3.8. Myricetin 3-O-(2″,6″-di-O-α-rhamnosyl)-β-glucoside (**3**)

A dark-yellow amorphous powder. UV (MeOH) λ_{max} nm (log ϵ): 356 (4.27), 256 (4.30); NaOMe: 400, 264 (intensity not decreased); $AlCl_3$: 428, 310, 270; $AlCl_3$/HCl: 400, 368, 308, 271; NaOAc: 387, 320, 270; NaOAc/H_3BO_3: 373, 339. ^1H and ^{13}C NMR: see Tables 1 and 2. Differential NOE experiment data: enhancement of H-2 of glucose (11.0% increase) and H-2 of 2″-rhamnose (6.3%) on irradiation of H-1 of 2″-rhamnose; enhancement of H-6a and-6b of glucose (1.6 and 2.1% increase, respectively) and H-2 of 6″-rhamnose (8.6%) on irradiation of H-1 of 6″-rhamnose. HR-MS: *m/z* 795.1951 $[M+Na]^+$, calc. for $C_{33}H_{40}O_{21}Na$, 795.1960. ESI–MS: *m/z* 773 $[M+H]^+$, 771 $[M-H]^-$. MS/MS of *m/z* 773: 627 $[M-\text{rhamnosyl}+H]^+$, 481 $[M-(2\times\text{rhamnosyl})+H]^+$, 319 $[M-(2\times\text{rhamnosyl})-\text{glucosyl}+H]^+$.

3.9. Myricetin 3-neohesperidoside (**8**)

Dark-yellow amorphous powder. UV (MeOH) λ_{max} nm (log ϵ): 361 (4.14), 255 (4.18); NaOMe: 401, 321, 267 (intensity not decreased); $AlCl_3$: 433, 270; $AlCl_3$/HCl: 402, 360, 308, 271; NaOAc: 364, 265; NaOAc/H_3BO_3: 381. ^1H and ^{13}C NMR: see Tables 1 and 2. Differential NOE experiment data: enhancement of H-2 of glucose (7.7% increase) and H-2 of 2″-rhamnose (5.5%) on irradiation of H-1 of 2″-rhamnose. HR-MS: *m/z* 649.1374 $[M+Na]^+$, calc. for $C_{27}H_{30}O_{17}Na$, 649.1381. ESI-MS: *m/z* 627 $[M+H]^+$, 625 $[M-H]^-$. MS/MS of *m/z* 627: 481 $[M-\text{rhamnosyl}+H]^+$, 319 $[M-\text{rhamnosyl}-\text{glucosyl}+H]^+$.

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