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PARVIFLORENE J, A CYTOTOXIC SESQUITERPENE DIMER WITH A NEW REARRANGED SKELETON FROM *CURCUMA PARVIFLORA*

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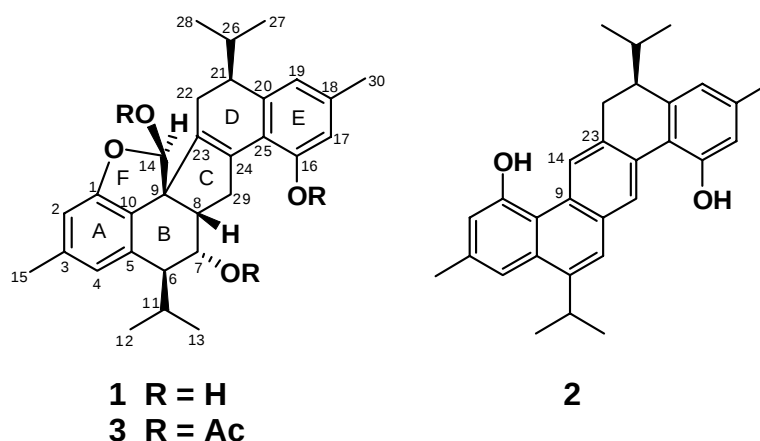
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Abstract – A new sesquiterpenoid dimer, parviflorene J, was isolated from *Curcuma parviflora*. The structure was elucidated using spectroscopic methods. Parviflorene J possessed an unprecedented backbone-rearranged skeleton and showed cytotoxicity against cultured tumor cell lines.

In our continuing research on the isolation of bioactive components from tropical plants,¹ we carried out phytochemical investigation on *Curcuma parviflora* Wall. (Zingiberaceae) collected in Thailand and isolated novel sesquiterpenoid dimers parviflorenes A-I, reported previously.²⁻⁴ Further separation of the extract afforded another new sesquiterpenoid dimer with an unprecedented backbone-rearranged skeleton, which was named as parviflorene J (**1**). In this paper, we report the isolation, structure elucidation, and cytotoxic activity of compound (**1**).

Parviflorenes A-I were previously isolated from the combined EtOAc and *n*-BuOH-soluble fractions of the MeOH extract of *C. parviflora*.²⁻⁴ Further fractionation of the remained materials using ODS HPLC and Sephadex LH-20 column chromatography yielded another new compound parviflorene J (**1**).

Parviflorene J (**1**) was obtained as an amorphous solid, $[\alpha]_D^{22}$ –116 (*c* 1.0, MeOH), and its molecular formula, C₃₀H₃₆O₄, was established by the observed peak at *m/z* 483.2490 (calcd for C₃₀H₃₆O₄Na [M+Na]⁺, 483.2511, Δ –2.1 mmu) in HRFABMS. Although the NMR spectral data showed some similarities with those of parviflorenes A-I,²⁻⁴ significant differences were also observed. The ¹H NMR



spectrum of **1** showed signals for two tertiary methyls attached on sp^2 carbons [δ_H 2.20 (3H, s, H₃-30) and 2.33 (3H, s, H₃-15)] and four aromatic ring protons (Table 1). The ^{13}C NMR spectrum aided by the HMQC experiment showed 14 sp^2 carbons and 16 sp^3 carbons (Table 1). Since 7 out of 13 unsaturation degrees were accounted for by the 14 sp^2 carbons, **1** was implied to have six rings. The 1H NMR also showed signals due to four secondary methyl groups, which were assigned to two isopropyl groups from the analysis of the 1H - 1H COSY spectrum (H₃-12/H-11/H₃-13 and H₃-27/H-26/H₃-28). Additionally, signals of a hemiacetal proton at δ_H 5.60 (s, H-14) and an sp^3 oxymethine at δ_H 4.19 (br s, H-7) were observed in the 1H NMR spectrum, corresponding to the carbons resonating at δ_C 107.6 and 73.8, respectively, in the ^{13}C NMR spectrum as shown in the HMQC spectrum. The long-range 1H - ^{13}C correlations in the HMBC spectrum together with the 1H - 1H COSY correlation data are summarized in Figure 1 and Table 1, analysis of which led to the planar structure of compound (**1**) as follows. The HMBC correlations were observed for H₃-15/C-2, H₃-15/C-3, H₃-15/C-4, H-2/C-1, H-2/C-10, and H-4/C-10; these data showed the presence of a tetra-substituted benzene (ring A) with a methyl group (C-15) attached on C-3. Then, the COSY correlations for H-6/H-7 and H-7/H-8 together with the HMBC correlation for H-6/C-4, H-6/C-5, H-7/C-9, H-8/C-9, H-8/C-14, and H-14/C-10 suggested the presence of a cyclohexene ring (ring B) connected with the ring A through the C-5/C-10 double-bond; an isopropyl group was located on C-6 (COSY: H-6/H-11; HMBC: H₃-12/C-6 and H₃-13/C-6), and the hemiacetal carbon [C-14: δ_C 107.6 and δ_H 5.60 (1H, s)] was attached on the C-9 quaternary carbon (δ_C 61.7). Another tetra-substituted benzene ring (ring E) was constructed in the same way from the HMBC correlations for H₃-30/C-17, H₃-30/C-18, H₃-30/C-19, H-17/C-16, H-17/C-25, H-19/C-25, H-21/C-19, and H-21/C-20, with a methyl group (C-30) attached on C-18. The COSY correlation for H-21/H₂-22 together with the HMBC correlations for H-22a/C-24, H-21/C-23, and H-21/C-25 suggested that a cyclohexadiene ring (ring D) was connected with ring E through the C-20/C-25 double-bond, and an isopropyl group was attached on C-21 as shown by the COSY (H-21/H-26) and HMBC (H₃-27/C-21 and H₃-28/C-21) correlations. All of the 14 sp^2 carbons were thus accounted for by two benzene rings A and

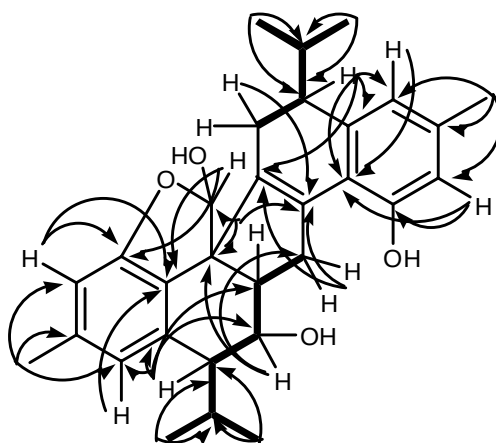


Figure 1. ^1H - ^1H COSY (bold lines) and key HMBC (arrows) correlations of parviflorene J (**1**)

Table 1. ^1H and ^{13}C NMR data and HMBC and NOESY correlations of compound (**1**) in CDCl_3

No.	δ_{H} (J in Hz)	δ_{C}	HMBC	NOESY
1		156.0		
2	6.61 s	109.3	C-1, C-4, C-5 ^a , C-10, C-15	H ₃ -15
3		139.0		
4	6.64 s	119.6	C-1 ^a , C-2, C-10, C-15	H-6, H ₃ -12, H ₃ -13, H ₃ -15
5		135.9		
6	2.31 m	44.4	C-3, C-4, C-5, C-7, C-8, C-11, C-13	H-4, H ₃ -12, H ₃ -13, H-22a
7	4.19 s	73.8	C-5, C-9, C-11, C-29	H-6, H-8, H ₃ -12, H ₃ -13, H-29a
8	2.97 dd 9.8, 4.0	42.7	C-6, C-7, C-9, C-14, C-24, C-29	H-7, H-29b
9		61.7		
10		124.4		
11	2.25 m	25.7	C-5, C-7, C-12, C-13	H ₃ -12, H ₃ -13
12	1.07 d 6.8	20.0	C-6, C-11, C-13	H-6, H-7, H-11
13	1.17 d 6.8	23.1	C-6, C-11, C-12	H-4, H-6, H-7, H-11
14	5.60 s	107.6	C-1, C-10	H-22b, H-27
15	2.33 s	22.3	C-2, C-3, C-4	H-2, H-4
16		151.9		
17	6.36 s	115.1	C-16, C-19, C-25, C-30	H ₃ -30
18		137.0		
19	6.47 s	123.0	C-16 ^a , C-17, C-21, C-25, C-30	H-21, H ₃ -28, H ₃ -30
20		141.3		
21	2.24 m	46.0	C-19, C-20, C-23, C-26	H-19, H-22a, H ₃ -27, H ₃ -28
22	(a) 1.90 m (b) 2.40 m	23.3	C-24	H-6, H-21 H-6, H-14
23		135.5		
24		130.9		
25		117.0		
26	1.83 m	29.8	C-21, C-22, C-27, C-28	H ₃ -27, H ₃ -28
27	0.92 d 6.8	20.2	C-21, C-26, C-28	H-14, H-21, H-22b, H-26
28	0.71 d 6.8	21.6	C-21, C-26, C-27	H-21, H-26
29	(a) 2.83 dd 17.6, 4.0 (b) 3.31 dd 17.6, 9.8	37.8	C-7, C-8, C-23, C-24 C-23, C-24	H-7 H-8
30	2.20 s	21.1	C-17, C-18, C-19	H-17, H-19

^a): 4-bonds correlations

E and a double bond at C-23/C-24 position (δ_{C} 135.5 and 130.9, respectively). The COSY correlation for H-8/H₂-29 and the HMBC correlations for H-8/C-9, H-8/C-24, H₂-29/C-23, and H₂-29/C-24 suggested that the C-8 (ring B) and the C-24 (ring D) was connected through an sp^3 methylene carbon [C-29: δ_{C} 37.8 and δ_{H} 2.83 (1H, dd, $J=17.6$ and 4.0 Hz) and 3.31 (1H, dd, $J=17.6$ and 9.8 Hz)]. Since the HMBC correlation was clearly observed from the H-14 hemiacetal proton to the C-1 aromatic carbon (δ_{C} 156.0), whose chemical shift implied that this C-1 aromatic carbon was oxygenated, a dihydrofuran ring (ring F) was constructed by C-1, C-10, C-9, C-14, and an ether oxygen atom. Since compound (**1**) was implied to have six rings (vide supra), the remaining two quaternary carbons (C-9 on ring B and C-23 on ring D) had to be connected by process of elimination to give rise to a cyclopentene ring (ring C). The whole planar structure of parviflorene J (**1**) was thus constructed, having an unprecedented bis-cadinane skeleton. The C-14/C-23 bond contained in most of parviflorenes [*e.g.*, parviflorene A (**2**)] was migrated to C-9/C-23 bond; thus parviflorene J (**1**) possesses a 23(14 $\rightarrow$ 9)*abeo*-skeleton. Acetylation of compound (**1**) afforded a triacetate (**3**, m/z 586 [M^+] in EI-MS). The ^1H NMR spectral data of **3** showed three acetyl methyl signals, and significant downfield shifts were observed for two acetoxy-bearing methine protons of **3** [δ_{H} 5.12 (H-7) and δ_{H} 6.48 (H-14)], compared to those of **1** [δ_{H} 4.19 (H-7) and 5.60 (H-14)].

The relative stereochemistry of parviflorene J (**1**) was elucidated by combination of NOESY and differential NOE experiments (Figure 2). The NOESY data were summarized in Table 1. Irradiation of H-7 showed NOEs in H-8 and H₃-12, and additionally, irradiation of H-8 showed NOE in H-7, indicating that these hydrogens were oriented in the same side in space. Since the H-7 signal was observed almost as a singlet in the ^1H NMR spectrum, its dihedral angles with both of H-6 and H-8 were almost 90 degrees and the hydroxyl group on C-7 was therefore axial. On the other hand, irradiation of H-14 showed NOEs in H-22b and H₃-27, while NOE was observed at H-14 on irradiation of H₃-27, thus

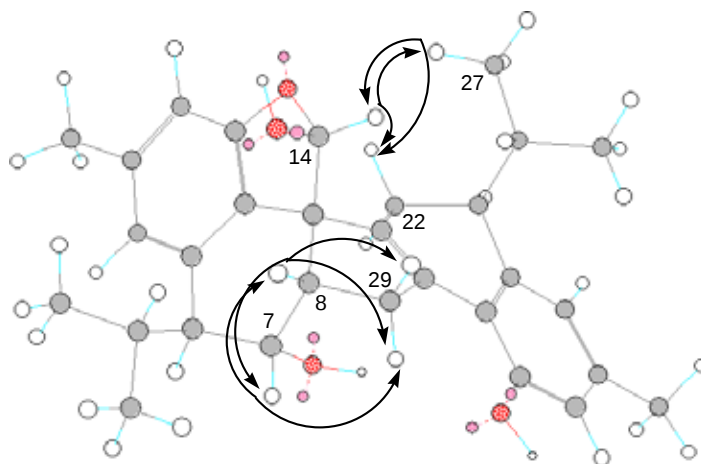


Figure 2. Key NOEs observed for parviflorene J (**1**)

suggesting that the H-14 was oriented close to ring D and the C-14 hydroxyl group oriented to ring B side. The B/C ring juncture at C-8 and C-9 had to be *cis* on the basis of the model consideration. Thus, the relative stereochemistry of parviflorene J was suggested as shown in structure formula (1). Although the CD spectral data of parviflorene J (1) (see, experimental section) were not similar to those of other parviflorenes such as 2,²⁻⁴ the absolute stereochemistry of these biscadinanes obtained from the same plant were assumed to be corresponding to each other.

The new compound, parviflorene J (1), exhibited cytotoxicity against Jurkat,⁵ and vincristine-resistant P388/VCR and KB/VJ-300⁶ cell lines with IC₅₀ values of 3.0, 0.54, and 2.4 µg/mL, respectively.

EXPERIMENTAL

Optical rotation was measured with a JASCO P-1020 polarimeter. CD spectrum was obtained in a JASCO J-720WI spectropolarimeter. EIMS was measured on a JEOL GC-Mate and HRFABMS on a JEOL HX-110A spectrometer. NMR spectra were recorded on JEOL JNM A500 and ECP600 spectrometers with a deuterated solvent which chemical shift was taken as an internal standard.

Extraction and isolation: The plant *Curcuma parviflora* was collected at Khon Kaen, Thailand. A voucher specimen has been deposited at Faculty of Agriculture, Khon Kaen University. Previous TLC examination revealed that the parviflorene-related compounds were contained in the underground part of this plant.³ The air-dried underground part (280 g) was extracted with MeOH and acetone. The combined extract (12.6 g) suspended in water (200 mL) was partitioned against EtOAc (400 mL×2 and 200 mL) and *n*-BuOH (200 mL×2). The EtOAc-soluble fraction (8.0 g) and previously obtained EtOAc and *n*-BuOH-soluble fractions (2.9 g) from the whole plant² were combined, and subjected to a silica gel column chromatography (column A, 4.5 × 57 cm) eluted with 0-100% EtOAc in hexane. The fraction (0.9 g) of column A eluted with 33-50% EtOAc in hexane was further separated by second silica gel column chromatography (3.5 × 21 cm) eluted with 0-100% EtOAc in hexane. From a fraction (126 mg) eluted with 33% EtOAc in hexane, a part (88 mg) was purified by an ODS HPLC with 85% MeOH (Mightysil RP18GP, 10 × 250 mm) to give compound (1) (10 mg). Compound (1) (9.6 mg) was also obtained from another fraction (412 mg) of column A eluted with 33-50% EtOAc in hexane after separation by a Sephadex LH-20 column chromatography (1.5 × 63 cm) eluted with MeOH followed by purification by HPLC under the same conditions as above.

Parviflorene J (1): amorphous solid; $[\alpha]_D^{22}$ -116° (*c* 1.0, MeOH); CD (0.743 mM, MeOH, 24°C) $\Delta\epsilon$ (nm): -4.9 (308), 0 (292), 14.1 (273), 0 (249), -16.0 (235), -8.6 (225) and -37.4 (210); UV $\lambda_{\max}$ (MeOH) 309 (log ϵ 4.0), 285 (4.1), 276 (4.1) and 204 (4.6); IR (film) $\nu_{\max}$ 3288, 2958, 2871, 1713, 1614, 1575 and 1456 cm⁻¹; EIMS *m/z* 460 (M⁺); HRFABMS (NBA/PEG) *m/z* 483.2490 (calcd for C₃₀H₃₆O₄Na 483.2511, Δ -2.1 mmu); ¹H and ¹³C NMR (Table 1).

Acetylation of 1: Compound (**1**) (1 mg) was treated with acetic anhydride (500 μ L) and pyridine (500 μ L) under stirring for 24 h at room temperature. After usual work up, 0.7 mg compound (**3**) was obtained. ^1H NMR (CDCl_3 , 500 MHz) δ : 0.82 (3H, d J = 6.8 Hz), 0.92 (3H, d J = 6.8 Hz), 0.93 (3H, d J = 6.8 Hz), 1.18 (3H, d J = 6.8 Hz), 1.78 (3H, s), 1.88 (1H, m), 2.00 (3H, s), 2.28 (3H, s), 2.35 (3H, m), 2.36 (3H, s), 2.35-2.43 (4H, m), 2.58 (1H, brd J = 17.0 Hz), 2.99 (1H, brd J = 9.4 Hz), 3.26 (1H, dd, J = 17.0, 9.0 Hz), 5.12 (1H, s), 6.48 (1H, s), 6.64 (1H, s), 6.67 (1H, s), 6.70 (1H, s), 6.80 (1H, s); EIMS m/z 586 (M^+).

Cytotoxic activity: For Jurkat cells, 3.5×10^5 cells/mL of the cells were seeded in 95 μ L of culture medium per well in 96-well microtitre plates, and were treated with 5 μ L of graded concentrations of samples in the absence or presence of 0.5 $\mu\text{g/mL}$ of TRAIL, and were then incubated for 42 h at 37°C in a 5% CO_2 -95% air atmosphere. Cell viability was determined by the colorimetric assay using alamer blue.⁵ KB/VJ-300 cells (1.2×10^4) in 195 μ L of culture medium were seeded in 96-well plates, and were pre-incubated for 24 h at 37 °C. The cells were treated with 5 μ L of graded concentrations of samples, and then incubated at 37 °C for 72 h. Cell viability was determined by the colorimetric assay using MTT.⁶

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