Kaempulchraols A–H, Diterpenoids from the Rhizomes of *Kaempferia pulchra* Collected in Myanmar

Nwet Nwet Win,[†‡] Takuya Ito,[†] Simayijiang Aimaiti,[†] Hiroshi Imagawa,[§] Hla Ngwe,[‡] Ikuro Abe,[⊥] and Hiroyuki Morita*[†]

[†]Institute of Natural Medicine, University of Toyama, 2630-Sugitani, Toyama 930-0194, Japan

[‡]Department of Chemistry, University of Yangon, Yangon 11041, Myanmar

[§]Faculty of Pharmaceutical Sciences, Tokushima Bunri University, Yamashiro-cho, Tokushima 770-8514, Japan

[⊥]Graduate School of Pharmaceutical Sciences, The University of Tokyo, 7-3-1 Hongo, Bunkyo-ku, Tokyo 113-0033, Japan

ABSTRACT

Eight new diterpenoids, kaempulchraols A–H (**1**–**8**), along with five known analogues were isolated from the $CHCl_3$ soluble extract of rhizomes of *Kaempferia pulchra* of Myanmar. The structures of these compounds were elucidated using extensive spectroscopic techniques including X-ray diffraction analysis. All the isolates were tested for their antiproliferative activity against a panel of five human cancer cell lines (A549, human lung cancer; HeLa, human cervix cancer; PANC-1 and PSN-1, human pancreatic cancer, MDA-MB-231, human breast cancer) and TIG-3, normal human primary fibroblast cells. Kaempulchraol F (**6**) exhibited weak activity against human pancreatic PSN-1 cell line with an IC_{50} value of 12.3 μM.

Cancer is a leading cause of death worldwide.[1] In anticancer drug discovery, natural products characterized from medicinal plants play an important role. Vincristine, irinotecan, etoposide, and paclitaxel are examples of plant-derived compounds that are being employed in cancer treatment.[2] According to GLOBOCAN 2012 report, the five most frequent cancers in Myanmar are lung, breast, cervix uteri, stomach, and liver.[1] Many of Myanmar medicinal plants have been used to prevent, alleviate, and cure human disease by the Myanmar people since time immemorial. In our ongoing research for the discovery of anticancer agents from Myanmar medicinal plants,[3–6] we screened the crude extracts against a panel of five human cancer cell lines (A549, human lung cancer; HeLa, human cervix cancer; PANC-1 and PSN-1, human pancreatic cancer, MDA-MB-231, human breast cancer) and TIG-3, normal human primary fibroblast cells. The $CHCl_3$ soluble fraction of rhizomes of *Kaempferia pulchra* exhibited reasonable antiproliferative activity against the tested cancer cell lines. *Kaempferia pulchra* Ridl., a perennial herb of the Zingiberaceae family is cultivated in some tropical countries including Myanmar, Indonesia, Malaysia, and Thailand. It is commonly known as "Shan-pan-oot" in Myanmar, and has been extensively used for cough, blood stimulation, carminative, quenching heat, deodorant, urinary tract infection, diuretic, and mellitus diabetes.[7] It has been reported to possess anti-inflammatory[8] and antitumor activities.[9] The rhizomes have been used locally for the self-medication by cancer and AIDS patients in Myanmar. Previous studies reported the presence of sandaracopimaradiene diterpenoids and ethyl 4-methoxy-*trans*-cinnamate.[8, 10] Herein, the isolation, structural elucidation, and antiproliferative activity of the isolated compounds are reported.

RESULTS AND DISCUSSION

The chloroform extract of *K. pulchra* exhibited reasonable antiproliferative activity against a panel of five human cancer cell lines (A549, human lung cancer; HeLa, human cervix cancer;

PANC-1 and PSN-1, human pancreatic cancer, MDA-MB-231, human breast cancer). Thus, it was subjected to a series of chromatographic separations which furnished eight new isopimarane diterpenoids, named kaempulchraols A–H (**1**–**8**) together with five known ones [9α-hydroxyisopimara-8(14),15-dien-7-one (**9**),[11] 7β, 9α-dihydroxypimara-8(14),15-diene (**10**),[12] 1α, 11α-dihydroxypimara-8(14),15-diene (**11**),[13] 1α, 2α-dihydroxypimara-8(14),15-diene (**12**),[8] and (2*R*)-*ent*-2hydroxyisopimara-8(14),15-diene (**13**)].[14]

Compound **1** was obtained as colorless needles, and its molecular formula was determined as $C_{21}H_{34}O_2$ via ^{13}C NMR and HREIMS data. The IR spectrum of **1** showed absorption bands of hydroxy and olefinic groups at 3519 and 1634 cm^{-1}, respectively. The ^{1}H NMR spectrum (Table 1) displayed signals due to terminal vinyl protons [δ_H 6.07, dd, (*J* = 16.5, 12.0 Hz, H-15), 5.07, dd (*J* = 16.5, 1.5 Hz, H-16a), 5.05, dd (*J* = 12.0, 1.5 Hz, H-16b)], three methines including two oxygenated ones [δ_H 4.54, t (*J* = 5.0 Hz, H-6), 3.13, s (H-14), 1.27, br s (H-5)], six methylene groups, one methoxy group [δ_H 3.46, s (OMe-14)], and four tertiary methyls [δ_H 1.03 (H_3-17), 1.23 (H_3-18), 0.98 (H_3-19), 1.38 (H_3-20)]. The ^{13}C NMR spectrum (Table 3) revealed 21 signals including four olefinic carbons (δ_C 144.4, 140.7, 123.7, 112.4), two oxygenated methines (δ_C 88.9, 66.1), one methine (δ_C 53.6), three quaternary carbons (δ_C 40.17, 37.8, 34.3), six methylenes (δ_C 43.3, 40.7, 40.18, 31.4, 20.4, 19.4), one methoxy (δ_C 62.1), and four methyls (δ_C 33.7, 24.3, 22.9, 21.9). These data suggested **1** to be an isopimarane diterpenoid similar to those reported by Tuchinda et al.[8] However, significant differences included the absence of the trisubstituted $\Delta^{8(14)}$ double bond of the sandaracopimaradiene skeleton and the presence of a tetrasubstituted $\Delta^{8(9)}$ double bond in **1**, which was confirmed from the ^{1}H–^{13}C HMBC correlations of H_3-20 (δ_H 1.38, s) and H_2-12 (δ_H 1.39, 1.69, both m) to C-9 (δ_C 140.7) and those of H_2-7 to C-8 (δ_C 123.7) and C-9 (Supporting Information Figure S2). Thus, the skeleton of **1** was considered to be an isopimara-8(9),15-diene. The presence of two oxygenated methine protons and one methoxy signal suggested the presence of a hydroxy and a methoxy group. The HMBC correlations of

H-6 (δ_H 4.54) to C-5/C-7/C-8/C-10, of H-14 (δ_H 3.13) to C-8/C-9/C-13/C-15/C-17, and of OMe-14 (δ_H 3.46) to C-14 confirmed the position of hydroxy group at C-6 and that of methoxy group at C-14. The relative configuration of **1** was assigned on the basis of a 2D NOESY experiment. The NOESY correlations between H-6 and H-5/H_3-19 and between H-14 and H_3-17 suggested the orientation of OH-6 and OMe-14 as β and α, respectively. The X-ray crystallographic pattern (Figure 1) confirmed the structure of **1** as 6β-hydroxy-14α-methoxyisopimara-8(9),15-diene and named kaempulchraol A.

Compound **2** was obtained as colorless needles having a molecular formula of $C_{21}H_{34}O_2$. The ^{1}H and ^{13}C NMR spectroscopic data (Tables 1and 3) of **2** were similar to those of **1**. The difference was the orientation of methoxy group at C-14 (δ_H 3.50, s) as β, which was confirmed from the 2D NOESY correlations of OMe-14 to H_3-17 (δ_H 1.10, s) and X-ray diffraction analysis (Figure 1). Thus, the structure of **2** was assigned as 6β-hydroxy-14β-methoxyisopimara-8(9),15-diene and named kaempulchraol B.

Compound **3** was obtained as colorless needles and its molecular formula was shown to be $C_{20}H_{32}O_2$ by HREIMS and ^{13}C NMR data. The ^{1}H and ^{13}C NMR spectroscopic data of **3** (Tables 1and 3) were also similar to those of **1** except for the absence of the C-14 methoxy group (δ_H 3.46, δ_C 62.1) in **3**. Instead of the methoxy group, the presence of a hydroxy group in **3** was in agreement with its HMBC correlations between the oxygenated methine (δ_H 3.61, d, J = 6.1 Hz, H-14) and C-7/C-9/C-15/C-17 (Supporting Information Figure S2). On the basis of X-ray diffraction analysis data (Figure 1), the relative configuration of **3** was similar to that of **1**. Accordingly, **3** was identified as 6β, 14α-dihydroxyisopimara-8(9),15-diene and named kaempulchraol C.

Compound **4** was isolated as colorless needles. The HREIMS of **4** exhibited an $[M]^+$ ion at *m/z* 304.2407 corresponding to the molecular formula $C_{20}H_{32}O_2$. Its ^{1}H and ^{13}C NMR spectroscopic data (Tables 1 and 3) as well as HMBC correlations were similar to those of **3** and thus **4** was assumed to be an isomer of **3**. The significant differences were the splitting

patterns of H-14 [**3**: 3.61, d (J = 6.1 Hz); **4**: δ_H 3.37, br s] and X-ray diffraction patterns (Figure 1). Thus, the orientation of C-14 hydroxy group in **4** was concluded to be opposite to that of **3**. Hence, the structure of **4** was elucidated as 6β, 14β-dihydroxyisopimara-8(9),15-diene and named kaempulchraol D.

Compound **5** was obtained as colorless plates. The molecular formula, $C_{20}H_{32}O_2$ was determined by HREIMS and ^{13}C NMR data. The IR spectrum revealed absorption bands at 3388, 2939, and 1634 cm^{-1} ascribable to be the presence of hydroxy and olefinic groups. The ^{1}H NMR spectrum (Table 2) of **5** displayed signals for a sandaracopimaradiene type skeleton including terminal vinylic protons [δ_H 5.77, dd (J = 17.7, 10.3 Hz, H-15), 4.92, dd (J = 17.7, 1.5 Hz, H-16a), 4.90, dd (J = 10.3, 1.5 Hz, H-16b)], an olefinic methine [δ_H 5.43, br t (J = 1.7 Hz, H-14)] and four methyl singlets [δ_H 1.09 (H_3-17, H_3-20), 1.26 (H_3-18), 1.03 (H_3-19)]. Analyses of the HMQC and ^{13}C NMR spectroscopic data (Table 3) of **5** indicated the presence of four olefinic carbons, four methines including two oxygenated ones, three quaternary carbons, five methylenes, and four methyls. These data revealed **5** to possess a dihydroxysandaracopimaradiene skeleton. The HMBC correlations (Supporting Information Figure S2) between H-1 (δ_H 3.64, br s) and C-3/C-5/C-9/C-20 and between H-6 (δ_H 4.36, d, J = 2.3 Hz) and C-4/C-8/C-10 showed the attachment of these hydroxy groups at C-1 and C-6. The 2D NOESY correlations of H-1 and H_3-20, of H-6 and H-5/H-9/H_3-19 and the X-ray diffraction pattern (Figure 1) established the relative configuration of **5** as shown in Chart 1. Consequently, its structure was elucidated as 1α, 6β-dihydroxyisopimara-8(14),15-diene and named kaempulchraol E.

Compound **6** was obtained as colorless needles and its molecular formula was shown as $C_{20}H_{32}O_2$ by HREIMS and ^{13}C NMR data. The ^{1}H and ^{13}C NMR spectroscopic data (Tables 2 and 3) of **6** were similar to those of reported sandaracopimaradienes.[8] The presence of two oxygenated methines at δ_H 3.75 and δ_H 3.57 suggested **6** to be a dihydroxy derivative of sandaracopimaradiene. The HMBC correlations (Supporting Information Figure S2) between

H-1 (δ_H 3.75, br s) and C-3/C-5/C-9/C-20 and between H-3 (δ_H 3.57, br s) and C-1/C-5/C-18/C-19 confirmed the position of these two hydroxy groups at C-1 and C-3, respectively. The orientations of the C-1 and C-3 hydroxy groups were determined by NOESY correlations of H-1 and H_3-20, and of H-3 and H_3-18. Thus, **6** was identified as 1α, 3α-dihydroxyisopimara-8(14),15-diene and named kaempulchraol F.

Compound **7** was obtained as an amorphous solid and its molecular formula was observed to be $C_{20}H_{30}O_2$ by HREIMS and ^{13}C NMR data. The IR spectrum exhibited absorption bands at 3530, 1742, and 1647 cm^{-1} due to the presence of hydroxy, carbonyl, and olefinic groups, respectively. The ^{1}H and ^{13}C NMR spectroscopic data of **7** (Tables 2 and 3) were similar to those of **4**. The only difference involves the presence of carbonyl carbon (δ_C 202.1) in **7** instead of an oxygenated methine (δ_C 74.8, C-14) of **4**. The HMBC correlations of H-15 (δ_H 5.80, dd, J = 17.5, 10.7 Hz), H-7α (δ_H 2.41, m) and H-7β (δ_H 2.54, m) to this carbonyl carbon (Supporting Information Figure S2) supported its C-14 location. The β orientation of the C-6 hydroxy group was established on the basis of NOESY correlations of H-6 to H-5α and H_3-19, respectively. Hence, the structure of **7** was concluded to be 6β-hydroxy-14-oxoisopimara-8(9),15-diene and named kaempulchraol G.

Compound **8** was isolated as an amorphous solid and its HREIMS (*m/z* 320.2356) and ^{13}C NMR data suggested it to have the molecular formula $C_{20}H_{32}O_3$. Its ^{1}H and ^{13}C NMR data (Tables 2 and 3) were similar to those of **6**. The only difference involves the presence of an additional oxygenated methine (δ_H 4.33, d, J = 2.5 Hz, δ_C 69.1) in **8**. Thus, **8** was proposed to be a trihydroxy derivative of sandaracopimaradiene. On the basis of HMBC correlations of H-1 (δ_H 3.64, br s) to C-3/C-5/C-9/C-20, of H-3 (δ_H 3.52, br s) to C-1/C-5/C-18/C-19 and of H-6 to C-4/C-8/C-10, the locations of the three hydroxy groups were at C-1, C-3, and C-6, respectively (Supporting Information Figure S2). The orientations of the C-1, C-3, and C-6 hydroxy groups were determined by NOESY correlations of H-1 to H_3-20 (δ_H 1.08, s), of H-3 to H_3-18 (δ_H 1.27, s), and of H-6 to H-5 (δ_H 1.78, d, J = 1.7 Hz) and H_3-19 (δ_H 1.12, s),

respectively. Consequently, the structure of **8** was elucidated as 1α, *3*α, *6*β-trihydroxyisopimara-8(14),15-diene and named kaempulchraol H.

The structure of the known compounds **9–13** (Supporting Information Figure S1) were identified by comparison of their observed and reported NMR data. Based on the present study, the rhizomes of *K. pulchra* were found to be a rich source of 6β-hydroxyisopimarane diterpenoids. This is the first report for the presence of 8(9),15-isopimarane diterpenoids in the rhizomes of *K. pulchra* which were collected from the middle eastern part of Myanmar.

All the isolated compounds **1–13** were subjected for antiproliferative activity against five human cancer cell lines. Among the tested compounds, only kaempulchraol F (**6**) exhibited weak activity against human pancreatic PSN-1 cell line with an IC_{50} value of 12.3 μM.

EXPERIMENTAL SECTION

General Experimental Procedures. The melting points were determined on a Yanaco micro melting point apparatus and are uncorrected. Optical rotations were recorded on a JASCO P2100 polarimeter. Infrared spectra were recorded as KBr pellets on a Jasco FT/IR-460 Plus spectrometer. NMR spectra were recorded at 600 MHz (^{1}H NMR) and 150 MHz (^{13}C NMR), respectively, on a Varian UNITY 600 spectrometer. Chemical shift values were expressed in δ (ppm) downfield from TMS as internal standard. The mass spectra, including high-resolution mass spectra, were recorded on a JEOL MStation JMS-700 spectrometer. Open column chromatography was performed with normal-phase silica gel (silica gel 60N, Spherical, neutral, 40–50 μm, Kanto Chemical Co., Inc, Japan) and cosmosil 75C18-OPN (Nacalai Tesque Inc., Kyoto, Japan). MPLC was done with a Büchi Sepacore system (Büchi Labortechnik AG, Flawil, Switzerland). TLC was carried out on precoated silica gel 60F_{254} and RP-18 F_{254} plates (Merck, 0.25 or 0.50 mm thickness). The cell lines such as A549 (human lung cancer), HeLa (human cervix cancer), PANC-1 and PSN-1

(human pancreatic cancer), MDA-MB-231 (human breast cancer), and TIG-3 (normal human primary fibroblast cell) were available and maintained in our laboratory. Cell culture flasks and 96-well plates were from Corning Inc. (Corning, NY, USA). The SH-1200 Microplate Reader® (Corona, Hitachinaka, Japan) was used to measure the absorbance of the cells in the antiproliferative activity assay.

Plant Material. Rhizomes of *Kaempferia pulchra* Ridl. were collected from Pindaya Township, Shan State, Myanmar in September 2013 and identified by the authorized botanist of the Department of Botany, University of Yangon. A voucher specimen (TMPW 28301) was deposited at the Museum for Materia Medica, Analytical Research Center for Ethnomedicines, Institute of Natural Medicine, University of Toyama, Japan.

Extraction and Isolation. The rhizomes of *K. pulchra* (500 g) were extracted with $CHCl_3$ under sonication (1L, 90 min, ×3) at 35 °C and the solvent was evaporated under reduced pressure to give 30 g of extract.

The chloroform extract (30 g) was chromatographed on silica gel with an EtOAc–*n*-hexane solvent system to give seven fractions [1: EtOAc–*n*-hexane (10:90) eluate, 0.96 g; 2: EtOAc–*n*-hexane (15:85) eluate, 1.99 g; 3: EtOAc–*n*-hexane (20:80) eluate, 6.43 g; 4: EtOAc–*n*-hexane (25:75) eluate, 3.52 g; 5: EtOAc–*n*-hexane (30:70) eluate, 3.84 g; 6: EtOAc–*n*-hexane (40:60) eluate, 1.34 g; 7: EtOAc–*n*-hexane (50:50) eluate, 3.94 g].

Fractions 1 and 2 were oily substances. Fraction 3 (6 g) was rechromatographed on cosmosil 75C18-OPN with MeCN–acetone–MeOH–H_2O (2:2:1:1) to give three subfractions [3-1: 0.5 g; 3-2: 4 g; 3-3: 0.5 g]. Subfraction 3-2 (4 g) was separated by MPLC with *n*-hexane–CH_2Cl_2–EtOAc (100:100:7) [column: polypropylene (∅ 40 mm × 150 mm); flow rate: 20 mL/min] to give kaempulchraols A (**1**, 40 mg), B (**2**, 55 mg), C (**3**, 70 mg), D (**4**, 10 mg), and E (**5**, 473 mg) and 9α-hydroxyisopimara-8(14),15-dien-7-one (**9**, 15 mg).[11]

The 7β, 9α-dihydroxypimara-8(14),15-diene[12] (**10**, 1.2 g) and 1α, 11α-dihydroxypimara-8(14),15-diene[13] (**11**, 1.1 g) were directly obtained as pure needles

from fraction 4.

Fraction 5 (3.84 g) was rechromatographed on cosmosil 75C18-OPN with MeCN–acetone–MeOH–H_2O (2:1:1:1) to afford four subfractions [5-1: 2.3 g; 5-2: 0.6 g; 5-3: 0.5 g; 5-4: 162 mg]. Subfraction 5-1 (2.3 g) was separated by MPLC [column: polypropylene (∅ 40 mm × 150 mm); flow rate: 20 mL/min] with *n*-hexane–CH_2Cl_2–EtOAc (4.5:4.5:1) followed by purification on cosmosil 75C18-OPN with MeOH–H_2O (3:1) to give 1α, 2α-dihydroxypimara-8(14),15-diene[8] (**12**, 354 mg), kaempulchraol F (**6**, 700 mg), and mixture (349 mg). That mixture (349 mg) was subjected to normal-phase preparative TLC with *n*-hexane– CH_2Cl_2–acetone (4:4:1) to give kaempulchraols C (**3**, 50.5 mg) and G (**7**, 15.2 mg), and **11** (144 mg) and (2*R*)-*ent*-2hydroxyisopimara-8(14),15-diene[14] (**13**, 10 mg).

Fraction 6 (1.34 g) was rechromatographed on cosmosil 75C18-OPN with MeCN–acetone–MeOH–H_2O (2:1:1:1) to afford three subfractions [6-1: 1.15 g; 6-2: 74 mg; 6-3: 63 mg]. Further purification of subfraction 6-1 (1.15 g) by MPLC [column: polypropylene (∅ 40 mm × 150 mm); flow rate: 20 mL/min] with $CHCl_3$:EtOAc (95:5) afforded kaempulchraols D (**4**, 56 mg) and H (**8**, 892 mg).

*Kaempulchraol A (**1**)*: colorless needles (*n*-hexane:CH_2Cl_2, 9:1); mp 130–133 °C; $[\alpha]^{25}_D$ –43 (*c* 0.1, MeOH); IR (KBr) ν_{max} 3519, 2937, 2363, 1829, 1662, 1634, 1460, 1219, 1155 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 1 and 3; EIMS *m/z* 318 $[M]^+$ (17); HREIMS *m/z* 318.2559 $[M]^+$ (calcd for $C_{21}H_{34}O_2$ 318.2559).

*Kaempulchraol B (**2**)*: colorless needles (*n*-hexane:CH_2Cl_2, 9:1); mp 95–98 °C; $[\alpha]^{25}_D$ +121 (*c* 0.1, MeOH); IR (KBr) ν_{max} 3427, 2918, 1637, 1459, 1224, 1090, 1003, 940, 917 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 1 and 3; EIMS *m/z* 318 $[M]^+$ (18); HREIMS *m/z* 318.2567 $[M]^+$ (calcd for $C_{21}H_{34}O_2$ 318.2559).

*Kaempulchraol C (**3**)*: colorless needles (acetone); mp 110–113 °C; $[\alpha]^{25}_D$ –16 (*c* 0.1, MeOH); IR (KBr) ν_{max} 3339, 2920, 1655,1640, 1458, 1288, 1259, 1089, 1009, 984 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 1 and 3; EIMS *m/z* 304 $[M]^+$ (6); HREIMS *m/z* 304.2396 $[M]^+$

(calcd for $C_{20}H_{32}O_2$ 304.2402).

*Kaempulchraol D (**4**)*: colorless needles (acetone); mp 120–123 °C; $[\alpha]^{25}_D$ +93 (*c* 0.05, MeOH); IR (KBr) ν_{max} 3465, 3360, 2916, 1634, 1457, 1213, 1089, 970 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 1 and 3; EIMS *m/z* 304 $[M]^+$ (6); HREIMS *m/z* 304.2407 $[M]^+$ (calcd for $C_{20}H_{32}O_2$ 304.2402).

*Kaempulchraol E (**5**)*: colorless plates (*n*-hexane:CH_2Cl_2, 9:1); mp 145–150 °C; $[\alpha]^{25}_D$ +51 (*c* 0.1, MeOH); IR (KBr) ν_{max} 3388, 2939, 1634, 1449, 1224, 1194, 979, 947, 912 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 1 and 3; EIMS *m/z* 304 $[M]^+$ (18); HREIMS *m/z* 304.2425 $[M]^+$ (calcd for $C_{20}H_{32}O_2$ 304.2402).

*Kaempulchraol F (**6**)*: colorless needles (CH_2Cl_2); mp 165–170 °C; $[\alpha]^{25}_D$ –19 (*c* 0.1, MeOH); IR (KBr) ν_{max} 3293, 2946, 1636, 1457, 1064, 998 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 1 and 3; EIMS *m/z* 304 $[M]^+$ (15); HREIMS *m/z* 304.2395 $[M]^+$ (calcd for $C_{20}H_{32}O_2$ 304.2402).

*Kaempulchraol G (**7**)*: amorphous solid; $[\alpha]^{25}_D$ +93 (*c* 0.1, MeOH); IR (KBr) ν_{max} 3530, 2924, 1742, 1647, 1618, 1457, 1221, 1060, 1018, 923 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 2 and 3; EIMS *m/z* 302 $[M]^+$ (28); HREIMS *m/z* 302.2240 $[M]^+$ (calcd for $C_{20}H_{30}O_2$ 302.2246).

*Kaempulchraol H (**8**)*: amorphous solid; $[\alpha]^{25}_D$ –55 (*c* 0.1, MeOH); IR (KBr) ν_{max} 3568, 3278, 2952, 1635, 1450, 1193, 1066, 981 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 2 and 3; EIMS *m/z* 320 $[M]^+$ (8); HREIMS *m/z* 320.2356 $[M]^+$ (calcd for $C_{20}H_{32}O_3$ 320.2351).

X-ray Crystallographic Analysis of Kaempulchraols A–E (1–5). X-ray data for compounds **1–5** were collected on a Bruker APEX 2 CCD area detector diffractometer with a multi-layered confocal mirror Helios ($\phi-\omega$ scans), Mo *Kα* radiation (λ = 0.71069 Å) and the radiation source is Bruker TXS fine-focus rotating anode. Bruker *APEX 2* was used for cell refinement and data reduction. The programs *SHELXL*-97, *SHELXL*-2014, and *PLATON* were used for structure solution, structure refinement, and ORTEP plot, respectively.[15–17]

Crystallographic data for the structures for compounds **1–5** have been deposited with the Cambridge Crystallographic Data Centre (Deposition numbers: CCDC 1046089–1046093). Copies of these data can be obtained, free of charge, on application to the Director, CCDC, 12 Union Road, Cambridge CB2 1EZ, UK (fax: +44-(0)1223-336033 or e-mail: deposit@ccdc.cam.ac.uk).

*Crystal Data of Kaempulchraol A (**1**)*: $C_{21}H_{34}O_2$, M = 318.49, orthorhombic, space group $P2_12_12_1$, a = 6.108 (2) Å, b = 11.902 (3) Å, c = 24.407 (9) Å, V = 1774.2 (11) Å^3, Z = 4, D_{calcd} = 1.140 g/cm^3, T = 100 K, $F(000)$ = 672, and μ (Mo $K\alpha$) = 0.071 mm^{-1}. A total of 10461 reflections (4495 unique, R_{int} = 0.0557) were collected from 1.66° to 29.45° in θ and index ranges $8 \geq h \geq -8$, $8 \geq k \geq -16$, $30 \geq l \geq -33$. The final stage converged to R_1 = 0.0640 (wR_2 = 0.1546) for 4264 observed reflections [with $I > 2\sigma(I)$] and 214 variable parameters and R_1 = 0.0669 (wR_2 = 0.1564) for all unique reflections and GoF = 1.111.

*Crystal Data of Kaempulchraol B (**2**)*: $C_{21}H_{34}O_2$, M = 318.49, monoclinic, space group $P2_1$, a = 7.4283 (4) Å, b = 21.5310 (11) Å, c = 12.2627 (6) Å, β = 104.9149° (6)**,** V = 1895.20 (17) Å^3, Z = 4, D_{calcd} = 1.067 g/cm^3, T = 100 K, $F(000)$ = 672, and μ (Mo $K\alpha$) = 0.066 mm^{-1}. A total of 11747 reflections (8692 unique, R_{int} = 0.0137) were collected from 1.71° to 29.23° in θ and index ranges $10 \geq h \geq -9$, $27 \geq k \geq -29$, $16 \geq l \geq -7$. The final stage converged to R_1 = 0.0295 (wR_2 = 0.0762) for 8555 observed reflections [with $I > 2\sigma(I)$] and 427 variable parameters and R_1 = 0.0300 (wR_2 = 0.0767) for all unique reflections and GoF = 1.011.

*Crystal Data of Kaempulchraol C (**3**)*: $C_{20}H_{32}O_2$, M = 304.46, monoclinic, space group $P2_1$, a = 11.3576 (8) Å, b = 12.9484 (9) Å, c = 24.0498 (17) Å, β = 89.9595° (9)**,** V = 3536.8 (4) Å^3, Z = 8, D_{calcd} = 1.144 g/cm^3, T = 100 K, $F(000)$ = 1344, and μ (Mo $K\alpha$) = 0.071 mm^{-1}. A total of 22053 reflections (16184 unique, R_{int} = 0.0221) were collected from 0.84° to 29.28° in θ and index ranges $15 \geq h \geq -15$, $16 \geq k \geq -17$, $32 \geq l \geq -20$. The final stage converged to R_1 = 0.0368 (wR_2 = 0.0853) for 15714 observed reflections [with $I > 2\sigma(I)$] and 817 variable parameters and R_1 = 0.0379 (wR_2 = 0.0856) for all unique reflections and GoF =

2.926.

*Crystal Data of Kaempulchraol D (**4**)*: $C_{20}H_{32}O_2$, M = 304.46, orthorhombic, space group $P2_12_12$, a = 20.378 (2) Å, b = 22.837 (3) Å, c = 11.6754 (13) Å, V = 5433.4 (11) Å^3, Z = 12, D_{calcd} = 1.117 g/cm^3, T = 100 K, $F(000)$ = 2016, and μ (Mo $K\alpha$) = 0.070 mm^{-1}. A total of 29692 reflections (11243 unique, R_{int} = 0.0446) were collected from 1.33° to 26.52° in θ and index ranges $25 \geq h \geq -25$, $28 \geq k \geq -28$, $14 \geq l \geq -8$. The final stage converged to R_1 = 0.0579 (wR_2 = 0.1457) for 9809 observed reflections [with $I > 2\sigma(I)$] and 613 variable parameters and R_1 = 0.0667 (wR_2 = 0.1516) for all unique reflections and GoF = 0.945.

*Crystal Data of Kaempulchraol E (**5**)*: $C_{20}H_{32}O_2$, M = 304.46, monoclinic, space group $P2_1$, a = 7.5976 (5) Å, b = 11.1628 (7) Å, c = 42.287 (3) Å, α = 90°, β = 90°, γ = 90°, V = 3586.4 (4) Å^3, Z = 8, D_{calcd} = 1.128 g/cm^3, T = 100 K, $F(000)$ = 1344, and μ (Mo $K\alpha$) = 0.070 mm^{-1}. A total of 22137 reflections (15455 unique, R_{int} = 0.0371) were collected from 0.96° to 29.21° in θ and index ranges $10 \geq h \geq -10$, $14 \geq k \geq -14$, $39 \geq l \geq -57$. The final stage converged to R_1 = 0.0650 (wR_2 = 0.1681) for 14617 observed reflections [with $I > 2\sigma(I)$] and 817 variable parameters and R_1 = 0.0679 (wR_2 = 0.1707) for all unique reflections and GoF = 0.987.

In Vitro Antiproliferative Activity. The cell lines used were A549 (human lung cancer), HeLa (human cervix cancer), TIG-3 (normal human primary fibroblast cell), PANC-1 and PSN-1 (human pancreatic cancer), and MDA-MB-231 (human breast cancer). The α-minimum essential medium with L-glutamine and phenol red (α-MEM, Wako) was used for the first three cell lines whereas the high glucose Dulbecco's modified Eagle's medium with L-glutamine, phenol red, and sodium pyruvate (DMEM, Wako) were used for the latter ones. Both media were supplemented with 10% fetal bovine serum (FBS, Nichirei Bioscience) and 1% antibiotic antimycotic solution (Sigma-Aldrich).

The in vitro antiproliferative activity of crude extracts and isolated compounds was determined by the procedure as described previously.[18] Briefly, each cell line was seeded in

96-well plates (2×10^3 per well) and incubated either in α-MEM or DMEM at 37 °C under 5% CO_2 and 95% air for 24 h. After the cells were washed with PBS (Nissui Pharmaceuticals), serial dilutions of the tested samples were added. After 72 h incubation, the cells were washed with PBS, and 100 μL of α-MEM or DMEM containing 10% WST-8 cell counting kit (Dojindo; Kumamoto, Japan) solution was added to the wells. After 2 h incubation, the absorbance at 450 nm was measured. The different concentrations of serial dilution of tested samples were 100–3.125 μg/mL for crude extract, 100–3.125 μM for isolated compounds, and 10–0.3125 μM for positive control, respectively. Cell viability was calculated from the mean values of data from three wells by using the following equation and antiproliferative activity was expressed as IC_{50} (50% inhibitory concentration) value. 5-Fluorouracil was used as positive control. The IC_{50} values for the antiproliferative activity of 5-fluorouracil against A549, HeLa, PANC-1, PSN-1, MDA-MB-231, and TIG-3 were 2.8, 5.8, 3.7, 4.4, 5.2, and 8.4 μM respectively.

$$(\%)\ \text{Cell viability} = 100 \times [\{Abs_{(\text{test samples})} - Abs_{(\text{blank})}\}/\{Abs_{(\text{control})} - Abs_{(\text{blank})}\}]$$

ASSOCIATED CONTENT

Supporting Information

The structures of reported compounds **9–13** (Figure S1), the COSY and key HMBC ($^1H \rightarrow {}^{13}C$) correlations of compounds **1–8** (Figure S2), ^1H and ^{13}C NMR, $^1H^1$–H COSY, HMQC, HMBC, and NOESY spectra of compounds **1–8** (Figure S3–Figure S50). CIF files and X-ray crystallographic data of kaempulchraols A–E (**1–5**). This material is available free of charge via the internet at http://pubs.acs.org.

AUTHOR INFORMATION

Corresponding Author

*Tel: +81 76 434 7625. Fax: +81 76 434 5059. E-mail: hmorita@inm.u-toyama.ac.jp (H.

Morita).

Notes

The authors declare no competing financial interest.

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Table 1. ^{1}H NMR Spectroscopic Data (600 MHz, $CDCl_3$) of Kaempulchraols A–D (1–4), (δ in ppm and *J* Values in (Hz) in Parentheses)

Position	1	2	3	4
1α	1.14, m	1.02, m	1.12, m	1.03, m
1β	1.69, m	1.67[a], m	1.71, m	1.67[a], m
2α	1.49, m	1.47, m	1.50[a], m	1.48, m
2β	1.65, m	1.67[a], m	1.67, m	1.67[a], m
3α	1.16, m	1.14, m	1.17, m	1.17, m
3β	1.39[a], m	1.38[a], m	1.40, m	1.39, m
5α	1.27, br s	1.13, br s	1.23[a], m	1.16, br s
6α	4.54, t (5.0)	4.50, d (5.0)	4.54, br s	4.53, d (5.1)
7α	2.78, m	2.30, m	2.75, m	2.31, m
7β	1.94[a], m	2.36, m	2.01[a], m	2.50, m
11α	2.13, m	1.91, m	2.17, m	1.99[a], m
11β	1.94[a], m	1.99, m	2.01[a], m	1.99[a], m
12α	1.69, m	1.62, m	1.74, m	1.43, m
12β	1.39[a], m	1.38[a], m	1.50[a], m	1.58, m
14α		2.93, br s		3.37, br s
14β	3.13, s		3.61, d (6.1)	
15	6.07, dd (16.5, 12.0)	5.77, dd (17.6, 11.1)	5.97, dd (17.7, 10.9)	5.74, dd (17.6, 11.1)
16-a	5.07, dd (16.5, 1.5)	4.94, dd (17.6, 1.3)	5.15, dd (17.7, 1.5)	4.96, dd (17.6, 1.4)
16-b	5.05, dd (12.0, 1.5)	4.96, dd (11.1, 1.3)	5.20, dd (10.9, 1.5)	4.97, dd (11.1, 1.4)
17	1.03, s	1.10, s	1.05, s	1.07, s
18	1.23, s	1.23, s	1.23[a], s	1.22, s
19	0.98, s	0.99, s	0.98, s	0.99, s
20	1.38, s	1.37, s	1.39, s	1.36, s
OMe-14	3.46, s	3.50, s		

[a]Overlapping resonances within the same column, δ values were measured from HMQC spectrum.

Table 2. ^{1}H NMR Spectroscopic Data (600 MHz, $CDCl_3$) of Kaempulchraols E−H (5−8), (δ in ppm and *J* Values in (Hz) in Parentheses)

Position	**5**	**6**	**7**	**8**
1α			1.10, m	
1β	3.64, br s	3.75, br s	1.74[a], m	3.64, br s
2α	1.56, dq (14.4, 3.6)	2.05[a], q (2.9)	1.53, m	2.01, dt (15.1, 2.7)
2β	1.92, tq (14.4, 2.2)	2.05[a], q (2.9)	1.74[a], m	2.10, dt (15.1, 3.3)
3α	1.70, td (13.8, 3.5)		1.16, m	
3β	1.14, dt (13.4, 3.4)	3.57, br s	1.42, m	3.52, br s
5α	1.47, d (1.7)	1.77, dd (12.5, 2.7)	1.12, br s	1.78, d (1.7)
6α	4.36, d (2.3)	1.60, m	4.60, d (4.05)	4.33, d (2.5)
6β		1.43, m		
7α	2.33[a], m	2.10, m	2.41, m	2.40, dq (14.4, 1.9)
7β	2.23[a], m	2.28, m	2.54, m	2.26, dd (14.4, 2.7)
9 α	2.37, br d (7.1)	2.41, t (7.2)		2.52, t (7.0)
11α	1.66, m	1.67, m	2.32[a], m	1.66, m
11β	1.60, m	1.50, m	2.32[a], m	1.57, m
12α	1.42, m	1.42−1.48[a], m	1.95, m	1.42, m
12β	1.49, m	1.42−1.48[a], m	1.84, m	1.49, m
14	5.43, br t (1.7)	5.28, br s		5.44, br s
15	5.77, dd (17.7, 10.3)	5.79, dd (17.4, 10.5)	5.80, dd (17.5, 10.7)	5.77, dd (17.7, 10.3)
16-a	4.92, dd (17.7, 1.5)	4.92, dd (17.4, 1.4)	4.90, dd (17.5, 0.8)	4.93, dd (17.7, 1.4)
16-b	4.90, dd (10.3, 1.5)	4.89, dd (10.5, 1.4)	5.03, dd (10.7, 0.8)	4.91, dd (10.3, 1.4)
17	1.09[a], s	1.05, s	1.18, s	1.09, s
18	1.26, s	0.89, s	1.24, s	1.27, s
19	1.03, s	1.03, s	1.00, s	1.12, s
20	1.09[a], s	0.79, s	1.47, s	1.08, s

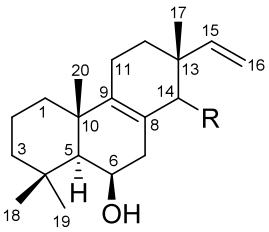
[a]Overlapping resonances within the same column, δ values were measured from HMQC spectrum.

Table 3. ^{13}C NMR Spectroscopic Data (150 MHz, $CDCl_3$) of Kaempulchraols A–H (1–8), (δ in ppm)

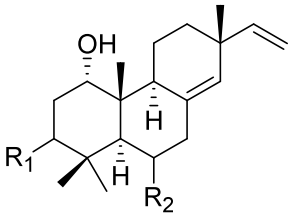
Position	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**
1	40.18	39.7	40.1	39.7	73.4	73.8	38.9	75.3
2	19.4	19.2	19.3	19.4	26.1	30.0	19.1	29.9
3	43.3	43.2	43.3	43.3	36.0	78.1	42.9	79.4
4	34.3	34.2	34.2	34.3	34.1	38.0	34.3	38.8
5	53.6	53.9	53.7	53.9	50.1	42.6	53.1	44.4
6	66.1	65.9	65.8	65.9	68.9	22.4	64.9	69.1
7	40.7	40.7	40.5	40.6	45.8	35.8	35.8	45.9
8	123.7	122.7	123.9	123.9	134.0	137.6	126.0	133.9
9	140.7	141.5	140.8	141.8	42.7	43.5	164.4	42.6
10	37.8	37.4	37.7	37.7	40.0	42.5	39.1	43.5
11	20.4	21.2	20.4	21.3	18.0	18.3	22.1	17.9
12	31.4	30.4	30.6	29.4	34.3	34.6	35.5	34.3
13	40.17	40.5	40.3	39.8	37.9	37.6	47.7	37.9
14	88.9	85.2	77.5	74.8	133.0	129.3	202.1	133.1
15	144.4	144.5	142.9	144.1	148.4	149.2	140.9	148.4
16	112.4	112.2	115.5	112.8	110.9	110.3	114.5	110.9
17	22.9	23.3	23.0	23.6	26.8	26.2	24.5	26.8
18	24.3	23.8	24.1	24.1	24.4	22.7	24.1	24.2
19	33.7	33.6	33.7	33.8	33.9	28.7	33.6	28.8
20	21.9	21.4	21.6	21.4	19.0	15.3	20.7	18.6
OMe-14	62.1	61.8						

δ Values were measured from HMQC spectrum.

Chart 1. Structures of kaempulchraols A–H (**1**–**8**).

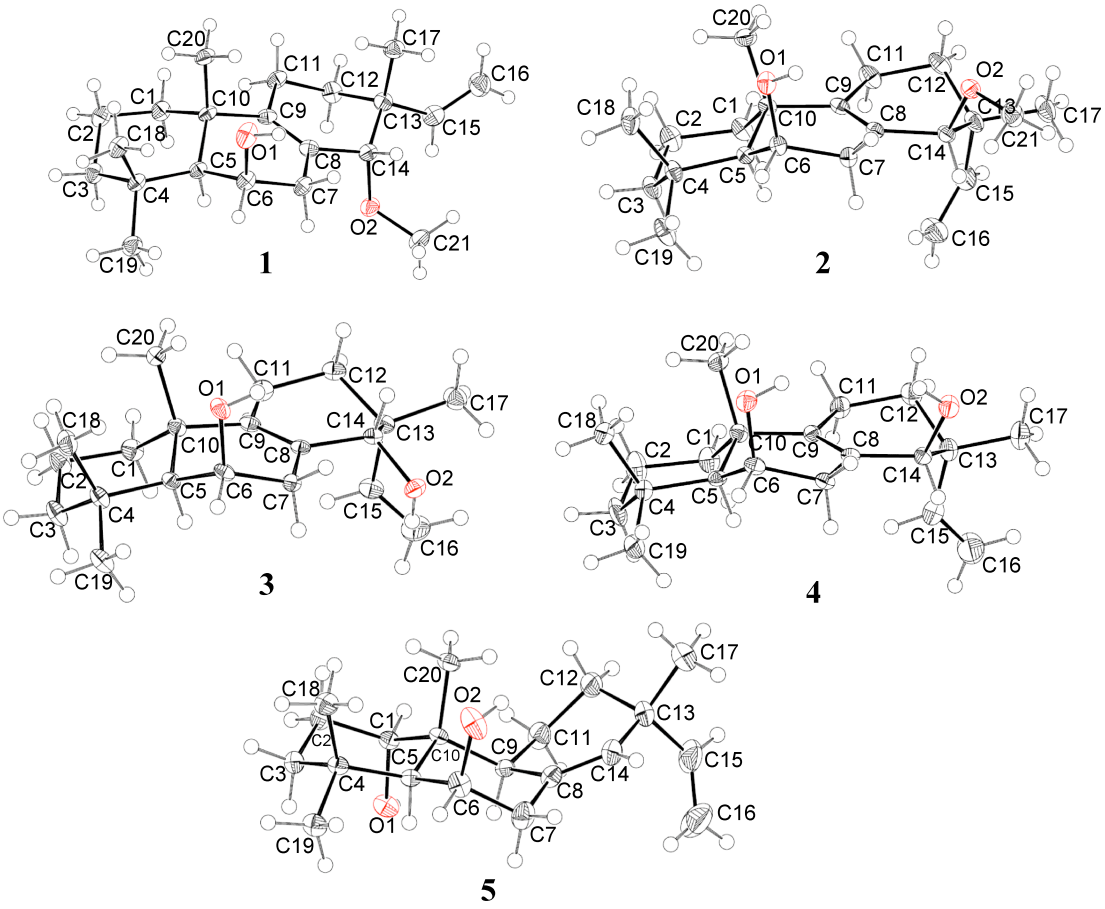


	R
1	α-OMe
2	β-OMe
3	α-OH
4	β-OH
7	=O



	R_1	R_2
5	H	β-OH
6	α-OH	H
8	α-OH	β-OH

Figure 1. X-ray crystallographic structures of kaempulchraols A–E (**1**–**5**).



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