Natural Product Sciences
26(2) : 151-157 (2020)
https://doi.org/10.20307/nps.2020.26.2.151

Phytochemical Constituents of *Phyllanthus urinaria*

Joon Min Cha[1], Jong Eel Park[1], Sang Un Choi[2], and Kang Ro Lee[1,*]

[1]*Natural Products Laboratory, School of Pharmacy, Sungkyunkwan University, 2066 Seobu-Ro, Jangan-gu, Suwon, Gyeonggi-do 440-746, Republic of Korea*
[2]*Korea Research Institute of Chemical Technology, Daejeon 34114, Korea*

Abstract – Extensive column chromatography separation of the MeOH extract from the aerial parts of *Phyllanthus urinaria* afforded seventeen compounds (**1 - 17**). The structures of the compounds were elucidated by physicochemical and spectroscopic methods to be 5′-β-D-glucopyranosyloxyjasmonic butyl ester (**1**), (+)-cucurbic acid (**2**), dendranthemoside B (**3**), boscialin 4′-*O*-β-D-glucoside (**4**), 4,5-dihydroblumenol A (**5**), (6*R*,9*R*)-megastigman-4-ene-9,13-diol (**6**), (3*S*,5*R*,6*S*,9*R*)-3,6-dihydroxy-5,6-dihydro-β-ionol (**7**), (6*S*,9*R*)-roseoside (**8**), mallophenol B (**9**), icariside B_5 (**10**), corchoinoside B (**11**), canangaionoside (**12**), 5,6-epoxy-3-hydroxy-7-megastigmen-9-one (**13**), icariside B_2 (**14**), (7*E*)-2β,3β-dihydroxy-megastigm-7-en-9-one (**15**), betulalbuside A (**16**), and loliolide (**17**). The compounds **1**, and **3 - 16** were isolated for the first time from this plant. The absolute stereochemistry of compound **1** was newly determined. The isolated compounds were tested for cytotoxic activity against four human tumor cell lines *in vitro* using a Sulforhodamin B bioassay, but all the compounds showed weak cytotoxic activities.
Keywords – *Phyllanthus urinaria*, Euphorbiaceae, Cytotoxicity

Introduction

Phyllanthus urinaria L. (Euphorbiaceae) is an annual plant widely distributed throughout East Asia.[1] It has long been used as a traditional medicine for treating enteritis, diarrhea, and dropsy.[2] Previous phytochemical investigation of this plant reported the isolation of tannins, flavonoids, and phenolic compounds.[3-5] In continuation of our search for biologically active compounds from Korean medicinal plants, we investigated the constituents of the aerial parts of *P. urinaria*. Column chromatographic purification of MeOH extract led to the isolation of two jasmonic acid derivatives (**1 - 2**), together with fifteen megastigmane derivatives (**3 - 17**) (Fig. 1). The structures of the compounds were elucidated by physicochemical and spectroscopic methods including 1D NMR (^{1}H and ^{13}C NMR) and MS data. The isolated compounds were tested for cytotoxicity against four human cancer cell lines (A549, SK-OV-3, SK-MEL-2, and HCT15 cells) *in vitro* using a SRB bioassay.

*Author for correspondence
Kang Ro Lee, Natural Products Laboratory, School of Pharmacy, Sungkyunkwan University, 2066 Seobu-Ro, Jangan-ku, Suwon, Gyeonggi-do 440-746, Republic of Korea
Tel: +82-31-290-7710; E-mail: krlee@skku.ac.kr

Experimental

General experimental procedures – Optical rotations were measured on a Jasco P-1020 polarimeter in MeOH. IR spectra were recorded on a Bruker IFS-66/S FT-IR spectrometer. Circular dichroism (CD) spectra were recorded with a JASCO J-1500 CD spectrometer. HR-MS spectra were obtained on a JEOL JMS700 mass spectrometer. NMR spectra were recorded on a Bruker AVANCE III 700 NMR spectrometer at 700 MHz (^{1}H) and 175 MHz (^{13}C). Preparative high performance liquid chromatography (HPLC) was conducted using a Gilson 306 pump with Shodex refractive index detector and YMC Triart C_{18} column (250 × 10.00 mm). Low-pressure liquid chromatography (LPLC) was carried out on a Merck LiChroprep Lobar®-A RP-C_{18} and Si 60 column (240 × 10 mm) with an FMI QSY-0 pump (ISCO). Silica gel 60 (Merck, 70 - 230 and 230 - 400 mesh) and RP-C_{18} silica gel (Merck, 230 - 400 mesh) were used for column chromatography. The packing material for molecular sieve column chromatography was Sephadex LH-20 (Pharmacia Co.). TLC was performed using Merck precoated Silica gel F_{254} plates and RP-18 F_{254}s plates. Spots were detected by thin layer chromatography (TLC) under UV light or by heating after spraying with anisaldehyde–sulfuric acid.

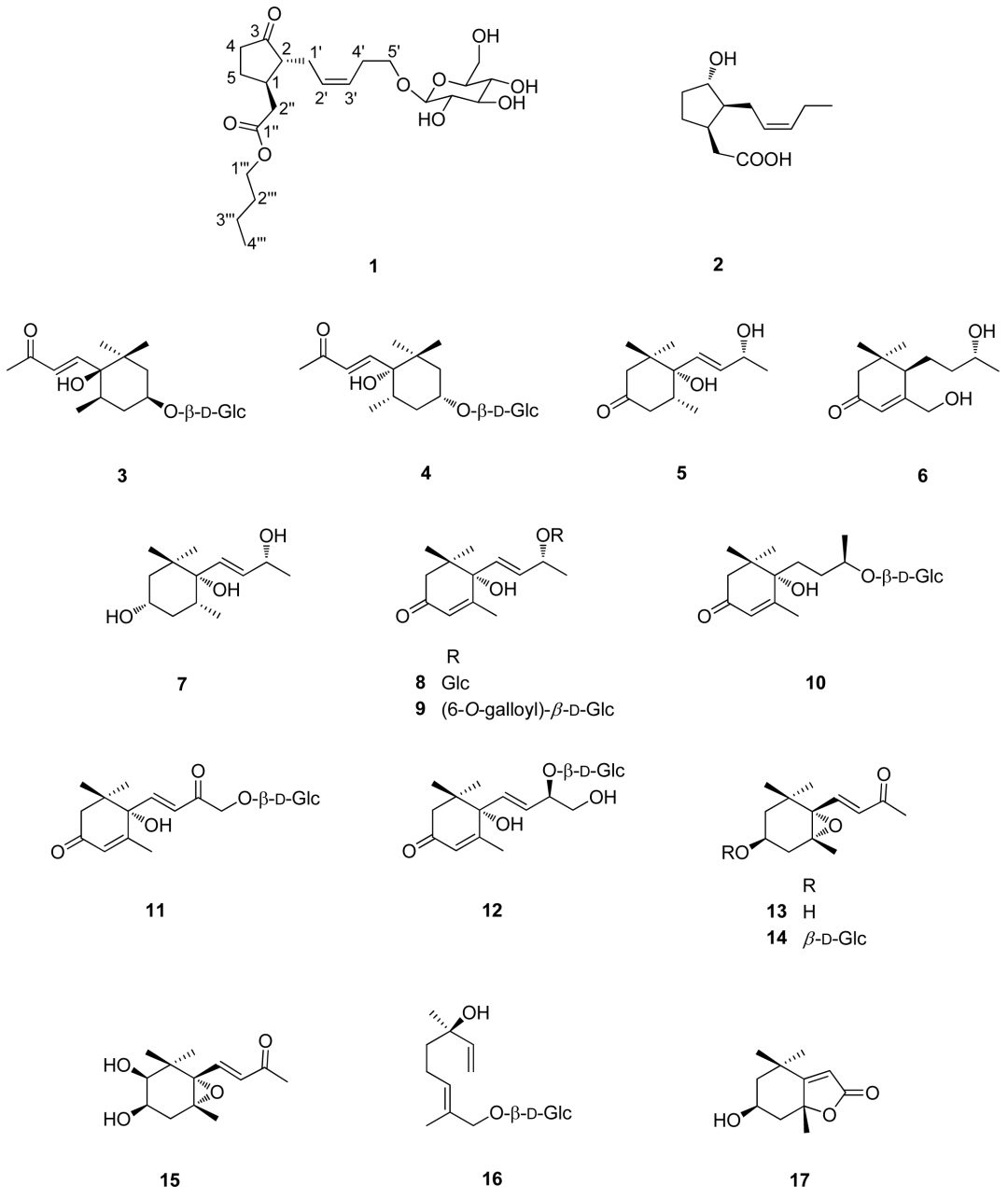

Fig. 1. The structures of compounds **1** - **17** isolated from *P. urinaria.*

Plant materials – The dried aerial parts of *P. urinaria* (13.5 kg) were collected at Goesan-gun in Chungcheongbuk-Do province in November 2013 and identified by one of the authors (K.R.L.). A voucher specimen (SKKU-NPL-1405) of the plant was deposited at the School of Pharmacy at Sungkyunkwan University, Suwon, Korea.

Extraction and isolation – The aerial parts of *P. urinaria* (13.5 kg) were extracted with 80% MeOH at room temperature and filtered. The filtrate was concentrated under reduced pressure to obtain a crude extract (320 g), which was suspended in water and solvent-partitioned successively to yield *n*-hexane (35 g), $CHCl_3$ (10 g), EtOAc (47 g), and *n*-BuOH (77 g). The $CHCl_3$ soluble fraction (9 g) was separated over a silica gel column ($CHCl_3$: MeOH = 50 : 1) to yield six fractions (C1-C6). Fraction C5 (500 mg) was chromatographed on an RP-C_{18} silica gel column (40% MeOH) and purified with RP-C_{18} semi-prep. HPLC (37% MeCN) to afford compounds **5** (14 mg, R_t = 21.0 min), **7** (5 mg, R_t = 18.3 min), **6** (6 mg, R_t = 23.0 min), and **13** (7 mg, R_t = 20.1

min). Fraction C6 (2 g) was chromatographed on an RP-C_{18} silica gel column (45% MeOH) to give eight subfractions (C61-C68). Subfraction C67 (310 mg) was purified with RP-C_{18} semi-prep. HPLC (23% MeCN) to obtain compounds **15** (4 mg, R_t = 21.0 min) and **17** (12 mg, R_t = 32.0 min). The EtOAc layer (15 g) was chromatographed on a silica gel column ($CHCl_3$: MeOH : H_2O = 5 : 1 : 0.1) to yield five fractions (E1-E5). Fraction E3 (900 mg) was purified with RP-C_{18} semi-prep. HPLC (37% MeOH) to afford compounds **3** (16 mg, R_t = 21.5 min), **4** (6 mg, R_t = 23.0 min), and **10** (2 mg, R_t = 28.0 min). Fraction E4 (2 g) was separated over an RP-C_{18} silica gel column (30% MeOH) to give five subfractions (E41-E45). Subfraction E44 (500 mg) was purified with a Sephadex LH-20 column (100% MeOH) and RP-C_{18} semi-prep. HPLC (50% MeOH) to afford compounds **8** (7 mg, R_t = 18.0 min) and **14** (4 mg, R_t = 26.3 min). The *n*-BuOH soluble fraction (20 g) was subjected to a silica gel column with a solvent system of $CHCl_3$: MeOH : H_2O = 4 : 1 : 0.1 to give nine fractions (A1-A9). Fraction A1 (3 g) was chromatographed on an RP-C_{18} silica gel column (30% MeOH) to give nine subfractions (A11-A19). Subfraction A12 (635 mg) was purified by an RP-C_{18} semi-prep. HPLC (32% MeOH) to afford compound **4** (5 mg, R_t = 35.0 min). Fraction A2 (7 g) was fractionated over an RP-C_{18} silica gel column (30% MeOH) to give six subfractions (A21-A26). Subfration A26 (980 mg) was purified by RP-C_{18} semi-prep. HPLC (23% MeOH) to afford compounds **1** (13 mg, R_t = 22.0 min) and **9** (5 mg, R_t = 37.0 min). Fraction A3 (7 g) was subjeted to an RP-C_{18} silica gel column (30% MeOH) to give nine subfractions (A31-A39). Subfraction A33 (210 mg) was purified with a Si 60 silica Lobar®-A ($CHCl_3$: MeOH : H_2O = 5 : 1 : 0.1) and RP-C_{18} semi-prep. HPLC (21% MeOH) to afford compounds **11** (5 mg, R_t = 21.2 min) and **12** (5 mg, R_t = 25.0 min). Compound **16** (4 mg, R_t = 31.0 min) was obtained by purification of subfraction A36 (1 g) using an RP-C_{18} semi-prep. HPLC (32% MeOH).

5′-β-D-Glucopyranosyloxyjasmonic butyl ester (1) – Colorless gum; $[\alpha]_D^{25}$: −5.2 (*c* 0.18, MeOH); IR ν_{max} (MeOH): 3350, 2941, 1748, 1078, 1019 cm^{-1}; CD (MeOH) λ_{max} ($\Delta\varepsilon$) 297 (−18.41); ^{1}H NMR (CD_3OD, 700 MHz): δ 5.54 (1H, dtt, *J* = 10.9, 7.5, 1.5 Hz, H-3′), 5.43 (1H, dtt, *J* = 10.9, 7.3, 1.6 Hz, H-2′), 4.30 (1H, d, *J* = 7.8 Hz, Glc-1), 4.12 (2H, dt, *J* = 6.6, 1.2 Hz, H-1‴), 3.89 (1H, m, H-5′b), 3.87 (1H, m, Glc-6b), 3.69 (1H, m, Glc-6a), 3.59 (1H, dt, *J* = 9.5, 7.2 Hz, H-5′a), 3.28 (2H, m, Glc-3 and 5), 3.21 (1H, m, Glc-4), 3.19 (1H, dd, *J* = 9.1, 7.9 Hz, Glc-2), 2.73 (1H, dd, *J* = 14.9, 4.3 Hz, H-2″a), 2.40 (2H, m, H-4′), 2.39 (1H, m, H-2″b), 2.32 (1H, m, H-4b), 2.30 (1H, m, H-1), 2.27 (2H, m, H-1′), 2.18 (1H, m, H-5b), 2.12 (1H, m, H-4a), 2.01 (1H, m, H-2), 1.65 (2H, dt, *J* = 14.6, 6.6 Hz, H-2‴), 1.55 (1H, m, H-5a), 1.43 (2H, m, H-3‴), 0.98 (3H, t, *J* = 7.4 Hz, CH_3-4‴); ^{13}C NMR (CD_3OD, 175 MHz): δ 220.8 (C-3), 174.3 (C-1″), 129.1 (C-3′), 128.9 (C-2′), 104.5 (Glc-1), 78.3 (Glc-3), 78.1 (Glc-5), 75.2 (Glc-2), 71.8 (Glc-4), 70.4 (C-5′), 65.6 (C-1‴), 62.9 (Glc-6), 55.2 (C-2), 40.0 (C-2″), 39.4 (C-1), 38.7 (C-4), 29.2 (C-2‴), 29.1 (C-4′), 28.3 (C-5), 26.6 (C-1′), 20.4 (C-3‴), 14.2 (C-4‴); ESI-MS *m/z*: 445.2 $[M+H]^+$.

(+)-Cucurbic acid (2) – Colorless gum; $[\alpha]_D^{25}$: +26.3 (*c* 0.25, MeOH); ^{1}H NMR (CD_3OD, 700 MHz): δ 5.37 (1H, dt, *J* = 11.1, 7.0 Hz, H-3′), 5.24 (1H, dt, *J* = 11.1, 6.8 Hz, H-2′), 4.05 (1H, dt, *J* = 7.1, 3.2 H-3), 2.61 (1H, m, H-1), 2.39 (1H, dd, *J* = 15.3, 7.0 Hz, H-2″b), 2.24 (1H, dd, *J* = 15.3, 9.0 Hz, H-2″a), 2.10 (1H, m, H-2), 2.07 (1H, m, H-4b), 2.00 (2H, m, H-4′), 1.94 (1H, m, H-1′b), 1.82 (1H, ddd, *J* = 10.3, 6.8, 3.7 Hz, H-5b), 1.70 (1H, ddd, *J* = 15.1, 10.4, 7.2 Hz, H-1′a), 1.57 (1H, m, H-4a), 1.28 (1H, dt, *J* = 10.3, 3.2 Hz, H-5a), 0.97 (3H, t, *J* = 7.2 Hz, CH_3-5′); ^{13}C NMR (CD_3OD, 175 MHz): δ 177.4 (C-1″), 134.1 (C-3′), 128.4 (C-2′), 77.5 (C-3), 48.7 (C-2), 36.7 (C-1), 35.4 (C-2″), 31.8 (C-4), 29.3 (C-5), 26.0 (C-1′), 21.3 (C-4′), 14.1 (C-5′); ESI-MS *m/z*: 213.1 $[M+H]^+$.

Dendranthemoside B (3) – Colorless gum; $[\alpha]_D^{25}$: −49.2 (*c* 0.25, MeOH); ^{1}H NMR (CD_3OD, 700 MHz): δ 6.84 (1H, d, *J* = 16.0 Hz, H-7), 6.32 (1H, d, *J* = 16.0 Hz, H-8), 4.31 (1H, d, *J* = 8.0 Hz, Glc-1), 3.92 (1H, m, H-3), 3.86 (1H, dd, *J* = 11.5, 3.2 Hz, Glc-6b), 3.68 (1H, dd, *J* = 11.5, 6.5 Hz, Glc-6a), 3.28 (1H, m, Glc-3), 3.26 (1H, m, Glc-5), 3.17 (1H, m, Glc-4), 3.22 (1H, dd, *J* = 9.1, 7.9 Hz, Glc-2), 2.29 (3H, s, CH_3-10), 2.09 (1H, m, H-5), 1.83 (1H, d, *J* = 11.8 Hz, H-4b), 1.70 (1H, m, H-2a), 1.57 (1H, dt, *J* = 12.3, 5.6 Hz, H-2b), 1.53 (1H, d, *J* = 11.8 Hz, H-4a), 1.53 (1H, d, *J* = 11.8 Hz, H-4a), 1.17 (3H, s, CH_3-11), 0.86 (3H, s, CH_3-12), 0.80 (3H, d, *J* = 7.3 Hz, CH_3-13); ^{13}C NMR (CD_3OD, 175 MHz): δ 199.9 (C-9), 153.2 (C-7), 128.9 (C-8), 104.2 (Glc-1), 79.2 (C-6), 78.3 (Glc-3), 78.0 (Glc-5), 75.6 (C-3), 75.1 (Glc-2), 71.8 (Glc-4), 62.7 (Glc-6), 42.6 (C-2), 40.0 (C-1), 37.2 (C-4), 35.1 (C-5), 26.3 (C-10), 25.4 (C-12), 25.1 (C-11), 15.8 (C-13); ESI-MS *m/z*: 389.2 $[M+H]^+$.

Boscialin 4′-*O*-β-D-glucoside (4) – Colorless gum; $[\alpha]_D^{25}$: −27.1 (*c* 0.25, MeOH); ^{1}H NMR (CD_3OD, 700 MHz): δ 6.87 (1H, d, *J* = 16.0 Hz, H-7), 6.35 (1H, d, *J* = 16.0 Hz, H-8), 4.35 (1H, d, *J* = 8.0 Hz, Glc-1), 4.01 (1H, m, H-3), 3.85 (1H, dd, *J* = 11.3, 3.2 Hz, Glc-6b), 3.63 (1H, dd, *J* = 11.3, 6.2 Hz, Glc-6a), 3.28 (1H, m, Glc-3), 3.25 (1H, m, Glc-5), 3.12 (1H, m, Glc-4), 3.22 (1H,

dd, J = 9.3, 7.8 Hz, Glc-2), 2.29 (3H, s, CH_3-10), 2.10 (1H, m, H-5), 1.83 (1H, m, H-4b), 1.71 (1H, m, H-2b), 1.57 (1H, m, H-2a), 1.53 (1H, m, H-4a), 1.02 (3H, s, CH_3-11), 0.86 (3H, s, CH_3-12), 0.82 (3H, d, J = 7.0 Hz, CH_3-13); ^{13}C NMR (CD_3OD, 175 MHz): δ 199.6 (C-9), 1530 (C-7), 130.1 (C-8), 102.1 (Glc-1), 78.1 (C-6), 77.1 (Glc-3), 76.1 (Glc-5), 74.1 (C-3), 74.1 (Glc-2), 71.1 (Glc-4), 62.5 (Glc-6), 41.2 (C-2), 40.1 (C-1), 37.0 (C-4), 33.8 (C-5), 26.3 (C-10), 24.9 (C-12), 24.1 (C-11), 15.3 (C-13); ESI-MS *m/z*: 389.2 $[M+H]^+$.

4,5-Dihydroblumenol A (5) – Colorless gum; $[\alpha]_D^{25}$: −20.1 (*c* 0.08, MeOH); ^{1}H NMR (CD_3OD, 700 MHz): δ 5.78 (1H, dd, J = 15.8, 6.1 Hz, H-8), 5.62 (1H, d, J = 15.8 Hz, H-7), 4.28 (1H, m, H-9), 2.87 (1H, d, J = 13.1 Hz, H-2b), 2.38 (1H, d, J = 13.6 Hz, H-4b), 2.36 (1H, m, H-5), 2.21 (1H, dd, J = 13.6, 2.4, H-4a), 1.85 (1H, dd, J = 13.1, 2.8 Hz, H-2a), 1.27 (3H, d, J = 6.4 Hz, CH_3-10), 0.98 (3H, s, CH_3-11), 0.91 (3H, s, CH_3-12), 0.90 (3H, d, J = 6.6 Hz, CH_3-13); ^{13}C NMR (CD_3OD, 175 MHz): δ 214.5 (C-3), 134.1 (C-8), 134.0 (C-7), 78.3 (C-6), 69.1 (C-9), 51.8 (C-2), 46.2 (C-4), 43.5 (C-1), 38.1 (C-5), 26.2 (C-11), 25.2 (C-12), 24.8 (C-10), 16.1 (C-13); ESI-MS *m/z*: 227.1 $[M+H]^+$.

(6*R*,9*R*)-Megastigman-4-ene-9,13-diol (6) – Colorless gum; $[\alpha]_D^{25}$: +15.5 (*c* 0.20, MeOH); ^{1}H NMR (CD_3OD, 700 MHz): δ 7.12 (1H, d, J = 16.0 Hz, H-7), 6.20 (1H, d, J = 16.0 Hz, H-8), 3.82 (1H, dddd, J = 11.8, 9.7, 7.4, 4.3 Hz, H-3), 2.17 (3H, s, CH_3-10), 2.12 (1H, ddd, J = 15.2, 7.3, 2.1 Hz, H-4b), 1.69 (1H, dd, J = 15.2, 10.1 Hz, H-4a), 1.42 (1H, dd, J = 12.2, 12.1 Hz, H-2b), 1.28 (1H, ddd, J = 12.2, 4.2, 2.1 Hz, H-2a), 1.22 (3H, s, CH_3-11) 1.15 (3H, s, CH_3-13), 0.92 (3H, s, CH_3-12); ^{13}C NMR (CD_3OD, 175 MHz): δ 171.1 (C-5), 142.2 (C-7), 135.1 (C-8), 79.4 (C-6), 78.3 (C-9), 64.1 (C-3), 44.1 (C-2), 39.7 (C-4), 35.7 (C-1), 27.5 (C-12), 26.9 (C-10), 25.1 (C-11), 20.4 (C-13); ESI-MS *m/z*: 227.1 $[M+H]^+$.

(3*S*,5*R*,6*S*,9*R*)-3,6-Dihydroxy-5,6-dihydro-β-ionol (7) – Colorless gum; $[\alpha]_D^{25}$: −40.5 (*c* 0.25, MeOH); ^{1}H NMR (CD_3OD, 700 MHz): δ 5.62 (1H, dd, J = 15.2, 6.3, H-8), 5.52 (1H, d, J = 15.2, H-7), 4.31 (1H, t, J = 6.3, H-9), 3.72 (1H, m, H-3), 1.87 (1H, m, H-5), 1.72 (1H, d, J = 11.8, H-4b), 1.59 (1H, t, J = 12.1, H-2a), 1.52 (1H, m, H-2b), 1.30 (1H, d, J = 11.8, H-4a), 1.29 (3H, d, J = 6.3, CH_3-10), 0.96 (3H, s, CH_3-11), 0.82 (3H, s, CH_3-12), 0.77 (3H, d, J = 6.3, CH_3-13); ^{13}C NMR (CD_3OD, 175 MHz): δ 134.4 (C-8), 132.8 (C-7), 76.9 (C-6), 68.4 (C-9), 66.6 (C-3), 45.1 (C-2), 39.4 (C-4), 39.1 (C-1), 34.0 (C-5), 23.8 (C-10), 25.1 (C-11), 24.5 (C-12), 15.8 (C-13); ESI-MS *m/z*: 229.2 $[M+H]^+$.

(6*S*,9*R*)-Roseoside (8) – Colorless gum; $[\alpha]_D^{25}$: +85.2 (*c* 0.15, MeOH); ^{1}H NMR (CD_3OD, 700 MHz): δ 5.83 (1H, m, H-7), 5.80 (1H, m, H-4), 5.78 (1H, m, H-7), 4.32 (1H, m, H-9), 4.29 (1H, d, J = 7.3 Hz, Glc-1), 3.36 (1H, dd, J = 11.3, 3.2 Hz, Glc-6b), 3.28 (1H, dd, J = 11.3, 6.2 Hz, Glc-6a), 3.20-3.13 (3H, m, Glc-3, 4, and 5), 3.10 (1H, m, Glc-2), 2.39 (1H, d, J = 16.4 Hz, H-2a), 2.07 (1H, d, J = 16.4 Hz, H-2b), 1.90 (3H, d, J = 1.5 Hz, CH_3-13), 1.20 (3H, d, J = 6.4 Hz, CH_3-10), 0.98 (3H, s, CH_3-11), 0.97 (3H, s, CH_3-12); ^{13}C NMR (CD_3OD, 125 MHz): δ 200.5 (C-3), 165.1 (C-5), 132.1 (C-8), 130.8 (C-7), 127.1 (C-4), 102.5 (Glc-1), 80.4 (C-6), 78.2 (Glc-3), 78.0 (Glc-5), 77.2 (C-9), 75.4 (Glc-2), 72.1 (Glc-4), 62.6 (Glc-6), 50.1 (C-2), 42.8 (C-1), 25.3 (C-12), 23.4 (C-11), 20.9 (C-10), 19.2 (C-13); ESI-MS *m/z*: 387.2 $[M+H]^+$.

Mallophenol B (9) – Colorless gum; $[\alpha]_D^{25}$: +87.5 (*c* 0.25, MeOH); ^{1}H NMR (CD_3OD, 700 MHz): δ 7.09 (2H, s, H-2′ and 6′) 5.82 (1H, s, H-4), 5.80 (2H, m, H-7 and 8), 4.51 (1H, dd, J = 12.0, 2.1 Hz, Glc-6b), 4.42 (1H, d, J = 8.1, Glc-1), 4.37 (1H, m, H-9), 4.36 (1H, m, Glc-6a), 3.41 (1H, m, Glc-3), 3.38 (1H, m, Glc-4), 3.26 (1H, m, Glc-5), 3.14 (1H, m, Glc-2), 2.37 (1H, d, J = 17.1, H-2b), 2.13 (1H, d, J = 17.1, H-2a), 1.87 (3H, d, J = 1.3, CH_3-13), 1.25 (3H, d, J = 6.3, CH_3-10), 1.01 (3H, s, CH_3-11), 0.95 (3H, s, CH_3-12); ^{13}C NMR (CD_3OD, 175 MHz): δ 201.1 (C-3), 168.7 (C-7′), 168.0 (C-5), 145.8 (C-3′, 5′), 140.1 (C-4′), 135.1 (C-8), 130.8 (C-7), 126.8 (C-4), 120.3 (C-1′), 110.4 (C-2′, 6′), 102.7 (Glc-1), 79.8 (C-6), 77.8 (Glc-3), 77.1 (C-9), 75.1 (Glc-2), 74.8 (Glc-5), 71.5 (Glc-4), 64.5 (Glc-6), 51.0 (C-2), 41.4 (C-1), 25.0 (C-12), 23.1 (C-11), 21.5 (C-10), 19.3 (C-13); ESI-MS *m/z*: 539.2 $[M+H]^+$.

Icariside B_5 (10) – Colorless gum; $[\alpha]_D^{25}$: +12.1 (*c* 0.20, MeOH); ^{1}H NMR (CD_3OD, 700 MHz): δ 5.82 (1H, s, H-4), 4.30 (1H, d, J = 7.8, Glc-1), 3.85 (1H, dd, J = 12.1, 2.5 Hz, Glc-6b), 3.79 (1H, d, J = 7.4, H-9), 3.66 (1H, dd, J = 12.1, 6.3 Hz, Glc-6a), 3.34 (1H, m, Glc-3), 3.27 (1H, m, Glc-4), 3.26 (1H, m, Glc-5), 3.11 (1H, m, Glc-2), 2.60 (1H, d, J = 17.6, H-2b), 2.13 (1H, d, J = 17.6, H-2a), 2.11 (1H, dd, J = 13.0, 5.1, H-7a), 2.08 (3H, d, J = 1.3, CH_3-13), 1.84 (1H, dd, J = 13.0, 3.6, H-7b), 1.76 (1H, m, H-8a), 1.48 (1H, dt, J = 17.1, 6.6, H-8b), 1.21 (3H, d, J = 6.3, CH_3-10), 1.12 (3H, s, CH_3-12), 1.03 (3H, s, CH_3-11); ^{13}C NMR (CD_3OD, 175 MHz): δ 201.1 (C-3), 170.8 (C-5), 126.9 (C-4), 103.8 (Glc-1), 79.3 (C-6), 78.1 (C-9), 78.0 (Glc-3), 77.6 (Glc-5), 75.1 (Glc-2), 71.4 (Glc-4), 62.4 (Glc-6), 50.8 (C-2), 43.0 (C-1), 34.1 (C-7), 32.6 (C-8), 24.1 (C-12), 24.0 (C-11), 22.3 (C-10), 21.7 (C-13); ESI-MS *m/z*: 389.2 $[M+H]^+$.

Corchoinoside B (11) – Colorless gum; $[\alpha]_D^{25}$: +98.5 (*c* 0.20, MeOH); ^{1}H NMR (CD_3OD, 700 MHz): δ 7.07

(1H, d, J = 14.8, H-7), 6.52 (1H, d, J = 14.8, H-8), 5.89 (1H, d, J = 1.4, H-4), 4.68 (2H, m, H-10), 4.31 (1H, d, J = 7.8, Glc-1), 3.85 (1H, dd, J = 12.1, 3.1 Hz, Glc-6b), 3.62 (1H, dd, J = 12.1, 6.1 Hz, Glc-6a), 3.31 (1H, m, Glc-3), 3.25 (1H, m, Glc-4), 3.23 (1H, m, Glc-5), 3.21 (1H, m, Glc-2), 2.62 (1H, d, J = 17.3, H-2b), 2.21 (1H, d, J = 17.3, H-2a), 1.91 (3H, d, J = 1.3, CH_3-11), 1.07 (3H, s, CH_3-12), 1.03 (3H, s, CH_3-13); ^{13}C NMR (CD_3OD, 175 MHz): δ 200.1 (C-3), 198.1 (C-9), 165.1 (C-5), 147.1 (C-7), 127.9 (C-4), 127.3 (C-8), 104.1 (Glc-1), 79.8 (C-6), 78.0 (Glc-5), 77.6 (Glc-3), 75.3 (Glc-2), 74.8 (C-10), 71.1 (Glc-4), 62.7 (Glc-6), 50.1 (C-2), 43.1 (C-1), 24.6 (C-13), 23.1 (C-12), 20.7 (C-11); ESI-MS *m*/*z*: 401.1 $[M+H]^+$.

Canangaionoside (12) – Colorless gum; $[\alpha]_D^{25}$: +50.5 (*c* 0.25, MeOH); ^{1}H NMR (CD_3OD, 700 MHz): δ 6.12 (1H, dd, J = 16.1, 1.4 Hz, H-7), 5.86 (1H, m, H-4), 5.81 (1H, dd, J = 16.1, 7.2 Hz, H-8), 4.52 (1H, m, H-9), 4.26 (1H, d, J = 7.6 Hz, Glc-1), 3.79 (1H, dd, J = 12.3, 2.5 Hz, Glc-6a), 3.59 (1H, dd, J = 12.3, 6.2 Hz, Glc-6b), 3.61 (1H, dd, J = 13.1, 4.2 Hz, H-10a), 3.56 (1H, dd, J = 13.1, 6.5 Hz, H-10b), 3.30 (3H, m, Glc-2, 3, and 4), 3.17 (1H, m, Glc-5), 2.61 (1H, d, J = 17.1 Hz, H-2a), 2.15 (1H, d, J = 17.1 Hz, H-2b), 1.92 (3H, d, J = 1.6 Hz, CH_3-13), 1.04 (3H, s, CH_3-11), 1.01 (3H, s, CH_3-12); ^{13}C NMR (CD_3OD, 175 MHz): δ 201.0 (C-3), 166.7 (C-5), 134.1 (C-7), 129.3 (C-8), 128.1 (C-4), 102.1 (Glc-1), 80.8 (C-6), 80.1 (C-9), 78.1 (Glc-3), 78.0 (Glc-5), 75.1 (Glc-2), 71.1 (Glc-4), 66.8 (C-10), 62.9 (Glc-6), 50.7 (C-2), 42.1 (C-1), 24.6 (C-12), 23.7 (C-11), 19.4 (C-13); ESI-MS *m*/*z*: 408.2 $[M+H]^+$.

5,6-Epoxy-3-hydroxy-7-megastigmen-9-one (13) – Colorless gum; $[\alpha]_D^{25}$: −52.1 (*c* 0.20, MeOH); ^{1}H NMR (CD_3OD, 700 MHz): δ 7.21 (1H, d, J = 15.6 Hz, H-7), 6.18 (1H, d, J = 15.6 Hz, H-8), 3.75 (1H, m, H-3), 2.31 (1H, dd, J = 8.7, 5.1 Hz, H-4a), 2.28 (3H, s, CH_3-10), 1.71 (1H, dd, J = 13.7, 8.7 Hz, H-4b), 1.64 (1H, dd, J = 13.1, 4,1 Hz, H-2a), 1.32 (1H, dd, J = 13.1, 11.0 Hz, H-2b), 1.24 (3H, s, CH_3-13), 1.20 (3H, s, CH_3-11), 0.98 (3H, s, CH_3-12); ^{13}C NMR (CD_3OD, 175 MHz): δ 200.1 (C-9), 143.7 (C-7), 133.1 (C-8), 69.5 (C-6), 66.8 (C-5), 62.8 (C-3), 46.7 (C-4), 40.1 (C-2), 35.2 (C-1), 30.1 (C-11), 28.4 (C-10), 22.8 (C-12), 19.1 (C-13); ESI-MS *m*/*z*: 225.1 $[M+H]^+$.

Icariside B_2 (14) – Colorless gum; $[\alpha]_D^{25}$: −63.5 (*c* 0.05, MeOH); ^{1}H NMR (CD_3OD, 700 MHz): δ 7.21 (1H, d, J = 16.0 Hz, H-7), 6.14 (1H, d, J = 16.0 Hz, H-8), 4.37 (1H, d, J = 8.0 Hz, Glc-1), 3.81 (1H, dd, J = 13.2, 2.5 Hz, Glc-6b), 3.76 (1H, m, H-3), 3.61 (1H, dd, J = 13.2, 6.2 Hz, Glc-6a), 3.28 (1H, m, Glc-3), 3.24 (1H, m, Glc-4), 3.21 (1H, m, Glc-5), 3.15 (1H, m, Glc-2), 2.27 (3H, s, CH_3-10), 1.80 (1H, m, H-2b), 1.68 (1H, m, H-4a), 1.41 (1H, m, H-2a), 1.20 (3H, s, CH_3-13), 1.15 (3H, s, CH_3-12), 0.98 (3H, s, CH_3-11); ^{13}C NMR (CD_3OD, 175 MHz): δ 199.2 (C-9) 143.7 (C-7), 133.1 (C-8), 102.1 (Glc-1), 73.1 (Glc-3), 76.5 (Glc-5), 74.1 (Glc-2), 72.1 (C-3), 70.3 (Glc-4), 69.6 (C-6), 66.8 (C-5), 61.3 (Glc-6), 43.8 (C-2), 37.2 (C-4), 34.8 (C-1), 27.9 (C-11), 26.1 (C-10), 24.3 (C-12), 19.1 (C-13); ESI-MS *m*/*z*: 387.1 $[M+H]^+$.

(7*E*)-2β,3β-Dihydroxy-megastigm-7-en-9-one (15) – Colorless gum: $[\alpha]_D^{25}$: −87.5 (*c* 0.25, MeOH); ^{1}H NMR (CD_3OD, 700 MHz): δ 7.02 (1H, d, J = 16.1 Hz, H-7), 6.48 (1H, d, J = 16.1 Hz, H-8), 4.01 (1H, m, H-3), 3.42 (1H, m, H-2), 2.24 (3H, s, CH_3-10), 1.22 (3H, s, CH_3-12) 0.96 (3H, s, CH_3-10); ^{13}C NMR (CD_3OD, 175 MHz): δ 198.1 (C-9), 142.7 (C-7), 133.5 (C-8), 76.1 (C-2), 72.8 (C-3), 69.5 (C-6), 66.8 (C-5), 39.2 (C-1), 33.7 (C-4), 28.1 (C-10), 23.5 (C-11), 23.1 (C-12), 19.8 (C-13); ESI-MS *m*/*z*: 241.1 $[M+H]^+$.

Betulalbuside A (16) – Colorless gum, $[\alpha]_D^{25}$: −15.2 (*c* 0.20, MeOH); ^{1}H NMR (CD_3OD, 700 MHz): δ 6.01 (1H, dd, J = 17.9, 11.2 Hz, H-2), 5.52 (1H, dd, J = 7.3, 1.3 Hz, H-6), 5.20 (1H, dd, J = 17.9, 2.3 Hz, H-1b), 5.02 (1H, dd, J = 11.2, 2.3 Hz, H-1a), 4.21 (1H, d, J = 7.6 Hz, Glc-1), 4.18 (1H, d, J = 12.3 Hz, H-8b), 3.98 (1H, d, J = 12.3 Hz, H-8a), 3.82 (1H, dd, J = 12.3, 2.3 Hz, Glc-6b), 3.63 (1H, dd, J = 12.3, 6.5 Hz, Glc-6a), 3.28 (1H, m, Glc-3), 3.26 (1H, m, Glc-4), 3.21 (1H, m, Glc-5), 3.17 (1H, m, Glc-2), 2.12 (2H, m, H-4), 1.66 (3H, br s, CH_3-10), 1.51 (2H, m, H-5), 1.21 (3H, s, CH_3-9); ^{13}C NMR (CD_3OD, 175 MHz): δ 145.9 (C-2), 134.3 (C-7), 131.9 (C-6), 111.7 (C-1), 102.8 (Glc-1), 78.1 (Glc-3), 77.2 (Glc-5), 76.4 (C-8), 75.0 (Glc-2), 73.2 (C-3), 71.1 (Glc-4), 62.1 (Glc-6), 43.1 (C-4), 27.7 (C-9), 21.1 (C-5), 13.7 (C-10); ESI-MS *m*/*z*: 333.1 $[M+H]^+$.

Loliolide (17) – Colorless gum; $[\alpha]_D^{25}$: −50.5 (*c* 0.25, MeOH); ^{1}H NMR (CD_3OD, 700 MHz): δ 5.72 (1H, s, H-3), 4.18 (1H, m, H-6), 2.38 (1H, td, J = 13.3 Hz, H-7a), 1.97 (1H, td, J = 13.6 Hz, H-5a), 1.72 (3H, s, CH_3-10), 1.74 (1 H, d, J = 13.3 Hz, H-7b), 1.48 (1 H, d, J = 13.6 Hz, H-5b), 1.42 (3H, s, CH_3-8), 1.30 (3H, s, CH_3-9); ^{13}C NMR (CD_3OD, 175 MHz): δ 184.2 (C-2), 174.5 (C-7b), 112.9 (C-3), 88.9 (C-7a), 68.3 (C-6), 48.1 (C-5), 45.1 (C-7), 37.1 (C-4), 32.0 (C-9), 27.5 (C-10), 27.1 (C-8); ESI-MS *m*/*z*: 197.1 $[M+H]^+$.

Acid hydrolysis of 1 and sugar analysis – Compound **1** (3.0 mg) was stirred with 1 mL of 1 N HCl for 1 h at 90 °C. The hydrolysate was extracted with EtOAc. The EtOAc layer afforded (3*R*,7*R*)-tuberonic acid (**1a**, 1.0 mg), and the aqueous layer was neutralized using an Amberlite IRA-67 column to yield the sugar. The sugar

was dissolved in anhydrous pyridine (0.5 mL), and 2.0 mg of L-cysteine methyl ester hydrochloride (Sigma) was added. The mixture was stirred at 60 ºC for 1.5 h and trimethylsilylated through adding 0.1 mL of 1-trimethylsilylimidazole (Sigma) for 2 hrs. The mixture was partitioned with *n*-hexane and H_2O (1.0 mL), and the *n*-hexane layer (1.0 μL) was analyzed through GC/MS. Identification of D-glucopyranose {$[\alpha]_D^{25}$: +53.2 (*c* 0.03, H_2O)} was performed by co-injection of the hydrolysate with authentic sample, giving a single peak at 9.723 min. An authentic sample (Sigma) treated in the same way displayed a single peak at 9.721 min.

(3*R*,7*R*)-Tuberonic acid (1a) – Colorless gum; $[\alpha]_D^{25}$: −50.1 (*c* 0.03, MeOH); ^{1}H NMR (CD_3OD, 700 MHz): δ 5.51 (1H, dtt, *J* = 10.9, 7.5, 1.5 Hz, H-3′), 5.41 (1H, dtt, *J* = 10.9, 7.3, 1.6 Hz, H-2′), 3.43 (2H, m, H-5′), 2.65 (1H, dd, *J* = 16.9, 7.2 Hz, H-2″a), 2.39 (1H, m, H-2″b), 2.37 (2H, m, H-1′), 2.31-2.28 (4H, m, H-1, H-4a, and H-4′), 2.23 (1H, m, H-5a), 2.20 (1H, m, H-2), 2.12 (1H, m, H-4b), 1.56 (1H, m, H-5b).

Cytotoxicity assay – A sulforhodamine B bioassay (SRB) was used to determine the cytotoxicity of each compound against four cultured human cancer cell lines.[6] The assays were performed at the Korea Research Institute of Chemical Technology. The cell lines used were A549 (non small cell lung adenocarcinoma), SK-OV-3 (ovarian cancer cells), SK-MEL-2 (skin melanoma), and HCT15 (colon cancer cells). Doxorubicin (Sigma Chemical Co., ≥98%) was used as a positive control.

Result and Discussion

The compounds **1 - 17** were determined as 5′-β-D-glucopyranosyloxyjasmonic butyl ester (**1**),[7] (+)-cucurbic acid (**2**),[8] dendranthemoside B (**3**),[9] boscialin 4′-*O*-β-D-glucoside (**4**),[10] 4,5-dihydroblumenol A (**5**),[11] (6*R*,9*R*)-megastigman-4-ene-9,13-diol (**6**),[12] (3*S*,5*R*,6*S*,9*R*)-3,6-dihydroxy-5,6-dihydro-β-ionol (**7**),[13] (6*S*,9*R*)-roseoside (**8**),[14] mallophenol B (**9**),[15] icariside B_5 (**10**),[16] corchoinoside B (**11**),[17] canangaionoside (**12**),[18] 5,6-epoxy-3-hydroxy-7-megastigmen-9-one (**13**),[19] icariside B_2 (**14**),[20] (7*E*)-2β,3β-dihydroxy-megastigm-7-en-9-one (**15**),[21] betulalbuside A (**16**),[22] and loliolide (**17**)[23] based on spectroscopic and physical data compared with their previously reported data. The compound **1** was isolated from *Lavandula augustifolia*,[7] but the stereochemistry was not clearly reported. We described the structural identification of compound **1** including the absolute configuration.

Compound **1** was obtained as a colorless gum. The molecular formula was determined to be $C_{22}H_{36}O_9$ from the molecular ion peak $[M+H]^+$ at *m/z* 445.2 (calcd for $C_{22}H_{37}O_9$, 445.2359) in the positive-ion ESI-MS. The IR spectrum of **1** indicated the presence of hydroxy (3350 cm^{-1}) and ketone groups (1748 cm^{-1}). The ^{1}H NMR showed two olefinic protons [δ_H 5.54 (1H, dtt, *J* = 10.9, 7.5, 1.5 Hz, H-3′), and 5.43 (1H, dtt, *J* = 10.9, 7.3, 1.6 Hz, H-2′)], three oxyenated methylenes [δ_H 3.89 (1H, m, H-5′b), 3.59 (1H, dt, *J* = 9.5, 7.2 Hz, H-5′a), 2.73 (1H, dd, *J* = 14.9, 4.3 Hz, H-2″a), 2.39 (1H, m, H-2″b), 2.32 (1H, m, H-4b), and 2.12 (1H, m, H-4a)], two methines [δ_H 2.30 (1H, m, H-1), and 2.01 (1H, m, H-2)], and three methylene protons at [δ_H 2.40 (2H, m, H-4′), 2.27 (2H, m, H-1′), 2.18 (1H, m, H-5b), 1.55 (1H, m, H-5a)]. The ^{13}C NMR spectrum contained 12 carbon signals, including a ketone carbon [δ_C 220.8 (C-3)], a carbonyl carbon [δ_C 174.3 (C-1″)], two olefinic carbons [δ_C 129.1 (C-3′), and 128.9 (C-2′)], two oxygenated methylenes [δ_C 70.4 (C-5′), and 40.0 (C-2″)], two methines [δ_C 55.2 (C-2), and 39.4 (C-1)], and four methylene carbons [δ_C 38.7 (C-4), 29.1 (C-4′), 28.3 (C-5), and 26.6 (C-1′)]. These spectral data implied that **1** was to be a jasmonic acid derivative.[7] Additionally, one glycopyranosyl signals at [δ_H 4.30 (1H, d, *J* = 7.8 Hz, Glc-1), 3.87 (1H, m, Glc-6b), 3.69 (1H, m, Glc-6a), 3.28 (2H, m, Glc-3, 5), 3.21 (1H, m, Glc-4), and 3.19 (1H, dd, *J* = 9.1, 7.9 Hz, Glc-2), and one *n*-butanol group at [δ_H 4.12 (2H, dt, *J* = 6.6, 1.2 Hz, H-1‴), 1.65 (2H, dt, *J* = 14.6, 6.6 Hz, H-2‴), 1.43 (2H, m, H-3‴), 0.98 (3H, t, *J* = 7.4 Hz, CH_3-4‴) in the ^{1}H NMR spectrum were appeared. The locations of glucose and *n*-butanol moieties were confirmed at C-5′ and C-1″, respectively, based on the HMBC correlations from Glc-1 [δ_H 4.30 (1H, d, *J* = 7.8 Hz)] with C-5′ (δ_C 70.4), and H-1‴ [δ_H 4.12 (2H, dt, *J* = 6.6, 1.2 Hz)] with C-1″ (δ_C 174.3) (Fig. 2). Glucose was identified as *β*-form by the coupling constant (*J* = 7.8 Hz) of the anomeric proton signal.[24] Acid hydrolysis of **1** afforded D-glucopyranose, which was identified by its specific rotation {$[\alpha]_D^{25}$: +53.2 (*c* 0.03, H_2O)} and GC/MS analysis.[25] The relative configuration of **1** could be deduced from comparison with ^{1}H and ^{13}C-NMR data on 5′-β-D-glucopyranosyloxy-jasmonic butyl ester.[7] The *cis*-olefinic functionality at C-2′ and C-3′ was determined by the chemical shifts of olefinic protons at [δ_H 5.54 (1H, dtt, *J* = 10.9, 7.5, 1.5 Hz, H-3′), and 5.43 (1H, dtt, *J* = 10.9, 7.3, 1.6 Hz, H-2′)].[26-28] The absolute configuration of **1** was identified as 1*R* and 2*R* from the negative cotton effect at 297 nm in the circular dichroism (CD) spectrum.[29] Thus, the structure of **1** was determined to be (1*R*,2*R*,2′*Z*)-5′-β-D-glucopyranosyloxy-jasmonic butyl ester.[7]

The cytotoxicities of compounds **1 - 17** against the

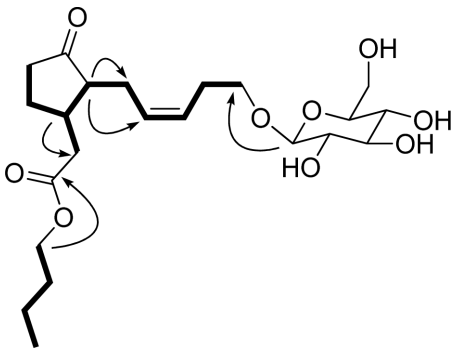

Fig. 2. Key correlations of ^{1}H-^{1}H COSY (bold lines) and HMBC (arrows) of **1**.

A549 (a non small cell lung carcinoma), SK-OV-3 (ovary malignant ascites), SK-MEL-2 (skin melanoma), and HCT15 (colon adenocarcinoma) human cancer cell lines were evaluated using the SRB assay. But all the compounds showed little cytotoxicity against tested cell lines (IC_{50} >30 μM).

In conclusion, two jasmonic acid derivatives (**1 - 2**), along with fifteen megastigmane derivatives (**3 - 17**) were isolated from the aerial parts of *P. urinar*. The absolute configuration of compound **1** was first determined. The isolated compounds showed little cytotoxicities against tested four cell lines (IC_{50} >30 μM).

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 (2016R1A2B2008380). We are grateful to the Korea Basic Science Institute (KBSI) for the NMR spectrum data.

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Received March 17, 2020
Revised April 28, 2020
Accepted April 29, 2020