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Phytochemical Constituents of *Carpesium macrocephalum* F_R. et S_{AV}.

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From the methanol extract of the whole plants of *Carpesium macrocephalum* F_R. et S_{AV}., five sesquiterpene lactones (**1**: carabron, **2**: tomentosin, **3**: ivalin, **4**: 4*H*-tomentosin, **5**: carabrol) and three terpenoids (**6**: loliolide, **7**: vomifoliol, **8**: citrusin C) were isolated. The structures and stereochemistry of compounds **1-8** were established on the basis of chemical analysis as well as 1D- and 2D-NMR spectroscopy. Among them, compounds **2**, **4**, and **6-8** were isolated for the first time from *Carpesium* species.

Key words: *Carpesium macrocephalum*, Compositae, Sesquiterpene lactone, Terpenoid

INTRODUCTION

Carpesium macrocephalum F_R. et S_{AV}. (Compositae) is a plant, which is rare in Korea, and it has been used in folk medicine as antipyretic, analgesic, vermifuge, insecticide, for pain-relief and antiinflammatory treatment in Korea (Lee, 1993).

An earlier investigation on the phytochemical constituents of the *Carpesium* species revealed them to be a rich source of sesquiterpene lactones; 11(13)-dehydroivaxillin (Maruyama *et al.*, 1983) and carpesiolin (Maruyama *et al.*, 1977) from *Carpesium abrotanoides*; divaricin A, B, and C (Maruyama, 1990) from *Carpesium divaricatum*; ineupatorolide A and B (Maruyama *et al.*, 1995) from *Carpesium glossophyllum*; two new guaianolides (Kim *et al.*, 2002) from *Carpesium macrocephalum*; divaricin analogues (Kim *et al.*, 1999) from *Carpesium triste* var. *manshuricum*.

In our continuing research for sesquiterpene lactones and other constituents from the whole plants of *C. macrocephalum*, we have isolated carabron (**1**), tomentosin (**2**), ivalin (**3**), 4*H*-tomentosin (**4**), carabrol (**5**), loliolide (**6**), vomifoliol (**7**), and citrusin C (**8**). Among them, compounds **2**, **4**, and **6-8** were reported for the first time from *Carpesium* species. In this paper, we describe the isolation of the compounds and their subsequent structural determination by spectroscopic analysis.

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MATERIALS AND METHODS

General procedure

The optical rotations were measured with a JASCO DIP-1000 digital polarimeter. UV spectra were obtained on a JASCO UV-530 UV/VIS Spectrophotometer. The EI-MS (70 eV) spectra were recorded on a JEOL JMS-AX 505H mass spectrometer. The NMR spectra were recorded on a Bruker DMX 600 spectrometer. The chemical shifts are expressed in parts per million (ppm) relative to TMS as the internal standard, and the coupling constants (*J*) are given in hertz (Hz). The 2D NMR spectra were recorded by using Brukers standard pulse program. Column chromatography was carried out with Kieselgel 60 (70-230 and 230-400 mesh, Merck) and polyamide gel (0.07 mm, MN polyamide SC6, MACHEREY-NAGEL). TLC was carried out on pre-coated Merck Kieselgel 60 F_{254} (art. 5715) and RP-18 F_{254}s (art. 15389) plates. The Lobar column chromatography and preparative HPLC were performed on Lichroprep® Si60 (40-63 μm) and a Hichrom RPB (5 μm, 10×250 mm, Hichrom Ltd.), respectively.

Plant material

The whole plants of *Carpesium macrocephalum* were collected in July 1999, at Odaesan, Kangwondo, Korea and were identified by Dr. Jae-Gil Kim. A voucher specimen

was deposited in the Herbarium of the College of Pharmacy, Chungbuk National University (CBNU-99-007).

Extraction and isolation

The air-dried whole plants of *C. macrocephalum* (1.58 kg) were finely ground and extracted with 90% aqueous MeOH (5 L×3) at room temperature for 2 weeks (×3). The MeOH solution was evaporated to dryness. The MeOH extract (165 g) was suspended in water (1.5 L), and then fractionated successively with equal volumes of *n*-Hexane, CH_2Cl_2, and EtOAc. The *n*-Hexane extract (28 g) was column chromatographed on a silica gel (650 g, 9×85 cm) column with an *n*-hexane-EtOAc (50:1 → 1:1). Fractions were combined based on their TLC profiles to yield subfraction designated as H1-H8. The subfraction, H8 (1.75 g) was further purified by column chromatography over a silica gel (170 g, 4×70 cm) eluting with a $CHCl_3$-MeOH (300:1 → 10:1) solvent system to afford four subfractions (H8.1-H8.4). Among these subfractions, H8.2 (250 mg) were carried out in Lobar column chromatography (MeOH-H_2O, 45:55) and preparative HPLC (MeOH-*i*-PrOH-H_2O, 5:4:1) to yield compound **1** (79 mg, R_f=0.48). The subfraction, H8.3 (167 mg) was further purified by polyamide column chromatography (55 g, 1.7×65 cm, MeOH → *i*-PrOH → DMF → *n*-hexane) and preparative HPLC (MeOH-H_2O, 1:1) to yield compound **2** (4.1 mg, R_f=0.45). The CH_2Cl_2 extract (6.6 g) was chromatographed on a silica gel (370 g, 6×75 cm) column with an *n*-hexane-EtOAc (50:1 → 1:1) and $CHCl_3$-MeOH (50:1 → 5:1). Fractions were combined based on their TLC profiles to yield subfraction designated as C1-C4. The subfraction, C1 (1.1 g) was rechromatographed on a silica gel (150 g, 3×68 cm) using an isocratic system of *n*-hexane-EtOAc (1:1) to give two subfractions (C1.1-C1.2). The subfraction, C1.2 (180 mg) was further purified by chromatography on semi-preparative HPLC (MeOH-H_2O, 50:50) and recrystallization from $CHCl_3$-MeOH (2:1) to yield compound **3** (40 mg, R_f=0.46) and **6** (6 mg, R_f=0.44). The subfraction, C2 (2 g) was rechromatographed on Lobar column (*n*-hexane-$CHCl_3$-MeOH, 40:40:1) to give three subfractions (C2.1-C2.3). The subfraction, C2.3 (850 mg) was rechromatographed on preparative HPLC (MeOH-H_2O, 60:40) to yield compound **4** (26 mg, R_f=0.30) and **5** (34 mg, R_f=0.32). The subfraction, C3 (984 mg) was rechromatographed on a silica gel (230 g, 2.8×80 cm) using a stepwise gradient of *n*-hexane-EtOAc-Me_2CO (5:1:1 → 1:1:1) to give nine subfractions (C3.1-C3.9). The subfraction, C3.5 (161.3 mg) was rechromatographed on preparative HPLC (MeOH-H_2O, 48:52) to yield compound **7** (7 mg, R_f=0.43). The subfraction, C4 (421 mg) was rechromatographed on a silica gel (130 g, 2.3×70 cm) using a stepwise gradient of *n*-hexane-EtOAc-Me_2CO (5:1:1 → 1:2:2) and $CHCl_3$-MeOH (10:1 → 1:1) to give two subfractions (C4.1-C4.2). The subfraction, C4.1 (250 mg) was rechromatographed on preparative HPLC (MeOH-H_2O, 38:62) to yield compound **8** (40 mg, R_f= 0.15).

TLC was performed on precoated Kieselgel 60 F_{254} plate developed with *n*-hexane-EtOAc-Me_2CO (1:1:1=A) and *n*-hexane-$CHCl_3$-MeOH (5:5:1=B, 3:2:1=C). A 10% H_2SO_4 reagent (in EtOH) was sprayed for detection and then heated.

Carabrone (1)

Yellowish oil; UV (MeOH) λ_{max} 213 nm; EI-MS (rel.int.%) *m/z* 248 (M^+, 8.3), 230 (8.5), 190 (81.9), 145 (81), 79 (60.3), 43 (100); ^{1}H-NMR ($CDCl_3$, 300 MHz) δ : 6.24 (1H, d, *J*=2.8 Hz, H-13α), 5.55 (1H, d, *J*=2.4 Hz, H-13β), 4.77 (1H, m, H-8), 3.15 (1H, m, H-7), 2.53 (2H, t, *J*=7.5 Hz, H_2-3), 2.33 (2H, m, H_2-6), 2.16 (3H, s, H-15), 1.61 (2H, m, H_2-9), 1.17 (3H, s, H-14), 0.93 (2H, m, H_2-2), 0.46 (1H, m, H-1), 0.40 (1H, m, H-5); ^{13}C-NMR ($CDCl_3$, 75 MHz) δ : 208.68 (C-4), 170.67 (C-12), 138.93 (C-11), 122.57 (C-13), 75.57 (C-8), 43.55 (C-3), 37.69 (C-7), 37.25 (C-9), 34.19 (C-1), 30.70 (C-6), 30.07 (C-15), 23.30 (C-2), 22.86 (C-5), 18.19 (C-14), 17.13 (C-10).

Tomentosin (2)

Colorless oil; UV (MeOH) λ_{max} 210 nm; EI-MS (rel.int.%) *m/z* 248 (M^+, 4.6), 205 (22.4), 190 (92.8), 145 (71.6), 107 (50.6), 79 (48.7), 43 (100); ^{1}H-NMR ($CDCl_3$, 300 MHz) δ : 6.26 (1H, d, *J*=2.8 Hz, H-13α), 5.52 (1H, d, *J*=2.2 Hz, H-13β), 5.44 (1H, t, H-5), 4.64 (1H, m, H-8), 3.31 (1H, m, H-7), 2.52 (2H, m, H_2-2), 2.36 (2H, m, H_2-6), 2.25 (2H, t, *J*=7.5 Hz, H_2-9), 2.16 (3H, s, H-15), 1.99 (1H, m, H-10), 1.63 (2H, m, H_2-3), 1.14 (3H, d, *J*=2.2 Hz, H-14); ^{13}C-NMR ($CDCl_3$, 75 MHz) δ : 208.09 (C-4), 170.31 (C-12), 138.94 (C-11), 122.19 (C-13), 120.28 (C-5), 79.24 (C-8), 42.10 (C-7), 42.09 (C-3), 36.62 (C-9), 35.43 (C-10), 30.39 (C-2), 29.91 (C-15), 29.54 (C-6), 20.92 (C-14).

Ivalin (3)

White crystals; $[\alpha]_D^{25}$ +133.52° (c 1.0, $CHCl_3$); UV (MeOH) λ_{max} 210 nm; IR ν_{max} (KBr) cm^{-1}: 3308 (OH), 1774 (α-methylene-γ-lactone moiety), 1723 (C=O), 1646 (C=C), 1138 (C-O); EI-MS (rel.int.%) *m/z* 248 (M^+, 10), 230 (98.5), 215 (32.5), 215 (98.5), 119 (100), 91 (77.5); ^{1}H-NMR ($CDCl_3$, 300 MHz) δ : 6.15 (1H, d, *J*=3.0 Hz, H-13α), 5.61 (1H, d, *J*=3.0 Hz, H-13β), 4.89 (1H, d-like, H-15a), 4.57 (1H, d-like, H-15b), 4.51 (1H, t-like, H-8), 3.84 (1H, m, H-2), 3.01 (1H, H-7), 2.69 (1H, dd, *J*=2.7, 12.3 Hz, H-3a), 2.27 (1H, dd, *J*=15.6 Hz, H-9a), 2.00 (1H, t, *J*=11.7 Hz, H-3b), 1.82 (2H, m, H-1a, 5), 1.79 (1H, d, *J*=6.9 Hz, H-6a), 1.54 (1H, dd, *J*=4.8, 15.3 Hz, H-9b), 1.39 (1H, q, H-6b), 1.19 (1H, t, *J*=11.7 Hz, H-1b), 0.85 (3H, s, H-14); ^{13}C-NMR ($CDCl_3$, 75 MHz) δ : 170.46 (C-12), 145.98 (C-4),

141.91 (C-11), 120.44 (C-13), 109.35 (C-15), 76.56 (C-8), 67.10 (C-2), 50.90 (C-1), 46.31 (C-3), 45.64 (C-5), 41.17 (C-9), 40.56 (C-7), 33.91 (C-10), 27.32 (C-6), 18.74 (C-14).

4H-tomentosin (4)

Colorless oil; $[\alpha]_D^{25}$ +10.2° (c 1.0, MeOH); UV (MeOH) λ_{max} 205 nm; EI-MS (rel.int.%) *m/z* 250 (M^+, 1), 232 (33), 217 (17), 203 (10), 193 (28.5), 175 (26.5), 161 (19), 147 (45.5), 134 (45), 121 (100), 107 (48.5), 93 (59), 67 (29), 41 (28.5); ^{1}H-NMR (CD_3OD, 300 MHz) δ : 6.10 (1H, d, *J*=3.2 Hz, H-13α), 5.56 (1H, d, *J*=2.8 Hz, H-13β), 5.45 (1H, m, H-5), 4.65 (1H, m, H-8), 3.61 (1H, m, H-7), 3.32 (1H, m, H-4), 2.36 (2H, m, H_2-6), 2.15 (1H, m, H-9a), 1.96 (3H, m), 1.88 (1H, m, H-9b), 1.42 (2H, m, H-3), 1.07 (3H, d, *J*=6.2 Hz, H-15), 1.06 (3H, d, *J*=6.9 Hz, H-14); ^{13}C-NMR (CD_3OD, 75 MHz) δ : 172.88 (C-12), 147.32, 141.41 (C-11), 123.20 (C-13), 121.54 (C-5), 81.83 (C-8), 68.78, 43.86 (C-7), 39.62, 38.21, 36.80, 34.44, 28.05, 23.73 (C-15), 21.80 (C-14).

Carabrol (5)

Colorless oil; $[\alpha]_D^{25}$ +98.9° (c 1.0, MeOH); UV (MeOH) λ_{max} 208 nm; EI-MS (rel.int.%) *m/z* 250 (M^+, 0.5), 232 (2.5), 217 (3), 206 (2), 190 (6.5), 175 (7.5), 161 (6), 145 (17), 131 (14.5), 119 (18), 105 (17.5), 85 (100), 79 (23), 68 (14), 43 (14), 41 (13.5); ^{1}H-NMR (CD_3OD, 300 MHz) δ : 6.12 (1H, d, *J*=2.8 Hz, H-13α), 5.63 (1H, d, *J*=2.4 Hz, H-13β), 4.79 (1H, m, H-8), 3.71 (1H, m, H-4), 3.20 (1H, m, H-7), 2.36 (2H, q, *J*=7.0 Hz, H_2-3), 2.24 (2H, dd, *J*=6.1, 13.5 Hz, H_2-6), 1.46 (2H, m, H_2-9), 1.31 (2H, m), 1.12 (3H, *J*=6.3 Hz, H-15), 0.97 (3H, s, H-14), 0.92 (1H, m, H_2-2), 0.46 (1H, m, H-1), 0.38 (1H, m, H-5); ^{13}C-NMR (CD_3OD, 75 MHz) δ : 173.11(C-12), 141.47 (C-11), 123.55 (C-13), 78.12 (C-8), 68.41, 40.5 (C-7), 39.30, 38.64, 36.32, 32.14, 26.72, 24.68, 23.98, 18.88 (C-14), 18.48 (C-15).

Loliolide (6)

White crystals; $[\alpha]_D^{25}$ -57.32° (c 0.25, MeOH); UV (MeOH) λ_{max} 213 nm; EI-MS (rel.int.%) *m/z* 196 (M^+, 27), 178 (55), 163 (32), 111 (100); ^{1}H-NMR (CD_3OD, 600 MHz) δ : 5.75 (1H, s, H-3), 4.21 (1H, m, H-6), 2.41 (1H, td, *J*=13.8 Hz, H-7α), 1.97 (1H, td, *J*=14.4 Hz, H-5α), 1.76 (3H, s, H-10), 1.74 (1H, d, *J*=3.3 Hz, H-7β), 1.53 (1H, d, *J*=3.6 Hz, H-5β), 1.46 (3H, s, H-8), 1.27 (3H, s, H-9); ^{13}C-NMR (CD_3OD, 150 MHz) δ : 185.43 (C-2), 174.55 (C-7b), 113.47 (C-3), 88.90 (C-7a), 67.34 (C-6), 47.93 (C-5), 46.55 (C-7), 37.28 (C-4), 31.16 (C-9), 27.57 (C-10), 26.98 (C-8).

Vomifoliol (7)

Colorless oil; UV (MeOH) λ_{max} 230 nm; ^{1}H-NMR (CD_3OD, 600 MHz) δ : 5.82 (1H, s, H-4), 5.80 (1H, d, *J*=3.7 Hz, H-8), 5.78 (1H, H-7), 4.31 (1H, q, H-9), 2.21 (1H, H-2a), 2.17 (1H, H-2b), 1.38 (3H, H-10), 1.04 (3H, s, H-11), 1.01 (3H, s, H-12); ^{13}C-NMR (CD_3OD, 150 MHz) δ : 200.86 (C-3), 161.54 (C-5), 136.98 (C-8), 130.08 (C-7), 125.94 (C-4), 79.98 (C-6), 68.72 (C-9), 49.92 (C-2), 42.26 (C-1), 24.74 (C-12), 23.84 (C-10), 23.55 (C-11), 20.05 (C-13).

Citrusin C (8)

White crystals; $[\alpha]_D^{25}$ -43.6° (c 1.0, MeOH,); UV (MeOH) λ_{max} 275 nm; IR ν_{max} (KBr) cm^{-1}: 3368 (OH), 2860 (arom. OCH_3), 1636 (C=C), 1076, 1029; ^{1}H-NMR (CD_3OD, 600 MHz) δ : 7.08 (1H, d, *J*=8.2 Hz, H-6), 6.82 (1H, d, *J*=1.9 Hz, H-3), 6.72 (1H, dd, *J*=1.9, 8.2 Hz, H-5), 5.94 (1H, dd, *J*=9.6, 16.9, Hz, H-8), 5.07 (1H, dd, *J*=1.6, 18.6 Hz, H-9a), 5.03 (1H, dd, *J*=1.6, 9.4 Hz, H-9b), 4.84 (1H, d, *J*=7.3 Hz, H-1'), 3.86 (1H, dd, *J*=6.0, 12.2 Hz, H_{glc}-6a), 3.83 (3H, s, H-10), 3.69 (1H, dd, *J*=5.1, 12.0 Hz, H_{glc}-6b), 3.47 (2H, m, H_{glc}-2, 5), 3.39 (2H, m, H_{glc}-3, 4), 3.32 (2H, d, *J*=6.7 Hz, H-7); ^{13}C-NMR (CD_3OD, 150 MHz) δ : 150.85 (C-2), 146.31 (C-1), 138.92 (C-4), 136.58 (C-8), 122.19 (C-5), 118.30 (C-3), 115.97 (C-9), 114.29 (C-6), 103.18 (C_{glc}-1), 78.16 (C_{glc}-5), 77.80 (C_{glc}-3), 74.97 (C_{glc}-2), 71.41 (C_{glc}-4), 62.53 (C_{glc}-6), 56.74 (10-OCH_3), 40.86 (C-7).

RESULTS AND DISCUSSION

The MeOH extract of the whole plants of *Carpesium macrocephalum* was suspended in water and then consecutively partitioned with *n*-Hexane, CH_2Cl_2, and EtOAc. The soluble parts of the *n*-Hexane and CH_2Cl_2 extract were purified by column chromatography using silica gel, as well as a combination of polyamide column chromatography and preparative HPLC, to yield the eight known compounds (**1-8**, Fig. 1).

Among them, compounds **2**, **4**, and **6-8** have been isolated for the first time from *Carpesium* species.

Compound **1** was obtained as yellowish oil. The EI-MS spectrum showed the molecular ion peak at *m/z* 248. The ^{1}H-NMR spectrum showed two doublet signals at δ 6.24 (1H, d, *J*=2.8 Hz, H-13α) and 5.55 (1H, d, *J*=2.4 Hz, H-13β), which are characteristic of the exocyclic methylene protons of an α-methylene-γ-lactone group (Yoshioka *et al.*, 1973). Moreover, two up-field signals showed at δ 2.16 (3H, s) and 1.17 (3H, s) were assigned to methyl protons at C-15 and 14, respectively, in the HMQC spectrum. The ^{13}C-NMR spectrum showed three signals at δ 170.67, 138.93, and 122.57 (C-12, 11, and 13, respectively) due to the α-methylene-γ-lactone moiety, carbonyl group at δ 208.68 (C-4), and two methyl signals at δ 30.07 and 18.19 (C-15 and 14, respectively). Based on the foregoing observations and a comparison of the data with the literature (Maruyama and Omura, 1977), compound **1** was determined to be carabrone.

Compound **2** was obtained as colorless oil. The EI-MS

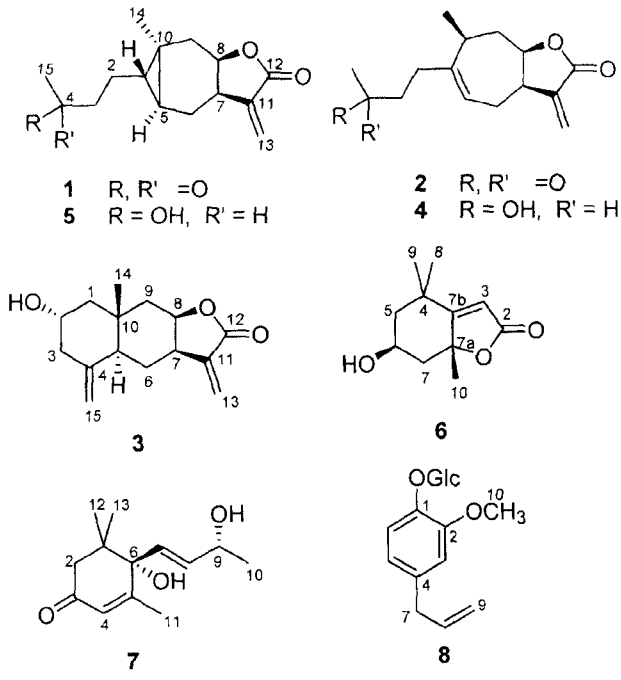

Fig. 1. Chemical structures of compounds **1-8** from *C. macrocephalum*

spectrum showed the molecular ion peak at *m/z* 248. In the ^{1}H- and ^{13}C-NMR spectra showed typical signals due to the α-methylene-γ-lactone moiety (δ_H 6.26, 1H, d, *J*=2.8 Hz, H-13α: 5.52, 1H, d, *J*=2.2 Hz, H-13β; δ_C 170.31, C-12: 138.94, C-11: 122.19, C-13) (Yoshioka *et al.*, 1973). Moreover, two up-field signals at δ 2.16 (3H, s) and 1.14 (3H, d, *J*=2.2 Hz), and one down-field signal at δ 5.44 (1H, t) were assigned to methyl protons and olefinic proton at C-15, 14, and 5, respectively, in the HMQC spectrum. Down-field signal at δ 208.09 was assigned to carbonyl carbon at C-4 in the ^{13}C-NMR spectrum. Based on the foregoing observations and a comparison of the data with the literature (Bohlman *et al.*, 1978), compound **2** was determined to be tomentosin (2,3-dihydro-4-one-1(5), 11(13)-xanthadien-8β,12-olide).

Compound **3** was obtained as white crystals, showed the absorbance band at 3502 (OH), 1773 (α-methylene-γ-lactone moiety), 1723 (C=O), and 1646 (C=C) cm^{-1} in the IR spectrum. The EI-MS spectrum showed the molecular ion peak at *m/z* 248. The ^{1}H-NMR spectrum was also showed two doublet signals at δ 6.15 (1H, d, *J*=3.0 Hz, H-13α) and 5.61 (1H, d, *J*=3.0 Hz, H-13β), which are characteristic of the exocyclic methylene protons of an α-methylene-γ-lactone group (Yoshioka *et al.*, 1973), two down-field signals at δ 4.89 (1H, d-like, H-15a) and 4.57 (1H, d-like, H-15b) were assigned to methylene protons. The ^{13}C-NMR spectrum showed three signals at δ 170.46, 141.91, and 120.44 (C-12, 11, and 13, respectively) due to the α-methylene-γ-lactone moiety, two signals due to the C-15 and C-4 carbons at δ 145.98 and 109.35, respectively, and one methyl signal due to the C-14 carbon at δ 18.74. Based on the forgoing observations and a comparison of the data with the literature (Walter *et al.*, 1976), compound **3** was determined to be ivalin (2α-hydroxy-4(15),11(13)-eudesmadien-8β,12-olide).

Compound **4** was obtained as colorless oil. The EI-MS spectrum showed the molecular ion peak at *m/z* 250. The ^{1}H-NMR spectrum showed of **4** was similar to that of **2**; their only difference was that a methyl singlet at δ 2.16 (3H, H-15) in the spectrum of **2** replaced by a 3H doublet at δ 1.07 (d, *J*=6.2 Hz, H-15) and a 1H multiplet at δ 3.32 (H-4) in the spectrum of **4**. These facts suggested that **4** had a secondary hydroxyl group in place of carbonyl group of **2**. However, the stereochemistry of OH group at C-4 could not be determined by comparison of the ^{1}H-NMR data of literature (Bohlmann *et al.*, 1978). Based on the foregoing observations and a comparison of the data with the literature (Bohlmann *et al.*, 1978), compound **4** was determined to be 4*H*-tomentosin (2,3-dihydro-4-hydroxy-1(5),11(13)-xanthadien-8β,12-olide).

Compound **5** was obtained as colorless oil. The EI-MS spectrum showed the molecular ion peak at *m/z* 250. The ^{1}H-NMR spectrum showed of **5** was similar to that of **1**; their only difference was that a methyl singlet at δ 2.16 (3H, H-15) in the spectrum of **1** replaced by a 3H doublet at δ 1.12 (d, *J*=6.3 Hz, H-15) and a 1H multiplet at δ 3.71 (H-4) in the spectrum of **5**. These facts suggested that **5** had a secondary hydroxyl group in place of carbonyl group of **1**. However, the stereochemistry of OH group at C-4 could not be determined by comparison of the ^{1}H-NMR data of literature (Maruyama *et al.*, 1983). Based on the foregoing observations and a comparison of the data with the literature (Maruyama *et al.*, 1983), compound **5** was determined to be carabrol.

Compound **6** was obtained as white crystals, showed the absorbance band at 213 nm in the UV spectrum. The EI-MS spectrum showed the molecular ion peak at *m/z* 196. The ^{1}H-NMR spectrum showed two germinal methyl protons at δ 1.46 (3H, s, H-8) and 1.27 (3H, s, H-9). The ^{13}C-NMR spectrum showed carbonyl group at δ 185.43 (C-2). Based on the NMR spectral evidence, and a comparison of the data with the literature (Valdes III, 1986), compound **6** was determined to be loliolide (5,6,7,7a-tetrahydro-6-hydroxy-4,4,7a-trimethyl-2(4*H*)-benzofuranone).

Compound **7** was obtained as white crystals, showed the absorbance band at 230 nm. The ^{1}H-NMR spectrum showed germinal methyl protons at δ 1.04 (3H, s, H-12) and 1.01 (3H, s, H-13), and *trans* olefinic protons at δ 5.80 (1H, d, *J*=3.7 Hz, H-8) and 5.78 (1H, H-7). The ^{13}C-NMR spectrum exhibited a carbonyl carbon at δ 200.86 (C-3), four methyl carbons at δ 24.74 (C-12), 23.84 (C-10), 23.55 (C-11), and 20.05 (C-13). Based on the NMR

spectral evidence, and a comparison of the data with the literature (Okamura *et al.*, 1981), compound **7** was determined to be vomifoliol (6,9-dihydroxy-4,7-megastigmadien-3-one).

Compound **8** was obtained as white crystals, showed the absorbance band at 275 nm. Its IR spectrum showed absorption bands due to hydroxyl groups (3368, 1076, and 1029 cm^{-1}) and olefinic group (1636 cm^{-1}). The ^{1}H-NMR spectrum showed four olefinic protons at δ 7.08 (1H, d, *J*=8.2 Hz, H-6), 6.82 (1H, d, *J*=1.9 Hz, H-3), 6.72 (1H, dd, *J*=1.9, 8.2 Hz, H-5), and 5.94 (1H, dd, *J*=9.6, 16.9 Hz, H-8), four methylene protons at δ 5.07 (1H, dd, *J*=1.6, 8.6 Hz, H-9a), 5.03 (1H, dd, *J*=1.6, 9.4 Hz, H-9b), and 3.32 (2H, d, *J*=6.7 Hz, H_2-7), and methoxy group at δ 3.83 (3H, s, H-10). Moreover, three signals due to a glucosyl moiety were observed at δ 4.84 (1H, d, *J*=7.3 Hz, H_{glc}-1), 3.86 (1H, dd, *J*=6.0, 12.2 Hz, H_{glc}-6a), and 3.69 (1H, dd, *J*=5.1, 12.0 Hz, H_{glc}-6b), of which the coupling constant indicated the β-linkage (Kim *et al.*, 2004) with the aglycone. The ^{13}C-NMR spectrum showed signals due to D-glucopyranoside, trisubstituted olefinic carbons at δ 150.85 (C-2), 146.31 (C-1), and 138.92 (C-4), another olefinic carbons at δ 122.19 (C-5), 118.30 (C-3), and 114.29 (C-6), and methoxy carbon at δ 56.74 (C-10). Based on the NMR spectral evidence, and a comparison of the data with the literature (Tomoyuki and Mitsuru, 1992), compound **8** was determined to be citrusin C (eugenyl *O*-β-D-glucopyranoside).

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