Cadinane Sesquiterpenes from *Curcuma parviflora*

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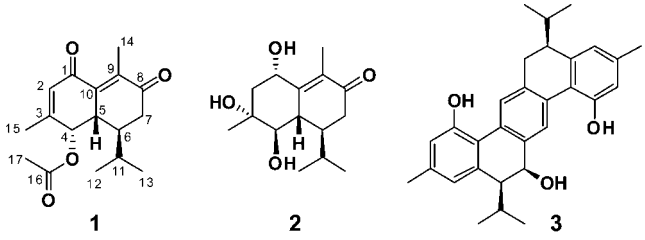
Two new cadinane sesquiterpenes (**1** and **2**) were isolated from *Curcuma parviflora*, and their structures were elucidated by spectroscopic data. Compound **1**, 4α-acetoxycadina-2,9-diene-1,8-dione, possesses two conjugated enone chromophores, while compound **2**, 1α,3α,4*β*-trihydroxy-9-cadinen-8-one, has an enone moiety with three hydroxy groups. Isolation of these cadinane monomers may reasonably suggest that parviflorenes are biogenetically classified as cadinane dimers.

During our search for bioactive natural products,[1] we recently performed a phytochemical investigation of *Curcuma parviflora* Wall. (Zingiberaceae) collected in Thailand. This led to the isolation of new sesquiterpenoid dimers, parviflorenes A−J,[2−5] and revealed that the major constituent, parviflorene F (**3**), induced apoptosis through increased death-receptor enhancement activity.[6] In order to get insight into the biogenesis of cadinane dimers,[7,8] we further separated this plant extract to obtain two new cadinane sesquiterpenes, 4α-acetoxycadina-2,9-diene-1,8-dione (**1**) and 1α,3α,4*β*-trihydroxy-9-cadinen-8-one (**2**). In this paper, we report the isolation and structure elucidation of these new compounds.



1 **2** **3**

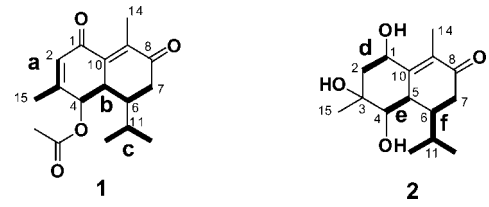
Parviflorenes A−J were previously isolated from the combined EtOAc- and *n*-BuOH-soluble fractions of the MeOH extract of the underground parts of *C. parviflora*.[2−5] Materials in the remaining fractions were further fractionated using silica gel chromatography as well as HPLC to afford two new compounds, **1** and **2**.

Compound **1** was obtained as an amorphous solid, $[\alpha]^{21}_{D}$ −13 (*c* 1.0, MeOH), and its molecular formula, $C_{17}H_{22}O_4$, was established by the HRFABMS data observed at *m/z* 291.1593 (calcd for $C_{17}H_{23}O_4$ $[M + H]^+$, 291.1596, Δ −0.3 mmu). The UV spectrum of **1** showed absorption maxima at 276 and 367 nm, indicating the presence of conjugated system(s), while IR absorption bands at 1739 and 1664 cm^{-1} were suggestive of the presence of two or more carbonyl groups. The ^{13}C NMR spectrum of **1** (Table 1) showed signals due to two ketone (δ_C 200.8 and 190.1) and one ester (δ_C 170.6) carbonyl group, one sp^2 methine (δ_C 131.5) and three quaternary sp^2 carbons (δ_C 154.7, 141.6, and 140.4), four sp^3 methines including one oxymethine (δ_C 68.4, 43.6, 39.7, and 27.5), one methylene (δ_C 36.1), and five methyls (δ_C 22.0, 21.0, 20.8, 16.4, and 13.2). The ^1H NMR spectrum showed signals for an olefinic proton (δ_H 6.14), one oxymethine proton (δ_H 5.65), a sharp methyl singlet due to an acetyl group (δ_H 2.08, 3H), two broad allylic methyls (δ_H 2.15 and 2.04, each 3H), and two secondary methyl groups (δ_H 0.92 and 0.86, each 3H, doublet, $J = 7.0$ and 6.9 Hz, respectively). The $^1H-^1H$ COSY spectrum showed partial structures (Figure 1) from H-2 to CH_3-15 (partial structure **a**), from H-4 to H_2-7 (partial structure **b**), and from H-11 to CH_3-12 and CH_3-13 (an isopropyl group; partial structure **c**).

Table 1. ^1H and ^{13}C NMR Data of Compounds **1** and **2** (δ in ppm, *J* in Hz; **1** in $CDCl_3$ and **2** in CD_3OD)

	1		**2**	
no.	δ_H (*J* in Hz)	δ_C	δ_H (*J* in Hz)	δ_C
1		190.1	4.42 dd (11.3, 4.4)	73.1
2	6.14 br s	131.5	(α) 1.73 dd (12.3, 11.3)	49.0[a]
			(*β*) 2.12 dd (12.3, 4.4)	
3		154.7		74.1
4	5.65 d (3.2)	68.4	3.53 d (11.3)	79.0
5	3.00 dd (8.9, 3.2)	43.6	2.14 m	45.0
6	1.92 m	39.7	2.08 m	40.1
7	(α) 2.50 dd (14.9, 4.3)	36.1	(α) 2.58 dd (16.2, 5.8)	37.3
	(*β*) 2.26 dd (14.9, 12.9)		(*β*) 2.43 dd (16.2, 2.2)	
8		200.8		202.5
9		141.6		129.6
10		140.4		157.9
11	2.02 m	27.5	1.59 m	31.0
12	(3H) 0.92 d (7.0)	21.0	(3H) 0.91 d (6.6)	21.4
13	(3H) 0.86 d (6.9)	16.4	(3H) 0.88 d (6.5)	20.9
14	(3H) 2.15 br s	13.2	(3H) 2.05 s	10.4
15	(3H) 2.04 br s	22.0	(3H) 1.35 s	21.8
16		170.6		
17	(3H) 2.08 s	20.8		

[a] Overlapped with the signal of CD_3OD.



1 **2**

Figure 1. Partial structures of **1** and **2** indicated by their COSY spectra.

In partial structure **b**, an acetoxy group was shown to be attached to C-4 from the low-field resonance of H-4 as well as the HMBC correlations observed for H-4/C-16 and H_3-17/C-16, while a C-6 isopropyl group was indicated by HMBC cross-peaks for H_3-12/C-6, H_3-13/C-6, and H_2-7/C-11. The presence of two enone moieties (C-1/C-2/C-3/C-15 and C-8/C-9/C-10/C-14) was revealed from the

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following HMBC cross-peaks: H-2/C-3, H-2/C-15, H_3-15/C-3, H_3-15/C-2, and H_3-15/C-1 (4-bond correlation); H_3-14/C-8, H_3-14/C-9, and H_3-14/C-10. The C-1/C-2/C-3/C-15 enone moiety (including part **a**) was connected to part **b** through the C-3/C-4 bond, which was indicated by the HMBC correlations for H-4/C-3, H-4/C-2, H-4/C-15, and H_3-15/C-4. The two enone moieties (C-1/C-2/C-3/C-15 and C-8/C-9/C-10/C-14) were connected by the C-1/C-10 bond from the HMBC correlations observed for H-2/C-10 and H_3-14/C-1 (4-bond correlation). The C-8/C-9/C-10/C-14 enone moiety was connected with part **b** through C-7/C-8 and C-5/C-10 bonds, indicated by H_2-7/C-8, H_2-7/C-9, H-5/C-10, and H-4/C-10. From these observations, the planar structure of compound **1** was elucidated to contain a cadinane carbon nucleus with one acetoxy and two enone functions. The relative configuration of C-4, C-5, and C-6 was deduced as H-4/H-5 *cis* and H-5/H-6 *trans* from the coupling constants ($J_{4,5}$ = 3.2 Hz, $J_{5,6}$ = 8.9 Hz, $J_{6,7\alpha}$ = 4.3 Hz, and $J_{6,7\beta}$ = 12.9 Hz) as well as NOE correlations (H-4/H-5, H-5/H-7β, and H-6/H-7α).

Compound **2**, a colorless amorphous solid, $[\alpha]^{24}_D$ −45 (*c* 0.54, MeOH), showed quasi-molecular ion peaks at *m/z* 269 $(M + H)^+$ and 307 $(M + K)^+$ in its positive ion FAB mass spectrum. The molecular formula was revealed as $C_{15}H_{24}O_4$ by HRFABMS data [*m/z* 269.1764, $(M + H)^+$, Δ +1.1 mmu]. The UV spectrum of **2** showed an absorption maximum at 231 nm, indicating the presence of conjugated system(s). In the ^{13}C NMR spectrum (Table 1) 15 carbons signals were observed and were assigned to one ketone (δ_C 202.5), two quaternary sp^2 carbons (δ_C 157.9 and 129.6), one quaternary sp^3 carbon (δ_C 74.1), five sp^3 methines, including two oxymethines (δ_C 79.0, 73.1, 45.0, 40.1, and 31.0), two methylenes (δ_C 49 and 37.3), and four methyls (δ_C 21.8, 21.4, 20.9, and 10.4). The ^1H NMR spectrum showed signals for two methyl singlets (δ_H 2.05 and 1.35, each 3H), two secondary methyls (δ_H 0.91 and 0.88, each 3H, doublet), and two oxymethines (δ_H 4.42 and 3.53). The presence of three partial structures (**d**−**f**, Figure 1) was suggested by analysis of the ^1H−^1H COSY spectrum, while the HMBC correlations observed from H_3-14 to C-8, C-9, and C-10 revealed the presence of an enone moiety (C-8/C-9/C-10/C-14). The connection of three partial structures (**d**−**f**), the enone moiety (C-8/C-9/C-10/C-14), and the sp^3 quaternary carbon (C-3) resonating at δ_C 74.1 was elucidated by HMBC correlations (H-1 to C-9, C-10; H_3-15 to C-2, C-3, C-4; H-5 to C-6, C-7, C-9, C-11; H-6 to C-8, C-10; H_2-7 to C-5, C-6, C-8), leading to construction of the cadinane skeleton for **2**. Since four unsaturation degrees were thus accounted for by an enone moiety and two rings, and one oxygen atom was contained in the enone group, the remaining three oxygen atoms were attributed to three hydroxy groups, which were located on two oxymethines (C-1 and C-4) and one sp^3 quaternary carbon (C-3). Thus, the structure of compound **2** was revealed as 1,3,4-trihydroxy-9-cadinen-8-one. Analysis of coupling constants ($J_{1,2\alpha}$ = 11.3 Hz, $J_{1,2\beta}$ = 4.4 Hz, $J_{4,5}$ = 11.3 Hz, $J_{5,6}$ = ca. 0 Hz, $J_{6,7\alpha}$ = 5.8 Hz, and $J_{6,7\beta}$ = 2.2 Hz) as well as NOESY correlation data (H-1/H-2β, H-1/H_3-15, H-1/H-5, H-2β/H_3-15, H-2α/H-4, H_3-15/H-5, H-4/H-7α, H-5/H_3-12, H-6/H-7α, and H-7β/H_3-13) led to assignment of the relative configuration, i.e., H-1, β-axial; CH_3-3 (H_3-15), β-axial; H-4, α-axial; H-5, β-axial; H-6, α-equatorial.

From this Zingiberaceous plant, *C. parviflora*, we have isolated a series of C_{30} compounds named parviflorenes, such as **3**,[2−5] which were assumed to be generated by dimerization of two cadinane sesquiterpene molecules. Although we have previously also isolated cadinane monomers from this plant,[2] the isolation of two more cadinane monomers here may provide further support of our biogenetical view of parviflorenes. We assume the absolute configurations of **1** and **2** corresponded to those of parviflorene, which however was not secured experimentally.

Experimental Section

General Experimental Procedures. Optical rotations were measured with a JASCO P-1020 polarimeter. CD spectra were obtained on 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, whose chemical shift was taken as an internal standard.

Plant Material. The plant *C. parviflora* was collected at Khon Kaen, Thailand, and was identified by one of the authors (T. Kowithayakorn). A voucher specimen has been deposited in our laboratory.

Extraction and Isolation. The air-dried underground parts (280 g) were extracted with MeOH and acetone. The combined extract (12.6 g) suspended in H_2O (200 mL) was partitioned with EtOAc (2 × 400 and 200 mL) and *n*-BuOH (2 × 200 mL). 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 silica gel column chromatography (column A, 4.5 × 57 cm) eluted with 0−100% EtOAc in *n*-hexane and 100% acetone. The fraction (0.9 g) of column A eluted with 20−33% EtOAc in *n*-hexane was further separated by silica gel column chromatography (4.0 × 33 cm) eluted with EtOAc/*n*-hexane (2:8), followed by reversed-phase HPLC with 90% MeOH (Develosil C30-UG-5, 10 × 250 mm) to give compound **1** (3.0 mg). Another fraction (590 mg) of column A eluted with 100% acetone was further separated by silica gel column chromatography (4.3 × 21.5 cm) eluted with $CHCl_3$/MeOH (9:1) followed by reversed-phase HPLC with 67% MeOH (YMC Pak ODS-AM, 10 × 250 mm) to give compound **2** (5.4 mg).

Compound 1 (4α-acetoxycadina-2,9-diene-1,8-dione): amorphous solid; $[\alpha]^{21}_D$ −13 (*c* 1.0, MeOH); CD (0.188 mM, MeOH, 24 °C) $\Delta\epsilon$ (λ_{ext} nm) 2.6 (284), −5.5 (245), −4.2 (225), and −7.9 (208); UV (MeOH) λ_{max} nm (log ϵ) 367 (3.2), 276 (4.1), and 202 (4.4); IR (film) ν_{max} 2960, 1739, 1664, 1373, and 1226 cm^{-1}; EIMS *m/z* 290 (M^+); HRFABMS *m/z* 291.1593 (calcd for $C_{17}H_{23}O_4$ $[M + H]^+$, 291.1596); ^1H and ^{13}C NMR (Table 1).

Compound 2 (1α,3α,4β-trihydroxy-9-cadinen-8-one): amorphous solid; $[\alpha]^{24}_D$ −45 (*c* 0.54, MeOH); CD (0.081 mM, MeOH, 24 °C) $\Delta\epsilon$ (λ_{ext} nm) 0 (311), 0.3 (288), −4.4 (250), and 0 (208); UV (MeOH) λ_{max} nm (log ϵ) 231 (3.8); FABMS *m/z* 269 $(M + H)^+$ and 307 $(M + K)^+$; HRFABMS *m/z* 269.1764 (calcd for $C_{15}H_{25}O_4$ $[M + H]^+$, 269.1753); ^1H and ^{13}C NMR (Table 1).

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