# Isolation of a New Saponin and Cytotoxic Effect of Saponins from the Root of *Platycodon grandiflorum* on Human Tumor Cell Lines

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**Abstract**

A novel triterpenoid saponin, deapioplatycoside E (**1**) was isolated from the root extract of *Platycodon grandiflorum*, together with the seven known saponins **2** – **8**, i.e., platycoside E (**2**), deapioplatycodin $D_3$ (**3**), platycodin $D_3$ (**4**), polygalacin $D_2$ (**5**), platycodin $D_2$ (**6**), deapioplatycodin D (**7**) and platycodin D (**8**). The structure of the new saponin **1** was determined on the basis of spectral analysis and chemical evidence. The crude saponin fraction ($ED_{50}$: ca. 10 – 15 $\mu$g/mL) and compounds **6** – **8** ($ED_{50}$: ca. 4 – 18 $\mu$g/mL) exhibited significant inhibition on the proliferation of five kinds of cultured human tumor cell lines, i.e., A549 (non-small cell lung), SK-OV-3 (ovary), SK-MEL-2 (melanoma), XF498 (central nerve system) and HCT-15 (colon), *in vitro*.

The species, *Platycodon grandiflorum* A. DC (Campanulaceae), is a perennial herb commonly known as balloon flower widely spread in northeast Asia. It is often cultivated on the farmyard and the two- or three-year-old root of the species is used as a common food and frequently employed as a folk remedy for bronchitis, asthma and pulmonary tuberculosis, hyperlipidemia, diabetes and inflammatory diseases [1], [2]. Recently, it has been reported that the root extract of the species cultivated over 20 years [3] prevented hypercholesterolemia and hyperlipidemia [4] and also enhanced some of the functions of macrophages, such as their proliferation, spreading ability, phagocytosis, NO secretion, and the gene expression of TNF$\alpha$, IL-1$\beta$, and IL-6 [5], [6].

During our ongoing search for potent antitumor substances from natural resources, we have found that the crude saponin fraction B of the root extract of *P. grandiflorum* had exhibited a marked inhibition on the proliferation of cultured human tumor cell lines *in vitro*. Thus, a phytochemical investigation has been un-

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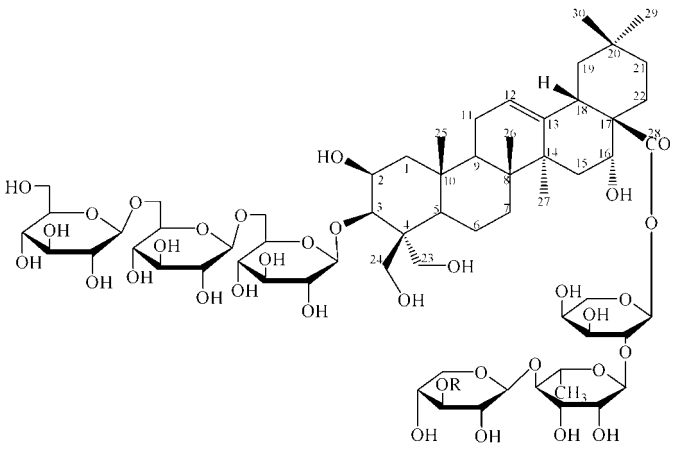
dertaken for the isolation of active constituents from the crude saponin fraction of the extract. By the serial chromatography of the saponin fraction, eight triterpenoid saponins (**1** – **8**) were isolated and their chemical structures were established by comparison of their spectral data with published ones. Among the isolates, compound **1** (Fig. **1**) was found to be a new component which had not been reported previously.

Compound **1**, an amorphous powder, $[\alpha]_D^{20}$: –22 (*c*, 0.1 in EtOH), possessed the molecular formula $C_{64}H_{104}O_{34}$ as determined by MALDI-TOF-MS (*m/z* = 1439: $[M + Na]^+$; Voyager; PE Biosystems USA). The $^1$H-NMR and $^{13}$C-NMR spectral data of **1** indicated that it had the sapogenin, 2*β*,3*β*,16*α*,23,24-pentahydroxyolean-12-en-28-oic acid and oligosaccharide moieties at C-3 and C-28.

Particularly, the $^{13}$C-NMR spectrum of **1** is quite imposable with that of platycoside E (**2**; Fig. **1**) except for some typical signals ($\delta$ = 111.7, 81.0 and 65.9) due to the apiose moiety of **2**. These results suggested that **l** be a congener of **2** (platycoside E), which has been found recently in this species [7]. By the scrutiny of the spectral data of **1** with those of **2**, the chemical structure of **1** was established to be 3-*O*-*b*-D-glucopyranosyl-(1→6)-*β*-D-glucopyranosyl-(1→6)-*β*-D-glucopyranosyl-2*β*,3*β*,16*α*,23,24-pentahydroxyolean-12-ene-28-oic acid 28-*O*-*β*-D-xylopyranosyl-(1→4)-*α*-L-rhamnopyranosyl-(1→2)-*α*-L-arabinopyranoside, which bis the deapio analogue of platycoside E (**2**). Compound **2** was found to be converted slowly to **1** by mild acid hydrolysis in 0.1 N HCl.

All of the isolated saponins were examined for the cytotoxicity against five cultured human tumor cell lines [8], the currently used cell lines in the National Cancer Institute (USA) for their *in vitro* anti-cancer drug screening program, i.e., A549 (non-small cell lung), SK-OV-3 (ovary), SK-MEL-2 (melanoma), XF498 (central nerve system) and HCT-15 (colon), *in vitro* [9]. When the examined tumor cells were exposed continuously to each of compounds **1** – **8** for 48 hours, their proliferation was significantly decreased in a dose-dependent manner. The $ED_{50}$ values of each component (**1** – **8**) on the proliferation of the five human tumor cells are summarized in Table **1**.



**1**: R = –H **2**: R = –apiofuranosyl

Fig. **1** Structures of saponins **1** and **2**.

Therefore, it could be concluded that the cytotoxic effect of the root extract of *P. grandiflorum* on cultured human tumor cell lines might be attributed predominantly to the saponin fraction (1.0 – 2.0% of the extract), particularly to the components, platycodin $D_2$ (**6**), deapioplatycodin D (**7**), and platycodin D (**8**).

## Materials and Methods

*Extraction and isolation:* The species *P. grandiflorum* cultivated for three years in Kangwon Province, Korea was harvested in September 2000. The roots were cut into slices and dried. For future reference, a voucher specimen (herbarium No. KM-00024) has been preserved at the Herbarium of Kookmin University, Institute of Forest Science, Korea.

The dried roots (1.0 kg) were soaked in methanol at room temperature for 7 days. Concentration of the solvent gave 220 g of a brown syrupy MeOH extract which was suspended in 2.2 L of $H_2O$ and poured into a Diaion HP-20 column (Ø = 5.0 × 100 cm), which was stabilized with $H_2O$. The column was eluted with additional 10 L of 20% MeOH. The eluate was combined and concentrated under reduced pressure to give 190 g of syrupy residue (Fr. A). The Diaion HP-20 column was further washed with additional 10 L of 85% MeOH. The eluate was concentrated under reduced pressure to give 24 g of brown powder (Fr. B). The column was finally washed out with MeOH. The washings were concentrated under reduced pressure to give 0.8 g of oily residue (Fr. C) [10]. The crude MeOH extract of the root and each fraction obtained from the MeOH extract by the Diaion HP-20 column chromatography (Fr. A – Fr. C) were evaluated for cytotoxic effects on the cultured human tumor cell lines. Among the tested, only the Fr. B exhibited a moderate cytotoxicity upon each of the tested cell lines. Therefore, the Fr. B was purified by repeated preparative HPLC (Futecs NS-3000i system equipped with GROM-SIL 120 ODS-4 HE column; 250 cm × 20 mm) with 26% acetonitrile in 20 mM $KH_2PO_4$ as eluent, which led to the isolation of eight triterpenoidal saponins (**1** – **8**), i.e., 22 mg of **1** ($t_R$ = 12.8 min, GROM-SIL 120 ODS-5, ST column; 250 cm × 5 mm, flow rate 0.7 mL/min), 150 mg of **2** ($t_R$ = 14.4 min), 15 mg of **3** ($t_R$ = 24.3 min), 28 mg of **4** ($t_R$ = 27.9 min), 14 mg of **5** ($t_R$ = 37.3 min), 20 mg of **6** ($t_R$ = 65.3 min), 24 mg of **7** ($t_R$ = 74.7 min) and 42 mg of **8** ($t_R$ = 77.4 min). The structure of the new saponin **1** was determined on the basis of spectral analysis and chemical evidences. The detailed chemical shifts of **1** in the $^{13}$C-NMR (in pyridine-$d_5$) spectrum are as follows (the data in the parenthesis are those for the corresponding signals of **2** [10]); C1 : 45.3 (45.3), C2 : 68.7 (68.8), C3 : 88.8 (88.8), C4 : 48.2 (48.2), C5 : 47.6 (47.6), C6 : 19.4 (19.5), C7 : 33.6 (33.5), C8 : 40.5 (40.6), C9 : 45.0 (45.0), C10 : 38.0 (38.0), C11 : 24.1 (24.1), C12 : 123.1 (123.3), C13 : 144.4 (144.7), C14 : 42.5 (42.5), C15 : 36.1 (36.2), C16 : 74.0 (73.9), C17 : 49.7 (49.7), C18 : 41.6 (41.7), C19 : 47.2 (47.2), C20 : 31.0 (31.0), C21 : 36.1 (36.1), C22 : 32.2 (32.2), C23 : 63.5 (63.7), C24 : 67.3 (67.3), C25 : 19.2 (19.2), C26 : 17.7 (17.7), C27 : 27.1 (27.1), C28 : 176.0 (176.1), C29 : 33.4 (33.3), C30 : 24.8 (24.8), *i*G1 (*inner glucose*): 106.1 (106.1), *i*G2 : 74.9 (74.9), *i*G3 : 78.4 (78.5), *i*G4 : 72.4 (72.3), *i*G5 : 76.5 (76.6), *i*G6 : 70.2 (70.7), *c*G1 (*central glucose*): 105.0 (105.0), *c*G2 : 75.4 (75.4), *c*G3 : 78.5 (78.4), *c*G4 : 71.1 (71.3), *c*G5 : 77.2 (77.2), *c*G6 : 70.2 (70.2), *t*G1 (*terminal glucose*): 105.7 (105.6), *t*G2 : 75.2 (75.2), *t*G3 : 78.7 (78.7),

Table **1** Inhibition of tumor cell proliferation by saponins from *Platycodon grandiflorum*

| | $ED_{50}$ (μg/mL)[a] | | | | |
|---|---|---|---|---|---|
| | *A549* | *SK-OV-3* | *SK-MEL-2* | *XF498* | *HCT15* |
| MeOH extract | > 50 | > 50 | > 50 | > 50 | > 50 |
| Fr. A | > 50 | > 50 | > 50 | > 50 | > 50 |
| Fr. B | 10.8 ± 0.3[b] | 6.2 ± 0.3 | 13.2 ± 0.2 | 15.2 ± 0.2 | 12.1 ± 0.3 |
| Fr. C | 30.2 ± 0.6 | 24.2 ± 0.5 | 42.2 ± 0.2 | 35.2 ± 0.5 | 32.1 ± 0.6 |
| 1 | > 50 | > 50 | > 50 | > 50 | > 50 |
| 2 | > 50 | > 50 | > 50 | > 50 | > 50 |
| 3 | > 50 | > 50 | > 50 | > 50 | > 50 |
| 4 | > 50 | > 50 | > 50 | > 50 | > 50 |
| 5 | > 50 | > 50 | > 50 | > 50 | > 50 |
| 6 | 3.7 ± 0.1 | 3.6 ± 0.2 | 3.8 ± 0.2 | 3.9 ± 0.3 | 3.8 ± 0.2 |
| 7 | 13.2 ± 0.1 | 12.9 ± 0.2 | 17.4 ± 0.2 | 17.1 ± 0.3 | 17.5 ± 0.2 |
| 8 | 3.8 ± 0.2 | 3.7 ± 0.2 | 3.9 ± 0.2 | 3.9 ± 0.3 | 3.8 ± 0.2 |
| cisplatin | 1.4 ± 0.1 | 0.9 ± 0.3 | 0.8 ± 0.2 | 0.9 ± 0.3 | 2.2 ± 0.4 |

[a] $ED_{50}$ value of compound against each cancer cell line, which was defined as the concentration that caused 50% inhibition of cell proliferation *in vitro*.
[b] Data are mean ± S.E.M. of three distinct experiments.



*t*G4 : 71.0 (70.9), *t*G5 : 77.7 (77.7), *t*G6 : 62.7 (62.7), A1 (*arabinose*): 93.7 (93.7), A2 : 75.3 (75.3), A3 : 71.3 (71.6), A4 : 66.4 (66.6), A5 : 63.1 (63.1), R1 (*rhamnose*): 101.2 (101.2), R2 : 72.0 (71.9), R3 : 72.8 (72.8), R4 : 83.6 (84.0), R5 : 68.7 (68.6), R6 : 18.5 (18.4), X1 (*xylose*): 106.9 (106.8), X2 : 76.1 (76.1), X3 : 78.6 (84.8), X4 : 71.6 (69.5), X5 : 67.5 (67.0). The other components **2** – **8** had been identified by direct comparison of their physical and spectral properties ($^1$H-NMR and $^{13}$C-NMR) with those in the literature [7], [11], [12]. Details of the work-up procedure and copies of the original spectra of **1** – **8** are obtainable from the author of correspondence.

*Preparation of* **1** *by acid hydrolysis of* **2**: One mg of **2** was dissolved in 0.2 mL of 0.1 N HCl at room temperature and an aliquot of reaction mixture was subjected to HPLC analysis (GROM-SIL 120 ODS-5, ST column; 250 cm × 5 mm at 40 °C) every 8 hours; it was observed that **2** ($t_R$ = 14.4 min) was slowly converted to **1** ($t_R$ = 12.8 min) in a time-dependent manner. More than 90% of **2** were converted to **1** after 48 hours.

*Cytotoxicity assessment:* The cytotoxicity of the compounds against cultured human tumor cell lines was evaluated by the SRB method [8], [9]. Briefly, each tumor cell line was inoculated over a series of standard 96-well flat-bottom microplates and were then preincubated for 24 h at 37 °C in a humidified atmosphere of 5% $CO_2$. The attached cells were then incubated with serially diluted saponin samples (**1** – **8**). After continuous exposure to the compounds for 48 h, the culture medium was removed from each well and the cells fixed with 10% cold trichloroacetic acid at 4 °C for 1 h. After washing with tap water, the cells were stained with 0.4% SRB dye and incubated for 30 min at room temperature. The cells were washed again and then solubilized with 10 mM unbuffered Tris bases solution (pH 10.5). The absorbance was measured spectrophotometrically at 520 nm with a microtiter plate reader.

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