^{1}H-NMR CHEMICAL SHIFT OF THE FLAVONOL 5-HYDROXY PROTON AS A CHARACTERIZATION OF 6- OR 8-ISOPRENOID SUBSTITUTION[1]

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Abstract —— ^{1}H Nmr examination of 6- or 8-isoprenoid substituted flavonols has shown that the location of isoprenoid side chain on A ring can be deduced from the chemical shift of the 5-hydroxy proton. The application of this ^{1}H nmr technique to identification of the isoprenoid substituted flavonols is discussed. The proposed structures for glepidotin A and two flavonols were revised with this method as well as chemical method.

In the EI-mass spectrometry, 6-prenylated or 8-prenylated flavonol, as well as flavone and isoflavone, gives characteristic fragment ions (6-prenyl group; *intense* $[M-43]^+$, $[M-55]^+$ *ions*, 8-*prenyl group; intense* $[M-15]^+$, $[M-55]^+$, $[M-68]^+$ ions).[2,3] However, the geranylated or diprenylated flavonoids could not gave the corresponding intense ions,[4~6] and also some 8-isoprenoid substituted flavonols show positive Gibbs test as well as coumarin derivatives,[7] so that the complete characterization of 6- or 8-isoprenoid substituted flavonol is necessary to support with other methods such as observing ^{13}C nmr spectra[10] or deriving to pyrano compound and then observing acetylation shifts of olefinic proton signals on pyran ring.[11]

In our previous ^{1}H nmr studies on the isoprenoid substituted isoflavone, flavone, and flavanone,[3,5,12] the signal of hydrogen-bonded hydroxy proton

(OH-5) of the 6-isoprenoid (e.g. prenyl, geranyl, γ-hydroxyisoamyl group) substituted flavonoid appears at more downfield (0.25-0.30 ppm) compared with that of the 6-nonsubstituted flavonoid having same B and C rings (in acetone-d_6). On the other hand, the OH-5 signal of the 8-isoprenoid substituted flavonoid is observed at more upfield (0.04-0.07 ppm, in acetone-d_6) compared with that of the flavonoid having same B and C rings and no side chain. Recently, the prenylation effects on isoflavone derivatives were also reported by Tahara *et al.*[13] In this paper, we report the *C*-prenylation effects for the OH-5 signal of flavonol derivatives and also describe the revisions of the structures proposed for glepidotin A (reported as **4c**) and other two flavonols (characterized as **7** and **9**). ^{1}H Nmr data of OH-5 signal of naturally occurring flavonols and synthetic flavonols listed in Tables 1 and 2 were measured in acetone-d_6. The prenylation at C-6 position of kaempferol (**1a**) and quercetin (**1b**) produced a downfield shift for the OH-5 signal (0.25 ppm). On the other hand, an upfield shift of the OH-5 signal was observed with prenylation at C-8 position of kaempferol (0.07 ppm) and quercetin (0.06 ppm). These prenylation effects for the OH-5 signals were the same effects on flavone, isoflavone, and flavanone derivatives. The *C*-prenylation induced shifts for the OH-5 signal regard to be a useful method for the structure identification of 6- or 8-isoprenoid substituted flavonols.

Glepidotin A was isolated from *Glycyrrhiza lepidota*, and the structure was proposed as 8-prenylgalangin (**4c**).[14] The OH-5 signal of glepidotin A (δ 12.30, in acetone-d_6) is more downfield compared with that of galangin (**1c**, δ 12.09). The downfield shift of the OH-5 signal of glepidotin A indicates that the structure of the natural compound is 6-prenylgalangin. To elucidate the structure of glepidotin A, 6-prenylgalangin (**3c**) and 8-prenylgalangin (**4c**) were synthesized. The prenylation of 3,7-di-*O*-methoxymethylgalangin (**1f**) with 1-bromo-3-methyl-2-butene gave 6-prenylated and 8-prenylated compounds (**3f** and **4f**) as well as 5-*O*-prenylated compound (**2f**). The structures of the *C*-prenylated flavonols were identified with the

Table 1. Chemical shifts of OH-5 signal at 23°C (in acetone-d_6, 400 MHz)

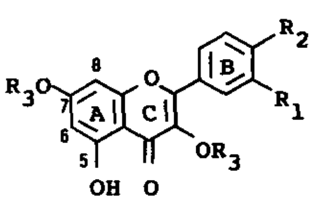
	chemical shift of OH-5	prenylation shift	isoprenoid group on A ring	(trivial name)
1a	12.18		none	(kaempferol)[a]
3a	12.43	−0.25	6-prenyl	(licoflavonol)
4a	12.11	+0.07	8-prenyl	(des-O-methylanhydro-icaritin)
7	12.08	+0.10	8-(γ-hydroxy-isoamyl	(noricaritin)
4h	12.09[b]		8-prenyl	(anhydroicaritin)[c]
1b	12.17		none	(quercetin)[a]
3b	12.42	−0.25	6-prenyl	(gancaonin P)
4b	12.11	+0.06	8-prenyl	
1c	12.09		none	(galangin)[d]
3c	12.35	−0.26	6-prenyl	
4c	12.03	+0.06	8-prenyl	
1g	12.25 (12.14)[e]		none	(kaempferol triacetate)
3g	12.63 (12.42)[e]	−0.38 (−0.28)	6-prenyl	(licoflavonol triacetate)
4g	12.16 (12.04)[e]	+0.09 (+0.10)	8-prenyl	(des-O-methylanhydro-icaritin triacetate)

a: Commercial reagent, supplied by Tokyo Kasei Kogyo Co., LTD. b: When measured at 35°C and 50°C, the signal was observed at δ 12.05 and 12.00, respectively. c: Obtained from *Epimedium grandiflorum* (ref. 8).
d: Commercial reagent, supplied by Aldrich Chemical Company, Inc.
e: Measured in $CDCl_3$ at 23°C.

Table 2. Chemical shifts of OH-5 signal of pyranoflavonol (**9** and **10**) and pyranoflavanone (**11** and **13**) at 23°C (in acetone-d_6, 400 MHz)

	Chemical shift of OH-5	induced shift	A,D-ring pattern	(trivial name)
9	11.91	+0.27[a]	angular	(des-O-methyl-β-anhydro-icaritin)
10	12.48	−0.30[a]	linear	
11	11.88	+0.30[b]	angular	
13	12.55	−0.37[b]	linear	

a: Difference of chemical shifts of OH-5 signal between **1a** and **9** (**10**).
b: Difference of chemical shifts of OH-5 signal between **12** and **11** (**13**).



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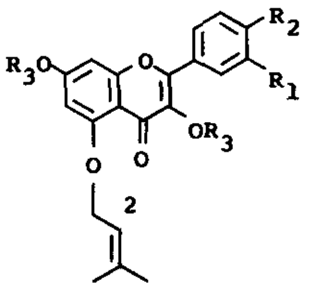

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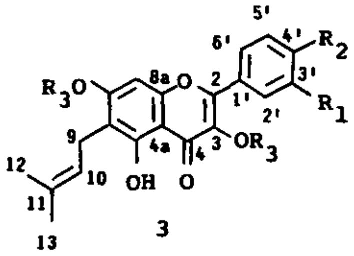

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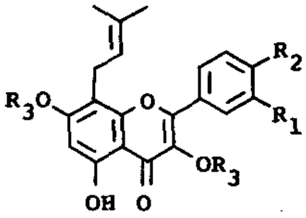
a: $R_1=R_3=H$, $R_2=OH$
b: $R_1=R_2=OH$, $R_3=H$
c: $R_1=R_2=R_3=H$
d: $R_1=H$, $R_2=OCH_2OCH_3$,
$R_3=CH_2OCH_3$
e: $R_1=R_2=OCH_2OCH_3$,
$R_3=CH_2OCH_3$
f: $R_1=R_2=H$,
$R_3=CH_2OCH_3$
g: $R_1=H$, $R_2=OCOCH_3$,
$R_3=COCH_3$



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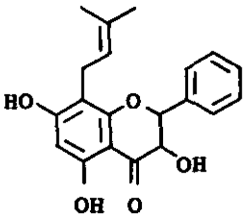

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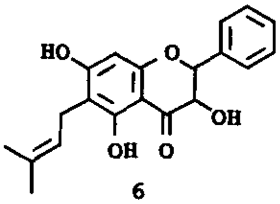
h: $R_1=R_3=H$, $R_3=OCH_3$



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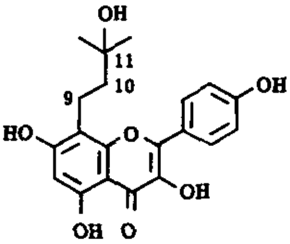

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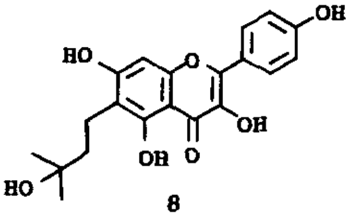

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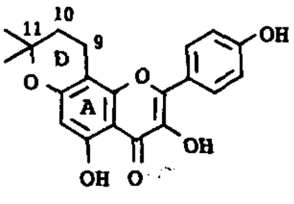

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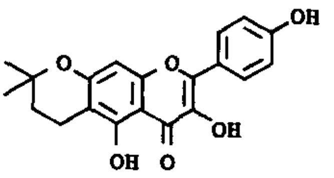

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chemical shifts and coupling patterns of C-6, C-8, and C-8a carbon signals as shown in Table 3. The prenylated flavonols (**3f** and **4f**) were treated with ion-exchange resin (Dowex 50 [H-form]) to give 6-prenylgalangin (**3c**) and 8-prenylgalangin (**4c**), respectively. Glepidotin A was identified as 6-prenylgalangin (**3c**) by direct comparison between the natural compound and the synthetic flavonols (**3c** and **4c**). In the earlier paper,[14] the structure of glepidotin A was elucidated with comparison between the spectral and physical data of glepidotin A triacetate and 6-prenylgalangin triacetate synthesized by Jain and Zutshi.[15] Therefore, the structure of the earlier synthesized *C*-prenylgalangin must be 8-prenylgalangin (**4a**). On the other hand, the structure of glepidotin B was proposed as 5,7-dihydroxy-8-prenyldihydroflavonol (**5**).[14] The compound was derived to glepidotin A by oxidation reaction. Thus, the structure of glepidotin B must be revised to 5,7-dihydroxy-6-prenyldihydroflavonol (**6**).

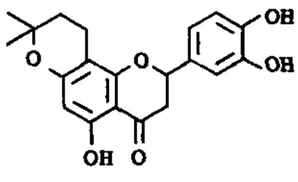
Souza *et al*. reported the isolation of 8-(γ-hydroxyisoamyl)-4',5,7-trihydroxyflavonol (**7**, noricaritin) from *Bursera leptophloeos*.[16] The chemical shift of OH-5 signal of the natural compound (δ 12.40, in acetone-d_6) is more downfield than that of kaempferol (**1a**, δ 12.18). The OH-5 signal of synthetic noricaritin (**7**) was observed at δ 12.08. Although, the effort to synthesize 6-(γ-hydroxyisoamyl)kaempferol was failure, the chemical shift of OH-5 signal of the natural compound indicates unambiguously that the structure of the compound is 6-(γ-hydroxyisoamyl)kaempferol (**8**). The isolation of dihydropyranoflavonol, des-*O*-methyl-β-anhydroicaritin (**9**), from the same plant was also reported by Souza *et al*.[16] The OH-5 signal of the flavonol (δ 12.46, in acetone-d_6) is more downfield than that of kaempferol (**1a**, δ 12.18). In the case of angular type dihydropyranoflavanone (**11**),[12] the chemical shift of OH-5 signal is more upfield (δ 11.88) than that of eriodictyol (**12**, δ 12.18). On the other hand, the OH-5 signal of linear type dihydropyranoflavanone (**13**)[12] was observed at more downfield (δ 12.55) compared with that of eriodictyol (**12**). Thus, the downfield shift of OH-5 signal of the natural dihydropyranoflavonol indicates that the structure of

Table 3. ^{13}C Nmr data of **3d**, **e**, **f**, and **4d**, **e**, **f** (in $CDCl_3$, 100 MHz)

C	**3d**	**3e**	**3f**	**4d**	**4e**	**4f**
2	156.59	156.23	156.96	156.75	156.26	157.13
3	135.56	135.72	136.01	135.30	135.49	135.74
4	178.65	178.66	179.03	178.97	179.01	179.05
4a	106.47	106.47	106.55	106.36	106.34	106.41
5	158.52	158.50	158.45	160.14	160.14	160.10
6	113.30^{a}	113.33^{a}	113.33^{a}	97.60^{e}	97.83^{g}	97.61^{h}
7	160.61	160.61	160.68	160.31	160.35	160.39
8	92.24^{b}	92.24^{d}	92.29^{b}	108.60^{a}	108.68^{a}	108.63^{a}
8a	154.97^{c}	154.94^{c}	155.07^{c}	153.74^{f}	153.67^{f}	153.82^{f}
9	21.63	21.65	21.62	21.90	21.89	21.85
10	122.09	122.06	121.96	122.37	122.26	122.24
11	131.80	131.84	131.93	131.90	131.93	131.94
12	17.81	17.82	17.82	17.97	17.94	17.94
13	25.78	25.79	25.80	25.73	25.17	25.76
1'	124.19	124.70	130.73	124.42	124.96	130.95
2'	130.63	117.96	128.38	130.67	118.15	128.42
3'	115.95	146.67	128.99	116.01	146.84	129.05
4'	159.22	149.67	130.73	159.28	149.76	130.77
5'	115.95	115.81	128.99	116.01	115.92	129.05
6'	130.63	123.94	128.38	130.67	124.00	128.42
$-OCH_2O-$	94.15 94.29	94.13 95.19 95.75	94.10	94.33 94.40	94.40 95.26 95.85	94.31
$3-OCH_2O-$	97.85	97.89	97.79	97.86	97.92	97.79
$-OCH_3$	56.21 56.25	56.29 56.37 56.40	56.28	56.26 56.32	56.28(2C) 56.43	56.35
$3-OCH_3$	57.68	57.71	57.51	57.72	57.77	57.55

The coupling pattern and coupling constants of C-6, C-8, and C-8a signals: a; multiplet of singlet or broad singlet, b; d, J =165 Hz, c; d, J = 5 Hz, d; d, J = 166 Hz, e; dd, J = 8 and 164 Hz, f; t, J = 4 Hz, g; dd; J = 7 and 163 Hz, h; dd, J = 8 and 161 Hz.

Long-range selective proton decoupling (irradiation at OH-5 signal): **3d**: C-4a; t(J=5 Hz) ⟶ d(J=5 Hz), C-5; dt(J=4 and 5 Hz) ⟶ t(J=5 Hz), C-6; multiplet ⟶ changed, **4d**, C-4a; t(J=5 Hz) ⟶ d(J=5 Hz), C-5; t(J=4 Hz) ⟶ d(J=4 Hz), C-6; dd ⟶ d(J=164 Hz).



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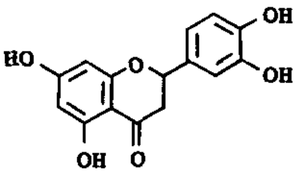

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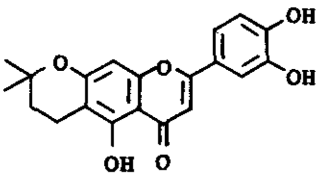

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the compound is linear type dihydropyranoflavonol (**10**). To elucidate the structure of the natural flavonol, compounds (**9** and **10**) were synthesized by cyclization of 6-prenylkaempferol (**3a**) and 8-prenylkaempferol (**4a**) with acidic condition. The ^{1}H nmr data of the natural dihydropyranoflavonol were agreement with those of the synthetic linear type flavonol (**10**) but not agreement with those of the angular type flavonol (**9**).

In our knowledge, these two flavonols (**8** and **10**) isolated from Bursera leptophloeos are first examples as natural products.

Souza et al. also reported the isolation of des-O-methylanhydroicaritin as triacetate [δ 12.42 (5-OH, in $CDCl_3$), mp 166-168 °C]. The ^{1}H nmr data and melting point of the triacetate are agreement with those of licoflavonol triacetate (**3g**) but not agreement with those of des-O-methylanhydroicaritin triacetate (**4g**), respectively.[17]

EXPERIMENTAL

The general procedures and the instruments used are described in our previous paper.[12] For preparative tlc (silica gel), Wakogel B-5F was used.

3,4',7-Trimethoxymethylkaempferol (1d)

A mixture of kaempferol (**1a**, 300 mg, 1.0 mmol), chloromethyl methyl ether (0.4 ml, 5.3 mmol), and dry K_2CO_3 (1 g, 7.2 mmol) in acetone (20 ml) was allowed to stand at room temperature for 8 h. The reaction mixture was treated as usual, and the product was purified by preparative tlc (n-hexane-EtOAc=2:1) to give **1d** (105 mg, 24%). The compound showed the following data: mp 74-75 °C (pale yellow prisms, crystallized from methanol). $FeCl_3$ test: brown. EI-Ms, m/z 418 $[M]^+$. Anal. Calcd for $C_{21}H_{22}O_9$; C, 60.29; H, 5.30. Found: C, 60.26; H, 5.37. ^{1}H Nmr ($CDCl_3$, 90 MHz): δ 3.22 (3H, s, $-OCH_3$), 3.50 (6H, s, $-OCH_3 x2$), 5.17, 5.21, 5.24 (each 2H, s, $-OCH_2O-$), 6.42 (1H, d, J = 2 Hz, C-6-H), 6.59 (1H, d, J = 2 Hz, C-8-H), 7.11 (2H, d, J = 9 Hz, C-3'-H and C-5'-H), 8.00 (2H, d, J = 9 Hz, C-2'-H and C-6'-H), 12.40 (1H, s, 5-OH).

Prenylation of 1d (Formation of 2d, 3d, and 4d)

A mixture of **1d** (100 mg, 0.2 mmol), 1-bromo-3-methyl-2-butene (0.1 ml, 0.9 mmol), and dry K_2CO_3 (2 g, 14.5 mmol) in methanol (30 ml) was allowed to stand at room temperature for 10 h. The reaction mixture was treated as usual, and the product was purified by preparative tlc (n-hexane-EtOAc=2:1, $CHCl_3$ only) to give **2d** (28 mg, 24%), **3d** (13 mg, 11%), **4d** (16 mg, 14%), and the starting material (**1d**, 47 mg, 47%). Compound (**2d**) showed the following data: mp 119-120 °C (colorless prisms, from methanol). $FeCl_3$ test: negative. EI-Ms, m/z 486 $[M]^+$. Anal. Calcd for $C_{26}H_{30}O_9$: C, 64.19; H, 6.22. Found: C, 63.82; H, 6.21. ^1H Nmr ($CDCl_3$, 90 MHz): δ 1.78 (6H, br s, C-11-CH_3x2), 3.21 (3H, s, -OCH_3), 3.56 (6H, s, -OCH_3x2), 4.68 (2H, br d, J = 6 Hz, C-9-Hx2), 5.20 (2H, s, -OCH_2O-), 5.23 (4H, s, -OCH_2O-x2), 5.40-5.70 (1H, br, C-10-H), 6.41 (1H, d, J = 2.5 Hz, C-6-H), 6.68 (1H, d, J = 2.5 Hz, C-8-H), 7.10 (2H, d, J = 9 Hz, C-3'-H and C-5'-H), 8.00 (2H, d, J = 9 Hz, C-2'-H and C-6'-H). Compound (**3d**) showed the following data: mp 78-80 °C (pale yellow prisms, from methanol). $FeCl_3$ test: green. HR-Ms, m/z 486.1876 $[M]^+$ ($C_{26}H_{30}O_9$ requires: 486.1890). ^1H Nmr ($CDCl_3$, 400 MHz): δ 1.68, 1.80 (each 3H, br s, C-11-CH_3), 3.21, 3.49, 3.50 (each 3H, s, -OCH_3), 3.39 (2H, br d, J = 6 Hz, C-9-Hx2), 5.17, 5.24, 5.28 (each 2H, s, -OCH_2O-), 5.23 (1H, br t, J = 6 Hz, C-10-H), 6.68 (1H, s, C-8-H), 7.15 (2H, d, J = 9 Hz, C-3'-H and C-5'-H), 8.02 (2H, d, J = 9 Hz, C-2'-H and C-6'-H), 12.70 (1H, s, 5-OH). Compound (**4d**) showed the following data: mp 105-108 °C (yellow needles, from methanol). $FeCl_3$ test: green. HR-Ms, m/z 486.1902 $[M]^+$ ($C_{26}H_{30}O_9$ requires: 486.1890). ^1H Nmr ($CDCl_3$, 400 MHz): δ 1.69, 1.78 (each 3H, br s, C-11-CH_3), 3.22, 3.48, 3.51 (each 3H, s, -OCH_3), 3.51 (2H, br d, J = 7 Hz, C-9-Hx2), 5.12, 5.26, 5.27 (each 2H, s, -OCH_2O-), 6.59 (1H, s, C-6-H), 7.16 (2H, d, J = 9 Hz, C-3'-H and C-5'-H), 8.04 (2H, d, J = 9 Hz, C-2'-H and C-6'-H), 12.55 (1H, s, 5-OH).

6-Prenylkaempferol (Licoflavonol, 3a)

A mixture of **3d** (13 mg) and Dowex 50 [H-form] (70 mg) in methanol (3 ml) was allowed to stand at 40 °C for 24 h. The reaction mixture was treated as usual, and the product was purified by preparative tlc (n-hexane-acetone=2:1) to give **3a** (3 mg, 32%). The compound showed the following data: mp 185-190 °C (yellow prisms, from methanol, lit.[18] mp 185-187 °C) $FeCl_3$ test: dark green. EI-Ms, m/z 354 $[M]^+$.

8-Prenylkaempferol (Des-O-methylanhydroicaritin, 4a)

A mixture of **4d** (30 mg) and Dowex 50 [H] (100 mg) in methanol (7 ml) was allowed to stand at 40 °C for 24 h. The reaction mixture was treated as usual, and the product was purified by preparative tlc (n-hexane-EtOAc=2:1) to give **4a** (18 mg, 82%). The compound showed the following data: mp 223-225 °C (yellow prisms, from acetone-$CHCl_3$, lit.[19] mp 226 °C). $FeCl_3$ test: dark green. EI-Ms, m/z 354 $[M]^+$.

Preparation of 7 (Noricaritin) and 9 (Des-O-methyl-ß-anhydroicaritin, Nor-ß-anhydroicaritin)

A mixture of **4a** (9 mg) and 3N HCl (0.6 ml) in dioxane (2 ml) was allowed to stand at 80 °C for 5 h. The reaction mixture was treated as usual, and the product was purified by preparative tlc (n-hexane-EtOAc=1:1) to give **7** (noricaritin,[20] 0.2 mg, 2%) and **9** (2 mg, 22%). Compound (**7**) showed the following data: amorphous powder. $FeCl_3$ test: green. EI-Ms, m/z 372 $[M]^+$. ^1H Nmr (acetone-d_6, 400 MHz): δ 1.31 (6H, s, C-11-CH_3x2), 1.78 (2H, m, C-10-Hx2), 3.00 (2H, m, C-9-Hx2), 6.34 (1H, s, C-6-H), 7.02 (2H, d, J = 9 Hz, C-3'-H and C-5'-H), 8.29 (2H, d, J = 9 Hz, C-2'-H and C-6'-H), 12.08 (1H, s, 5-OH). Compound (**9**) showed the following data: mp 309-311°C (decomp., yellow needles, from acetone, lit.[21] mp 308-309°C). $FeCl_3$ test: green. EI-Ms, m/z 354 $[M]^+$. ^1H Nmr (acetone-d_6, 400 MHz): δ 1.39 (6H, s, C-11-CH_3x2), 1.95 (2H, t, J = 7 Hz, C-10-Hx2), 2.95 (2H, t, J = 7 Hz, C-9-Hx2), 6.14 (1H, s, C-6-H), 7.05 (2H, d, J = 9 Hz, C-3'-H and C-5'-H), 8.21 (2H, d, J = 9 Hz, C-2'-H and C-6'-H), 8.08, 9.08 (each 1H, br s, OH), 11.91 (1H, s, 5-OH).

Cyclization of **3a** (Formation of **10**)

A mixture of **3a** (2 mg) and 3N HCl (0.3 ml) in dioxane (1 ml) was allowed to stand at 80°C for 5 h. The reaction mixture was treated as usual and the product was purified by preparative tlc (n-hexane-EtOAc=1:1) to give **10** (0.4 mg, 20%). The compound showed the following data: mp 220-225°C (decomp., yellow prisms, from acetone). $FeCl_3$ test: green. EI-Ms, m/z 354 $[M]^+$. ^1H Nmr (acetone-d_6, 400 MHz): δ 1.38 (6H, s, C-11-CH_3x2), 1.89 (2H, t, J = 7 Hz, C-10-Hx2), 2.71 (2H, t, J = 7 Hz, C-9-Hx2), 6.46 (1H, s, C-8-H), 7.02 (2H, d, J = 9 Hz, C-3'-H and C-5'-H), 8.17 (2H, d, J = 9 Hz, C-2'-H and C-6'-H), 7.99, 9.05 (each 1H, br s, OH), 12.48 (1H, s, 5-OH).

3,3',4',7-Tetramethoxymethylquercetin (**1e**)

A mixture of quercetin (**1b**, 2 g, 6.6 mmol), chloromethyl methyl ether (2 ml, 26.5 mmol), and dry K_2CO_3 (25 g, 180.9 mmol) in acetone (150 ml) was allowed to stand at room temperature for 2 h. The reaction mixture was treated as usual, and the product was purified by silica-gel column chromatography (benzene-acetone=200:1) to give **1e** (1.2 g, 38%). The compound showed the following data: mp 72-73°C (pale yellow prisms, from methanol). $FeCl_3$ test: brown. HR-Ms, m/z 478.1548 $[M]^+$ ($C_{23}H_{26}O_{11}$ requires: 478.1476). ^1H Nmr ($CDCl_3$, 90 MHz): δ 3.25, 3.48, 3.52, 3.54 (each 3H, s, $-OCH_3$), 5.18, 5.22, 5.27, 5.29 (each 2H, s, $-OCH_2O-$), 6.41 (1H, d, J = 2 Hz, C-6-H), 6.58 (1H, d, J = 2 Hz, C-8-H), 7.23 (1H, d, J = 8.5 Hz, C-5'-H), 7.68 (1H, dd, J = 2 and 8.5 Hz, C-6'-H), 7.89 (1H, d, J = 2 Hz, C-2'-H), 12.39 (1H, s, 5-OH).

Prenylation of **1e** (Formation of **2e**, **3e**, and **4e**)

A mixture of **1e** (1.1 g, 2.3 mmol), 1-bromo-3-methyl-2-butene (1.2 ml, 10.8 mmol), and dry K_2CO_3 (4 g, 28.9 mmol) in methanol (400 ml) was allowed to stand at room temperature for 3 h. The reaction mixture was purified by preparative tlc (n-hexane-EtOAc=2:1, $CHCl_3$-benzene=3:1, $CHCl_3$ only) to give **2e** (72 mg, 6%), **3e** (25 mg, 2%), **4e** (57 mg, 5%), and the starting material (**1e**, 417 mg, 38%). Compound (**2e**) showed the following data: mp 81-82 °C (colorless needles, from methanol). $FeCl_3$ test: negative. HR-Ms, m/z 546.2119 [M]$^+$ ($C_{28}H_{34}O_{11}$ requires: 546.2102). ^{1}H Nmr ($CDCl_3$, 90 MHz): δ 1.77 (6H, br s, C-11-CH_3x2), 3.23, 3.49, 3.51, 3.53 (each 3H, s, -OCH_3), 4.69 (2H, br d, J = 7 Hz, C-9-Hx2), 5.21, 5.27 (each 4H, s, -OCH_2O-x2), 5.56 (1H, br t, J = 7 Hz, C-10-H), 6.41 (1H, d, J = 2 Hz, C-6-H), 6.65 (1H, d, J = 2 Hz, C-8-H), 7.22 (1H, d, J = 9 Hz, C-5'-H), 7.69 (1H, dd, J = 2 and 9 Hz, C-6'-H), 7.89 (1H, d, J = 2 Hz, C-2'-H). Compound (**3e**) showed the following data: mp 99-100 °C (pale yellow needles, from methanol). $FeCl_3$ test: green. EI-Ms, m/z: 546 [M]$^+$. Anal. Calcd for $C_{28}H_{34}O_{11}$: C, 61.53; H, 6.27. Found: C, 61.38; H, 6.31. ^{1}H Nmr ($CDCl_3$, 400 MHz): δ 1.68, 1.81 (each 3H, br s, C-11-CH_3), 3.24, 3.49, 3.54, 3.55 (each 3H, s, -OCH_3), 3.39 (2H, br d, J = 7 Hz, C-9-Hx2), 5.17, 5.29, 5.30, 5.32 (each 2H, s, -OCH_2O-), 5.23 (1H, br t, J = 7 Hz, C-10-H), 6.68 (1H, s, C-8-H), 7.27 (1H, d, J = 8.5 Hz, C-5'-H), 7.70 (1H, dd, J = 2 and 8.5 Hz, C-6'-H), 7.91 (1H, d, J = 2 Hz, C-2'-H), 12.69 (1H, s, 5-OH). Compound (**4e**) showed the following data: mp 77-78 °C (pale yellow needles, from methanol). $FeCl_3$ test: green. HR-Ms, m/z 546.2106 [M]$^+$ ($C_{28}H_{34}O_{11}$ requires: 546.2102). ^{1}H Nmr ($CDCl_3$, 90 MHz): δ 1.63, 1.77 (each 3H, br s, C-11-CH_3), 3.27, 3.40 (each 3H, s, -OCH_3), 3.54 (6H, s, -OCH_3x2), 5.18, 5.24, 5.27, 5.30 (each 2H, s, -OCH_2O-), 6.57 (1H, s, C-6-H), 7.22 (1H, d, J = 9 Hz, C-5'-H), 7.72 (1H, dd, J = 2 and 9 Hz, C-6'-H), 7.96 (1H, d, J = 2 Hz, C-2'-H), 12.39 (1H, s, 5-OH).

6-Prenylquercetin (Gancaonin P, 3b)

A mixture of **3e** (10 mg) and Dowex 50 [H] (20 mg) in methanol (4 ml) was allowed to stand at 55 °C for 10 h. The reaction mixture was treated as usual, and the product was purified by preparative tlc (benzene-$CHCl_3$-methanol=6:2:1) to give **3b** (2 mg, 30%). The synthetic compound was identified as gancaonin P[3] by direct comparison.

8-Prenylquercetin (4b)

A mixture of **4e** (33 mg) and Dowex 50 [H] (100 mg) in methanol (7 ml) was allowed to stand at 55 °C for 10 h. The reaction mixture was treated as usual, and the product was purified by preparative tlc (benzene-$CHCl_3$-methanol=6:2:1, benzene-EtOAc=4:3) to give **4b** (7 mg, 30%). The compound showed the following data: mp 221-224 °C (yellow needles, from acetone). $FeCl_3$ test: green. HR-Ms, m/z 370.1049 [M]$^+$ ($C_{20}H_{18}O_7$ requires: 370.1052). ^{1}H Nmr (acetone-d_6, 400 MHz), δ 1.66, 1.82 (each 3H, br s, C-11-CH_3), 3.57 (2H, br d, J = 7 Hz, C-9-Hx2), 5.29 (1H, br t, J = 7 Hz, C-10-H), 6.36 (1H, s, C-6-H), 7.02 (1H, d, J = 8.5 Hz, C-5'-H), 7.73 (1H, dd, J = 2 and 8.5 Hz, C-6'-H), 7.88 (1H, d, J = 2 Hz, C-2'-H), 12.11 (1H, s, 5-OH).

3,7-Dimethoxymethylgalangin (1f)

A mixture of galangin (**1c**, 94 mg, 0.4 mmol), chloromethyl methyl ether (0.1 ml, 1.3 mmol), and dry K_2CO_3 (1 g, 7.2 mmol) in acetone (10 ml) was allowed to stand at room temperature for 2 h. The reaction mixture was treated as usual, and the product was purified by preparative tlc (n-hexane-EtOAc=4:1) to give **1f** (36 mg, 29%). The compound showed the following data: mp 109°C (pale yellow prisms, from methanol). $FeCl_3$ test: brown. HR-Ms, m/z 358.1048 $[M]^+$ ($C_{19}H_{18}O_7$ requires: 358.1053). ^1H Nmr ($CDCl_3$, 90 MHz): δ 3.12, 3.48 (each 3H, s, $-OCH_3$), 5.16, 5.21 (each 2H, s, $-OCH_2O-$), 6.43 (1H, d, J = 2 Hz, C-6-H), 6.60 (1H, d, J = 2 Hz, C-8-H), 7.4-7.6 (3H, m, C-3'-H, C-4'-H, and C-5'-H), 7.9-8.1 (2H, m, C-2'-H and C-6'-H), 12.36 (1H, s, 5-OH).

Prenylation of 1f (Formation of 2f, 3f, and 4f)

A mixture of **1f** (50 mg, 0.1 mmol), 1-bromo-3-methyl-2-butene (0.1 ml, 0.9 mmol), and dry K_2CO_3 (1 g, 7.2 mmol) in methanol (15 ml) was allowed to stand at room temperature for 8 h. The reaction mixture was treated as usual, and the product was purified by preparative tlc (n-hexane-EtOAc=5:1, benzene only) to give **2f** (5 mg, 8%), **3f** (2 mg, 3%), **4f** (4 mg, 7%), and the starting material (**1f**, 28 mg, 56%). Compound (**2f**) showed the following data: colorless oil. $FeCl_3$ test: negative. HR-Ms, m/z 426.1687 $[M]^+$. ($C_{24}H_{26}O_7$ requires: 426.1679). ^1H Nmr ($CDCl_3$, 400 MHz): δ 1.77, 1.79 (each 3H, br s, C-11-CH_3), 3.10, 3.51 (each 3H, s, $-OCH_3$), 4.76 (2H, br d, J = 6.5 Hz, C-9-Hx2), 5.19, 5.24 (each 2H, s, $-OCH_2O-$), 5.58 (1H, br t, J = 6.5 Hz, C-10-H), 6.44 (1H, d, J = 2 Hz, C-6-H), 6.71 (1H, d, J = 2 Hz, C-8-H), 7.44-7.52 (3H, m, C-3'-H, C-4'-H, and C-5'-H), 8.01-8.05 (2H, m, C-2'-H and C-6'-H). Compound (**3f**) showed the following data: mp 95-97°C (pale yellow needles, from methanol). $FeCl_3$ test: dark green. HR-Ms, m/z 426.1671 $[M]^+$ ($C_{24}H_{26}O_7$ requires: 426.1679). ^1H Nmr ($CDCl_3$, 400 MHz): δ 1.68, 1.81 (each 3H, br s, C-11-CH_3), 3.10, 3.49 (each 3H, s, $-OCH_3$), 3.40 (2H, br d, J = 7 Hz, C-9-Hx2), 5.15, 5.28 (each 2H, s, $-OCH_2O-$), 5.23 (1H, br t, J = 7 Hz, C-10-H), 6.70 (1H, s, C-8-H), 7.47-7.53 (3H, m, C-3'-H, C-4'-H, and C-5'-H), 7.98-8.03 (2H, m, C-2'-H and C-6'-H), 12.65 (1H, s, 5-OH). Compound (**4f**) showed the following data: mp 71-74°C (pale yellow needles, from methanol). $FeCl_3$ test: dark green. HR-Ms, m/z 426.1678 $[M]^+$ ($C_{24}H_{26}O_7$ requires: 426.1679). ^1H Nmr ($CDCl_3$, 400 MHz): δ 1.68, 1.75 (each 3H, br s, C-11-CH_3), 3.12, 3.49 (each 3H, s, $-OCH_3$), 3.50 (2H, br d, J = 7 Hz, C-9-Hx2), 5.16, 5.27 (each 2H, s, $-OCH_2O-$), 5.18 (1H, br t, J = 7 Hz, C-10-H), 7.49-7.54 (3H, m, C-3'-H, C-4'-H, and C-5'-H), 8.01-8.06 (2H, m, C-2'-H and C-6'-H), 12.50 (1H, s, 5-OH).

6-Prenylgalangin (3c)

A mixture of **3f** (2 mg) and Dowex 50 [H] (50 mg) in methanol (5 ml) was allowed to stand at 40°C for 24 h. The reaction mixture was treated as usual, and the product was purified by preparative tlc (n-hexane-acetone=3:1) to give **3c** (1 mg, 60%). The compound showed the following data: mp 204-206°C (yellow prisms, from benzene-acetone, glepidotin A: lit.[14] mp

200-201°C). $FeCl_3$ test: dark green. HR-Ms, m/z 338.1167 $[M]^+$ ($C_{20}H_{18}O_5$ requires: 338.1154). ^1H Nmr (acetone-d_6, 400 MHz): δ 1.65, 1.79 (each 3H, br s, C-11-CH_3), 3.37 (2H, br d, J = 7 Hz, C-9-Hx2), 5.29 (1H, br t, J = 7 Hz, C-10-H), 6.65 (1H, s, C-8-H), 7.51 (1H, br t, J = 7 Hz, C-4'-H), 7.57 (2H, br t, J = 7 Hz, C-3'-H and C-5'-H), 8.24 (2H, br d, J = 7 Hz, C-2'-H and C-6'-H), 10.05 (1H, br s, OH), 12.35 (1H, s, 5-OH).

8-Prenylgalangin (**4c**)

A mixture of **4f** (11 mg) and Dowex 50 [H] (50 mg) in methanol (5 ml) was allowed to stand at 40°C for 24 h. The reaction mixture was treated as usual, and the product was purified by preparative tlc (n-hexane-acetone=4:1) to give **4c** (5 mg, 57%). The compound showed the following data: mp 227-229°C (pale yellow prisms, from benzene-acetone). $FeCl_3$ test: dark green. HR-Ms, m/z 338.1160 $[M]^+$ ($C_{20}H_{18}O_5$ requires: 338.1154). ^1H Nmr (acetone-d_6, 400 MHz): δ 1.67, 1.82 (each 3H, br s, C-11-CH_3), 3.58 (2H, br d, J = 7 Hz, C-9-Hx2), 5.29 (1H, br t, J = 7 Hz, C-10-H), 6.39 (1H, s, C-6-H), 7.52 (1H, br t, J = 7 Hz, C-4'-H), 7.59 (2H, br t, J = 7 Hz, C-3'-H and C-5'-H), 8.27-8.31 (2H, m, C-2'-H and C-6'-H), 9.77 (1H, br s, OH), 12.03 (1H, s, 5-OH).

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