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Four new ginsenosides from red ginseng with inhibitory activity on melanogenesis in melanoma cells

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ABSTRACT

During a search for novel melanogenesis inhibitors originating from nature sources, four new ginsenosides, including three dammarane-type triterpenoid saponins, 20(*S*)-ginsenoside-Rf-1a (**1**), 20*Z*-ginsenoside-Rs_4 (**2**), 23-*O*-methylginsenoside-Rg_{11} (**3**), and one oleanane-type saponin, ginsenoside-Ro-6′-*O*-butyl ester (**4**) were isolated from red ginseng (the steamed ginseng) to evaluate their protective effects against melanogenesis. Compounds **2** and **3** exhibited potent inhibitory effects against both melanin synthesis and tyrosinase activity in a dose-dependent manner in the α-MSH-stimulated B16 melanoma cells, and were more potent than the positive control arbutin, a well-known tyrosinase inhibitor. The results indicated that just the two carbon-20(22) double-bond-type ginsenosides showed strong inhibiting activity on melanogenesis through reducing tyrosinase activity. Thus, ginsenosides with such similar chemical structure in red ginseng may be potential natural products as tyrosinase inhibitors against malignant melanoma.

Ginseng, the roots and rhizomes of *Panax ginseng* C. A. Mey. (Araliaceae), is widely used as one of the most famous traditional Chinese medicine in Asian region for over 2000 years. It possesses various biological effects including anticancer, antitumor, antioxidative, antiaging, neurovascular modulatory and other activities.[1–3] Red ginseng, as one of the most common commercial ginseng, is manufactured by steaming the raw ginseng. This steaming process may lead to significant variations in the chemical constituents especially for ginsenosides which are considered to be the principal active components in ginseng.[4] A large number of studies have demonstrated well that many ginsenosides only exist in red ginseng such as ginsenosides-Rg_3, -Rg_5, -Rg_6, -Rh_1, -Rh_2, -Rk_1–Rk_3 and -Rs_3–Rs_7, and fortunately, some of them have remarkable biological activities.[5–8]

The control of melanin formation is quite significant in the treatment of abnormal skin pigmentation, which may transform to malignant melanoma. Melanogenesis is a complex pathway involving endogenous substances such as alpha-melanocyte stimulating hormone (α-MSH),[9] as well as exogenous substances including drugs, herbicides, carcinogens,[10] and UV radiation,[11,12] etc. Tyrosinase is a key enzyme in the melanin biosynthesis and its expression closely correlates with melanogenesis.[13] Therefore, the effective inhibitory action on tyrosinase activity is greatly significant for the prevention and treatment of pigmentation. Since several tyrosinase inhibitors such as kojic acid and arbutin were found to be either toxic or have side effects, safe natural sources against tyrosinase activity were worth being explored.[14,15] Recently, the oral administration of red ginseng powder was found to be an effective treatment for patients with melasma.[16] Here, four new ginsenosides, including three dammarane-type triterpenoid saponins, 20(*S*)-ginsenoside-Rf-1a (**1**), 20*Z*-ginsenoside-Rs_4 (**2**), 23-*O*-methylginsenoside-Rg_{11} (**3**), and one oleanane-type saponin, ginsenoside-Ro-6′-*O*-butyl ester (**4**) were isolated from red ginseng (the steamed ginseng). We examined whether these novel ginsenosides had protective effects against melanogenesis in the α-MSH-stimulated B16 melanoma cells.

Compound **1** was obtained as a white powder and assigned the molecular formula $C_{42}H_{72}O_{14}$ on the basis of 42 carbon signals in the ^{13}C NMR and a quasi-molecular ion peak at *m/z* 845.4872 $[M+HCOOH-H]^-$ in the HR-ESI-MS. Its IR spectrum exhibited strong absorption bands at 3367, 1647 and 1030 cm^{-1} due to hydroxyl, double bond and ether functions. The presence of a dammarane-type triterpene aglycone with four oxygenated carbons and one double bond, β-glucopyranosyl ($J_{H\text{-}1'}$ = 7.4 Hz) and α-glucopyranosyl ($J_{H\text{-}1''}$ = 3.7 Hz) groups was deduced from the ^1H, ^{13}C NMR and HSQC spectra. The NMR data of **1** (Table 1) was found to be similar with those of 20(*S*)-ginsenoside-Rf[17] except the glucopyranosyl chain unit. Further comparative studies revealed that the characteristic C-1′ (δ_C 103.9) and C-4′ (δ_C 71.7)

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Table 1
^{1}H NMR (400 MHz) and ^{13}C NMR (100 MHz) data for compounds **1**–**4** in py-d_5[a]

Pos.	1		2		3		4	
	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C
1	0.99 (m), 1.66 (m)	39.3t	1.52 (m), 0.78 (m)	39.3t	1.52 (m), 0.79 (m)	39.2t	1.43 (m), 0.82 (m)	38.7t
2	1.81 (m), 1.85 (m)	27.9t	1.83 (m), 2.19 (m)	26.8t	1.95 (m), 1.53 (m)	29.3t	2.08 (m), 1.83 (m)	26.6t
3	3.49 (dd, 11.6, 4.5)	78.5d	3.28 (dd, 11.6, 4.4)	89.2d	3.27 (dd, 11.4, 4.4)	88.9d	3.23 (dd, 11.6, 4.4)	89.3d
4	—	40.3s	—	39.8s	—	39.7s	—	39.5s
5	1.39 (d, 10.5)	61.3d	0.71 (br d, 11.5)	56.4d	0.68 (br d, 11.0)	56.3d	0.68 (br d, 11.6)	55.7d
6	4.35 (m)	80.2d	1.32 (m), 1.46 (m)	18.5t	1.33 (m), 1.45 (m)	18.4t	1.42 (m), 1.31 (m)	18.5t
7	1.88 (m), 2.40 (m)	45.0t	1.22 (m), 1.47 (m)	35.4t	1.22 (m), 1.45 (m)	35.3t	1.78 (m), 1.31 (m)	33.1t
8	—	41.0s	—	40.3s	—	40.2s	—	39.9s
9	1.52 (m)	50.1d	1.56 (m)	50.9d	1.40 (m)	50.8d	1.58 (m)	48.0d
10	—	39.6s	—	37.1s	—	37.0s	—	36.9s
11	1.52 (m), 2.02 (m)	32.0t	1.69 (m), 1.93 (m)	32.6t	1.68 (m), 1.92 (m)	32.6t	2.06 (m), 1.92 (m)	23.8t
12	3.88 (m)	71.0d	3.92 (m)	72.5d	3.91 (m)	72.0d	5.40 (br s)	122.8d
13	2.03 (m)	48.2d	2.06 (m)	50.9d	2.05 (m)	51.0d	—	144.1s
14	—	51.6s	—	51.2s	—	51.0s	—	42.1s
15	1.03 (m), 1.62 (m)	31.2t	1.09 (m), 1.42 (m)	32.7t	1.08 (m), 1.42 (m)	32.8t	2.32 (m), 1.16 (m)	28.2t
16	1.39 (m), 1.82 (m)	26.8t	1.70 (m), 2.04 (m)	28.4t	1.81 (m), 2.19 (m)	26.7t	2.06 (m), 1.95 (m)	23.4t
17	2.27 (m)	54.7d	2.06 (m)	50.2d	2.83 (m)	50.6d	—	47.0s
18	1.16 (s)	17.6q	1.13 (s)	15.8q	1.03 (s)	15.8q	3.17 (m)	41.7d
19	0.98 (s)	17.7q	1.05 (s)	16.5q	0.85 (s)	16.4q	1.74 (m), 1.22 (m)	46.2t
20	—	72.9s	—	139.8s	—	146.8s	—	30.7s
21	1.38 (s)	27.0q	1.90 (s)	19.9q	1.91 (s)	14.0q	1.35 (m), 1.06 (m)	34.0t
22	1.68 (m)	35.8t	5.25 (t, 7.0)	123.7d	5.52 (d, 9.6)	120.5d	1.30 (m), 0.93 (m)	32.5t
23	2.28 (m), 2.60 (m)	23.0t	2.92 (t, 7.0)	27.1t	4.05 (dd, 9.6, 7.8)	77.9d	1.23 (s)	28.1q
24	5.31 (t, 7.3)	126.3d	5.26 (t, 6.9)	124.6d	3.11 (d, 7.8)	66.6d	1.07 (s)	16.7q
25	—	130.7s	—	130.8s	—	57.0s	0.83 (s)	15.5q
26	1.64 (s)	25.8q	1.63 (s)	25.7q	1.27 (s)	24.9q	1.07 (s)	17.4q
27	1.63 (s)	17.3q	1.59 (s)	17.7q	1.34 (s)	19.9q	1.23 (s)	26.1q
28	2.08 (s)	31.7q	1.34 (s)	28.0q	1.28 (s)	28.1q	—	176.4s
29	1.58 (s)	16.3q	0.85 (s)	16.5q	1.10 (s)	16.5q	0.86 (s)	23.6q
30	0.78 (s)	16.8q	0.97 (s)	17.0q	0.96 (s)	17.0q	0.89 (s)	33.1q
1′	4.91 (d, 7.4)	105.7d	4.89 (d, 7.3)	104.9d	4.91 (d, 7.6)	105.1d	4.95 (d, 7.6)	105.4d
2′	4.55 (m)	74.8d	4.15 (m)	84.3d	4.22 (m)	83.4d	4.30 (m)	82.6d
3′	4.32 (m)	78.8d	4.21 (m)	78.1d	4.30 (m)	78.2d	4.31 (m)	77.6d
4′	4.30 (m)	81.3d	4.12 (m)	71.0d	4.32 (m)	71.6d	4.43 (m)	72.7d
5′	3.70 (m)	76.4d	3.90 (m)	78.0d	4.05 (m)	78.0d	4.49 (m)	76.9d
6′	4.47 (m), 4.33 (m ov.)	62.2t	4.50 (br d, 11.0), 4.34 (m ov.)	62.9t	4.34 (m), 4.46 (m ov.)	62.6t	—	169.9s
1″	5.88 (d, 3.7)	103.0d	5.31 (d, 7.7)	106.2d	5.35 (d, 7.6)	106.0d	5.38 (d, 7.6)	105.9d
2″	4.15 (m)	74.4d	4.12 (m)	76.8d	4.09 (m)	77.1d	4.11 (m)	77.0d
3″	4.03 (m)	75.3d	4.27 (m)	78.6d	3.91 (m)	78.3d	4.25 (m)	77.9d
4″	4.13 (m)	71.9d	4.32 (m)	71.4d	4.12 (m)	71.6d	4.30 (m)	71.7d
5″	3.99 (m)	75.5d	4.00 (m)	75.4d	4.12 (m)	78.0d	3.91 (m)	78.3d
6″	4.52 (m), 4.33 (m ov.)	62.8t	4.92 (m), 4.78 (dd, 11.6, 4.6)	64.8t	4.55 (br d, 10.5), 4.46 (m ov.)	62.8t	4.46 (m ov.), 4.35 (m)	62.7t
1‴			—	171.0s	3.46 (s)	55.6q	6.32 (d, 8.0)	95.7d
2‴			2.05 (s)	20.9q			4.20 (m)	74.1d
3‴							4.32 (m)	79.3d
4‴							4.33 (m)	71.1d
5‴							4.02 (m)	78.9d
6‴							4.52 (m), 4.45 (m ov.)	62.2t
1⁗							4.24 (t, 6.3)	65.0t
2⁗							1.56 (m)	30.8t
3⁗							1.31 (m)	19.2t
4⁗							0.75 (t, 7.3)	13.7q

[a] In ^{1}H NMR, coupling constants in parenthesis are given in hertz (s: single peak; d: double peaks; dd: double-double peaks; br s: broad single peak; br d: broad double peaks; m: multipeaks; t: triplet peaks; ov.: overlapped). In ^{13}C NMR, C-multiplicities were established by a HSQC experiment (s: C; d: CH; t: CH_2, q: CH_3).

signals of the glucopyranosyl group in 20(*S*)-ginsenoside-Rf[18] significantly downfield-shifted to δ_C 105.7 and δ_C (81.3) in **1**, respectively, while the C-2′ signal (δ_C 79.7) in 20(*S*)-ginsenoside-Rf was upfield-shifted to δ_C 74.8 in **1**, and the HMBC correlation (Fig. 1) observed between δ_H 5.88 (H-1″, from the α-glucopyranosyl group) and δ_C 81.3 (C-4′) indicated the α-glucopyranosyl group was located at C-4′ of β-glucopyranosyl unit, which was located at C-2′ of β-glucopyranosyl unit in 20(*S*)-ginsenoside-Rf. On the other hand, the HMBC correlation observed between δ_H 4.91 (H-1′, J = 7.4) and δ_C 80.2 (C-6) confirmed the β-glucopyranosyl group substituted at C-6 of the aglycone. Ultimately, compound **1** was unambiguously identified as 3β,6α,12β,20(*S*)-tetrahydroxydammar-24-ene-6-*O*-α-D-glucopyranosyl-(1→4)-β-D-glucopyranoside. It was a new compound and given the trivial name 20(*S*)-ginsenoside Rf-1a.

Compound **2** was also obtained as a white powder. The molecular formula $C_{44}H_{72}O_{13}$ was determined by 44 carbon signals in the ^{13}C NMR spectrum and the accurate mass measurement of $[M+HCOOH-H]^-$ at *m/z* 853.4938 in the HR-ESI-MS. Its IR spectrum exhibited strong absorption bands at 3369, 1735 and 1031 cm^{-1} due to hydroxyl and ether carbonyl functional groups. The ^{1}H and ^{13}C NMR spectra showed resonances for two double bonds, two D-glucopyranosyl units, one carbonyl group with a dammarane-type triterpene aglycone.[2] The NMR data of **2** (Table 1) was found to be very similar with those of (*E*)-20(22)-ginsenoside-Rs$_4$.[5] The characteristic signals (δ_H 1.82 and δ_C 13.2) of C-21 from the (*E*)-20(22)-type double-bond was disappeared, whereas the characteristic signals (δ_H 1.90 and δ_C 19.9) of C-21 from the (*Z*)-20(22)-type double-bond[19] was observed in **2**. This conclusion was further supported by the correlations between H-17 (δ_H 2.06),