Cyclohexylethanoids from the Flower of *Campsis grandiflora*

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Campsis grandiflora K. Schum. (Bignoniaceae) is a climbing plant cultivated in the fields of Korea, China and Japan. The flower of this plant is used as a traditional medicine in Korea and China, and as an ornamental plant in Japan.[1] Constituents, including an iridoid glucoside and a phenylpropanoid glycoside,[2,3] have been isolated from the genus *Campsis*. The chemical components such as essential oils, iridoid, cyclohexyethanoids, triterpenoids and lipids[4-9] from *C. grandiflora* have been studied by some researchers including the authors. We report here the isolation of new cyclohexylethanoids, campsion and campsiketalin, from the flowers of *C. grandiflora*.

Repeated silica gel and ODS column chromatography of the EtOAc fraction from the flower of *C. grandiflora* separated six cyclohexylethanoids **1**-**6**.

Compounds **1**-**4** were identified as halleridone [4-hydroxy-3,4-(epoxyethano)-5-cyclohexenone], 4-hydroxy-5-methoxy-3,4-(epoxyethano)-cyclohexanone, 4-hydroxy-4-(2-hydroxyethyl)-cyclohexanone, and hallerone [4-hydroxy-4-(2-hydroxyethyl)-2,5-cyclohexadienone], respectively.[10,11] The optical rotations of compounds **1** and **2** of nearly zero suggest that they might be racemic mixtures.[12] This is the first report for the isolation of these four compounds from the *C. grandiflora*.

Compound **5** was obtained as colorless syrup. The IR spectrum showed an absorption characteristic of hydroxyl (3354 cm^{-1}), carbonyl (1740 cm^{-1}) and double bond (1668 cm^{-1}). A molecular formula of $C_{16}H_{22}O_{10}$ was determined by HRFABMS ($[M+H]^+$, *m/z* 375.1295, calcd 375.1291 for $C_{16}H_{23}O_{10}$). NMR signals for compound **5** indicated the presence of hallerone moiety (compound **4**), such as δ_C 185.09 (C-1), δ_C 152.98 (C-3 and C-5), δ_C 126.54 (C-2 and C-6), δ_C 67.35 (C-4), δ_C 63.61 (C-2'), δ_C 40.15 (C-1'), δ_H 7.17 (2H, m, H-3 and H-5), δ_H 6.26 (2H, m, H-2 and H-6), δ_H 4.33 (1H, ddd, *J* = 9.6, 4.0, 3.2 Hz, H-2'a), δ_H 4.07 (1H, ddd, *J* = 9.6, 4.0, 3.2 Hz, H-2'b), δ_H 2.21 (1H, br. dd, *J* = 4.0, 4.0 Hz, H-1'a), and δ_H 2.17 (1H, br. dd, *J* = 3.2, 3.2 Hz, H-1'b). The other signals indicated the presence of one 2,3,3-trihydroxypropanoic acid methyl ester [δ_C 171.74 (C-1''), δ_C 72.68 (C-2''), δ_C 103.09 (C-3''), δ_C 51.20 (-OMe), δ_H 4.86 (1H, d, *J* = 5.6 Hz, H-2''), δ_H 5.41(1H, d, *J* = 5.6 Hz, H-3''), δ_H 3.73 (3H, s, -OMe)] and one 2,3-dihydroxypropanoic acid methyl ester [δ_C 171.39 (C-1'''), δ_C 77.06 (C-2'''), δ_C 62.38 (C-3'''), δ_C 51.20 (-OMe), δ_H 5.00 (1H, dd, *J* = 6.4, 4.4 Hz, H-2'''), δ_H 4.31 (1H, dd, *J* = 12.0. 6.4 Hz, H-3'''a), δ_H 4.29 (1H, dd, *J* = 12.0, 4.4 Hz, H-3'''b), δ_H 3.66 (3H, s, -OMe)]. The overlaps of some signals in the ^{1}H-NMR spectrum of compound **5** led to acetylation of the compound to obtain more clear NMR spectrum. ^{1}H and ^{13}C NMR data for the triacetate of compound **5** (compound **5a**) were assigned on the basis of the COSY, HSQC and HMBC spectra of **5a**. The HMBC spectrum[13] of compound **5a** revealed the presence of cross peaks between an oxygenated methine carbon (δ_C 73.88) of the 2,3-dihydroxypropanoic acid moiety (C-2''') and an acetal proton (δ_H 5.41) of the 2,3,3-trihydroxypropanoic acid moiety (H-3''), and between the acetal carbon (δ_C 103.09) of the 2,3,3-trihydroxy-propanoic acid moiety (C-3'') and an oxygenated methylene protons (δ_H 4.33, δ_H 4.07) of the 4-hydroxy-4-(2'-hydroxy-ethyl)-2,5-cyclohexadienone moiety (H-2') (Figure 1). Accordingly, we confirmed the 2,3,3-trihydroxypropanoic acid methyl ester moiety was linked to the 4-hydroxy-4-(2'-hydroxyethyl)-2,5-cyclohexadienone through an ether bond between C-2' and C-3'', and the 2,3-dihydroxypropanoic acid methyl ester moiety was linked to the 2,3,3-trihydroxy-propanoic acid methyl ester through an ether bond between C-3'' and C-2'''. Finally, the chemical structure of compound **5** was determined to be a 4-hydroxy-4-{[(methyl 2,3-di-hydroxypropionate) (2-*O*-3)-methyl 2,3,3-trihydroxypro-pionate] (3-*O*-2)-2-hydroxyethyl}-2,5-cyclohexadienone, a new compound, and was named campsione.

Compound **6** was obtained as colorless oil. The IR spectrum showed an absorption characteristic of hydroxyl (3390 cm^{-1}). A molecular formula of $C_{10}H_{20}O_4$ was deter-mined by HREIMS ($[M]^+$, *m/z* 204.1364, calcd 204.1361 for $C_{10}H_{20}O_4$). The NMR data for compound **6** were similar to those for compound **3**, with the exception of additional resonances due to the presence of one ketal (δ 99.78, C) and two methoxy groups [δ_H 3.13 (3H, s) δ_H 3.08 (3H, s), δ_C 47.64 (CH_3), δ_C 47.54 (CH_3)], instead of a ketone. In the

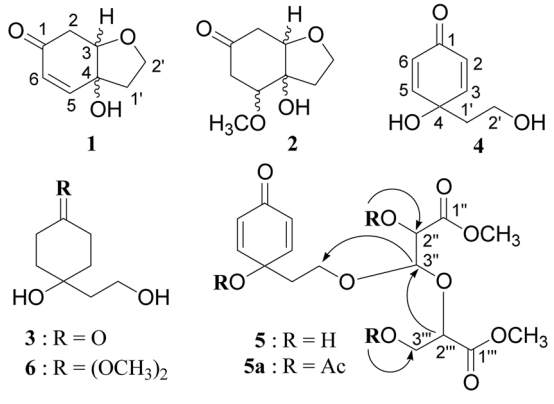

Figure 1. Chemical structures of cyclohexylethnoids from the flower of *Campsis grandiflora*. The arrows indicate the long range correlation from carbon to proton signals in the HMBC spectrum.

^{13}C-NMR spectrum, one oxygenated quaternary (δ_C 71.43), one oxygenated methylene (δ_C 59.17), and five methylenes (δ_C 41.92, 33.76 (x2), 27.81 (x2)] were observed. The ^{1}H-NMR spectrum showed one oxygenated methylene (δ_H 3.80, 2H, t, J = 6.0 Hz), one non-cyclic aliphatic methylene (δ_H 1.65, 2H, t, J = 6.0 Hz), and four cyclic methylenes [(δ_H 1.70-1.66, 4H, m), [(δ_H 1.62-1.58, 2H, m), [(δ_H 1.47-1.39, 2H, m)]. Thus, compound **6** was determined to be a 4-hydroxy-4-(2-hydroxyethyl)-1,1-dimethoxycyclohexane, a new compound, and was named campsiketalin.

Cyclohexylethanoids such as compounds **1** and **4** have been identified in the genera *Millingtonia*[11] and *Halleria*[10,14] of the families Bignoniaceae and Scrophulariaceae, respectively. Chemicals like compounds **2** and **3** have been identified in the genus *Oroxylum*[15] of the family Bignoniaceae.

Experimental Section

General Experimental Procedures. Uncorrected melting points were determined on a Fisher-John apparatus. Optical rotations were measured on a JASCO P-1010 digital polarimeter. EI-MS, CI-MS (butane) and FAB-MS were recorded on a JEOL JMS700. IR spectra were run on a Perkin Elmer Spectrum One FT-IR spectrometer. ^{1}H NMR (400 MHz) and ^{13}C NMR (100 MHz) spectra were taken on a Varian Unity Inova AS 400 FT-NMR spectrometer and tetramethyl silane (TMS) was used for reference peak.

Plant Material. *Campsis grandiflora* K. Schum. was imported from China in March, 2005, and identified by Prof. Dae-Keun Kim, Woosuk University, Jeonju. A voucher specimen (KHU05027) was reserved at the Laboratory of Natural Products Chemistry, KyungHee University, Suwon, Korea.

Extraction and Isolation. The dried and powdered flower of *C. grandiflora* (6 kg) was extracted three times at r.t. with 80% aqueous MeOH (45 L × 3). The extracts were partitioned with water (4 L), EtOAc (4 L × 3) and *n*-BuOH (4 L × 3). The EtOAc extract (114 g) was subjected to silica gel (70-230 mesh, Merck) column (9 × 20 cm) chromatography (c.c.) and the column was eluted with *n*-hexane-$CHCl_3$-MeOH (30:10:1, 3 L) and $CHCl_3$-MeOH (50:1, 30:1, 10:1, 5:1 and 3:1, each 1 L) and monitored by thin layer chromatography (TLC, Kiesel gel 60 F_{254}, Merck) to produce the 15 fractions (CGE1 to CGE15). The seventh fraction (CGE7, 26 g) was loaded onto silica gel column (7 × 14 cm) and eluted with $CHCl_3$-EtOH (35:1, 25:1 and 15:1, each 2 L) to afford 18 fractions (CGE7-1 to CGE7-18). The sixth fraction obtained (CGE7-6, 1.2 g) was subjected to ODS (230-400 mesh, Merck) c.c. (4.5 × 10 cm) and the column was eluted with MeOH-H_2O (2:1, 1.5 L) to produce 22 fractions (CGE7-6-1 to CGE7-6-22). The first fraction (CGE7-6-1, 154 mg) was then loaded onto another silica gel column (3.8 × 11 cm) which was eluted with $CHCl_3$-MeOH (50:1 and 30:1, each 500 mL) to ultimately produce the purified compounds **1** (47 mg) and **2** (21 mg). The tenth fraction of the seventh fraction (CGE7-10, 707 mg) was applied to silica gel column (4.5 × 13 cm) and eluted with $CHCl_3$-EtOH (11:1, 2 L) to afford 12 fractions (CGE7-10-1 to CGE7-10-12). The fraction containing cyclohexylethanoids (CGE7-10-5, 221 mg) was subjected to ODS c.c. (4.5 × 13 cm) and the column was eluted with MeOH-H_2O (1:1, 2:1, each 1 L) to give three fractions (CGE7-10-5-1 to CGE7-10-5-3) and the first fraction (CGE7-10-5-1, 161 mg) was purified by NH_2 silica gel (230-400 mesh, Merck) c.c. (3.8 10 cm) using $CHCl_3$-MeOH (30:1, 1 L) as eluent to yield compounds **3** (60 mg) and **6** (70 mg). The fraction CGE7-4 (858 mg) was subjected to silica gel c.c (4.5 × 11 cm) and the column was eluted with $CHCl_3$-MeOH (8:1, 2 L) to afford five fractions (CGE7-4-1 to CGE7-4-5). The fraction containing cyclohexylethanoid (CGE7-4-4, 56 mg) was purified by silica gel c.c. (2.8 × 10 cm) using $CHCl_3$-EtOH (8:1, 800 mL) as eluent to yield compound **4** (5 mg). The fraction CGE7-14 (818 mg) was purified by ODS c.c (4.5 × 60 cm) using MeOH-H_2O (1:6, 1.5 L) as eluent to yield the purified compound **5** (32 mg).

4-Hydroxy-4-{[(methyl 2,3-dihydroxypropionate) (2-*O*-3)-methyl 2,3,3-trihydroxypropionate] (3-*O*-2)-2-hydroxyethyl}-2,5-cyclohexadienone (5): Colorless syrup ($CHCl_3$-MeOH); $[\alpha]_D^{25}$ +13.2 (*c* 1.2, $CHCl_3$-MeOH); IR ($CHCl_3$) υ_{max} 3354, 2954, 1740, 1668 cm^{-1}; ^{1}H NMR (400 MHz, pyridine-d_5) δ 7.17 (2H, m, H-3 and H-5), 6.26 (2H, m, H-2 and H-6), 5.41 (1H, d, J = 5.6 Hz, H-3"), 5.00 (1H, dd, J = 6.4, 4.4 Hz, H-2'"), 4.86 (1H, d, J = 5.6 Hz, H-2"), 4.33 (1H, ddd, J = 9.6, 4.0, 3.2 Hz, H-2'a), 4.31 (1H, dd, J = 12.0, 6.4 Hz, H-3'"a), 4.29 (1H, dd, J = 12.0, 4.4 Hz, H-3'"b), 4.07 (1H, ddd, J = 9.6, 4.0, 3.2 Hz, H-2'b), 3.73 (3H, s, OMe), 3.66 (3H, s, OMe), 2.21 (1H, br. dd, J = 4.0, 4.0 Hz, H-1'a), 2.17 (1H, br. dd, J = 3.2, 3.2 Hz, H-1'b); ^{13}C NMR (100 MHz, $CDCl_3$) δ 185.09 (C, C-1), 171.74 (C, C-1"), 171.39 (C, C-1'"), 152.98 (x2, CH, C-3 and C-5), 126.54 (x2, CH, C-2 and C-6), 103.09 (CH, C-3"), 77.06 (CH, C-2'"), 72.68 (CH, C-2"), 67.35 (C, C-4), 63.61 (CH_2, C-2'), 62.38 (CH_2, C-3'"), 51.20 (x2, CH_3, OMe), 40.15 (CH_2, C-1'); pos. FABMS *m/z* 375 $[M+H]^+$, 344, 314, 285, 109; HRFABMS *m/z* 375.1295 (Calcd for $C_{16}H_{23}O_{10}$, 375.1291).

4-Hydroxy-4-(2-hydroxyethyl)-1,1-dimethoxycyclohexane (6): Colorless oil ($CHCl_3$-MeOH); IR ($CHCl_3$) υ_{max} 3390,

2947 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 3.80 (2H, t, J = 6.0 Hz, H-2'), 3.13 (3H, s, OMe), 3.08 (3H, s, OMe), 1.70-1.66 (4H, m, H-3 and H-5), 1.65 (2H, t, J = 6.0 Hz, H-1'), 1.62-1.58 (2H, m, H-2a and H-6a), 1.47-1.39 (2H, m, H-2b and H-6b); ^{13}C NMR (100 MHz, $CDCl_3$) δ 99.78 (C, C-1), 71.43 (C, C-4), 59.17 (CH_2, C-2'), 47.64 (CH_3, OMe), 47.54 (CH_3, OMe), 41.92 (CH_2, C-1'), 33.76 (x2, CH_2, C-2 and C-6), 27.81 (x2, CH_2, C-3 and C-5). EIMS *m/z* 204 $[M]^+$, 187, 186, 174, 157, 100, 87, 64, 59; HREIMS *m/z* 204.1364 (Calcd for $C_{10}H_{20}O_4$, 204.1361).

Acetylation of Compound 5. Compound **5** (14 mg) was treated with acetic anhydride (5 mL) in pyridine (5 mL) in an ice bath and stirred at room temperature overnight. The reaction mixture was put into ice water (50 mL) and extracted with EtOAc (50 mL × 2). The organic layer was washed with 5% HCl solution (50 mL), saturated $NaHCO_3$ water (50 mL), and saturated brine (50 mL) followed by dehydration using anhydrous magnesium sulfate and concentration using rotary vacuum evaporator. The concentrate was subjected to the silica gel c.c. (3 × 10 cm) and the column was eluted with $CHCl_3$-MeOH (16:1, 1 L) to afford the triacetate (**5a**, 19 mg).

4-Hydroxy-4-{[(methyl 2,3-dihydroxypropionate) (2-*O*-3)-methyl 2,3,3-trihydroxypropionate] (3-*O*-2)-2-hydroxyethyl}-2,5-cyclohexadienone triacetate (5a): Colorless syrup (*n*-hexane-$CHCl_3$); $[\alpha]_D^{25}$ −1.80 (*c* 0.9, $CHCl_3$); IR ($CHCl_3$) υ_{max} 2962, 1743 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.09 (2H, m, H-3 and H-5), 6.44 (2H, d, J = 10.4 Hz, H-2 and H-6), 5.75(1H, d, J = 4.2 Hz, H-2''), 5.48 (1H, d, J = 4.2 Hz, H-3''), 5.01 (1H, dd, J = 5.6, 3.6 Hz, H-2'''), 4.71 (1H, dd, J = 11.6, 3.6 Hz, H-3'''a), 4.60 (1H, dd, J = 11.6, 5.6 Hz, H-3'''b), 4.14 (1H, ddd, J = 10.0, 4.0, 3.2 Hz, H-2'a), 3.95 (1H, ddd, J = 10.4, 4.0, 3.2 Hz, H-2'b), 3.75 (3H, s, OMe), 3.68 (3H, s, OMe), 2.22 (1H, br. dd, J = 4.0, 4.0 Hz, H-1'a), 2.16 (1H, br. dd, J = 3.2, 3.2 Hz, H-1'b), 2.11 (3H, s, acetyl-Me), 2.00 (3H, s, acetyl-Me), 1.97 (3H, s, acetyl-Me); ^{13}C NMR (100 MHz, $CDCl_3$) δ 184.49 (C, C-1), 169.73 (C, 3'''-$OCOCH_3$), 169.39 (x2, C, 2'' -$OCOCH_3$ and C-1'''), 168.66 (C, 4-$OCOCH_3$), 167.07 (C, C-1''), 148.39 (x2, CH, C-3 and C-5), 128.19 (x2, CH, C-2 and C-6), 100.94 (CH, C-3''), 75.01 (C, C-4), 73.88 (CH, C-2'''), 73.06 (CH, C-2''), 63.99 (CH_2, C-2'), 63.35 (CH_2, C-3'''), 51.95 (CH_3, OMe), 51.79 (CH_3, OMe), 39.18 (CH_2, C-1'), 20.50, 20.08, 19.92 (CH_3, Ac-Me3).

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