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Anticomplement Activities of Oleanolic Acid Monodesmosides and Bisdesmosides Isolated from *Tiarella polyphylla*

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Seven known oleanolic acid glycosides (**1-7**) were isolated from the MeOH extract of *Tiarella polyphylla*. The structures were identified to be 3-*O*-(β-D-glucopyranosyl) oleanolic acid (**1**), 3-*O*-[β-D-glucopyranosyl-(1→3)-β-D-glucopyranosyl] oleanolic acid (**2**), 3-*O*-[β-D-glucopyranosyl-(1→2)-β-D-glucopyranosyl] oleanolic acid (**3**), 3-O-[β-D-glucopyranosyl-(1→3)-β-D-glucopyranosyl] oleanolic acid 28-*O*-β-D-glucopyranosyl ester (**4**), 3-*O*-[β-D-glucopyranosyl-(1→2)-β-D-glucopyranosyl] oleanolic acid 28-*O*-β–D-glucopyranosyl ester (**5**), 3-O-[a-L-rhamnopyranosyl-(1→3)-β-D-glucuronopyranosyl] oleanolic acid (**6**), and 3-*O*-[α-L-rhamnopyranosyl-(1→3)-β-D-glucuronopyranosyl] oleanolic acid 28-*O*-β-D-glucopyranosyl ester (**7**) on the basis of physicochemical and spectral data. These triterpene glycosides were tested for the anticomplement activity and hemolytic activity. Bisdesmosidic saponins, **4**, **5**, and **7**, showed anticomplement activity; in contrast, monodesmosidic saponins, **1-3**, and **6**, showed direct hemolytic activity. Methyl esterified monodesmosidic saponins showed anticomplement activity at a low concentration and hemolytic activity at a high concentration.

Key words : *Tiarella polyphylla*, Saxifragaceae, Saponins, Anti-complement activity

INTRODUCTION

The complement system is a major effector of humoral immunity involved in the host defense mechanism. The sequence of complement activation is initiated by antigen-antibody complexes (classical pathway, CP) or binding to the surfaces of infectious organisms (alternative pathway, AP). Activation of complement system leads to the formation of the membrane attack complex, which causes osmotic lysis of cells (Kuby, 1994). During the complement cascades, biologically active complement fragments are produced, which are successively associated with various diseases (Rother *et al.*, 1985). Therefore, specific inhibitors to modulate the complement activation should be useful in therapies of diseases caused by excessive complement activation.

In search for biologically active compounds from traditional medicines, the MeOH extract of *Tiarella polyphylla* D. Don (Saxifragaceae) was found to have a potent anti-complement activity. *T. polyphylla* is the only species among this genus distributed in Korea, and occurs wildly in Ullung island. The whole plant of *T. polyphylla* has been traditionally used for the treatment of asthma (Kim, 1984). However, any chemical constituents have not been so far revealed. In the previous paper, we reported the isolation and structure determination of a new triterpene, tiarellic acid, along with three known triterpenes, oleanolic acid, corosolic acid and tormentic acid (unpublished observation). In the present paper we report the isolation and structure elucidation of seven oleanolic acid glycosides from the BuOH fraction of *T. polyphylla* extract, and their anticomplement activity.

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MATERIALS AND METHODS

General procedure

Silica gel (230-400 mesh) was purchased from Merck Co. (Germany) and reversed phase (RP-18) silica gel (70-230 mesh, YMC GEL ODS-A) was purchased from YMC Co. (Japan). The ^{1}H-NMR (300 or 600 MHz) and ^{13}C-NMR (75 or 150 MHz) spectra were recorded using

Varian Unity 300 or Brucker DRX-600 spectrometer. Solvent systems used for TLC were $CHCl_3$-MeOH-H_2O (7:3:1, lower phase, system A) and $CHCl_3$-MeOH-H_2O (6:4:1, system B).

Plant material

The whole plant of *T. polyphylla* was collected in September 1997, Ullung island, Korea. A voucher specimen (NDC-209) is deposited in our laboratory.

Extraction and isolation

Dried and chopped whole plants (1 kg) were ex-tracted with MeOH (4 L × 5). The extract was concentrated *in vacuo* to yield a dark green residue (120 g), which was suspended in water and successively parti-tioned with EtOAc and BuOH, respectively. The BuOH layer was concentrated *in vacuo*, and the extract (20 g) was subjected to RP-18 column and eluted with water MeOH mixture (1:1→0:1) to yield six subfractions. Subfraction II was purified by silica gel column chromatography using EtOAc-MeOH-water (100:16.5:13.5) to obtain **6** and **7**. Subfraction III was subjected to silica gel column with eluting solvent $CHCl_3$-MeOH-H_2O (7:3:1, lower phase) to yield **4** and **5**. Subfraction IV was separated by silica gel column chromatography using $CHCl_3$-MeOH-H_2O (8:2:1, lower phase) to yield **2** and **3**. Compound **1** was obtained from subfraction V by silica gel column chromatography using the solvent system $CHCl_3$-MeOH (4:1).

3-*O*-(β-D-Glucopyranosyl) oleanolic acid (1) R_f: 0.64 (system A), 0.94 (system B). ^{13}C NMR : 88.8 (C-3), 180.1 (C-28), 106.8 (C-1′), 75.7 (C-2′), 78.7 (C-3′), 71.8 (C-4′), 78.1 (C-5′), 63.0 (C-6′).

3-*O*-[β-D-Glucopyranosyl-(1→3)-β-D-glucopyranosyl] oleanolic acid (2) R_f: 0.47 (system A), 0.74 (system B). ^{13}C NMR : 89.0 (C-3), 180.2 (C-28), 106.0 (C-1′), 75.6 (C-2′), 89.0 (C-3′), 70.0 (C-4′), 78.7 (C-5′), 62.7 (C-6′), 106.4 (C-1″), 74.5 (C-2″), 78.3 (C-3″), 71.7 (C-4″), 78.0 (C-5″), 62.6 (C-6″).

3-*O*-[β-D-Glucopyranosyl-(1→2)-β-D-glucopyranosyl] oleanolic acid (3) R_f : 0.36 (system A), 0.67 (system B). ^{13}C NMR : 89.0 (C-3), 180.2 (C-28), 105.0 (C-1′), 83.3 (C-2′), 78.4 (C-3′), 71.7 (C-4′), 78.2 (C-5′), 62.8 (C-6′), 106.0 (C-1″), 77.1 (C-2″), 78.0 (C-3″), 71.6 (C-4″), 77.9 (C-5″), 62.7 (C-6″).

3-*O*-[β-D-Glucopyranosyl-(1→3)-β-D-glucopyranosyl] oleanolic acid 28 - *O*-β-D-glucopyranosyl ester (4) R_f : 0.28 (system A), 0.65 (system B). ^{13}C NMR : 88.8 (C-3), 176.4 (C-28), 105.9 (C-1′), 75.4 (C-2′), 89.0 (C-3′), 69.8 (C-4′), 78.6 (C-5′), 62.5 (C-6′), 106.3 (C-1″), 74.4 (C-2″), 78.2 (C-3″), 71.5 (C-4″), 77.8 (C-5″), 62.4 (C-6″), 95.7 (C-1‴), 74.1 (C-2‴), 79.2 (C-3‴), 71.1 (C-4‴), 78.8 (C-5‴), 62.2 (C-6‴).

3-*O*-[β-D-Glucopyranosyl-(1→2)-β-D-glucopyranosyl] oleanolic acid 28-*O*-β-D-glucopyranosyl ester (5) R_f: 0.19 (system A), 0.60 (system B). ^{13}C NMR : 89.0 (C-3), 176.4 (C-28), 105.0 (C-1′), 83.3 (C-2′), 78.3 (C-3′), 71.7 (C-4′), 78.2 (C-5′), 62.8 (C-6′), 105.9 (C-1″), 77.0 (C-2″), 78.0 (C-3″), 71.6 (C-4″), 77.9 (C-5″), 62.7 (C-6″) 95.7 (C-1‴), 74.1 (C-2‴), 79.2 (C-3‴), 71.1 (C-4‴), 78.8 (C-5‴), 62.2 (C-6‴).

3-*O*-[α-L-Rhamnopyranosyl-(1→3)-β-D-glucuronopyranosyl] oleanolic acid (6) R_f: 0.11 (system A), 0.51 (system B). ^{13}C NMR : 89.4 (C-3), 180.3 (C-28), 106.5 (C-1′), 75.8 (C-2′), 82.1 (C-3′), 72.6 (C-4′), 76.9 (C-5′), 176.0 (C-6′), 102.3 (C-1″), 72.0 (C-2″), 72.5 (C-3″), 74.3 (C-4″), 69.6 (C-5″), 18.7 (C-6″).

3-*O*-[α-L-Rhamnopyranosyl-(1→3)-β-D-glucuronopyranosyl] oleanolic acid 28-*O*-β-D-glucopyranosyl ester (7) R_f: 0.06 (system A), 0.43 (system B). ^{13}C NMR : 89.3 (C-3), 176.4 (C-28), 106.4 (C-1′), 76.0 (C-2′), 81.9 (C-3′), 72.7 (C-4′), 76.2 (C-5′), 176.2 (C-6′), 102.2 (C-1″), 72.2 (C-2″), 72.5 (C-3″), 74.0 (C-4″), 69.5 (C-5″), 18.7 (C-6″), 95.7 (C-1‴), 74.3 (C-2‴), 79.3 (C-3‴), 71.1 (C-4‴), 78.8 (C-5‴), 62.2 (C-6‴).

Methyl esterification of saponins

Methanol solutions of oleanolic acid glycosides having free carboxylic acid were treated with excess etherial diazomethane and the esterification was monitored by TLC. The resultant products obtained after removal of the solvent under reduced pressure were tested for anti-complement assay without further purification.

Acid hydrolysis of saponins

Saponin fraction was refluxed with 5% HCl in 60% aqueous dioxane for 3 hr. The resulting solution was dried under reduced pressure, and the hydrolysate was extracted with ether. This final extract was evaporated to yield oleanolic acid, which was identified by direct comparison of TLC with authentic sample.

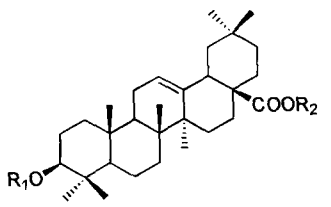
Determination of anti-complement activity through the classical pathway

Anti-complement activity was determined by the modified method of Mayer as described previously (Oh

Table 1. Anti-complement activity of saponins on classical pathway

Compounds[a]	IC_{50} (μM)
Glc(1→3)Glc-OA-Glc **4**	12.8
Glc(1→2)Glc-OA-Glc **5**	21.2
Rha(1→3)GlcA-OA-Glc **7**	41.2
7a (methyl ester of **7**)	29.2

[a]Glc ; β-$_D$-glucopyranosyl
GlcA ; β-$_D$-glucuronopyranosyl
Rha ; α-$_L$-rhamnopyranosyl
OA ; oleanolic acid



1. R_1 = -Glc	R_2 = H	1a. R_1 = -Glc	R_2 = Me
2. R_1 = -Glc^3-Glc	R_2 = H	2a. R_1 = -Glc^3-Glc	R_2 = Me
3. R_1 = -Glc^2-Glc	R_2 = H	3a. R_1 = -Glc^2-Glc	R_2 = Me
4. R_1 = -Glc^3-Glc	R_2 = Glc		
5. R_1 = -Glc^2-Glc	R_2 = Glc		
6. R_1 = -GlcA^3-Rha	R_2 = H	6a. R_1 = -MeGlcA^3-Rha	R_2 = Me
7. R_1 = -GlcA^3-Rha	R_2 = Glc	7a. R_1 = -MeGlcA^3-Rha	R_2 = Glc

et al., 1996; Kabat *et al.*, 1961). For the classical pathway assay, a diluted solution of normal human serum (80 μl) was mixed with gelatin veronal buffer (80 μl) with or without sample. The mixture was preincubated at 37°C for 30 min, then sensitized sheep red blood cells (40 μl) were added. After incubation under the same condi-tions, the mixture was centrifuged and the optical density of the supernatant (100 μl) was measured at 405 nm. Anti-complement activity was determined as a mean of triplicates.

RESULTS AND DISCUSSION

The dried whole plant of *T. polyphylla* was extracted with MeOH and successively partitioned with EtOAc and BuOH, respectively. The BuOH fraction was subjected to RP-18 and silica gel column chromatography to give seven saponins. On acid hydrolysis of all the isolates, the aglycone was found to be oleanolic acid by comparison of TLC with authentic sample. The ^{13}C-NMR spectra of compounds **1-3** and **6** showed the signals of C-3 at δ 88.8 89.4 and C-28 at d 180.1 180.3 and those of **4**, **5** and **7** showed the signals of C-3 at δ 89.0 89.3 and C-28 at d 176.4, which indicated compounds **1-3** and **6** were mono-desmosides and **4**, **5** and **7** were bisdes-mosides. Anomeric proton of sugar moiety of **1** were shown at δ 4.93 (1H, d, J = 7.7 Hz) in ^{1}H-NMR spectrum. In ^{13}C-NMR spectrum, sugar signals of **1** were identical to those of β-D-gluco-pyranoside (Sati *et al.*, 1990). Therefore, compound **1** was identified as 3-*O*-(β-D-glucopyranosyl) oleanolic acid. Compounds **2** and **3** showed two anomeric proton signals in sugar moieties at d 4.88 (1H, d, J = 7.5 Hz, H-1′) and 5.27 (1H, d, J = 7.9 Hz, H-1″), and d 4.90 (1H, d, J = 7.4 Hz, H-1′) and 5.30 (1H, d, J = 7.5 Hz, H-1″), respectively. In ^{13}C-NMR spectrum, all the sugar signals of **2** were in agreement with those of β-D-gluco-pyranosyl-(1→3)-β-D-gluco-pyranoside. In addition, down-field shift at C-3′ (δ 89.0) due to glycosylation indicated the glycoside linkage between two glucose moieties was β(1→3). Thus, compound **2** was identified as 3-*O*-[β-D-glucopyranosyl-(1→3)-β-D-gluco-pyranosyl] oleanolic acid (Sotheeswaran *et al.*, 1989). The two glucose moieties of **3** were found in NMR spectra and the linkage between two sugar moieties was proven to be β(1→2) by a comparison of reported NMR data. Thus, compound **3** was identified as 3-*O*-[β-D-glucopyranosyl-(1→2)-β-D-glucopyranosyl] oleanolic acid (Nagao *et al.*, 1991). Compound **6** showed two anomeric proton signals at d 4.90 (1H, d, J=7.6 Hz, H-1′) and 6.33 (1H, s, H-1″) in ^{1}H-NMR spectrum which signals were corresponding to glucuronic acid and rhamnose (Borel *et al.*, 1987). In ^{13}C-NMR spectrum, sugar signals of **6** were in good agreement with those of α-L-rhamnosyl-(1→3)-β-D-glucuronopyranoside, and down field shift of C-3′ (δ 82.1) confirmed linkage between rhamnose and glu-curonic acid was α(1→3). Thus, compound **6** was identified as 3–*O*–[α–L–rhamnosyl-(1→3)–β–D–glucurono-pyranosyl] oleanolic acid. Compound **4** was a bis-desmoside in which 28-*O*-glucosyl moiety was attached to **2**. In ^{13}C-NMR spectrum, additional signals due to 28-*O*-glucosyl attachment to **2** were appeared. Three anomeric proton signals of sugar moieties were appeared at d 4.85 (1H, d, J = 7.6 Hz, H-1′), 5.25 (1H, d, J = 7.8 Hz, H-1″) and 6.28 (1H, d, J = 7.8 Hz, H-1‴) in ^{1}H-NMR spectrum. Thus, all of the sugar moieties in compound **4** were found to be β-D-gluconopyranose, and compound **4** was identified to be 3-*O*-[β-D-gluco-pyranosyl-(1→3)-β-D-glucopyranosyl] oleanolic acid 28-*O*-β-D-gluco-pyranosyl ester. 28-*O*-α-D-Glucopyranosyl ester (d 5.70, 1H, d, J = 4.5 Hz, H-1‴) of compound **2** have been previously reported from *Anchusa officinalis* (Romussi et *al.*, 1979). However, 28-O-β-D-glucopyranosyl ester (**4**) was first report from natural source. Compounds **5** showed three anomeric proton signals at d 4.88 (1H, d, J = 7.4 Hz, H-1′), 5.35 (1H, d,

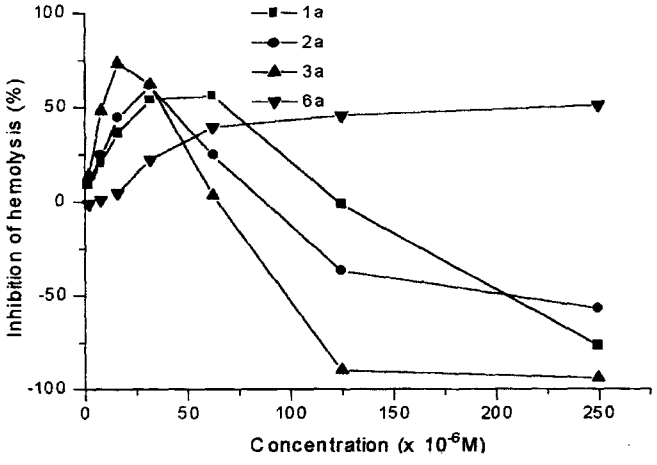


Fig. 1. Anti-complement effect of methyl esterified saponins from *T.polyphylla* on the CP of complement system.

J = 7.6 Hz, H-1″) and 6.29 (1H, d, J = 7.9 Hz, H-1‴). In ^{13}C-NMR spectrum, one additional glucose signal to that for compound **3** were appeared. Thus, compound **5** was identified as 3-*O*-[β-D-gluco-pyranosyl-(1→2)-β-D-glucopyranosyl] oleanolic acid 28-*O*-β-D-glucopyranosyl ester (Davidyants *et al.*, 1984). Compounds **7** was identified as 3-*O*-[α-L-rhamno-pyranosyl-(1→3)-β-D-glucuronopyranosyl] oleanolic acid 28-*O*-β-D-glucopyranosyl ester, a bisdesmoside glycosylated at C-28 of **6**, by comparison with reported NMR spectral data (Borel *et al.*, 1987).

Bisdesmosidic saponins, **4**, **5** and **7**, inhibited hemolysis (IC_{50} = 12.8, 21.2 and 41.2 μM, respectively) in a dose-dependent manner on the CP of the complement system. However, monodesmosidic saponins, **1-3** and **6**, caused direct hemolysis. Methyl esters of monodesmosidic saponins, **1a-3a**, showed biphasic action on the CP of the complement system. These saponins showed anti-complement activity at lower concentrations than 62.5 μM but showed hemolytic activity at higher concentrations. Compound **6a** showed the moderate anti-complement activity (Fig. 1). Moreover, methyl esterification of glucuronic acid of compound **7** enhanced anti-complement activity (IC_{50} = 29.3 μM). A similar result has been also reported in the case of ginsenoside Ro (Kim *et al.*, 1998).

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