



Research article

Complete $^1\text{H-NMR}$ and $^{13}\text{C-NMR}$ spectral analysis of the pairs of 20(S) and 20(R) ginsenosidesHeejung Yang¹, Jeom Yong Kim², Sun Ok Kim², Young Hyo Yoo², Sang Hyun Sung^{3,*}¹ College of Pharmacy, Kangwon National University, Chuncheon, Korea² Greencrosshs, Suntech City, Sungnam, Korea³ College of Pharmacy and Research Institute of Pharmaceutical Science, Seoul National University, Seoul, Korea

ARTICLE INFO

Article history:

Received 8 October 2013

Received in Revised form

11 March 2014

Accepted 12 March 2014

Available online 27 May 2014

Keywords:

less polar ginsenosides

nuclear magnetic resonance spectroscopy

Panax ginseng

protopanaxadiol

protopanaxatriol

ABSTRACT

Background: Ginsenosides, the major ingredients of *Panax ginseng*, have been studied for many decades in Asian countries as a result of their wide range of pharmacological properties. The less polar ginsenosides, with one or two sugar residues, are not present in nature and are produced during manufacturing processes by methods such as heating, steaming, acid hydrolysis, and enzyme reactions. $^1\text{H-NMR}$ and $^{13}\text{C-NMR}$ spectroscopic data for the identification of the less polar ginsenosides are often unavailable or incomplete.

Methods: We isolated 21 compounds, including 10 pairs of 20(S) and 20(R) less polar ginsenosides (**1**–**20**), and an oleanane-type triterpene (**21**) from a processed ginseng preparation and obtained complete $^1\text{H-NMR}$ and $^{13}\text{C-NMR}$ spectroscopic data for the following compounds, referred to as compounds **1**–**21** for rapid identification: 20(S)-ginsenosides Rh2 (**1**), 20(R)-Rh2 (**2**), 20(S)-Rg3 (**3**), 20(R)-Rg3 (**4**), 6'-O-acetyl-20(S)-Rh2 [20(S)-AcetylRh2] (**5**), 20(R)-AcetylRh2 (**6**), 25-hydroxy-20(S)-Rh2 (**7**), 25-hydroxy-20(S)-Rh2 (**8**), 20(S)-Rh1 (**9**), 20(R)-Rh1 (**10**), 20(S)-Rg2 (**11**), 20(R)-Rg2 (**12**), 25-hydroxy-20(S)-Rh1 (**13**), 25-hydroxy-20(R)-Rh1 (**14**), 20(S)-AcetylRg2 (**15**), 20(R)-AcetylRg2 (**16**), Rh4 (**17**), Rg5 (**18**), Rk1 (**19**), 25-hydroxy-Rh4 (**20**), and oleanolic acid 28-O- β -D-glucopyranoside (**21**).

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1. Introduction

Ginsenosides, major components in *Panax ginseng* Meyer, are mainly classified into two groups of the dammarane-type triterpenes: protopanaxadiol (PPD) and protopanaxatriol (PPT) [1]. The substitution of sugar chains at C-3 or C-20 in PPD, or at C-3, C-6, and C-20 in PPT gives rise to a wide range of ginsenosides [2]. The PPD type typically includes the ginsenosides Rb1, Rb2, Rc, and Rd, whereas the PPT type includes Re, Rf, Rg1, and Rg2, which have three to five sugar moieties, in harvested ginseng. During processing by steaming with heat and acidic solutions, or in microbial reactions, these polar ginsenosides decrease and the less polar ginsenosides, such as Rg2, Rg3, Rh1, and Rh2, increase [3–5]. It has been suggested that they could be generated by the elimination of sugar chains or by dehydroxylation [6]. These reactions can also generate the irregular $\Delta 20(21)$ and $\Delta 20(22)$ ginsenosides, such as Rg5, Rh3, Rh4, and Rk1, which are rarely

found in nature [7]. In particular, the 20(R)-ginsenosides, including 20(R)-Rh2 and 20(R)-Rg3, are derived by selective deglycosylation and dehydroxylation at C-20, followed by biotransformation by reaction with a hydroxyl group [8,9]. The acetylated ginsenosides are generated by decarboxylation from the malonylated ginsenosides, including malonyl (Mal)-Rb1, Rb2, Rc, Rd, and Re [10]. As the less polar ginsenosides can be easily absorbed into blood vessels and act as the pharmacological agents with potential as drug candidates, the mass production or isolation of the less polar ginsenosides is of much interest in the ginseng industry [5].

Recent improvements in chromatographic techniques have led to the analysis and isolation of the stereoisomers of minor ginsenosides in ginseng preparations [11]. The structure–activity relationships between the diverse ginsenosides isolated by these improved techniques has been studied in both cancer cells and noncancer cells [12].

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In this study, we isolated 21 minor ginsenosides from a processed ginseng preparation and unequivocally determined their structures by one-dimensional and two-dimensional NMR spectroscopy and compared these results with previously published data. The NMR data obtained for these minor ginsenosides will be useful in studying the structure–activity relationships between structural modifications such as the number of sugar groups, the sugar linkage at C-6, the number of hydroxyl groups, and the stereoisomers of 20(*S*) and 20(*R*), as well as in the identification of stereoisomers of ginsenosides.

2. Materials and methods

2.1. General procedure

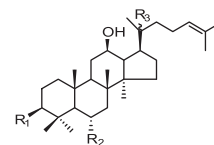
Column chromatography (CC) was carried out using Kiesgel 60 silica gel (40–60 μm, 230–400 mesh, Merck, USA), YMC-GEL ODS-A (5–150 μm, YMC), and Sephadex LH-20 (25–100 μm, Pharmacia, NJ, USA) columns. Thin-layer chromatography was carried out using Kiesgel 60 F₂₅₄ coated normal silica gel and RP-18 F₂₅₄ coated reversed-phase (RP) silica gel columns. The ¹H-NMR and ¹³C-NMR, ¹H-¹H COSY, HSQC, and HMBC spectra were recorded on a Bruker AMX 500 or 600 spectrometer in pyridine-*d*₅. The solvent signals were used as internal standards. The high-performance liquid chromatography (HPLC) system consisted of a G-321 pump (Gilson, USA), a G-151 UV detector (Gilson), and a YMC-Pack Pro C₁₈ column (250 mm × 10 mm i.d.; 5 μm); and all chromatograms were monitored at 210 nm. HPLC-grade solvents (Fisher Scientific, USA) were used in the MeOH–H₂O or MeCN–H₂O system.

2.2. Ginseng preparation

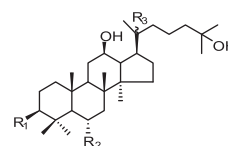
The processed ginseng preparation was gifted from Green-cross (Sungnam, Korea). It was prepared using patented technology and a previously reported method [13]. Briefly, the harvested ginseng was repeatedly extracted with ethanol, followed by reaction with an enzyme containing ginsenoside-β-glucosidase. After acid hydrolysis of the residue, the reactant was purified with HP-20 resin followed by washing out with distilled water and, finally, 95% ethanol.

2.3. Isolation of ginsenosides from the processed ginseng preparation

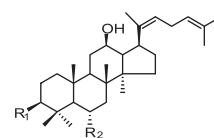
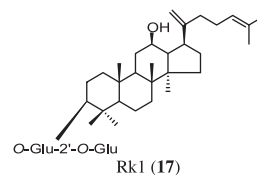
Powders of the processed ginseng extract (GE) (90 g) were each subjected to normal silica CC (20 × 5 cm column) with a gradient elution of solvents (CHCl₃:MeOH = 10:1, 7:1, 5:1, 3:1, 0:1; all 1-L volumes) and 24 sub-fractions (GE1–24) were obtained. 20(*S/R*)-AcetylRh2 (**5**, **6**) (20 mg, *R*_t = 14.1 min) were obtained from the GE-5 (2.8 g) sub-fraction by RP silica gel CC (20 × 5 cm; MeOH:H₂O = 9:1, 1 L), followed by preparative HPLC (MeOH:H₂O = 65:35, 4 mL/min). Oleanolic acid 28-*O*-β-D-glucopyranose (**21**) (200 mg) was isolated by recrystallization (100% MeOH) from the sub-fraction separated from the GE-7 (6.5 g) sub-fraction by RP silica gel CC (10 × 3 cm; MeOH:H₂O = 7:3, 2 L). Five sub-fractions (GE8–10 A–E) were obtained from GE8–10 (12.1 g) by RP silica gel CC (MeOH:H₂O = 8.5:1.5, 4 L). Rh4 (**17**) (5 mg, *R*_t = 19.1 min) was isolated from GE8–10 B, and 20(*S*)-Rh2 (**1**) (300 mg, *R*_t = 5.7 min) and 20(*R*)-Rh2 (**2**) (210 mg, *R*_t = 6.1 min) were isolated from GE8–10 C by preparative HPLC (MeCN:H₂O = 55:45, 13 mL/min). The mixtures of 25-hydroxy-Rh4 (**20**) (35 mg, *R*_t = 11.1 min), 20(*S/R*)-Rh1 (**9**, **10**) (90 mg, *R*_t = 13.2 min), 25-hydroxy-20(*S*)-Rh2 (**7**) (28 mg, *R*_t = 23.1 min), and 25-hydroxy-20(*R*)-Rh2 (**8**) (100 mg, *R*_t = 23.3 min) were prepared from GE12–14 (8.2 g) and were isolated by RP silica gel CC (10 × 3 cm;



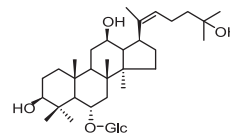
Name	R ₁	R ₂	R ₃
20(<i>S</i>)-Rh2 (1)	<i>O</i> -Glu	H	β-OH
20(<i>R</i>)-Rh2 (2)	<i>O</i> -Glu	H	α-OH
20(<i>S</i>)-Rg3 (3)	<i>O</i> -Glu-2'- <i>O</i> -Glu	H	β-OH
20(<i>R</i>)-Rg3 (4)	<i>O</i> -Glu-2'- <i>O</i> -Glu	H	α-OH
20(<i>S/R</i>)-AcetylRh2 (5 and 6)	<i>O</i> -AcetylGlu	H	α- and β-OH
20(<i>S</i>)-Rh1 (9)	OH	<i>O</i> -Glu	β-OH
20(<i>R</i>)-Rh1 (10)	OH	<i>O</i> -Glu	α-OH
20(<i>S</i>)-Rg2 (11)	OH	<i>O</i> -Glu-2'- <i>O</i> -Rha	β-OH
20(<i>R</i>)-Rg2 (12)	OH	<i>O</i> -Glu-2'- <i>O</i> -Rha	α-OH
20(<i>S</i>)-AcetylRg2 (15)	OH	<i>O</i> -AcetylGlu-2'- <i>O</i> -Rha	β-OH
20(<i>R</i>)-AcetylRg2 (16)	OH	<i>O</i> -AcetylGlu-2'- <i>O</i> -Rha	α-OH



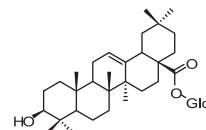
Name	R ₁	R ₂	R ₃
25-Hydroxy-20(<i>S</i>)-Rh2 (7)	<i>O</i> -Glc	H	α-OH
25-Hydroxy-20(<i>R</i>)-Rh2 (8)	<i>O</i> -Glc	H	α-OH
25-Hydroxy-20(<i>S/R</i>)-Rh1 (13 and 14)	OH	<i>O</i> -Glc	α- and β-OH



Name	R ₁	R ₂
Rh4 (18)	OH	<i>O</i> -Glu
Rg5 (20)	<i>O</i> -Glu-2'- <i>O</i> -Glu	H



25-Hydroxy-Rh4 (**17**)



Oleanolic acid 28-*O*-β-D-glucopyranose (**21**)

Fig. 1. Structures of compounds **1–21** isolated from the processed ginseng extract. Glu, β-D-Glucose; AcetylGlu, β-D-6'-*O*-Acetyl-glucose; Rha, α-L-Rhamnose.

Table 1
¹H-NMR Spectroscopic Data for Compounds **1–8** in Pyridine-*d*₅

No.	20(S)-Rh2 ¹⁾ (1)	20(R)-Rh2 ¹⁾ (2)	20(S)-Rg3 ²⁾ (3)	20(R)-Rg3 ²⁾ (4)	20(S/R)-AcetylRh2 ^{2),3)} (5 and 6)	25-Hydroxy-20(S)-Rh2 ²⁾ (7)	25-Hydroxy-20(R)-Rh2 ²⁾ (8)
<i>δ</i> _H (<i>J</i> in Hz)							
1a	1.49 (1H, m)	1.49 (1H, m)	1.46 (1H, m)	1.47 (1H, m)	1.58 (1H, m)	1.49 (1H, m)	1.49 (1H, m)
1b	0.79 (1H, m)	0.79 (1H, m)	0.72 (1H, m)	0.72 (1H, m)	0.88 (1H, m)	0.75 (1H, m)	0.75 (1H, m)
2a	2.19 (1H, m)	2.20 (1H, m)	2.16 (1H, m)	2.17 (1H, m)	2.12 (1H, m)	2.20 (1H, m)	2.18 (1H, m)
2b	1.78 (1H, m)	1.79 (1H, m)	1.79 (1H, m)	1.81 (1H, m)	1.78 (1H, m)	1.38 (1H, m)	1.36 (1H, m)
3	3.35 (1H, dd, <i>J</i> = 4.6, 11.9)	3.36 (1H, dd, <i>J</i> = 3.7, 11.5)	3.26 (1H, dd, <i>J</i> = 11.75, 4.35)	3.26 (1H, dd, <i>J</i> = 11.75, 4.35)	3.24 (1H, m)	3.36 (1H, dd, <i>J</i> = 4.4, 11.7)	3.35 (1H, dd, <i>J</i> = 4.4, 11.7)
5	0.72 (1H, d, <i>J</i> = 11.9)	0.73 (1H, d, <i>J</i> = 11.5)	0.65 (1H, d, <i>J</i> = 11.4)	0.66 (1H, d, <i>J</i> = 11.5)	0.71 (1H, m)	0.73 (1H, m)	0.71 (1H, m)
6a	1.48 (2H, m)	1.50 (2H, m)	1.49 (1H, m)	1.52 (1H, m)	1.48 (2H, m)	1.58-1.32 (2H, m)	1.52-1.36 (2H, m)
6b			1.35 (1H, m)	1.40 (1H, m)			
7a	1.47 (1H, m)	1.49 (1H, m)	1.42 (1H, m)	1.41 (1H, m)	1.45 (1H, m)	1.47 (1H, m)	1.48 (1H, m)
7b	1.21 (1H, m)	1.23 (1H, m)	1.19 (1H, m)	1.21 (1H, m)	1.20 (1H, m)	1.22 (1H, m)	1.22 (1H, m)
9	1.40 (1H, m)	1.42 (1H, m)	1.37 (1H, m)	1.37 (1H, m)	1.41 (1H, m)	1.41 (1H, m)	1.41 (1H, m)
11a	1.58 (1H, m)	1.58 (1H, m)	2.02 (1H, m)	2.00 (1H, m)	1.52 (1H, m)	2.05 (1H, m)	2.03 (1H, m)
11b	1.11 (1H, m)	1.13 (1H, m)	1.55 (1H, m)	1.55 (1H, m)	1.02 (1H, m)	1.54 (1H, m)	1.52 (1H, m)
12	3.89 (1H, m)	3.91 (1H, m)	3.90 (1H, m)	3.91 (1H, m)	3.82 (1H, m)	3.90 (1H, m)	3.90 (1H, m)
13	2.01 (1H, m)	2.00 (1H, m)	2.00 (1H, m)	1.97 (1H, m)	1.94 (1H, m)	2.06 (1H, m)	2.00 (1H, m)
15a	2.01 (1H, m)	2.11 (1H, m)	1.50 (1H, m)	1.56 (1H, m)	1.96 (1H, m)	1.58 (1H, m)	1.57 (1H, m)
15b	1.49 (1H, m)	1.51 (1H, m)	1.05 (1H, m)	1.04 (1H, m)	1.42 (1H, m)	1.02 (1H, m)	1.02 (1H, m)
16a	1.88 (1H, m)	1.91 (1H, m)	1.87 (1H, m)	1.93 (1H, m)	1.84 (1H, m)	1.92 (1H, m)	1.91 (1H, m)
16b	1.39 (1H, m)	1.35 (1H, m)	1.38 (1H, m)	1.35 (1H, m)	1.44 (1H, m)	1.81 (1H, m)	1.80 (1H, m)
17	2.35 (1H, m)	2.38 (1H, m)	2.33 (1H, m)	2.38 (1H, m)	2.26 (1H, m)	2.30 (1H, m)	2.34 (1H, m)
18	0.77 (3H, s)	0.80 (3H, s)	0.94 (3H, s)	0.99 (3H, s)	0.94 (3H, s)	0.91 (3H, s)	0.80 (3H, s)
19	0.94 (3H, s)	1.00 (3H, s)	0.77 (3H, s)	0.80 (3H, s)	0.78 (3H, s)	1.01 (3H, s)	1.00 (3H, s)
21	1.40 (3H, s)	1.38 (3H, s)	1.40 (3H, s)	1.37 (3H, s)	1.32 (3H, s)	1.41 (3H, s)	1.38 (3H, s)
22a	2.01 (1H, m)	1.70 (2H, m)	2.01 (1H, m)	1.71 (2H, m)	1.90 (1H, m)	1.62 (2H, m)	2.00 (1H, m)
22b	1.68 (1H, m)		1.68 (1H, m)		1.58 (1H, m)		1.63 (1H, m)
23a	2.57 (1H, m)	2.52 (1H, m)	2.58 (1H, m)	2.52 (1H, m)	2.46 (1H, m)	2.48 (1H, m)	2.16 (1H, m)
23b	2.29 (1H, m)	2.45 (1H, m)	2.26 (1H, m)	2.47 (1H, m)	2.16 (1H, m)	2.42 (1H, m)	1.82 (1H, m)
24	5.29 (1H, t-like)	5.30 (1H, t-like)	5.28 (1H, t, <i>J</i> = 6.95)	5.30 (1H, t-like)	5.24 (1H, m)	5.25 (1H, m)	1.71 (2H, m)
26	1.63 (3H, s)	1.68 (3H, s)	1.60 (3H, s)	1.68 (3H, s)	1.62 (3H, s)	1.37 (3H, s)	1.40 (3H, s)
27	1.60 (3H, s)	1.63 (3H, s)	1.60 (3H, s)	1.64 (3H, s)	1.55 (3H, s)	1.38 (3H, s)	1.40 (3H, s)
28	1.30 (3H, s)	1.30 (3H, s)	1.27 (3H, s)	1.27 (3H, s)	1.20 (3H, s)	1.30 (3H, s)	1.30 (3H, s)
29	0.97 (3H, s)	0.98 (3H, s)	1.08 (3H, s)	1.09 (3H, s)	0.88 (3H, s)	0.98 (3H, s)	0.98 (3H, s)
30	0.94 (3H, s)	0.98 (3H, s)	0.92 (3H, s)	0.96 (3H, s)	0.96 (3H, s)	0.94 (3H, s)	0.94 (3H, s)
3- <i>O</i> -β-D-Glucopyranosyl							
1'	4.92 (1H, d, <i>J</i> = 7.8)	4.92 (1H, d, <i>J</i> = 7.60)	4.90 (1H, d, <i>J</i> = 7.55)	4.91 (1H, d, <i>J</i> = 7.6)	4.74 (1H, m)	4.93 (1H, d, <i>J</i> = 7.8)	4.91 (1H, d, <i>J</i> = 7.8)
2'	4.02 (1H, m)	4.02 (1H, m)	4.23 (1H, m)	4.22 (1H, m)	3.92 (1H, m)	4.02 (1H, m)	4.01 (1H, m)
3'	4.23 (1H, t, <i>J</i> = 8.7)	4.22 (1H, m)	4.21 (1H, m)	4.20 (1H, m)	4.06 (1H, m)	4.25 (1H, m)	4.22 (1H, t, <i>J</i> = 8.8)
4'	4.18 (1H, t, <i>J</i> = 8.7)	4.18 (1H, m)	4.11 (1H, m)	4.13 (1H, m)	3.88 (1H, m)	4.20 (1H, m)	4.18 (1H, t, <i>J</i> = 8.8)
5'	3.90 (1H, m)	3.98 (1H, m)	3.88 (1H, m)	3.89 (1H, m)	3.87 (1H, m)	3.99 (1H, m)	3.98 (1H, m)
6'a	4.56 (1H, d, <i>J</i> = 11.9)	4.57 (1H, d, <i>J</i> = 11.9)	4.53 (1H, m)	4.54 (1H, m)	4.79 (1H, m)	4.57 (1H, dd, <i>J</i> = 2.2, 11.7)	4.56 (1H, dd, <i>J</i> = 1.9, 11.7)
6'b	3.67 (1H, dd, <i>J</i> = 5.5, 11.9)	4.37 (1H, dd, <i>J</i> = 5.5, 11.9)	4.33 (1H, m)	4.32 (1H, m)	4.67 (1H, dd, <i>J</i> = 6.42, 11.88)	4.37 (1H, dd, <i>J</i> = 5.4, 11.7)	4.37 (1H, dd, <i>J</i> = 5.4, 11.7)
2'- <i>O</i> -β-D-Glucopyranosyl							
1''			5.35 (1H, d, <i>J</i> = 7.65)	5.36 (1H, d, <i>J</i> = 7.65)			
2''			4.10 (1H, m)	4.12 (1H, m)			
3''			4.29 (1H, m)	4.28 (1H, m)			
4''			4.32 (1H, m)	4.31 (1H, m)			
5''			3.91 (1H, m)	3.93 (1H, m)			
6''a			4.46 (1H, m)	4.46 (1H, m)			
6''b			4.45 (1H, m)	4.45 (1H, m)			
COCH ₃					1.93 (3H, s)		

¹⁾ ¹H-NMR data measured at 600 MHz

²⁾ ¹H-NMR data measured at 500 MHz

³⁾ 20(S/R)-AcetylRh2; 6'-*O*-acetyl-20(S/R)-Rh2

Table 2
¹³C-NMR Spectroscopic Data for Compounds **1–8** in Pyridine-*d*₅

No.	20(S)-Rh2 ¹⁾ (1)	20(R)-Rh2 ¹⁾ (2)	20(S)-Rg3 ²⁾ (3)	20(R)-Rg3 ²⁾ (4)	20(S/R)-AcetylRh2 ^{2), 3)} (5 and 6)	25-Hydroxy-20(S)-Rh2 ²⁾ (7)	25-Hydroxy-20(R)-Rh2 ²⁾ (8)
δ _c multiplicity							
1	39.1 t	39.1 t	39.1 t	39.1 t	38.9 t	39.1 t	39.1 t
2	26.7 t	26.6 t	26.7 t	26.6 t	26.6 t	26.7 t	26.7 t
3	88.7 d	88.7 d	88.9 d	88.9 d	89.0 d	88.8 d	88.7 d
4	39.6 s	39.6 s	39.6 s	39.6 s	39.4 s	39.7 s	39.6 s
5	56.3 d	56.3 d	56.3 d	56.3 d	56.2 d	56.4 d	56.3 d
6	18.4 t	18.4 t	18.4 t	18.4 t	18.2 t	18.4 t	18.4 t
7	35.1 t	35.1 t	35.1 t	35.1 t	34.9 t	35.2 t	35.1 t
8	40.0 s	40.0 s	39.9 s	40.0 s	39.8 s	40.0 s	40.0 s
9	50.3 d	50.3 d	50.3 d	50.3 d	50.2 d	50.4 d	50.3 d
10	36.9 s	36.9 s	36.8 s	36.9 s	36.8 s	37.0 s	36.9 s
11	31.3 t	31.4 t	31.3 t	31.4 t	31.1 t	31.2 t	32.1 t
12	70.9 d	70.8 d	70.9 d	70.8 d	70.7 d	70.6 d	70.8 d
13	48.5 d	49.2 d	48.5 d	49.2 d	48.9 d	48.2 d	48.6 d
14	51.7 s	51.7 s	51.6 s	51.7 s	51.5 s	51.7 s	51.7 s
15	32.0 t	32.1 t	32.0 t	32.1 t	31.7 t	31.8 t	31.4 t
16	26.8 t	26.7 t	26.8 t	26.7 t	26.4 t	27.2 t	26.6 t
17	54.7 d	50.6 d	54.7 d	50.6 d	54.5 d	50.3 d	54.7 d
18	16.3 q	16.3 q	15.8 q	15.8 q	15.6 q		16.8 q
19	15.8 q	15.8 q	16.3 q	16.3 q	16.1 q		15.8 q
20	72.9 s	72.9 s	72.9 s	72.9 s	72.8 s		73.3 s
21	27.0 q	22.7 q	27.0 q	22.7 q	26.7 q	22.4 q	26.9 q
22	35.8 t	43.2 t	35.8 t	43.2 t	35.6 t	42.9 t	36.5 t
23	22.9 t	22.6 t	23.0 t	22.5 t	22.3 t		19.1 t
24	126.3 d	126 d	126.2 d	126.0 d	126.0 d	125.8 d	45.7 t
25	130.7 s	130.7 s	130.7 s	130.7 s	130.5 s		69.6 s
26	25.8 q	25.8 q	25.7 q	25.8 q	25.7 q	25.6 q	30.2 q
27	17.6 q	17.7 q	17.0 q	17.2 q	17.1 q	16.8 q	29.9 q
28	28.1 q	28.1 q	28.1 q	28.1 q	27.9 q		28.1 q
29	16.7 q	16.7 q	16.5 q	16.5 q	16.5 q		16.4 q
30	17.0 q	17.3 q	17.6 q	17.6 q	17.5 q		17.0 q
3-O-β-D-Glucopyranosyl							
1'	106.9 d	106.9 d	105.0 d	105.1 d	106.6 d	106.9 d	106.9 d
2'	75.7 d	75.7 d	83.4 d	83.4 d	74.5 d	75.8 d	75.7 d
3'	78.7 d	78.7 d	77.9 d	77.9 d	78.1 d	78.7 d	78.7 d
4'	71.8 d	71.8 d	71.6 d	71.6 d	71.3 d	71.9 d	71.8 d
5'	78.3 d	78.3 d	78.2 d	78.2 d	75.1 d	78.3 d	78.3 d
6'	63.0 t	63.0 t	62.8 t	62.8 t	64.4 t	63.1 t	63.0 t
2'-O-β-D-Glucopyranosyl							
1''			106.0 d	106.0 d			
2''			77.1 d	77.1 d			
3''			78.3 d	78.3 d			
4''			71.6 d	71.6 d			
5''			78.0 d	78.1 d			
6''			62.7 t	62.7 t			
COCH ₃					170.5 s		
COCH ₃					20.6 q		

Multiplicity of ¹³C-NMR data was determined by DEPT experiments

¹⁾ ¹³C-NMR data measured at 150 MHz

²⁾ ¹³C-NMR data measured at 125 MHz

³⁾ 20(S/R)-AcetylRh2; 6'-O-acetyl-20(S/R)-Rh2

MeOH:H₂O = 7:3, 4 L) followed by preparative HPLC (MeCN:H₂O = 50:50, 70:30, 13 mL/min). GE15–18 (10.1 g) were subjected to RP silica gel CC (MeOH:H₂O = 6:4, 4 L) to give five sub-fractions (GE15–18 A–E). 20S-AcetylRg2 (**15**) (15 mg, R_t = 24.7 min) and 20R-AcetylRg2 (**16**) (8 mg, R_t = 25.1 min) were isolated from GE15–18 B. Rk1 (**19**) (25 mg, R_t = 19.9 min) and Rg5 (**18**) (31 mg, R_t = 20.3 min) were obtained from GE15–18 D by preparative HPLC (MeOH:H₂O = 7:3, 10 mL/min), respectively. 20(S/R)-Rg2 (**11, 12**) (50 mg), 20(S)-Rg3 (**3**) (400 mg), and 20(R)-Rg3 (**4**) (400 mg) were obtained from GE19–20 (8.1 g) sub-fractions by RP silica gel CC (10 × 3 cm) with a mixture of MeOH:H₂O (3:1, 5 L). 20(S)-Rg2 (**11**) (10 mg, R_t = 13.1 min) and 20(R)-Rg2 (**12**) (15 mg, R_t = 13.4 min) were purified using preparative HPLC (MeCN:H₂O = 35:65, 10 mL/min). GE21–22 (3.1 g) sub-fractions were further isolated to give the mixture of 25-hydroxy-20(S/R)-Rh1 (**13, 14**) (30 mg).

3. Results and discussion

The structures of compounds **1–21** were unequivocally determined by comparing the one-dimensional and two-dimensional NMR spectrometry and mass spectrometry data with previously published values. These were: 20(S)-ginsenosides Rh2 (**1**) [14], 20(R)-Rh2 (**2**) [15], 20(S)-Rg3 (**3**) [16], 20(R)-Rg3 (**4**) [16], 6'-O-acetyl-20(S)-Rh2 (20(S)-AcetylRh2) (**5**) [16], 20(R)-AcetylRh2 (**6**) and 25-hydroxy-20(S)-Rh2 (**7**) [13], 25-hydroxy-20(R)-Rh2 (**8**) [13], 20(S)-Rh1 (**9**) [17], 20(R)-Rh1 (**10**) [17], 20(S)-Rg2 (**11**) [17], 20(R)-Rg2 (**12**) [18], 25-hydroxy-20(S)-Rh1 (**13**) [19], 25-hydroxy-20(R)-Rh1 (**14**) [19], 20(S)-AcetylRg2 (**15**) [20], 20(R)-AcetylRg2 (**16**) [20], Rk1 (**17**) [21], Rh4 (**18**) [17], 25-hydroxy-Rh4 (**19**) [18], Rg5 (**20**) [21], and oleanolic acid 28-O-β-D-glucopyranoside (**21**) [22] (Fig. 1). Of these compounds, compound **6** had not been reported previously.

Table 3
¹H-NMR Spectroscopic Data for Compounds **9–16** in Pyridine-*d*₅

No.	20(S)-Rh1 ¹⁾ (9)	20(R)-Rh1 ¹⁾ (10)	20(S)-Rg2 ¹⁾ (11)	20(R)-Rg2 ²⁾ (12)	25-Hydroxy-20(S/R)-Rh1 ¹⁾ (13 and 14)	20(S)-Acetyl-Rg2 ^{1,3)} (15)	20(R)-Acetyl-Rg2 ^{1,4)} (16)
<i>δ</i> _H (<i>J</i> in Hz)							
1a	1.66 (1H, m)	1.69 (1H, m)	1.61 (1H, m)	1.61 (1H, m)	1.67 (1H, m)	1.65 (1H, m)	1.64 (1H, m)
1b	1.02 (1H, m)	1.01 (1H, m)	0.92 (1H, m)	0.92 (1H, m)	1.02 (1H, m)	0.98 (1H, m)	0.97 (1H, m)
2a	1.89 (1H, m)	1.90 (1H, m)	1.83 (1H, m)	1.82 (1H, m)	1.89 (1H, m)	1.82 (1H, m)	1.82 (1H, m)
2b	1.80 (1H, m)	1.80 (1H, m)	1.76 (1H, m)	1.76 (1H, m)	1.82 (1H, m)	1.76 (1H, m)	1.76 (1H, m)
3	3.50 (1H, m)	3.50 (1H, br d)	3.43 (1H, m)	3.35 (1H, br s)	3.50 (1H, m)	3.46 (1H, m)	3.46 (1H, dd, <i>J</i> = 4.1, 11.2)
5	1.40 (1H, m)	1.42 (1H, d, <i>J</i> = 10.5)	1.37 (1H, m)	1.39 (1H, m)	1.42 (1H, m)	1.37 (1H, m)	1.39 (1H, m)
6	4.40 (1H, td, <i>J</i> = 2.8, 10.5)	4.43 (1H, td, <i>J</i> = 2.9, 10.5)	4.64 (1H, m)	4.68 (1H, m)	4.42 (1H, m)	4.75 (1H, s)	4.70 (1H, td, <i>J</i> = 3.3, 10.6)
7a	2.50 (1H, m)	2.51 (1H, m)	2.22 (1H, m)	2.23 (1H, m)	2.51 (1H, m)	2.14 (1H, m)	2.15 (1H, m)
7b	1.91 (1H, m)	1.93 (1H, m)	1.95 (1H, m)	1.96 (1H, m)	1.93 (1H, m)	1.97 (1H, m)	1.98 (1H, m)
9	1.53 (1H, m)	1.57 (1H, m)	1.51 (1H, m)	1.52 (1H, m)	1.58 (1H, m)	1.55 (1H, s)	1.56 (1H, s)
11a	2.11 (1H, m)	2.13 (1H, m)	2.04 (1H, m)	2.09 (1H, m)	2.13 (1H, m)	2.14 (1H, m)	2.15 (1H, m)
11b	1.56 (1H, m)	1.52 (1H, m)	1.51 (1H, m)	1.54 (1H, m)	1.56 (1H, m)	1.56 (1H, m)	1.57 (1H, m)
12	3.88 (1H, m)	3.91 (1H, m)	3.89 (1H, m)	3.90 (1H, m)	3.89 (1H, m)	3.93 (1H, m)	3.95 (1H, m)
13	2.01 (1H, m)	2.00 (1H, m)	1.97 (1H, m)	1.96 (1H, m)	2.02 (1H, m)	2.04 (1H, m)	2.01 (1H, m)
15a	1.59 (1H, m)	1.59 (1H, m)	1.51 (1H, m)	1.50 (1H, m)	1.63 (1H, m)	1.62 (1H, m)	1.62 (1H, m)
15b	1.07 (1H, m)	1.11 (1H, m)	0.83 (1H, m)	0.91 (1H, m)	0.91 (1H, m)	0.98 (1H, m)	1.02 (1H, m)
16a	1.76 (1H, m)	1.80 (1H, m)	1.73 (1H, m)	1.82 (1H, m)	1.32 (2H, m)	1.82 (1H, m)	1.88 (1H, m)
16b	1.30 (1H, m)	1.28 (1H, m)	1.28 (1H, m)	1.22 (1H, m)		1.38 (1H, m)	1.30 (1H, m)
17	2.26 (1H, m)	2.32 (1H, m)	2.25 (1H, m)	2.34 (1H, m)	2.28 (1H, m)	2.31 (1H, m)	2.37 (1H, m)
18	1.16 (3H, s)	1.22 (3H, s)	1.18 (3H, s)	1.22 (3H, s)	1.03 (3H, s)	1.22 (3H, s)	1.25 (3H, s)
19	1.00 (3H, s)	1.04 (3H, s)	0.93 (3H, s)	0.96 (3H, s)	1.25 (3H, s)	0.99 (3H, s)	1.02 (3H, s)
21	1.37 (3H, s)	1.37 (3H, s)	1.38 (3H, s)	1.36 (3H, s)	1.38 (3H, s)	1.35 (3H, m)	1.35 (3H, s)
22a	2.01 (1H, m)	1.68 (2H, m)	1.98 (1H, m)	2.01 (1H, m)	2.00 (1H, m)	2.04 (1H, m)	1.68 (2H, m)
22b	1.66 (1H, m)		1.62 (1H, m)	1.68 (1H, m)	1.63 (1H, m)	1.67 (1H, m)	
23a	2.56 (1H, m)	2.48 (1H, m)	2.58 (1H, m)	2.57 (1H, m)	2.13 (1H, m)	2.57 (1H, m)	2.49 (1H, m)
23b	2.25 (1H, m)	2.41 (1H, m)	2.23 (1H, m)	2.29 (1H, m)	1.86 (1H, m)	2.25 (1H, m)	2.41 (1H, m)
24	5.30 (1H, t-like)	5.28 (1H, t-like)	5.31 (1H, t-like)	5.29 (1H, t-like)	1.70 (2H, t-like)	5.29 (1H, t-like)	5.28 (1H, t-like)
26	1.63 (3H, s)	1.67 (3H, s)	1.63 (3H, s)	1.67 (3H, s)	1.38 (3H, s)	1.62 (3H, s)	1.68 (3H, m)
27	1.60 (3H, s)	1.61 (3H, s)	1.60 (3H, s)	1.62 (3H, s)	1.40 (3H, s)	1.59 (3H, s)	1.60 (3H, s)
28	2.05 (3H, s)	2.06 (3H, s)	2.06 (3H, s)	2.09 (3H, s)	2.05 (3H, s)	2.05 (3H, m)	2.03 (3H, m)
29	1.57 (3H, s)	1.59 (3H, s)	1.31 (3H, s)	1.34 (3H, s)	1.58 (3H, s)	1.29 (3H, s)	1.28 (3H, s)
30	0.79 (3H, s)	0.84 (3H, s)	0.91 (3H, s)	0.95 (3H, s)	0.82 (3H, s)	0.97 (3H, s)	1.00 (3H, s)
6- <i>O</i> -β-D-glucopyranosyl							
1'	5.00 (1H, m)	5.03 (1H, m)	5.23 (1H, d, <i>J</i> = 6.9)	5.26 (1H, m)	5.02 (1H, m)	5.22 (1H, d, <i>J</i> = 7.0)	5.22 (1H, d, <i>J</i> = 7.0)
2'	4.08 (1H, m)	4.09 (1H, m)	4.32 (1H, m)	4.32 (1H, m)	4.07 (1H, m)	4.33 (1H, m)	4.32 (1H, m)
3'	4.23 (1H, m)	4.25 (1H, m)	4.33 (1H, m)	4.36 (1H, m)	4.23 (1H, m)	4.29 (1H, m)	4.29 (1H, m)
4'	4.19 (1H, m)	4.20 (1H, m)	4.19 (1H, m)	4.19 (1H, m)	4.07 (1H, m)	3.92 (1H, m)	3.94 (1H, m)
5'	3.92 (1H, m)	3.95 (1H, m)	3.93 (1H, m)	3.95 (1H, m)	3.94 (1H, m)	4.01 (1H, t-like)	4.03 (1H, t-like, <i>J</i> = 8.2)
6'a	4.51 (1H, m)	4.52 (1H, dd, <i>J</i> = 1.9, 11.4)	4.49 (1H, m)	4.50 (1H, m)	4.51 (1H, m)	5.00 (1H, m)	4.90 (1H, m)
6'b	4.34 (1H, m)	4.35 (1H, dd, <i>J</i> = 5.3, 11.4)	4.36 (1H, m)	4.37 (1H, m)	4.34 (1H, m)	4.61 (1H, m)	4.63 (1H, m)
2'- <i>O</i> -α-L-rhamnopyranosyl							
1''			6.47 (1H, br s)	6.47 (1H, s)		6.47 (1H, s)	6.47 (1H, s)
2''			4.75 (1H, m)	4.78 (1H, m)		4.68 (1H, dt, <i>J</i> = 3.2, 10.6)	4.75 (1H, m)
3''			4.63 (1H, m)	4.66 (1H, m)		4.64 (1H, m)	4.64 (1H, m)
4''			4.30 (1H, m)	4.31 (1H, m)		4.34 (1H, m)	4.33 (1H, m)
5''			4.92 (1H, m)	4.94 (1H, m)		4.98 (1H, m)	4.80 (1H, m)
6''			1.76 (3H, d, <i>J</i> = 6.2)	1.78 (3H, br s)		1.76 (3H, d, <i>J</i> = 6.2)	1.77 (3H, d, <i>J</i> = 6.1)
COCH ₃							
						2.04 (3H, s)	2.08 (3H, s)

¹⁾ ¹H-NMR data measured at 500 MHz

²⁾ ¹H-NMR data measured at 600 MHz

³⁾ 20(S)-AcetylRg2; 6'-*O*-acetyl-20(S)-Rg2

⁴⁾ 20(R)-AcetylRg2; 6'-*O*-acetyl-20(R)-Rg2

Table 4
¹³C-NMR Spectroscopic Data for Compounds **9–16** in Pyridine-*d*₅

No.	20(S)-Rh1 ¹⁾ (9)	20(R)-Rh1 ¹⁾ (10)	20(S)-Rg2 ¹⁾ (11)	20(R)-Rg2 ²⁾ (12)	25-Hydroxy-20(S/R)-Rh1 ¹⁾ (13 and 14)	20(S)-Acetyl-Rg2 ^{1),3)} (15)	20(R)-Acetyl-Rg2 ^{1),4)} (16)
δ _C multiplicity							
1a	39.3 t	39.3 t	39.5 t	39.6 t	39.6 t	39.5 t	39.5 t
2	27.8 t	27.9 t	27.7 t	27.7 t	27.9 t	27.6 t	27.6 t
3	78.5 d	78.5 d	78.3 d	78.3 d	78.5 d	78.2 d	78.1 d
4	40.3 s	40.3 s	41.1 s	39.9 s	40.3 s	39.8 s	39.8 s
5	61.4 d	61.4 d	60.7 d	60.8 d	61.4 d	60.5 d	60.5 d
6	80.0 d	80.0 d	74.2 d	74.3 d	80.0 d	72.2 d	73.3 d
7	45.2 t	45.1 t	46.0 t	46.0 t	45.2 t	45.1 t	46.1 t
8	41.0 s	41.1 s	41.1 s	41.1 s	41.0 s	39.2 s	39.2 s
9	50.1 d	50.1 d	49.7 d	49.7 d	50.2 d	49.6 d	49.6 d
10	39.6 s	39.6 s	39.9 s	39.9 s	39.3 s	41.1 s	41.1 s
11	32.0 t	32.2 t	32.0 t	32.1 t	32.1 t	32.0 t	32.0 t
12	71.0 d	70.9 d	71.0 d	70.9 d	71.0 d	70.9 d	70.8 d
13	48.2 d	48.8 d	48.1 d	48.8 d	48.2 d	48.9 d	48.8 d
14	51.6 s	51.7 s	51.6 s	51.7 s	51.6 s	51.6 s	51.6 s
15	31.2 t	31.3 t	31.2 t	31.3 t	31.3 t	31.2 t	31.3 t
16	26.7 t	26.6 t	26.8 t	26.6 t	26.8 t	26.7 t	26.5 t
17	54.7 d	50.5 d	54.6 d	50.5 d	54.6 d	50.7 d	54.7 d
18	17.3 q	17.3 q	17.6 q	17.6 q	17.6 q	17.0 q	17.1 q
19	17.6 q	17.6 q	17.5 q	17.5 q	17.3 q	17.5 q	17.4 q
20	72.9 s	73.0 s	72.9 s	72.9 s	73.3 s	72.9 s	72.9 s
21	26.9 q	22.7 q	27.0 q	22.7 q	27.1 q	22.7 q	26.9 q
22	35.8 t	43.2 t	35.7 t	43.2 t	36.4 t	43.9 t	35.8 t
23	22.9 t	22.5 t	22.9 t	22.5 t	19.1 t	18.6 t	22.9 t
24	126.2 d	126.0 d	126.3 d	126.0 d	45.7 t	126.2 d	125.9 d
25	130.7 s	130.7 s	130.7 s	130.7 s	69.7 s	130.7 s	130.7 s
26	25.7 q	25.8 q	25.8 q	25.8 q	30.1 q	25.7 q	25.7 q
27	17.6 q	17.6 q	17.6 q	17.6 q	17.6 q	17.6 q	17.6 q
28	31.6 q	31.7 q	32.1 q	32.1 q	31.7 q	31.9 q	32.0 q
29	16.3 q	16.3 q	16.8 q	17.2 q	16.8 q	17.4 q	17.5 q
30	16.7 q	17.0 q	17.1 q	17.1 q	17.0 q	16.9 q	17.0 q
6-O-α-L-Rhamnopyranosyl							
1'	106.0 d	106.0 d	101.9 d	101.9 d	105.9 d	101.2 d	101.2 d
2'	75.4 d	75.4 d	79.4 d	79.4 d	75.4 d	78.2 d	78.2 d
3'	79.6 d	79.6 d	78.5 d	78.5 d	79.6 d	79.0 d	79.0 d
4'	71.8 d	71.8 d	72.4 d	72.4 d	71.8 d	72.3 d	72.3 d
5'	78.1 d	78.1 d	78.3 d	78.3 d	78.1 d	75.3 d	75.3 d

(continued on next page)

Table 4 (continued)

No.	20(S)-Rh1 ¹⁾ (9)	20(R)-Rh1 ¹⁾ (10)	20(S)-Rg2 ¹⁾ (11)	20(R)-Rg2 ²⁾ (12)	25-Hydroxy-20(S/R)-Rh1 ¹⁾ (13 and 14)	20(S)-Acetyl-Rg2 ^{1,3)} (15)	20(R)-Acetyl-Rg2 ^{1,4)} (16)
6'a	63.0 t	63.0 t	63.0 t	63.1 t		64.8 t	64.8 t
	63.0 t						
2'-O- α -L-Rhamnopyranosyl							
1'			101.7 d	101.7 d		102.0 d	102.0 d
2''			72.2 d	72.2 d		73.3 d	72.2 d
3''			72.5 d	72.6 d		72.2 d	72.2 d
4''			74.1 d	74.1 d		74.0 d	74.0 d
5''			69.4 d	69.4 d		69.3 d	69.3 d
6''			18.7 q	18.7 q		18.6 q	18.6 q
COCH ₃						170.7 s	170.7 s
COCH ₃						20.8 q	20.8 q

Multiplicity of ¹³C-NMR data was determined by DEPT experiments

¹⁾ ¹³C-NMR data measured at 125 MHz

²⁾ ¹³C-NMR data measured at 150 MHz

³⁾ 20(S)-AcetylRg2; 6'-O-acetyl-20(S)-Rg2

⁴⁾ 20(R)-AcetylRg2; 6'-O-acetyl-20(R)-Rg2

Table 5

¹H-NMR Spectroscopic Data for Compounds 17–21 in Pyridine-d₅

No.	Rk1 (17)	Rh4 (18)	25-Hydroxy-Rh4 (19)	Rg5 (20)	Oleanolic acid 28-O- β -D-glu (21)
δ_H (J in Hz)					
1a	1.49 (1H, m)	1.67 (1H, m)	1.68 (1H, m)	1.47 (1H, m)	1.50 (1H, m)
1b	0.74 (1H, m)	1.01 (1H, m)	1.03 (1H, m)	0.75 (1H, m)	0.97 (1H, m)
2a	2.18 (1H, m)	1.85 (1H, m)	1.88 (1H, m)	2.18 (1H, m)	1.80 (2H, m)
2b	1.80 (1H, m)	1.80 (1H, m)	1.82 (1H, m)	1.78 (1H, m)	
3	3.27 (1H, dd, J = 4.3, 11.7)	3.49 (1H, dd, J = 4.7, 11.6)	3.50 (1H, dd, J = 11.6, 4.6)	3.27 (1H, dd, J = 4.3, 11.6)	3.42 (1H, dd, J = 5.2, 10.8)
5	0.67 (1H, d, J = 11.2)	1.40 (1H, m)	1.41 (1H, m)	0.67 (1H, d, J = 11.1)	0.83 (1H, m)
6a	1.47 (1H, m)	4.40 (1H, td, J = 3.2, 10.3)	4.41 (1H, td, J = 10.6, 2.8)	1.51 (1H, m)	1.51 (1H, m)
6b	1.36 (1H, m)			1.36 (1H, m)	1.34 (1H, m)
7a	1.47 (1H, m)	2.49 (1H, m)	2.51 (1H, dd, J = 12.7, 2.8)	1.43 (1H, m)	1.52 (1H, m)
7b	1.24 (1H, m)	1.92 (1H, m)	1.93 (1H, m)	1.21 (1H, m)	1.40 (1H, m)
9	2.80 (1H, m)	1.53 (1H, m)	1.55 (1H, m)	1.38 (1H, m)	1.64 (1H, m)
11a	1.91 (1H, m)	1.95 (1H, m)	1.56 (1H, m)	1.91 (1H, m)	2.08 (2H, m)
11b	1.40 (1H, m)	1.41 (1H, m)	1.46 (1H, m)	1.41 (1H, m)	5.44 (1H, m)
12	3.89 (1H, m)	3.88 (1H, m)	3.88 (1H, m)	3.90 (1H, m)	
13	2.06 (1H, m)	2.71 (1H, m)	1.97 (1H, m)	2.77 (1H, m)	
15a	1.45 (1H, m)	1.52 (1H, m)	1.71 (1H, m)	1.64 (1H, m)	2.35 (1H, m)
15b	1.06 (1H, m)	1.11 (1H, m)	1.18 (1H, m)	1.09 (1H, m)	1.16 (1H, m)
16a	2.06 (1H, m)	1.45 (2H, m)	1.46 (2H, m)	1.98 (1H, m)	2.36 (1H, m)
16b	1.57 (1H, m)	1.96 (1H, m)	2.72 (1H, m)	1.52 (1H, m)	1.92 (1H, m)
17	1.40 (1H, m)	1.20 (3H, s)	0.81 (3H, s)	1.98 (1H, m)	
18	1.01 (3H, s)	1.01 (3H, s)	1.02 (3H, s)	1.01 (3H, s)	3.19 (1H, dd, J = 2.8, 10.8)
19a	0.80 (3H, s)			0.81 (3H, s)	1.74 (1H, m)
19b					1.27 (1H, m)
21a	5.14 (2H, s)	1.77 (3H, s)	1.79 (3H, s)	1.81 (3H, s)	1.33 (1H, m)
21b					1.05 (1H, m)
22a	2.48 (1H, m)	5.43 (1H, t, J = 7.0)	5.55 (1H, t, J = 6.7)	5.50 (1H, t, J = 6.6)	1.83 (1H, m)
22b	2.38 (1H, m)				1.74 (1H, m)
23	2.32 (1H, m)	2.72 (2H, m)	2.33 (2H, m)	2.77 (2H, m)	1.22 (3H, s)
24	5.28 (1H, m)	5.18 (1H, m)	1.71 (2H, m)	5.22 (1H, t, J = 7.2)	1.01 (3H, s)
25					0.87 (3H, s)
26	1.66 (3H, s)	1.59 (3H, s)	1.33 (3H, s)	1.62 (3H, s)	1.12 (3H, s)
27	1.59 (3H, s)	1.56 (3H, s)	1.33 (3H, s)	1.58 (3H, s)	1.21 (3H, s)
28	1.27 (3H, s)	2.02 (3H, s)	2.04 (3H, s)	1.28 (3H, s)	
29	1.09 (3H, s)	1.55 (3H, s)	1.58 (3H, s)	1.10 (3H, s)	0.91 (3H, s)
30	0.95 (3H, s)	0.80 (3H, s)	1.22 (3H, s)	0.95 (3H, s)	0.89 (3H, s)
3-O- β -D-Glucopyranosyl					
1'	4.89 (1H, m)	4.98 (1H, m)	5.01 (1H, d, J = 7.8)	4.91 (1H, d, J = 7.5)	6.31 (1H, d, J = 8.1)
2'	4.20 (1H, m)	4.04 (1H, m)	4.06 (1H, m)	4.22 (1H, m)	4.18 (1H, m)
3'	4.21 (1H, m)	4.20 (1H, m)	4.23 (1H, m)	4.23 (1H, m)	4.01 (1H, m)
4'	4.11 (1H, m)	4.16 (1H, m)	4.19 (1H, m)	4.13 (1H, m)	4.33 (1H, m)
5'	3.89 (1H, m)	3.91 (1H, m)	3.92 (1H, m)	3.90 (1H, m)	4.26 (1H, m)
6'a	4.53 (1H, m)	4.48 (1H, dd, J = 2.6, 11.6)	4.51 (1H, dd, J = 11.5, 2.5)	4.55 (1H, dd, J = 2.0, 11.7)	4.45 (1H, m)
6'b	4.32 (1H, m)	4.32 (1H, dd, J = 5.4, 11.6)	4.33 (1H, dd, J = 11.5, 5.4)	4.32 (1H, m)	4.37 (1H, m)
2'-O- β -D-Glucopyranosyl					
1''	5.33 (1H, d, J = 7.6)			5.35 (1H, d, J = 7.6)	
2''	4.09 (1H, m)			4.12 (1H, m)	
3''	4.28 (1H, m)			4.32 (1H, m)	
4''	4.28 (1H, m)			4.30 (1H, m)	
5''	3.89 (1H, m)			3.90 (1H, m)	
6''a	4.47 (1H, m)			4.46 (2H, m)	
6''b	4.43 (1H, m)				

¹H-NMR data measured at 500 MHz

Table 6
¹³C-NMR Spectroscopic Data for Compounds **17–21** in Pyridine-*d*₅

No.	Rk1 (17)	Rh4 (18)	25-hydroxy-Rh4 (19)	Rg5 (20)	Oleanolic acid 28-O-β-D-glucopyranoside (21)
δ _C multiplicity					
1	39.3 t	39.4 t	39.5 t	39.3 t	39.0 t
2	26.7 t	28.7 t	27.9 t	26.7 t	28.1 t
3	88.9 d	78.5 d	78.5 d	88.9 d	78.1 d
4	39.7 s	40.3 s	40.3 s	40.2 s	39.4 s
5	56.4 d	61.4 d	61.4 d	56.4 d	55.8 d
6	18.4 t	80.0 d	80.0 d	18.4 t	18.8 t
7	35.3 t	45.2 t	45.3 t	35.3 t	33.1 t
8	40.2 s	41.3 s	41.3 s	39.7 s	40.0 s
9	48.2 d	50.5 d	50.5 d	50.8 d	48.1 d
10	37.0 s	39.7 s	39.7 s	37.0 s	37.4 s
11	32.6 t	32.2 t	32.2 t	32.2 t	23.4 t
12	72.4 d	72.5 d	72.6 d	72.6 d	122.9 d
13	52.4 d	50.3 d	50.8 d	50.4 d	144.1 s
14	51.2 s	50.6 s	50.6 s	50.9 s	42.1 s
15	32.6 t	32.5 t	32.5 t	32.6 t	28.3 t
16	30.7 t	27.8 t	28.7 t	28.1 t	23.6 t
17	50.8 d	50.7 d	50.5 d	51.0 d	47.0 s
18	15.8 q	17.3 q	16.8 q	15.8 q	41.8 d
19	16.4 q	17.6 q	17.7 q	16.6 q	46.2 t
20	155.5 s	140.0 s	139.5 s	140.2 s	30.8 s
21	108.1 t	13.0 q	13.0 q	13.1 q	34.0 t
22	33.8 t	123.5 d	125.5 d	123.2 d	32.5 t
23	27.0 t	27.4 t	23.6 t	27.4 t	28.8 q
24	125.3 d	123.8 d	44.2 t	123.5 d	16.5 q
25	131.2 s	131.2 s	69.5 s	131.2 s	15.6 q
26	25.7 q	25.6 q	29.9 q	25.7 q	17.5 q
27	17.7 q	17.6 q	29.7 q	17.7 q	26.1 q
28	28.1 q	31.6 q	31.7 q	28.8 q	176.4 s
29	16.5 q	16.3 q	16.3 q	16.4 q	33.2 q
30	17.0 q	16.7 q	17.4 q	17.0 q	23.8 q
	3-O-β-D-Glucopyranosyl	6-O-β-D-Glucopyranosyl	6-O-β-D-Glucopyranosyl	6-O-β-D-Glucopyranosyl	28-O-β-D-Glucopyranosyl
1'	105.1 d	105.9 d	106.0 d	105.1 d	95.7 d
2'	83.4 d	75.3 d	75.4 d	83.4 d	74.1 d
3'	77.9 d	79.5 d	79.5 d	78.2 d	79.3 d
4'	71.6 d	71.7 d	71.8 d	71.6 d	71.1 d
5'	78.2 d	78.0 d	78.1 d	77.9 d	78.9 d
6'	62.8 t	63.0 t	63.1 t	62.7 t	62.2 t
	2'-O-β-D-Glucopyranosyl			2'-O-β-D-Glucopyranosyl	
1''	106.0 d			106.0 d	
2''	77.0 d			77.1 d	
3''	78.3 d			78.3 d	
4''	71.6 d			71.7 d	
5''	78.0 d			78.1 d	
6''	62.7 t			62.8 t	

Multiplicity of ¹³C NMR data was determined by DEPT experiments
¹³C-NMR data measured at 125 MHz

Compounds **5** and **6**, and **13** and **14** were isolated as mixtures of the stereoisomers and were not purified to individual stereoisomers. Compounds **1–21** were categorized by their backbones (PPD type **1–6**; PPD-derived type, **7, 8, 18**, and **19**; PPT type, **9–12, 15**, and **16**; PPT-derived type, **13, 14**, and **17**; and an oleanane-type triterpene, **21**). The ¹H-NMR and ¹³C-NMR spectral data are given in Tables 1–6.

The comprehensive ¹H-NMR and ¹³C-NMR spectral data of compounds **1–21** are worth determining for the structures of the less polar ginsenosides as some of their ¹H-NMR and ¹³C-NMR spectroscopic data are not available. Other data are either scattered throughout published papers, or dated, therefore it is hard to compare the structures of the isolated compounds. In the study, the results were assigned using one-dimensional and two-dimensional NMR spectroscopic methods and were also confirmed by comparison with previously published data. Some signals, such as those for the methyl groups of C-26–C-30 and the saturated methylenes, which have not been reported previously, were unambiguously determined using two-dimensional NMR spectra including ¹H-¹H COSY, HSQC and HMBC spectra.

The ¹³C-NMR spectral data suggested the following information for the structural elucidation of the ginsenosides isomers. First, the chemical shifts of the characteristic peaks between the 20(*S*) and 20(*R*) ginsenosides provided information for the identification of the stereoisomers. In particular, changes in the chemical shifts between the *S*- and *R*- forms at C-17, C-21 and C-22 in the ¹³C-NMR spectra were approximately Δδ (δ_S – δ_R) +4.1 ± 0.1, +4.3 ± 0.1, and –7.4 ± 0.1 ppm, respectively (Tables 2 and 4). Next, the presence of the signal (δ_C 88.8 ± 0.1 ppm) of the hydroxyl carbon at C-3, which did not overlap with other hydroxyl groups in the backbone and the sugar moieties, easily indicated whether it was a PPD- (**1–8, 17**, and **20**) or PPT-type (**9–16** and **18**). In addition, the signals at δ_C 170.6 ± 0.1 showed the existence of the acetyl groups (**5, 6, 15**, and **16**) (Tables 2 and 4). It was assumed that they were produced from the malonyl moiety by decarboxylation during the manufacturing process and were located at C-6 in the glucose group (**5, 6, 15**, and **16**) [23]. Finally, the chemical shifts of the down-field signals indicated the type of backbones. The values for a double bond at Δ24(25) in 3,12,20-trihydroxydammar-24-ene and 3,6,12,20-tetrahydroxydammar-24-ene (**1–6, 9–12, 15**, and **16**) were δ_C 126.1 ± 0.2

(C-24) and 130.1 ± 0.1 (C-25), respectively (Tables 2 and 4). However, they were shifted to δ_C 124.2 ± 1.0 and 131.2 ± 0.0 as a result of the dehydration at $\Delta 20(21)$ (**17**) or $\Delta 20(22)$ (**18** and **20**) (Table 6). The differences between the chemical shifts of δ_C 155.5 and 108.1, and of δ_C 140.1 ± 0.1 and 123.4 ± 0.2 ppm indicated the discrimination of 3,12-dihydroxydammar-20, 24-diene (**17**) and 3,12-dihydroxydammar-20(22),24-diene (**18** and **20**). These results were in perfect agreement with previously published values [21,24,25]. Compound **21**, an oleanane-type triterpene, might be produced by the selective hydrolysis of sugar residues at C-3 in ginsenoside Ro [26] (Table 6).

Conflicts of interest

All the contributing authors declare no conflicts of interest.

Acknowledgments

This research was supported equally by the Basic Science Research Program through the National Research Foundation of Korea (NRF), funded by the Ministry of Education, Science and Technology (501100002701) (2012R1A1A2039102), and the Global Leading Technology Program of the Office of Strategic R&D Planning (OSP grant 10039320), funded by the Ministry of Knowledge Economy, Korea (501100002994).

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