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Parviflorene A, a novel cytotoxic unsymmetrical sesquiterpene–dimer constituent from *Curcuma parviflora*

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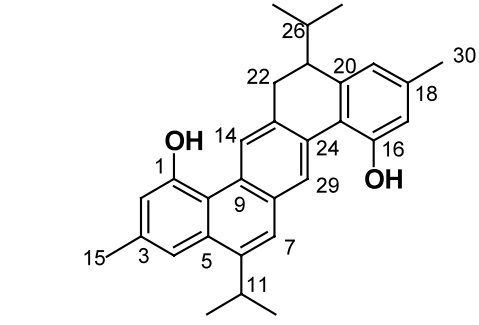
Abstract—A novel natural sesquiterpene–dimer-type compound, named parviflorene A (**1**), was isolated from the extract of a tropical Zingiberaceous plant, *Curcuma parviflora*, collected in Thailand, and its structure was elucidated by spectral data. Parviflorene A (**1**) possesses an unprecedented unsymmetrical bis-cadinane skeleton and exhibited cytotoxic activity against murine leukemia P388 and B16 melanoma cells.

Curcuma parviflora Wall. (Zingiberaceae) is widely distributed over the forest area of the northern part of Thailand,[1] and is used as an ornamental plant. It is edible, and also it has been said to be used for detoxification of scorpion bites in certain areas. During our search for bioactive natural products from tropical plants,[2] we investigated the chemical constituents of *C. parviflora* collected in Thailand. Here we describe the isolation and structure elucidation of a novel sesquiterpene–dimer-type compound, parviflorene A (**1**), together with two known sesquiterpenes, cadalenequinone (**2**)[3] and 8-hydroxycadalene (**3**),[4] corresponding to monomers for **1**. Parviflorene A (**1**) showed brine shrimp (*Artemia salina*) toxicity as well as cytotoxicity against murine leukemia P388 and B16 melanoma cells and possesses a previously unknown bis-cadinane carbon-skeleton.

The whole plant of *C. parviflora*, collected in Thailand, was extracted with MeOH, and the extract showed potent toxicity against *Artemia salina*[5,6] (almost 100% of the shrimps were killed at 1 mg/mL). The MeOH extract was subjected to solvent partitioning and repeated chromatographies on silica gel and Sephadex LH-20, guided by toxicity tests against *A. salina*, to give parviflorene A (**1**, 0.007% yield), together with cadalenequinone (**2**, 0.004% yield) and 8-hydroxy



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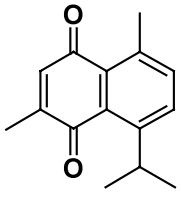

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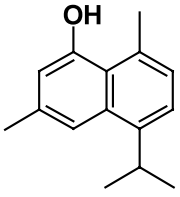

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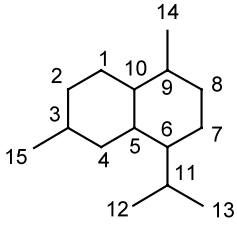

A

Keywords: Zingiberaceous plant; *Curcuma parviflora*; sesquiterpene; dimer; cytotoxic activity.

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cadalene (**3**, 0.002% yield). Cadalenequinone (**2**) was previously isolated from a Zingibraceous plant, *Stahlianthus campanulatus*,[3] whereas 8-hydroxycadalene (**3**) was known as a synthetic compound[4] and it has not been reported before as a natural product.

Parviflorene A (**1**) was obtained as a colorless amorphous solid; $[\alpha]_D^{24} = +58$ (*c* 0.20, MeOH), and was suggested to have the molecular formula $C_{30}H_{32}O_2$ by its HRFABMS data (*m*/*z* 424.2373, M^+, Δ −2.9 mmu). The UV spectrum of **1** showed absorption maxima at λ_{max} (MeOH) 225, 282, and 310 nm, indicating the presence of conjugated systems. The ^{1}H NMR spectrum of **1** showed signals due to two singlet methyls and four doublet methyls; the chemical shifts of the singlet methyls [δ_H 2.54 (3H, s) and 2.36 (3H, s)] implied that these two methyl groups were attached on sp^2 carbons. The ^{13}C NMR spectrum aided by HMQC experiments revealed the presence of twenty sp^2 carbons as well as ten sp^3 carbons. Since ten out of fifteen unsaturation degrees were thus accounted for, **1** was deduced to have five rings. The ^{1}H NMR of **1** showed seven proton signals of low-field resonance assignable to aromatic ring protons [δ_H 6.68 (1H, s), 6.72 (1H, s), 6.84 (1H, s), 7.60 (1H, s), 7.66 (1H, s), 8.47 (1H, s), and 9.40 (1H, s)]. The ^{1}H–^{1}H COSY spectrum of **1** suggested the presence of two isopropyl groups (H_3-12/H-11/H_3-13 and H_3-27/H-26/H_3-28). The methine proton (H-26) of the latter isopropyl group further showed a cross-peak to an sp^3 methine [H-21; δ_H 2.42 (1H, m)], which, in turn, showed connectivity to an sp^3 methylene group [H_2-22; δ_H 3.17 and 3.18 (each 1H, dd, J = 14.8 and 3.3 Hz)] in the ^{1}H–^{1}H COSY spectrum. The HMBC spectrum of **1** afforded long-range ^{1}H–^{13}C correlations as shown in Figure 1 and Table 1, which suggested that **1** consisted of four aromatic benzene

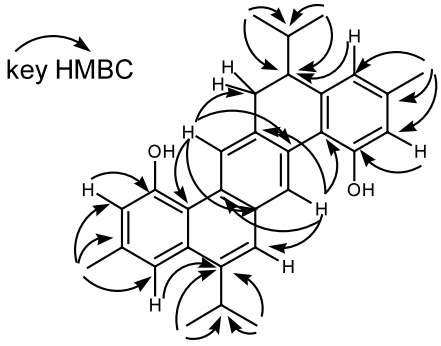

Figure 1. Key HMBC correlations.

Table 1. ^{1}H and ^{13}C NMR spectral data of compound **1** in $CDCl_3$

Position	δ_C	δ_H[a]	HMBC (C–H)	NOE (H–H)
1	154.7	–	H-2	–
2	114.2	6.84 s	H-4, H_3-15	H_3-15
3	135.9	–	H-2, H_3-15	–
4	116.8	7.60 s	H-2, H_3-15	H-11, H_3-12, H_3-13, H_3-15
5	133.9	–	H-4, H-7, H-11	–
6	141.1	–	H-4, H-11, H_3-12, H_3-13	–
7	123.9	7.66 s	H-11, H-29	H_3-12, H_3-13, H-29
8	131.3	–	H-14	–
9	128.1	–	H-7, H-29	–
10	117.6	–	H-2, H-4, H-14	–
11	28.9	3.68 m	H-7, H_3-12, H_3-13	H-4, H_3-12, H_3-13
12	23.4	1.44[b] d	H-11, H_3-13	H-4, H-7, H-11
13	23.3	1.48[b] d	H-11, H_3-12	H-4, H-7, H-11
14	127.5	9.40 s	H_2-22	H_2-22
15	21.7	2.54[b] s	H-2, H-4	H-2, H-4
16	153.0	–	H-17	–
17	115.7	6.72 s	H-19, H_3-30	H_3-30
18	137.7	–	H-17, H-19, H_3-30	–
19	123.0	6.68 s	H-17, H-21, H_3-30	H-21, H_3-27, H_3-30
20	144.1	–	H-19, H-21, H_2-22, H-26	–
21	47.3	2.42 m	H-19, H_2-22, H-26, H_3-27, H_3-28	H-19, H_2-22, H_3-27, H_3-28
22	33.9	(α) 3.18 dt (β) 3.17 dt	H-14	H-14, H-21, H_3-27, H_3-28
23	134.6	–	H-21, H_2-22, H-29	–
24	130.2	–	H-14, H_2-22	–
25	118.1	–	H-17, H-19, H-21, H-29	–
26	28.3	1.37 m	H-21, H_2-22, H_3-27, H_3-28	H-19, H_3-27, H_3-28, H_3-30
27	21.8	0.78[b] d	H-26, H_3-28	H-19, H-21, H-26, H_3-28
28	20.7	0.89[b] d	H-26, H_3-27	H-14, H-21, H_2-22, H-26, H_3-27
29	124.7	8.47 s	H-7	H-7
30	21.2	2.36[b] s	H-17, H-19	H-17, H-19

OH-1 and OH-16: δ_H 5.62 (1H, br s) and 5.76 (1H, br s).

[a] J-values in Hz: $J_{11,12} = 7.0$; $J_{11,13} = 7.0$; $J_{21,22\alpha} = 3.3$; $J_{21,22\beta} = 3.3$; $J_{22\alpha,22\beta} = 14.8$; $J_{21,26} = 9.9$; $J_{26,27} = 7.0$; $J_{26,28} = 7.0$.

[b] 3H.

rings (rings A, B, C, and E) and one cyclohexadiene (ring D). The positions of two methyl, two hydroxyl, and two isopropyl groups were clearly assignable by the HMBC correlations (Fig. 1 and Table 1). Particularly, the HMBC correlations for H-4/C-6 and H-19/C-21 as well as NOE observations for H-4/H_2-12, H-4/H_3-13, and H-19/H_3-27 provided evidence for the connections of the A/B and D/E rings. The pentacyclic skeleton suggested for **1** corresponded to an unsymmetric dimer of cadinane sesquiterpene (**A**); two new bonds are each formed between C-8 and C-14 of two cadinanes. The H-14 and H-29 were very close to the hydroxyl protons of C-1 and C-16, respectively, and they had extremely low-field resonances [δ_H 9.40 (H-14) and 8.47 (H-29)] in the ^{1}H NMR spectrum. The methine proton on C-21 was deduced to be equatorially oriented from the two small coupling constants with the vicinal methylene protons on C-22 ($J_{21,22\alpha} = J_{21,22\beta} = 3.3$ Hz), and the axial orientation of the isopropyl group (C-26/C-27/C-28) was also implied from the observation of NOE between H_3-28 and H-14. Although the CD spectrum of **1** exhibited a unique curve [λ_{ext} 327 nm ($\Delta\varepsilon$ −12.3), 275 (+18.5), 250 (−6.8), 233 (+17.2), and 214 (−3.7)], the absolute stereochemistry of the C-21 position remained undefined.

From the extract of *C. parviflora* two known cadinane sesquiterpenes, cadalenequinone (**2**) and 8-hydroxycadalene (**3**), were also isolated and identified by comparison of their spectral data with those in the literature.[3,4] 8-Hydroxycadalene (**3**) was previously known as a synthetic compound and was first isolated here as a natural product. Parviflorene A (**1**) was a novel bis-cadinane type compound with an unprecedented carbon skeleton, and the cadinane sesquiterpenes (**2** and **3**) may correspond to monomers of **1**. Parviflorene A (**1**) showed toxicity against *Artemia salina* (brine shrimp) with the LC_{50} value of ca. 10 μg/mL. Compound **1** also exhibited cytotoxity against murine leukemia P388 cells, and the IC_{50} values against vincristine (VCR)-resistant P388 cells in the presence and absence of 12.5 ng/mL of VCR were 3.2 and 3.0 μg/mL, respectively, while the IC_{50} value against a sensitive P388 strain was 3.2 μg/mL, indicating that compound **1** had no reversal effect of multidrug resistance.[7] Compound **1** also exhibited cytotoxicity against B16 melanoma cell with the IC_{50} value of 4.1 μg/mL.

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