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Two new cyclopentenones and a new furanone from *Baeckea frutescens* and their cytotoxicities

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ABSTRACT

Two new cyclopentenones, frutescencenones A (**1**) and B (**2**), and a new furanone derivative, frutescencenone C (**3**), together with two known cyclopentenones (**4** and **5**), were isolated from the leaves of *Baeckea frutescens*. Their structures were deduced by comprehensive spectroscopic analyses, including 1D and 2D NMR, and HREIMS data. Frutescencenone A (**1**) showed moderate growth inhibitory activity against human lung A549, pancreatic PSN-1, and breast MDA-MB-231 cancer cell lines, with IC_{50} values of 36.3 μM, 38.2 μM, and 29.3 μM, respectively. In contrast, frutescencenone C (**3**) showed selective cytotoxic activity against PSN-1, with an IC_{50} value of 20.1 μM.

1. Introduction

Baeckea frutescens L. (Myrtaceae), popularly known as "Jungrahab", is a commonly used folk medicine in Indonesia [1]. The aerial parts of *B. frutescens* are traditionally employed as remedies for influenza, malaria, fever, headache, abdominal pain, and dysentery [2–4]. In Hong Kong, *B. frutescens* is reportedly effective in external usage, such as for rheumatism and snake bites [5]. This plant is known as a rich source of sesquiterpenes, flavonoids, flavanones, phloroglucinols, chromones, and chromanones [4–12]. Previous studies on the biological activities of the isolated compounds have revealed their biological effects, such as the cytotoxic activities of the phloroglucinol derivative, BF-2, and the flavanone derivatives, BF-4 and BF-5 [6–7], the anti-inflammatory activity of the *C*-methylated biflavonoid, beackenin I [8], and the copper-induced low-density lipoprotein oxidation inhibitory activity of biflavonoid glycosides [9]. In addition, our previous phytochemical investigation of this species led to the isolation of new phloroglucinols, including baeckenone B with antibacterial activity against *Bacillus subtilis* [13] and baeckenone F with cytotoxicities against human pancreatic (PSN-1), lung (A549), and breast (MDA-MB-231) cancer cell lines [14]. In our ongoing research for the discovery of new bioactive constituents from *B. frutescens* collected in Indonesia, two new cyclopentenones, frutescencenones A (**1**) and B (**2**), and a new furanone, frutescencenone C (**3**), were isolated from the leaves of *B. frutescens*, together with two known cyclopentenones (**4** and **5**) (Fig. 1) [5,15]. Herein, we report the isolation and structure determination of these compounds, as well as their cytotoxic activities against human cancer cell lines.

1.1. General experimental procedures

IR spectra were measured using a JASCO IR-460 plus spectrophotometer (Japan Spectroscopic Co., Ltd., Tokyo, Japan). UV–vis spectroscopy spectra were recorded on a NanoDrop™ 2000C spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA). Optical rotations were determined using a JASCO DIP-140 digital polarimeter. NMR spectra were recorded on a Varian UNITY 600 MHz spectrometer in $CDCl_3$, with TMS (Wako Pure Chemical Industries, Ltd., Osaka, Japan) as an internal standard. The HREIMS data were measured on a JEOL MStation JMS-700 spectrometer (JEOL Ltd., Tokyo, Japan). Medium Pressure Liquid Chromatography (MPLC) was performed on a Büchi Sepacore system (Büchi Labortechnik AG, Flawil, Switzerland) with silica gel (60 N, spherical, neutral, 40–50 μm, Kanto Chemical Co., Inc., Tokyo, Japan) and Cosmosil 75C_{18}-OPN (Nacalai Tesque Inc., Kyoto, Japan). TLC was performed on precoated silica gel

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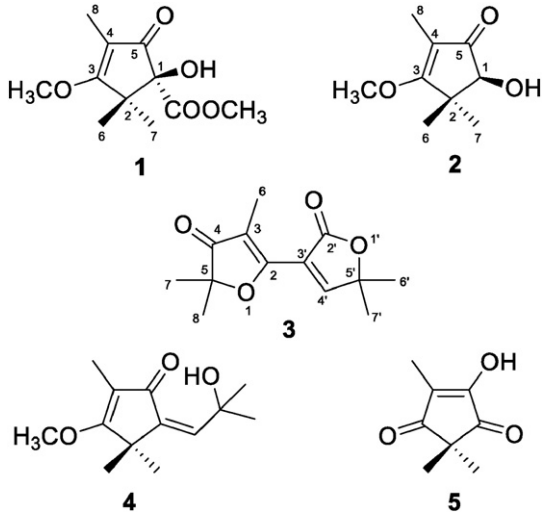

Fig. 1. The structures of **1**–**5**.

60F_{254} plates (0.25 or 0.50 mm thickness, Merck KGaA, Darmstadt, Germany). Preparative TLC was performed in vertical type rectangular chambers (Yazawa Scientific Co., Ltd., Tokyo, Japan), under conditions saturated with the developing solvent. The cell lines, PSN-1 (human pancreatic cancer), A549 (human lung cancer), and MDA-MB-231 (human breast cancer), were available and maintained in our laboratory. Cell culture flasks and 96-well plates were purchased from Corning, Inc. (Corning, NY, USA). An SH-1200 microplate reader (Corona Electric Co., Ltd., Hitachinaka, Japan) was used to measure the absorbance of the cells in the cytotoxic activity assay.

1.2. Plant material

The leaves of *B. frutescens* were purchased in Pasar Gedhe Market (Solo, Indonesia) and identified by Saifudin Azis, Ph.D. (School of Pharmacy, Universitas Muhammadiyah Surakarta, Indonesia). The voucher specimen (28298) was deposited at the Museum of Materia Medica, Analytical Research Center for Ethnomedicines, Institute of Natural Medicine, University of Toyama, Toyama, Japan.

1.3. Extraction and isolation

Dried leaves of *B. frutescens* were ground to a fine powder (425 g), and were exhaustively macerated with $CHCl_3$ (3 × 1.5 L) in an ultrasonic bath, for 90 min each at room temperature. After filtration of the suspension, the resulting solution was evaporated under reduced pressure to give the $CHCl_3$ extract (41 g). The extract was subjected to normal phase MPLC (1.85 kg; 40–50 μm; 100 × 460 mm; flow rate = 25 mL min^{-1}), eluted with *n*-hexane–EtOAc by gradually increasing the polarity system (from 1:0 to 0:1), to give ten fractions (Fr. 1–10). Fr. 3 (1.6 g) was separated by reversed phase MPLC (120 g; 36 × 160 mm), eluted with an isocratic mobile phase (MeCN–MeOH–H_2O = 4:3:3, v/v), and then purified by normal phase preparative TLC (200 × 200 mm, 185 mm separation distance, *n*-hexane–EtOAc = 1:1) to afford compounds **1** (1.5 mg) and **2** (3.7 mg). Fr. 5 (15.6 g) was further separated by reversed phase MPLC (120 g; 36 × 160 mm), eluted with an isocratic mobile phase (MeCN–MeOH–H_2O = 4:3:3, v/v), and reversed phase preparative TLC (200 × 200 mm, 185 mm separation distance, MeCN–MeOH–H_2O = 4:3:3) to furnish compounds **3** (2.3 mg) and **5** (7.0 mg). Fr. 4 (1.6 g) was subjected to reversed phase MPLC (120 g; 36 × 160 mm), eluted with an isocratic mobile phase (MeCN–MeOH–H_2O = 4:3:3, v/v), and then further separated by normal phase preparative TLC (200 × 200 mm, 185 mm separation distance, *n*-hexane–EtOAc = 1:1) to obtain compound **4** (7.0 mg). The structures of the known compounds **4** and **5** were elucidated using 1D and 2D NMR spectra, and MS, and compared with published data.

Frutescencenone A (**1**): Pale yellow amorphous solid; $[\alpha]_D^{25}$ +31 (*c* 0.1, $CHCl_3$); UV (MeOH): 233, 270 (nm). IR (KBr) *ν* (cm^{-1}): 3421, 2926, 1740, 1609, 1383, 1334, 1125, 595. HREIMS: *m/z* 228.1002 $[M]^+$ (calcd. for $C_{11}H_{16}O_5$, 228.0998); ^1H and ^{13}C NMR (see Table 1).

Frutescencenone B (**2**): Pale yellow amorphous solid; $[\alpha]_D^{25}$ +26 (*c* 0.1, $CHCl_3$); UV (MeOH): 257 (nm). IR (KBr) *ν* (cm^{-1}): 3421, 1734, 1607, 1388, 1313, 1026, 1003. HREIMS: *m/z* 170.1528 $[M]^+$ (calcd. for $C_9H_{14}O_3$, 170.1518); ^1H and ^{13}C NMR (see Table 1).

Frutescencenone B (**3**): Pale yellow powder; UV (MeOH): 221, 308 (nm). IR (KBr) *ν* (cm^{-1}): 1752, 1699, 1408, 1211, 1113, 787. HREIMS: *m/z* 236.1048 $[M]^+$ (calcd. for $C_{13}H_{16}O_4$, 236.1049); ^1H and ^{13}C NMR (see Table 2).

1.4. Assay for cytotoxic activity against cancer cell lines

Cell viability in the presence or absence of the test compounds was determined using the standard WST-8 assay, as described previously [13,16]. Briefly, the exponentially growing cells were harvested, and 2×10^3 cells were suspended in 100 μL of medium (α-MEM or DMEM at 37 °C, under a 5% CO_2 and 95% air atmosphere) in each well of a 96-well plate. After the cells were incubated for 24 h and washed with PBS (Nissui Pharmaceuticals Co., Ltd., Tokyo, Japan), serial dilutions of the samples to be tested were added. After a 72 h incubation at 37 °C, the cells were washed with PBS, and 100 μL of α-MEM or DMEM containing 10% WST-8 cell counting kit solution (Dojindo Laboratories, Kumamoto, Japan) was added to the wells. The absorbance at 450 nm was measured after a 2 h incubation at 37 °C. The concentrations of the serial dilutions of the tested samples were 100–3.125 μM for the isolated compounds and 10–0.3125 μM for the positive control. Cell viability was calculated from the mean values of data from three wells by using the following equation, and cell viability was expressed as the IC_{50} (50% inhibitory concentration) value.

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

2. Results and discussion

Compound **1** was obtained as a pale yellow amorphous solid. The HREIMS of **1** exhibited a molecular ion peak at *m/z* 228.1002 $[M]^+$, corresponding to the molecular formula $C_{11}H_{16}O_5$ with four degrees of unsaturation. The IR spectrum showed absorption bands at 3421, 1740 and 1609 cm^{-1}, assignable to hydroxy, carbonyls, and double bond, respectively. The ^1H NMR spectral data of **1** (Table 1) displayed signals for two methoxy groups [δ_H 4.12 (s, 3-OC*H*$_3$) and 3.58 (s, –COOC*H*$_3$)], an allylic methyl group [δ_H 1.83 (s, H_3-8)], *gem*-dimethyl groups [δ_H 1.04 (s,

Table 1
^1H (600 MHz) and ^{13}C NMR (150 MHz) data of **1** and **2** (δ in ppm).

	1 in DMSO-d_6		**2** in $CDCl_3$	
No.	δ_C	δ_H, (mult, *J* in Hz)	δ_C	δ_H, (mult, *J* in Hz)
1	84.3		79.6	3.90 (s)
2	47.6		44.9	
3	186.8		187.0	
4	108.7		107.4	
5	200.8		205.6	
6	24.9	1.04 (s)	22.2	1.10 (s)
7	20.6	0.96 (s)	23.7	1.26 (s)
8	8.4	1.83 (s)	8.1	1.94 (s)
$COOCH_3$	172.3			
3-OCH_3	60.3	4.12 (s)	59.5	4.15 (s)
$COOCH_3$	52.4	3.58 (s)		
1-OH		6.12 (s)		