Chemical Studies on Crude Drug Processing. VI.[1] Chemical Structures of Malonyl-ginsenosides Rb_1, Rb_2, Rc, and Rd Isolated from the Root of *Panax ginseng* C. A. MEYER

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Four malonylated dammarane-type triterpene oligoglycosides, named malonyl-ginsenosides Rb_1, Rb_2, Rc, and Rd, were isolated from the water-soluble portion of the dried root of *Panax ginseng* C. A. MEYER (Araliaceae), together with previously known ginsenosides. On the basis of chemical and physicochemical evidence including metastable ion analysis of liquid secondary ion mass spectra, the structures of malonyl-ginsenosides Rb_1, Rb_2, Rc, and Rd have been elucidated as 3-*O*-[6-*O*-malonyl-β-D-glucopyranosyl(1→2)-β-D-glucopyranosyl]-20-*O*-[β-D-glucopyranosyl(1→6)-β-D-glucopyranosyl]-20(*S*)-protopanaxadiol, 3-*O*-[6-*O*-malonyl-β-D-glucopyranosyl(1→2)-β-D-glucopyranosyl]-20-*O*-[α-L-arabinopyranosyl(1→6)-β-D-glucopyranosyl]-20(*S*)-protopanaxadiol, 3-*O*-[6-*O*-malonyl-β-D-glucopyranosyl(1→2)-β-D-glucopyranosyl]-20-*O*-[α-L-arabinofuranosyl(1→6)-β-D-glucopyranosyl]-20(*S*)-protopanaxadiol, and 3-*O*-[6-*O*-malonyl-β-D-glucopyranosyl(1→2)-β-D-glucopyranosyl]-20-*O*-[β-D-glucopyranosyl]-20(*S*)-protopanaxadiol, respectively.

Keywords malonyl-ginsenoside Rb_1; malonyl-ginsenoside Rb_2; malonyl-ginsenoside Rc; malonyl-ginsenoside Rd; dammarane-type triterpene oligoglycoside malonylated; ginsenoside metastable ion analysis; ginsenoside linked scan liquid SIMS; *Panax ginseng*; Ginseng Radix

In traditional Chinese medicine, the root of *Panax ginseng* C. A. MEYER (Araliaceae) has been used in both processed and unprocessed forms such as Ginseng Radix Rubra (red ginseng) and Ginseng Radix (white ginseng). The biologically active constituents of these ginsengs have been pursued extensively[2] and in recent years, various ginsenosides, the dammarane-type triterpene oligoglycosides, have been characterized as the principal ingredients of white ginseng.[3]

As a part of our chemical studies on the processing of crude drugs,[4] we have chemically compared in detail the constituents of white ginseng and red ginseng of various origins.[5] In those studies, we have isolated several then new compounds such as 20(*R*)-ginsenoside Rg_2, 20(*S*)-ginsenoside Rg_3, ginsenoside Rh_2, and panaxytriol from the lipophilic portion of red ginseng and have elucidated their chemical structures.[6] Among these characteristic constituents of red ginseng, ginsenoside Rh_2 has been shown specifically to exhibit growth-inhibitory activity against cultured tumor-cells.[7] Afterwards, we have further analyzed in more detail the lipophilic constituents, such as glycero-galactolipids, steryl glucoside fatty acid esters, and various acetylene-alcohol constituents, in fresh ginseng root, white ginseng, and red ginseng.[1]

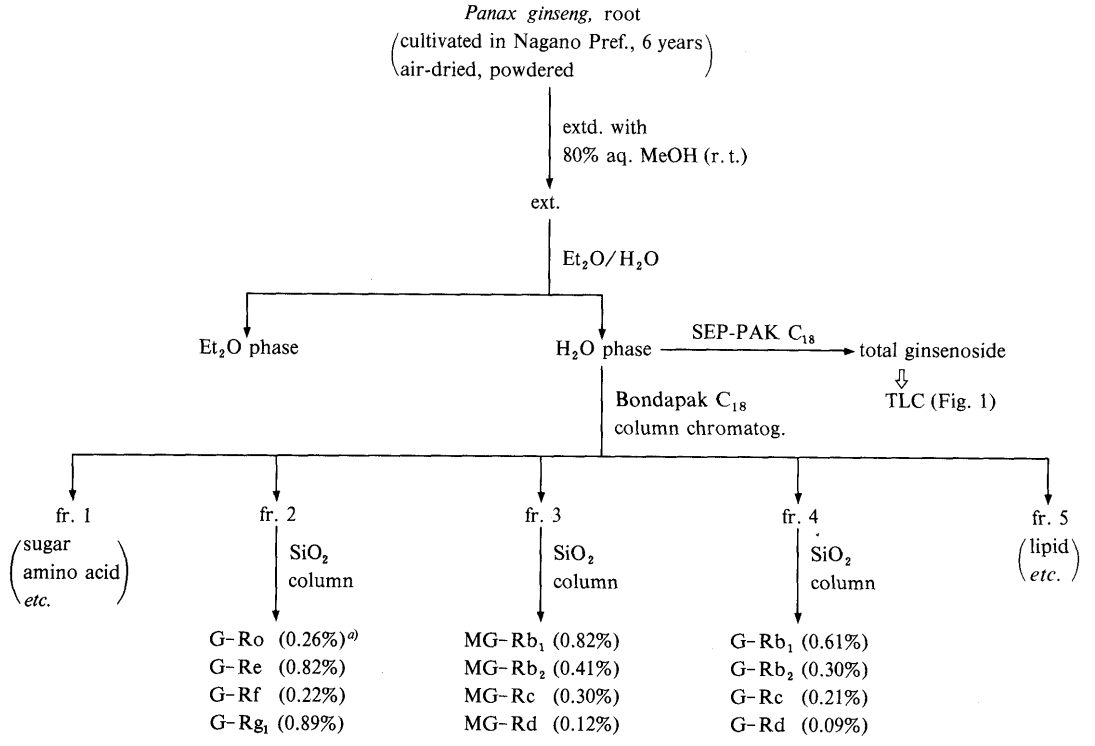


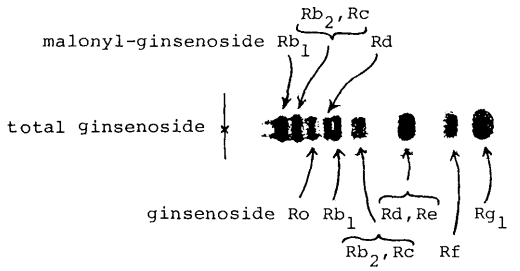
Chart 1. Isolation Procedure for Ginsenosides and Malonyl-ginsenosides

In continuing studies, we have comparatively investigated the water-soluble constituents of white ginseng and red ginseng and have found, in white ginseng, considerable amounts of four malonylated ginsenosides named malonyl-ginsenosides Rb_1 (**1**), Rb_2 (**3**), Rc (**5**), and Rd (**7**), which behave as acidic saponins due to the half ester forms of their malonyl residues. In this paper, we present a full account of the structure elucidation of these malonyl-ginsenosides.[8)]

The air-dried root of ginseng, cultivated for 6 years in Nagano Prefecture, was extracted with 80% aqueous MeOH and the extract was subjected to separation and purification through the procedures shown in Chart 1. Thin-layer chromatography (TLC) of total ginsenosides showed the presence of a large quantity of malonyl-ginsenosides (Fig. 1). After silica gel column chromatography of Fr. 3, malonyl-ginsenosides Rb_1 (**1**), Rb_2 (**3**), Rc (**5**), and Rd (**7**) were obtained in 0.82, 0.41, 0.30, and 0.12% yields, respectively, from the air-dried root.

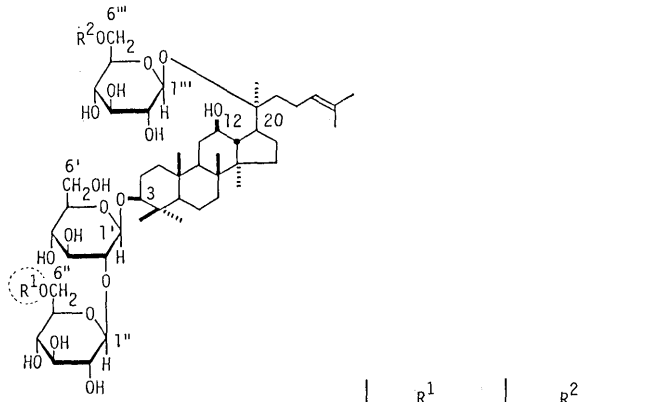
Malonyl-ginsenoside Rb_1 (1) The infrared (IR) spectrum of malonyl-ginsenoside Rb_1 (**1**) showed absorption bands ascribable to hydroxyl, ester carbonyl, and carboxyl functions. Alkaline hydrolysis of **1** provided ginsenoside Rb_1 (**2**) and malonic acid, whereas methylation of **1** with ethereal diazomethane in MeOH furnished the monomethyl ester (**1a**). The proton nuclear magnetic resonance (^{1}H-NMR) spectrum of **1a** showed signals assignable to a methylated malonyl group [δ 3.63 (3H, s), 3.69 (2H, s)]. Upon enzymatic hydrolysis using β-glucosidase, **1a** yielded malonyl-ginsenoside Rd methyl ester (**7a**) (*vide infra*) which liberated ginsenoside Rd (**8**), malonic acid, and monomethyl malonate after alkaline treatment. Comparison in detail of the carbon-13 nuclear magnetic resonance (^{13}C-NMR) data for **1a** and **7a** with those [3)] for **2** and **8** have led us to consider that the malonyl residue in **1** is attached to a primary (6′ or 6″) hydroxyl group of the D-glucosyl moiety (Table I). The location of the malonyl residue attached to the 6″-hydroxyl function in **1** has been presumed on the basis of the following metastable ion (MI) analysis[9)] of liquid secondary ion mass spectra (liquid SIMS) of **1**, **1a**, and **2**.

The liquid SIMS (glycerol matrix + NaCl) of **1**, **1a**, and **2** showed the respective quasimolecular ion peaks $(M+Na)^+$ [**1**, *m/z* 1217; **1a**, *m/z* 1231; **2**, *m/z* 1131] together with numerous fragment ion peaks (Fig. 2). The structures of these fragment ions were estimated by use of the B/E (B, magnetic field; E, electric field) linked scan technique by which the MI may be selectively recognized. The MI spectrum from the quasimolecular ion of **1a** (*m/z* 1231) clearly showed the characteristic fragment ion peaks at *m/z* 465 (**b**), 821 (**d**), 889 (**f**), 907 (**h**), 1069 (**k**), and 1097 (**n**), together with the fragment ion peaks at *m/z* 365 (**a′**), 789 (**e′**), 807 (**g′**), and 997 (**m′**) (Fig. 3) which were assigned as shown (underlined) in Chart 3. On the other hand, the fragment ion peaks of *m/z* 365 (**a**, **a′**), 721 (**c**), 789 (**e**, **e′**), 807 (**g**, **g′**), 951 (**i**), 969 (**j**, **j′**), and 997 (**m**, **m′**) were observed in the MI spectrum from the quasimolecular ion of **2** (*m/z* 1131) and some fragment ions of *m/z* 365, 789, 807, 969, and 997 were observed at identical *m/z* with those from **1a** (Fig. 3). Furthermore, in the MI spectra from the fragment ions **c** ($C_{37}H_{62}O_{12}Na$) and **d** ($C_{41}H_{66}O_{15}Na$), whose elemental compositions were determined by high-resolution MS, the fragment ions characteristically formed from each oligosaccharide moiety [from **c**, *m/z* 145 (**o**-H_2O), 163 (**o**), 365 (**a**); from **d**, *m/z* 245 (**p**-H_2O), 263 (**p**), 465 (**b**)]

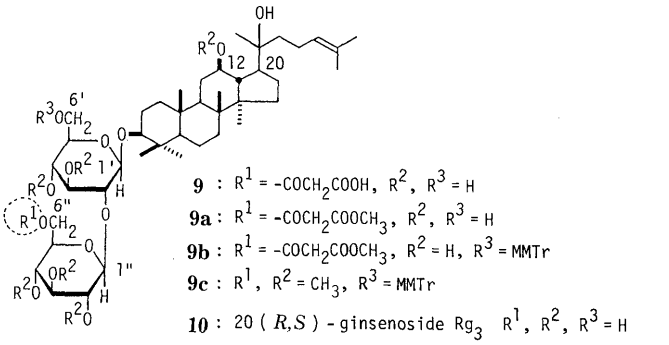


solvent: $CHCl_3$–MeOH–H_2O (65 : 35 : 10, lower phase)
adsorbent: pre-coated Silica gel 60 F_{254} (0.25 mm, Merck)

Fig. 1. Result of TLC of Total Ginsenoside



	R^1	R^2
1 : malonyl-ginsenoside Rb_1	$-COCH_2COOH$	
1a : MG-Rb_1 methyl ester	$-COCH_2COOCH_3$	
2 : ginsenoside Rb_1	H	
3 : malonyl-ginsenoside Rb_2	$-COCH_2COOH$	
3a : MG-Rb_2 methyl ester	$-COCH_2COOCH_3$	
4 : ginsenoside Rb_2	H	
5 : malonyl-ginsenoside Rc	$-COCH_2COOH$	
5a : MG-Rc methyl ester	$-COCH_2COOCH_3$	
6 : ginsenoside Rc	H	
7 : malonyl-ginsenoside Rd	$-COCH_2COOH$	
7a : MG-Rd methyl ester	$-COCH_2COOCH_3$	H
8 : ginsenoside Rd	H	



9 : R^1 = $-COCH_2COOH$, R^2, R^3 = H
9a : R^1 = $-COCH_2COOCH_3$, R^2, R^3 = H
9b : R^1 = $-COCH_2COOCH_3$, R^2 = H, R^3 = MMTr
9c : R^1, R^2 = CH_3, R^3 = MMTr
10 : 20 (*R,S*) - ginsenoside Rg_3 R^1, R^2, R^3 = H

Chart 2. Structures of Malonyl-ginsenosides and Their Degradation Products

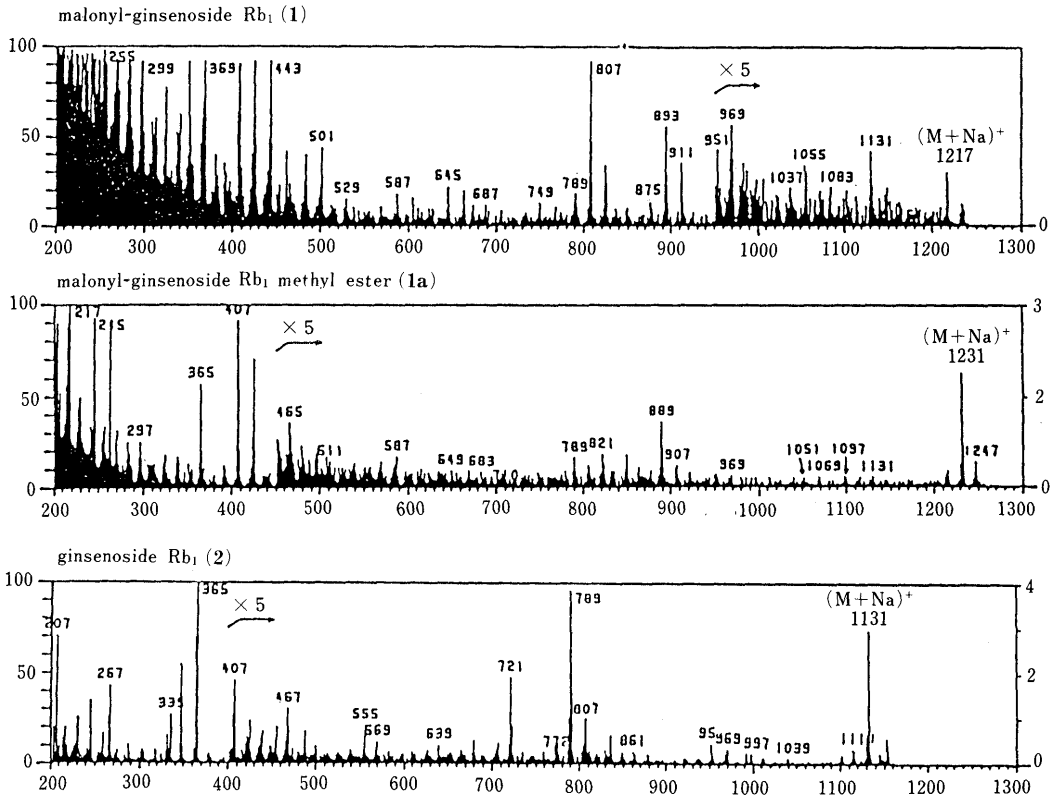


Fig. 2. Liquid SIMS of **1**, **1a**, and **2**

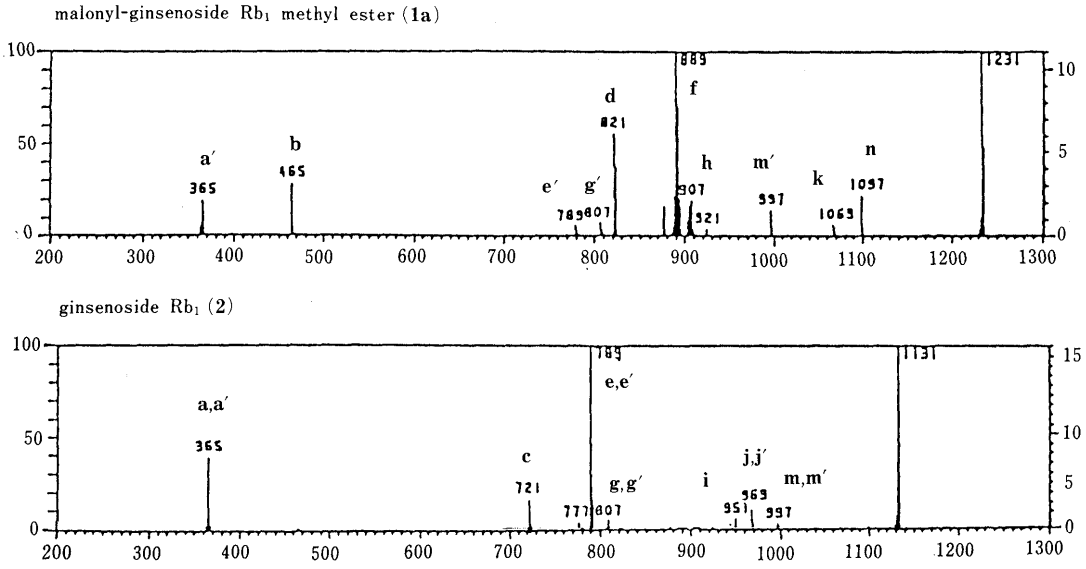


Fig. 3. MI Spectra of Quasimolecular Ions of **1a** and **2**

were observed with the fragment ion peaks at *m/z* 407 (**q**) and 425 (**r**) which may be derived commonly from the aglycone moiety of **c** and **d** (Fig. 4) (Chart 4).

Based on these comparisons in detail of the fragment ions in the liquid SIMS, malonyl-ginsenoside Rb_1 (**1**) has been assumed to be a malonylated derivative of ginsenoside Rb_1 (**2**) having the malonyl residue at the primary 6″-OH of the terminal D-glucose moiety. This assumption was finally verified by the following chemical evidence.

Partial hydrolysis of **1a** with 40% aqueous AcOH at 75 °C[6] furnished a 20-*O*-desglycosyl derivative (**9a**) which, on alkaline hydrolysis, yielded 20(*R*, *S*)-ginsenoside Rg_3 (**10**), malonic acid and monomethyl malonate. Treatment of **9a** with *p*-anisylchlorodiphenylmethane (MMTrCl) in pyridine at room temperature furnished the 6′-*O*-MMTr derivative (**9b**) which was then subjected to alkaline hydrolysis and subsequent methylation with CH_3I/DMSO/NaH[10] to provide a hepta-*O*-methyl derivative (**9c**). The IR spectrum of **9c** showed the preservation of a hydroxyl group and the ^{1}H-NMR spectrum showed signals ascribable to seven methoxyl groups, one MMTr group, and the 20-hydroxyl proton. Methanolysis of **9c** with 9% hydro-

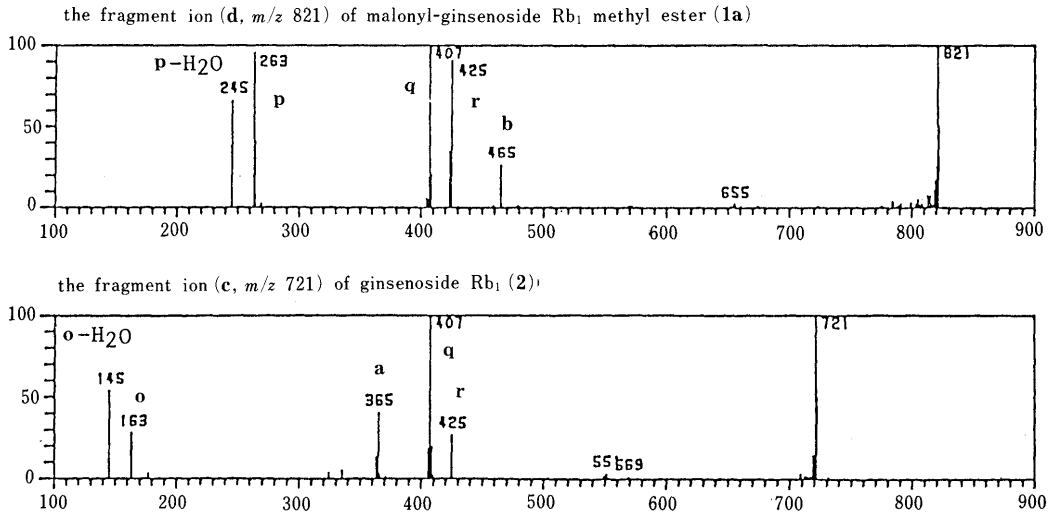


Fig. 4. MI Spectra of the Fragment Ions (**c**, **d**) from the Quasimolecular Ions

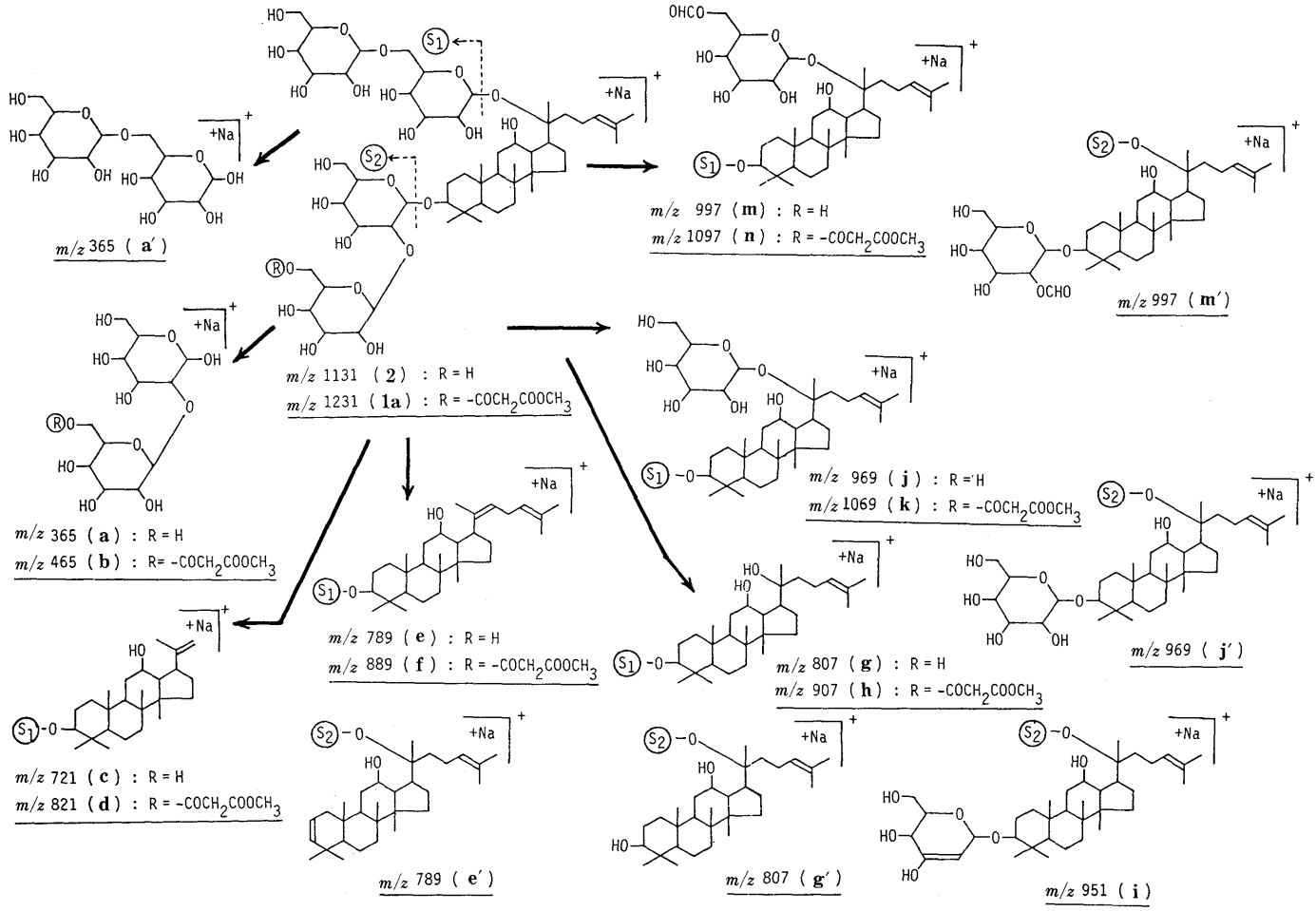


Chart 3. Mass Spectral Fragmentation Patterns of Malonyl-ginsenoside Rb_1 Methyl Ester (**1a**), and Ginsensoside Rb_1 (**2**)

gen chloride in dry MeOH liberated methyl 2,3,4,6-tetra-*O*-methylglucopyranoside and methyl 3,-4-di-*O*-methylglucopyranoside. Consequently, it has become clear that the MMTr group in **9c** is connected at the 6′-OH moiety and the malonyl residues in **9a** and **9b** are at the respective 6″-OH moieties. Based on the above evidence, the structure of malonyl-ginsenoside Rb_1 (**1**) has been determined to be as shown.

Malonyl-ginsenoside Rb_2 (3) The IR spectrum of malonyl-ginsenoside Rb_2 (**3**) also showed the presence of hydroxyl, carboxyl, and ester groups. Alkaline hydrolysis of **3** provided ginsenoside Rb_2 (**4**) and malonic acid. On diazomethane methylation, **3** furnished the monomethyl ester (**3a**). The ^{1}H-NMR spectrum of **3a** showed signals ascribable to a methylated malonyl group. The ^{13}C-NMR data for **3a** have been assigned as given in Table I, in

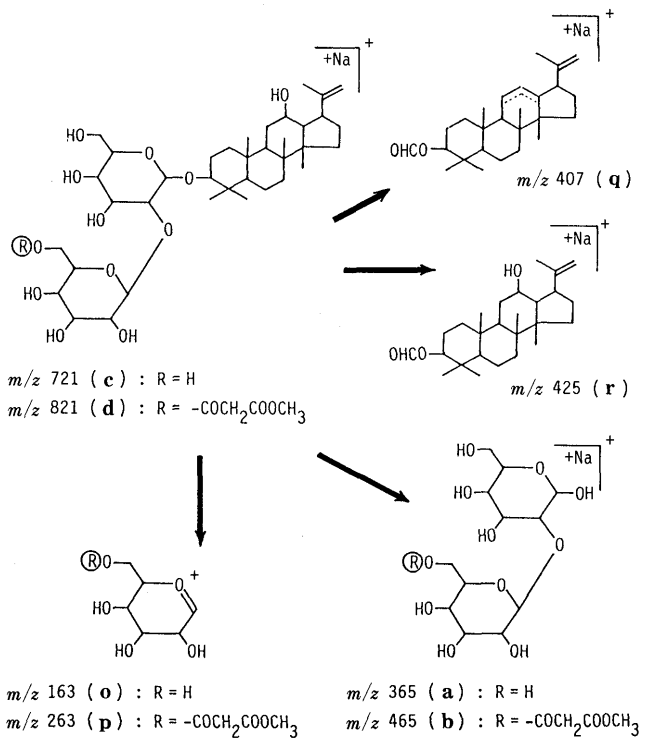


Chart 4. Fragmentation Patterns of Fragment Ions (**c**, **d**)

the same manner as in the case of malonyl-ginsenoside Rb_1 methyl ester (**1a**). The liquid SIMS of **3**, **3a**, and **4** gave respective quasimolecular ions $(M+Na)^+$ at *m/z* 1187, *m/z* 1201, and *m/z* 1101 (Fig. 5). Comparisons of the fragment ion peaks in the MI spectra from the quasimolecular ions of **3a** (**b**, **d**, **f**, **h**, **s**, **u**, **w**) and **4** (**c**, **e**, **e′**, **g**, **g′**, **s**, **u**, **w**) have led us to assign the location of the malonyl residue in **3** at 6″-OH of the terminal D-glucose moiety of **4**. On partial hydrolysis with aqueous AcOH as described above for **1a**, **3a** provided **9a**. Based on these above-mentioned evidence, the structure of malonyl-ginsenoside Rb_2 (**3**) has been elucidated as shown.

Malonyl-ginsenoside Rc (5) and Malonyl-ginsenoside Rd (7) The structures of malonyl-ginsenosides Rc (**5**) and Rd (**7**) have been determined in the same manner as described for malonyl-ginsenosides Rb_1 (**1**) and Rb_2 (**3**). The IR spectra of **5** and **7** were very similar to the spectrum of **3**. On alkaline hydrolysis, **5** liberated ginsenoside Rc (**6**) and malonic acid, while diazomethane methylation of **5** provided the monomethyl ester (**5a**) which, on partial hydrolysis with aqueous AcOH gave **9a**. The liquid SIMS spectra of **5**, **5a**, and **6** gave respective quasimolecular ions at *m/z* 1187, *m/z* 1201, and *m/z* 1101 and, in the MI spectra of the quasimolecular ions of **5a** and **6**, the fragment ion peaks (**b**, **d**, **f**, **g″**, **h**, **s′**, **u′**, and **w′** from **5a** and **c**, **e**, **g**, **s′**, **u′**, and **w′** from **6**) were observed (Fig. 6). Finally, comparison in detail of the ^{13}C-NMR data for **5a** with those for **6**[3] has led to the formulation of malonyl-ginsenoside Rc as **5**.

On the other hand, diazomethane methylation of **7** furnished the monomethyl ester (**7a**) which was found to be identical with a product obtained above by β-glucosidase hydrolysis of **1a** (*vide supra*). Thus, the structure of **7a** has been determined to be as shown, and this was further

TABLE I. ^{13}C-NMR Data for Malonyl-ginsenoside Methyl Esters[a,b]

		1a	**3a**	**5a**	**7a**	**9a**
Sapogenol	C-3	89.3	89.3	89.3	89.3	89.2
	C-12	70.2	70.2	70.3	70.2	70.9
	C-20	83.5	83.5	83.4	83.3	73.0
3-*O*-β-D-Gluco-pyranosyl	C-1′	105.3	104.9	104.8	104.8	104.8
	C-2′	84.2	84.2	84.1	84.1	84.1
	C-3′	77.9[c]	78.0[c]	77.9[c]	78.4[c]	78.4[c]
	C-4′	71.7	72.1	72.1	71.6	71.4
	C-5′	78.3[c]	78.6[c]	77.9[c]	79.0[c]	77.9[c]
	C-6′	62.8	62.9	62.6	62.8	62.8
2′-*O*-β-D-Gluco-pyranosyl	C-1″	106.1	106.1	106.0	106.0	106.0
	C-2″	77.0	76.6	76.6	76.6	76.5
	C-3″	79.1[c]	79.1[c]	78.8[c]	79.6[c]	79.6[c]
	C-4″	71.0	71.0	70.9	70.9	70.9
	C-5″	75.2	75.2	75.1	75.1	75.1
	C-6″	65.6	65.5	65.4	65.5	65.5
20-*O*-β-D-Gluco-pyranosyl	C-1‴	98.1	98.1	98.1	98.2	
	C-2‴	74.9	74.7	75.0	75.1	
	C-3‴	78.3[c]	78.0[c]	78.4[c]	78.1[c]	
	C-4‴	71.7	71.5	71.4	71.6	
	C-5‴	76.6	76.6	76.4	77.9[c]	
	C-6‴	71.6	69.2	68.4	62.8	
6‴-*O*-β-D-Gluco-pyranosyl	C-1⁗	104.9				
	C-2⁗	74.9				
	C-3⁗	78.3[c]				
	C-4⁗	71.7				
	C-5⁗	78.3[c]				
	C-6⁗	62.8				
6‴-*O*-α-L-Arabinosyl	C-1⁗		104.5	110.0		
	C-2⁗		71.8	83.1		
	C-3⁗		73.8	79.0		
	C-4⁗		68.4	86.0		
	C-5⁗		65.4	62.6		
6″-O–CO		167.1	167.2	167.2	167.2	167.1
CH_2		41.6	41.6	41.6	41.6	41.6
CO		167.1	167.2	167.2	167.2	167.1
OCH_3		52.2	52.2	52.2	52.2	52.2

a) Measured at 22.5 MHz in pyridine-d_5 at 25 °C. Chemical shifts are in δ_C. *b*) The characterizations of prim-C, sec-C, tert-C, and quat-C were based on INEPT (insensitive nuclei enhanced by polarization transfer) experiments. *c*) The assignments may be interchangeable within the same column.

supported by comparison of the liquid SIMS of **7** and **7a** with that of ginsenoside Rd (**8**). Thus, the quasimolecular ions $(M+Na)^+$ [**7**: *m/z* 1055, **7a**: *m/z* 1069, **8**: *m/z* 969] and their fragment ion peaks [**b**, **g″**, **d**, **f**, **h**, **t**, and **v** from **7a**; and **a**, **c**, **e**, **g**, and **t** from **8**] were observed respectively in their liquid SIMS and MI spectra.

Recently, Tanaka and his group reported the solubilizing properties of several oleanene-type saponins.[11] It is interesting to note that malonyl-ginsenosides are not only more soluble in water as compared with corresponding ginsenosides but also remarkably increase the water solubility of the ginsenosides.[12] As we reported previously,[1] the malonyl residues in malonyl-ginsenosides are readily eliminated through hydrolysis or methanolysis by treatment with hot water or hot methanol. Recently, it has been found that malonyl-ginsenosides are partially hydrolyzed under acidic conditions such as by treatment with 40% aqueous AcOH or artificial gastric juice, to provide malonyl 20(*R*, *S*)-ginsenoside Rg_3 (**9**). Thus, it has become clear that the malonyl residues in malonylginsenosides may be preserved under acidic conditions when the glycosyl residues attached to the 20-OH in ginsenosides are hydrolyzed, whereas the malonyl residues are readily lost by

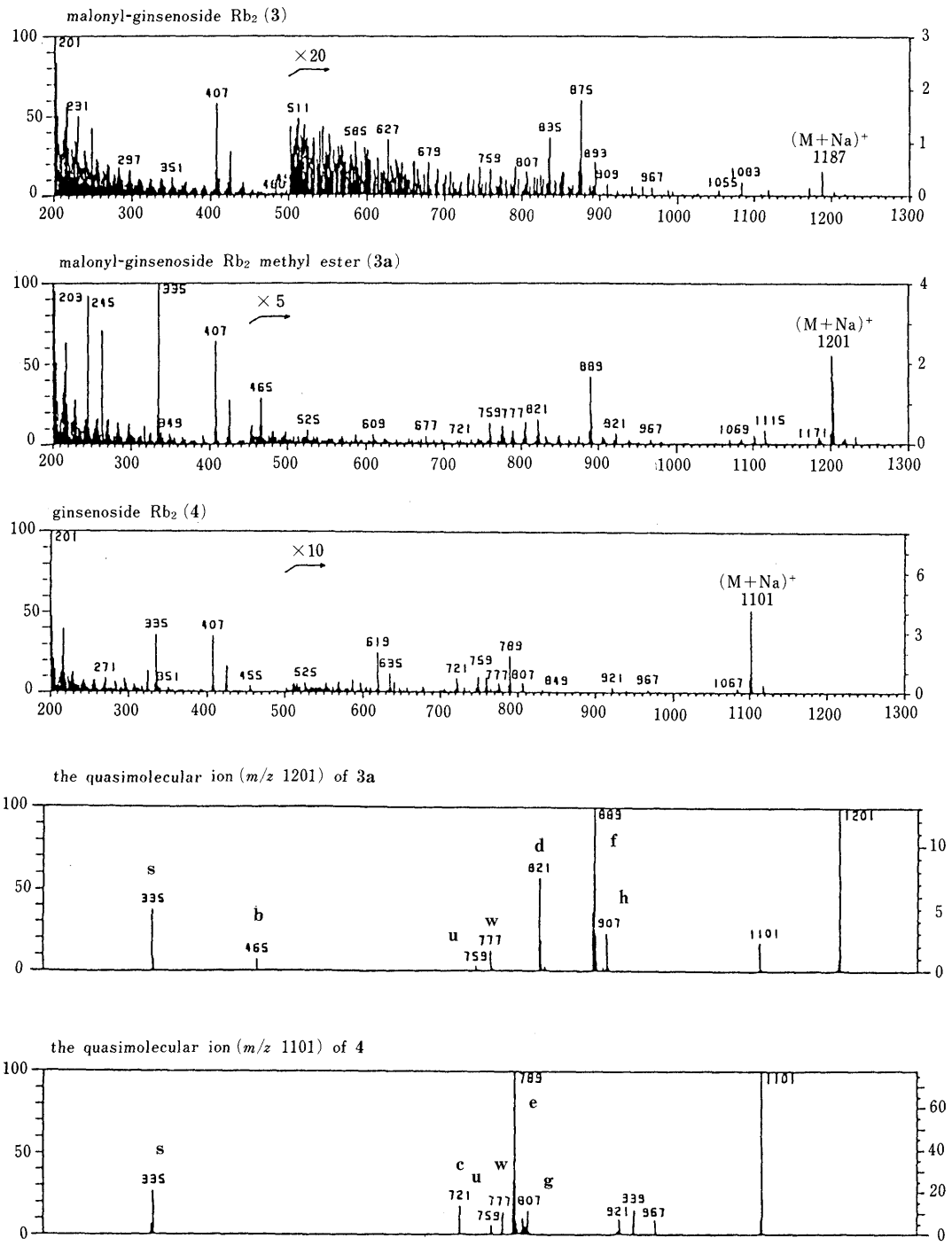


Fig. 5. Liquid SIMS and MI Spectra of **3**, **3a**, and **4**

mild alkaline treatment or even during decoction.

Experimental

The instruments used to obtain physical data and the experimental conditions for chromatography were the same as described in our previous paper[1] except for the following. The liquid SIMS were taken with a Hitachi M-68 double-focusing mass spectrometer. The primary ion was Xe^+ and the accelerating voltages of primary and secondary ions were 10 and 3 kV, respectively. The MI spectra were obtained by the B/E linked scan technique using a Hitachi M-003 datalyzer. The MeOH solution of each sample (1—2 μl, 2 mg/ml) was loaded on a silver substrate and NaCl–glycerol mixture was added to the sample on the substrate. For the accurate mass measurements, polyethylene glycol (#1000, Nakarai Chemical) was used [Calcd for $C_{37}H_{62}O_{12}Na$ (c): 721.4136, $C_{41}H_{66}O_{15}Na$ (**d**): 821.4296. Found: 721.4165, 821.4259].

Isolation of Malonyl-ginsenosides Rb_1 (1), Rb_2 (3), Rc (5), and Rd (7) and Ginsenosides Ro, Rb_1 (2), Rb_2 (4), Rc (6), Rd (8), Re, Rf, and Rg_1 The air-dried roots of *Panax ginseng* (1.0 kg), cultivated for 6 years in Nagano Prefecture, were powdered and extracted with 80% aqueous MeOH five times (5 l each) with occasional stirring at room temperature (25 °C). The aqueous MeOH extract (187 g), obtained after removal of the solvent at below 40 °C under reduced pressure, was partitioned into an ether–water (1 l each) mixture to give the water-soluble portion. Column chromatography of the water-soluble portion over reversed-phase silica gel [Bondapak C_{18} 1.0 kg, H_2O–MeOH (4:1→1:9) gradient elution] furnished four fractions after removal of the solvent under reduced pressure: Fr. 1 (sugar, amino acid, *etc.*, 112.0 g), Fr. 2 (23.0 g), Fr. 3 (17.4 g), Fr. 4 (13.4 g), and Fr. 5 (lipids, *etc.*, 3.2 g (Chart 1).[13]

Fraction 2 (6.0 g) was purified by column chromatography [SiO_2 600 g, $CHCl_3$–MeOH–H_2O (7:3:1, lower phase)→(65:35:10, lower phase)→6:4:1] to furnish ginsenosides Rg_1 (2.32 g), Rf (0.57 g), Re (2.14 g), and

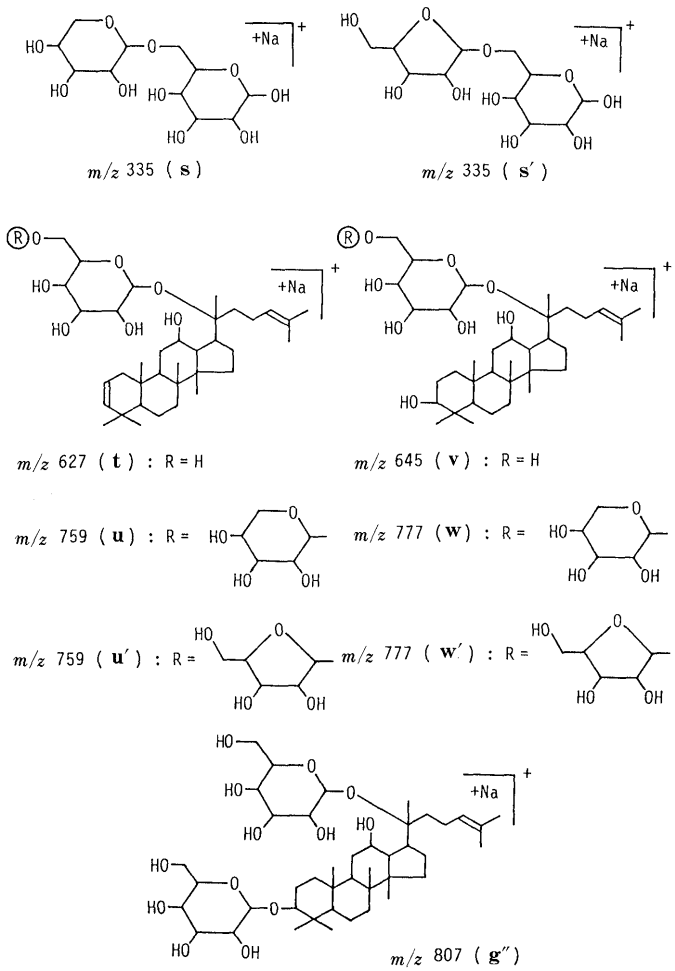


Chart 5

Ro (0.68 g). Fraction 3 (5.8 g) was first purified by column chromatography [SiO_2 600 g, $CHCl_3$–MeOH–H_2O (6:4:1)] and each separated compound was dissolved in MeOH and treated with Dowex 50W×8 (H^+ form). After removal of the resin by filtration, the solvent was evaporated off to furnish malonyl-ginsenosides Rb_1 (**1**, 2.73 g) and Rd (**7**, 0.40 g), and a mixture (2.47 g) of malonyl-ginsenosides Rb_2 (**3**) and Rc (**5**). The mixture of **3** and **5** was purified further by column chromatography [SiO_2 300 g, 1-BuOH–AcOEt–MeOH–H_2O (4:2:1:1) and subsequent treatment of the separated compounds with Dowex 50W×8 (H^+ form) as described above furnished malonyl-ginsenosides Rb_2 (**3**, 1.37 g) and Rc (**5**, 1.00 g). Fraction 4 (6.7 g) was subjected repeatedly to column chromatography [SiO_2 600 g, $CHCl_3$–MeOH–H_2O (65:35:10, lower phase) and then SiO_2 400 g, 1-BuOH–AcOEt–H_2O (4:1:5, upper phase)] to furnish ginsenosides Rb_1 (**2**, 3.05 g), Rb_2 (**4**, 1.50 g), Rc (**6**, 1.05 g), and Rd (**8**, 0.45 g).

Malonyl-ginsenoside Rb_1 (**1**), mp 150–152 °C (colorless fine crystals from aqueous EtOH), $[\alpha]_D^{20}$ +10.2° (c=1.0, MeOH). *Anal.* Calcd for $C_{57}H_{94}O_{26}\cdot 3H_2O$: C, 54.80; H, 8.06. Found: C, 54.55; H, 8.41. IR ν_{max}^{KBr} cm^{-1}: 3489 (br), 2925, 1730, 1628, 1380, 1071. Liquid SIMS: Fig. 2.

Malonyl-ginsenoside Rb_2 (**3**), mp 148—150 °C (colorless fine crystals from aqueous EtOH), $[\alpha]_D^{20}$ +11.5° (c=0.9, MeOH). *Anal.* Calcd for $C_{56}H_{92}O_{25}\cdot 2H_2O$: C, 55.99; H, 8.05. Found: C, 55.83; H, 8.14. IR ν_{max}^{KBr} cm^{-1}: 3381 (br), 2928, 1730, 1628, 1380, 1067. Liquid SIMS: Fig. 5.

Malonyl-ginsenoside Rc (**5**), mp 150—152 °C (colorless fine crystals from aqueous EtOH), $[\alpha]_D^{20}$ +1.7° (c=1.0, MeOH). *Anal.* Calcd for $C_{56}H_{92}O_{25}\cdot H_2O$: C, 56.84; H, 8.01. Found: C, 56.82; H, 8.11. IR ν_{max}^{KBr} cm^{-1}: 3381 (br), 2934, 1733, 1630, 1381, 1071. Liquid SIMS: Fig. 6.

Malonyl-ginsenoside Rd (**7**), mp 158—161 °C (colorless fine crystals from aqueous EtOH), $[\alpha]_D^{20}$ +16.4° (c=0.5, MeOH). *Anal.* Calcd for $C_{51}H_{84}O_{21}\cdot 2H_2O$: C, 57.29; H, 8.30. Found: C, 57.27; H, 8.55. IR ν_{max}^{KBr} cm^{-1}: 3383 (br), 2937, 1738, 1632, 1383, 1074. Liquid SIMS: Fig. 7.

Alkaline Hydrolysis of Malonyl-ginsenoside Rb_1 (1) A solution of **1** (150 mg) in MeOH (5 ml) was treated with 5% KOH–MeOH (0.2 ml) and the whole mixture was stirred at room temperature (22 °C) for 30 min. The reaction mixture was neutralized with Dowex 50W×8 (H^+ form) and the resin was removed by filtration. Removal of the solvent from the filtrate under reduced pressure gave a product which was purified by column chromatography with reversed-phase silica gel (Bondapak C_{18} 5 g, H_2O–MeOH (1:1)→MeOH] to furnish ginsenoside Rb_1 (**2**, 114 mg) and a mixture (15 mg) of malonic acid and monomethyl malonate, which was secondarily formed during the neutralization with resin. **2** was determined to be identical with an authentic sample by TLC comparison [$CHCl_3$–MeOH–H_2O (65:35:10, lower phase), 1-BuOH–AcOEt–H_2O (4:1:5, upper phase)], mixed melting-point determination, and IR (KBr) and ^{13}C-NMR spectral comparisons. The mixture containing malonic acid (15 mg) was dissolved in MeOH (2 ml) and treated with excess CH_2N_2–Et_2O. The reaction mixture was left standing for 12 h and the whole was concentrated under reduced pressure. The product was subjected to gas-liquid chromatography (GLC) to identify dimethyl malonate: GLC [5% butane-1,4-diol succinate (BDS) on Uniport B (80—100 mesh); 3 mm × 2 m glass column; N_2 flow rate, 25 ml/min; column temperature, 130 °C; t_R, 3 min 15 s.

Methylation of 1 with Diazomethane A solution of **1** (120 mg) in MeOH (10 ml) was treated with excess CH_2N_2–Et_2O solution and the whole mixture was left standing at room temperature (22 °C) for 30 min, then the reaction was quenched by adding AcOH. Removal of the solvent from the whole mixture under reduced pressure gave a product, which was crystallized from EtOH to furnish **1a** (110 mg).

1a: mp 178—182 °C (colorless fine crystals), $[\alpha]_D^{20}$ +9.8° (c=0.7, MeOH). *Anal.* Calcd for $C_{58}H_{96}O_{26}\cdot 3H_2O$: C, 55.14; H, 8.14. Found: C, 54.93; H, 8.42. IR ν_{max}^{KBr} cm^{-1}: 3402 (br), 2926, 1737, 1627, 1072. ^{1}H-NMR (pyridine-d_5, δ): 0.87 (3H), 0.98 (9H), 1.15, 1.34 (3H each) (all s, *tert* $CH_3\times 6$), 1.61, 1.67 (3H each, all s, vinyl.$CH_3\times 2$), 3.63 (3H, s, $COOCH_3$), 3.69 (2H, s, –$COCH_2CO$–). ^{13}C-NMR: Table I. Liquid SIMS: Fig. 2.

Enzymatic Hydrolysis of 1a with β-Glucosidase A solution of **1a** (100 mg) in water (5 ml) was treated with β-glucosidase (100 mg, from almond, Sigma) and the whole mixture was stirred at 37 °C for 3 d. The reaction mixture was diluted with water and the whole was extracted with 1-BuOH. After removal of the solvent under reduced pressure, the product was purified by column chromatography [SiO_2 10 g, $CHCl_3$–MeOH–H_2O (7:3:1, lower phase)] to furnish **7a** (45 mg) and **1a** (37 mg) (recovered).

7a: mp 182—183 °C (colorless fine crystals from EtOH), $[\alpha]_D^{20}$ +20.8° (c=0.8, MeOH). *Anal.* Calcd for $C_{52}H_{86}O_{21}\cdot 2H_2O$: C, 57.66; H, 8.37. Found: C, 57.55; H, 8.40. IR ν_{max}^{KBr} cm^{-1}: 3390 (br), 2933, 1744, 1627, 1077. ^{1}H-NMR (pyridine-d_5, δ): 0.86 (3H), 0.96 (9H), 1.14, 1.33 (3H each) (all s, *tert* $CH_3\times 6$), 1.59 (6H, s, vinyl. $CH_3\times 2$), 3.63 (3H, s, $COOCH_3$), 3.69 (2H, s, –$COCH_2CO$–). ^{13}C-NMR: Table I. Liquid SIMS: Fig. 7.

Alkaline Hydrolysis of 7a A solution of **7a** (40 mg) in MeOH (3 ml) was treated with 5% KOH–MeOH (0.1 ml) and the whole mixture was stirred at room temperature (22 °C) for 30 min. The reaction mixture was neutralized with Dowex 50W×8 (H^+ form) and filtered. After removal of the solvent from the filtrate under reduced pressure, the product was purified by reversed-phase silica gel column chromatography [Bondapak C_{18} 3 g, H_2O–MeOH (1:1)→MeOH] to furnish ginsenoside Rd (**8**, 34 mg) and a mixture of malonic acid and monomethyl malonate (4 mg). **8** was found to be identical with an authentic sample by TLC comparison (under same conditions as described above for identification of **2**), mixed melting-point determination, and IR (KBr) and ^{13}C-NMR (pyridine-d_5) spectral comparisons. The mixture of malonic acid and monomethyl malonate (4 mg) was dissolved in MeOH (1 ml) and the whole mixture was treated with excess CH_2N_2–Et_2O for 12 h. The product was subjected to GLC to identify dimethyl malonate (as described above in connection with the alkaline hydrolysis of **1**).

Partial Hydrolysis of 1a with Aqueous AcOH A solution of **1a** (450 mg) in 40% aqueous AcOH (15 ml) was stirred at 75 °C for 3 h. The reaction mixture was diluted with water and the whole was extracted with a 1:1 mixture of 1-BuOH and AcOEt. The 1-BuOH–AcOEt extract was washed with water and then dried over $MgSO_4$. After removal of the solvent from the extract under reduced pressure, the product was purified by column chromatography [SiO_2 20 g, $CHCl_3$–MeOH–H_2O (15:3:1, lower phase)] to furnish **9a** (104 mg).

9a: IR ν_{max}^{KBr} cm^{-1}: 3386 (br), 2939, 1740, 1627, 1076. ^{1}H-NMR (pyridine-d_5, δ): 0.87 (3H), 0.98 (9H), 1.15, 1.35 (3H, each) (all s, *tert* $CH_3\times 6$), 1.67 (6H, s, vinyl. $CH_3\times 2$), 3.63 (3H, s, $COOCH_3$), 3.69 (2H, s, –$COCH_2CO$–). ^{13}C-NMR: Table I. Liquid SIMS (Xe^+, glycerol matrix, m/z): 907 $(M+Na)^+$, 889 (**f**), 821 (**d**), 465 (**b**), 263 (**o**).

Alkaline Hydrolysis of 9a A solution of **9a** (50 mg) in MeOH (3 ml) was treated with 5% KOH–MeOH (0.2 ml) and the whole mixture was stirred at room temperature (20 °C) for 30 min. The reaction mixture was

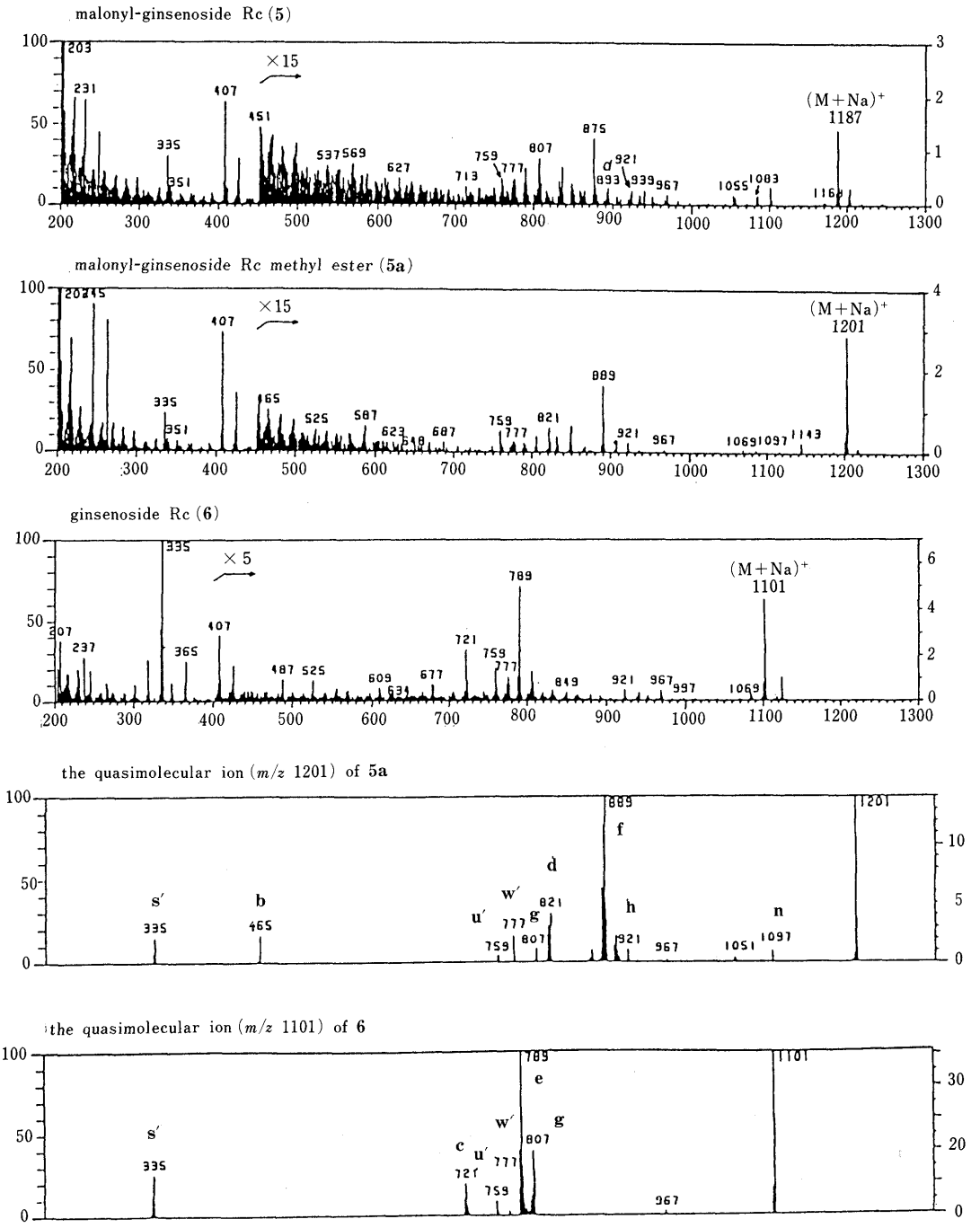


Fig. 6. Liquid SIMS and MI Spectra of **5**, **5a**, and **6**

neutralized with Dowex 50W × 8 (H^+ form) and filtered. The product, obtained by removal of the solvent from the filtrate under reduced pressure, was purified by column chromatography [Bondapak C_{18} 3 g, H_2O–MeOH (1 : 1)→MeOH] to furnish 20 (*R*, *S*)-ginsenoside Rg_3 (**10**, 45 mg) and a mixture of malonic acid and monomethyl malonate (5 mg). **10** was found to be identical with an authentic sample by TLC [$CHCl_3$—MeOH–H_2O (7 : 3 : 1, lower phase), 1-BuOH–AcOEt–H_2O (4 : 1 : 5, upper phase)] and ^{13}C-NMR (pyridine-d_5) spectral comparisons. The mixture of malonic acid and monomethyl malonate was derived to dimethyl malonate with CH_2N_2–Et_2O as described above in connection with the alkaline hydrolysis of **7a**. Dimethyl malonate thus obtained was found to be identical with an authentic sample by GLC comparison (as described above).

Monomethoxytritylation of 9a A solution of **9a** (80 mg) in pyridine (5 ml) was treated with MMTrCl (100 mg) and the whole mixture was stirred under an N_2 atmosphere at room temperature (22 °C) for 18 h. The reaction mixture was diluted with water and the whole was extracted with AcOEt. After work-up of the AcOEt extract in the usual manner, the product was purified by column chromatography [SiO_2 10 g, $CHCl_3$–MeOH (10 : 1)] to furnish **9b** (72 mg).

9b: IR ν_{max}^{KBr} cm^{-1}: 3384 (br), 2937, 1748, 1604, 1505, 1074. ^{1}H-NMR ($CDCl_3$, δ): 3.73 (2H, s, $-COCH_2CO-$), 3.80 (6H, s, OCH_3, $COOCH_3$), 6.70—7.40 (14H, m, aromatic H).

Alkaline Treatment of 9b Followed by Complete Methylation A solution of **9b** (72 mg) in MeOH (3 ml) was treated with 5% KOH–MeOH (0.1 ml) and the whole mixture was stirred at room temperature (22 °C) for 30 min. The reaction mixture was neutralized with Dowex 50W × 8 (H^+ form) and the resin was removed by filtration. The filtrate was worked up as described above for alkaline treatment of **7a**. The product (50 mg), obtained by removal of the solvent under reduced pressure, was dissolved in DMSO (4 ml) and treated with dimsyl carbanion (4 ml). The reaction mixture was stirred under an N_2 atmosphere at room temperature (20 °C) for 1 h and then treated with CH_3I (2 ml) with stirring in the dark for 3 h. The reaction mixture was poured into ice water and the whole was extracted with AcOEt. The AcOEt extract was washed with 10% aqueous $Na_2S_2O_3$ and water, then dried over $MgSO_4$. Removal of the solvent from

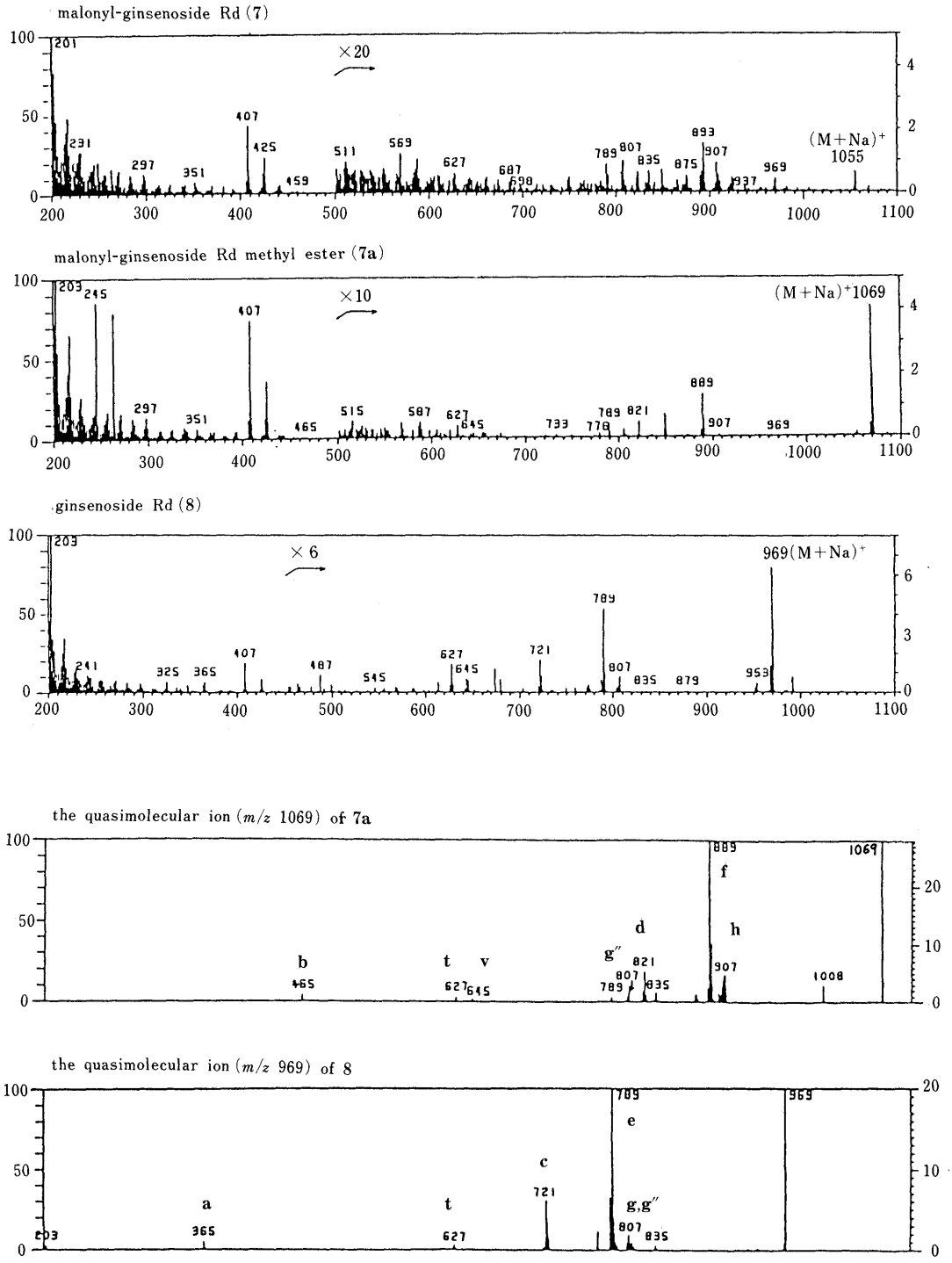


Fig. 7. Liquid SIMS and MI Spectra of **7**, **7a**, and **8**

the extract under reduced pressure gave a product, which was purified by column chromatography [SiO_2 5 g, benzene–acetone (20 : 1)] to furnish **9c** (34 mg).

9c: IR $\nu_{max}^{CCl_4}$ cm^{-1}: 3379 (br), 2920, 1704, 1594, 1497, 1129. ^{1}H-NMR ($CDCl_3$, δ): 3.27, 3.37, 3.52, 3.58, 3.61, 3.63, 3.77 (3H each, all s, $OCH_3 \times 7$), 4.33, 4.69 (1H each, all d, $J=7$ Hz, anomeric H × 2), 5.38 , 5.67 [1/2H each, both br s, D_2O exchangeable, 20 (*S*) and 20 (*R*)-OH], 6.70—7.40 (14H, m, aromatic H).

Methanolysis of 9c A solution of **9c** (5 mg) in 9% HCl–dry MeOH (2 ml) was heated under reflux for 1 h. The reaction mixture was neutralized with Ag_2CO_3 powder and the inorganic precipitate was removed by filtration. After removal of the solvent from the filtrate under reduced pressure, the product was subjected to TLC and GLC analyses to identify methyl 2,3,4,6-tetra-*O*-methylglucopyranoside (**a**) and methyl 3,4-di-*O*-methylglucopyranoside (**b**). TLC: benzene–acetone (2 : 1), *n*-hexane–AcOEt (1 : 2), $CHCl_3$–MeOH (10 : 1). GLC: i) 5% BDS on Uniport B (80—100 mesh); 3 mm × 1 m glass column; N_2 flow rate, 36 ml/min; column temperature, 180 °C. t_R: **a**, 3 min 31 s, 4 min 43 s (major), **b**, 23 min 26 s (major), 27 min 41 s. ii) 15% polyneopentyl glycol succinate (NPGS) on Chromosorb WAW (80—100 mesh); 3 mm × 2 m glass column; N_2 flow rate, 36 ml/min; column temperature, 190 °C. t_R: **a**, 3 min 52 s, 5 min 13 s (major), **b**, 21 min 50 s (major), 25 min 20 s.

Alkaline Hydrolysis of Malonyl-ginsenoside Rb_2 (3) A solution of **3** (30 mg) in MeOH (3 ml) was treated with 5% KOH–MeOH (0.1 ml) and the whole mixture was stirred at room temperature (22 °C) for 30 min. The reaction mixture was neutralized with Dowex 50W × 8 (H^+ form) and filtered. Work-up of the filtrate as described above for the alkaline hydrolysis of **1** furnished the product, which was purified by column chromatography [Bondapak C_{18} 3 g, H_2O–MeOH (1 : 1)→MeOH] to furnish ginsenoside Rb_2 (**4**, 24 mg) and a mixture of malonic acid and

monomethyl malonate (3 mg). **4** was found to be identical with an authentic sample by TLC comparison (under the same conditions as described above for **2**), mixed melting-point determination, and IR (KBr) and ^{13}C-NMR (pyridine-d_5) spectral comparisons. Malonic acid and monomethyl malonate were derived by CH_2N_2 methylation to dimethyl malonate, which was identified by GLC comparison as described above in connection with the alkaline hydrolysis of **1**.

Methylation of 3 with Diazomethane A solution of **3** (50 mg) in MeOH (95 ml) was treated with excess CH_2N_2–Et_2O and the whole mixture was left standing for 30 min. The product, obtained by evaporation of the solvent from the reaction mixture under reduced pressure, was crystallized from EtOH to furnish **3a** (48 mg).

3a: mp 179—182 °C (colorless fine crystals), $[\alpha]_D^{20}$ +11.2° (c=0.6, MeOH). *Anal.* Calcd for $C_{57}H_{94}O_{25}\cdot 2H_2O$: C, 56.33; H, 8.13. Found: C, 56.13; H, 8.32. IR ν_{max}^{KBr} cm^{-1}: 3382 (br), 2940, 1739, 1628, 1074. ^{1}H-NMR (pyridine-d_5, δ): 0.91 (3H), 0.98 (9H), 1.14, 1.34 (3H each) (all s, *tert* $CH_3\times 6$), 1.63, 1.66 (3H each, both s, vinyl. $CH_3\times 2$), 3.63 (3H, s, $COOCH_3$), 3.70 (2H, s, $-COCH_2CO-$). ^{13}C-NMR: Table I. Liquid SIMS: Fig. 5.

Partial Hydrolysis of 3a with Aqueous AcOH A solution of **3a** (90 mg) in 40% aqueous AcOH (5 ml) was stirred at 75 °C for 30 min. The reaction mixture was diluted with water and the whole was extracted with 1-BuOH–AcOEt (1 : 1). Work-up of the 1-BuOH–AcOEt extract as described above for partial acidic hydrolysis of **1a** gave a product, which was purified by column chromatography [SiO_2 5 g, $CHCl_3$–MeOH–H_2O (15 : 3 : 1, lower phase)] to furnish **9a** (34 mg). **9a** was found to be identical with an authentic sample by TLC [$CHCl_3$–MeOH–H_2O (7 : 3 : 1, lower phase) and 1-BuOH–AcOEt–H_2O (1 : 1 : 1, upper phase)] and ^{13}C-NMR (pyridine-d_5) spectral comparisons.

Alkaline Hydrolysis of Malonyl-ginsenoside Rc (5) A solution of **5** (40 mg) in MeOH (3 ml) was treated with 5% KOH–MeOH (0.1 ml) and the whole mixture was stirred at room temperature (22 °C) for 30 min. The reaction mixture was neutralized with Dowex 50W×8 (H^+ form) and filtered. Work-up of the filtrate as described above in connection with the alkaline hydrolysis of **1** furnished a product, which was purified by column chromatography [Bondapak C_{18} 3 g, H_2O–MeOH (1 : 1)→MeOH] to furnish ginsenoside Rc (**6**, 32 mg) and a mixture of malonic acid and monomethyl malonate (5 mg). **6** thus obtained was shown to be identical with an authentic sample by TLC comparison (as described above for **2**), mixed melting-point determination, and IR (KBr) and ^{13}C-NMR (pyridine-d_5) spectral comparisons. Malonic acid and monomethyl malonate were derived by CH_2N_2 methylation to dimethyl malonate, which was found to be identical with an authentic sample by GLC comparison as described above in connection with the alkaline hydrolysis of **1**.

Methylation of 5 with Diazomethane A solution of **5** (50 mg) in MeOH (5 ml) was treated with excess CH_2N_2–Et_2O and the whole mixture was left standing for 30 min. Work-up of the reaction mixture as described above in the diazomethane methylation of **1** furnished a product, which was crystallized from EtOH to furnish **5a** (48 mg).

5a: mp 159—163 °C (colorless fine crystals), $[\alpha]_D^{20}$ +1.6° (c=0.8, MeOH). *Anal.* Calcd for $C_{57}H_{94}O_{25}\cdot 2H_2O$: C, 56.33; H, 8.13. Found: C, 56.25; H, 8.11. IR ν_{max}^{KBr} cm^{-1}: 3392 (br), 2945, 1736, 1638, 1071. ^{1}H-NMR (pyridine-d_5, δ): 0.80 (3H), 0.96 (9H), 1.12, 1.32 (3H each) (all s, *tert* $CH_3\times 6$), 1.63, 1.67 (3H each, both s, vinyl. $CH_3\times 2$), 3.65 (3H, s, $COOCH_3$), 3.75 (2H, s, $-COCH_2CO-$). ^{13}C-NMR: Table I. Liquid SIMS: Fig. 6.

Partial Hydrolysis of 5a with Aqueous AcOH A solution of **5a** (100 mg) in 40% aqueous AcOH (5 ml) was stirred at 75 °C for 3 h. The reaction mixture was worked up as described above in connection with the partial acidic hydrolysis of **1a** and the product was purified by column chromatography [SiO_2 5 g, $CHCl_3$–MeOH–H_2O (15 : 3 : 1, lower phase)] to furnish **9a** (40 mg). **9a** was found to be identical with an authentic sample by TLC (as described above) and ^{13}C-NMR (pyridine-d_5) spectral comparisons.

Methylation of 7 with Diazomethane A solution of **7** (40 mg) in MeOH (5 ml) was treated with excess CH_2N_2–Et_2O and the whole mixture was left standing for 30 min. Work-up of the reaction mixture as described above for the diazomethane methylation of **1** gave a product, which was purified by crystallization from EtOH to furnish **7a** (40 mg). **7a** was shown to be identical with an authentic sample obtained above by β-glucosidase hydrolysis of **1a** by TLC comparison [as described above for **1a**], mixed melting-point determination, and IR (KBr) and ^{13}C-NMR (pyridine-d_5) spectral comparisons.

Treatment of Malonyl-ginsenoside Mixture with Aqueous AcOH A solution of a malonyl-ginsenoside mixture (100 mg) in 40% aqueous AcOH (5 ml) was stirred at 75 °C for 3 h. The reaction mixture was worked up as described above for the partial hydrolysis of **1a** and the product was purified by column chromatography [Bondapak C_{18} 10 g, MeOH–H_2O (1 : 1)→MeOH] to furnish **9** (35 mg).

9: IR ν_{max}^{KBr} cm^{-1}: 3390 (br), 2937, 1733, 1625, 1070. Liquid SIMS (Xe^+, glycerol matrix, m/z): 893 $(M+Na)^+$.

Treatment of Malonyl-ginsenoside Mixture with Artificial Gastric Juice A solution of malonyl-ginsenoside mixture (90 mg) in artificial gastric juice[14] (10 ml) was stirred at 40 °C for 2 d. The reaction mixture was neutralized with saturated aqueous $NaHCO_3$ and the whole mixture was purified by column chromatography [Bondapak C_{18} 10 g, MeOH–H_2O (1 : 1)→MeOH] to furnish **9** (40 mg).

References and Notes

1) Part V: I. Kitagawa, T. Taniyama, H. Shibuya, T. Noda, and M. Yoshikawa, *Yakugaku Zasshi*, **107**, 495 (1987).
2) *a*) O. Tanaka, "Metabolism and Disease," Vol. 10 (Special Issue for Wakan-yaku), Nakayama Shoten, Tokyo, 1973, pp. 548—555; *b*) B. H. Han, "Korean Ginseng," ed. by H. W. Base, Korean Ginseng Research Institute, Seaul, 1978, pp. 77—108; *c*) S. Shibata, *Gendai Tōyō Igaku*, **3**, No. 3, 62 (1982).
3) *a*) J. Shoji, "Panax Ginseng," ed. by H. Oura, A. Kumagai, S. Shibata, and K. Takagi, Kyoritsu Shuppan, Tokyo, 1981, pp. 10—41; *b*) O. Tanaka, *ibid.*, pp. 42—58; *c*) O. Tanaka and R. Kasai, "Progress in the Chemistry of Organic Natural Products," ed. by W. Herz, H. Grisebach, G. W. Kirby, and Ch. Tamm, Springer-Verlag, Vienna, New York, 1984, pp. 1—76; *d*) O. Tanaka, *Yakugaku Zasshi*, **105**, 323 (1985).
4) *a*) I. Kitagawa, Z. L. Chen, M. Yoshihara, and M. Yoshikawa, *Yakugaku Zasshi*, **104**, 848 (1984); *b*) I. Kitagawa, Z. L. Chen, M. Yoshihara, K. Kobayashi, M. Yoshikawa, N. Ono, and Y. Yoshimura, *ibid.*, **104**, 858 (1984); *c*) I. Kitagawa, Z. L. Chen, M. Yoshihara, and M. Yoshikawa, *ibid.*, **104**, 867 (1984); *d*) I. Kitagawa, Y. Fukuda, T. Taniyama, and M. Yoshikawa, *Chem. Pharm. Bull.*, **34**, 1399 (1986); *e*) M. Yoshikawa, Y. Fukuda, T. Taniyama, and I. Kitagawa, *ibid.*, **34**, 1403 (1986); *f*) M. Yoshikawa, Y. Fukuda, T. Taniyama, B. C. Cha, and I. Kitagawa, *ibid.*, **34**, 2294 (1986); *g*) I. Kitagawa and M. Yoshikawa, *Gendai Tōyō Igaku*, **7**, No. 3, 55 (1986).
5) *a*) I. Kitagawa, *The Ginseng Review*, **1**, 21 (1983); *b*) I. Kitagawa, Proceedings of the 4th International Ginseng Symposium, Daejeon, Korea, Sept. 1984, Symposium Paper, pp. 159—168; *c*) I. Kitagawa and M. Yoshikawa, *Gendai Tōyō Igaku*, **6**, No. 4, 101 (1985).
6) I. Kitagawa, M. Yoshikawa, M. Yoshihara, T. Hayashi, and T. Taniyama, *Yakugaku Zasshi*, **103**, 612 (1983).
7) *a*) S. Odashima, T. Ohta, H. Kohno, T. Matsuda, I. Kitagawa, H. Abe, and S. Arichi, *Cancer Res.*, **45**, 2781 (1985); *b*) T. Ota, K. Fujiwara-Yamamoto, Z. Zong, M. Yamazaki, S. Odashima, I. Kitagawa, H. Abe, and S. Arichi, *ibid.*, **47**, 3863 (1987).
8) This work was partly reported in our preliminary communications: *a*) I. Kitagawa, T. Taniyama, T. Hayashi, and M. Yoshikawa, *Chem. Pharm. Bull.*, **31**, 3353 (1983); *b*) Y. Ikenishi, K. Iwatani, Y. Nakagawa, and I. Kitagawa, "Secondary Ion Mass Spectrometry," Proceedings of the 4th International Conference, Osaka, Japan, Nov. 1983, ed. by A. Benninghoven, J. Okano, R. Shimizu, H. W. Werner, Springer-Verlag, Berlin, Heidelberg, New York, Tokyo, 1984, pp. 415—418.
9) A. P. Bruins, K. R. Jennings, and S. Evans, *Int. J. Mass Spectrom. Ion Phys.*, **26**, 395 (1978).
10) S. Hakomori, *J. Biochem.* (Tokyo), **55**, 205 (1964).
11) *a*) H. Kimata, N. Sumida, N. Matsufuji, T. Morita, K. Ito, N. Yata, and O. Tanaka, *Chem. Pharm. Bull.*, **33**, 2849 (1985); *b*) Y. Sasaki, K. Mizutani, R. Kasai, and O. Tanaka, *ibid.*, **36**, 3491 (1988).
12) I. Kitagawa and T. Taniyama, to be published.
13) Total ginsenoside was obtained from the water-soluble portion by column chromatography [Sep-Pak C_{18} (Waters), H_2O→40% aqueous MeOH→MeOH].
14) Nippon Koteisho Kyokai ed., "The Comment Book of Pharmacopoeia Japonica Edition Undecima," Hirokawa Publishing Co., Tokyo, 1986, p. B-349.