Acylated Protopanaxadiol-Type Ginsenosides from the Root of *Panax ginseng*

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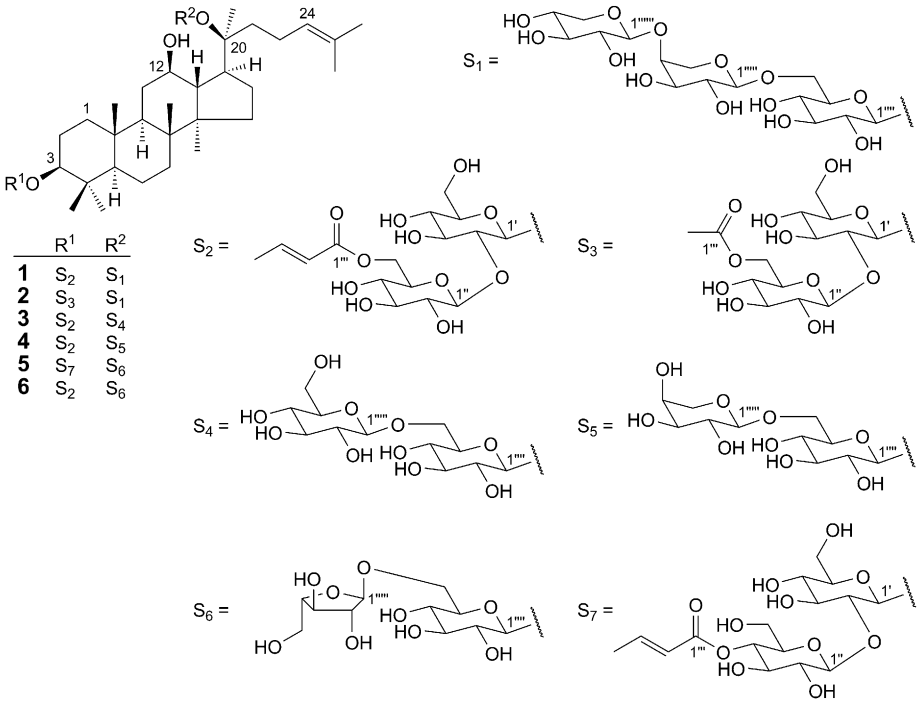
Six new protopanaxadiol-type ginsenosides, named ginsenosides Ra_4–Ra_9 (**1**–**6**, resp.), along with 14 known dammarane-type triterpene saponins, were isolated from the root of *Panax ginseng*, one of the most important Chinese medicinal herbs. The structures of the new compounds were determined by spectroscopic methods, including 1D- and 2D-NMR, HR-MS, and chemical transformation as (20*S*)-3-*O*-{β-D-6-*O*-[(*E*)-but-2-enoyl]glucopyranosyl-(1→2)-β-D-glucopyranosyl}-20-*O*-[β-D-xylopyranosyl-(1→4)-α-L-arabinopyranosyl-(1→6)-β-D-glucopyranosyl]protopanaxadiol (**1**), (20*S*)-3-*O*-[β-D-6-*O*-acetylglucopyranosyl-(1→2)-β-D-glucopyranosyl]-20-*O*-[β-D-xylopyranosyl-(1→4)-α-L-arabinopyranosyl-(1→6)-β-D-glucopyranosyl]protopanaxadiol (**2**), (20*S*)-3-*O*-{β-D-6-*O*-[(*E*)-but-2-enoyl]glucopyranosyl-(1→2)-β-D-glucopyranosyl}-20-*O*-[β-D-glucopyranosyl-(1→6)-β-D-glucopyranosyl]protopanaxadiol (**3**), (20*S*)-3-*O*-{β-D-6-*O*-[(*E*)-but-2-enoyl]glucopyranosyl-(1→2)-β-D-glucopyranosyl}-20-*O*-[α-L-arabinopyranosyl-(1→6)-β-D-glucopyranosyl]protopanaxadiol (**4**), (20*S*)-3-*O*-{β-D-4-*O*-[(*E*)-but-2-enoyl]glucopyranosyl-(1→2)-β-D-glucopyranosyl}-20-*O*-[α-L-arabinofuranosyl-(1→6)-β-D-glucopyranosyl]protopanaxadiol (**5**), (20*S*)-3-*O*-{β-D-6-*O*-[(*E*)-but-2-enoyl]glucopyranosyl-(1→2)-β-D-glucopyranosyl}-20-*O*-[α-L-arabinofuranosyl-(1→6)-β-D-glucopyranosyl]protopanaxadiol (**6**). The sugar moiety at C(3) of the aglycone of each new ginsenoside is butenoylated or acetylated.

Introduction. – *Panax ginseng* C. A. MEYER (Araliaceae) is a well-known oriental medicinal plant that has been used as a general tonic for thousands of years. Previous phytochemical studies on *P. ginseng* led to the isolation of ginsenosides (triterpenoid saponin glycosides) [1–5], polyacetylenes [6][7], sesquiterpenoids [8], flavonoids [9], and polysaccharides [10]. Protopanaxadiol- and protopanaxatriol-type ginsenosides are the major biologically active components of *P. ginseng* that contribute to its diverse pharmacological effects on the central nervous system, cardiovascular system, endocrine secretion, immune-modulation, metabolism, stress, aging, and cancer [11–13]. Due to the continued interests in the chemical diversity and structure–bioactivity relationship of ginsenosides [14][15], we reinvestigated the root of *P. ginseng* and obtained six new acylated protopanaxadiol-type ginsenosides, **1**–**6**, and 14 known protopanaxadiol-type ginsenosides. Here, we report the isolation and structure elucidation of these new acylated protopanaxadiol-type ginsenosides.

Results and Discussion. – The concentrated 70% EtOH extract of *P. ginseng* root was sequentially partitioned with petroleum ether/H_2O, AcOEt/H_2O, and BuOH/H_2O. The BuOH-soluble fraction was subjected to a series of column chromatography on



	R^1	R^2
1	S_2	S_1
2	S_3	S_1
3	S_2	S_4
4	S_2	S_5
5	S_7	S_6
6	S_2	S_6

silica gel, macroporous resin, and reversed-phase *RP18*. After purification by high-performance liquid chromatography (HPLC), six new acylated protopanaxadiol-type ginsenosides, **1**–**6**, along with 14 known ginsenosides, *i.e.*, ginsenosides Ra_1 [1], Ra_2 [1], Rb_1 [3], Rb_2 [4], Rb_3 [16], Rc [16], Rd [17], Rs_1 [5], Rs_2 [5], malonylginsenoside Rb_1 [4], gypenoside XVII [17], pseudoginsenoside RC_1 [18], quinquenoside R_1 [19], vinaginsenoside R_{16} [20], were identified. Known compounds were identified by comparing their MS and NMR-spectroscopic properties with published data.

Compound **1** was isolated as a white amorphous powder with a molecular formula $C_{62}H_{102}O_{27}$ determined by HR-ESI-MS spectrum (*m/z* 1277.6519 ($[M-H]^-$). The IR spectrum of **1** showed strong absorption bands at 3392 and 1078 cm^{-1}, suggesting an oligoglycosidic structure, together with absorption bands at 1716 and 1654 cm^{-1} due to an α,β-unsaturated ester C=O and olefin group. The UV spectrum showed significant absorptions at 210 and 253 nm, which also indicated the presence of α,β-unsaturated ester. The ^{1}H-NMR spectrum of **1** (*Table 1*) displays signals due to eight tertiary Me groups at δ(H) 0.82–1.65, an olefin at δ(H) 5.29, and five anomeric H-atoms at δ(H) 4.89 (*d*, $J=7.2$, H–C(1′)), 5.32 (*d*, $J=7.6$, H–C(1″)), 5.10 (*d*, $J=8.0$, H–C(1‴)), 4.92 (*d*, $J=6.8$, H–C(1⁗)), and 5.09 (*d*, $J=8.0$, H–C(1⁗′)). In the ^{13}C-NMR spectrum of **1**, 30 C-atom signals assigned to the aglycone and five sets of signals due to three β-glucopyranosyl, one α-arabinopyranosyl, and one β-xylopyranosyl groups were observed (*Table 2*). Comparison of the ^{13}C-NMR chemical shifts of the aglycone moiety in **1** with those of (20*S*)-protopanaxadiol and its glycosides [1][3][21] suggests that **1** is a (20*S*)-

protopanaxadiol-type ginsenoside, which was confirmed by HMBC and NOESY analysis (*Figs. 1* and *2*). On comparison of the ^{1}H- and ^{13}C-NMR signals of **1** with those of ginsenoside Ra_1 (**7**) [1], an additional set of signals due to a butenoyl unit at δ(C) 166.7, 123.3, 144.8, and 17.8, and δ(H) 5.96 (*dd*, J=15.6, 1.6, H–C(2‴)), 7.03 (*dq*, J= 15.6, 6.8, H–C(3‴)), and 1.64 (*d*, J=6.8, Me(4‴)) were observed. Based on the typical *AB*-system coupling constant (J=15.6) of H–C(2‴) and H–C(3‴), the configuration of the butenoyl unit was determined as (*E*). HMBC Correlation between H–C(6″) (δ(H) 5.00 and 4.88) and C(1‴) (δ(C) 166.7) as well as a downfield shift for C(6″) (δ(C) 64.4) of the glucosyl unit indicates that the butenoyl group is attached to C(6″) of terminal glucopyranosyl of disaccharide moiety linked at C(3) of the aglycone. The linkage sites and sequences of the sugars and of the aglycone were determined by HMBC long-range correlations H–C(1′)/C(3), H–C(1″)/C(2′), H–C(1‴″)/C(20), H–C(1‴″″)/C(6‴″), and H–C(1‴″″′)/C(4‴″″). Furthermore, the alkaline hydrolysis of **1** with MeONa liberated ginsenoside Ra_1 (**7**) which confirmed that **1** is an acylated

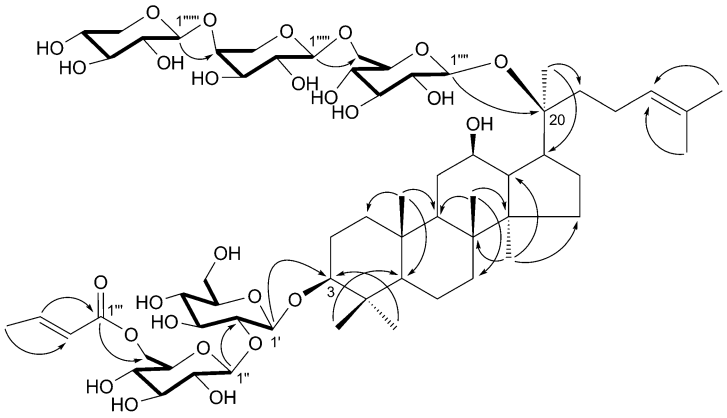


Fig. 1. *Selected ^{1}H,^{1}H-COSY* (—) *and HMBC* (H→C) *correlations of compound* **1**

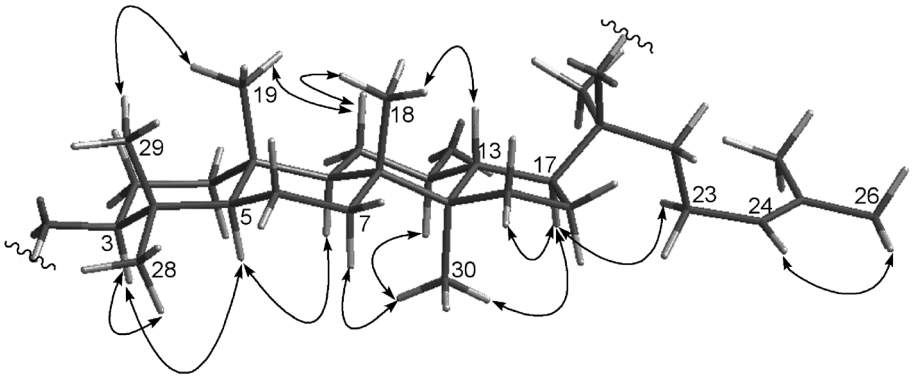


Fig. 2. *Selected NOESY correlations of compound* **1**

Table 1. *^{1}H-NMR* (400 MHz, C_5D_5N) *Data of Compounds* **1**–**6**[a])

	1	**2**	**3**	**4**	**5**	**6**
CH_2(1)	1.54, 0.74	1.55, 0.74	1.54, 0.72 (*t*, *J* = 12.8)	1.52, 0.72 (*t*, *J* = 12.4)	1.52, 0.70 (*t*, *J* = 12.4)	1.51, 0.71 (*t*, *J* = 13.2)
CH_2(2)	2.19, 1.83	2.21, 1.84	2.19, 1.81	2.18, 1.82	2.17, 1.82	2.18, 1.82
H–C(3)	3.25 (*dd*, *J* = 11.2, 4.4)	3.25 (*dd*, *J* = 11.2, 4.4)	3.24 (*dd*, *J* = 11.6, 4.4)	3.24 (*dd*, *J* = 11.6, 4.4)	3.23 (*dd*, *J* = 11.2, 4.4)	3.24 (*dd*, *J* = 11.6, 4.4)
H–C(5)	0.69	0.68	0.67 (*d*, *J* = 10.8)	0.67 (*d*, *J* = 11.6)	0.64 (*d*, *J* = 10.8)	0.66 (*d*, *J* = 11.6)
CH_2(6)	1.55, 1.37	1.54, 1.35	1.52, 1.38	1.51, 1.36	1.50, 1.30	1.51, 1.33
CH_2(7)	1.46, 1.19	1.47, 1.18	1.47, 1.20	1.46, 1.19	1.45, 1.18	1.46, 1.18 (*d*, *J* = 10.8)
H–C(9)	1.36	1.36	1.37	1.35	1.34	1.34
CH_2(11)	1.96, 1.49	1.96, 1.49	1.96, 1.49	1.95, 1.47	1.96, 1.47	1.95, 1.46
H–C(12)	4.19	4.19	4.18	4.18	4.12	4.11
H–C(13)	1.98	1.98	1.99	1.97	1.97	1.96
CH_2(15)	1.56, 0.98	1.56, 0.98	1.55, 0.99	1.56, 0.97	1.55, 0.97	1.55, 0.98 (*t*, *J* = 10.4)
CH_2(16)	1.81, 1.35	1.83, 1.35	1.82, 1.34	1.79, 1.33	1.75, 1.33	1.82, 1.33
H–C(17)	2.58	2.58	2.57	2.55	2.53	2.52
Me(18)	0.94 (*s*)	0.94 (*s*)	0.96 (*s*)	0.94 (*s*)	0.94 (*s*)	0.94 (*s*)
Me(19)	0.82 (*s*)	0.82 (*s*)	0.81 (*s*)	0.81 (*s*)	0.79 (*s*)	0.80 (*s*)
Me(21)	1.62 (*s*)	1.62 (*s*)	1.64 (*s*)	1.62 (*s*)	1.63 (*s*)	1.62 (*s*)
CH_2(22)	2.37, 1.79	2.38, 1.81	2.38, 1.85	2.37, 1.81	2.36, 1.84	2.36, 1.83
CH_2(23)	2.57, 2.35	2.59, 2.36	2.58, 2.36	2.56, 2.35	2.55, 2.33	2.54, 2.32
H–C(24)	5.29	5.29	5.30	5.30	5.30 (*t*, *J* = 6.0)	5.30
Me(26)	1.60 (*s*)	1.60 (*s*)	1.59 (*s*)	1.60 (*s*)	1.60 (*s*)	1.60 (*s*)
Me(27)	1.65 (*s*)	1.65 (*s*)	1.65 (*s*)	1.64 (*s*)	1.65 (*s*)	1.65 (*s*)
Me(28)	1.31 (*s*)	1.31 (*s*)	1.31 (*s*)	1.30 (*s*)	1.24 (*s*)	1.30 (*s*)
Me(29)	1.11 (*s*)	1.11 (*s*)	1.10 (*s*)	1.10 (*s*)	1.08 (*s*)	1.09 (*s*)
Me(30)	0.97 (*s*)	0.97 (*s*)	0.96 (*s*)	0.94 (*s*)	0.93 (*s*)	0.94 (*s*)
	3-Glc	3-Glc	3-Glc	3-Glc	3-Glc	3-Glc
H–C(1′)	4.89	4.90 (*d*, *J* = 7.2)	4.89 (*d*, *J* = 7.8)	4.89	4.89 (*d*, *J* = 7.6)	4.89 (*d*, *J* = 7.2)
H–C(2′)	4.15	4.13	4.14	4.14	4.25	4.14
H–C(3′)	4.28	4.27	4.28	4.29	4.27	4.27
H–C(4′)	4.12	4.13	4.11	4.13	4.11	4.12
H–C(5′)	3.91	3.90	3.92	3.90	3.92	3.90
CH_2(6′)	4.54 (*d*, *J* = 11.6), 4.33	4.55 (*d*, *J* = 11.2), 4.32	4.53 (*d*, *J* = 13.2), 4.32	4.55 (*d*, *J* = 11.2), 4.32	4.56 (*d*, *J* = 11.2), 4.33	4.55 (*d*, *J* = 10.0), 4.34
	2′-Glc	2′-Glc	2′-Glc	2′-Glc	2′-Glc	2′-Glc
H–C(1″)	5.32 (*d*, *J* = 7.6)	5.31 (*d*, *J* = 7.6)	5.31 (*d*, *J* = 8.0)	5.32 (*d*, *J* = 7.6)	5.43 (*d*, *J* = 7.6)	5.31 (*d*, *J* = 8.0)
H–C(2″)	4.13	4.12	4.13	4.13	4.15	4.12

Table 1 (cont.)

	1	**2**	**3**	**4**	**5**	**6**
H–C(3″)	4.18	4.20	4.23	4.22	4.34	4.20
H–C(4″)	4.17	4.12	4.15	4.17	5.79 (*t*, *J* = 9.6)	4.18
H–C(5″)	4.03	4.00	4.02	4.03	3.99	4.04
CH_2(6″)	5.00 (*d*, *J* = 11.6), 4.88	4.93, 4.78 (*dd*, *J* = 11.6, 4.4)	4.99 (*d*, *J* = 10.8), 4.87	4.99, 4.88	4.24, 4.14	4.99 (*d*, *J* = 10.8), 4.86
	6″-Bu	6″-Ac	6″-Bu	6″-Bu	4″-Bu	6″-Bu
H–C(2‴)	5.96 (*dt*, *J* = 15.6, 1.6)	2.03 (*s*)	5.96 (br. *d*, *J* = 15.6)	5.95 (*dt*, *J* = 15.6, 1.6)	5.89 (*dd*, *J* = 15.6, 2.0)	5.95 (*dd*, *J* = 15.6, 1.2)
H–C(3‴)	7.03 (*dq*, *J* = 15.6, 6.8)		7.03 (*dq*, *J* = 15.6, 6.8)	7.03 (*dq*, *J* = 15.6, 6.8)	6.97 (*dq*, *J* = 15.6, 6.8)	7.03 (*dq*, *J* = 15.6, 6.8)
Me(4‴)	1.64		1.65	1.63 (*d*, *J* = 6.8)	1.57 (*d*, *J* = 6.8)	1.64 (*d*, *J* = 6.8)
	20-Glc	20-Glc	20-Glc	20-Glc	20-Glc	20-Glc
H–C(1⁗)	5.10 (*d*, *J* = 8.0)	5.10 (*d*, *J* = 7.6)	5.11 (*d*, *J* = 8.0)	5.12 (*d*, *J* = 8.0)	5.14 (*d*, *J* = 7.6)	5.13 (*d*, *J* = 8.0)
H–C(2⁗)	3.90	3.90	3.91	3.93	3.95	3.96
H–C(3⁗)	4.14	4.14	4.19	4.18	4.18	4.16
H–C(4⁗)	4.00	4.01	4.03	4.03	3.97	3.98
H–C(5⁗)	3.99	4.00	4.03	4.03	4.02	4.00
CH_2(6⁗)	4.69 (*d*, *J* = 11.2), 4.22	4.69 (*d*, *J* = 11.2), 4.22	4.70 (*d*, *J* = 11.6), 4.31	4.68 (*d*, *J* = 10.8), 4.23	4.66 (*d*, *J* = 10.8), 4.10	4.65 (*d*, *J* = 9.6), 4.08
	6⁗-Ara(p)	6⁗-Ara(p)	6⁗-Glc	6⁗-Ara(p)	6⁗-Ara(f)	6⁗-Ara(f)
H–C(1⁗)	4.92	4.92 (*d*, *J* = 6.8)	5.08 (*d*, *J* = 8.0)	4.98 (*d*, *J* = 7.2)	5.65 (br. *s*)	5.65 (br. *s*)
H–C(2⁗)	4.39	4.39	4.03	4.44 (*t*, *J* = 7.2)	4.86	4.86
H–C(3⁗)	4.21	4.21	4.14	4.20	4.79	4.79 (*dd*, *J* = 6.4, 3.6)
H–C(4⁗)	4.35	4.36	4.20	4.35	4.75	4.73
CH_2(5⁗)	4.43, 3.79 (*d*, *J* = 12.0)	4.42, 3.79 (*d*, *J* = 12.0)		4.30, 3.77 (*d*, *J* = 11.2)	4.30, 4.20	4.31, 4.20
H–C(5⁗)			3.90			
CH_2(6⁗)			4.49 (*d*, *J* = 13.2), 4.33			
	4⁗″-Xyl	4⁗″-Xyl				
H–C(1⁗″)	5.09 (*d*, *J* = 8.0)	5.10 (*d*, *J* = 7.6)				
H–C(2⁗″)	4.02	4.03				
H–C(3⁗″)	4.10	4.10				
H–C(4⁗″)	4.17	4.18				
CH_2(5⁗″)	4.29, 3.64 (*t*, *J* = 10.4)	4.29, 3.64 (*t*, *J* = 10.4)				

[a]) Data were assigned on the basis of HSQC, HMBC, ^{1}H,^{1}H-COSY, and NOESY experiments. The chemical shifts are in ppm, and coupling constants (*J* in Hz) are in parentheses. Overlapped signals are reported without designated multiplicities. Glc = Glucopyranosyl; Ara(p) = arabinopyranosyl; Ara(f) = arabinofuranosyl; Xyl = xylopyranosyl.

Table 2. *^{13}C-NMR* (100 MHz, C_5D_5N) *Chemical Shift Assignments for Compounds* **1**–**6**

	1	**2**	**3**	**4**	**5**	**6**
C(1)	39.3	39.2	39.3	39.3	39.2	39.2
C(2)	26.7	26.7	26.7	26.7	26.7	26.7
C(3)	89.3	89.3	89.3	89.2	89.1	89.2
C(4)	39.8	39.8	39.8	39.8	39.7	39.8
C(5)	56.5	56.5	56.5	56.5	56.4	56.5
C(6)	18.6	18.5	18.6	18.6	18.5	18.6
C(7)	35.2	35.2	35.3	35.2	35.2	35.2
C(8)	40.1	40.1	40.1	40.1	40.0	40.1
C(9)	50.3	50.3	50.3	50.3	50.2	50.2
C(10)	37.0	37.0	37.0	37.0	36.9	37.0
C(11)	30.9	30.9	30.8	30.8	30.8	30.8
C(12)	70.2	70.2	70.3	70.2	70.3	70.3
C(13)	49.6	49.6	49.6	49.5	49.5	49.5
C(14)	51.4	51.4	51.5	51.5	51.5	51.5
C(15)	30.8	30.8	30.8	30.8	30.7	30.7
C(16)	26.9	26.9	26.9	26.9	26.7	26.9
C(17)	51.5	51.5	51.7	51.7	51.7	51.7
Me(18)	16.3	16.3	16.4	16.3	16.3	16.3
Me(19)	16.1	16.1	16.1	16.1	16.1	16.0
Me(20)	83.5	83.5	83.6	83.6	83.4	83.4
Me(21)	22.3	22.3	22.5	22.4	22.4	22.4
C(22)	36.2	36.2	36.3	36.2	36.2	36.2
C(23)	23.2	23.2	23.3	23.3	23.2	23.2
C(24)	126.0	126.0	126.0	126.0	126.1	126.1
C(25)	131.2	131.2	131.1	131.2	131.0	131.1
Me(26)	25.9	25.9	25.9	25.9	25.8	25.8
Me(27)	17.9	17.9	18.0	17.9	17.9	17.9
Me(28)	28.0	28.0	28.1	28.0	28.1	28.0
Me(29)	16.6	16.5	16.6	16.6	16.7	16.6
Me(30)	17.5	17.5	17.5	17.4	17.4	17.4
	3-Glc	3-Glc	3-Glc	3-Glc	3-Glc	3-Glc
C(1′)	105.0	105.0	105.0	105.0	105.0	105.0
C(2′)	84.4	84.4	84.5	84.4	83.2	84.4
C(3′)	78.5	78.5	78.6	78.6	78.4	78.6
C(4′)	71.5	71.5	71.5	71.5	71.7	71.5
C(5′)	78.2	78.2	78.0	78.0	78.2	78.0
C(6′)	62.9	62.9	62.9	62.9	62.9	62.9
	2′-Glc	2′-Glc	2′-Glc	2′-Glc	2′-Glc	2′-Glc
C(1″)	106.3	106.3	106.3	106.3	105.7	106.3
C(2″)	76.9	76.8	76.9	76.8	77.1	76.9
C(3″)	78.0	78.0	78.2	78.2	75.4	78.2
C(4″)	71.0	71.1	71.0	71.0	72.4	71.0
C(5″)	75.6	75.5	75.6	75.6	76.3	75.6
C(6″)	64.4	64.8	64.5	64.4	62.2	64.4
	6″-Bu	6″-Ac	6″-Bu	6″-Bu	4″-Bu	6″-Bu
C(1‴)	166.7	171.1	166.7	166.7	166.1	166.7
C(2‴)	123.3	21.0	123.3	123.3	123.2	123.3
C(3‴)	144.8		144.8	144.8	145.2	144.7
Me(4‴)	17.8		17.9	17.8	17.8	17.8

Table 2 (cont.)

	1	**2**	**3**	**4**	**5**	**6**
	20-Glc	20-Glc	20-Glc	20-Glc	20-Glc	20-Glc
C(1‴)	98.1	98.1	98.2	98.2	98.1	98.2
C(2‴)	74.9	74.9	74.9	75.0	75.1	75.1
C(3‴)	79.4	79.4	79.3	79.3	79.3	79.3
C(4‴)	71.9	71.9	71.6	71.8	72.2	72.2
C(5‴)	76.9	76.9	77.1	76.9	76.6	76.6
C(6‴)	69.9	69.8	70.3	69.3	68.5	68.6
	6‴-Ara(p)	6‴-Ara(p)	6‴-Glc	6‴-Ara(p)	6‴-Ara(f)	6‴-Ara(f)
C(1⁗)	105.2	105.2	105.4	104.7	110.2	110.2
C(2⁗)	73.0	73.0	75.3	72.2	83.4	83.4
C(3⁗)	74.0	74.0	78.5	74.2	78.9	78.9
C(4⁗)	78.8	78.8	71.8	68.7	86.0	86.0
C(5⁗)	65.8	65.8	78.4	65.7	62.7	62.7
C(6⁗)			62.9			
	4⁗-Xyl	4⁗-Xyl				
C(1⁗)	107.0	107.1				
C(2⁗)	75.5	75.4				
C(3⁗)	78.6	78.6				
C(4⁗)	71.1	71.1				
C(5⁗)	67.4	67.4				

ginsenoside Ra_1. All signals of the ^{1}H- and ^{13}C-NMR were assigned based on 2D-NMR data including COSY, HSQC, HMBC, and NOESY. On the basis of the above results, the structure of **1** was determined as (20*S*)-3-*O*-{β-D-6-*O*-[(*E*)-but-2-enoyl]glucopyranosyl-(1→2)-β-D-glucopyranosyl}-20-*O*-[β-D-xylopyranosyl-(1→4)-α-L-arabinopyranosyl-(1→6)-β-D-glucopyranosyl]protopanaxadiol, and named ginsenoside Ra_4.

Compound **2** was obtained as a white amorphous powder and showed absorption bands at 3392, 1739, 1636, and 1079 cm^{-1} due to OH, ester, and olefin groups in its IR spectrum. The molecular formula $C_{60}H_{99}O_{27}$ was verified from its negative-ion-mode HR-ESI-MS (*m*/*z* 1251.6356 ($[M-H]^-$)). The alkaline hydrolysis of **2** with MeONa also yielded ginsenoside Ra_1 suggesting that **2** is also an acylated ginsenoside Ra_1. The ^{1}H- and ^{13}C-NMR spectra (*Tables 1* and *2*) of **2** are very similar to those of **1** and ginsenoside Ra_1 [1], except for the signals due to an Ac group at δ(C) 171.1 and 21.0, and δ(H) 2.03 (*s*, 3 H), which suggest that **2** is an acetyl derivative of ginsenoside Ra_1. The location of Ac group was assigned to C(6″) of terminal glucopyranosyl moiety linked at C(3) of the aglycone on the basis of an acetylation shift effect on C-atom signals around C(6″) (δ(C) 64.8) and HMBC correlation between H–C(6″) (δ(H) 4.93 and 4.78) and C(1‴) (δ(C) 171.1). Thus, the structure of **2** was determined as (20*S*)-3-*O*-[β-D-6-*O*-acetylglucopyranosyl-(1→2)-β-D-glucopyranosyl]-20-*O*-[β-D-xylopyranosyl-(1→4)-α-L-arabinopyranosyl-(1→6)-β-D-glucopyranosyl]protopanaxadiol, and named ginsenoside Ra_5.

Compound **3** was isolated as a white amorphous powder. Its molecular formula was established as $C_{58}H_{96}O_{24}$ on the basis of its HR-ESI-MS (*m*/*z* 1175.6223 ($[M-H]^-$), 1221.6277 ($[M+HCOO]^-$)). The alkaline hydrolysis of **3** yielded ginsenoside Rb_1

suggesting that **3** is an acylated ginsenoside Rb_1. The ^1H- and ^{13}C-NMR data (*Tables 1* and *2*) of **3** were found to be identical to those of ginsenoside Rb_1 [3], except for the signals ascribed to a (*E*)-butenoyl group at δ(C) 166.7, 123.3, 144.8, and 17.9, and δ(H) 5.96 (br. *d*, $J = 15.6$, H–C(2‴)), 7.03 (*dq*, $J = 15.6$, 6.8, H–C(3‴)), 1.65 (Me(4‴)). The acyl group was determined to be at C(6″) on the basis of three-bond HMBC correlation between H–C(6″) (δ(H) 4.87 and 4.99) and C(1‴) (δ(C) 166.7), as well as the acylation shift of the C(6″) signal. All signals of the ^1H- and ^{13}C-NMR were assigned based on 2D-NMR techniques; thus **3** was determined as (20*S*)-3-*O*-{β-D-6-*O*-[(*E*)-but-2-enoyl]glucopyranosyl-(1→2)-β-D-glucopyranosyl}-20-*O*-[β-D-glucopyranosyl-(1→6)-β-D-glucopyranosyl]protopanaxadiol and was named ginsenoside Ra_6.

The molecular formula of compound **4** was determined as $C_{57}H_{93}O_{23}$ from the quasimolecular-ion peaks at *m/z* 1145.6084 ($[M-H]^-$) and 1191.6142 ($[M+HCOO]^-$) in its negative-ion-mode HR-ESI-MS. The IR spectrum of **4** showed a typical absorption band at 1716 cm^{-1} due to an α,β-unsaturated ester group which was confirmed by UV absorptions at 210 and 253 nm. The alkaline hydrolysis of **4** with MeONa liberated ginsenoside Rb_2, suggesting that **4** is an ester of ginsenoside Rb_2. The ^1H- and ^{13}C-NMR spectra of **4** (*Tables 1* and *2*) showed signals assignable to a ginsenoside Rb_2 moiety [4] and a (*E*)-butenoyl part. This was fully supported by 2D-NMR data including COSY, HSQC, HMBC, and NOESY. In HMBC spectrum of **4**, a long-range correlation was observed between H–C(6″) (δ(H) 4.99 and 4.87) and C(1‴) (δ(C) 166.7), which suggests that the butenoyl group is at C(6″) of terminal glucopyranosyl in C(3)-linked disaccharide moiety. Accordingly, the structure of **4** was determined as (20*S*)-3-*O*-{β-D-6-*O*-[(*E*)-but-2-enoyl]glucopyranosyl-(1→2)-β-D-glucopyranosyl]-20-*O*-[α-L-arabinopyranosyl-(1→6)-β-D-glucopyranosyl]protopanaxadiol, and was named ginsenoside Ra_7.

Compound **5** and **6** have the same molecular formula, $C_{57}H_{94}O_{23}$, as determined from their HR-ESI-MS data. The alkaline hydrolysis of **5** and **6** yielded the same deacyl product ginsenoside Rc, suggesting that **5** and **6** are the ester derivatives of ginsenoside Rc. Detailed analysis of ^1H- and ^{13}C-NMR spectra (*Tables 1* and *2*) of **5** and **6** revealed that both compounds possess a ginsenoside Rc part and a (*E*)-butenoyl moiety, but there is a difference between the butenoyl moiety linkage position in **5** and **6**. Comparison of the ^{13}C-NMR data (*Table 2*) of **5** with those of ginsenoside Rc [16] revealed an esterification downfield shift at C(4″) (δ(C) 72.4) in **5**, which suggests that the butenoyl group is at C(4″) of the terminal glucopyranosyl of disaccharide moiety linked at C(3) of the aglycone. This was confirmed by the three-bond correlation between H–C(4″) (δ(H) 5.79 (*t*, $J = 9.6$)) and C(1‴) (δ(C) 166.1) in HMBC spectrum of **5**. Hence, compound **5** was characterized as (20*S*)-3-*O*-{β-D-4-*O*-[(*E*)-but-2-enoyl]glucopyranosyl-(1→2)-β-D-glucopyranosyl}-20-*O*-[α-L-arabinofuranosyl-(1→6)-β-D-glucopyranosyl]protopanaxadiol, named ginsenoside Ra_8. On the other hand, the typical acylation downfield shift of C(6″) signal at δ(C) 64.4 in ^{13}C-NMR spectrum of **6** and HMBC correlation between H–C(6″) (δ(H) 4.99 and 4.86) and C(1‴) (δ 166.7) suggests that the butenoyl moiety in **6** is at C(6″) of terminal glucopyranosyl of C(3)-attached disaccharide. Consequently, the structure of **6** was determined as (20*S*)-3-*O*-{β-D-6-*O*-[(*E*)-but-2-enoyl]glucopyranosyl-(1→2)-β-D-glucopyranosyl}-20-*O*-[α-L-arabinofuranosyl-(1→6)-β-D-glucopyranosyl]protopanaxadiol, which is named ginsenoside Ra_9.

Compared to malonyl ginsenosides from *P. ginseng* [22], ginsenosides Ra_4–Ra_9 (**1**–**6**, resp.) reported in this article are very minor acylated ginsenosides. Occurrence of these minor components illustrates the chemical diversity and complexity of saponins in *P. ginseng*.

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Experimental Part

General. Column chromatography (CC): silica gel (SiO_2, 200–300 mesh, *Qingdao Haiyang Chemical Group Co.*, Qingdao, P. R. China), *D101* macroporous resin (*Tianjin Pesticide Co.*, Tianjin, P. R. China), and *RP18* (20–45 μm, *Merck*). TLC: Precoated SiO_2 *60* F_{254} (*Merck*) and *RP18* F_{254} (*Merck*) reversed-phase (RP) plates. Semiprep. HPLC: *Perkin–Elmer Series 200* separation system equipped with *253C* diode array detector and *YMC-Pack ODS-A* semiprep. column (10 μm, 250 × 10 mm) with a flow rate of 5.0 ml/min. Optical rotations: *Jasco P-1010* polarimeter (Na 589 nm). UV Spectra: *Jasco V-530* spectrophotometer; λ_{max} (log ε) in nm. IR Spectra (KBr): *Perkin–Elmer Spectrum One* FT-IR spectrometer; $\tilde{\nu}$ in cm^{-1}. NMR: *Bruker DMX-400* NMR spectrometer; δ in ppm using the lowest field signals of (D_5)pyridine (^1H, δ(H) 8.71; ^{13}C, δ(C) 149.9) as an internal ref., *J* in Hz. 2D-NMR (COSY, HSQC, HMBC, and NOESY): standard *Bruker* potocols. ESI-MS: *Thermo Finnigan LCQ Advantage* mass spectrometer; in *m*/*z*. UPLC/HR-ESI-MS: *Acquity Ultra Performance LC System (Waters)-Bruker micrOTOF* mass spectrometer.

Plant Material. The root of *Panax ginseng* C. A. Meyer was collected in the Jilin Province, P. R. China, in October 2007. Specimens were verified by Prof. *Zhong-Zhen Zhao*, School of Chinese Medicine, Hong Kong Baptist University. A voucher specimen (PG-0710) was deposited with the Research and Development Division, School of Chinese Medicine, Hong Kong Baptist University.

Extraction and Isolation. Air-dried, milled roots of *Panax ginseng* C. A. Meyer (7.0 kg) were extracted with 70% EtOH (3 × 21 l) under reflux, and the extracts were evaporated to obtain a brown residue (*ca.* 1.8 kg). The residue was dissolved in H_2O (7 l) and partitioned successively with petroleum ether (3 × 7 l), AcOEt (3 × 7 l), and BuOH (3 × 7 l) to give a petroleum ether-soluble fraction (110 g), an AcOEt-soluble fraction (120 g), and BuOH-soluble fraction (185 g), resp. The BuOH extract (180 g) was separated by CC (SiO_2; $CHCl_3$/MeOH 10:1 → 3:7). Various fractions were combined according to their TLC behavior to obtain nine fractions, *Frs. A* → *I*. *Fr. D* (17 g) was subjected to CC (SiO_2; AcOEt/MeOH/H_2O 50:8:1; and *RP-18*; MeCN/H_2O 20:80 → 40:60) and finally purified by HPLC (MeCN/H_2O 35:65 → 38:62) to obtain compounds **5** (4.5 mg), **6** (39 mg), ginsenoside Rd (100 mg), ginsenoside Rs_2 (20 mg), pseudoginsenoside RC_1 (6 mg), and vinaginsenoside R_{16} (5.5 mg). *Fr. E* (10 g) was repeatedly purified by CC (SiO_2; AcOEt/MeOH/H_2O 50:8:1; and *RP-18*; MeCN/H_2O 20:80 → 40:60), and HPLC (MeCN/H_2O 35:65) to yield **4** (15 mg), ginsenoside Rs_1 (25 mg), and gypenoside XVII (15 mg). *Fr. F* (14 g) was subjected to CC (SiO_2; AcOEt/MeOH/H_2O 50:10:1; and *RP-18*; MeCN/H_2O 35:65) and finally purified by HPLC (MeCN/H_2O 30:70 → 32:68) to afford **3** (25 mg), ginsenoside Rc (350 mg), and quinquenoside R_1 (8 mg). *Fr. G* (15 g) was subjected to CC (*D101* macroporous resin; H_2O, 20% EtOH, and 50% EtOH). The 50% EtOH fraction was purified by CC (*RP-18*; MeCN/H_2O 35:65) and HPLC (MeCN/H_2O 3:7) to give **2** (6.5 mg), ginsenosides Rb_2 and Rb_3 (90 and 20 mg, resp.). *Fr. H* (45 g) was separated by CC (*D101* macroporous resin; H_2O, 20% EtOH, and 50% EtOH). The 50% EtOH fraction was then purified by CC (*RP-18*; MeCN/H_2O 25:75 → 35:65, MeOH/H_2O 65:35), and finally by HPLC (MeCN/H_2O 27:73 and 38:62) to afford **1** (8 mg), and ginsenosides Ra_1, Ra_2, and Rb_1 (45, 40, and 110 mg, resp.). *Fr. I* (13 g) was separated by CC (*D101* macroporous resin; H_2O, 20% EtOH, and 50% EtOH). The 50% EtOH fraction was then purified by CC (*ODS*; MeCN/H_2O 25:75 → 35:65) and then by HPLC (MeCN/0.1% AcOH 3:7) to obtain malonylginsenoside Rb_1 (10 mg).

Alkaline Hydrolysis of Compounds **1**–**6**. MeOH Solns. of **1**–**6** (*ca.* 0.2 mg/ml each) were hydrolyzed with MeONa (20 mM) for 12 h at r.t. The reaction mixtures of **1a**–**6a** were neutralized with HCOOH

(20 mM) and then subjected to UPLC/HR-ESI-MS analysis under the following conditions: column, *Acquity UPLC BEH C18* (2.1 × 100 mm, 1.7 μm); solvent, 0.1% HCOOH in MeCN (*A*) and 0.1% HCOOH in H_2O (*B*); gradient elution from 10 to 40% *A* over 6 min at a flow rate of 0.35 ml/min; HR-ESI-MS (neg.; *Nebulizer*, 2.0 bar; dry heater, 180°; dry gas, 8.0 l/min; cap., 4000 V; end plate offset, −500 V; and collision cell RF, 550.0 Vpp). The deacyl saponins were identified by comparing their UPLC retention times and HR-MS data with those of authentic samples. Compounds **1a** (t_R 3.954 min; HR-ESI-MS: 1209.6266 ($[M-H]^-$)) and **2a** (t_R 3.948 min; HR-ESI-MS: 1209.6236 ($[M-H]^-$)) were identical to ginsenoside Ra_1 (t_R 3.955 min; HR-ESI-MS: 1209.6274 ($[M-H]^-$, $C_{58}H_{97}O_{26}^-$; calc. 1209.6274). Compound **3a** (t_R 3.862 min; HR-ESI-MS: 1107.5977 ($[M-H]^-$)) was identified as ginsenoside Rb_1 (t_R 3.866 min; HR-ESI-MS: 1107.5956 ($[M-H]^-$, $C_{54}H_{91}O_{23}^-$; calc. 1107.5957). Compound **4a** (t_R 4.078 min; HR-ESI-MS: 1077.5878 ($[M-H]^-$), 1123.5865 ($[M+HCOO]^-$)) was determined as ginsenoside Rb_2 (t_R 4.072 min; HR-ESI-MS: 1077.5867 ($[M-H]^-$, $C_{53}H_{89}O_{22}^-$; calc. 1077.5851), 1123.5877 ($[M+HCOO]^-$, $C_{54}H_{91}O_{24}^-$; calc. 1123.5906)). Compounds **5a** (t_R 3.972 min; HR-ESI-MS: 1077.5873 ($[M-H]^-$), 1123.5883 ($[M+HCOO]^-$)) and **6a** (t_R 3.963 min; HR-ESI-MS: 1077.5854 ($[M-H]^-$), 1123.5863 ($[M+HCOO]^-$)) were determined as ginsenoside Rc (t_R 3.971 min; HR-ESI-MS: 1077.5870 ($[M-H]^-$, $C_{53}H_{89}O_{22}^-$; calc. 1077.5851; and 1123.5876 ($[M+HCOO]^-$, $C_{54}H_{91}O_{24}^-$; calc. 1123.5906).

Ginsenoside Ra4 (=*(20S)-3*-O-*{β*-D-*6*-O-*[(*E*)-But-2-enoyl]glucopyranosyl-(1→2)-β*-D-*glucopyranosyl}-20*-O-*[β*-D-*xylopyranosyl-(1→4)-α*-L-*arabinopyranosyl-(1→6)-β*-D-*glucopyranosyl]protopanaxadiol*=*(3β,12β)-12-Hydroxy-20-{[β*-D-*xylopyranosyl-(1→4)-α*-L-*arabinopyranosyl-(1→6)-β*-D-*glucopyranosyl]oxy}dammar-24-en-3-yl 2*-O-*{6*-O-*[(2*E*)-But-2-enoyl]-β*-D-*glucopyranosyl}-β*-D-*glucopyranoside*; **1**). A white amorphous powder. $[\alpha]_D^{25} = +15.3$ (*c* = 0.3, MeOH). UV (MeOH): 210 (3.84), 252 (3.43). IR (KBr): 3392, 2944, 2878, 1716, 1654, 1552, 1445, 1417, 1385, 1312, 1265, 1165, 1078, 1043, 921, 894, 839, 785, 647, 578, 535. ^{1}H- and ^{13}C-NMR: *Tables 1* and *2*, resp. HR-ESI-MS: 1277.6519 ($[M-H]^-$, $C_{62}H_{101}O_{27}^-$; calc. 1277.6536).

Ginsenoside Ra5 (=*(20S)-3*-O-*[β*-D-*6*-O-*Acetylglucopyranosyl-(1→2)-β*-D-*glucopyranosyl]-20*-O-*[β*-D-*xylopyranosyl-(1→4)-α*-L-*arabinopyranosyl-(1→6)-β*-D-*glucopyranosyl]protopanaxadiol*=*(3β,12β)-12-Hydroxy-20-{[β*-D-*xylopyranosyl-(1→4)-α*-L-*arabinopyranosyl-(1→6)-β*-D-*glucopyranosyl]oxy}dammar-24-en-3-yl 2*-O-*(6*-O-*Acetyl-β*-D-*glucopyranosyl)-β*-D-*glucopyranoside*; **2**). A white amorphous powder. $[\alpha]_D^{25} = +9.5$ (*c* = 0.4, MeOH). IR (KBr): 3392, 2943, 2878, 1739, 1637, 1551, 1453, 1385, 1369, 1307, 1250, 1202, 1164, 1079, 1041, 920, 892, 841, 786, 648, 624, 575, 535. ^{1}H- and ^{13}C-NMR: *Tables 1* and *2*, resp. HR-ESI-MS: 1251.6356 ($[M-H]^-$, $C_{60}H_{99}O_{27}^-$; calc. 1251.6379).

Ginsenoside Ra6 (=*(20S)-3*-O-*{β*-D-*6*-O-*[(*E*)-But-2-enoyl]glucopyranosyl-(1→2)-β*-D-*glucopyranosyl}-20*-O-*[β*-D-*glucopyranosyl-(1→6)-β*-D-*glucopyranosyl]protopanaxadiol*=*(3β,12β)-20-{[6*-O-*(β*-D-*Glucopyranosyl)-β*-D-*glucopyranosyl]oxy}-12-hydroxydammar-24-en-3-yl 2*-O-*{6*-O-*[(2*E*)-But-2-enoyl]-β*-D-*glucopyranosyl}-β*-D-*glucopyranoside*; **3**). A white amorphous powder. $[\alpha]_D^{25} = +17.6$ (*c* = 0.6, MeOH). UV (MeOH): 212 (3.83), 252 (2.87). IR (KBr): 3400, 2944, 2879, 1715, 1656, 1553, 1445, 1384, 1314, 1231, 1168, 1076, 923, 892, 838, 627, 579, 535. ^{1}H- and ^{13}C-NMR: *Tables 1* and *2*, resp. HR-ESI-MS: 1175.6223 ($[M-H]^-$, $C_{58}H_{95}O_{24}^-$; calc. 1175.6219), 1221.6277 ($[M+HCOO]^-$, $C_{59}H_{97}O_{26}^-$; calc. 1221.6274).

Ginsenoside Ra7 (=*3*-O-*{β*-D-*6*-O-*[(*E*)-But-2-enoyl]glucopyranosyl-(1→2)-β*-D-*glucopyranosyl}-20*-O-*[α*-L-*arabinopyranosyl-(1→6)-β*-D-*glucopyranosyl]protopanaxadiol*=*(3β,12β)-20-{[6*-O-*(α*-L-*Arabinopyranosyl)-β*-D-*glucopyranosyl]oxy}-12-hydroxydammar-24-en-3-yl 2*-O-*{6*-O-*[(2*E*)-But-2-enoyl]-β*-D-*glucopyranosyl}-β*-D-*glucopyranoside*; **4**). A white amorphous powder. $[\alpha]_D^{25} = +14.0$ (*c* = 0.6, MeOH). UV (MeOH): 214 (3.83), 252 (3.18). IR (KBr): 3400, 2944, 2878, 1717, 1655, 1552, 1542, 1445, 1384, 1314, 1174, 1078, 921, 889, 863, 839, 780, 647, 579, 536. ^{1}H- and ^{13}C-NMR: *Tables 1* and *2*, resp. HR-ESI-MS: 1145.6084 ($[M-H]^-$, $C_{57}H_{93}O_{23}^-$; calc. 1145.6113), 1191.6142 ($[M+HCOO]^-$, $C_{58}H_{95}O_{25}^-$; calc. 1191.6168).

Ginsenoside Ra8 (=*(20S)-3*-O-*{β*-D-*4*-O-*[(*E*)-But-2-enoyl]glucopyranosyl-(1→2)-β*-D-*glucopyranosyl}-20*-O-*[α*-L-*arabinofuranosyl-(1→6)-β*-D-*glucopyranosyl]protopanaxadiol*=*(3β,12β)-20-{[6*-O-*(α*-L-*Arabinofuranosyl)-β*-D-*glucopyranosyl]oxy}-12-hydroxydammar-24-en-3-yl 2*-O-*{4*-O-*[(2*E*)-But-2-enoyl]-β*-D-*glucopyranosyl}-β*-D-*glucopyranoside*; **5**). A white amorphous powder. $[\alpha]_D^{25} = -19.3$ (*c* = 0.2, MeOH). UV (MeOH): 211 (3.98), 252 (3.03). IR (KBr): 3392, 2944, 2879, 1717, 1651, 1445, 1384, 1309,

1267, 1231, 1172, 1078, 1043, 890, 839, 810, 647, 575. ^{1}H- and ^{13}C-NMR: *Tables 1* and *2*, resp. HR-ESI-MS: 1145.6095 ($[M-H]^-$, $C_{57}H_{93}O_{23}^-$; calc. 1145.6113), 1191.6127 ($[M+HCOO]^-$, $C_{58}H_{95}O_{25}^-$; calc. 1191.6168).

Ginsenoside Ra$_9$ (=*(20*S*)-3*-O-*{β*-D-*6*-O-*[(*E*)-But-2-enoyl]glucopyranosyl-(1→2)-β*-D-*glucopyranosyl}-20*-O-*[α*-L-*arabinofuranosyl-(1→6)-β*-D-*glucopyranosyl]protopanaxadiol=(3β,12β)-20-{[6*-O-*(α*-L-*Arabinofuranosyl)-β*-D-*glucopyranosyl]oxy}-12-hydroxydammar-24-en-3-yl 2*-O-*{6*-O-*[(2*E*)-But-2-enoyl]-β*-D-*glucopyranosyl}-β*-D-*glucopyranoside*; **6**). A white amorphous powder. $[\alpha]_D^{25} = +0.7$ ($c = 1.2$, MeOH). UV (MeOH): 217 (3.65), 252 (2.99). IR (KBr): 3392, 2944, 2879, 1710, 1655, 1542, 1445, 1384, 1314, 1233, 1197, 1076, 924, 889, 839, 808, 647, 576, 535. ^{1}H- and ^{13}C-NMR: *Tables 1* and *2*, resp. HR-ESI-MS: 1145.6092 ($[M-H]^-$, $C_{57}H_{93}O_{23}^-$; calc. 1145.6113), 1191.6152 ($[M+HCOO]^-$, $C_{58}H_{95}O_{25}^-$; calc. 1191.6168).

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