Flavonoid Glycosides from *Adina racemosa* and Their Inhibitory Activities on Eukaryotic Protein Synthesis

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From the dried leaves, flowers, and twigs of *Adina racemosa*, five new flavonoid glycosides, quercetin 3-*O*-α-L-rhamnopyranosyl(1→6)−(3-*O*-*trans*-*p*-coumaroyl)-*β*-D-galactopyranoside (**1**), quercetin 3-*O*-α-L-rhamnopyranosyl(1→6)-[(4-*O*-*trans*-*p*-coumaroyl)-α-L-rhamnopyranosyl(1→2)]-(4-*O*-*trans*-*p*-coumaroyl)-*β*-D-galactopyranoside (**2**), kaempferol 3-*O*-α-L-rhamnopyranosyl(1→6)-[(4-*O*-*trans*-*p*-coumaroyl)-α-L-rhamnopyranosyl(1→ 2)]-(4-*O*-*trans*-*p*-coumaroyl)-*β*-D-galactopyranoside (**3**), quercetin 3-*O*-α-L-rhamnopyranosyl(1→6)-[(4-*O*-*trans*-*p*-coumaroyl)-α-L-rhamnopyranosyl(1→2)]-(3-*O*-*trans*-*p*-coumaroyl)-*β*-D-galactopyranoside (**4**), and quercetin 3-*O*-α-L-rhamnopyranosyl(1→6)-[(4-*O*-*trans*-caffeoyl)-α-L-rhamnopyranosyl-(1→2)]-(3-*O*-*trans*-*p*-coumaroyl)-*β*-D-galactopyranoside (**5**), and eight known compounds were isolated. The structures of the new compounds were determined by spectroscopic and chemical means. Their inhibitory activities on protein synthesis were assessed. The new glycosides were found to be inhibitors of eukayrotic, but not prokaryotic, protein synthesis.

In the course of our phytochemical studies on glycosidic constituents of Rubiaceae plants, we have recently examined the leaves, flowers, and twigs of *Adina racemosa* (Sieb. et Zucc.) Miq. and isolated several new iridoid glycosides.[1] Further investigation of this plant material led to the isolation of five new flavonoid glycosides **1**−**5**, along with eight known glycosides, (5*S*)-5-carboxystrictosidine,[2] loganic acid,[3] nicotiflorin,[4] rutin,[5] kaempferol 3-*O*-α-L-rhamnopyranosyl(1→6)-*β*-D-galactopyranoside,[5] quercetin 3-*O*-α-L-rhamnopyranosyl(1→6)-*β*-D-galactopyranoside (**6**),[5] quercetin 3-*O*-α-L-rhamnopyranosyl(1→6)-(4-*O*-*trans*-*p*-coumaroyl)-*β*-D-galactopyranoside (**7**),[6] and kaempferol 3-*O*-α-L-rhamnopyranosyl(1→6)-(4-*O*-*trans*-*p*-coumaroyl)-*β*-D-galactopyranoside (**8**).[6] These known glycosides were isolated for the first time from this plant species. The structures of the five new glycosides **1**−**5** were determined and their biological activities assessed. These glycosides were found to be inhibitors of eukayrotic, but not prokaryotic, protein synthesis.

Results and Discussion

Compound **1** was isolated as a yellow amorphous powder and showed the molecular formula $C_{36}H_{36}O_{18}$ from its HR-SIMS. Its ^{1}H NMR spectrum exhibited a pair of *meta*-coupled aromatic protons at *δ* 6.22 and 6.42 (each d, J = 2.0 Hz) and an AMX spin system at *δ* 6.89 (d, J = 8.5 Hz), 7.62 (dd, J = 8.5, 2.0 Hz), and 7.86 (d, J = 2.0 Hz). These spectral data, together with its UV and IR spectra, indicated a quercetin moiety as a basic skeleton. Furthermore, the ^{1}H and ^{13}C NMR spectral data of **1** showed signals for a *β*-galactopyranosyl, an α-rhamnopyranosyl, and a *trans*-*p*-coumaroyl unit (Tables 1 and 2), suggesting its structural similarity to **7**. An acid hydrolysis of **1** liberated D-galactose and L-rhamnose, which were identified by GLC analysis of their thiazolidine derivatives.[7] The HMBC correlations between H-1 of the galactosyl unit and C-3 of the quercetin unit, between H-1 of the rhamnosyl unit and C-6 of the galactosyl unit, and between H-6 of the galactosyl unit and C-1 of the rhamnosyl unit demonstrated the glycosidic linkages as in quercetin 3-*O*-α-L-rhamnopyranosyl(1→6)-*β*-D-galactopyranoside (**6**) and **7**. Acylation of the hydroxyl group at C-3 of the galactosyl unit in **1** was determined by the downfield shifts of C-3 and H-3 of the galactosyl unit as well as the upfield shits of C-2 and C-4 of the galactosyl unit in **1** relative to those of **6**. Thus, compound **1** is quercetin 3-*O*-α-L-rhamnopyranosyl(1→6)-(3-*O*-*trans*-*p*-coumaroyl)-*β*-D-galactopyranoside.

Compound **2**, $C_{51}H_{52}O_{24}$, was also obtained as a yellow amorphous powder. Its ^{1}H and ^{13}C NMR spectral data were similar to those of quercetin 3-*O*-α-L-rhamnopyranosyl-(1→6)-(4-*O*-*trans*-*p*-coumaroyl)-*β*-D-galactopyranoside (**7**) except for additional signals of an α-rhamnopyranosyl and a *trans*-*p*-coumaroyl unit (Tables 1 and 2). Attachment of the second rhamnosyl group to the hydroxyl group at C-2 of the galactosyl unit was shown by the HMBC correlations between H-1 of rhamnose-II and C-2 of the galactosyl unit and the ROESY correlation between H-1 of rhamnose-II and H-2 of the galactosyl unit. Attachment of the second *trans*-*p*-coumaroyl unit at the hydroxyl group at C-4 of rhamnose-II was also shown by the HMBC correlation between H-4 of rhamnose-II and the carbonyl carbon of the second *trans*-*p*-coumaroyl unit. Accordingly, compound **2** is quercetin 3-*O*-α-L-rhamnopyranosyl(1→6)-[(4-*O*-*trans*-*p*-coumaroyl)-α-L-rhamnopyranosyl(1→2)]-(4-*O*-*trans*-*p*-coumaroyl)-*β*-D-galactopyranoside.

The spectral features of compound **3** were similar to those of **2** and suggested that **3** possessed *O*-α-L-rhamnopyranosyl(1→6)-[(4-*O*-*trans*-*p*-coumaroyl)-α-L-rhamnopyranosyl(1→2)]-(4-*O*-*trans*-*p*-coumaroyl)-*β*-D-galactopyranose as a glycosidic moiety. We noted differences between **2** and **3** in the proton and carbon signals of the aglycone moiety. The ^{1}H NMR spectrum of **3** showed an AA′BB′ spin system [*δ* 6.93 (2H, d, J = 9.0 Hz), 8.12 (2H, d, J = 9.0 Hz)] instead of the AMX spin system observed in **2**.

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Table 1. ^{1}H NMR Spectral Data of **1**−**5** in CD_3OD at 500 MHz

H	**1**			**2**			**3**			**4**			**5**		
flavonol															
6	6.22	d	(2.0)	6.24	d	(2.0)	6.24	d	(2.0)	6.22	d	(1.5)	6.24	d	(2.0)
8	6.42	d	(2.0)	6.40	d	(2.0)	6.42	d	(2.0)	6.39	d	(1.5)	6.40	d	(2.0)
2′	7.86	d	(2.0)	7.71	d	(2.0)	8.12	d	(9.0)	7.79	d	(2.0)	7.77	d	(2.0)
3′							6.93	d	(9.0)						
5′	6.89	d	(8.5)	6.90	d	(8.5)	6.93	d	(9.0)	6.88	d	(8.5)	6.88	d	(8.5)
6′	7.62	dd	(8.5, 2.0)	7.69	dd	(8.5, 2.0)	8.12	d	(9.0)	7.59	dd	(8.5, 2.0)	7.59	dd	(8.5, 2.0)
galactose															
1	5.26	d	(8.0)	5.60	d	(7.0)	5.62	d	(7.0)	5.80	d	(7.5)	5.80	d	(7.5)
2	4.13	dd	(10.0, 8.0)	4.02	dd	(9.5, 7.0)	3.96	dd	(9.5, 7.0)	4.31	dd	(10.0, 7.5)	4.30	dd	(10.0, 7.5)
3	4.93	dd	(10.0, 3.5)	3.99	dd	(9.5, 3.5)	3.99	dd	(9.5, 3.0)	5.14	dd	(10.0, 3.0)	5.12	dd	(10.0, 3.5)
4	4.08	brd	(3.5)	5.36	brd	(3.5)	5.35	dd	(3.0, 0.5)	4.14	brd	(3.0)	4.13	brd	(3.5)
5	3.79	brt	(6.0)	3.86	brt	(6.5)	3.87	brt	(6.5)	3.87	brt	(6.5)	3.85	brt	(6.5)
6	3.40	dd	(9.0, 5.5)	3.22	dd	(10.5, 6.5)	3.22	dd	(10.5, 6.5)	3.46	dd	(10.0, 6.5)	3.46	dd	(10.0, 6.5)
6	3.76	dd	(9.0, 6.0)	3.49	dd	(10.5, 6.0)	3.49	dd	(10.5, 6.0)	3.77	dd	(10.0, 6.5)	3.76	dd	(10.0, 6.5)
rhamnose-I															
1	4.55	d	(1.5)	4.46	d	(1.5)	4.46	d	(1.5)	4.56	d	(1.5)	4.56	d	(1.5)
2	3.61	dd	(3.5, 1.5)	3.60	dd	(3.5, 1.5)	3.59	dd	(3.5, 1.5)	3.65	dd	(3.5, 1.5)	3.64	dd	(3.5, 1.5)
3	3.51	dd	(9.5, 3.5)	3.52	dd	(9.5, 3.5)	3.51	dd	(9.5, 3.5)	3.53	dd	(9.5, 3.5)	3.53	dd	(9.5, 3.5)
4	3.30	m		3.23	t	(9.5)	3.23	t	(9.5)	3.29	t	(9.5)	3.29	t	(9.5)
5	3.52	dq	(9.5, 6.0)	3.38	dq	(9.5, 6.0)	3.37	dq	(9.5, 6.0)	3.54	dq	(9.5, 6.5)	3.54	dq	(9.5, 6.5)
6	1.19	d	(6.0)	1.05	d	(6.0)	1.05	d	(6.0)	1.19	d	(6.5)	1.19	d	(6.5)
rhamnose-II															
1				5.24	d	(1.5)	5.24	d	(1.5)	5.12	d	(1.5)	5.10	d	(1.5)
2				4.07	dd	(3.5, 1.5)	4.06	dd	(3.5, 1.5)	3.89	dd	(3.5, 1.5)	3.87	dd	(3.5, 1.5)
3				4.17	dd	(9.5, 3.5)	4.16	dd	(9.5, 3.5)	4.10	dd	(10.0, 3.5)	4.07	dd	(10.0, 3.5)
4				5.03	t	(9.5)	5.02	t	(9.5)	4.97	t	(10.0)	4.97	t	(10.0)
5				4.52	dq	(9.5, 6.0)	4.49	dq	(9.5, 6.5)	4.40	dq	(10.0, 6.5)	4.37	dq	(10.0, 6.5)
6				0.96	d	(6.0)	0.94	d	(6.5)	0.88	d	(6.5)	0.87	d	(6.5)
acyl-I															
2	7.49	d	(8.5)	7.54	d	(8.5)	7.52	d	(8.5)	7.51	d	(8.5)	7.52	d	(8.5)
3	6.82	d	(8.5)	6.86	d	(8.5)	6.86	d	(8.5)	6.82	d	(8.5)	6.82	d	(8.5)
5	6.82	d	(8.5)	6.86	d	(8.5)	6.86	d	(8.5)	6.82	d	(8.5)	6.82	d	(8.5)
6	7.49	d	(8.5)	7.54	d	(8.5)	7.52	d	(8.5)	7.51	d	(8.5)	7.52	d	(8.5)
α	6.46	d	(16.0)	6.46	d	(16.0)	6.38	d	(16.0)	6.47	d	(16.0)	6.47	d	(16.0)
β	7.72	d	(16.0)	7.67	d	(16.0)	7.66	d	(16.0)	7.74	d	(16.0)	7.74	d	(16.0)
acyl-II															
2				7.34	d	(8.5)	7.34	d	(8.5)	7.28	d	(8.5)	7.00	d	(1.5)
3				6.76	d	(8.5)	6.76	d	(8.5)	6.75	d	(8.5)			
5				6.76	d	(8.5)	6.76	d	(8.5)	6.75	d	(8.5)	6.72	d	(8.5)
6				7.34	d	(8.5)	7.34	d	(8.5)	7.28	d	(8.5)	6.74	dd	(8.5, 1.5)
α				6.27	d	(16.0)	6.27	d	(16.0)	6.21	d	(16.0)	6.17	d	(16.0)
β				7.50	d	(16.0)	7.50	d	(16.0)	7.44	d	(16.0)	7.40	d	(16.0)

Furthermore, the molecular formula, $C_{51}H_{52}O_{23}$, obtained from its HR-SIMS was one oxygen less than that of **2**. These spectral data indicated that compound **3** had kaempferol as an aglycone moiety. Thus, compound **3** is kaempferol 3-*O*-α-L-rhamnopyranosyl(1→6)-[(4-*O*-*trans*-*p*-coumaroyl)-α-L-rhamnopyranosyl(1→2)]-(4-*O*-*trans*-*p*-coumaroyl)-*β*-D-galactopyranoside.

Compound **4** was recognized as an isomer of **2** from its HR-SIMS. The spectral features were quite similar to those of **2** except for the chemical shifts of proton and carbon signals arising from the galactose moiety. A downfield-shifted proton at *δ* 5.36 (br d, *J* = 3.5 Hz) was assigned to H-4 of galactose in **2**, whereas a downfield-shifted proton at *δ* 5.14 (dd, *J* = 10.0, 3.0 Hz) was assigned to H-3 of galactose in **4** by 2D NMR experiments, suggesting that the acyl group was attached to the hydroxyl group at C-3 of galactose in **4** instead of C-4 of galactose as in **2**. Accordingly, compound **4** is quercetin 3-*O*-α-L-rhamnopyranosyl(1→6)-[(4-*O*-*trans*-*p*-coumaroyl)-α-L-rhamnopyranosyl(1→2)]-(3-*O*-*trans*-*p*-coumaroyl)-*β*-D-galactopyranoside.

Compound **5**, $C_{51}H_{52}O_{25}$, was also obtained as a yellow powder. The spectral features of **5** were similar to those of **4**, with differences ascribed to the acyl unit. In the ^{1}H NMR spectrum of **5**, signals for one *trans*-*p*-coumaroyl unit [*δ* 6.47 (1H, d, *J* = 16.0 Hz), 6.82 (2H, d, *J* = 8.5 Hz), 7.52 (2H, d, *J* = 8.5 Hz), 7.74 (1H, d, *J* = 16.0 Hz)] and one *trans*-caffeoyl unit [*δ* 6.17 (1H, d, *J* = 16.0 Hz), 6.72 (1H, d, *J* = 8.5 Hz), 6.74 (1H, dd, *J* = 8.5, 1.5 Hz), 7.00 (1H, d, *J* = 1.5 Hz), 7.40 (1H, d, *J* = 16.0 Hz)] were observed, instead of the signals obtained for the two *trans*-*p*-coumaroyl units present in **4**. The linkage of the *trans*-caffeoyl unit to the hydroxyl group at C-4 of rhamnose-II was shown by its HMBC correlation between H-4 of rhamnose-II [*δ* 4.97 (1H, t, *J* = 10.0 Hz)] and the carbonyl carbon of the *trans*-caffeoyl unit (*δ* 169.0). Therefore, compound **5** is quercetin 3-*O*-α-L-rhamnopyranosyl(1→6)-[(4-*O*-*trans*-caffeoyl)-α-L-rhamnopyranosyl(1→2)]-(3-*O*-*trans*-*p*-coumaroyl)-*β*-D-galactopyranoside.

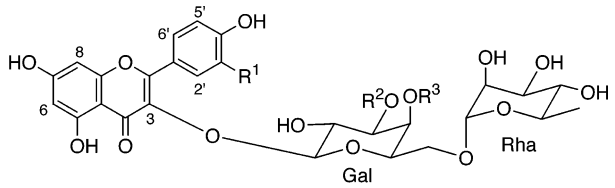
Flavonoids have so far been isolated from *Adina cordifolia*.[8−10] Our investigation thus represents the second report of flavonoid glycosides from the genus *Adina*. Given the fact that many carbohydrate-containing compounds, such as aminoglycosides, are known protein synthesis inhibitors, the activity of these novel flavonoid glycosides on in vitro protein synthesis was analyzed. Titrations were performed in translation extracts prepared from mouse Krebs cells in the presence of a reporter transcript, FF/HCV/Ren mRNA, in which 5′ cap-dependent initiation is monitored by the production of firefly luciferase production and internal initiation is monitored by the production of renilla luciferase (Figure 2A). The results indicate that whereas **1**, **7**, and **8** partially inhibit synthesis (∼2-fold) of both firefly and renilla luciferase at 100 *μ*M final concentrations, **2**, **3**, **4**, and **5** are more potent inhibitors (Figure

Table 2. ^{13}C NMR Spectral Data of **1**−**5** in CD_3OD at 125 MHz

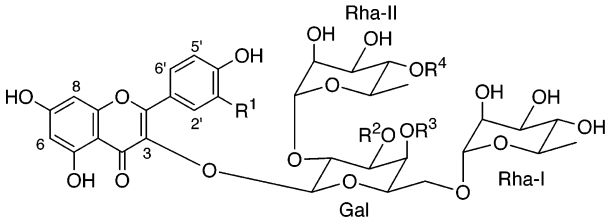
C	1	2	3	4	5
flavonol					
2	159.0	159.3	159.4	158.7	158.7
3	135.8	134.5	134.4	134.6	134.5
4	179.4	179.1	179.2	179.0	179.0
5	163.0	163.2	163.2	163.1	163.2
6	100.0	100.0	100.0	100.0	100.0
7	166.2	165.9	165.9	165.9	165.8
8	94.9	94.9	94.9	94.8	94.8
9	158.5	158.5	158.5	158.4	158.4
10	105.6	105.9	105.9	105.9	105.9
1′	122.8	123.6	123.2	123.1	123.2
2′	117.9	117.6	132.2	117.5	117.5
3′	145.9	146.0	116.1	145.8	145.8
4′	150.1	149.7	161.5	149.8	149.8
5′	116.2	115.9	116.1	116.2	116.2
6′	123.1	123.4	132.2	123.0	123.0
galactose					
1	105.7	101.4	101.4	101.7	101.6
2	70.8	78.0	78.0	75.3	75.4[c]
3	77.5	74.1	74.1	77.8	77.7
4	67.8	72.0	72.0	68.0	68.0[d]
5	75.0	73.6	73.7	74.7	74.8
6	66.7	67.1	67.1	66.6	66.6
rhamnose-I					
1	101.9	102.2	102.3	101.9	101.9
2	72.1	72.1[a]	72.1[b]	72.0	72.1[e]
3	72.3	72.2[a]	72.2[b]	72.2	72.3[e]
4	73.9	73.8	73.7	73.8	73.9
5	69.8	69.9	69.9	69.7	69.8
6	18.0	17.9	17.9	18.0	18.0
rhamnose-II					
1		102.6	102.6	102.4	102.5
2		72.5	72.5	72.5	72.6
3		70.5	70.4	70.3	70.4
4		75.7	75.7	75.5	75.5[c]
5		67.9	67.9	68.0	68.1[d]
6		17.4	17.4	17.3	17.3
acyl-I					
1	127.3	127.3	127.2	127.1	127.1
2	131.3	131.5	131.4	131.4	131.5
3	116.9	116.9	116.9	116.9	116.9
4	161.4	161.3	161.4	161.5	161.5
5	116.9	116.9	116.9	116.9	116.9
6	131.3	131.5	131.4	131.4	131.5
α	115.3	115.0	114.9	114.7	114.7
β	147.0	147.4	147.3	147.6	147.6
CO	168.7	168.9	168.6	168.3	168.3
acyl-II					
1		127.2	127.2	127.1	127.7
2		131.2	131.2	131.2	114.8
3		116.8	116.8	116.8	146.7
4		161.1	161.2	161.1	149.5
5		116.8	116.8	116.8	116.4
6		131.2	131.2	131.2	123.3
α		115.4	115.4	115.4	115.3
β		146.5	146.5	146.4	146.9
CO		169.0	169.0	168.9	169.0

[a−e] Values with the same superscript are interchangeable.

2B). Additionally, **4** and **5** appear to inhibit translation more than 10-fold at 100 μM, whereas an approximately 5-fold effect was observed with **2** and **3** at the same concentration (Figure 2B). These results indicate that flavonoid glycosides with three carbohydrate residues are more potent than those containing two carbohydrate moieties and that a *trans*-*p*-coumaroyl linked to the hydroxyl group of C-3 of galactose leads to higher inhibitory activity. No inhibition on bacterial protein synthesis was observed by **5** in an *E. coli* S30 translation extract (Figure 2C), indicating that the activity of the flavonoid glycosides is specific for the eukaryotic process. To determine if the inhibition observed by **5** was at the level of translation



1: R^1=OH, R^2=*trans*-*p*-coumaroyl, R^3=H
6: R^1=OH, R^2=R^3=H
7: R^1=OH, R^2=H, R^3=*trans*-*p*-coumaroyl
8: R^1=R^2=H, R^3=*trans*-*p*-coumaroyl



2: R^1=OH, R^2=H, R^3=R^4=*trans*-*p*-coumaroyl
3: R^1=R^2=H, R^3=R^4=*trans*-*p*-coumaroyl
4: R^1=OH, R^2=R^4=*trans*-*p*-coumaroyl, R^3=H
5: R^1=OH, R^2=*trans*-*p*-coumaroyl, R^3=H, R^4=*trans*-caffeoyl

Figure 1. Structures of compounds **1**−**8**.

initiation, we performed ribosome binding experiments in which we analyzed 80S initiation complex formation by sedimentation velocity (Figure 2D). In the presence of cycloheximide, a known inhibitor of translation elongation, an 80S complex can be trapped on the initiation codon of a [^{32}P]radiolabeled reporter mRNA (Figure 2D). When complexes are formed in the presence of **5** and cycloheximide, no inhibition of 80S initiation complex formation was observed (Figure 2D), indicating that **5** does not inhibit translation initiation and most likely acts downstream on translation elongation.

Experimental Section

General Experimental Procedures. UV spectra were recorded on a Shimadzu UV-240 spectrophotometer and IR spectra on a Shimadzu FTIR-8200 spectrophotometer. Optical rotations were measured on a Jasco DIP-370 digital polarimeter. ^1H (500 MHz) and ^{13}C (125 MHz) NMR spectra were recorded on Varian VXR-500 spectrometers with TMS as an internal standard. MS and HRMS were obtained with a Hitachi M-4100 mass spectrometer. Glycerol was used as the matrix for SIMS and HRSIMS. MPLC was carried out with Wakogel LP-40 C18. TLC was performed on precoated Kieselgel 60F_{254} plates (Merck).

Plant Material. The leaves, flowers, and twigs of *Adina racemosa* were collected in Heng-Chun Tropical Botanical Garden, Taiwan, in May 1997. A voucher specimen (KPFY-972) is deposited in the laboratory of Kobe Pharmaceutical University.

Extraction and Isolation. Dried leaves, flowers, and twigs (1.27 kg) of *A. racemosa* were extracted with hot MeOH, and the extract was fractionated as described previously.[1] Fraction 14 in ref 1 was submitted to reversed-phase MPLC with MeOH−H_2O. The residue (571.2 mg) from the 40% MeOH eluate was further purified by preparative HPLC (μBondasphere 5 μ C18-100 Å, MeOH−H_2O, 9:1 and 1:1) to afford quercetin (76.0 mg) and (5*S*)-5-carboxystrictosidine (12.4 mg). The 50% MeOH residue (478.0 mg) was also subjected to preparative HPLC (MeOH−H_2O, 11:9 and 3:2) and preparative TLC ($CHCl_3$−MeOH−H_2O, 70:30:0.3) to yield **1** (7.5 mg), **7** (22.1 mg), **8** (11.2 mg), quercetin (60.6 mg), and kaempferol (38.8 mg). Fractions 15−17 in ref 1 were further purified by a combination of reversed-phase MPLC (H_2O−MeOH, 17:3−3:2), preparative HPLC (MeOH−H_2O, 2:3, 9:11, 1:1, 11:9, or 3:2),

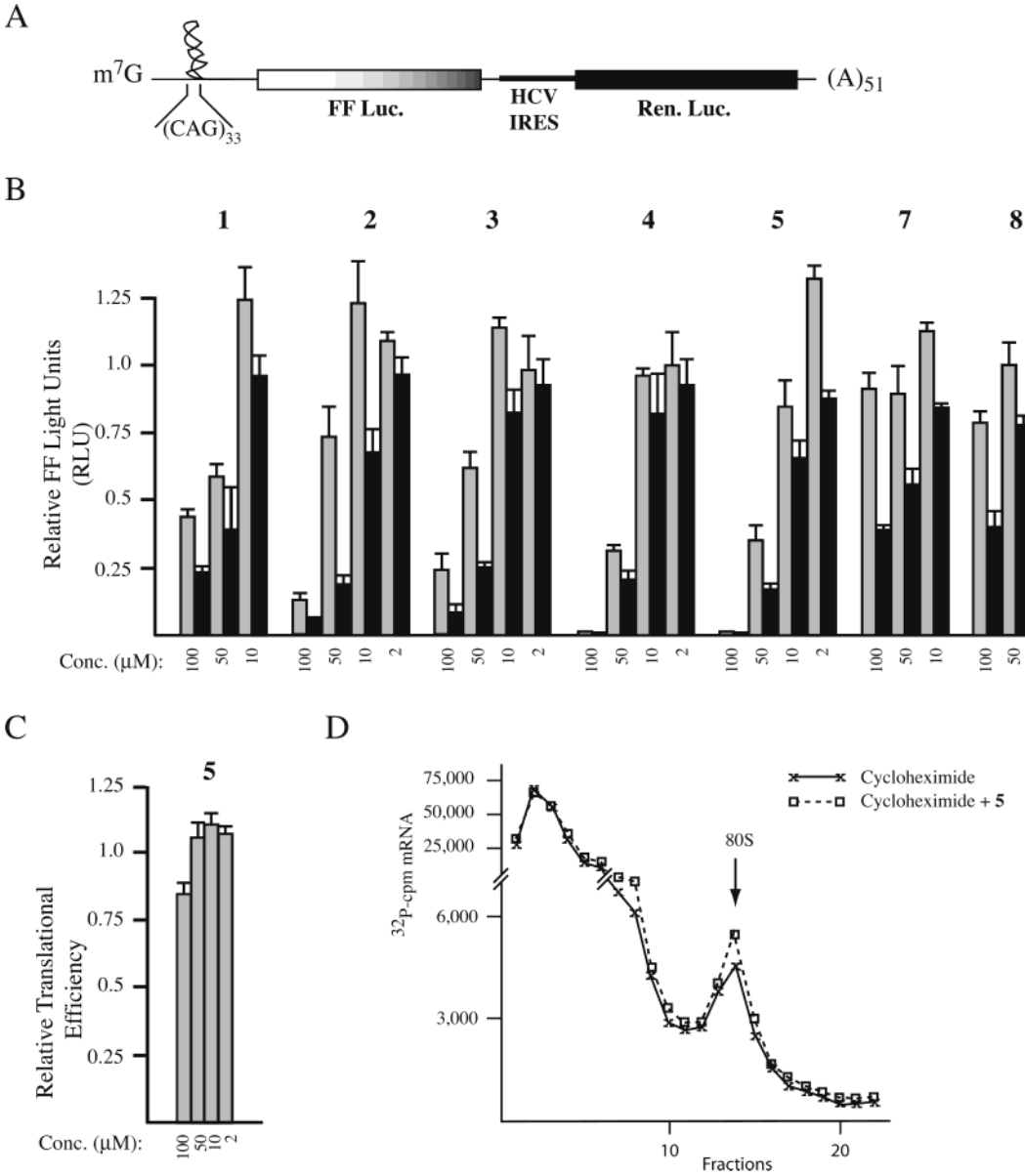


Figure 2. Inhibition of eukaryotic protein synthesis by flavonoid glycosides. (A) Schematic diagram of $(CAG)_{33}$/FF/HCV/Ren. To generate mRNA for in vitro transcriptions, the plasmid is linearized with BamH I. The firefly luciferase coding region is denoted by a shaded box, whereas the renilla coding region is denoted by a blackened box. The HCV IRES (indicated by a thickened line) allows for internal initiation upstream of the renilla ATG. (B) Titration of compounds in Krebs extracts. Translations were performed in the presence of the indicated amounts of compound and at a final mRNA and K^+ concentration of 5 μg/mL and 100 mM, respectively. Since the final concentration of DMSO in the compound additions was 1%, control translation reactions contained 1% DMSO. The obtained luciferase activities are normalized to the activity obtained in the control translations (which were set at one). The relative firefly luciferase values are represented by gray bars, and the relative renilla values are represented by black bars. Translations were performed two times, and the average values are presented along with the error of the mean. (C) Titration of **5** in *E. coli* S30 translation extracts. Translation reactions utilizing *E. coli* S30 extracts (Promega) were programmed with pBEST/luc plasmid and ^{35}S-met. The incorporation of ^{35}S-methionine into protein was monitored by TCA precipitation and normalized to the activity obtained in control reactions (which were set to one). The average of duplicate translations is shown, as well as the error of the mean. (D) **5** does not inhibit binding to mRNA templates. ^{32}P-labeled CAT mRNA was incubated in rabbit reticulocyte lysates in the presence of 600 μM cycloheximide, 600 μM cycloheximide, and 50 μM **5**. Following centrifugation, fractions of each sucrose gradient were collected using a Brandel tube piercer connected to an ISCO fraction collection and were individually counted. The position of the 80S complex is indicated.

and preparative TLC ($CHCl_3$−MeOH−H_2O, 70:30:0.3), respectively. Fraction 15 yielded **7** (33.5 mg), **8** (5.6 mg), loganic acid (93.5 mg), (5*S*)-5-carboxystrictosidine (11.0 mg), quercetin (11.4 mg), and kaempferol (0.7 mg); fraction 16, **4** (20.9 mg), **5** (17.4 mg), **6** (74.4 mg), **7** (91.6 mg), **8** (21.4 mg), quercetin (76.2 mg), kaempferol (25.8 mg), (5*S*)-5-carboxystrictosidine (84.3 mg), loganic acid (94.2 mg), kaempferol 3-*O*-α-L-rhamnopyranosyl-(1→6)-*β*-D-galactopyranoside (17.1 mg), and nicotiflorin (14.4 mg); fraction 17, **2** (98.3 mg), **3** (27.5 mg), **5** (18.3 mg), **6** (4.3 mg), **7** (32.4 mg), **8** (17.7 mg), quercetin (47.9 mg), kaempferol (15.8 mg), and rutin (61.6 mg).

Quercetin 3-*O*-α-L-rhamnopyranosyl(1→6)-(3-*O*-*trans*-*p*-coumaroyl)-*β*-D-galactopyranoside (1): yellow powder; $[\alpha]^{22}_D$ −86° (*c* 0.6, MeOH); UV (MeOH) λ_{max} (log ϵ) 224sh (4.43), 257 (4.30), 268 (4.29), 299sh (4.43), 315 (4.49), 369sh (4.14) nm; IR (KBr) ν_{max} 3394, 1690, 1655, 1605, 1514 cm^{-1}; ^{1}H NMR, Table 1; ^{13}C NMR, Table 2; NOESY, H-6 of galactose (δ 3.40)/H-1 of rhamnose; HMBC, H-2′ → C-2, H-6′ → C-2, H-1 of galactose → C-3, H-6 of galactose (δ 3.40) → C-1 of rhamnose, H-1 of rhamnose → C-6 of galactose, H_2-2, 6 of acyl → H-β, H-β → C_2-2, 6 of acyl, H-β → CO of acyl, H-α → CO of acyl; SIMS *m*/*z* 755 $[M - H]^-$, 609, 301; HRSIMS *m*/*z* 775.1837 (calcd for $C_{36}H_{35}O_{18}$, 755.1824).

Quercetin 3-*O*-α-L-rhamnopyranosyl(1→6)-[(4-*O*-*trans*-*p*-coumaroyl)-α-L-rhamnopyranosyl(1→2)]-(4-*O*-*trans*-*p*-coumaroyl)-*β*-D-galactopyranoside (2): yellow powder; $[\alpha]^{25}_D$ −300° (*c* 0.5, MeOH); UV (MeOH) λ_{max} (log ϵ) 226sh (4.56), 255sh (4.37), 269 (4.43), 299sh (4.62), 314 (4.68), 362sh (4.20)

nm; IR (KBr) ν_{max} 3394, 1695, 1655, 1605, 1514 cm^{-1}; ^{1}H NMR, Table 1; ^{13}C NMR, Table 2; ROESY, H-2 of galactose/H-1 of rhamnose-II, H-6 of galactose (δ 3.22) /H-1 of rhamnose-I; HMBC, H-2′ → C-2, H-1 of galactose → C-3, H-1 of rhamnose-II → C-2 of galactose, H-6 of galactose (δ 3.22) → C-1 of rhamnose-I, H-1 of rhamnose-I → C-6 of galactose, H-4 of galactose → CO of acyl-I, H-4 of rhamnose-II → CO of acyl-II; SIMS *m/z* 1047 [M − H]$^-$, 901, 755, 301; HRSIMS *m/z* 1047.2761 (calcd for $C_{51}H_{51}O_{24}$, 1047.2772).

Kaempferol 3-*O*-α-L-rhamnopyranosyl(1→6)-[(4-*O*-*trans*-*p*-coumaroyl)-α-L-rhamnopyranosyl(1→2)]-(4-*O*-*trans*-*p*-coumaroyl)-*β*-D-galactopyranoside (3): yellow powder; $[\alpha]^{25}_D$ −274° (*c* 0.3, MeOH); UV (MeOH) λ_{max} (log ϵ) 224 (4.56), 268 (4.44), 300sh (4.64), 314 (4.70), 364sh (4.09) nm; IR (KBr) ν_{max} 3389, 1697, 1655, 1605, 1514 cm^{-1}; ^{1}H NMR, Table 1; ^{13}C NMR, Table 2; ROESY, H-2 of galactose/H-1 of rhamnose-II; HMBC, H-1 of rhamnose-II → C-2 of galactose, H-1 of rhamnose-I → C-6 of galactose, H-4 of galactose → CO of acyl-I, H-4 of rhamnose-II → CO of acyl-II; SIMS *m/z* 1031 [M − H]$^-$, 885, 739, 285; HRSIMS *m/z* 1031.2804 (calcd for $C_{51}H_{51}O_{23}$, 1031.2823).

Quercetin 3-*O*-α-L-rhamnopyranosyl(1→6)-[(4-*O*-*trans*-*p*-coumaroyl)-α-L-rhamnopyranosyl(1→2)]-(3-*O*-*trans*-*p*-coumaroyl)-*β*-D-galactopyranoside (4): yellow powder; $[\alpha]^{29}_D$ −114° (*c* 1.0, MeOH); UV (MeOH) λ_{max} (log ϵ) 226sh (4.54), 258 (4.37), 270 (4.40), 300sh (4.62), 315 (4.68), 365sh (4.18) nm; IR (KBr) ν_{max} 3405, 1697, 1655, 1605, 1516 cm^{-1}; ^{1}H NMR, Table 1; ^{13}C NMR, Table 2; ROESY, H-6 of galactose (δ 3.46)/H-1 of rhamnose-I; HMBC, H-2′ → C-2, H-6′ → C-2, H-1 of galactose → C-3, H-2 of galactose → C-1 of rhamnose-II, H-1 of rhamnose-II → C-2 of galactose, H$_2$-6 of galactose → C-1 of rhamnose-I, H-1 of rhamnose-I → C-6 of galactose, H-4 of rhamnose-II → CO of acyl-II; SIMS *m/z* 1047 [M − H]$^-$, 901, 301, 163; HRSIMS *m/z* 1047.2778 (calcd for $C_{51}H_{51}O_{24}$, 1047.2772).

Quercetin 3-*O*-α-L-rhamnopyranosyl(1→6)-[(4-*O*-*trans*-caffeoyl)-α-L-rhamnopyranosyl(1→2)]-(3-*O*-*trans*-*p*-coumaroyl)-*β*-D-galactopyranoside (5): yellow powder; $[\alpha]^{28}_D$ −117° (*c* 0.5, MeOH); UV (MeOH) λ_{max} (log ϵ) 222sh (4.56), 234sh (4.46), 253 (4.39), 269 (4.34), 299sh (4.51), 321 (4.59) nm; IR (KBr) ν_{max} 3400, 1695, 1655, 1605, 1514 cm^{-1}; ^{1}H NMR, Table 1; ^{13}C NMR, Table 2; ROESY, H$_2$-2, 6 of acyl-I/H-α of acyl-I, H$_2$-2, 6 of acyl-I/H-*β* of acyl-I, H-2 of acyl-II/H-α of acyl-II, H-2 of acyl-II/H-*β* of acyl-II, H-6 of acyl-II/H-α of acyl-II, H-6 of acyl-II/H-*β* of acyl-II; HMBC, H-2′ → C-2, H-1 of galactose → C-3, H-2 of galactose → C-1 of rhamnose-II, H-1 of rhamnose-II → C-2 of galactose, H-1 of rhamnose-I → C-6 of galactose, H-α of acyl-I → CO of acyl-I, H-*β* of acyl-I → CO of acyl-I, H$_2$-2, 6 of acyl-I → C-*β* of acyl-I, H-*β* of acyl-I → C$_2$-2, 6, H-4 of rhamnose-II → CO of acyl-II, H-α of acyl-II → CO of acyl-II, H-*β* of acyl-II → CO of acyl-II, H-2 of acyl-II → C-*β* of acyl-II, H-*β* of acyl-II → C-2 of acyl-II, H-*β* of acyl-II → C-6 of acyl-II; SIMS *m/z* 1063 [M − H]$^-$, 301, 179; HRSIMS *m/z* 1063.2737 (calcd for $C_{51}H_{51}O_{25}$, 1063.2721).

Acid Hydrolysis of Compounds 1−5. Each compound (1 mg) was heated at 95 °C with dioxane (0.5 mL) and 5% H_2SO_4 (0.5 mL) for 1 h. After neutralization with Amberlite IRA-400 (OH^- form), the reaction mixture was concentrated and the residue was passed through a Sep-Pak C_{18} cartridge with H_2O. The eluate was concentrated, and the residue was treated with L-cysteine methyl ester hydrochloride (1 mg) in pyridine (0.125 mL) at 60 °C for 1 h. The solution was then treated with *N*,*O*-bis(trimethylsilyl)trifluoroacetamide (0.05 mL) at 60 °C for 1 h. The supernatant was applied to GLC; GLC conditions: column, Supelco SPB-1, 30 m × 0.25 mm; column temperature, 230 °C; N_2 flow rate, 0.8 mL/min; t_R of derivatives, D-galactose 13.7 min, L-galactose 14.6 min, L-rhamnose 9.0 min. D-Galactose and L-rhamnose were detected from **1**−**5**.

In Vitro Translations. In vitro translation assays were performed with a bicistronic mRNA reporter, (CAG)$_{33}$/FF/HCV/Ren, in which the first cistron encodes the firefly (FF) luciferase (luc) protein and the second cistron encodes the renilla (Ren) luc protein (Figure 2A). Expression of the second cistron is driven by HCV IRES sequences, and 33 (CAG) trinucleotide repeats are present within the 5′ UTR of the FF luc cistron. Construction and details of this reporter will be presented elsewhere, but the essential details are shown in Figure 2A. In vitro transcriptions were performed as previously described[11] using BamH I linearized templates. Translations were performed in Krebs extracts as previously reported.[12] Translations in *E. coli* S30 extracts were performed as recommended by the manufacturer (Promega Corp). Firefly and renilla luciferase activity (RLU) were measured on a Berthold Lumat LB 9507 luminometer as previously reported.[13]

Ribosome Binding Assays. Ribosome binding assays were performed as previously described.[11] Essentially, ^{32}P-labeled CAT mRNA was incubated in 25 μL of rabbit reticulocyte lysate at 20 °C for 10 min in the presence of 0.6 mM cycloheximide and 50 μM of **5**. The final KOAc concentration was adjusted to be 150 mM. Initiation complexes formed were analyzed by sedimentation through 10%−30% glycerol gradients. Centrifugation was for 3.5 h at 39 000 rpm at 4 °C in an SW40 rotor. Fractions of 500 μL were collected, and radioactivity was determined by scintillation counting.

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