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

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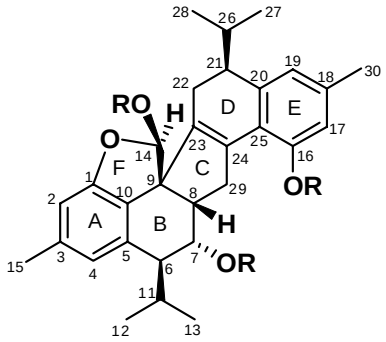
Abstract – A new sesquiterpenoid dimer, parviflorene J, was isolated from *Curcuma parviflora*. The structure was elucidated using spectroscopic methods. Parviflorene J possessed an unprecedented backbone-rearranged skeleton and showed cytotoxicity against cultured tumor cell lines.

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, $C_{30}H_{36}O_4$, was established by the observed peak at *m/z* 483.2490 (calcd for $C_{30}H_{36}O_4Na$ $[M+Na]^+$, 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



1 R = H
3 R = Ac

2

spectrum of **1** showed signals for two tertiary methyls attached on sp^2 carbons [δ_H 2.20 (3H, s, H_3-30) and 2.33 (3H, s, H_3-15)] 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 (H_3-12/H-11/H_3-13 and H_3-27/H-26/H_3-28). 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 H_3-15/C-2, H_3-15/C-3, H_3-15/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: H_3-12/C-6 and H_3-13/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 costructed in the same way from the HMBC correlations for H_3-30/C-17, H_3-30/C-18, H_3-30/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/H_2-22 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 (H_3-27/C-21 and H_3-28/C-21) correlations. All of the 14 sp^2 carbons were thus accounted for by two benzene rings A and

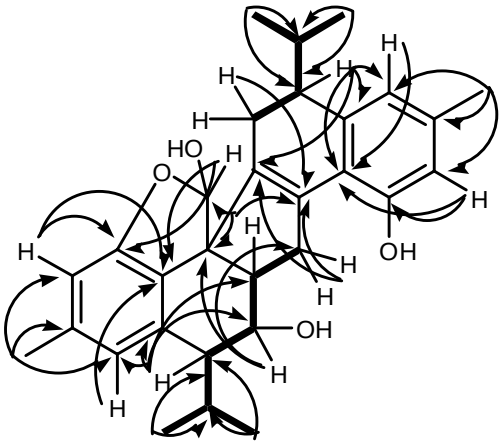


Figure 1. ^{1}H-^{1}H COSY (bold lines) and key HMBC (arrows) correlations of parviflorene J (**1**)

Table 1. ^{1}H 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/H_2-29 and the HMBC correlations for H-8/C-9, H-8/C-24, H_2-29/C-23, and H_2-29/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→9)*abeo*-skeleton. Acetylation of compound (**1**) afforded a triacetate (**3**, *m/z* 586 [M^+] in EI-MS). The ^{1}H 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 H_3-12, 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 ^{1}H 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 H_3-27, while NOE was observed at H-14 on irradiation of H_3-27, thus

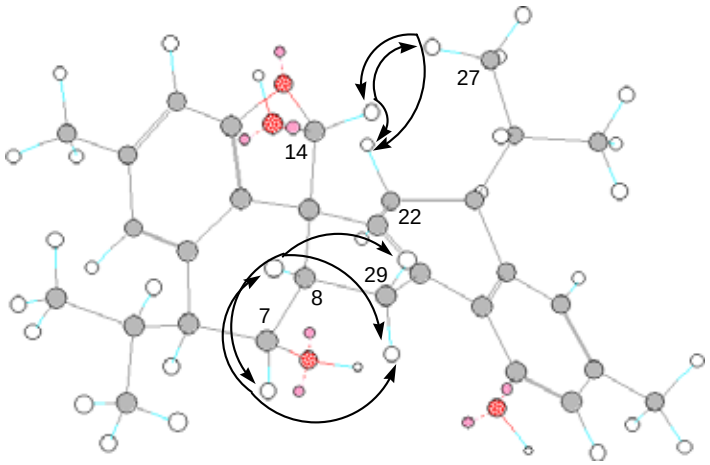


Figure 2. Key NOEs observed for parviflorene J (**1**)

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-300[6] cell lines with IC_{50} 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 plant[2] 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) Δε (nm): –4.9 (308), 0 (292), 14.1 (273), 0 (249), –16.0 (235), –8.6 (225) and –37.4 (210); UV λ_{max}(MeOH) 309 (logε 4.0), 285 (4.1), 276 (4.1) and 204 (4.6); IR (film) ν_{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 $C_{30}H_{36}O_4Na$ 483.2511, Δ –2.1 mmu); ^1H and ^{13}C NMR (Table 1).

Acetylation of 1: Compound (**1**) (1 mg) was treated with acetic anhydride (500 μL) and pyridine (500 μL) under stirring for 24 h at room temperature. After usual work up, 0.7 mg compound (**3**) was obtained. ^{1}H 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 μL of culture medium per well in 96-well microtitre plates, and were treated with 5 μL of graded concentrations of samples in the absence or presence of 0.5 μg/mL of TRAIL, and were then incubated for 42 h at 37°C in a 5% CO_2-95% air atomosphere. Cell viability was determined by the colorimetric assay using alamer blue.[5] KB/VJ-300 cells (1.2×10^4) in 195 μL 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 μL 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]

ACKNOWLEDGEMENTS

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