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Labdane diterpenoids from *Curcuma amada* rhizomes collected in Myanmar and their antiproliferative activities

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ABSTRACT

Four new labdane diterpenoids, 12*β*-hydroxy-15-norlabda-8(17),13(14)-dien-16-oic acid (**1**), (*E*)-15-ethoxy-15-methoxylabda-8(17),12-dien-16-al (**2**), (*E*)-15*α*-ethoxy-14*α*-hydroxylabda-8(17),12-dien-16-olide (**3**), and 15-ethoxy-12*β*-hydroxylabda-8(17),13(14)-dien-16,15-olide (**4**) were isolated from the methanol extract of *Curcuma amada* rhizomes collected in Myanmar, together with 13 known analogs. Their structures were elucidated by extensive spectroscopic techniques. All of the isolates were evaluated for their antiproliferative activities against a small panel of five different human cancer cell lines (A549, human lung cancer; HeLa, human cervical cancer; MCF7, human breast cancer; PANC-1 and PSN-1, human pancreatic cancer). Among the isolates, compounds **2** – **4**, **7**, **8**, **12**, and **17** showed mild antiproliferative activities with IC_{50} values ranging from 19.7 to 96.1 μM. (*E*)-14-Hydroxy-15-norlabda-8(17),12-dien-16-al (**11**) exhibited strong antiproliferative activities selectively against HeLa, PANC-1, and PSN-1 cells, with IC_{50} values of 5.88, 1.00, and 3.98 μM, respectively. These potencies were comparable to those of the positive control, 5-fluorouracil.

1. Introduction

Medicinal herbs belonging to the Zingiberaceae family are being increasingly recognized as useful complementary treatments for the prevention of cancer and HIV. One of the richest and most diverse regions for Zingiberaceae is Myanmar, with 24 genera and about 155 species. Among them, 24 species of *Curcuma* plants are widely distributed throughout Myanmar, and are used as food, spices, medicines, and aesthetics. *Curcuma amada* Roxb. is an important member of this genus and is commonly known as mango ginger, due to the raw mango-like aroma of the rhizome. This herbaceous perennial plant is locally known as Thayetkin [1], and has a long history of traditional uses ranging from folk medicine to culinary preparations. The fresh rhizomes of *C. amada* are consumed as a dipping vegetable. The rhizomes are also utilized in many herbal medicines and ethnomedicines for the treatment of skin diseases, stomach ailments, cough, inflammation, and rheumatism. Previous phytochemical studies of *C. amada* revealed the presence of labdane diterpenoids, *β*-sitosterol, and curcumin [2]. Most of the phytochemicals of *C. amada* are found in the genera *Curcuma* [3–7], *Alpinia* [8–17], *Hedychium* [18–22], *Zingiber* [23], and *Aframomum* [24–25]. The various pharmacological actions, such as antioxidant, antibacterial, antifungal, anti-inflammatory, antiallergic, biopesticide, hypoglyceredemic, and cytoxic activites of *C. amada* have been reported [26]. In our ongoing research for the discovery of bioactive compounds from Myanmar's natural resources [27–33], we performed biological and phytochemical studies of *C. amada*. Herein, the isolation, structural elucidation, and antiproliferative activities of the isolated compounds are described.

2. Experimental

2.1. General experimental procedures

Optical rotations were recorded on a Jasco P2100 polarimeter. UV spectra were measured on a Shimadzu UV-160A spectrophotometer. 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) on a Varian UNITY 600 spectrometer. Chemical

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shift values were expressed in δ (ppm) downfield from TMS as an 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 60 N, spherical, neutral, 40 − 50 μm, Kanto Chemical Co., Inc., Japan) and Cosmosil 75C18-OPN (Nacalai Tesque Inc., Kyoto, Japan). TLC was performed on precoated silica gel 60F_{254} and RP-18 F_{254} plates (Merck, 0.25 or 0.50 mm thickness). The cell lines, including A549, human lung cancer; HeLa, human cervical cancer; MCF7, human breast cancer; and PANC-1 and PSN-1, human pancreatic cancer, were available and maintained in our laboratory. Cell culture flasks and 96-well plates were from Corning Inc. (Corning, NY, USA). An SH-1200 Microplate Reader® (Corona, Hitachinaka, Japan) was used to measure the absorbance of the cells in the antiproliferative activity assay.

2.2. Plant material

The fresh rhizomes of *C. amada* (2.5 kg) were collected from Kyaikmaraw Township, Mon State, Myanmar, in September 2016 and identified by Dr. Tin Nwe Ni, a lecturer in the Department of Botany, University of Yangon. The rhizomes were cut into small pieces and dried at room temperature (300 g). A voucher specimen (TMPW 27645) was deposited at the Museum for Materia Medica, Analytical Research Center for Ethnomedicines, Institute of Natural Medicine, University of Toyama, Japan.

2.3. Extraction and isolation

The dried rhizomes of *C. amada* (300 g) were sonicated in MeOH (2 L, 90 min, × 3) at 30 °C, and the solvent was evaporated under reduced pressure to give 35 g of extract. The methanol extract (34 g) was chromatographed on silica gel with an EtOAc − *n*-hexane solvent system to give six fractions [1: EtOAc − *n*-hexane (0:100) eluate, 1.00 g; 2: EtOAc − *n*-hexane (10:90) eluate, 1.50 g; 3: EtOAc − *n*-hexane (20:80) eluate, 5.50 g; 4: EtOAc − *n*-hexane (30:70) eluate, 5.34 g; 5: EtOAc − *n*-hexane (50:50) eluate, 10.6 g; 6: EtOAc − *n*-hexane (100: 0) eluate, 3.13 g]. Fraction 1 was an oily substance. Fraction 2 (1.50 g) was rechromatographed on Cosmosil 75C18-OPN with acetone − MeCN − MeOH − H_2O (2:1:1:1) to give three subfractions [2-1: 315 mg; 2-2: 77 mg; 2-3: 101 mg]. Compounds **5** (4.7 mg) and **6** (2.1 mg) were obtained from silica gel column chromatography of subfraction 2-1 (315 mg) with *n*-hexane − EtOAc (9:1), followed by normal-phase preparative TLC (pTLC) using benzene − CH_2Cl_2 (2:1) as the mobile phase. Fraction 3 (5.50 g) was rechromatographed on Cosmosil 75C18-OPN with acetone − MeCN − MeOH − H_2O (2:1:1:1) to afford four subfractions [3-1: 84.5 mg; 3-2: 2.87 g; 3–3: 284 mg; 3-4: 1.5 g]. Subfraction 3-2 (2.87 g) was separated by silica gel column chromatography with CH_2Cl_2 − EtOAc (9:1, 8:2, 7:3) to give three subfractions [3-2-1: 250 mg; 3-2-2: 64 mg; 3-2-3: 240 mg]. Purification of subfraction 3-2-1 (250 mg) by normal-phase pTLC, using *n*-hexane − EtOAc (9:1), gave **7** (15 mg) and **8** (15 mg). Compounds **9** (11 mg) and **10** (35 mg) were obtained from the purification of subfraction 3-2-2 (64 mg) by normal-phase pTLC with *n*-hexane − EtOAc (9:1). Purification of subfraction 3–2-3 (240 mg) by normal-phase pTLC, using *n*-hexane − EtOAc (9:1), afforded **2** (9.7 mg), **11** (2.0 mg), and **12** (2.1 mg). Fraction 4 (5.34 g) was rechromatographed on Cosmosil 75C18-OPN with acetone − MeCN − MeOH − H_2O (2:1:1:1) to afford three subfractions [4-1: 94 mg; 4-2: 429 mg; 4-3: 1.15 g]. Rechromatographic separation of subfraction 4-2 (429 mg) by silica gel column chromatography using *n*-hexane − EtOAc (4:1), followed by reverse-phase pTLC using MeOH − H_2O (9:1), afforded **1** (2.0 mg) and **13** (15 mg). Subfraction 4-3 (1.15 g) was rechromatographed on a silica gel column, using *n*-hexane − acetone (9:1, 7:1, 4:1), to give three subfractions [4-3-1: 385 mg; 4-3-2: 282 mg; 4-3-3: 238 mg]. Further purification of subfraction 4-3-2 (282 g) by normal-phase pTLC, using *n*-hexane − CH_2Cl_2-acetone (1:1:0.1), yielded **3** (2 mg), **14** (1.5 mg), **15** (2.0 mg), **4** (2.0 mg), **16** (6.0 mg), and **17** (6.3 mg).

Table 1
^{1}H (600 MHz, $CDCl_3$) and ^{13}C (150 MHz, $CDCl_3$) NMR spectroscopic data for **1** (δ in ppm and *J* values in (Hz) in parentheses).

Position	δ_H	δ_C
1α	1.08, m	38.9
1β	1.71, m	
2α	1.50, m	19.3
2β	1.57, m	
3α	1.20, m	42.0
3β	1.39, m	
4		33.5
5α	1.18, d (2.6)	55.4
6α	1.75, m	24.3
6β	1.34, m	
7α	2.05, m	38.2
7β	2.42, m	
8		148.7
9α	2.09, dd (13.4, 6.8)	52.2
10		39.2
11α	1.73,[a] m	31.1
11β	1.73,[a] m	
12α	4.47, dd (6.8, 5.9)	69.9
13		142.6
14a	6.35, br t (1)	126.7
14b	5.92, br t (1)	
16		169.8
17a	4.87, br t (1)	106.7
17b	4.60, br t (1)	
18	0.81, s	21.8
19	0.89, s	33.5
20	0.68, s	14.6

[a] Overlapping resonances within the same column.

12β-Hydroxy-15-norlabda-8(17),13(14)-dien-16-oic acid (**1**): colorless oil; $[\alpha]_D^{25}$ + 14 (*c* 0.1, $CHCl_3$); UV (MeOH) λ_{max} (log ε) 206 (3.77) nm; IR (KBr) ν_{max} 3445, 2932, 1705, 1680 cm^{-1}; ^{1}H and ^{13}C NMR data, see Table 1; EIMS *m/z* 306 $[M]^+$ (4); HREIMS *m/z* 306.2186 $[M]^+$ (calcd. for $C_{19}H_{30}O_3$: 306.2195).

(*E*)-15-Ethoxy-15-methoxylabda-8(17),12-dien-16-al (**2**): colorless oil; $[\alpha]_D^{25}$ + 11 (*c* 0.1, MeOH); UV (MeOH) λ_{max} (log ε) 227 (4.03) nm; IR (KBr) ν_{max} 2930, 1745, 1677, 1461, 1364, 1255, 1120 cm^{-1}; ^{1}H and ^{13}C NMR data, see Table 2; EIMS *m/z* 362 $[M]^+$ (5); HREIMS *m/z* 362.2823 $[M]^+$ (calcd. for $C_{23}H_{38}O_3$: 362.2821).

(*E*)-15α-Ethoxy-14α-hydroxylabda-8(17),12-dien-16-olide (**3**): colorless oil; $[\alpha]_D^{25}$ + 103 (*c* 0.1, MeOH); UV (MeOH) λ_{max} (log ε) 206 (3.91) nm; IR (KBr) ν_{max} 3469, 2935, 2386, 1767, 1657, 1461, 1341, 1194 cm^{-1}; ^{1}H and ^{13}C NMR data, see Table 2; EIMS *m/z* 362 $[M]^+$ (7); HREIMS *m/z* 362.2459 $[M]^+$ (calcd. for $C_{22}H_{34}O_4$: 362.2457).

15-Ethoxy-12β-hydroxylabda-8(17),13(14)-dien-16,15-olide (**4**): colorless oil; $[\alpha]_D^{25}$ + 39 (*c* 0.1, MeOH); UV (MeOH) λ_{max} (log ε) 225 (4.08) nm; IR (KBr) ν_{max} 3449, 2932, 1745, 1677, 1460, 1336, 1125, 1037 cm^{-1}; ^{1}H and ^{13}C NMR data, see Table 3; EIMS *m/z* 362 $[M]^+$ (7); HREIMS *m/z* 362.2451 $[M]^+$ (calcd. for $C_{22}H_{34}O_4$: 362.2457).

2.4. In vitro antiproliferative activity

The *in vitro* antiproliferative activities of the crude extracts and the isolated compounds against the A549 (human lung cancer), HeLa (human cervix cancer), MCF7 (human breast cancer), and PANC-1 and PSN-1 (human pancreatic cancer) cell lines were evaluated by the procedure described previously [27]. Briefly, each cell line was seeded in 96-well plates (2 × 10^3 per well) and cultured in either α-Minimum Essential Medium (α-MEM) or Dulbecco's Modified Eagle Medium (DMEM) at 37 °C, under a 5% CO_2 and 95% air atmosphere for 24 h. α-MEM with L-glutamine and phenol red (Wako) was used for the A549, HeLa, and MCF7 cell lines, and DMEM was used for the PANC-1 and PSN-1 cell lines. All media were supplemented with 10% fetal bovine