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MALONYL-GINSENOSIDES Rb_1, Rb_2, Rc, AND Rd, FOUR NEW MALONYLATED DAMMARANE-TYPE TRITERPENE OLIGOGLYCOSIDES FROM GINSENG RADIX

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Four new malonylated dammarane-type triterpene oligoglycosides, named malonyl-ginsenosides Rb_1 (1), Rb_2 (2), Rc (3), and Rd (4), were isolated from Ginseng Radix, the root of *Panax ginseng*, and their structures were elucidated on the basis of chemical and physicochemical evidence.

KEYWORDS —— Ginseng Radix; malonyl-ginsenoside Rb_1; malonyl-ginsenoside Rb_2; malonyl-ginsenoside Rc; malonyl-ginsenoside Rd; dammarane-type triterpene oligoglycoside; ^{13}C-NMR of ginsenoside; SIMS of malonylated triterpene oligoglycoside

Ginseng Radix, the root of *Panax ginseng* C. A. Meyer (Araliaceae), is one of the best known Chinese crude drugs, and it has been investigated extensively in search of its bioactive principles.[1] Especially the oligoglycosidic constituents, which are the principal ingredients of Ginseng Radix, have been the subjects of many investigations and various ginsenosides have been characterized.[1,2]

In the course of our chemical studies on the precession of naturally occurring drug materials, we have compared the chemical constituents of Ginseng Radix (white ginseng) and Ginseng Radix Rubra (red ginseng) of various origins. We have found that red ginseng contains several characteristic bioactive compounds, *e.g.* antitumor-active ginsenoside Rh_2 in its lipophilic portion, and have elucidated their chemical structures.[3] In continuing studies, we have been examining the water-soluble constituents of both white and red ginseng and have found that white ginseng contains a considerable amount of four new malonylated ginsenosides named malonyl-ginsenosides Rb_1 (1), Rb_2 (2), Rc (3), and Rd (4) together with already known ginsenosides. It should be mentioned here that red ginseng contains only a trace amount of these malonyl-ginsenosides. This paper deals with the evidence for their chemical structures.

The aq. 80% MeOH extract (prepared at 25°C) of white ginseng (cultivated for 6 years at Nagano Prefecture)[4] was partitioned into an ether-water mixture. Purification of the water soluble portion by reversed phase SiO_2 column chromatography (Bondapak C_{18}; MeOH-H_2O) furnished total ginsenoside (6.21% from white ginseng).

Repeated separation of total ginsenoside by reversed-phase and ordinary-phase SiO_2 column chromatography furnished, together with known ginsenosides,[5] malonyl-ginsenoside Rb_1 (1, 0.82%), malonyl-ginsenoside Rb_2 (2, 0.41%), malonyl-ginsenoside Rc (3, 0.30%), and malonyl-ginsenoside Rd (4, 0.12%).[6]

Upon alkaline hydrolysis of malonyl-ginsenoside Rb_1 (1), mp 150-152°C, $[\alpha]_D^{23}$ +10.2° (MeOH), $C_{57}H_{94}O_{26}\cdot 3H_2O$,[7] IR (KBr): 3489, 1730 cm^{-1}, Secondary Ion MS (SIMS, Xe^+, glycerol matrix, *m/z*): 1217 $[(M+Na)^+]$, furnished ginsenoside Rb_1 (1b) and malonic acid. Methylation of 1 with ethereal CH_2N_2 in MeOH provided the monomethyl ester (1a), mp 178-182°C, $[\alpha]_D^{23}$ +9.8° (MeOH), $C_{58}H_{96}O_{26}\cdot 3H_2O$, IR (KBr): 3402, 1737 cm^{-1}, ^{1}H-NMR (d_5-pyr., δ): 0.87 (3H), 0.98 (9H), 1.15, 1.34 (3H each) (all s, *tert*-CH_3 x 6), 1.61, 1.67 (3H each, both s, vinyl. CH_3 x 2), 3.63 (3H, s, $COOCH_3$), 3.69 (2H, s, $-COCH_2CO-$), SIMS (*m/z*): 1231 $[(M+Na)^+]$. Enzymatic hydrolysis of 1a with β-glucosidase (from almond, Sigma) furnished 4a, mp 182-183°C, $[\alpha]_D^{23}$ +20.8° (MeOH), $C_{52}H_{86}O_{21}\cdot 2H_2O$, IR (KBr): 3390, 1744 cm^{-1}, ^{1}H-NMR (d_5-pyr., δ): 0.86 (3H), 0.96 (9H), 1.14, 1.33 (3H each) (all s, *tert*-CH_3 x 6), 1.59 (6H, s, vinyl. CH_3 x 2), 3.63 (3H, s, $COOCH_3$), 3.69 (2H, s, $-COCH_2CO-$), SIMS (*m/z*): 1069 $[(M+Na)^+]$, which, on subsequent alkaline hydrolysis, provided ginsenoside Rd (4b) and malonic acid.

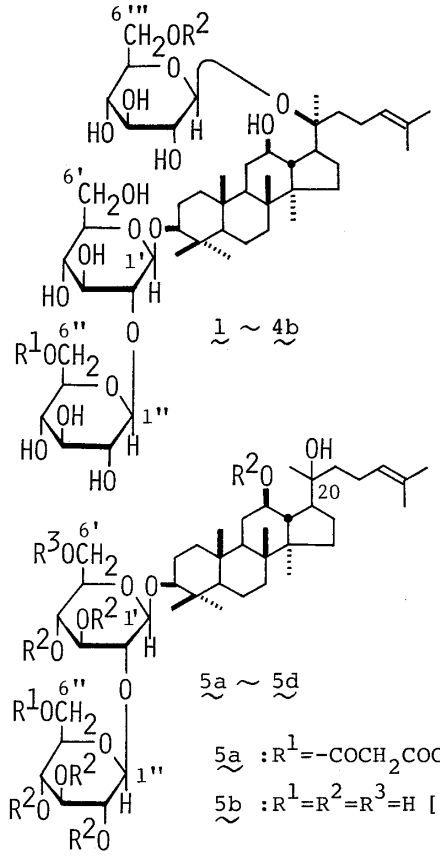
Based on these findings, and the ^{13}C-NMR examinations of 1a and 4a in comparison with the data for 1b and 4b[1b] (Table), malonyl-ginsenoside Rb_1 (1) has been assumed to be a malonylated derivative of ginsenoside Rb_1 (1b) at the primary 6'-OH or 6"-OH.

Partial hydrolysis of 1a with aq. 40% AcOH at 75°C for 3 h[3] furnished 5a (a mixture of 20R and 20S isomers), IR (KBr): 3386, 1740 cm^{-1}, ^{1}H-NMR (d_5-pyr., δ): 0.87 (3H), 0.98 (9H), 1.15, 1.35 (3H each) (all s, *tert*-CH_3 x 6), 1.67 (6H, s, vinyl. CH_3 x 2), 3.63 (3H, s, $COOCH_3$), 3.69 (2H, s, $-COCH_2CO-$), SIMS (*m/z*): 907 $[(M+Na)^+]$, which, on alkaline treatment, liberated 20(R,S)-ginsenoside Rg_3 (5b) and malonic acid. Here again, the location of the malonyl residue in 5a has been assumed to be either at the primary 6-OH or 6"-OH of the diglucoside moiety.

Treatment of 5a with *p*-anisylchlorodiphenylmethane (MMTrCl) at 22°C for 18 h furnished the monoMMTr derivative (5c), IR (KBr): 3384, 1745, 1604, 1505 cm^{-1}, ^{1}H-NMR ($CDCl_3$, δ): 3.73 (2H, s, $-COCH_2CO-$), 3.80 (6H, s, OCH_3, $COOCH_3$), 6.70-7.40 (14 H, m, arom. H), which, on alkaline hydrolysis followed by methylation with CH_3I and dimsyl carbanion,[8] was converted to 5d, IR (CCl_4): 3379 cm^{-1}, ^{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 x 7), 4.33 (1H, d, J=7 Hz), 4.69 (1H, d, J=7 Hz) (anom. H x 2), 6.70-7.40 (14H, m, arom. H). Methanolysis of 5d with 9% HCl-dry MeOH yielded methyl 2,3,4,6-tetra-O-methylglucopyranoside and methyl 3,4-di-O-methylglucopyranoside. Therefore, it has become clear that the MMTr residue in 5d is connected at the 6'-OH.

This proves that the malonyl residues in 4a, 5a, and 5c are at the respective 6"-OH, and consequently the structure of malonyl-ginsenoside Rb_1 (1) has been determined.

The structures of the other three malonyl-ginsenosides have been elucidated in the same way. Upon alkaline hydrolysis, malonyl-ginsenoside Rb_2 (2), mp 148-150° C, $[\alpha]_D^{23}$ +11.5° (MeOH), $C_{56}H_{92}O_{25}\cdot 2H_2O$, IR (KBr): 3381, 1730 cm^{-1}, SIMS (*m/z*): 1187 $[(M+Na)^+]$, provided ginsenoside Rb_2 (2b) and malonic acid. Methylation of 2 with CH_2N_2 as described above for 1 furnished the monomethyl ester (2a), mp 179-182°C,



1 ~ 4b

5a ~ 5d

1 :R^1=-$COCH_2COOH$, R^2=β-D-glucopyranosyl (malonyl-ginsenoside Rb_1)
1a :R^1=-$COCH_2COOCH_3$, R^2=β-D-glucopyranosyl
1b :R^1=H, R^2=β-D-glucopyranosyl (ginsenoside Rb_1)
2 :R^1=-$COCH_2COOH$, R^2=α-L-arabinopyranosyl (malonyl-ginsenoside Rb_2)
2a :R^1=-$COCH_2COOCH_3$, R^2=α-L-arabinopyranosyl
2b :R^1=H, R^2=α-L-arabinopyranosyl (ginsenoside Rb_2)
3 :R^1=-$COCH_2COOH$, R^2=α-L-arabinofuranosyl (malonyl-ginsenoside Rc)
3a :R^1=-$COCH_2COOCH_3$, R^2=α-L-arabinofuranosyl
3b :R^1=H, R^2=α-L-arabinofuranosyl (ginsenoside Rc
4 :R^1=-$COCH_2COOH$, R^2=H (malonyl-ginsenoside Rd)
4a :R^1=-$COCH_2COOCH_3$, R^2=H
4b :R^1=R^2=H (ginsenoside Rd)

5a :R^1=-$COCH_2COOCH_3$, R^2=R^3=H
5b :R^1=R^2=R^3=H [20(R,S)-ginsenoside Rg_3]
5c :R^1=-$COCH_2COOCH_3$, R^2=H, R^3=MMTr
5d :R^1=R^2=CH_3, R^3=MMTr

Table. ^{13}C NMR Data for Malonyl-Ginsenosides (d_5-pyridine, δc)[a]

		1a	2a	3a	4a	5a
Aglycone moiety	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
	C-1'	105.3	104.9	104.8	104.8	104.8
3-O-β-D-Gluco-pyranosyl moiety	C-2'	84.2	84.2[b]	84.1[b]	84.1[b]	84.1[b]
	C-3'	77.9[b]	78.0[b]	77.9[b]	78.4[b]	78.4[b]
	C-4'	71.7	72.1[b]	72.1[b]	71.6[b]	71.4[b]
	C-5'	78.3[b]	78.6[b]	77.9[b]	79.0[b]	77.9[b]
	C-6'	62.8	62.9	62.6	62.8	62.8
	C-1"	106.1	106.1	106.0	106.0	106.0
2'-O-β-D-Gluco-pyranosyl moiety	C-2"	77.0	76.6	76.6	76.6	76.5
	C-3"	79.1[b]	79.1[b]	78.8[b]	79.6[b]	79.6[b]
	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
	C-1'"	98.1	98.1	98.1	98.2	
20-O-β-D-Gluco-pyranosyl moiety	C-2'"	74.9[b]	74.7[b]	75.0[b]	75.1[b]	
	C-3'"	78.3[b]	78.0[b]	78.4[b]	78.1[b]	
	C-4'"	71.7	71.5	71.4	71.6[b]	
	C-5'"	76.6	76.6	76.4	77.9[b]	
	C-6'"	71.6	69.2	68.4	62.8	

		1a	2a	3a	4a	5a
6'"-O-β-D-Gluco-pyranosyl moiety	C-1'""	104.9				
	C-2'""	74.9[b]				
	C-3'""	78.3[b]				
	C-4'""	71.7[b]				
	C-5'""	78.3[b]				
	C-6'""	62.8				
6'"-O-α-L-Arabino-syl moiety	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) The characterizations of *prim*-C, *sec*-C, *tert*-C, and *quat*-C were based on the INEPT (Insensitive Nuclei Enhanced by Polarization Transfer) experiments.

b) The assignments for these signals within the same column may be interchanged.

$[\alpha]_D^{23}$ +11.2° (MeOH), $C_{57}H_{94}O_{25}\cdot 2H_2O$, IR (KBr): 3382, 1739 cm^{-1}: ^{1}H-NMR (d_5-pyr., δ) : 0.91 (3H), 0.98 (9H), 1.14, 1.34 (3H each)(all s, *tert*−CH_3 x 6), 1.63, 1.66 (3H each, both s, vinyl. CH_3 x 2), 3.63 (3H, s, $COOCH_3$), 3.70 (2H, s, $-COCH_2CO-$), SIMS (*m/z*): 1201 [$(M+Na)^+$]. Partial hydrolysis of 2a with aq. AcOH as described above for 1a provided 5a. Finally, the ^{13}C-NMR examination of 2a has led to the formulation of malonyl-ginsenoside Rb_2 as 2.

Malonyl-ginsenoside Rc (3), mp 150-152°C, $[\alpha]_D^{23}$ +1.7° (MeOH), $C_{56}H_{92}O_{25}\cdot H_2O$, IR (KBr): 3381, 1733 cm^{-1}, SIMS (*m/z*): 1187 [$(M+Na)^+$], furnished ginsenoside Rc (3b) and malonic acid upon alkaline hydrolysis. CH_2N_2 methylation of 3 gave the monomethyl ester (3a), mp 159-163°C, $[\alpha]_D^{23}$ +1.6° (MeOH), $C_{57}H_{94}O_{25}\cdot 2H_2O$, IR (KBr): 3392, 1736 cm^{-1}, ^{1}H-NMR (d_5-pyr., δ): 0.80 (3H), 0.96 (9H), 1.12, 1.32 (3H each) (all s, *tert*−CH_3 x 6), 1.63, 1.67 (3H each, both s, vinyl. CH_3 x 2), 3.65 (3H, s, $COOCH_3$), 3.75 (2H, s, $-COCH_2CO-$), SIMS (*m/z*): 1201 [$(M+Na)^+$], which, on partial acidic hydrolysis as described above, gave 5a. Based on these findings and the ^{13}C-NMR data for 3a, the structure of malonyl-ginsenoside Rc (3) has been determined.

CH_2N_2 methylation of malonyl-ginsenoside Rd (4), mp 158-161°C, $[\alpha]_D^{23}$ +16.4° (MeOH), $C_{51}H_{84}O_{21}\cdot 2H_2O$, IR (KBr): 3363, 1738 cm^{-1}: SIMS (*m/z*): 1055 [$(M+Na)^+$], furnished 4a. Thus, the structure of 4 has been substantiated.

We have also analyzed comparatively the oligoglycosidic constituents (total ginsenosides) of various white ginsengs of different origins and of the fresh root of *Panax ginseng* cultivated at Shimane Prefecture. We have found that the above-mentioned malonyl-ginsenosides are commonly distributed in those white ginsengs in considerable amount, but only in trace amounts in red ginsengs of various origins. The biological activity of these malonyl-ginsenosides is an interesting subject for future investigation.

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REFERENCES AND NOTES

1) a) O. Tanaka, "Metabolism and Disease", Vol. 10 (Special Issue for Wakan-Yaku), Nakayama Shoten, Tokyo, 1973, pp. 548-555; b) J. Shoji, "Panax Ginseng", ed. by H. Oura, A. Kumagai, S. Shibata, and K. Takagi, Kyoritsu Shuppan, Tokyo, 1981, pp. 10-41; c) S. Shibata, J. Traditional Sino-Japanese Medicine, 3, 62 (1982).
2) a) H. Besso, R. Kasai, Y. Saruwatari, T. Fuwa, and O. Tanaka, Chem. Pharm. Bull., 30, 2380 (1982); b) H. Koizumi, S. Sanada, Y. Ida, and J. Shoji, ibid., 30, 2393 (1982).
3) I. Kitagawa, M. Yoshikawa, M. Yoshihara, T. Hayashi, and T. Taniyama, Yakugaku Zasshi, 103, 612 (1983).
4) After extraction of the white ginseng 5 times with aq. 80% MeOH at 25°C for 5 h each, we successively extracted the white ginseng with boiling MeOH for 3 h. However, no oligoglycoside was found in this hot MeOH extract.
5) As known ginsenosides, ginsenoside Ro (0.26%), Rb_1 (0.61%), Rb_2 (0.30%), Rc (0.21%), Rd (0.09%), Re (0.82%), Rf (0.22%), and Rg_1 (0.89%) have been isolated so far.
6) The yields were calculated from the white ginseng. According to the usual n-BuOH-H_2O fractionation of the MeOH extract, these malonyl-ginsenosides are mostly fractionated into the water soluble portion.
7) Compounds given with the chemical formulae gave the satisfactory analytical values.
8) S. Hakomori, J. Biochem. (Tokyo), 55, 205 (1964).

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