Dammarane Saponins of *Gynostemma pentaphyllum* MAKINO and Isolation of Malonylginsenosides-Rb_1, -Rd, and Malonylgypenoside V

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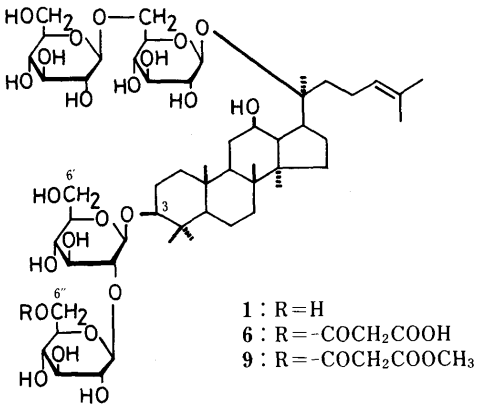
6″-Malonylginsenosides-Rb_1 (6), -Rd (7) and 6″-malonylgypenoside V (8) were isolated from the fresh leaves of *Gynostemma pentaphyllum* selected from wild plants. It was found that the saponin content of dry leaves was markedly reduced because of decomposition of saponins, by intracellular glycosidases.

Keywords *Gynostemma pentaphyllum*; 6″-malonylginsenoside-Rb_1; 6″-malonylginsenoside-Rd; 6″-malonylgypenoside V; ginsenoside-Rb_1; ginsenoside-Rd; gypenoside V; intracellular glycosidase; rhamnosidase

Gynostemma pentaphyllum MAKINO (amachazuru) (Cucurbitaceae) is a perennial liana growing wild throughout Japan. Takemoto *et al.*[1] first isolated some ginseng saponins, ginsenosides (G.) -Rb_1, -Rb_3, -Rd and -F_2, along with many other saponins, named gypenosides (Gy.), from the aerial parts of this plant collected in Kanazawa city in 1977, though Nagai *et al.*[2] had reported panaxadiol, one of the artificial sapogenins of ginseng saponins, before Takemoto. Since ginsenosides are the well-known biologically active principles in Korean ginseng, *G. pentaphyllum* has received much attention. Takemoto and his co-workers, however, did not particularly refer to ginseng saponins in their successive reports, and their extensive work so far has not focussed on evaluating this plant as a new resource of ginseng saponins but rather on investigating new compounds in materials collected in various districts in Japan.[3]

This paper deals with the screening of the wild plants of *G. pentaphyllum* containing ginseng saponins and the isolation of a new compound, 6″-malonylgypenoside V, 6″-malonylginsenosides-Rb_1 and -Rd from the leaves of cultivated plants.

Selection of the Wild Plants As Takemoto *et al.*[3] had demonstrated that the components of wild *G. pentaphyllum* vary markedly depending on the place where it grows, the authors first collected 813 plants from various districts for screening. The fresh or dry leaves were extracted with 80% (v/v) methanol and the extracts were subjected to thin layer chromatography (TLC) with G.-Rb_1 (**1**) as the index. As shown in Table I, 301 samples (37%) were chosen through the first screening with TLC (system 1), and the plants that



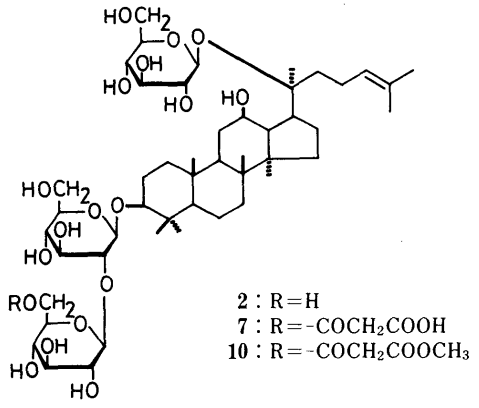
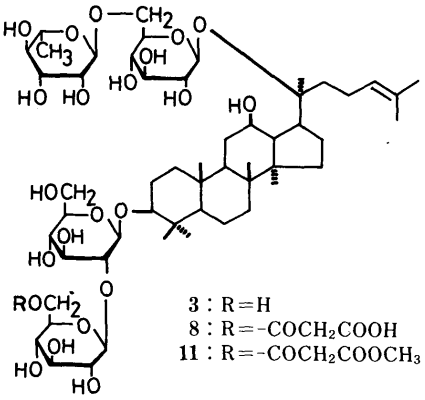
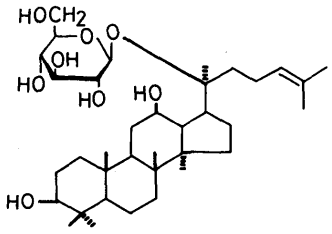


Chart 1

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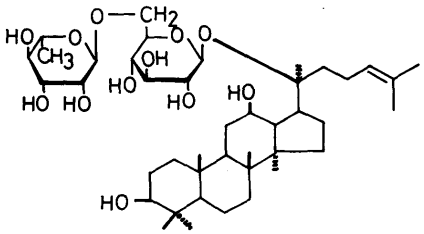
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Chart 2

TABLE I. Distribution of *G. pentaphyllum* Containing G.-Rb_1 (**1**)

Prefecture	Sample	1st (%)[a]	2nd (%)[b]
Ishikawa (Kanazawa)	100	50 (50.0)	43 (43.0)
Ishikawa (Kashima, Anamizu)	109	3 (2.8)	0
Fukui	44	20 (45.5)	3 (6.8)
Toyama	324	132 (40.1)	5 (1.5)
Shiga	24	2 (8.3)	1 (4.2)
Kyoto	18	1 (5.6)	0
Mie	36	3 (8.3)	1 (2.8)
Hyogo	14	1 (7.1)	1 (7.1)
Tokushima	1	0	—
Tottori	96	80 (83.3)	0
Okayama	16	0	—
Hiroshima	11	0	—
Yamaguchi	6	0	—
Aomori	1	0	—
Yamagata	4	4 (100)	2 (50.0)
Fukushima	5	5 (100)	1 (20.0)
Ibaraki	3	0	—
Chiba	1	0	—

a) 1st screening was carried out with TLC (system 1). *b*) 2nd screening was carried out with TLC (system 2) and HPLC.

TABLE II. Distribution of G.-Rb_1 (**1**), G.-Rd (**2**) and Gy. V (**3**) in Seedlings Grown in an Experimental Field (303 Samples)

Content (%)	1	2	3
—0.49	160 (52.8)	243 (80.2)	12 (4.0)
0.50—0.99	79 (26.1)	50 (16.5)	40 (13.2)
1.00—1.49	36 (11.9)	7 (2.3)	38 (12.5)
1.50—1.99	21 (6.9)	0	44 (14.5)
2.00—2.49	6 (2.0)	2 (0.7)	39 (12.0)
2.50—2.99	1 (0.3)	0	35 (11.5)
3.00—	0	1 (0.3)	95 (31.4)
Mean	0.615	0.313	2.455

Contents represent the values in terms of dry weight, and figures in parenthesis represent the incidence (%).

TABLE III. Saponin Contents of Cultivated *G. pentaphyllum* (%)[a]

		1	2	3	4	5	6	7	8	9	Mean
Leaf	**1**	0.80	0.98	1.20	0.65	1.09	1.53	0.80	1.22	0.77	1.00
	2	0.49	0.56	0.37	0.63	1.30	1.18	0.59	1.11	0.56	0.75
	3	2.30	2.84	2.22	2.09	1.79	2.01	1.93	3.33	2.83	2.39
Stem	**1**	0.19	0.27	0.26	0.17	0.26	0.39	0.17	0.26	0.20	0.24
	2	—	—	—	—	—	—	—	—	—	—
	3	0.38	0.62	0.58	0.40	0.47	0.43	0.54	0.58	0.62	0.51

a) Figures show the values in terms of dry weight.

showed a spot identical with **1** on TLC were concentrated in Ishikawa (Kanazawa), Fukui, Toyama, Tottori, Yamagata and Fukushima districts. Almost all the samples from Fukui, Toyama, Tottori, and Fukushima, however, were excluded by the second screening with TLC (system 2). It is interesting that the samples from Kanazawa city showed a particularly high incidence of **1**, because Takemoto *et al.*[3a] first isolated ginsenosides from materials collected in the same city. Finally, it was confirmed by high-performance liquid chromatography (HPLC) (system 1) that 57 samples contained **1**, but the maximum content was no more than 0.19%.

As it was difficult to proceed with our experiments with

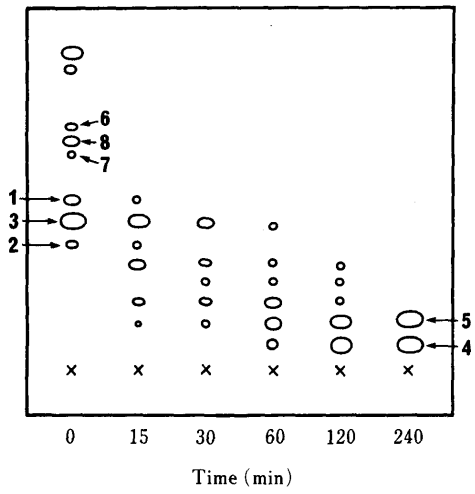

Fig. 1. Degradation of Saponins in a Homogenate of *G. pentaphyllum* at 40 °C

TLC plate, RP-8 F_{254}S (Merck); solvent, MeOH–H_2O (75:25).

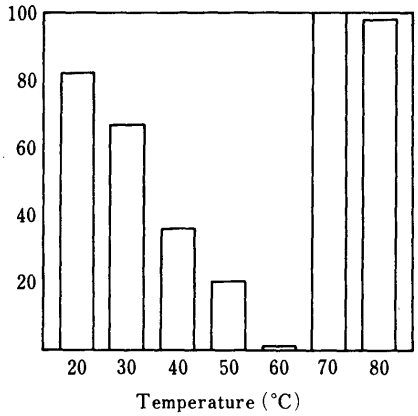

Fig. 2. Effect of Temperature on Hydrolysis of Gy. V (**3**) in a Homogenate of *G. pentaphyllum*

Recoveries are with respect to the initial value at 30 min.

the wild plants because of their low ginsenoside contents, the seeds of *G. pentaphyllum*, collected in Kanazawa city, were sown in an experimental field and screenings were carried out with 2136 seedlings that had grown for four months. In this screen, G.-Rd (**2**) and Gy. V (**3**) in addition to G.-Rb_1 (**1**), were used as indices, because they were contained in fairly large amounts in many samples. A few pieces of dry leaf of each individual plant were examined by TLC and HPLC as described above; 303 seedlings were found to contain more than 0.9% of **1**, 0.4% of **2** and/or 1.3% of **3** (Table II). Two hundred and fifty-four seedlings, however, were excluded because they were not vigorous and/or because of the existence of large amounts of unidentified components that were eluted close to **1**, **2**, or **3** in HPLC. Consequently, 49 seedlings were selected for propagation by cuttings; the saponin contents of 9 plants collected at random in September are shown in Table III. It was found that leaves are better than stems as a source.

It was then noticed that leaves dried at room temperature for 7 d had lost *ca.* 60% of the three saponins, whereas those dried at 50 °C for 10 h had lost no more than *ca.* 10% (Table IV). Moreover, the degradation of the saponin components started again when the dry leaves were left at

room temperature after rehydration. As these facts suggested that the degradation of saponins was caused by intracellular enzymes (glycosidases), an aqueous homogenate of fresh leaves was allowed to stand at 40 °C and the degradation of saponins was followed by TLC. Most of the saponins in the fresh leaves were gradually degraded to compound K (**4**) and Gy. XIV (**5**) (Fig. 1). The glucosidases in a homogenate showed the highest activity at 60 °C, and were deactivated completely at 70 °C (Fig. 2). The most useful dry materials were obtained by microwave irradiation of fresh leaves; there was no loss of saponins.

Isolation of Malonylated Saponins The fresh leaves of *G. pentaphyllum* were extracted with ethyl acetate and then methanol. The methanol extract was partitioned in ethyl acetate and water. From the aqueous layer, three acidic saponins, 6″-malonylginsenoside-Rb_1 (**6**), 6″-malonylginsenoside-Rd (**7**) and 6″-malonylgypenoside V (**8**) along with **1**, **2** and **3**, were isolated by repeated column chromatography on octadecyl silica (ODS) and silica gel as described in the experimental section.

6″-Malonylginsenoside-Rb_1 (**6**) was obtained as the sodium salt, a white powder, $C_{57}H_{93}NaO_{26}$, mp 198—200 °C, $[\alpha]_D$ +7.8° (c=1.0, MeOH); the secondary ion mass spectrum (SIMS) showed an $(M+H)^+$ ion peak at m/z 1217. Two absorptions at 1600 and 1730 cm^{-1} in the infrared (IR) spectrum suggested carboxylate and ester functions, and **6** gave G.-Rb_1 (**1**) on alkaline hydrolysis. These findings suggested the existence of a malonyl ester function, so **6** was methylated with ethereal diazomethane in methanol solution to give monomethyl ester (**9**) as a white powder, mp 176—179 °C, $[\alpha]_D$ +12.6° (c=1.0, MeOH). It was confirmed that **9** was identical with 6″-malonylginsenoside-Rb_1 methyl ester, mp 178—182 °C, $[\alpha]_D$ +9.8° (MeOH), provided by Kitagawa *et al.*,[4] based on ^{13}C-nuclear magnetic resonance (^{13}C-NMR) spectral comparison. The structure of **6**, therefore, was determined to be 6″-malonylginsenoside-Rb_1, which was first isolated from Ginseng Radix.

6″-Malonylginsenoside-Rd (**7**) was also obtained as the sodium salt, a white powder, $C_{51}H_{83}NaO_{21}$, mp 215—217 °C, $[\alpha]_D$ +14.3° (c=1.0, MeOH). On hydrolysis with sodium hydroxide, **7** gave **2**. From the $(M+H)^+$ ion peak at m/z 1055 in SIMS, and an absorption at 1720 cm^{-1} in the IR spectrum, it was assumed that **7** was a malonyl ester of G.-Rd, which had been isolated together with **6** from Ginseng Radix by Kitagawa *et al.* On methylation, **7** afforded the monomethyl ester (**10**) as a white powder, mp 179—181 °C, $[\alpha]_D$ +20.5° (c=1.0, MeOH); the ^{13}C-NMR spectrum was identical with that of 6″-malonylginsenoside-Rd methyl ester provided by Kitagawa *et al.*[4] Thus, the structure of **7** was elucidated as 6″-malonylginsenoside-Rd.

TABLE IV. Comparison of Saponin Contents in Dry Leaves of *G. pentaphyllum* (%)

	1	2	3	6	7	8
Fresh (initial)	0.75	0.54	1.76	0.30	0.24	0.65
Dried at room temperature (7 d)	0.32	0.21	0.71	0.15	0.12	0.27
Dried at 50 °C	0.67	0.46	1.58	0	0	0.23
Irradiated with microwaves	0.75	0.56	1.74	0.25	0.22	0.65

The initial values represent % of dry weight.

TABLE V. ^{13}C-NMR Data for Ginsenosides

		1	2	4	9	10
	C-3	88.2	88.9	78.0	89.2	89.3
3-O-Glc	1′	105.1	105.0		105.2	104.9
(inner)	2′	83.4	83.3		84.2	84.3
	3′	77.8[a]	77.9[a]		77.9[a]	78.2[a]
	4′	71.6	71.7		71.7	71.5
	5′	78.2[a]	78.2[a]		78.4[a]	78.5[a]
	6′	62.7	62.9		62.8	62.9
3-O-Glc	1″	105.9	106.0		106.0	106.1
(terminal)	2″	76.9	77.0		76.5	76.6
	3″	79.0[a]	79.2[a]		79.1[a]	79.2[a]
	4″	71.6	71.6		70.8	70.9
	5″	78.1[a]	78.1[a]		75.1	75.2
	6″	62.7	62.7		65.5	65.6
20-O-Glc	1‴	97.9	98.2	98.2	98.0	98.2
(inner)	2‴	75.1	75.0	75.1	75.1	75.1
	3‴	78.0[a]	78.0[a]	79.2[a]	78.2[a]	78.0[a]
	4‴	71.6	71.7	71.6	71.4	71.7
	5‴	76.9	77.9[a]	78.2[a]	76.9	77.9[a]
	6‴	71.5	62.9	62.6	70.1	62.9
20-O-Glc	1⁗	104.9			104.8	
(terminal)	2⁗	74.7			74.7	
	3⁗	79.0[a]			78.2[a]	
	4⁗	71.6			71.5	
	5⁗	77.8[a]			77.8[a]	
	6⁗	62.8			62.8	
Glc–O–CO					167.1	167.2
$-CH_2-$					41.5	41.6
(CH_3)–O–CO					167.1	167.2
OCH_3					52.2	52.2

δ ppm, pyridine-d_5. *a*) The indicated assignments in the same column may be interchanged.

TABLE VI. ^{13}C-NMR Data for Gypenosides

		3	5	8	11
	C-3	88.9	78.4	89.2	89.3
3-O-Glc	1′	104.9		104.7	104.9
(inner)	2′	83.3		83.8	84.3
	3′	77.8[a]		77.9[a]	78.5[a]
	4′	71.5		71.3	71.4
	5′	77.6[a]		77.4[a]	78.0[a]
	6′	62.7		62.7	62.9
3-O-Glc	1″	105.8		105.8	106.1
(terminal)	2″	76.8		76.6	76.7
	3″	78.9[a]		79.1[a]	79.2[a]
	4″	71.1		71.3	70.9
	5″	77.9[a]		75.1	75.2
	6″	62.6		64.8	65.6
20-O-Glc	1‴	98.0	98.1	98.1	98.2
	2‴	74.9	75.2	75.0	75.1
	3‴	78.0[a]	78.2	78.1[a]	77.9[a]
	4‴	71.5	71.6	70.9	71.4
	5‴	76.4	76.5	76.4	76.7
	6‴	66.6	66.7	66.8	66.9
20-O-Rham	1⁗	101.3	101.5	101.5	101.6
	2⁗	72.7	73.0	72.8	72.9
	3⁗	72.0	72.3	72.2	72.3
	4⁗	74.1	74.4	74.2	74.3
	5⁗	69.2	69.5	69.3	69.4
	6⁗	18.5	18.7	18.6	18.6
Glc–O–CO				169.6	167.2
$-CH_2-$				44.2	41.6
(Na, CH_3)–O–CO				171.4	167.2
OCH_3					52.2

δ ppm, pyridine-d_5. *a*) The indicated assignments in the same column may be interchanged.

6″-Malonylgypenoside V (**8**) was also obtained as the sodium salt, a white powder, $C_{57}H_{93}NaO_{25}$, mp 205—207 °C, $[\alpha]_D$ +6.7° (c=1.0, MeOH). Its IR spectrum (1730 cm^{-1}) also showed the presence of an ester function, and Gy. V was obtained by alkaline hydrolysis of **8**. Moreover, in the ^{13}C-NMR spectrum of **8**, three new signals appeared at 44.2, 169.6, and 171.4, showing that the acid moiety was composed of malonic acid. Although the other signals were very similar to those of **3**, one of the two signals corresponding to the 6′ and 6″ carbons was shifted downfield by *ca.* 2 ppm. These findings indicate that the structure of **8** was 6′- or 6″-malonylgypenoside V; this conclusion was supported by the $(M+H)^+$ ion peak at m/z 1201 in SIMS. After methylation of **8**, the monomethyl ester (**11**), a white powder, $C_{58}H_{96}O_{25}$, mp 181—183 °C, $[\alpha]_D$ +10.3° (c=1.0, MeOH), was subjected to hydrolysis with a rhamnosidase to compare the product with **10**. Rhamnosidase was obtained by culturing *Cryptococcus laurentii* var. *laurentii* (IFO 0609) in a liquid medium containing hesperidin alone as the carbon source. The rhamnosidase that accumulated in the culture broth showed strong activity toward the rhamnosidic linkage of **11** to give **10** in a good yield, but the glucosidases in the broth were scarcely active against the glucosidic moieties of the substrate when incubation was carried out pH 7.0 and at 40 °C. The structure of **8**, therefore, was elucidated as 6″-malonylgypenoside V, which is a new compound.

Thus, it was found that some saponins exist partly as malonyl esters in *G. pentaphyllum*, as in Ginseng Radix. The contents of malonylated components as well as other neutral saponins also varied in the dry leaves depending on the method of drying the fresh materials (Table IV).

Experimental

Melting points were determined on a Yanaco MP-S3 micro melting point apparatus and are uncorrected. Optical rotations were measured on a JASCO DIP-181 spectrometer. IR spectra were recorded on a Hitachi 260-10 spectrometer, and SIMS were taken on a Hitachi M-80A spectrometer. ^{13}C-NMR spectra were recorded on a JEOL GX-400 spectrometer (100.5 MHz) with tetramethylsilane as the internal standard; chemical shifts are given in δ values (ppm). Silica gel ODS-Q3 (Fuji gel) and Silica gel 60 (70—230 mesh, Merck) were used for column chromatography. HPLC was performed on an LC-6A liquid chromatograph (Shimadzu) with an SPD-6AV ultraviolet detector (Shimadzu). Microwaves (2450 MHz) were generated with a National NE-M 315 (500 W) generator (Matsushita).

Materials (a) The leaves of *Gynostemma pentaphyllum* MAKINO growing wild were collected for screening during May to September in 1983. The fresh leaves were dried in part at room temperature for no less than 7 d.

(b) Some leaves of seedlings obtained by the germination of seeds from Kanazawa city were collected in June, and 49 selected seedlings were propagated by taking cuttings at Fukuchiyama Experimental Farm of Takeda Chemical Industries (Kyoto). The aerial parts were collected every year from June to October.

General Procedures of TLC Fresh (2 g) leaves were ground in MeOH (2 ml), and ground dry leaves (50 mg) were extracted with 80% (v/v) MeOH (2 ml). After ultrasonication for 1 h, an aliquot of the extract (5 μl) was subjected to TLC. TLC was performed on a RP-8 F_{254}S TLC plate (Merck) using the following solvents: MeOH–H_2O (75:25) for system 1 and $CHCl_3$–CH_3CN–CH_3COOH–H_2O (1.5:40:1:60) for system 2. The saponin components on the TLC plate were detected by spraying with 10% H_2SO_4 followed by heating.

General Procedures of HPLC Fresh (4 g) leaves were ground in MeOH (7 ml), and ground dry leaves (100 mg) were extracted with 80% (v/v) MeOH (9 ml). After ultrasonication for 1 h, the suspension was diluted to 10 ml with 80% MeOH, and the supernatant (10 μl) was subjected to HPLC. HPLC was carried out with an ERC-ODS-1161 (3 μm, 6 × 100 mm) column (Erma) at 40 °C under the following conditions (mobile phase, flow rate and detection). System 1: CH_3CN–H_2O (35:65), 1 ml/min and 203 nm [t_R; *ca.* 6.1 (**1**), *ca.* 13.3 (**2**) and *ca.* 9.9 (**3**) min]; system 2: CH_3CN–H_2O (35:65) with added PIC A (Waters), 1 ml/min and 210 nm [t_R; *ca.* 7.0 (**6**), *ca.* 12.5 (**7**) and *ca.* 10.5 (**8**) min]; system 3: MeOH–H_2O (80:20), 1 ml/min and 203 nm [t_R; *ca.* 19.1 (**4**), *ca.* 14.6 (**5**) min].

Isolation of Compound K (4) and Gy. XIV (5) The ground leaves, dried at room temperature (200 g) were added to H_2O (1 l), and the suspension was allowed to stand at 40 °C. The changes of components in the suspension were followed by TLC (system 1) for 4 h as shown in Fig. 1. After addition of MeOH (3 l), the suspension, was extracted at room temperature for 3 h under ultrasonication and then filtered. The filtrate was concentrated *in vacuo* to remove MeOH, and the remaining aqueous suspension was partitioned between H_2O and EtOAc (1:1) (2 l). The EtOAc layer was column chromatographed on ODS (5 × 50 cm). After elution with H_2O (1 l) and 60% (v/v) MeOH (1 l), **4** (1.2 g) and **5** (2.8 g) were eluted with 80% (v/v) MeOH. **4**, a white powder (H_2O–MeOH), mp 159—161 °C, $[\alpha]_D$ +39.0° (c=1.0, MeOH). *Anal.* Calcd for $C_{36}H_{62}O_8 \cdot 0.5H_2O$: C, 68.43; H, 10.05. Found: C, 68.39; H, 10.26. It was identical with an authentic sample in terms of mixed melting point and, TLC, HPLC and ^{13}C-NMR comparisons. **5**, a white powder (H_2O–MeOH), mp 167—169 °C, $[\alpha]_D$ +27.0° (c=1.0, MeOH). *Anal.* Calcd for $C_{47}H_{72}O_{12} \cdot 2H_2O$: C, 62.66; H, 9.52. Found: C, 62.52; H, 9.70. It was identical with an authentic sample in terms of mixed melting point and, TLC, HPLC and ^{13}C-NMR comparisons.

Activities of Intracellular Enzymes in the Degradation of Gy. V (3) Fresh leaves (5 g) were ground in cold water (100 ml) with a blender under cooling with ice, and the homogenate was divided into ten 10 ml fractions. MeOH (40 ml) was added to one fraction, and the suspension was ultrasonicated for 1 h. An aliquot of the supernatant (10 μl) was subjected to HPLC (system 1) to give the initial value of **3**. The other fractions were allowed to stand at 20, 30, 40, 50, 60, 70 and 80 °C, respectively, for 30 min, and were analyzed individually by the same procedure as above. The recovery rates as % of the initial value were 82, 67, 36, 20, 1, 100 and 98, respectively.

Activities of Intracellular Enzymes in the Dry Leaves Fresh leaves, the saponin content of which had been determined previously, were dried at room temperature for 7 d, at 50 °C for 10 h and in a microwave generator for 10 min, and the saponin contents of the dry leaves were measured by HPLC (systems 1 and 2) as shown in Table IV. Afterwards, the ground dry materials (100 mg) in H_2O (2 ml) were left for 12 h at 40 °C. The saponins in each suspension were identified in comparison with authentic samples by TLC and HPLC. None of **1**, **2**, **3**, **6**, **7** and **8** was detected in the suspension of the material dried at room temperature or at 50 °C, but all saponins were recovered with the yields of 110% (**1**), 105% (**2**), 112% (**3**), 42% (**6**), 51% (**7**) and 40% (**8**) from the suspension of the material dried by microwave irradiation.

Isolation of Malonylated G.-Rb_1 (6), -Rd (7) and Gy. V (8) Cut fresh leaves (2.5 kg) were extracted with EtOAc (3 l) overnight, then with MeOH (6 l) for 2 d at room temperature. The MeOH solution was concentrated *in vacuo*, and the residue was dissolved in H_2O (1.5 l) and EtOAc (1 l). The aqueous solution was subjected to ODS column (6 × 100 cm) chromatography with H_2O (10 l), 50% (v/v) MeOH (1.5 l) and 80% (v/v) MeOH (2 l) as the eluents. The fraction eluted with 80% MeOH was concentrated *in vacuo* to *ca.* 400 ml to give precipitates (29.1 g). The precipitates were dissolved in a small amount of 10% (w/v) $NaHCO_3$ solution, and the solution was applied again to an ODS column (6 × 100 cm) with H_2O–MeOH in a gradient mode as the eluent to give 5 fractions. The fractions were further chromatographed individually on silica gel with $CHCl_3$–MeOH–H_2O (70:30:5) as the eluent to give **1** (2.1 g), **2** (1.0 g), **3** (4.3 g), **6** (0.5 g), **7** (0.9 g) and **8** (2.5 g). **1**, **2** and **3** were shown to be identical with authentic samples by TLC, HPLC, and ^{13}C-NMR comparisons. 6″-Malonylginsenoside-Rb_1 (**6**), a white powder (MeOH–EtOAc), mp 198—200 °C, $[\alpha]_D$ +7.8° (c=1.0, MeOH). *Anal.* Calcd for $C_{57}H_{93}NaO_{26} \cdot 3H_2O$: C, 53.85, H, 7.85. Found: C, 53.94; H, 7.70. IR (KBr): 1730 cm^{-1}. SIMS m/z: 1217 $(M+H)^+$. 6″-Malonylginsenoside-Rd (**7**), a white powder (MeOH–EtOAc), mp 215—217 °C, $[\alpha]_D$ +14.3° (c=1.0, MeOH). *Anal.* Calcd for $C_{51}H_{83}NaO_{21} \cdot 4H_2O$: C, 54.54; H, 7.95. Found: C, 54.33; H, 7.75. IR (KBr): 1720 cm^{-1}. SIMS m/z: 1055 $(M+H)^+$. 6″-Malonylgypenoside V (**8**) a white powder (MeOH–EtOAc), mp 205—207 °C, $[\alpha]_D$ +6.7° (c=1.0, MeOH). *Anal.* Calcd for $C_{57}H_{93}NaO_{25} \cdot 3H_2O$: C, 54.54; H, 7.95. Found: C, 54.33; H, 7.75. IR (KBr): 1730 cm^{-1}. SIMS m/z: 1201 $(M+H)^+$. ^{13}C-NMR: shown in Table VI.

Hydrolyses of 6, 7, and 8 with Sodium Hydroxide A few milligrams of **6**, **7**, or **8** was dissolved in 1 N NaOH (1 ml) and allowed to stand overnight at

room temperature. The reaction mixture was neutralized with HCl, and was subjected to TLC and HPLC to identify the product as **1**, **2**, or **3**.

Methylation of 6, 7, and 8 with Diazomethane Ethereal diazomethane was added dropwise to a MeOH solution (50 ml) of **6**, **7**, or **8** (200 mg). The reaction solution was kept below pH 6 by adding CH_3COOH. The solvent was evaporated off *in vacuo*, and the residue was dissolved in H_2O (200 ml) and BuOH (300 ml). The BuOH fraction was chromatographed on a silica gel column (4 × 50 cm) with $CHCl_3$–MeOH–H_2O (70 : 30 : 5) as the eluent to give **9** (90 mg), **10** (132 mg) or **11** (128 mg). 6′′-Malonylginsenoside-Rb_1 methyl ester (**9**) a white powder (EtOH–EtOAc), mp 176—179 °C, $[\alpha]_D$ +12.6° (c=1.0, MeOH). *Anal.* Calcd for $C_{58}H_{96}O_{26}\cdot 3.5H_2O$: C, 54.75; H, 8.16. Found: C, 54.65; H, 7.95. IR (KBr): 1740 cm^{-1}. SIMS m/z: 1231 $(M+Na)^+$. ^{13}C-NMR: shown in Table V. 6′′-Malonylginsenoside-Rd methyl ester (**10**), a white powder (EtOH–EtOAc), mp 179–181 °C, $[\alpha]_D$ + 20.5° (c=1.0, MeOH). *Anal.* Calcd for $C_{52}H_{86}O_{21}\cdot 3.5H_2O$: C, 56.25; H, 8.44. Found: C, 56.11; H, 8.27. IR (KBr): 1745 cm^{-1}, SIMS m/z: 1069 $(M+Na)^+$. ^{13}C-NMR (Table V) was identical with a spectral chart provided by Prof. Yoshikawa. 6′′-Malonylgypenoside V methyl ester (**11**), a white powder (EtOH–EtOAc), mp 181—183 °C, $[\alpha]_D$ +10.3° (c= 1.0, MeOH). *Anal.* Calcd for $C_{58}H_{96}O_{23}\cdot 2H_2O$: C, 56.66; H, 8.20. Found: C, 56.62; H, 7.99. IR (KBr): 1745 cm^{-1}. SIMS m/z: 1215 $(M+Na)^+$. ^{13}C-NMR: shown in Table VI.

Preparation of Rhamnosidase A basal medium (1 l), composed of $(NH_4)_2SO_4$ (10 g), $MgSO_4\cdot 2H_2O$ (0.5 g), KH_2PO_4 (1 g) and yeast extract (0.5 g), was adjusted with NaOH to pH 5.7. *Cryptococcus laurentii* var. *laurentii* (IFO 0609) was inoculated into the basal medium (5 ml) which contained 0.1% (w/v) sucrose, and the culture was shaken for 7 d at 28 °C to afford a seed. This was transferred to another medium (600 ml) containing 0.1% (w/v) hesperidin, and this culture was shaken for 7 d to give a broth which contained rhamnosidase. The broth was filtered through a cotton plug, and the filtrate was used for enzymatic hydrolysis of **11**.

Hydrolysis of 11 by Rhamnosidase A solution of **11** (200 mg) in a broth (pH 7.0) prepared by the above procedure (400 ml) was incubated for 5 h at 40 °C. The reaction mixture was applied to an XAD-2 column (4 × 20 cm), and the adsorbed substances were eluted with H_2O (1 l), 20% (v/v) MeOH (0.5 l) and MeOH (2 l). The fraction eluted with MeOH was rechromatographed on a silica gel column (4 × 20 cm) with $CHCl_3$–MeOH–H_2O (70 : 20 : 1) as the eluent to give **10** (80 mg), a white powder (EtOH–EtOAc), mp 178—180 °C, $[\alpha]_D$ +20.0° (c=1.0, MeOH). *Anal.* Calcd for $C_{52}H_{86}O_{21}\cdot 3H_2O$: C, 56.71; H, 8.42. Found: C, 56.67; H, 8.57. IR (KBr): 1745 cm^{-1}. SIMS m/z: 1069 $(M+Na)^+$. This product was identical with an authentic sample (mixed melting point, TLC, HPLC, and ^{13}C-NMR).

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