

Triterpenoids from *Trapa pseudoincisa*

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Trapa pseudoincisa Nakai, an aquatic plant, was extracted with 80% aqueous MeOH, and the concentrated extract was successively partitioned with EtOAc, *n*-BuOH, and H₂O. The EtOAc fraction gave three compounds, which were isolated through the repeated silica gel and ODS column chromatographies. Based on the spectroscopic data obtained from NMR, MS, and IR, the chemical structures of the compounds were determined as cycloeucalenol (1), ursolic acid (2), and 2β,3α,23-trihydroxyurs-12-en-28-oic acid (3). These triterpenoids were isolated for the first time from Hydrocaryaceae plants including *T. pseudoincisa* NAKAI.

Key words: cycloeucalenol, Hydrocaryaceae, *Trapa pseudoincisa*, 2β,3α,23-trihydroxyurs-12-en-28-oic acid, triterpenoid, ursolic acid

Trapa pseudoincisa Nakai (Hydrocaryaceae), water chestnut, is a genus belonging to the annual floating-leaved aquatic plants widely distributed in the East Asian countries [Takano and Kadono, 2005]. Five species of *Trapa* are distributed in Korea [Chung *et al.*, 1987], 16 in China [Diao, 1990], and 3 in Japan [Nakano, 1913]. The Korean *Trapa* is classified into two groups based on the number of horns. The first group, having the two horns, consists of two species, *T. japonica* and *T. bicornis* var. *coronus*, as classified by the cultivation of horns. The second group, having four horns, consists of five species, *T. natans*, *T. natans* var. *japonica*, *T. pseudoincisa*, *T. maximowiczii*, and *T. incise*, as classified by the size of nuts, presence or absence of herbs on the horns, and thickness of horns [Chung *et al.*, 1987]. *T. pseudoincisa* Nakai, known as 'Ae-gi-ma-reum' in Korea, has four horns and is distributed in Korea, Japan, and China [Jung and Shin, 1990]. Its fruit has been found to have antitumor [Irikura *et al.*, 1972] and anticancer [Kosuge *et al.*, 1986]

effects, and was also effective for the treatments of quadriplegia, gastric ulcer, and diarrhea [Jung and Shin, 1990]. Analysis results of ergosta-4,6,8(14),22-tetraen-3-one [Ciminiello *et al.*, 1989], 22,23-dihydrostigma-4-en-3,6-dione and β-sitosterol [Irikura *et al.*, 1972], and *cis*-hinokiresinol [Song *et al.*, 2007] from *T. pseudoincisa* were previously reported. This paper describes the isolation and the structural elucidation of three triterpene compounds reported for the first time from *T. pseudoincisa*.

Materials and Methods

Instruments. Melting points were determined on a Fisher-John Apparatus (Fisher Scientific, Chicago, IL) and uncorrected. Optical rotations were measured on a JASCO P-1010 digital polarimeter (Jasco, Tokyo, Japan). EIMS and FABMS were recorded on a JEOL JMS 700 (JEOL, Tokyo, Japan). IR spectra were run on a Perkin Elmer spectrum One FT-IR spectrometer (Perkin Elmer, Norwalk, CT). ¹H-NMR (400 MHz) and ¹³C-NMR (100 MHz) spectra were taken on a Varian Unity Inova AS 400 FT-NMR spectrometer (Varian, Palo Alto, CA).

Plant Materials. *T. pseudoincisa* NAKAI was collected from a stream located in Sunchang, Jeonbuk, Korea on September and identified by Prof. Dae-Keun Kim of Woosuk University, Jeonju, Korea. A Voucher specimen (KHU04031) was deposited in the Laboratory of Natural

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Abbreviations: EtOAc, ethyl acetate; *n*-BuOH, normal-butanol; MeOH, methanol; MS, mass spectrometry; NMR, nuclear magnetic resonance; ODS, octadecyl silica gel; TLC, thin layer chromatography.

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Isolation of triterpenoids. The dried whole plant of *T. pseudoincisa* (1.1 kg) was extracted with 80% aqueous MeOH (2 L × 3) and evaporated *in vacuo*. The extracts were successively partitioned with water (1 L), EtOAc (1 L × 2), and *n*-BuOH (1 L × 2). The concentrated EtOAc fraction (TIE, 30 g) was subjected to the silica gel (SiO₂) (200 g, 70-230 mesh, Merck, Darmstadt, Germany) column chromatography (cc) (Φ 6 × 12 cm) eluted with *n*-hexane-EtOAc [10 : 1 → 5 : 1 → 3 : 1 → 1 : 1, v/v, 300 mL each] and chloroform (CHCl₃)-MeOH (10 : 1 → 7 : 1 → 5 : 1 → 3 : 1 → 1 : 1, v/v, 300 mL each) to give 24 fractions (TIE1~TIE24). TIE11 [512 mg, Ve/Vt (elution volume/total volume) 0.45-0.52] was purified by SiO₂ (50 g) cc (Φ 3.5 × 10 cm) eluted with *n*-hexane-EtOAc (15 : 1, v/v, 1800 mL) to give 12 fractions (TIE11-1~TIE11-12). TIE11-5 (64 mg, Ve/Vt 0.23-0.34) was chromatographed by ODS (50 g, 70-230 mesh, Merck) cc (Φ 3.5 × 10 cm, MeOH-water = 10 : 1, v/v, 2200 mL) to give compound **1** (28 mg, Ve/Vt 0.35-0.43; ODS TLC R_f 0.4, MeOH-H₂O = 5 : 1). TIE13 (2.1 g, Ve/Vt 0.56-0.62) was chromatographed by ODS (50 g) cc (Φ 3.5 × 10 cm, MeOH-water = 1 : 1 → 3 : 1, v/v, 2000 mL each) to give 25 fractions (TIE13-1~TIE13-25). TIE13-23 [123 mg, Ve/Vt 0.84-0.92] was purified by SiO₂ (50 g) cc (Φ 3 × 11 cm) eluted with CHCl₃-MeOH (17 : 1, v/v, 1800 mL) to give compound **2** (103 mg, Ve/Vt 0.25-0.70; SiO₂ TLC R_f 0.4, CHCl₃-MeOH = 13 : 1). TIE15 (3.5 g, Ve/Vt 0.65-0.69) was applied to SiO₂ (75 g) cc (Φ 4 × 10 cm) eluted with CHCl₃ in MeOH (15 : 1, 3200 mL) to afford 12 fractions (TIE15-1~TIE15-12). TIE15-9 (310 mg, Ve/Vt 0.61-0.73) was subjected to SiO₂ cc (25 g, Φ 3 × 7 cm) eluted with *n*-hexane-EtOAc (1 : 1, v/v, 2000 mL) to give 8 fractions (TIE15-9-1~TIE15-9-8). TIE15-9-8 (250 mg, Ve/Vt 0.43-0.89) was purified by ODS (50 g) cc (Φ 3.5 × 10 cm) eluted with MeOH in water (3 : 1, v/v, 1000 mL) to give compound **3** (44 mg, Ve/Vt 0.56-0.66; ODS TLC R_f 0.5, MeOH-H₂O = 3 : 1).

Cycloeucaleanol (1). Colorless crystal; mp 138-139°C; [α]_D +45 (*c* 2.9, CHCl₃); EI/MS *m/z* 426 [M, 10]⁺, 411 (13), 408 (23), 393 (23), 327, 285, 269, 218, 203; IR (KBr) 3650, 3590, 1630, 895 cm⁻¹; ¹H-NMR (400 MHz, CDCl₃, δ) 4.69 (1H, br. s, H-30a), 4.63 (1H, br. s, H-30b), 3.18 (1H, m, H-3), 1.01 (3H, d, *J* = 7.2 Hz, H-26), 0.99 (3H, d, *J* = 6.8 Hz, H-27), 0.95 (3H, d, *J* = 6.0 Hz, H-29), 0.94 (3H, s, H-18), 0.87 (3H, d, *J* = 6.0 Hz, H-21), 0.87 (3H, s, H-28), 0.36 (1H, d, *J* = 4.0 Hz, H-19a), 0.11 (1H, d, *J* = 4.0 Hz, H-19b); ¹³C-NMR (100 MHz, CDCl₃, δ); See Table 1.

Ursolic acid (2). White powder; [α]_D +66 (*c* 1.2, MeOH); EI/MS *m/z* 456 [M⁺, 10], 438 (12), 248 (100), 207 (16), 203 (66), 189 (20), 133 (32); IR (KBr) 3440,

2940, 1700, 1690 cm⁻¹; ¹H-NMR (400 MHz, C₃D₅N, δ) 5.30 (1H, m, H-12), 3.42 (1H, dd, *J* = 10.8, 6.8 Hz, H-3), 1.21 (3H, d, *J* = 7.0 Hz, H-29), 1.01 (3H, d, *J* = 7.0 Hz, H-30), 0.99 (6H, s, H-23, 25), 0.93 (3H, s, H-26), 0.86 (6H, s, H-24, 27); ¹³C-NMR (100 MHz, C₃D₅N, δ); See Table 1.

2β,3α,23-Trihydroxyurs-12-en-28-oic acid (3). White powder; [α]_D +42 (*c* 0.1, C₃H₅N); IR (KBr) 3356 (br), 2925, 1726, 1687, 1451, 1365, 1311, 1271 cm⁻¹; positive FABMS *m/z* 511 [M+Na]⁺; ¹H-NMR (400 MHz, C₃D₅N, δ) 5.43 (1H, br. s, H-12), 4.20 (1H, m, H-2), 4.18 (1H, br. s, H-3), 4.17 (1H, d, *J* = 10.0 Hz, H-23a), 3.69 (1H, d, *J* = 10.0 Hz, H-23b), 2.59 (1H, d, *J* = 11.2 Hz, H-18), 2.27 (1H, dd, *J* = 12.0, 2.4 Hz, H-1a), 1.33 (1H, dd, *J* = 12.0, 6.4 Hz, H-1b), 1.12 (3H, s, H-27), 1.03 (9H, s, H-23, 25,

Table 1. ¹³C-NMR chemical shifts (100 MHz, δ_C) of triterpenoids from the whole plant of *T. pseudoincisa* NAKAI

No. of Carbon	compound 1*	Compound 2*	compound 3*
1	30.9	39.2	47.9
2	34.9	28.8	68.9
3	76.6	78.2	78.0
4	44.6	40.0	43.7
5	43.4	53.6	47.8
6	24.7	18.9	18.5
7	28.2	33.7	31.2
8	46.9	39.4	40.0
9	23.6	48.1	48.1
10	29.6	37.5	38.3
11	25.2	23.7	23.8
12	32.9	125.6	125.5
13	45.4	139.2	139.3
14	48.9	42.6	42.5
15	35.1	31.2	28.6
16	27.3	25.0	24.9
17	52.2	49.7	48.1
18	17.9	55.9	53.5
19	27.0	39.6	39.5
20	36.2	39.5	39.4
21	18.4	28.2	31.1
22	35.4	37.4	33.2
23	31.4	28.9	66.3
24	156.8	17.6	14.4
25	33.9	17.3	17.6
26	22.1	17.8	17.5
27	21.9	24.0	23.9
28	19.2	179.8	180.1
29	14.5	21.5	21.4
30	105.9	16.7	17.6

*Compound **1** was dissolved in CDCl₃ and compounds **2** and **3** in C₃D₅N.

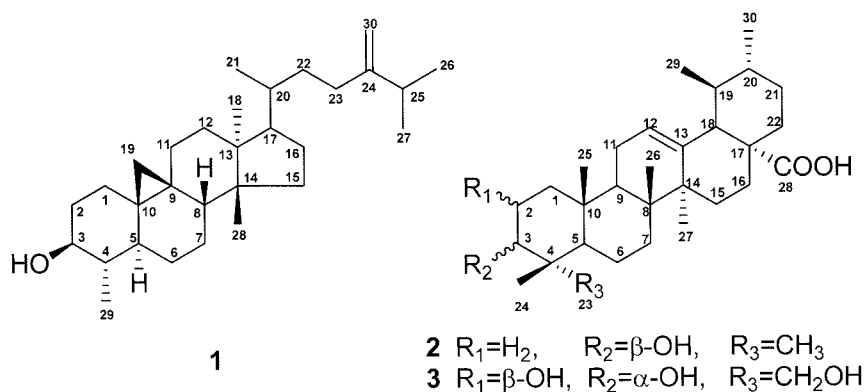


Fig. 1. Chemical structures of triterpenoids from the whole plant of *T. pseudoincisa* NAKAI.

26), 0.94 (3H, d, $J = 6.0$ Hz, H-29), 0.90 (3H, d, $J = 6.0$ Hz, H-30); $^{13}\text{C-NMR}$ (100 MHz, $\text{C}_5\text{D}_5\text{N}$, δ); See Table 1.

Results and Discussion

The whole plants of *T. pseudoincisa* was extracted with 80% aqueous MeOH, and the concentrated extract was successively partitioned with EtOAc, *n*-BuOH, and H₂O. Through successive repeated silica gel or ODS column chromatography of the EtOAc fraction, cycloeucalenol (yield; $2.7 \times 10^{-3}\%$), ursolic acid (yield; $9.4 \times 10^{-3}\%$), and 2 β ,3 α ,23-trihydroxyurs-12-en-28-oic acid (yield; $4.0 \times 10^{-3}\%$) were isolated. The chemical structures of the compounds were identified on the basis of several spectroscopic analyses including NMR and MS.

Compound **1**, colorless crystals, showed a characteristic absorption of hydroxyl (3650 and 3590 cm^{-1}) and olefine (1630 cm^{-1}) in the IR spectrum. The molecular ion peak was determined [M^+ , $\text{C}_{30}\text{H}_{50}\text{O}$] at m/z 426 (10) with 411 [M^+-CH_3 , 13], 408 [$\text{M}^+-\text{H}_2\text{O}$, 23], and 393 (23) by EIMS. The $^1\text{H-NMR}$ spectrum revealed the presence of two exomethylene protons at δ_{H} 4.69 (br. s) and δ_{H} 4.63 (br. s), one oxygenated methine proton at δ_{H} 3.18 (m), six methyl protons [δ_{H} 1.01 (d, $J = 7.2$ Hz), δ_{H} 0.99 (d, $J = 6.8$ Hz), δ_{H} 0.95 (d, $J = 6.0$ Hz), δ_{H} 0.94 (s), δ_{H} 0.87 (d, $J = 6.0$ Hz), and δ_{H} 0.87 (s)], and two methylene protons of a cyclopropyl group at δ_{H} 0.11 (d, $J = 4.0$ Hz) and δ_{H} 0.36 (d, $J = 4.0$ Hz). The $^{13}\text{C-NMR}$ spectrum showed 30 carbon signals consisting of one olefinic quaternary carbon at δ_{C} 156.8, one exomethylene carbon at δ_{C} 105.9, one oxygenated methine carbon at δ_{C} 76.6, and six methyl signals (δ_{C} 22.1, δ_{C} 21.9, δ_{C} 19.2, δ_{C} 18.4, δ_{C} 17.9 and δ_{C} 14.5). Through comparison of these NMR data with those in the literatures, compound **1** was identified as cycloeucalenol previously isolated from *Cedrela Odorata* [Paula *et al.*, 1997], *Pholidota chinensis* [Wen *et al.*, 1986], and *Tinopora crispa* [Kongkathip *et al.*, 2002].

Compound **2**, white powder, showed the characteristic

absorptions of hydroxyl (3440 and 2940 cm^{-1}), carbonyl (1700 cm^{-1}), and olefine (1690 cm^{-1}) groups in the IR spectrum. The molecular ion peak was recorded [M^+ , $\text{C}_{30}\text{H}_{48}\text{O}_3$] at m/z 456 (10) with fragments at m/z 438 (12), 248 (100), 207 (16), 203 (66), 189 (20), and 133 (32) in the EIMS. The $^1\text{H-NMR}$ spectrum exhibited the signals derived from one olefine methine proton at δ_{H} 5.30, one oxygenated methine proton at δ_{H} 3.42 (m), seven methyl protons [δ_{H} 1.21 (d, $J = 7.0$ Hz), δ_{H} 1.01 (d, $J = 7.0$ Hz), δ_{H} 0.99 (s, $\times 2$), δ_{H} 0.93 (s) and δ_{H} 0.86 (s, $\times 2$)], indicating compound **2** to be a pentacyclic triterpenoid of the ursane skeleton. The $^{13}\text{C-NMR}$ spectrum showed 30 carbon signals consisting of one carbonyl carbon at δ_{C} 179.8, one olefine quaternary carbon at δ_{C} 139.2, one olefine methine carbon at δ_{C} 125.9, one oxygenated methine carbon at δ_{C} 78.2, and seven methyl carbon (δ_{C} 28.9, δ_{C} 24.0, δ_{C} 21.5, δ_{C} 17.8, δ_{C} 17.6, δ_{C} 17.3, and δ_{C} 16.7) signals. Through comparison of these NMR data with those in the literatures [Chang *et al.*, 2001; Tkachev *et al.*, 1994; Hong *et al.*, 2003], compound **2** was identified as ursolic acid.

Compound **3**, white powder, showed a characteristic absorption of hydroxyl (3356 and 2925 cm^{-1}), carbonyl (1726 cm^{-1}) and olefine (1687 cm^{-1}) groups in the IR spectrum. Its positive FABMS showed a pseudomolecular ion at m/z 511 [$\text{M}+\text{Na}$] $^+$. The $^1\text{H-NMR}$ spectrum revealed the presence of one olefinic methine proton at δ_{H} 5.43, two oxygenated methine protons at δ_{H} 4.20 (m) and δ_{H} 4.18 (br. s.), two oxygenated methylene protons at δ_{H} 4.17 (d, $J = 10.0$ Hz) and δ_{H} 3.69 (d, $J = 10.0$ Hz), and six methyl protons at δ_{H} 1.12 (s), δ_{H} 1.03 (s, $\times 3$), δ_{H} 0.94 (d, $J = 7.0$ Hz), δ_{H} 0.90 (d, $J = 7.0$ Hz). The $^{13}\text{C-NMR}$ spectrum showed 30 carbon signals consisting of one carbonyl carbon at δ_{C} 180.1, one olefine quaternary carbon at δ_{C} 139.3, one olefine methine carbon at δ_{C} 125.5, two oxygenated methine carbons at δ_{C} 78.0 and δ_{C} 68.9, one oxygenated methylene carbon at δ_{C} 66.3, and six methyl carbon signals (δ_{C} 23.9, δ_{C} 21.4, δ_{C} 17.6, δ_{C} 17.6 δ_{C} 17.5, and δ_{C} 14.4). Through comparison of these NMR data

with those in the literature [Srivastava and Neraliya, 1995], compound **3** was identified as 2 β ,3 α ,23-trihydroxyurs-12-en-28-oic acid.

Three triterpenoids, cycloeucalenol (**1**), ursolic acid (**2**), and 2 β ,3 α ,23-trihydroxyurs-12-en-28-oic acid (**3**), were isolated from the whole plant of *T. pseudoincisa* for the first time by our group. Cycloeucalenol (**1**) was reported to show anti-inflammatory effects [Akihisa *et al.*, 2000], cardiogenic effects [Kongkathip *et al.*, 2002], and spasmolytic effect [Gomes *et al.*, 2005]. Ursolic acid (**2**) is a very famous ursane-skeleton triterpenoid and was reported to have various pharmacological effects such as anti-complementary activity [Thuong *et al.*, 2006], anti-HIV activity [Chien *et al.*, 2004; Zhang *et al.*, 2003], anticancer effect [Huang *et al.*, 2003; Sohn *et al.*, 1995], and anti-inflammatory effect [Ismaili *et al.*, 2000]. 2 β ,3 α ,23-Trihydroxyurs-12-en-28-oic acid (**3**) occurred so rarely in the natural sources that only one literature [Srivastava and Neraliya, 1995] reported that compound **3** isolated from *Nepeta hindostana* showed an insect-growth regulatory effect. Therefore, *T. pseudoincisa* NAKAI containing these triterpenoids having various biological activities might be good materials for the development of anti-cancer or anti-inflammatory drugs.

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