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Polyacetyleneginsenoside-Ro, a novel triterpene saponin from *Panax ginseng*

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Abstract—A new oleanolic acid-derived saponin, polyacetyleneginsenoside-Ro (**1**), was isolated along with the known ginsenosides-Ro methyl ester (**2**), -Rf, -Rg1, -Rg2, and gingerglycolipid B from the roots of *Panax ginseng* C. A. Meyer. The new saponin was identified as a ginsenoside-Ro derivative containing a polyacetylene side chain by spectroscopic means including 1D and 2D NMR, and was found to inhibit the replication of human immunodeficiency virus type 1 (HIV-1) with an IC_{50} value of 13.4 μg/mL (11.1 μM). © 2002 Elsevier Science Ltd. All rights reserved.

The root of *Panax ginseng* C. A. Meyer (Araliaceae), commonly known as Asian, Chinese, or Korean ginseng, is one of the oldest Chinese herbal medicines, having been recorded in the *Shen Nung Ben Cao Jing* in the 1st century AD.[1] Its history, medical uses, biological effects and chemistry have been extensively studied, documented, and reviewed.[1–5] In recent years, various formulations prepared from this plant material have been marketed, in varying degrees of quality, as dietary supplements in the USA under the Dietary Supplements Health Education Act (DSHEA) of 1994. A host of pharmacological properties of Asian ginseng are generally attributed to triterpene glycosides (saponins), called ginsenosides. More than 30 ginsenosides have been isolated and identified. Among them, ginsenosides-Rb_1, -Rb_2, -Rc, -Rg_1, -Re, and -Rd account for 90% of the total.[6] These compounds, along with the species specific ginsenoisde Rf, have been employed as marker compounds for the chemical analysis of *P. ginseng* products. As part of our continuing studies on the chemistry and biology of medicinal plants, we undertook the development of analytical high performance liquid chromatography (HPLC) and liquid chromatography-mass spectrometry (LC–MS) methods[7,8] for the analysis of ginsenosides, and for post-marketing quality control (profiling and quantitative analysis) of commercial ginseng products.[9,10] To facilitate these studies, the commercially unavailable ginsenoside Rf was isolated from a sample of *P. ginseng* root.[11] During the course of this phytochemical isolation, a new ginsenoside, which we named polyacetyleneginsenoside-Ro (**1**) (Fig. 1), was obtained, together with the known ginsenosides Ro methyl ester (**2**),[12] -Rf,[13] -Rg1,[14] -Rg2,[15] and gingerglycolipid B.[16] Compound **1** is composed of two sub-structural units: an oleanolic acid-derived triterpene saponin (ginsenoside) and a polyacetylene moiety, both of which had previously been isolated from *P. ginseng* as separate entities.[17,18]

Since polyacetyleneginsenoside-Ro (**1**) is a new natural product with an unknown profile of biological activity, it was subjected to a routine battery of bioassays available in our laboratory. Selected saponins have demonstrated anti-HIV activity in cell-based assays,[19–21] while others have been shown to possess inhibitory activity against the HIV-1 protease.[22] Consistent with the reported antiviral activities of the majority of oleanane-type saponins, polyacetyleneginsenoside-Ro (**1**) was found to inhibit the in vitro replication of HIV-1. In this paper, we report the isolation and characterization of this new saponin, as well as its anti-HIV activity in the HOG.R5 cell line. This reporter cell line was constructed specifically for quantitating HIV-1 replication in the microtiter format, and is based on the transactivation of a stably-integrated HIV-1 LTR-green fluorescent protein (GFP) transcription unit by the viral Tat protein.[23] The system was validated and adapted as a moderately high-throughput procedure for screening anti-HIV potential natural products.[24]

Keywords: *Panax ginseng*; Araliaceae; polyacetyleneginsenoside; HIV.

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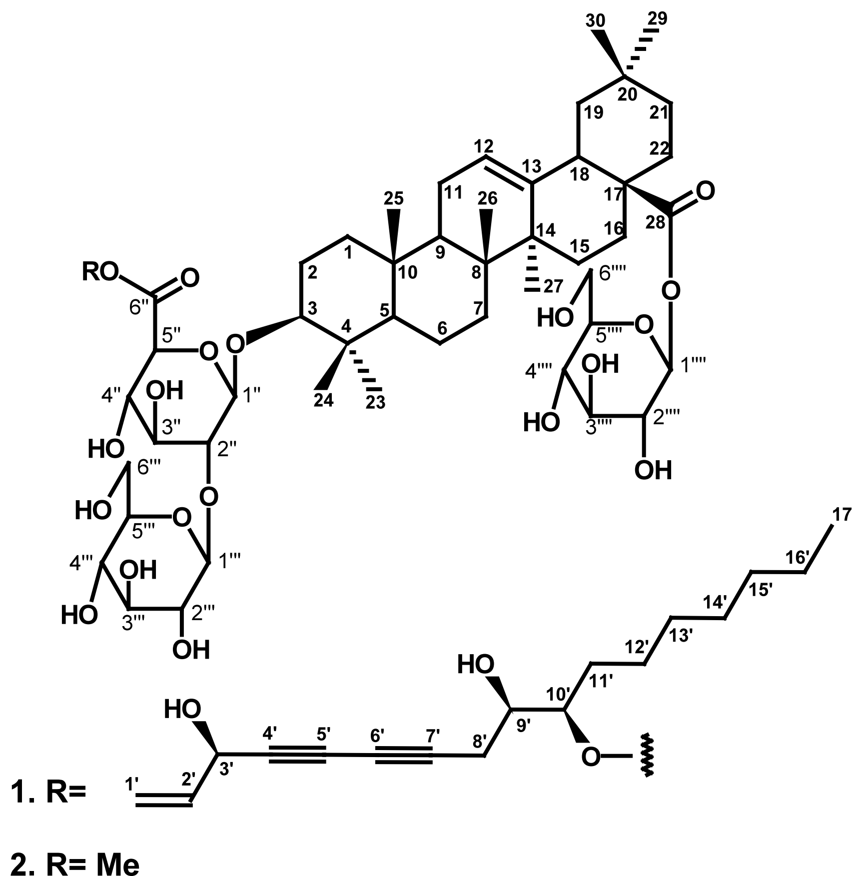

Figure 1. Structures of compounds **1** and **2**.

Polyacetyleneginsenoside-Ro (**1**) was isolated as a white powder from the methanol extract of *P. ginseng* C. A. Meyer (10 kg) by sequential column chromatography on Diaion HP-20 and silica gel, followed by preparative high performance liquid chromatography (HPLC).[25] HRMALDI-TOFMS gave $[M+Na]^+$ at *m*/*z* 1239.6670, corresponding to the molecular formula of $C_{65}H_{100}NaO_{21}$ (calcd for 1239.6655). Characteristic ^1H NMR signals for methyl groups [δ 1.26 (3H, s), 1.21 (3H, s), 1.10 (3H, s), 1.09 (3H, s), 0.91 (3H, s), 0.87 (6H, s); δ 33.18 (q), 28.01 (q), 26.12 (q), 23.69 (q), 17.51 (q), 16.68 (q), 15.61 (q)], in addition to the multi-methylene signals in the upfield region of ^1H and ^{13}C NMR spectra, revealed **1** to be an oleanolic acid triterpene derivative (Tables 1 and 2). Three sugar units were easily discerned from its NMR spectra by observing the relative lowfield signals of the anomeric protons and carbons [δ_H 6.34 (1H, d, J=8.08 Hz), 5.35 (1H, d, J=7.8 Hz), 4.89 (1H, d, J=7.5 Hz); δ_C 106. 05 (d), 105.57 (d), 95.80 (d)]. Comparison of the ^{13}C NMR data of **1** with that of ginsenoside-Ro methyl ester (**2**) revealed that the characteristic chemical shifts exhibited by **2** were also found in **1** (Tables 1 and 2). Thus, compound **1** was determined to be an oleanolic acid triterpene saponin with a ginsenoside-Ro unit. The remaining ^{13}C NMR signals of **1** were found to belong to a methyl carbon [δ 14.31 (q)], seven methylene carbons [δ 32.12 (t), 30.98 (t), 30.00 (t), 29.69 (t), 25.23 (2C, t), 22.98 (t)], three oxy-methine carbons [δ 76.94 (d), 70.88 (d), 63.01 (d)], two terminal double-bond carbons [δ 138.73 (d), 115.46 (t)], and two triple-bond carbons [δ 80.03 (s), 77.65 (s), 70.58 (s), 66.64 (s)]. Based on the long-range correlation observed in an HMBC experiment (Fig. 2), an oxy-methine group was assigned between the double bond and a triple-bond, which was coupled to a second triple-bond. The latter triple-bond was attached to a methylene carbon, and sequentially connected to two oxy-methine groups. A polyacetylene unit was, thus, elucidated. Alkaline hydrolysis of **1** with 3% Na_2CO_3 at room temperature for 2 h yielded a polyacetylene which was identified as the known panaxytriol by comparison with published spectral data.[17,26] The correlation between δ_H 5.57 (m, H-10′) and δ_C 169.53 (s, C-6″) in the HMBC spectrum fixed the coupling position of the oleanolic acid triterpene saponin and the polyacetylene units of **1**. The assignment of the absolute stereochemical configurations at C-9 and C-10 of panaxytriol had been a subject of debate, having been assigned as 3*R*, 9*R* and 10*R*[27–29] and as 3*R*, 9*S* and 10*S*.[30] Recently, Gurjar et al.[31] prepared four diasteromers of panaxytriol (3*R*,9*R*,10*R*-; 3*S*,9*R*,10*R*-; 3*R*,9*S*,10*S*- and 3*S*,9*S*,10*S*-) and their corresponding 9,10-acetonides. All four diasteromers of panaxytriol showed very similar NMR data to those of naturally occurring panaxytriol, but only the 3*R*,9*S*,10*S*-panaxytriol exhibited the same optical rotation ($[\alpha]_D$–25.5°)[31] as that of the naturally occurring panaxytriol ($[\alpha]_D$–25.4°).[28] The 3*S*,9*R*,10*R* and

Table 1. ^{1}H NMR data of compound **1** (500 MHz, pyridine-d_5, J in Hz)

H	1	H	1	H	1
Triterpene aglycone moiety	δ	Polyacetylene aglycone moiety	δ	Sugar moiety	δ
H_2-1	1.62 *m*	H-1′a	5.64 *dq* (17.0, 1.5)	H-1″	4.89 *d* (7.5)
H_2-2	1.57 *m*	H-1′b	5.23 *dq* (10.2, 1.5)	H-2″	4.19 *t* (8.0)
H-3	3.13 *dd* (12.1, 4.4)	H-2′	6.22 *ddd* (17.0, 10.2, 5.2)	H-3″	4.35 overlap
H-5	0.69 *brd* (11.7)	H-3′	5.34 *m*	H-4″	4.42 overlap
H-6a	1.42 *m*	H-8′a	2.99 *dd* (17.4, 8.2)	H-5″	4.53 *d* (9.7)
H-6b	1.31 *m*	H-8′b	2.90 *dd* (17.2, 4.8)	H-1‴	5.35 *d* (7.8)
H_2-7	1.80 *m*	H-9′	4.32 *m*	H-2‴	4.10 *t* (7.9)
H-9	1.65 overlap	H-10′	5.57 *m*	H-3‴	4.24 *t* (8.8)
H_2-11	1.97 *m*	H-11′a	2.10 *m*	H-4‴	4.30 *t* (8.8)
H-12	5.48 *brs*	H-11′b	1.97 overlap	H-5‴	3.92 *m*
H-15a	2.35 *m*	H_2-12′	1.19 *m*	H_2-6‴	4.47 overlap
H-15b	1.34 *m*	H_2-13′	1.19 *m*	H-1⁗	6.34 *d* (8.08)
H_2-16	1.97 *m*	H_2-14′	1.19 *m*	H-2⁗	4.22 *t* (7.6)
H-18	3.19 *brd* (13.5)	H_2-15′	1.19 *m*	H-3⁗	4.35 overlap
H-19a	1.76 *m*	H_2-16′	1.19 *m*	H-4⁗	4.32 *t* (9.2)
H-19b	1.30 *m*	Me-17′	0.81 *t* (7.30)	H-5⁗	4.04 *m*
H_2-21	1.39 *m*			H-6⁗a	4.47 overlap
H_2-22	1.18 *m*			H-6⁗b	4.43 overlap
Me-23	1.21 *s*				
Me-24	1.09 *s*				
Me-25	0.87 *s*				
Me-26	1.10 *s*				
Me-27	1.26 *s*				
Me-29	0.87 *s*				
Me-30	0.91 *s*				

3*S*,9*S*,10*S* isomers were dextro-rotatory, while the 3*R*,9*R*,10*R* polyacetylene showed an optical rotation of −12.3°. In addition, the four corresponding 9,10-acetonides of the panaxytriol diasteromers also showed very similar NMR data to those of the acetonide prepared from the naturally occurring panaxytriol, but only the 3*R*,9*S*,10*S*-panaxytriol acetonide ($[\alpha]_D$−28°)[31] exhibited a similar optical rotation to that of the naturally panaxytriol acetonide ($[\alpha]_D$−22.5°).[27] Based on these data, the naturally occurring panaxytriol must have the 3*R*,9*S*,10*S*-configuration.[31] Since the polyacetylene unit obtained by the alkaline hydrolysis of polyacetyleneginsenoside-Ro (**1**) showed similar optical rotation ($[\alpha]_D$−20.6°) to that of natural panaxytriol, the stereochemistry of the isolate must be 3′*R*,9′*S*,10′*S*. The structure of **1** was thus deduced, and given the trivial name of polyacetyleneginsenoside-Ro.

Polyacetyleneginsenoside-Ro (**1**) is the first example of a polyacetylene containing ginsenoside and was found to inhibit the replication of HIV-1 with an IC_{50} value of 13.4 μg/mL (11.1 μM). The compound was nontoxic toward the cultured cells up to a concentration of 20 μg/mL (16.4 μM). The reported role of the unusual monoterpenyl moieties of *Gleditsia* and *Gymnocladus* saponins[21] in modulating the anti-HIV activities of these compounds suggests that the polyacetylene moiety of **1** may well be responsible for the higher antiviral potency of this compound relative to that of the other published HIV-1 inhibitory saponins. Only one other oleanane-type saponin from *Panax*, zingibroside R1, has been reported to demonstrate anti-HIV activity.[20]

Little is known about the mechanism of action of these compounds. However, two mechanisms were proposed for the anti-HIV activity of glycyrrhizin: inhibition of viral adsorption to target cells by interference with protein kinase C activity and/or disruption of the initial stages of viral replication caused by non-specific interactions of the compound with the viral membrane.[32–34] None of the anti-HIV saponins reported in the literature, thus far, was found to affect the enzymatic activity of HIV-1 reverse transcriptase.[35,36] Further investigation into the mechanism of action of this class of compounds is warranted.

Acknowledgements

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Table 2. ^{13}C NMR data of compounds **1** and **2** (125 MHz, pyridine-d_5)

C	1	2	C	1	2	C	1	2
Triterpene aglycone moiety	δ	δ	Polyacetylene aglycone moiety	δ	δ	Sugar moiety	δ	δ
C-1	38.80 *t*	38.63 *t*	C-1′	115.46 *t*	–	C-1″	105.57 *d*	105.36 *d*
C-2	26.04 *t*	26.57 *t*	C-2′	138.73 *d*	–	C-2″	82.55 *d*	82.59 *d*
C-3	89.28 *d*	89.32 *d*	C-3′	63.01 *d*	–	C-3″	77.65 *d*	77.55 *d*
C-4	39.56 *s*	39.54 *s*	C-4′	77.65 *s*	–	C-4″	72.45 *d*	72.89 *d*
C-5	55.73 *d*	55.76 *d*	C-5′	70.58 *s*	–	C-5″	77.24 *d*	76.80 *d*
C-6	18.52 *t*	18.48 *t*	C-6′	66.64 *s*	–	C-6″	169.53 *s*	170.51 *s*
C-7	33.18 *t*	33.15 *t*	C-7′	80.03 *s*	–	C-1‴	106.05 *d*	105.97 *d*
C-8	39.94 *s*	39.89 *s*	C-8′	25.23 *t*	–	C-2‴	77.08 *d*	77.10 *d*
C-9	48.07 *d*	48.01 *d*	C-9′	70.88 *d*	–	C-3‴	78.01 *d*	77.99 *d*
C-10	37.01 *s*	36.91 *s*	C-10′	76.94 *d*	–	C-4‴	71.71 *d*	71.68 *d*
C-11	23.42 *t*	23.40 *t*	C-11′	30.98 *t*	–	C-5‴	78.41 *d*	78.36 *d*
C-12	123.03 *d*	122.88 *d*	C-12′	30.00 *t*	–	C-6‴	62.75 *t*	62.71 *t*
C-13	144.12 *s*	144.15 *s*	C-13′	29.69 *t*	–	C-1⁗	95.80 *d*	95.78 *d*
C-14	42.16 *s*	42.12 *s*	C-14′	25.23 *t*	–	C-2⁗	74.19 *d*	74.18 *d*
C-15	28.28 *t*	28.25 *t*	C-15′	32.12 *t*	–	C-3⁗	78.96 *d*	78.94 *d*
C-16	23.85 *t*	23.76 *t*	C-16′	22.98 *t*	–	C-4⁗	71.11 *d*	71.09 *d*
C-17	47.03 *s*	47.00 *s*	C-17′	14.31 *q*	–	C-5⁗	79.40 *d*	79.39 *d*
C-18	41.78 *d*	41.75 *d*				C-6⁗	62.21 *t*	62.20 *t*
C-19	46.27 *t*	46.20 *t*						
C-20	30.82 *s*	30.79 *s*						
C-21	34.01 *t*	34.00 *t*						
C-22	32.58 *t*	32.43 *t*						
C-23	28.01 *q*	28.12 *q*						
C-24	16.68 *q*	16.70 *q*						
C-25	15.61 *q*	15.54 *q*						
C-26	17.51 *q*	17.47 *q*						
C-27	26.12 *q*	26.10 *q*						
C-28	176.47 *s*	176.44 *s*						
C-29	23.69 *q*	23.65 *q*						
C-30	33.18 *q*	33.15 *q*						

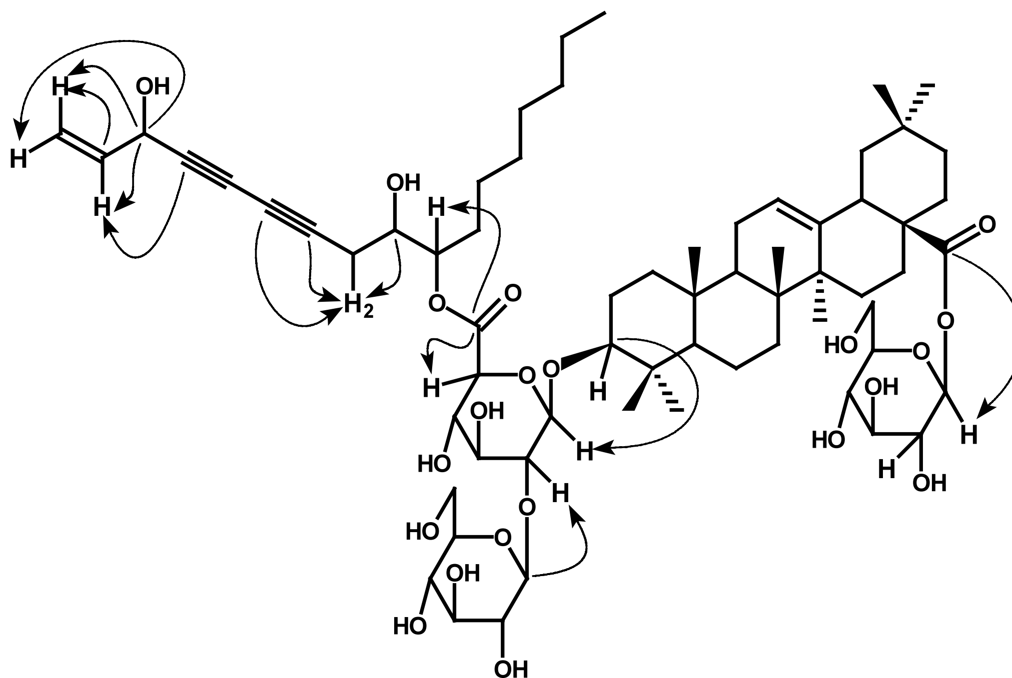

Figure 2. Selected HMBC correlations for polyacetyleneginsenoside-Ro (**1**) (Bruker DRX 500 MHz, pyridine-d_5).

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25. Polyacetyleneginsenoside-Ro (**1**): white power, $[\alpha]_D$ −13.9° (*c* 0.62, MeOH); UV (MeOH) λ_{max} (log ε) 207 (3.66), 242 (3.03), 256 (2.90) nm; IR (film) ν_{max} 3376 (br), 2927, 285, 2360, 2255, 1743, 1645, 1461, 1384, 1364, 1262, 1174, 1076, 1029, 892, 643, 597 cm^{-1}; HRMALDITOFMS *m/z* 1239.6670 $[M+Na]^+$ (calcd for $C_{65}H_{100}NaO_{21}$, 1239.6655, Δ+1.5 mmu).
26. Alkaline hydrolysis of polyacetyleneginsenoside-Ro (**1**). Compound **1** (10.16 mg) in 4 ml MeOH was stirred with 3% Na_2CO_3 solution (3.3 ml) at room temperature for 2 h. The solution was then neutralized with 1N HCl and concentrated to dry and subsequently extracted with $MeOH/CHCl_3$ (1:1). The extracted reaction mixture was subjected to preparative HPLC separation, eluted with $MeCN/H_2O$ (9:1) to yield corresponding panaxytriol (1.84 mg): $[\alpha]_D$ −20.6° (*c* 0.15, $CHCl_3$); ^1H NMR (pyridine-d_5, *J* in Hz) δ 7.12 (1H, brs, OH), 6.73 (1H, brs, OH), 6.22 (1H, ddd, $J=17.0$, 10.1, 5.1), 5.63 (1H, dt, $J=17.0$, 1.5), 6.06 (1H, brs, OH), 5.32 (1H, m), 5.21 (1H, dt, $J=10.1$, 1.4), 4.10 (1H, m), 3.99 (1H, pentalet, $J=4.1$), 3.02 (1H, ABd, $J=17.2$, 5.6), 2.90 (1H, ABd, $J=17.1$, 7.1), 1.82 (2H, m), 1.72 (1H, m), 1.51 (1H, m), 1.10–1.40 (11H, m), 0.85, (1H, m), 0.80 (3H, t, $J=7.1$); ^{13}C NMR (pyridine-d_5) δ 138.76 (d), 115.39 (t), 80.97 (s), 77.43 (s), 73.24 (2C, d), 70.66 (s), 66.40 (s), 63.02 (d), 34. 09 (t), 32.06 (t), 30.10 (t), 29.63 (t), 26.63 (t), 25.34 (t), 22.90 (t), 14.26 (q).
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