

A Genuine Dammarane Glycoside, (20E)-Ginsenoside F₄ from Korean Red Ginseng

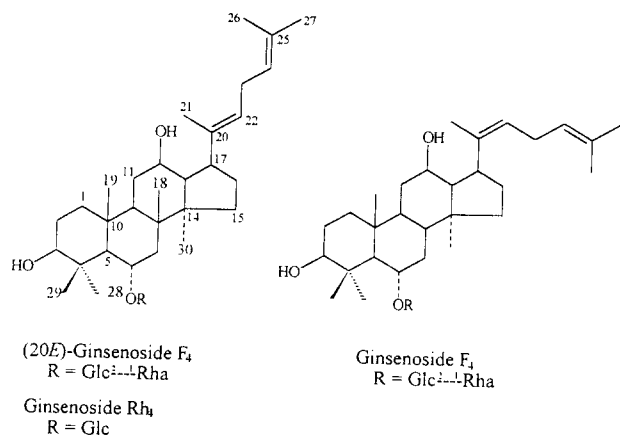
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In recent years, several new saponin constituents have been reported from Korean ginseng (Kim *et al.*, 1995a, Zhang *et al.*, 1990, Chen *et al.*, 1987). Especially, regarding with the anti-tumor activity of ginseng, ginsenoside Rh family has been studied extensively (Baek *et al.*, 1995). They are mostly found in Red Ginseng as the structure of partial hydrolysate or as the modified structure of having double bond between C-20 and C-22. These structures can be formed during the process of white ginseng to prepare Red Ginseng. And several trials have been performed to make these anti-tumor active ginsenosides by chemical treatment (Kim *et al.*, 1991, Kim *et al.*,



1995b). In continuing of our research for the new bioactive ginseng saponin, a new dammarane ginsenoside has been isolated from the heat treated ginseng. Its structure was identified as the geometric

Table I. ¹³C-NMR chemical shifts of (20E)-ginsenosid F₄, ginsenoside F₄, ginsenoside Rh₄^a

carbon no.	(20E)-F ₄ ^b	F ₄ ^c	Rh ₄ ^d
1	39.46	39.5	38.9
2	27.74	27.8	27.3
3	78.39	78.4	79.5
4	40.00	40.1	39.8
5	60.82	60.9	60.9
6	74.37	74.4	78.0
7	46.15	46.2	44.7
8	41.38	41.4	40.8
9	50.11	50.1	50.0
10	39.70	40.0	39.2
11	32.18	32.2	31.1
12	72.28	70.3	71.2
13	50.31	50.7	49.8
14	50.87	50.9	50.3
15	32.58	32.6	32.0
16	27.42	27.1	26.9
17	50.67	52.0	50.1
18	17.70	17.7	17.2
19	17.70	17.8	17.2
20	140.10	140.1	139.5
21	13.01	27.5	12.5
22	123.60	123.5	122.9
23	29.96	30.0	29.4
24	124.00	125.4	124.7
25	131.25	131.3	130.7
26	25.67	25.8	25.1
27	17.61	17.6	16.8
28	32.17	32.6	31.1
29	16.92	17.0	15.8
30	17.15	17.2	16.2
sugar moieties			
Glc 1	101.80	101.8	105.4
2	79.42	79.5	74.8
3	78.30	78.4	79.0
4	72.43	72.6	72.0
5	78.51	78.4	77.5
6	63.09	63.2	62.5
Rha 1	101.94	102.0	
2	72.57	72.3	
3	72.57	72.4	
4	74.17	74.2	
5	69.46	69.5	
6	18.75	18.8	

^aAll spectra were recorded in pyridine-d₅, chemical shift in ppm relative to internal TMS.

^bThe spectra were recorded at 125 MHz.

^cZhang *et al.*, 1990.

^dBaek *et al.*, 1996.

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isomer of ginsenoside F_4 isolated from the leaf of ginseng (Zhang *et al.*, 1990).

Six-year-old fresh ginseng (*Panax ginseng* C. A. Meyer) were heat-treated three times under 100°C for 3 hours using autoclave. After air drying, the material (300 g) was extracted with MeOH (1Lx3 times) with reflux. The MeOH extract (145 g) was partitioned between ether and water to remove lipid soluble fraction. The water layer was partitioned again with EtOAc and *n*-BuOH sequentially. The EtOAc soluble fraction (19 g) was applied to silica gel column with $CH_2Cl_2/MeOH$ (7:1→5:1→3:1) as eluent to yield subfraction containing compound **1** (2.3 g). It was further purified by HPLC on reverse phase column with 51% acetonitrile and yielded compound **1** (18 mg); amorphous powder, mp 177-181°C, $[\alpha]_D^{22}$ -7.93 (c=0.28, MeOH).

Compound **1** shows bands for hydroxyl groups at 3400 cm^{-1} , C=C at 1630 cm^{-1} and C-O at 1080 cm^{-1} in IR spectrum. The pattern of NMR spectra is very similar with those of ginsenoside F_4 (Zhang *et al.*, 1990). In 1H -NMR spectra (500 MHz, pyridine- d_5) of compound **1**, two olefinic [δ 5.45 (1H, t, J=6.9 Hz, H-22), 5.15 (1H, brt, J=7.3 Hz, H-24)], two anomeric [δ 5.26 (1H, d, J=6.7 Hz, anomeric H of glc), 4.79 (1H, brs, anomeric H of rha)] proton signals were observed. In 1H - 1H COSY spectra, peak at 2.75 (2H, H-23) correlated with two olefinic protons. And the compound **1** shows a $[M+Na]^+$ peak at m/z 789 in FAB mass spectrum (molecular formula, $C_{42}H_{70}O_{12}$), which shows that compound **1** is dehydrated structure of ginsenoside Rg_2 (molecular formula, $C_{42}H_{72}O_{13}$) like gindenoside F_4 . In the DEPT spectra (125 MHz, pyridine- d_5), two quaternary olefinic carbons (δ 140.1 and 131.25) and another two olefinic carbons (δ 124.00, C-24 and δ 123.60, C-22) were found. These NMR data is very similar with those of ginsenoside F_4 , but their configuration is different. As shown in Table 1, the chemical shift value of C-12, C-17, C-21, C-24 is different together. These spectral differences come from the configurational difference at the double bond between C-20 and C-22. Especially chem-

ical shift of C-21 of compound **1** shifted to downfield upto 14 ppm from ginsenoside F_4 . This spectral pattern is exactly same as ginsenoside Rh_4 (Baek *et al.*, 1996). The structure of compound **1** is confirmed as (20*E*)-3 β ,6 α ,12 β -trihydroxydammar-20(22),24-diene-6-O- α -L-rhamnopyranosyl-(1→2)- β -D-glucopyranoside. Compound **1** was also found in the commercial Korean Red Ginseng.

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