Triterpene Saponins from *Pleurospermum kamtschaticum* and Their Biological Activity

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Received March 26, 2012; accepted May 12, 2012

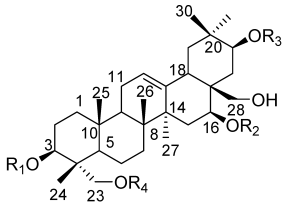
Eleven new triterpene saponins (1–11), together with fourteen known triterpene and triterpene saponins (12–25) were isolated from a MeOH extract of *Pleurospermum kamtschaticum* Hoffmann (Umbelliferae). The chemical structures of the new compounds (1–11) were determined by means of MS, ^{1}H-NMR, ^{13}C-NMR, correlated spectroscopy (COSY), heteronuclear multiple bond correlation (HMBC), total correlated spectroscopy (TOCSY) and nuclear Overhauser effect spectroscopy (NOESY) to be pleurosaponin A (1)–K (11). The isolated compounds were tested for their cytotoxicity against four human tumor cell lines (A549, SK-OV-3, SK-MEL-2, HCT15) *in vitro* using the sulforhodamine B bioassay (SRB) assay. All compounds showed little cytotoxicity against tested cell lines (IC_{50} >30 μM).

Key words *Pleurospermum kamtschaticum*; Umbelliferae; triterpene saponin; cytotoxicity

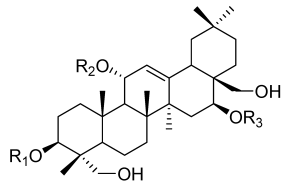
Pleurospermum kamtschaticum Hoffmann is a perennial herb that grows in the fields and mountains of Asian countries. The aerial parts of *P. kamtschaticum* have been used as Korean traditional medicine for the treatment of arthritis, atherosclerosis and impotence.[1)] Recent studies have demonstrated that the methanol extract of *P. kamtschaticum* and its component, buddlejasaponin IV, inhibited hyperlipodemia and hypercholesterolemia in rats.[2)] And anticarcinogenic activity of the methanol extract of *P. kamtschaticum* was reported in colon cancer cells.[3)] In our search for bioactive constituents from Korean medicinal plants, we investigated the methanol extract of *P. kamtschaticum*. Column chromatographic purification of EtOAc and *n*-BuOH-soluble fractions of the MeOH extract led to the isolation of 11 new triterpene saponins (**1–11**) and fourteen known triterpene and triterpene saponins (**12–25**). The structures of these new compounds were elucidated on the basis of 1D- and 2D-NMR spectroscopic data analysis (^{1}H-, ^{13}C-NMR, correlated spectroscopy (COSY), heteronuclear multiple quantum coherence (HMQC), heteronuclear multiple bond correlation (HMBC), total correlated spectroscopy (TOCSY) and, nuclear Overhauser effect spectroscopy (NOESY)), as well as chemical means. Compounds **1–25** were evaluated for cytotoxicity against four human cancer cell lines.

Results and Discussion

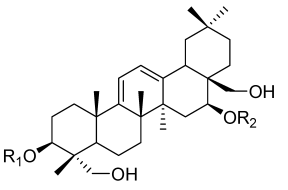
The dried aerial parts of *P. kamtschaticum* were extracted with 80% methanol at room temperature. The extract was suspended in H_2O and successively partitioned with *n*-hexane, EtOAc and *n*-BuOH. The EtOAc and *n*-BuOH fraction was separated by column chromatography to afford eleven new compounds (**1–11**) along with fourteen known compounds



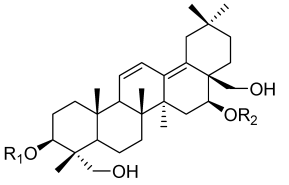
1. R_1=H, R_2=H, R_3=Glc, R_4=H
2. R_1=H, R_2=H, R_3=Glc, R_4=$COCH_3$
3. R_1=H, R_2=H, R_3=Glc(1-2)Glc R_4=H
4. R_1=H, R_2=H, R_3=Glc(1-2)Glc, R_4=$COCH_3$
5. R_1=Glc(1-2)[Glc(1-3)]Fuc, R_2=H, R_3=Glc, R_4=H



6. R_1=Fuc, R_2=H, R_3=H
6a. R_1=H, R_2=H, R_3=H
7. R_1=Glc(1-2)[Glc(1-3)]Fuc, R_2=H, R_3=Glc
8 . R_1=Glc(1-2)[Glc(1-3)]Fuc, R_2=CH_3, R_3=Glc



9. R_1=Glc(1-2)[Glc(1-3)]Fuc, R_2=H
9a. R_1=Glc(1-2)Fuc, R_2=H
10. R_1=Glc(1-2)[Glc(1-3)]Fuc, R_2=Glc



11. R_1=Glc(1-2)[Glc(1-3)]Fuc, R_2=Glc

Fig. 1. Structures of Compounds **1–11**

The authors declare no conflict of interest.

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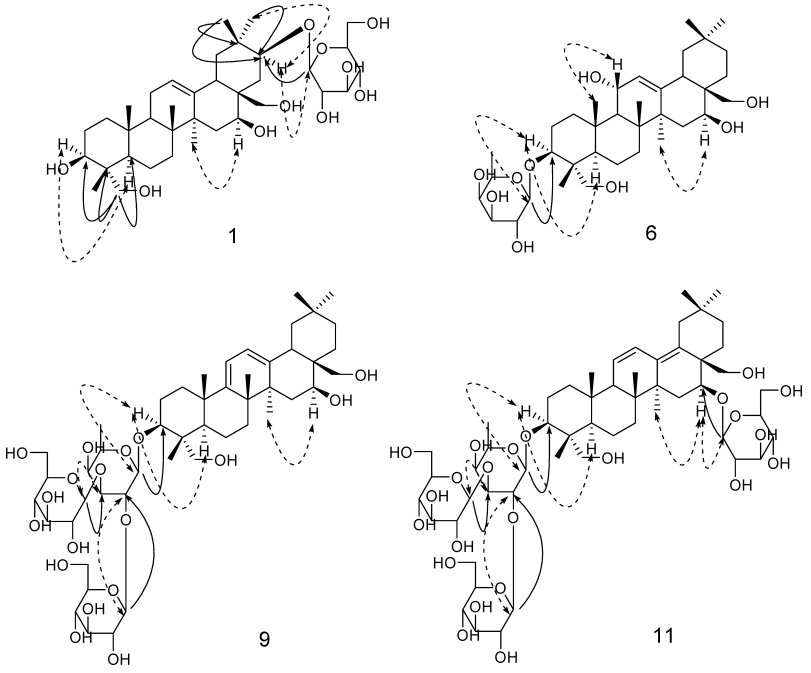

Fig. 2. Key HMBC (→) and NOESY (⇢) Correlations of **1**, **6**, **9** and **11**

(**12**–**25**). By comparison of ^{1}H-, ^{13}C-NMR and MS data with those reported in the literature, the structures of the known compounds (**12**–**25**) were identified as gymnestrogenin (**12**),[4] bepleuroside III (**13**),[5] saikosaponin b_3 (**14**),[6] 11*α*,16*β*,23,28-tetrahydroxyolean-12-en-3*β*-yl-[*β*-D-glucopyranosyl(1→2)]-[*β*-D-glucopyranosyl(1→3)]-*β*-D-fucopyranoside (**15**),[7] buddlejasaponin IVa (**16**),[8] saikosaponin b_1 (**17**),[9] buddlejasaponon IVb (**18**),[10] clinoposaponin IX (**19**),[11] saikogenin a (**20**),[8] buddlejasaponin IV (**21**),[12] clinoposaponin VIII (**22**),[8] 3-*O*-*β*-D-fucopyranosylsaikogenin F (**23**),[6] clinoposaponin XIV (**24**)[11] and clinoposaponin XVI (**25**).[11]

Pleurosaponin A (**1**), an amorphous powder, gave a sodiated molecular ion at *m/z* 675.4085 $[M+Na]^+$ (Calcd for 675.4084) in the high resolution (HR)-FAB-MS, which suggested the molecular formula $C_{36}H_{60}O_{10}Na$. The ^{1}H-NMR spectrum showed one olefinic proton signal at δ 5.22 (1H, brs), six tertiary methyl signals at δ_H 0.93 (3H, s), 0.97 (3H, s), 1.00 (3H, s), 1.11 (3H, s), 1.17 (3H, s), 1.25 (3H, s), oxymethine protons at δ_H 4.12 (1H, dd, *J*=11.0, 4.5 Hz) and 4.35 (1H, *d*, *J*=9.3 Hz), two oxymethylene protons at δ_H 3.64 (1H, d, *J*=10.4 Hz), 3.65 (1H, m), 4.10 (1H, d, *J*=11.0 Hz) and 4.28 (1H, d, *J*=10.4 Hz) and one sugar anomeric proton signal at δ_H 4.98 (1H, d, *J*=7.9 Hz). In the ^{13}C-NMR spectrum, 36 carbon signals appeared, which included six methyl carbon signals at δ_C 13.0, 16.1, 16.9, 18.3, 27.0 and 29.1, two olefinic carbon signals at δ_C 123.3 and 142.8, three oxygenated methine carbon signals at δ_C 68.1, 73.3 and 82.5, two oxygenated methylene carbon signals at δ_C 67.8 and 67.9, eight methylene carbon signals at δ_C 18.5, 23.9, 27.6, 32.6, 33.9, 36.2, 38.9 and 47.7, three methine carbon signals at δ_C 43.6, 47.2 and 48.5, six quaternary carbon signlas at δ_C 36.7, 36.9, 40.1, 42.9, 43.7 and 43.9, and six signals assignable to the sugar moiety δ_C 64.0, 72.6, 75.9, 78.4, 78.8 and 106.5. These NMR spectra were very similar with those of gymnestrogenin (**12**), which was isolated from *Gymnema sylvestre*.[4] The full NMR assignments and connectivities were determined by COSY, HMQC, and HMBC. The HMBC spectrum showed correlations between the δ_H 4.05 (1H, m, H-21) with δ_C 18.3 (C-30) and 29.1 (C-29), and δ_H 4.98 (1H, d, *J*=7.9 Hz, H-1′) with δ_C 82.5 (C-21), confirming the position of the sugar at C-21. The relative stereochemistry of the aglycone was assumed to be same with that of **12**, by comparing *J* values of **1** with those of **12**. That was also reconfirmed by NOESY spectrum (Fig. 2). The anomeric configuration for the sugar was to be a *β*-form from the coupling constant of 7.9 Hz.[13] Acid hydrolysis of **1** with 2 N HCl yielded gymnestrogenin (**12**) whose ^{1}H-NMR and MS data were in good agreement with values reported previously[4] and D-glucose ($[\alpha]_D^{25}$ +49.4° *c*=0.04 in H_2O), which was confirmed by GC and co-TLC (EtOAc–MeOH–H_2O=9 : 3 : 1, *Rf* value: 0.2) with a glucose standard (Aldrich Co., U.S.A.). Thus, the structure of **1** was established as 21-*O*-*β*-D-glucopyranosyl-3*β*,16*β*,21*β*,23,28-pentahydroxyolean-12-en, and was named pleurosaponin A.

Pleurosaponin B (**2**), an amorphous powder, gave a sodiated molecular ion at *m/z* 717.4190 $[M+Na]^+$ (Calcd for 717.4190) in the HR-FAB-MS, which suggested the molecular formula $C_{38}H_{62}O_{11}Na$. The NMR spectra of **2** were very similar to those of compound **1**. The differences in the ^{1}H-NMR spectrum of **2** showed the additional acetyl group; δ_H 1.96 (3H, s) in ^{1}H-NMR spectrum δ_C 20.7 and 170.8 in ^{13}C-NMR spectrum. In the HMBC spectrum, the long-range correlations between the oxygenated proton at δ_H 4.23 (1H, d, *J*=10.9 Hz, H-23*α*) and 4.39 (1H, d, *J*=10.9 Hz, H-23*β*) and the acetyl carbonyl carbon at δ_C 170.8, indicated the presence of an acetyl group at C-23 in **2**. The relative configuration of **2** was confirmed to be identical to that of **1** in the NOESY spectrum. Thus, the structure of **2** was established as 21-*O*-*β*-D-glucopyranosyl-23-acetoxyl-3*β*,16*β*,21*β*,28-tetrahydroxyolean-12-en, and was named pleurosaponin B.

Pleurosaponin C (**3**), an amorphous powder, gave a sodiated molecular ion at *m/z* 837.4615 $[M+Na]^+$ (Calcd for 837.4612) in the HR-FAB-MS, which suggested the molecular formula $C_{42}H_{70}O_{15}Na$. The NMR spectra of **3** were very similar to

those of compound **1**. The major differences were the additional glucose signals in the ^{1}H-NMR spectrum of **3**; δ_H 3.89 (1H, m), 3.96 (1H, m), 4.03 (1H, m), 4.29 (1H, m), 4.35 (1H, m), 4.40 (1H, m), 5.24 (1H, d, J=7.6 Hz). The position of the glucose attachment was confirmed by the HMBC correlations; δ_H 4.97 [1H, d, J=7.6 Hz, H-1 of glucose-I (H-1′)] to δ_C 82.8 (C-21) of aglycone, and δ_H 5.24 [1H, d, J=7.6 Hz, H-1 of glucose-II (H-1″)] to δ_C 84.3 [C-2 of glucose-I (C-2′). The position of glucose was further confirmed through NOESY correlations between H-1″/H-2′. Acid hydrolysis of **3** with 2 N HCl gave aglycone (**12**) and glucose, which were identified by GC and TLC comparison with authentic D-glucose samples. The relative configuration of **3** was confirmed to be identical to that of **1** in the NOESY spectrum. Thus, the structure of **3** was established as 21-*O*-*β*-D-glucopyranosyl(1→2)-*β*-D-glucopyranosyl-3*β*,16*β*,21*β*,23,28-pentahydroxyolean-12-en, and was named pleurosaponin C.

Pleurosaponin D (**4**), an amorphous powder, gave a sodiated molecular ion at *m/z* 879.4719 $[M+Na]^+$ (Calcd for 879.4718) in the HR-FAB-MS, which suggested the molecular formula $C_{44}H_{72}O_{16}Na$. The NMR spectra of **4** were very similar to those of compound **3**. The differences in the ^{1}H-NMR spectrum of **4** showed the additional acetyl group; δ_H 1.95 (3H, s) in ^{1}H-NMR spectrum δ_C 20.7 and 170.7 in ^{13}C-NMR spectrum. In the HMBC spectra, the long-range correlations between the oxygenated proton at δ_H 4.25 (1H, d, J=11.5 Hz, H-23*α*) and 4.39 (1H, d, J=11.5 Hz, H-23*β*) and the acetyl carbonyl carbon at δ_C 170.7 identified the presence of an acetyl group at C-23 in **3**. The relative configuration of **4** was confirmed to be identical to that of **1** in the NOESY spectrum. Thus, the structure of **4** was established as 21-*O*-*β*-D-glucopyranosyl(1→2)-*β*-D-glucopyranosyl-23-acetoxyl-3*β*,16*β*,21*β*,28-tetrahydroxyolean-12-en, and was named pleurosaponin D.

Pleurosaponin E (**5**), an amorphous powder, gave a sodiated molecular ion at *m/z* 1145.5718 $[M+Na]^+$ (Calcd for 1145.5720) in (1H, d, J=7.9 Hz), 4.73 (1H, d, J=7.5 Hz), 4.99 (1H, d, J=7.9 Hz) and 5.28 (1H, d, J=7.9 Hz), and anomeric carbon signals at δ_C 103.9, 103.9, 105.1 and 106.5, respectively. An anomeric proton at δ_H 5.28 (1H, d, J=7.9 Hz) and a methyl group at δ_H 1.09 (3H, d, J=6.1 Hz) suggest to be presence of D-fucopyranosyl moiety.[9] The position of attachment of sugars was confirmed by the HMBC correlations; δ_H 4.62 [1H, d, J=7.9 Hz, H-1 of fucose (H-1′)] to δ_C 82.7 (C-3) of aglycon, δ_H 5.28 [1H, d, J=7.9 Hz, H-1 of glucose (H-1″)] to δ_C 77.1 [C-2 of fucose (C-2′)], δ_H 4.99 [1H, d, J=7.9 Hz, H-1 of glucose-II (H-1‴)] to δ_C 84.7 [C-3 of fucose (C-3′)], δ_H 4.73 [1H, d, J=7.5 Hz, H-1 of glucose-III (H-1⁗)] to δ_C 82.6 (C-21) of aglycone. The relative configuration of **5** was confirmed to be identical to that of **1** in the NOESY spectrum. The position of attachment of glucose was further confirmed through NOESY correlation H-1′/H-3, H-1″/H-2′, H-1‴/H-3′, H-1⁗/H-21. Acid hydrolysis of **5** wi the HR-FAB-MS, which suggested the molecular formula $C_{54}H_{90}O_{24}Na$. The NMR spectra of **5** were similar to those of compound **1**. The differences were the chemical shifts in the ^{1}H-NMR spectrum [δ_H 4.12 (1H, dd, J=11.0, 4.5 Hz, H-3) in **5**, δ_H 3.80 (1H, m, H-3) in **1**], and in the ^{13}C-NMR spectrum [δ_C 73.3 (C-3) in **5**, δ_C 82.7 (C-3) in **1**], implying that **5** was also glycosylated at C-3. In ^{1}H- and ^{13}C-NMR spectra of **5** showed four anomeric proton signals at δ_H 4.62 th 2 N HCl yielded gymnestrogenin (**12**), whose ^{1}H-NMR and MS data were in good agreement with values reported previously,[4] and D-glucose ($[\alpha]_D^{25}$ +49.4°, c=0.04 in H_2O) and D-fucose ($[\alpha]_D^{25}$ +70.0°, c=0.04 in H_2O), which was confirmed by GC and co-TLC (EtOAc–MeOH–H_2O=9 : 3 : 1, *Rf* value: 0.2 and EtOAc–MeOH–H_2O=9 : 3 : 1, *Rf* value: 0.3, respectively) with standard (Aldrich Co., U.S.A.). Thus, the structure of **5** was established as 3-*O*-*β*-D-glucopyranosyl(1→2)-*β*-D-glucopyranosyl(1→3)-*β*-D-fucopyranosyl-21-*O*-*β*-D-glucopyranosyl-3*β*,16*β*,21*β*,23,28-pentahydroxyolean-12-en, and was named pleurosaponin E.

Pleurosaponin F (**6**), an amorphous powder, gave a sodiated molecular ion at *m/z* 659.4135 $[M+Na]^+$ (Calcd for 659.4136) in the HR-FAB-MS, which suggested the molecular formula $C_{36}H_{60}O_9Na$. The ^{1}H-NMR spectrum showed one olefinic proton signal at δ 5.54 (1H, d, J=3.0 Hz), six tertiary methyl signals at δ_H 0.80 (3H, s), 0.90 (3H, s), 0.95 (3H, s), 1.10 (3H, s), 1.19 (3H, s), 1.38 (3H, s), one secondary methyl signal at δ_H 1.45 (3H, d, J=6.7 Hz) and one sugar anomeric proton signal at δ_H 4.89 (1H, d, J=7.9 Hz). In the ^{13}C-NMR spectrum, 36 carbon signals appeared, which included seven methyl carbon signals at δ_C 13.6, 17.8, 18.4, 23.9, 26.8 and 33.2, two olefinic carbon signals at δ_C 128.1 and 145.2, three oxygenated methine carbon signals at δ_C 66.4, 66.8 and 82.0, two oxygenated methylene carbon signals at δ_C 64.7 and 68.7, eight methylene carbon signals at δ_C 18.3, 26.0, 26.4, 33.5, 34.2, 36.9, 41.5 and 46.5, three methine carbon signals at δ_C 43.9, 48.0 and 55.7, six quarternary carbon signals at δ_C 31.0, 38.2, 40.9, 43.8, 43.8, and 43.9, and six signals assignable to the sugar moiety δ_C 17.4, 71.2, 72.8, 73.0, 75.5 and 106.3. These NMR spectra showed similar features of an oleanane-type triterpene glycoside, bupleuroside III (**13**) from roots of *Bupleurum scorzonerifolium*.[5] The main differences were the ^{1}H-NMR spectrum showed anomeric proton signals at δ_H 5.27 (1H, d, J=7.6 Hz), 4.90 (1H, d, J=7.6 Hz) in bupleuroside III (**13**), and δ_H 5.27 (1H, d, J=7.6 Hz) in **6**. The HMBC spectrum showed key correlations between the δ_H 4.29 (1H, m, H-3) with δ_C 13.6 (C-24), 26.4 (C-2), 41.5 (C-1), 43.8 (C-4) and 64.7 (C-23), and δ_H 4.89 (1H, d, J=7.9 Hz, H-1′) with δ_C 82.0 (C-3), confirming the position of the sugar moiety at C-3. An anomeric proton at δ_H 4.89 (1H, d, J=7.9 Hz, H-1′) and a secondary methyl at δ_H 1.45 (3H, d, J=6.7 Hz, H-6′) was identified as a *β*-D-fucopyranosyl moiety.[8] The connectivity of the fucose unit and the stereochemistry at C-3 were confirmed by a NOESY experiment, where a correlation was observed between the fucosyl anomeric proton δ_H 4.89 and H-3*β* at δ_H 4.29. The *β*-orientation of the hydroxyl group at C-11 was confirmed by NOESY correlations with δ_H 4.42 (1H, br s, H-11) and 1.19 (3H, s, H-25). Acid hydrolysis of **6** with 2 N HCl yielded de-11-*O*-methyl saikosaponin b (**6a**)[7] and D-fucose ($[\alpha]_D^{25}$ 70.0°, c=0.04 in H_2O), which was confirmed by GC and co-TLC (EtOAc–MeOH–H_2O=9 : 3 : 1, *Rf* value: 0.3) with fucose standard (Aldrich Co., U.S.A.). Thus, the structure of **6** was established as 3-*O*-*β*-D-fucopyranosyl-3*β*,11*α*,16*β*,23,28-pentahydroxyolean-12-en, and was named pleurosaponin F.

Pleurosaponin G (**7**), an amorphous powder, gave a sodiated molecular ion at *m/z* 1145.5720 $[M+Na]^+$ (Calcd for 1145.5720) in the HR-FAB-MS, which suggested the molecular formula $C_{54}H_{90}O_{24}Na$. The NMR spectra of **7** were similar to those of compound **15**. The differences in the ^{1}H-NMR spectrum [δ_H 4.20 (1H, dd, J=10.0, 3.0 Hz, H-16) in **15**, δ_H 4.46 (1H, m, H-16) in **7**], and ^{13}C-NMR spectrum [δ_C 66.4 (C-16) in

15, δ_C 76.7 (C-16) in **7**], implying that **7** was also glucosylated at C-16. In ^{1}H- and ^{13}C-NMR spectra of **7** showed four anomeric proton signals at δ_H 4.63 (1H, d, J=7.9 Hz), 4.79 (1H, d, J=7.9 Hz), 5.01 (1H, d, J=7.9 Hz) and 5.31 (1H, d, J=7.9 Hz), and anomeric carbon signals at δ_C 104.0, 104.0, 105.1 and 106.4, respectively. The position of attachment of sugars was confirmed by the HMBC correlations; δ_H 4.63 (1H, d, J=7.9 Hz, H-1′) of fucose to δ_C 83.0 (C-3) of aglycone, δ_H 5.31 (1H, d, J=7.9 Hz, H-1″) of glucose-I to δ_C 77.1 (C-2′) of fucose, δ_H 5.01 (1H, d, J=7.9 Hz, H-1‴) of glucose-II to δ_C 84.8 (C-3) of fucose, δ_H 4.79 (1H, d, J=7.9 Hz, H-1⁗) of glucose-III to δ_C 76.7 (C-16) of aglycone. On mild methanolysis of **7** with AcCl–MeOH (1 : 20) yielded bepleuroside III (**13**),[8] whose ^{1}H-NMR and MS data were in good agreement with values reported previously[5] and, which were identified by GC and TLC comparison with authentic D-glucose samples. The relative configuration of **7** was confirmed to be identical to that of **6** in the NOESY spectrum. Thus, the structure of **7** was established as 3-*O*-*β*-D-glucopyranosyl(1→2)-*β*-D-glucopyranosyl(1→3)-*β*-D-fucopyranosyl-16-*O*-*β*-D-glucopyranosyl-3*β*,11*α*,16*β*,23,28-pentahydroxyolean-12-en, and was named pleurosaponin G.

Pleurosaponin H (**8**), an amorphous powder, gave a sodiated molecular ion at *m/z* 1159.5875 $[M+Na]^+$ (Calcd for 1159.5876) in the HR-FAB-MS, which suggested the molecular formula $C_{55}H_{92}O_{24}Na$. The NMR data of **8** resembled those of **7**, except for an additional signals of a methoxy group at δ_H 3.13 (3H, s) and δ_C 53.9 in **8**. The HMBC spectrum showed correlations from δ_H 3.13 (3H, s, –OCH_3) to δ_C 75.9 (C-11), and 5.47 (1H, d, J=3.0 Hz, H-12) to δ_C 43.6 (C-14), 51.9 (C-9) and 75.9 (C-11), suggesting the presence of a methoxyl at C-11 in the structure. Thus, the structure of **8** was established as 3-*O*-*β*-D-glucopyranosyl(1→2)-*β*-D-glucopyranosyl(1→3)-*β*-D-fucopyranosyl-16-*O*-*β*-D-glucopyranosyl-11*α*-methoxyl-3*β*,16*β*,23,28-tetrahydroxyolean-12-en, and was named pleurosaponin H.

Pleurosaponin I (**9**), an amorphous powder, gave a sodiated molecular ion at *m/z* 965.5083 $[M+Na]^+$ (Calcd for 965.5086) in the HR-FAB-MS, which suggested the molecular formula $C_{48}H_{78}O_{18}Na$. The NMR spectra of **9** were very similar to those of saikosaponin g (**9a**) from *Bupleurum falcatum*.[9] The major differences were the additional glucose unit signals δ_H 5.24 (1H, d, J=7.6 Hz), δ_C 104.0, 78.8, 77.4, 76.2, 72.1, 63.0. The position of attachment of glucose was confirmed by the HMBC correlations; δ_H 4.83 (1H, d, J=7.6 Hz, H-1′) of fucose to δ_C 82.4 (C-3) of aglycon, δ_H 5.52 (1H, d, J=7.6 Hz, H-1″) of glucose-II to δ_C 77.1 (C-2′) of fucose, and δ_H 5.52 (1H, d, J=7.6 Hz, H-1‴) of glucose-III to δ_C 84.6 (C-3′) of fucose. The connectivity of the glucose unit and the stereochemistry at the C-3 position were confirmed by a NOESY experiment, where a correlation was observed between the fucosyl anomeric proton δ_H 4.83 and H-3*β* at δ_H 4.07 (Fig. 2). On mild methanolysis of **9** with AcCl–MeOH (1 : 20) yielded saikogenin g (**9a**), whose ^{1}H-NMR and MS data were in good agreement with values reported previously.[9] Thus, the structure of **9** was established as 3-*O*-*β*-D-glucopyranosyl(1→2)-*β*-D-glucopyranosyl(1→3)-*β*-D-fucopyranosyl-3*β*,16*β*,23,28-tetrahydroxyolean-9,12-dien, and was named pleurosaponin I.

Pleurosaponin J (**10**), an amorphous powder, gave a sodiated molecular ion at *m/z* 1127.5615 $[M+Na]^+$ (Calcd for 1127.5614) in the HR-FAB-MS, which suggested the molecular formula $C_{54}H_{88}O_{23}Na$. Inspection of the ^{1}H-, ^{13}C-NMR data of **10** revealed that these data were very similar to those of **9**. Comparison of the ^{13}C-NMR data of **10** with that showed the downfield shift of C-16 (+10.3) and C-17 (+1.6), and upfield shift C-16 (−2.0) in **10**, indicating glycosylation at C-16. This linkage was confirmed by HMBC correlations between δ_H 4.76 (1H, d, J=7.9 Hz, H-1⁗) and δ_C 77.0 (C-16). On mild methanolysis of **10** with AcCl–MeOH (1 : 20) yielded saikogenin g (**9a**), whose ^{1}H-NMR and MS data were in good agreement with values reported previously.[9] Thus, the structure of **10** was established 3-*O*-*β*-D-glucopyranosyl(1→2)-*β*-D-glucopyranosyl(1→3)-*β*-D-fucopyranosyl-16-*O*-*β*-D-glucopyranosyl-3*β*,16*β*,23,28-tetrahydroxyolean-9,12-dien, and was named pleurosaponin J.

Pleurosaponin K (**11**), an amorphous powder, gave a sodiated molecular ion at *m/z* 1127.5615 $[M+Na]^+$ (Calcd for 1127.5614) in the HR-FAB-MS, which suggested the molecular formula $C_{54}H_{88}O_{23}Na$. The NMR spectra of **11** were similar to those of **18**. Comparison of the ^{1}H-NMR spectrum [δ_H 4.22 (1H, m, H-16) in **18**, δ_H 4.19 (1H, m, H-16) in **11**], and ^{13}C-NMR spectrum [δ_C 76.6 (C-16) in **18**, δ_C 86.1 (C-16) in **11**], implied that **11** was also glucosylated at C-16. This linkage was confirmed by HMBC correlations between δ_H 5.08 (1H, d, J=8.2 Hz, H-1⁗) and δ_C 86.1 (C-16). On mild methanolysis of **11** with AcCl–MeOH (1 : 20) yielded saikogenin b_1 (**17**), whose ^{1}H-NMR and MS data were in good agreement with values reported previously.[9] Thus, the structure of **11** was established as 3-*O*-*β*-D-glucopyranosyl(1→2)-*β*-D-glucopyranosyl(1→3)-*β*-D-fucopyranosyl-16-*O*-*β*-D-glucopyranosyl-3*β*,16*β*,23,28-tetrahydroxyolean-11,13-dien, and was named pleurosaponin K.

Cytotoxic activities of the isolated compounds (**1**–**25**) were evaluated by determining their inhibitory effects on human tumor cell lines (A549, SK-OV-3, SK-MEL-2, and HCT15) *in vitro* using the sulforhodamine B (SRB) assay.[14,15] Compounds **16**, **19**–**24** had mild cytotoxic activity against A549, SK-OV-3, SK-MEL-2 and HCT15 cell lines [IC_{50} (**21**): 12.37, 13.25, 10.76, 11.81, IC_{50} (**22**): 12.44, 12.93, 11.84, 12.28, IC_{50} (**24**): 11.82, 11.91, 11.27, 11.48, IC_{50} (**25**): 13.55, 14.12, 13.11, 11.53]. The other compounds were essentially noncytotoxic.

Experimental

General Experimental Procedures Melting points were determined on a Gallenkamp melting point apparatus and are uncorrected. IR spectra were recorded on a Bruker IFS-66/S FT-IR spectrometer. UV spectra were obtained on a Varian Cary 5000 UV-Vis-NIR spectrophotometer. NMR spectra were recorded on a Varian UNITY INOVA 500 NMR spectrometer operating at 500 MHz (^{1}H) or 125 MHz (^{13}C), with chemical shifts given in ppm (δ). FAB and HR-FAB mass spectra were obtained on a JEOL JMS700 mass spectrometer. GC (Gas Chromatography) was carried out on using a ZB-1MS capillary column (30 cm×0.25 mm×0.25 μm, Zebron); column temperature, 230°C; injection temperature, 250°C; carrier gas, He. Preparative HPLC was performed using a Gilson 306 pump with a Shodex refractive index detector. Chromatographic separation was performed on an Apollo Silica 5 μ column (250×10 mm i.d.) or Econosil RP-18–10 μ column (250×10 mm i.d.). Silica gel 60 (Merk Co., 70–230 mesh), RP-C18 silica gel (YMC GEL ODS-A, 12 nm, S-75 μm) and Sephadex LH-20 (Pharmacia) were used for column chromatography. TLC was performed using Merck pre-coated Silica

gel F254 plates and RP-18 F254s plates. Low-pressure liquid chromatography was performed over a LiChroprep Lobar-A RP-18 (240×10 mm i.d.) column with a FMI QSY-O pump (ISCO).

Plant Materials The aerial parts of *P. kamtschaticum* were collected at Goseong-gun in Gangwon-do province, Korea in June 2009 and the plant was identified by one of the authors (K. R. Lee). A voucher specimen (SKKU 2009-012) was deposited in the herbarium of the School of Pharmacy, Sungkyunkwan University, Suwon, Korea.

Extraction and Isolation The aerial parts of *P. kamtschaticum* (Umbelliferae) (2.0 kg) were extracted with 80% MeOH three times at room temperature. The resultant MeOH extracts (150 g) were suspended in distilled water (800 mL×3) and then successively partitioned with hexane, EtOAc and *n*-BuOH, yielding residues weighing 3 g, 16 g and 40 g, respectively. The *n*-BuOH-soluble fraction was chromatographed on a Diaion HP-20 column eluting with a gradient solvent system of 100% H_2O and 100% MeOH, yielding subfractions A and B. Fraction A (20 g) was purified using a RP-C18 silica gel (12 nm, S-75 μm, 300 g) column eluted with 60% MeOH to give subfractions BA1-BA9. Fraction BA5 (4 g) was loaded on a silica gel (230–400 mesh, 100 g) column and eluted with $CHCl_3$–MeOH–H_2O (10 : 4 : 0.2) to yield subfractions BA51–56. Fraction B52 was subjected to preparative reverse-phase HPLC using 80% MeOH as the eluant to give **1** (90 mg). Fraction BA54 (700 mg) was chromatographed on an RP-C18 silica gel (12 nm, S-75 μm, 100 g) and then reverse-phase HPLC using 65% MeOH as eluant to yield compound **3** (80 mg). Fraction BA55 (400 mg) was chromatographed on an LiChroprep Lobar-B RP-C18 column eluted with 60% MeOH and then reverse-phase HPLC using 80% MeOH as eluant to yield compounds **2** (10 mg) and **25** (16 mg). Fraction BA56 (500 mg) was chromatographed on a LiChroprep Lobar-B RP-C18 column eluted with 70% MeOH to yield subfractions BA561–565. Fraction BA 561 was subjected to preparative reverse-phase HPLC using 65% MeOH as eluant to yield compounds **5** (22 mg) and **6** (20 mg). Fraction BA 562 was subjected to preparative reverse-phase HPLC using 70% MeOH as eluant to yield compounds **7** (8 mg) and **8** (12 mg). Fraction BA 565 was subjected to preparative reverse-phase HPLC using 80% MeOH as eluant to yield compounds **10** (18 mg), **11** (45 mg) and **22** (87 mg). Fraction BA6 (4 g) was loaded on a silica gel (230–400 mesh, 100 g) column and eluted with $CHCl_3$–MeOH–H_2O (10 : 5 : 0.2) to yield subfractions BA61–63. Fraction BA62 (300 mg) was subjected to preparative reverse-phase HPLC using 70% MeOH as the eluant to give **16** (120 mg). Fraction BA7 (4 g) was chromatographed on an RP-C18 silica gel (12 nm, S-75 μm, 200 g) column eluted with 65% MeOH to give subfractions BA71–BA76. Fraction BA72 (350 mg) was chromatographed on a LiChroprep Lobar-A RP-C18 column eluted with 65% MeOH to give subfractions BA721–BA722. Fraction BA721 and 722 was subjected to preparative reverse-phase HPLC using 65% MeOH as the eluant to give **15** (31 mg) and **13** (27 mg), respectively. Fraction BA75 was subjected to preparative reverse-phase HPLC using 75% MeOH as the eluant to give **4** (10 mg) and **14** (43 mg). Fraction BA8 (2 g) was chromatographed on an RP-C18 silica gel (12 nm, 300 g) column eluted with 80% MeOH to give subfractions BA81–BA85. Fraction BA81 (400 mg) was chromatographed on a LiChroprep Lobar-B RP-C18 column eluted with 70% MeOH to give subfractions BA811–BA818. Fraction BA815 was subjected to preparative reverse-phase HPLC using 75% MeOH as the eluant to give **21** (66 mg). Fraction BA816 was subjected to preparative reverse-phase HPLC using 80% MeOH as the eluant to give **20** (52 mg). Fraction BA817 was subjected to preparative reverse-phase HPLC using 85% MeOH as the eluant to give **9** (41 mg). Fractions BA82 and BA83 were subjected to preparative reverse-phase HPLC using 80% and 85% MeOH as the eluant to give **17** (19 mg) and **18** (140 mg), respectively. The EtOAc-soluble fraction (16 g) was chromatographed on a Sephadex LH-20 column eluting with CH_2Cl_2–MeOH (1 : 1) to yield two subfractions E1–E8. Fraction E5 (0.9 g) was chromatographed on a Sephadex LH-20 column eluting with 80% MeOH to yield three subfractions E51–E53. Fraction E53 (60 mg) was subjected to preparative reverse-phase HPLC using 85% MeOH as eluant to yield compound **23** (10 mg). Fraction E6 (2.0 g) was subjected to preparative silica gel HPLC using $CHCl_3$–MeOH (7 : 1) as eluant to yield compound **12** (67 mg). Fraction E7 (1.5 g) was chromatographed on a Sephadex LH-20 column eluting with 80% MeOH to yield three subfractions E71–E73. Fraction E71 (550 mg) was chromatographed on a LiChroprep Lobar-B RP-C18 column eluted with 70% MeOH to give subfractions E711–E714. Fraction E714 was subjected to preparative reverse-phase HPLC using 80% MeOH as the eluant to give **24** (13 mg). Fraction E73 (440 mg) was subjected to preparative reverse-phase HPLC using 80% MeOH as eluant to yield compound **19** (230 mg).

Pleurosaponin A (**1**): White, amorphous powder; mp 305.5°C; IR (KBr) ν_{max} 3421, 2946, 1638, 1042 cm^{-1}; ^{1}H-NMR (500 MHz, C_5D_5N) δ: 0.93 (3H, s, H-25), 0.97 (3H, s, H-26), 1.00 (3H, s, H-24), 1.11 (3H, s, H-30), 1.17 (3H, s, H-27), 1.25 (3H, s, H-29), 1.64 (1H, t, J=9.1 Hz), 1.98 (1H, m, H-22α), 2.44 (1H, dd, J=14.0, 4.8 Hz, H-18), 3.47 (1H, dd, J=13.5, 4.2 Hz, H-22β), 3.64 (1H, d, J=10.4, H-28α), 3.65 (1H, d, J=11.0 Hz, H-23α), 4.00 (1H, m, H-21), 4.10 (1H, d, J=11.0 Hz, H-23β), 4.12 (1H, dd, J=11.0, 4.5 Hz, H-3), 4.28 (1H, d, J=10.4 Hz, H-28β), 4.35 (1H, d, J=9.3 Hz, H-16), 4.98 (1H, d, J=7.9 Hz, H-1′), 5.22 (1H, br s, H-12); ^{13}C-NMR data, see Table 1; FAB-MS m/z 675 $[M+Na]^+$; HR-FAB-MS m/z 675.4085 $[M+Na]^+$; (Calcd for $C_{36}H_{60}O_{10}Na$, 675.4084).

Pleurosaponin B (**2**): White, amorphous powder; mp 215.5°C; IR (KBr) ν_{max} 3360, 2946, 2833, 1724, 1451, 1031 cm^{-1}; ^{1}H-NMR (500 MHz, C_5D_5N) δ: 0.89 (3H, s, H-25), 0.91 (3H, s, H-24), 0.96 (3H, s, H-26), 1.13 (3H, s, H-30), 1.23 (3H, s, H-27), 1.27 (3H, s, H-29), 1.62 (1H, m, H-9), 1.96 (3H, s, CH_3CO), 2.00 (1H, m, H-22α), 2.47 (1H, dd, J=13.5, 4.8 Hz, H-18), 3.49 (1H, dd, J=13.0, 3.5 Hz, H-22β), 3.67 (1H, d, J=10.3 Hz, H-28α), 3.86 (1H, m, H-3), 4.09 (1H, m, H-21), 4.23 (1H, d, J=10.9 Hz, H-23α), 4.29 (1H, d, J=10.3 Hz, H-28β), 4.39 (1H, d, J=10.9 Hz, H-23β), 4.63 (1H, m, H-16), 5.24 (1H, br s, H-12); ^{13}C-NMR data, see Table 1; FAB-MS m/z 717 $[M+Na]^+$; HR-FAB-MS m/z 717.4190 $[M+Na]^+$; (Calcd for $C_{38}H_{62}O_{11}Na$, 717.4190).

Pleurosaponin C (**3**): White, amorphous powder; mp 272.6°C; IR (KBr) ν_{max} 3366, 2944, 1655, 1451, 1030 cm^{-1}; ^{1}H-NMR (500 MHz, C_5D_5N) δ: 0.92 (3H, s, H-25), 0.96 (3H, s, H-26), 0.98 (3H, s, H-24), 1.14 (3H, s, H-27), 1.24 (3H, s, H-30), 1.26 (3H, s, H-29), 1.62 (1H, t, J=9.1 Hz, H-9), 1.98 (1H, t, J=13.0 Hz, H-22α), 2.43 (1H, dd, J=13.5, 5.0 Hz, H-18), 3.42 (1H, dd, J=13.0, 3.6 Hz, H-22β), 3.64 (1H, d, J=10.5 Hz,

Table 1. ^{13}C-NMR Data of Compounds **1**–**11** (C_5D_5N, 125 MHz, δ in ppm)[a)]

C	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**
1	38.9	38.8	38.9	38.8	38.8	41.5	41.3	40.0	37.5	37.5	38.1
2	27.6	27.5	27.6	27.5	26.1	26.4	26.4	23.3	26.8	26.7	26.0
3	73.3	71.2	73.3	71.2	82.7	82.0	83.0	82.5	82.4	82.6	82.5
4	42.9	42.3	42.8	42.3	43.7	43.8	43.9	43.9	43.8	43.7	43.7
5	48.5	48.3	48.5	48.3	48.0	48.0	48.3	48.0	44.1	44.2	47.5
6	18.5	18.5	18.5	18.5	18.2	18.3	18.3	18.3	18.0	18.0	18.1
7	32.6	32.7	32.6	32.7	32.5	33.5	33.3	33.0	32.1	31.8	32.2
8	40.1	40.1	40.1	40.1	40.1	40.9	43.9	43.6	43.1	43.4	40.2
9	47.2	41.7	47.1	47.4	47.1	55.7	55.6	51.9	155.0	154.8	54.3
10	36.9	36.9	37.0	37.0	36.6	38.2	38.1	38.0	38.7	38.6	36.1
11	23.9	23.8	23.9	23.8	23.9	66.8	66.7	75.9	115.9	115.9	127.3
12	123.3	123.1	123.8	123.0	123.3	128.1	128.1	122.7	121.1	121.2	125.7
13	142.8	142.9	143.0	142.9	142.8	145.2	144.9	147.8	145.2	145.0	136.6
14	43.7	43.8	43.6	43.9	43.9	43.9	43.6	43.6	43.2	42.9	44.0
15	36.2	36.0	36.2	36.0	36.3	36.9	34.8	34.9	36.1	34.1	33.5
16	68.1	68.0	67.9	67.9	67.9	66.4	76.7	76.6	66.7	77.0	86.1
17	43.9	43.5	43.8	43.6	43.6	43.8	42.5	42.5	40.5	42.1	45.8
18	43.6	43.7	43.5	43.7	43.7	43.9	42.9	43.0	42.6	41.9	132.8
19	47.7	47.7	47.6	47.7	47.6	46.5	46.7	47.2	46.9	47.1	38.3
20	36.7	36.7	36.7	36.8	36.7	31.0	31.0	31.0	31.0	31.0	32.3
21	82.5	82.5	82.8	82.7	82.6	34.2	34.8	34.8	34.1	34.8	35.5
22	33.9	33.9	34.0	34.0	33.9	26.0	26.4	26.3	26.1	23.4	30.8
23	67.8	66.6	67.8	66.7	64.9	64.7	65.0	64.7	64.9	65.1	64.4
24	13.0	12.8	13.0	12.8	13.2	13.6	13.3	13.2	13.3	13.2	12.7
25	16.1	16.1	16.1	16.1	16.2	17.8	17.6	17.8	26.1	26.1	18.6
26	16.9	16.9	16.9	16.9	16.9	18.4	18.2	18.2	21.0	20.9	16.8
27	27.0	26.8	27.0	26.8	27.0	26.8	26.8	26.2	21.2	21.1	21.5
28	67.9	67.8	68.2	68.2	68.1	68.7	64.9	64.7	69.3	65.4	62.8
29	29.1	29.1	29.1	29.2	29.1	33.2	24.0	24.0	24.0	24.7	24.7
30	18.3	18.4	18.3	18.4	18.3	23.9	33.2	33.2	33.1	33.1	32.3
$-OCH_3$								53.9			
C=O		170.8		170.7							
CH_3		20.7		20.7							
1′	106.5	106.5	104.7	104.8	103.9	106.3	104.0	104.0	103.9	103.9	104.0
2′	75.9	75.9	84.3	84.4	77.1	73.0	77.1	77.2	77.1	77.1	77.2
3′	78.8	78.5	78.4	78.5	84.7	72.8	84.8	84.7	84.6	84.8	84.7
4′	72.6	72.6	72.4	72.5	71.9	75.5	71.9	72.1	71.9	71.9	71.8
5′	78.4	78.7	77.8	77.8	70.4	71.2	70.4	70.4	70.5	70.5	70.5
6′	64.0	63.9	63.7	63.8	17.2	17.4	17.1	17.2	17.2	17.2	17.2
1″			106.4	106.5	103.9		104.0	104.0	104.0	104.0	104.0
2″			77.3	77.4	76.2		76.2	76.2	76.2	76.2	76.3
3″			78.1	78.2	78.8		78.8	78.8	78.8	78.8	78.8
4″			71.4	71.4	72.1		72.1	72.1	72.1	72.1	72.2
5″			77.9	78.0	77.4		77.4	77.4	77.4	77.4	77.4
6″			62.4	62.4	63.1		63.0	63.1	63.0	63.0	63.1
1‴					105.1		105.1	105.1	105.1	105.1	105.1
2‴					75.3		75.3	75.4	75.3	75.3	75.3
3‴					78.4		78.4	78.5	78.4	78.5	78.5
4‴					71.5		71.7	71.9	71.6	71.8	71.5
5‴					75.5		78.5	78.4	78.5	78.4	78.5
6‴					62.5		62.5	62.5	62.4	62.5	62.5
1⁗					106.5		106.4	106.4		106.3	106.0
2⁗					75.9		75.7	75.8		75.8	75.6
3⁗					78.5		78.7	78.8		78.8	78.9
4⁗					72.6		71.5	71.7		71.5	72.0
5⁗					78.4		78.2	78.2		78.2	78.4
6⁗					64.0		62.8	62.8		62.8	62.8

a) The assignments were based on DEPT, HMQC, and HMBC experiments.

H-23α), 3.64 (1H, d, J=10.3 Hz, H-28α), 3.90 (1H, m, H-21), 4.08 (1H, m, H-23β), 4.10 (1H, m, H-3), 4.26 (1H, d, J=10.3 Hz, H-28β), 4.52 (1H, m, H-16), 4.97 (1H, d, J=7.6 Hz, H-1′), 5.21 (1H, br s, H-12), 5.24 (1H, d, J=7.6 Hz, H-1″); ^{13}C-NMR data, see Table 1; FAB-MS m/z 837 $[M+Na]^+$; HR-FAB-MS m/z 837.4615 $[M+Na]^+$; (Calcd for $C_{42}H_{70}O_{15}Na$, 837.4612).

Pleurosaponin D (**4**): White, amorphous powder; mp 272.6°C; IR (KBr) ν_{max} 3359, 2946, 2833, 1451, 1029 cm^{-1}; ^{1}H-NMR (500 MHz, C_5D_5N) δ: 0.89 (3H, s, H-25), 0.96 (3H, s, H-26), 0.98 (3H, s, H-24), 1.22 (3H, s, H-27), 1.28 (3H, s, H-30), 1.30 (3H, s, H-29), 1.62 (1H, m, H-9), 1.95 (3H, s, CH_3OH), 2.02 (1H, 1H, t, J=13.4 Hz, H-22α), 2.47 (1H, dd, J=14.0, 4.8 Hz, H-18), 3.47 (1H, dd, J=13.4, 3.4 Hz, H-23β), 3.64 (1H, d, J=10.0 Hz, H-28α), 3.88 (1H, m, H-3), 4.03 (1H, m, H-21), 4.25 (1H, d, J=11.5 Hz, H-23α), 4.30 (1H, d, J=10.0 Hz, H-28β), 4.39 (1H, d, J=11.5 Hz, H-23β), 4.59 (1H, m, H-16), 5.00 (1H, d, J=7.5 Hz, H-1′), 5.22 (1H, br d, H-12), 5.28 (1H, d, J=7.5 Hz, H-1″); ^{13}C-NMR data, see Table 1; FAB-MS m/z 879 $[M+Na]^+$; HR-FAB-MS m/z 879.4719 $[M+Na]^+$; (Calcd for $C_{44}H_{72}O_{16}Na$, 879.4718).

Pleurosaponin E (**5**): White, amorphous powder; mp 254.5°C; IR (KBr) ν_{max} 3362, 2946, 2833, 1451, 1029 cm^{-1}; ^{1}H-NMR (500 MHz, C_5D_5N) δ: 0.62 (3H, s, H-25), 0.68 (3H, s, H-26), 0.77 (3H, s, H-24), 0.87 (3H, s, H-30), 0.94 (3H, s, H-27), 1.01 (3H, s, H-29), 1.09 (3H, d, J=6.1 Hz, H-6′), 1.38 (1H, m, H-9), 1.74 (1H, t, J=12.8 Hz, H-22α), 2.22 (1H, dd, J=13.5, 4.3 Hz, H-18), 3.23 (1H, dd, J=12.8, 3.0 Hz, H-22β), 3.40 (1H, m, H-23α), 3.40 (1H, m, H-28α), 3.80 (1H, m, H-3), 3.80 (1H, m, H-21α), 4.01 (1H, m, H-23β), 4.02 (1H, m, H-28β), 4.35 (1H, m, H-16), 4.62 (1H, d, J=7.9 Hz, H-1′), 4.73 (1H, d, J=7.5 Hz, H-1‴), 4.95 (1H, s, H-12), 4.99 (1H, d, J=7.9 Hz, H-1‴), 5.28 (1H, d, J=7.9 Hz, H-1″); ^{13}C-NMR data, see Table 1; FAB-MS m/z 1145 $[M+Na]^+$; HR-FAB-MS m/z 1145.5718 $[M+Na]^+$; (Calcd for $C_{54}H_{90}O_{24}Na$, 1145.5720).

Pleurosaponin F (**6**): White, amorphous powder; mp 260.0°C; IR (KBr) ν_{max} 3364, 2947, 2834, 1654, 1451, 1028 cm^{-1}; ^{1}H-NMR (500 MHz, C_5D_5N) δ: 0.80 (3H, s, H-30), 0.90 (3H, s, H-29), 0.95 (3H, s, H-24), 1.10 (3H, s, H-26), 1.19 (3H, s, H-25), 1.38 (3H, s, H-27), 1.45 (3H, d, J=6.7 Hz, H-6′), 1.98 (1H, d, J=8.5 Hz, H-9), 2.40 (1H, dd, J=14.0, 4.8 Hz, H-18), 3.66 (1H, m, H-28α), 3.67 (1H, m, H-23α), 4.29 (1H, m, H-3), 4.31 (1H, m, H-23β), 4.36 (1H, d, J=10.4 Hz, H-28β), 4.42 (1H, br s, H-11), 4.57 (1H, m, H-16), 4.89 (1H, d, J=7.9 Hz, H-1′), 5.54 (1H, d, J=3.0 Hz, H-12); ^{13}C-NMR data, see Table 1; FAB-MS m/z 659 $[M+Na]^+$; HR-FAB-MS m/z 659.4135 $[M+Na]^+$; (Calcd for $C_{36}H_{60}O_9Na$, 659.4136).

Pleurosaponin G (**7**): White, amorphous powder; mp 251.5°C; IR (KBr) ν_{max} 3360, 2947, 2834, 1661, 1451, 1029 cm^{-1}; ^{1}H-NMR (500 MHz, C_5D_5N) δ: 0.57 (3H, s, H-30), 0.69 (3H, s, H-26), 0.72 (3H, s, H-29), 0.81 (3H, s, H-24), 0.85 (3H, s, H-25), 1.09 (3H, d, J=6.1 Hz, H-6′), 1.17 (3H, s, H-27), 1.65 (1H, m, H-9), 2.60 (1H, t, J=14.6 Hz, H-18), 3.43 (1H, d, J=10.4 Hz, H-23α), 3.60 (1H, d, J=10.3 Hz, H-28α), 3.90 (1H, m, H-3), 3.95 (1H, m, H-28β), 4.07 (1H, m, H-23β), 4.09 (1H, m, H-10), 4.46 (1H, m, H-16), 4.63 (1H, d, J=7.9 Hz, H-1′), 4.79 (1H, d, J=7.9 Hz, H-1‴), 5.01 (1H, d, J=7.9 Hz, H-1‴), 5.29 (1H, d, J=3.0, H-12), 5.31 (1H, d, J=7.9 Hz, H-1″); ^{13}C-NMR data, see Table 1; FAB-MS m/z 1145 $[M+Na]^+$; HR-FAB-MS m/z 1145.5720 $[M+Na]^+$; (Calcd for $C_{54}H_{90}O_{24}Na$, 1145.5720).

Pleurosaponin H (**8**): White, amorphous powder; mp 256.8°C; IR (KBr) ν_{max} 3357, 2946, 2833, 1451 cm^{-1}; ^{1}H-NMR (500 MHz, C_5D_5N) δ: 0.84 (3H, s, H-30), 0.85 (3H, s, H-26), 0.99 (3H, s, H-25), 1.00 (3H, s, H-29), 1.03 (3H, s, H-24), 1.32 (3H, d, J=6.1 Hz, H-6′), 1.38 (3H, s, H-27), 1.86 (1H, m, H-9), 2.93 (1H, m, H-18), 3.13 (3H, s, –OCH_3), 3.66 (1H, m, H-23α), 3.70 (1H, m, H-11), 3.85 (1H, m, H-28α), 4.09 (1H, m, H-3), 4.20 (1H, m, H-23β), 4.30 (1H, m, H-28β), 4.72 (1H, m, H-16), 4.87 (1H, d, J=7.9 Hz, H-1′), 5.02 (1H, d, J=7.9 Hz, H-1‴), 5.24 (1H, d, J=7.9 Hz, H-1‴), 5.47 (1H, d, J=3.0 Hz, H-12), 5.54 (1H, d, J=7.9 Hz, H-1″); ^{13}C-NMR data, see Table 1; FAB-MS m/z 1159 $[M+Na]^+$; HR-FAB-MS m/z 1159.5875 $[M+Na]^+$; (Calcd for $C_{55}H_{92}O_{24}Na$, 1159.5876).

Pleurosaponin I (**9**): White, amorphous powder; mp 271.0°C; UV λ_{max} (MeOH) nm (logε): 277 (3.9); IR (KBr) ν_{max} 3357, 2946, 2833, 1451, 1030 cm^{-1}; ^{1}H-NMR (500 MHz, C_5D_5N) δ: 0.82 (3H, s, H-30), 0.92 (3H, s, H-29), 1.05 (3H, s, H-24), 1.19 (3H, s, H-25), 1.20 (3H, s, H-27), 1.21 (3H, s, H-26), 1.33 (3H, d, J=6.5 Hz, H-6′), 2.48 (1H, dd, J=13.5, 4.7 Hz, H-18), 3.38 (1H, m, H-28α), 3.64 (1H, m, H-28β), 3.68 (1H, m, H-23α), 4.07 (1H, m, H-3), 4.30 (1H, m, H-23β), 4.51 (1H, m, H-16), 4.83 (1H, d, J=7.6 Hz, H-1′), 5.23 (1H, d, J=7.6 Hz, H-1‴), 5.52 (1H, d, J=7.6 Hz, H-1″), 5.56 (1H, d, J=5.8 Hz, H-12), 5.67 (1H, d, J=5.8 Hz, H-11); ^{13}C-NMR data, see Table 1; FAB-MS m/z 965 $[M+Na]^+$; HR-FAB-MS m/z 965.5083 $[M+Na]^+$; (Calcd for $C_{48}H_{78}O_{18}Na$, 965.5086).

Pleurosaponin J (**10**): White, amorphous powder; mp 255.7°C; UV λ_{max} (MeOH) nm (logε): 277 (4.1); IR (KBr) ν_{max} 3359, 2946, 2833, 1739, 1451, 1030 cm^{-1}; ^{1}H-NMR (500 MHz, C_5D_5N) δ: 0.56 (3H, s, H-30), 0.71 (3H, s, H-29), 0.75 (3H, s, H-24), 0.80 (3H, s, H-26), 0.86 (3H, s, H-25), 0.98 (3H, s, H-27), 1.05 (1H, d, J=6.1 Hz, H-6′), 2.67 (1H, dd, J=13.5, 4.2 Hz, H-18), 3.36 (1H, d, J=10.4, H-23α), 3.58 (1H, m, H-28α), 3.78 (1H, m, H-3), 3.94 (1H, m, H-28β), 3.98 (1H, m, H-23β), 4.43 (1H, dd, J=11.5, 4.8 Hz, H-16), 4.56 (1H, d, J=7.9 Hz, H-1′), 4.76 (1H, d, J=7.9 Hz, H-1‴), 4.94 (1H, d, J=7.5 Hz, H-1‴), 5.25 (1H, d, J=7.5 Hz, H-1″), 5.29 (1H, d, J=5.5 Hz, H-12), 5.36 (1H, d, J=5.5 Hz, H-11); ^{13}C-NMR data, see Table 1; FAB-MS m/z 1127 $[M+Na]^+$; HR-FAB-MS m/z 1127.5615 $[M+Na]^+$; (Calcd for $C_{54}H_{88}O_{23}Na$, 1127.5614).

Pleurosaponin K (**11**): White, amorphous powder; mp 265.3°C; UV λ_{max} (MeOH) nm (logε): 254 (4.1), 249 (3.8), 241 (3.6); IR (KBr) ν_{max} 3361, 2947, 2834, 1451, 1028 cm^{-1}; ^{1}H-NMR (500 MHz, C_5D_5N) δ: 0.67 (3H, s, H-26), 0.81 (3H, s, H-30), 0.84 (3H, s, H-25), 0.89 (3H, s, H-29), 0.97 (3H, s, H-24), 1.01 (3H, s, H-27), 1.34 (3H, d, J=6.5 Hz, H-6′), 1.93 (1H, m, H-9), 3.63 (1H, m, H-23α), 4.00 (1H, m, H-3), 4.02 (1H, m, H-28α), 4.13 (1H, m, H-28β), 4.19 (1H, m, H-16), 4.32 (1H, m, H-23β), 4.86 (1H, d, J=7.6 Hz, H-1′), 5.08 (1H, d, J=8.2 Hz, H-1‴), 5.25 (1H, d, J=7.6 Hz, H-1‴), 5.54 (1H, d, J=8.2 Hz, H-1″), 5.60 (1H, d, J=11.1 Hz, H-11), 6.43 (1H, d, J=11.1 Hz, H-12); ^{13}C-NMR data, see Table 1; FAB-MS m/z 1127 $[M+Na]^+$; HR-FAB-MS m/z 1127.5615 $[M+Na]^+$; (Calcd for $C_{54}H_{88}O_{23}Na$, 1127.5614).

Acid Hydrolysis of 1–6, and GC Analysis Compound **1** (5 mg) was shaken with 2 mL of 2 N HCl for 1 h at 90°C.[15] The hydrolysate was extracted with EtOAc, and the EtOAc extract was evaporated *in vacuo*. The EtOAc extract was purified using HPLC [Optimapak ODS-A, 250×4.6 mm; mobile phase: 85% MeOH; Detector: RI; flow rate: 2.0 mL/min] to yield gymnestrogenin (**12**, 2 mg). Compounds **2**–**5** (5 mg) were treated using the same method to gymnestrogenin (**12**, each

2.0 mg), and de-11-*O*-methylsaikosaponin b (**6a**, 2.0 mg) from **6**. The sugar obtained from the hydrolysis was dissolved in anhydrous pyridine (0.1 mL) and L-cysteine methyl ester hydrochloride (2.0 mg) was added. The mixture was stirred at 60°C for 1.5 h. After the reaction mixture was dried *in vacuo*, the residue was trimethylsilylated with 1-trimethylsilylimidazole (0.1 mL) for 2 h. The mixture was partitioned between *n*-hexane and H_2O (0.3 mL, each), and the organic layer (1 μL) was analyzed by gas chromatography (GC).[16] Identification of D-glucose and D-fucose were detected in each case by co-injection of the hydrolysate with standard silylated sugars.

Mild Methanolysis of 7–11 Each compound (5 mg) was refluxed with AcCl–MeOH (1 : 20, 3 mL) for 3 h. The reaction was concd to afford a residue and the residue was partitioned between EtOAc and H_2O.[8] The EtOAc layer was concd and subjected to HPLC [Optimapak ODS-A, 250×4.6 mm; mobile phase: 70% MeOH; Detector: RI; flow rate: 2.0 mL/min] to yield bepleuroside III (**13**, 2.0 mg) from **7**, saikogenin b_3 (**14**, 2.0 mg) from **8**, saikogenin g (**9a**, 2.0 mg) from **9** and **10**, and saikosaponin b_1 (**17**, 2.0 mg).

***In Vitro* Cytotoxicity Assay** A sulforhodamine B bioassay (SRB) was used to determine the cytotoxicity of the above 10 compounds. The cytotoxic activity of each compound against four cultured human tumor cells was examined *in vitro* at the Korea Research Institute of Chemical Technology. The tumor cell lines were A549 (non small cell lung adenocarcinoma), SK-OV-3 (ovarian cancer cells), SK-MEL-2 (skin melanoma) and HCT15 (colon cancer cells).[15] Doxorubicin was used as the positive control. The IC_{50} values for the cytotoxicity of doxorubicin were 0.0012, 0.0094, 0.0012 and 0.1836 μM in A549, SK-OV-3, SK-MEL-2 and HCT15 cells, respectively.

Acknowledgments This research was supported by the Basic Science Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Education, Science and Technology (20110028285). We thank Drs. E. J. Bang, S. G. Kim, and J. J. Seo at the Korea Basic Science Institute for their aid in obtaining the NMR and mass spectra.

References

1) Lee C. B., "Coloured Flora of Korean Medicinal Herbs," Koy-Hak Publishing Co., Korea, 1998, pp. 430–436.
2) Szepczynska K., Krolikowska M., *Acta Pol. Pharm.*, **48**, 55–57 (1991).
3) Mikolajczak K. L., Smith C. R. Jr., Wolff I. A., *Lipids*, **1**, 289–290 (1966).
4) Yang F., Su Y. F., Bi Y. P., Xu J., Zhu Z. Q., Tn G. Z., Gao X. M., *Helv. Chim. Acta*, **93**, 536–541 (2010).
5) Ahan D. K., "Illustrated Book of Korean Medicinal Herbs," Koy-Hak Publishing Co., Korea, 1998, p. 400.
6) Lee I. K., Kim K. H., Lee S. Y., Choi S. U., Lee K. R., *Chem. Pharm. Bull.*, **59**, 773–777 (2011).
7) Otsuka H., Yamasaki K., Yamauchi T., *Phytochemistry*, **28**, 3197–3200 (1989).
8) Park, S. Y., Kim, J. S., Lee, S. Y., Bae, K. H., Kang, S. S., *Nat. Prod. Sci.*, **14**, 281-288 (2008).
9) Koji S., Sakae A., Yukio O., *Chem. Pharm. Bull.*, **33**, 3349–3355 (1985).
10) Fico G., Braca A., Bilia A. R., Tomè F., Morelli I., *J. Nat. Prod.*, **63**, 1563–1565 (2000).
11) Khan A., Ahmad V. U., Farooq U., Bader S., Arshad S., *Chem. Pharm. Bull.*, **57**, 276–279 (2009).
12) Yoshida K., Hishida A., Iida O., Hosokawa K., Kawabata J., *J. Agric. Food Chem.*, **56**, 4367–4371 (2008).
13) Yoshida T., Saito T., Kadoya S., *Chem. Pharm. Bull.*, **35**, 97–107 (1987).
14) Lee J. S., Yoo H. S., Suh Y. G., Jung J. K., Kim J. W., *Planta Med.*, **74**, 1481–1487 (2008).
15) Skehan P., Stroreng R., Scudiero D., Monks A., Mcmahon J., Vistica D., Warren J. T., Bokesch H., Kenney S., Boyd M. R., *J. Nat. Cancer Inst.*, **82**, 1107–1112 (1990).
16) Ferreres F., Castaner M., Tomas-Barberan F. A., *Phytochemistry*, **45**, 1701–1705 (1997).