Isolation and Structures of Guaianolides from *Carpesium macrocephalum*

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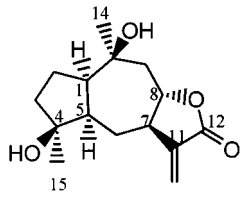
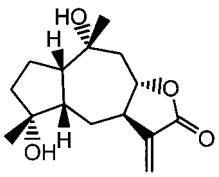
Received July 30, 2001

Two new guaianolides, 4α,10α-dihydroxy-1*β*(H),5*β*(H)-guai-11(13)-en-8α,12-olide (**2**) and 4*β*,10*β*-dihydroxy-5α(H)-1,11(13)-guaidien-8α,12-olide (**3**), from *Carpesium macrocephalum* were isolated, and their structures were elucidated on the basis of spectroscopic studies.

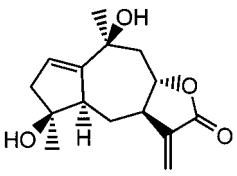
Carpesium macrocephalum French. et Sav. (Compositae) is a plant that is rare in Korea and has been used in folk medicine as an antipyretic, analgesic, vermifuge, and insecticide and for pain-relief and antiinflammatory treatment in Korea.[1,2] Several sesquiterpene lactones from the genus *Carpesium* have already been reported; those include granilin,[3] carabrone,[4] carabrol, ivaxillin, eriolin, 11(13)-dehydroivaxillin,[5] and ivalin from *C. abrotanoides*; divaricin A, B, and C[6,7] and cardivin A, B, C, and D[8] from *C. divaricatum*; ineupatorolide A and B[9] from *C. glossophyllum*; nepalolide A, B, C, and D[10] from *C. nepalense* and divaricin analogues[11] from *C. triste* var. *manshuricum*; carpelipine A and B[12] from *C. Lipskyi*, and carpesin[13] from *C. eximium*. However, there has been no report on components of *C. macrocephalum*, and in this paper, we present isolation and structure determination of two new guaianolides, 4α,10α-dihydroxy-1*β*(H),5*β*(H)-guai-11(13)-en-8α,12-olide (**2**) and 4*β*,10*β*-dihydroxy-5α(H)-1,11(13)-guaidien-8α,12-olide (**3**), along with 4*β*,10*β*-dihydroxy-1α(H),5α(H)-guai-11(13)-en-8α,12-olide (**1**).



1



2



3

The MeOH extract of *C. macrocephalum* was partitioned between H_2O and *n*-hexane. The resulting H_2O layer was extracted with CH_2Cl_2, EtOAc, and *n*-BuOH successively. The CH_2Cl_2 extract was chromatographed twice on a silica gel column, which afforded three sesquiterpene lactones, **1**, **2**, and **3**.

The structure of **1** was identified to be 4*β*,10*β*-dihydroxy-1α(H),5α(H)-guai-11(13)-en-8α,12-olide by comparison of ^{1}H and ^{13}C NMR, HMQC, HMBC, and NOESY data of **1** with those of the same compound reported from *Inula thapsoides*.[14]

Compound **2** showed the $[M + 1]^+$ peak at *m*/*z* 267 on FAB. The carbon shifts of **2** were similar to those of **1** except for C-7, C-8, and C-9. ^{1}H−^{1}H COSY, HMQC, and HMBC spectra established the structure as **2**. The NOESY spectrum of **2** showed a cross-peak between H-1 and H-5 but none between H-7 and H-8, which indicated 1,5-*cis* and 7,8-*trans* conformations. Signals of axial protons on C-6 and C-9 (H-6b and H-9b) could be distinguished from those of the equatorial ones (H-6a and H-9a) by the relative chemical shifts (1.21 ppm of H-6b vs 2.34 ppm of H-6a and 2.03 ppm of H-9b vs 2.41 ppm of H-9a) and their larger coupling constants ($J_{5-6b} = 12.3$ Hz vs $J_{5-6a} = 2.1$ Hz, $J_{8-9b} = 11.6$ Hz vs $J_{8-9a} = 1.7$ Hz). The relative configurations of protons on C-1, C-5, C-7, and C-8 were determined as *β*, *β*, α, *β*, respectively, by cross-peaks between H-1 and H-5, H-1 and H-8, H-5 and H-6a, and H-7 and H-9b and the absence of a cross-peak between H-7 and H-8. The relative configuration of the hydroxyl group on C-4 of **2** was α because of NOEs between H-15 and H-5, H-1 and H-6a; similarly H-14 was also α because of an NOE with H-1. Therefore, **2** was 4α,10α-dihydroxy-1*β*(H),5*β*(H)-guai-11-(13)-en-8α,12-olide.

Compound **3**, $[M + 1]^+$ at *m*/*z* 265 on FAB, had ^{1}H and ^{13}C NMR spectra similar to those of **1** except for a downfield signal of an olefinic proton (*δ* 5.79, 155.1, and 125.3). ^{1}H−^{1}H COSY, HMQC, and HMBC spectra of **3** permitted assignment of the proton and carbon signals. In the NOESY spectrum of **3**, cross-peaks between H-5 and H-7, H-6b and H-8, and H-7 and H-9b and the absence of a cross-peak between H-7 and H-8 indicated the relative configurations of C-5, C-7, and C-8 as α, α, and *β*, respectively. Configurations of the two hydroxyl groups on C-4 and C-10 were proposed to be both *β* based on NOE cross-peaks between H-15 and H-5 and between H-14 and H-5, respectively. Therefore, the structure of **3** was 4*β*,10*β*-dihydroxy-5α(H)-1,11(13)-guaidien-8α,12-olide.

Experimental Section

General Experimental Procedures. The NMR spectra were recorded on a Bruker 600 MHz (DMX 600) spectrometer. Samples dissolved in methanol-d_4 were reported in ppm downfield from TMS. The 2D NMR spectra were recorded by

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10.1021/np010388r CCC: $22.00
Published on Web 03/06/2002

using Bruker's standard pulse program. Silica gel 60 (70−230 and 230−400 mesh) and TLC plate (silica gel 60 F_{254}) were purchased from Merck (Germany). Semipreparative HPLC was carried out on a Hichrom RPB (5 μm, 10 × 250 mm, Hichrom Ltd., England) column. Optical rotations were measured with a JASCO DIP-1000 instrument. UV spectra were recorded in MeOH using a JASCO V-550 UV/vis spectrophotometer.

Plant Material. *Carpesium macrocephalum* was collected in July 1996, at Odae Mt., Kangwon-do, Korea, and dried at room temperature for three weeks. A voucher specimen was deposited in the herbarium of the College of Pharmacy, Chungbuk National University (CBNU-96-007).

Extraction and Isolation. The air-dried whole plant material (1.58 kg) was finely ground and extracted twice at room temperature with 90% aqueous MeOH (4 L) for 1 week. The MeOH solution was evaporated to dryness (165 g), which was partitioned between H_2O (1.6 L) and *n*-hexane (1.6 L). The resulting H_2O layer was extracted with CH_2Cl_2, EtOAc, and *n*-BuOH, successively. The CH_2Cl_2 extract (6.6 g) was loaded on a silica gel column. The column was eluted with a stepwise gradient of *n*-hexane−EtOAc (50:1→1:1, v/v) and $CHCl_3$−MeOH (50:1→5:1, v/v), and 50 mL fractions were collected to give seven subfractions (fr.1−fr.7). Fraction 5 (1.7 g) was chromatographed on a silica gel column with a solvent mixture of *n*-hexane−EtOAc (1:1, v/v) and then $CHCl_3$−MeOH (30:1→5:1, v/v) to give four fractions (fr.51−fr.54). Among these fractions, semipreparative HPLC (MeOH−H_2O, 50:50, v/v) of fr.52 afforded **1** (16 mg, 0.0011%), **2** (15 mg, 0.0010%), and **3** (2.5 mg, 0.0002%).

Compound 1: colorless oil (16 mg); $[\alpha]^{25}_D$ −16.5° (*c* 1.0, MeOH); UV (MeOH) λ_{max} (log ϵ) 207 (4.24); ^{1}H NMR (CD_3OD, 600 MHz) δ 6.12 (1H, d, J = 3.3 Hz, H-13a), 5.58 (1H, d, J = 3.1 Hz, H-13b), 4.19 (1H, ddd, J = 10.2, 10.2, 6.6 Hz, H-8), 3.25 (1H, brdd, J = 11.0, 10.2 Hz, H-7), 2.78 (1H, ddd, J = 11.7, 8.4, 8.4 Hz, H-1), 2.43 (1H, dd, J = 13.9, 6.6 Hz, H-9a), 2.21 (1H, ddd, J = 13.1, 8.4, 6.6 Hz, H-5), 2.16 (1H, ddd, J = 13.4, 6.6, 1.3 Hz, H-6a), 1.92 (1H, dd, J = 13.9, 10.2 Hz, H-9b), 1.83 (1H, m, H-2a), 1.81 (1H, m, H-3a), 1.71 (1H, ddd, J = 10.4, 6.6, 6.6 Hz, H-3b), 1.49 (1H, ddd, J = 11.7, 9.6, 9.6 Hz, H-2b), 1.29 (3H, s, H-14) 1.24 (3H, s, H-15), 1.16 (1H, ddd, J = 13.4, 13.1, 11.0 Hz, H-6b); ^{13}C NMR (CD_3OD, 150 MHz) δ172.6 (s, C-12), 142.1 (s, C-11), 119.7 (t, C-13), 84.2 (d, C-8), 81.6 (s, C-4), 73.8 (s, C-10), 52.9 (d, C-1), 51.3 (d, C-5), 45.5 (t, C-9), 44.6 (d, C-7), 39.1 (t, C-3), 30.4 (q, C-14), 29.0 (t, C-6), 25.6 (t, C-2), 23.9 (q, C-15); EIMS *m*/*z* 251 $[M - Me]^+$ (2.7), 248 (22), 230 (14.5), 190 (50), 95 (100), 81 (50), 71 (32.5); HRESIMS *m*/*z* 284.1863 (calcd for $C_{15}H_{22}O_4 \cdot NH_4$, 284.1862).

Compound 2: colorless oil (15 mg); $[\alpha]^{25}_D$ −6.38° (*c* 0.5, $CHCl_3$−MeOH = 1:1); UV (MeOH) λ_{max} (log ϵ) 207 (4.27); ^{1}H NMR (CD_3OD, 600 MHz) δ 6.07 (1H, d, J = 3.4 Hz, 13a), 5.56 (1H, d, J = 3.4 Hz, H-13b), 4.35 (1H, brdd, J = 10.7, 10.7 Hz, H-8), 2.59 (1H, ddddd, J = 12.3, 10.7, 3.4, 3.4, 4.0 Hz, H-7), 2.41 (1H, dd, J = 14.0, 1.7 Hz, H-9a), 2.34 (1H, ddd, J = 12.8, 4.2, 2.1 Hz, H-6a), 2.03 (1H, dd, J = 11.6, 14.0 Hz, H-9b), 1.95 (1H, ddd, J = 9.0, 8.8, 4.8 Hz, H-1), 1.81 (1H, ddd, J = 14.8, 6.2, 4.8 Hz, H-2a), 1.65 (1H, m, H-2b), 1.65 (1H, m, H-3a), 1.58 (1H, m, H-3b), 1.58 (1H, m, H-5), 1.28 (3H, s, H-14), 1.21 (1H, ddd, J = 12.3, 12.3, 12.3 Hz, H-6b), 1.17 (3H, s, H-15); ^{13}C NMR (CD_3OD, 150 MHz) δ 172.4 (s, C-12), 142.4 (s, C-11), 119.0 (t, C-13), 81.2 (d, C-8), 81.1 (s, C-4), 73.9 (s, C-10), 53.2 (d, C-1), 52.2 (d, C-7), 51.0 (d, C-5), 49.6 (t, C-9), 41.7 (t, C-3), 30.2 (t, C-6), 25.1 (q, C-14), 24.6 (t, C-2), 22.8 (q, C-15); EI$^+$-MS *m*/*z* (rel int), 248 $[M - H_2O]^+$ (28), 230 (61), 215 (14.5), 190 (73), 95 (100), 71 (58.7), 53 (45); HRESIMS *m*/*z* 284.1868 (calcd for $C_{15}H_{22}O_4 \cdot NH_4$, 284.1862).

Compound 3: white crystals (2.5 mg); mp 197−198 °C (dec); $[\alpha]^{25}_D$ −8.73° (*c* 0.6, MeOH); UV (MeOH) λ_{max} (log ϵ) 204 (3.66); ^{1}H NMR (CD_3OD, 600 MHz) δ 6.08 (1H, d, J = 3.3 Hz, H-13a), 5.79 (1H, dd, J = 2.5, 2.5 Hz, H-2), 5.57 (1H, d, J = 3.1 Hz, H-13b), 4.01 (1H, brdd, J = 10.4, 10.4 Hz, H-8), 2.73 (1H, dddd, J = 12.4, 10.4, 3.5, 3.1 Hz, H-7), 2.45 (1H, brd, J = 16.9 Hz, H-3a), 2.38 (1H, dd, J = 14.1, 1.4 Hz, H-9a), 2.33 (1H, dd, J = 12.6, 3.1 Hz, H-5), 2.27 (1H, ddd, J = 12.5, 3.5, 3.5 Hz, H-6a), 2.23 (1H, dd, J = 16.8, 3.2 Hz, H-3b), 2.11 (1H, dd, J = 14.1, 10.4 Hz, H-9b), 1.39 (3H, s, H-14), 1.37 (3H, s, H-15), 1.05 (1H, ddd, J = 12.4, 12.4, 11.9 Hz, H-6b); ^{13}C NMR (CD_3OD, 150 MHz) δ 172.4 (s, C12), 155.1 (s, C-1), 142.3 (s, C-11), 125.3 (d, C-2), 119.3 (t, C-13), 83.0 (s, C-4), 82.2 (d, C-8), 72.7 (s, C-10), 56.9 (d, C-5), 51.3 (d, C-7), 48.2 (t, C-9), 45.5 (t, C-3), 32.3 (t, C-6), 31.9 (q, C-14), 24.3 (q, C-15); EI$^+$-MS *m*/*z* (rel int) 264 $[M]^+$ (6), 246 (100), 231 (25), 228 (21), 203 (86), 188 (71.5), 93 (61), 53 (43); HREIMS *m*/*z* 264.1359 (calcd for $C_{15}H_{20}O_4$, 264.1362).

Acknowledgment. The authors are grateful to Dr. J. J. Jung and cooperators in Korea Basic Science Institute for measuring NMR spectra of these compounds. This work was supported in part by Grant No. 2000-2-216-001-3 from the Basic Research Program of the Korea Science & Engineering Foundation.

Supporting Information Available: Table of NOESY interactions and HMBC correlations for compounds **2** and **3**. This material is available free of charge via the Internet at http://pubs.acs.org.

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NP010388R