New Bioactive Clerodane Diterpenoids from the Bark of *Casearia grewiifolia*

Somdej Kanokmedhakul,* Kwanjai Kanokmedhakul, Tanika Kanarsa, and Mongkol Buayairaksa

Department of Chemistry, Applied Taxonomic Research Center, Faculty of Science, Khon Kaen University, Khon Kaen 40002, Thailand

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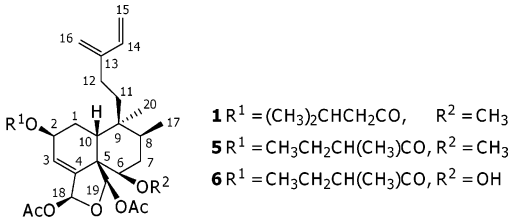
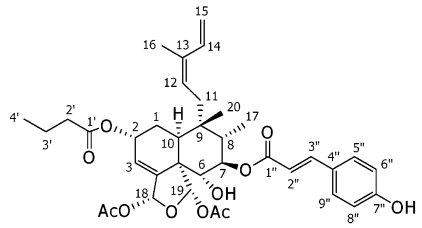
Bioactivity-guided fractionation of hexane and dichloromethane extracts of the bark of *Casearia grewiifolia* afforded four new clerodane diterpenes, caseargrewiins A−D (**1**−**4**), and two known clerodane diterpenes, *rel*-(2*S*,5*R*,6*R*,8*S*,9*S*,10*R*,18*S*,19*R*)-18,19-diacetoxy-18,19-epoxy-6-methoxy-2-(2-methylbutanoyloxy)cleroda-3,13(16),14-triene (**5**) and *rel*-(2*S*,5*R*,6*R*,8*S*,9*S*,10*R*,18*S*,19*R*)-18,19-diacetoxy-18,19-epoxy-6-hydroxy-2-(2-methylbutanoyloxy)cleroda-3,13(16),14-triene (**6**). The structures of **1**−**4** were established on the basis of the interpretation of their 1D and 2D NMR spectral data. The absolute configuration of **4** was determined by the modified Mosher's method. All compounds exhibited promising antimalarial and antimycobacterial activities but also cytotoxicity against three cancer cell lines.

Casearia grewiifolia Vent. (Flacourtiaceae) is a shrubby tree, 3−10 m in height, growing widely in the northern and northeastern parts of Thailand. It is known as "Kruai pa" or "Pha sam",[1] and decoctions of the bark and flowers are used traditionally as a tonic and a febrifuge, resectively.[2] Previous phytochemical and biological investigations on *Casearia* species have resulted in the isolation of several clerodane-type diterpenes.[3−19] However, no investigation of the phytochemical constituents and bioactivity of *C. grewiifolia* has yet been carried out. As part of our search for bioactive constituents from Thai plants, the hexane and CH_2Cl_2 extracts of the air-dried bark of *C. grewiifolia* were found to exhibit antiplasmodial activity against *Plasmodium falciparum* (IC_{50} 0.8 and 1.2 μg/mL, respectively), antimycobacterial activity against *Mycobacterium tuberculosis* (MIC 25 μg/mL for both), and cytotoxicity against several cancer cell lines (IC_{50} range 2−9 μg/mL). We report herein the isolation, characterization, and bioactivities of four new clerodane diterpenes, caseargrewiins A−D (**1**−**4**), together with the biological activity of two known clerodane diterpenes, **5** and **6**.





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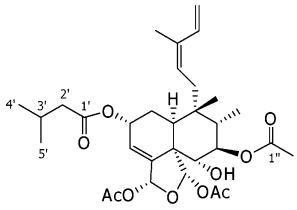

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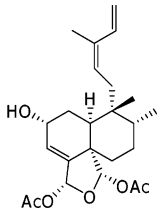

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Results and Discussion

Clerodane diterpenes **1**−**6** were isolated from hexane and CH_2Cl_2 extracts of the air-dried bark of *C. grewiifolia* using a combination of silica gel column chromatography and preparative TLC. The two known clerodane diterpene isolates were identified as *rel*-(2*S*,5*R*,6*R*,8*S*,9*S*,10*R*,18*S*,19*R*)-18,19-diacetoxy-18,19-epoxy-6-methoxy-2-(2-methylbutanoyloxy)cleroda-3,13(16),14-triene (**5**) and *rel*-(2*S*,5*R*,6*R*,8*S*,9*S*,10*R*,18*S*,19*R*)-18,19-diacetoxy-18,19-epoxy-6-hydroxy-2-(2-methylbutanoyloxy)cleroda-3,13(16),14-triene (**6**), respectively, by comparison of their spectral data with values reported in the literature.[10,16]

Caseargrewiin A (**1**) was obtained as a colorless amorphous solid and was assigned the molecular formula $C_{30}H_{44}O_8$, as deduced from the HRESITOFMS (observed *m/z* 555.2932 $[M + Na]^+$). The UV spectrum displayed an absorption maximum due to a conjugated diene at 232 nm. The IR spectrum showed the presence of three carbonyl ester groups (1757, 1739, 1722 cm^{-1}). The ^1H and ^{13}C NMR spectra (Tables 1 and 2) exhibited the presence of the 3-methylbutanoyl group from the resonances at δ_H/δ_C 2.26 and 2.09 (m, H_2-2′)/43.7; 2.12 (m, H-3′)/26.1; 1.01 (d, J = 6.3 Hz, H-4′)/22.4; and 1.00 (d, J = 6.3 Hz, H-5′)/22.3, and the carbonyl of the C-1′ ester at δ 172.5. The ^1H NMR spectrum displayed a methyl doublet at δ 0.94 (J = 6.8 Hz, CH_3-17) and a methyl singlet at δ 0.93 (CH_3-20), two oxymethines at δ 5.43 (brs, H-2) and 3.30 (overlapping, H-6), two acetal-acyloxy groups at δ 6.65 (s, H-18) and 6.43 (s, H-19), and a trisubstituted olefinic proton at δ 5.94 (brd, J = 3.9 Hz, H-3), consistent with the basic skeleton of clerodane diterpenes previously isolated from the genus *Casearia*.[7−18] The skeleton was also supported by COSY and HMBC correlations. The olefinic region showed two terminal methylene units in the six-carbon side chain (C-11 through C-16). One of these displayed classic *trans*/*cis* coupling at δ 5.17 (d, J = 17.5 Hz, 15a), 5.02 (d, J = 11.2 Hz, H-15b), and 6.43 (dd, J = 17.5, 11.2 Hz, H-14), while the other terminal methylene displayed two singlet signals at δ 5.05 (H-16a) and 4.93 (H-16b).

* To whom correspondence should be addressed. Tel: +66-43-202222-41, ext. 2243, 2370-4. Fax: +66-43-202373. E-mail: somdej@kku.ac.th.

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Table 1. ^{1}H NMR Spectral Data for Compounds **1**−**4** ($CDCl_3$, 400 MHz)[a]

position	1	2	3	4
1	1.92 m	1.92 m	1.94 m	1.91 m
2	5.43 brs	5.45 brs	5.46 brs	4.28 brs
3	5.94 brd (3.9)	5.95 d (3.9)	6.03 brs	5.93 d (3.5)
6	3.30[b]	3.79 d (10.5)	3.68 d (10.5)	1.48 m
7	1.50 m, 1.84, m	5.12[b]	4.97 t (10.5)	1.70 m, 1.42 m
8	1.70 m	1.88 m	1.88 m	1.67 m
10	2.35 dd (10.0, 2.9)	2.38[b]	2.38[b]	2.19 dd (11.7, 4.4)
11	1.56 m, 1.20, m	2.55 dd (16.0, 9.3)	2.50 dd (16.0, 9.3)	2.37 dd (16.1, 9.0)
		1.65 m	1.73 m	1.74 m
12	2.09 m	5.25 d (7.8)	5.25 brd (7.8)	5.36 brd (7.0)
14	6.43 dd (17.5, 11.2)	6.65 dd (16.8, 10.9)	6.62 dd (17.2, 10.9)	6.65 dd (17.2, 10.9)
15	5.17 d (17.5)	5.20 d (16.8)	5.21 d (17.2)	5.18 d (17.2)
	5.02 d (11.2)	5.15 d (10.9)	5.13 d (10.9)	5.09 d (10.9)
16	5.05 s, 4.93 s	1.81 s	1.81 s	1.90 s
17	0.94 d (6.8)	0.93 d (6.3)	0.92 d (6.6)	0.88 d (6.6)
18	6.65 s	6.70 s	6.72s	6.67 s
19	6.43 s	6.65 s	6.58 s	6.36 s
20	0.93 s	0.80 s	0.84 s	0.85 s
2′	2.26 m, 2.09 m	2.38 t (7.2)	2.26 m, 2.09 m	
3′	2.12 m	1.70 m	2.10 m	
4′	1.01 d (6.3)	1.01 t (7.2)	1.01 d (6.4)	
5′	1.00 d (6.3)		1.01 d (6.4)	
2″		6.25 d (16.0)		
3″		7.65 d (16.0)		
5″, 9″		6.83 d (8.2)		
6″, 8″		7.34 d (8.2)		
O*Me*-6	3.31 s			
*Me*CO-7			2.11 s	
*Me*CO-18	2.06 s	2.11 s	2.08 s	2.10 s
*Me*CO-19	1.87 s	2.10 s	1.99 s	2.00 s

[a] Figures in parentheses are coupling constants in Hz. [b] Coupling constant(s) not indicated because of overlapping with other protons.

The relative stereochemistry at all eight chiral centers of **1** was assigned on the basis of their coupling constants and the NOESY correlations together with the specific optical rotation (+65°), having the same sign as reported for structural analogues.[16,18] However, the coupling constant of H-6 to H-7 was not determined due to overlapping signals of H-6 with methoxy protons at C-6. A 1,3-diaxial NOESY correlation was observed for H-6 and H-8, which indicated the C-8 methyl group was in an equatorial orientation. The *J* values of 2.9 and 10.0 Hz for the coupling of H-10 to H-1 revealed that H-10 was in an axial position. The chemical shift at δ 66.3 for the C-2 oxymethine carbon was consistent with the C-2 substituent having a β-orientation, as reported for related compounds.[10,16,18] The acetal proton H-19 was also assigned with β-orientation on the basis of the NOESY correlations of H-19 to H-11 and H-7. The acetal protons H-18 and H-19 were found to be *cis* due to the NOESY correlation between these two protons. On the basis of the above evidence, the structure of **1** was established as *rel*-(2*S*,5*R*,6*R*,8*S*,9*S*,10*R*,18*S*,19*R*)-18,19-diacetoxy-18,19-epoxy-6-methoxy-2-(3-methylbutanoyloxy)-cleroda-3,13(16),14-triene, which has been named caseargrewiin A.

Caseargrewiin B (**2**) was obtained as a colorless amorphous solid and was assigned the molecular formula $C_{37}H_{46}O_{11}$, as deduced from the HRESITOFMS (observed *m/z* 689.3303 $[M + Na]^+$). The IR spectrum showed the presence of hydroxyl (3437 cm^{-1}) and carbonyl ester groups (1732 br cm^{-1}). The UV absorption displayed maxima at 233 and 308 nm, suggesting α,β-unsaturated carbonyl and aromatic units in **2**. The ^{1}H and ^{13}C NMR spectra (Tables 1 and 2) revealed the presence of butanoyloxy and *p*-hydroxycinnamoyloxy substituents in the structure. The ^{1}H NMR spectrum showed the presence of a methyl doublet at δ 0.93 (*J* = 6.3 Hz, CH_3-17), a methyl singlet at δ 0.80 (CH_3-20), three oxymethines at δ 5.45 (brs, H-2), 5.12 (overlapping, H-7), and 3.79 (d, *J* = 10.5 Hz, H-6), and two acetal-acyloxy groups at δ 6.70 (s, H-18) and 6.65 (s, H-19), consistent with the basic skeleton of clerodane diterpenes isolated earlier from the genus *Casearia*.[4−6] A six-carbon diene side chain was assigned to C-11 through C-16. A terminal double bond was indicated by *trans* and *cis* coupling at δ 5.20 (d, *J* = 16.8 Hz) and 5.15 (d, *J* = 10.9 Hz) for H_2-15, respectively, which were coupled to the methine proton at δ 6.65 (dd, *J* = 16.8, 10.9 Hz, H-14). The other double bond was trisubstituted, with a broad doublet methine proton signal observed at δ 5.25 (*J* = 7.8 Hz, H-12), which was confirmed from the COSY spectrum. The HMBC spectrum showed the correlations in this side chain of H-15 to C-13; H-14 to C-12, C-13, and C-16; and H-12 to C-13 and C-14. The configuration of the C-12, 13 double bond was found to be *Z*, on the basis of the observation of NOESY correlations from H-12 to H-16 and from H-11 to H-14. The butanoyloxy group was deduced from the resonances at δ 2.38 (2H, t, *J* = 7.2 Hz, H-2′), 1.70 (2H, m, H-3′), and 1.01 (3H, t, *J* = 7.2 Hz, H-4′). The COSY correlations between H-2′ and H-3′ and between H-3′ and H-4′ as well as the HMBC correlations of H-2′ to C-1′, C-3′, and C-4′; H-3′ to C-1′, C-2′, and C-4′; and H-4′ to C-2′ and C-3′ also supported the presence of this group. A *p*-hydroxy-substituted benzene ring was indicated by a pair of doublets in the ^{1}H NMR spectrum at δ 7.34 and 6.83, both with the same coupling constant (*J* = 8.2 Hz). The configuration of a double bond of the cinnamoyl group was determined as *E*, since large *trans* coupling constants of 16.0 Hz were observed at δ 7.65 and 6.25. The HMBC correlations supported the substitution pattern from the correlations between H-2″ and C-1″ and C-4″; H-3″ and C-1″, C-2″, and C-5″; H-5″ and C-7″; and H-6″ and C-4″, and it also confirmed the position of the *p*-hydroxy-cinnamoyl group attached to C-7 from the correlation between H-7 and C-1″.

The relative stereochemistry of **2** was assigned from the coupling constants together with the NOESY correlations

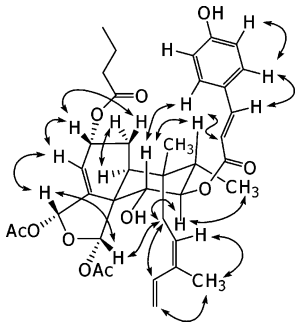


Figure 1. Selected NOESY correlations of **2**.

Table 2. ^{13}C NMR Spectral Data for Compounds **1**−**4** ($CDCl_3$, 100 MHz)

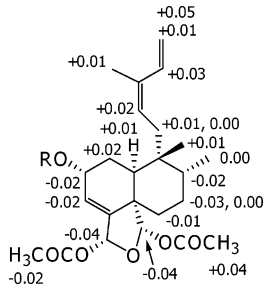
position	**1**	**2**	**3**	**4**
1	27.0 t[a]	26.8 t	26.7 t	28.9 t
2	66.3 d	66.0 d	66.0 d	64.2 d
3	121.3 d	122.1 d	122.1 d	123.8 d
4	146.0 s	144.3 s	144.6 d	145.2 d
5	53.1 s	53.6 s	53.6 s	49.2 s
6	81.9 d	74.8 d	74.9 d	28.9 d
7	31.1 t	75.2 d	75.4 d	27.4 t
8	36.9 d	41.1 d	41.1 d	33.7 d
9	37.5 s	39.2 s	39.3 s	33.7 s
10	36.5 d	36.0 d	36.0 d	36.5 d
11	27.8 t	30.1 t	30.1 t	29.5 t
12	23.8 t	125.6 t	125.6 t	126.9 t
13	145.1 s	133.9 s	134.0 s	133.4 s
14	140.5 d	133.3 d	133.3 d	133.6 d
15	112.2 t	114.6 t	114.8 t	114.0 t
16	115.6 t	20.0 t	20.0 t	20.3 t
17	15.9 q	11.0 q	11.0 q	21.3 q
18	96.2 d	95.8 d	95.6 d	94.7 d
19	98.4 d	97.5 d	97.5 d	99.2 d
20	25.5 q	25.3 q	25.4 q	15.7 q
1′	172.5 s	173.2 s	172.5 s	
2′	43.7 t	36.4 t	43.6 t	
3′	26.1 d	18.5 d	26.1 d	
4′	22.4 q	13.9 q	22.3 q	
5′	22.3 q		22.4 q	
1″		168.1 d		
2″		114.0 d		
3″		146.0 d		
4″		126.2 s		
5″, 9″		116.0 d		
6″, 8″		130.2 d		
7″		158.8 s		
O*Me*-6	57.1 t			
*Me*CO-7			20.9	
Me*CO*-7			171.6	
O*C*O-18	170.2 s	170.5 s	170.1 s	170.4 s
*Me*CO-18	21.6 q	20.1 q	21.2 q	25.6 q
O*C*O-19	169.8 s	169.0 s	168.8 s	169.6 s
*Me*CO-19	21.3 q	20.1 q	21.2 q	25.6 q

[a] Multipicities were determined by analysis of the DEPT spectra.

of those protons (Figure 1). The coupling constant observed between H-6 and H-7 (J = 10.5 Hz) and the lack of a correlation between these two protons in the NOESY spectrum indicated that H-6 and H-7 are 1,2-diaxial. A 1,3-diaxial NOESY correlation was also observed for H-6 and H-8, which enabled the H-17 methyl group to be placed in an equatorial position. However, the J value for the coupling of H-10 to H-1 was unable to be identified due to overlapping with other protons. The NOESY correlations from H-6 to H-2″ and H-5″, together with the correlation between H-7 and H-17 methyl group, indicated the β-orientation of the *p*-hydroxycinnamoyloxy group at C-7. The acetal proton H-19 was also assigned with β-orientation on the basis of the NOESY correlations of H-19 to H-11 and H-7. The acetal protons H-18 and H-19 were found to be *cis* due to the NOESY correlation between these two protons. On the basis of the above evidence, and comparison of the specific optical rotation (+65.5°) with those of known compounds,[4−6,13] the structure of **2** was elucidated as *rel*-(2*R*,5*S*,6*R*,7R,8S,9*S*,10*S*,18*R*,19*S*)-18,19-diacetoxy-18,19-epoxy-2-butanoyloxy-6-hydroxy-7-(*p*-hydroxycinnamoyloxy)cleroda-3,12(*Z*),14-triene and was named caseargrewiin B.

Caseargrewiin C (**3**) was obtained as a colorless amorphous solid and was assigned the molecular formula $C_{31}H_{44}O_{10}$, as deduced from the HRESITOFMS (observed *m/z* 599.2842 $[M + Na]^+$). The IR spectrum showed absorptions for hydroxyl (3489 cm^{-1}) and carbonyl groups (1754, 1732 br cm^{-1}). The ^1H NMR spectrum of **3** was similar to that of **2** except that the butanoyl and *p*-hydroxycinnamoyl groups were displaced by 3-methylbutanoyl and acetoxy groups at the C-2 and C-7 positions, respectively. The 3-methylbutanoyloxy unit was deduced from the ^1H NMR resonances at δ 2.26 and 2.09 (2H, m, H-2′), 2.10 (1H, m, H-3′), and 1.01 (6H, d, J = 6.4 Hz, H-4′ and H-5′). The COSY correlations of H-2′ to H-3′, and H-3′ to H-4′ and H-5′, together with the HMBC correlations of H-2′ to C-1′, C-4′, and C-5′; H-3′ to C-1′, C-4′, and C-5′; and H-4′ and H-5′ to C-2′ and C-3′ supported the presence of this group. The acetoxy moiety at C-7 showed signals at δ_H 2.11 (s, CH_3) and δ_C 20.9 and 171.6. The HMBC spectrum exhibited correlations between H-7 and C-1″, confirming the connection of the acetate unit at C-7. The complete ^1H and ^{13}C NMR spectral data assignments of **3** were established from the DEPT, COSY, HSQC, HMBC, and NOESY data (Tables 1 and 2). The relative stereochemistry of **3** was identical to that of **2**, on the basis of the same characteristic NOESY correlations and coupling constants of those protons, as well as the same sign of the specific optical rotation value (see Experimental Section). On the above evidence, **3** was assigned as *rel*-(2*R*,5*S*,6*R*,7R,8S,9*S*,-10*S*,18*R*,19*S*)-18,19-diacetoxy-18,19-epoxy-2-(3-methylbutanoyloxy)-6-hydroxy-7-acetoxycleroda-3,12(*Z*),14-triene and has been named caseargrewiin C.

Caseargrewiin D (**4**) was obtained as a colorless amorphous solid and exhibited the molecular formula $C_{24}H_{34}O_6$, as deduced from the HRESITOFMS (observed *m/z* 441.2253 $[M + Na]^+$). The IR spectrum of compound **4** showed the presence of a hydroxyl group (3503 cm^{-1}) and ester carbonyl groups (1732, 1722 cm^{-1}). The ^1H, ^{13}C NMR and DEPT spectral data indicated 24 carbons, attributable to six quaternary (including two carbonyl carbons), eight methine, five methylene, and five methyl carbons. The ^1H NMR spectrum showed the presence of a methyl doublet at δ 0.88 (J = 6.6 Hz, H-17), a methyl singlet at δ 0.85 (H-20), one oxymethine at δ 4.28 (brs, H-2), two acetalacyloxy methine protons at δ 6.67 (s, H-18) and 6.36 (s, H-19), and a trisubstituted olefinic proton at δ 5.93 (d, J = 3.5 Hz, H-3), consistent with the basic skeleton of a clerodane diterpene isolated from the genus *Casearia*.[10,16] The COSY spectrum also confirmed this basic clerodane structure by showing correlations between H-1 and H-2, H-1 and H-10, H-2 and H-3, H-6 and H-7, H-7 and H-8, and H-8 and the protons of the 17-methyl group. The six-carbon diene side chain C-11 through C-16 contained a terminal vinyl group, as evidenced by resonances at δ 5.18 (d, J = 17.2 Hz, H-15a) and 5.09 (d, J = 10.9 Hz, H-15b) and a trisubstituted double bond at δ 6.65 (dd, J = 17.2, 10.9 Hz, H-14); this was also confirmed by COSY correlations between H-11 and H-12, and H-14 and H-15. The HMBC spectrum exhibited correlations of H-12 to C-9 and C-14; H-11 to C-14; H-14 to C-12 and C-16; and H-15 to



4R, R = (*R*)-MTPA
4S, R = (*S*)-MTPA

Figure 2. $\Delta\delta$ values ($\Delta\delta = \delta_S - \delta_R$ in ppm) obtained for MTPA esters **4R** and **4S**.

Table 3. Biological Activities of Compounds **1**−**6**

compound	antimalarial (IC_{50}, μg/mL)	antimycobacterial (MIC, μg/mL)	cytotoxicity (IC_{50}, μg/mL) KB[a]	BC1[b]	NCl-H187[c]
1	2.9	12.5	8.7	4.6	0.9
2	2.4	12.5	2.3	1.9	1.5
3	3.0	25.0	1.9	2.0	0.3
4	3.3	12.5	1.3	0.1	1.5
5	3.2	12.5	7.6	4.0	0.8
6	3.1	12.5	2.8	2.5	5.3
artemisinin	0.001				
isoniazid		0.05			
kanamycin sulfate		2.5			
ellipticine			0.36	0.26	0.32

[a] Human oval epidermoid carcinoma. [b] Human breast cancer cells. [c] Human lung cancer cells.

C-13, revealing this side chain to be located at C-9. The configuration of the C-12, 13 double bond was found to be *Z*, on the basis of the NOESY correlations between H-12 and H-16, and H-11 and H-14.

The assignment of the absolute stereochemistry of C-2 was carried out by the modified Mosher ester method.[20−22] Reaction of **4** with (*R*)- and (*S*)-α-methoxy-α-(trifluoromethyl)phenylacetyl chloride (MTPA-Cl) gave the (*S*)- and (*R*)-MTPA esters, respectively. The differences in the ^{1}H NMR chemical shifts between the (*S*)- and (*R*)-MTPA ($\Delta\delta_H = \delta_S - \delta_R$) around the C-2 position were analyzed to determine the absolute configuration of this position, which was found to be *R* (Figure 2). The absolute stereochemistry of the rest of the molecule in **4** was elucidated on the basis of the coupling constants and the NOESY spectrum of these protons. The coupling constants of 11.7 and 4.4 Hz of H-10 revealed an axial orientation, so the A/B ring junction was *cis*. The NOESY correlations between H-11 and H-19, H-11, and the 17-methyl protons revealed the α-orientation of an acetate group at C-19, and the 17-methyl group was equatorial. The acetal-acyloxy protons H-18 and H-19 were assigned as β on the basis of NOESY interactions between H-18 and H-19 and between H-19 and H-11 and H-7$_{ax}$. On the basis of the above data, the structure of **4** was elucidated as (2*R*,5*S*,8R,9*R*,10*S*,18*R*,19*S*)-18,19-diacetoxy-18,19-epoxy-2-hydroxy-cleroda-3,12(*Z*),14-triene, which has been named caseargrewiin D.

The biological test results of the isolated compounds are shown in Table 3. All six compounds (**1**−**6**) exhibited activity against *P. falciparum*, with respective IC_{50} values of 2.9, 2.4, 3.0, 3.3, 3.2, and 3.1 μg/mL, and exhibited moderate activities against *M. tuberculosis*, with MIC values of 12.5, 12.5, 25.0, 12.5, 12.5, and 12.5 μg/mL, respectively. However, the potency of these responses are much lower than both the antimalarial drug artemisinin and the antimycobacterial drug isoniazid. In addition, **1**−**6** showed discernible cytotoxicity against three cancer cell lines (KB, BC1, and NCI-H187), with IC_{50} values ranging between 0.1 and 8.7 μg/mL. Among these, **3** and **4** have IC_{50} values against NCI-H187 and BC1 of 0.3 and 0.1 μg/mL, respectively, which are close to the control drug, ellipticine (Table 3).

Experimental Section

General Experimental Procedures. Melting points were determined using a Gallenkamp melting point apparatus and are uncorrected. Optical rotations were obtained using a JASCO DIP-1000 digital polarimeter. UV spectra were measured on an Agilent 8453 UV−visible spectrophotometer. IR spectra were carried out on a Perkin-Elmer Spectrum One spectrophotometer. NMR spectra were recorded in $CDCl_3$ on a Varian Mercury Plus 400 spectrometer, using residual $CHCl_3$ as an internal standard. HRESITOFMS were obtained using a Micromass LCT mass spectrometer, and the lock mass calibration was applied for the determination of accurate masses. Column chromatography and preparative TLC were carried out on silica gel 60 (230−400 mesh) and PF_{254}, respectively.

Plant Material. The bark of *Casearia grewiifolia* Vent. was collected in the campus of Khon Kaen University in September 2001 and was identified by Prof. Pranom Chantaranothai, Department of Biology, Khon Kaen University. A voucher specimen (S. Kanokmedhakul 3) was deposited at the herbarium of the Department of Biology, Faculty of Science, Khon Kaen University, Khon Kaen, Thailand.

Extraction and Isolation. Air-dried bark of *C. grewiifolia* (5.4 kg) was ground and extracted successively with hexane (4 L × 3) and CH_2Cl_2 (4 L × 3) at room temperature. The filtered samples were combined, and the solvents were evaporated in vacuo to yield crude hexane (28.5 g) and CH_2Cl_2 extracts (19.0 g). The hexane extract was initially separated by passage over silica gel (250 g) column chromatography and eluted with increasing concentrations of EtOAc in hexane followed by MeOH in EtOAc. Each fraction (75 mL) was monitored by TLC; fractions with similar TLC patterns were combined to yield nine major fractions (F_1−F_9). Fraction F_5 was further rechromatographed on a silica gel column, eluted with a gradient system of CH_2Cl_2−EtOAc, to furnish five subfractions designated as $F_{5/1}$−$F_{5/5}$. Subfraction $F_{5/2}$ (140 mg) was further separated by preparative TLC, eluted with acetone−CH_2Cl_2−hexane (10:10:80) (developed × 4), to yield **1** (R_f 0.43, 60 mg) and **5** (R_f 0.50, 50 mg). Fraction F_7 was further separated by preparative TLC, eluting with EtOAc−hexane (30:70), and then preparative TLC eluted with acetone−CH_2Cl_2−hexane (10:10:80) (developed × 4), to afford **6** (R_f 0.30, 104 mg).

The CH_2Cl_2 extract was subjected first to silica gel flash column chromatography eluted with the same gradient system as the hexane extract above to give eight fractions, F'_1−F'_8. Further separation of F'_4 by preparative TLC, eluting with acetone−hexane (10:90), and crystallization from EtOAc−hexane yielded an additional mixture of **1** and **5** (128 mg). Fraction F'_5 was rechromatographed on silica gel eluted with a gradient system of acetone−hexane to give four subfractions designated as $F'_{5/1}$−$F'_{5/4}$. Subfraction $F'_{5/2}$ was purified by preparative TLC, eluting with EtOAc−hexane (45:55) (developed × 3), and yielded **2** (65 mg). Subfraction $F'_{5/3}$ was further purified by silica gel column chromatography to give three subfractions, $F'_{5/3-1}$−$F'_{5/3-3}$. Subfraction $F'_{5/3-1}$ was purified by preparative TLC, eluted with CH_2Cl_2 (developed × 3), to yield **3** (58 mg). Fraction F'_7 was purified by preparative TLC, eluted with EtOAc−hexane (45:55), to give **4** (90 mg).

Caseargrewiin A (1): colorless crystals, yield 0.0023% w/w; R_f = 0.68 (EtOAc−hexane, 60:40); mp 191−193 °C; $[\alpha]^{25}_D$ +65.0° (*c* 0.45, MeOH); UV (MeOH) λ_{max} (log ϵ) 232 (3.88) nm; IR (KBr) ν_{max} 3093, 2964, 2883, 1757, 1739, 1722, 1629, 1595, 1454, 1373, 1228 1170 cm^{-1}; ^{1}H and ^{13}C NMR, see Tables 1

and 2; HRESITOFMS *m/z* 555.2932 $[M + Na]^+$ (calcd for $C_{30}H_{44}O_8$ + Na, 555.2934).

Caseargrewiin B (2): colorless amorphous solid, yield 0.0012% w/w; R_f = 0.41 (EtOAc−hexane, 60:40); mp 125−127 °C; $[\alpha]^{21}_D$ +65.5° (*c* 0.58, MeOH); UV (MeOH) λ_{max} (log ϵ) 233 (4.28), 308 (4.19) nm; IR (KBr) ν_{max} 3437, 3077, 2969, 1732, 1631, 1605, 1587, 1515, 1441, 1375, 1232, 1170, 1025, 834 cm^{-1}; ^1H and ^{13}C NMR, see Tables 1 and 2; HRESITOFMS *m/z* 689.3303 $[M + Na]^+$ (calcd for $C_{37}H_{46}O_{11}$ + Na, 689.3302).

Caseargrewiin C (3): colorless amorphous solid, yield 0.0011% w/w; R_f = 0.46 (EtOAc−hexane, 60:40); mp 85−87 °C; $[\alpha]^{29}_D$ +66.6° (*c* 0.42, MeOH); UV (MeOH) λ_{max} (log ϵ) 232 (3.70) nm; IR (KBr) ν_{max} 3489, 3080, 2967, 1754, 1732, 1640, 1597,1454, 1373, 1231, 1026, 926 cm^{-1}; ^1H and ^{13}C NMR, see Tables 1 and 2; HRESI-TOFMS *m/z* 599.2842 $[M + Na]^+$ (calcd for $C_{31}H_{44}O_{10}$ + Na, 599.2832).

Caseargrewiin D (4): colorless amorphous solid, yield 0.00167% w/w; R_f = 0.56 (EtOAc−hexane, 60:40); mp 119−121 °C; $[\alpha]^{28}_D$ −46.6° (*c* 0.25, MeOH); UV (MeOH) λ_{max} (log ϵ) 232 (3.98) nm; IR (KBr) ν_{max} 3503, 3090, 2968, 1732, 1722, 1620, 1597, 1456, 1373, 1231, 1073, 1026, 918 cm^{-1}; ^1H NMR and ^{13}C NMR, see Tables 1 and 2; HRESITOFMS *m/z* 441.2253 $[M + Na]^+$ (calcd for $C_{24}H_{34}O_6$ + Na, 441.2256).

Preparation of the (*R*)-α-Methoxy-α-(trifluoromethyl)-phenyl Acetate of 4. To a solution of the alcohol (−)-**4** (9.8 mg, 23.43 μM), in pyridine (1.0 mL), were added (dimethylamino)pyridine (2.0 mg) and (*S*)-(α-methoxy-α-(trifluoromethyl)phenylacetyl chloride (MPTA-Cl) (17.49 μL, 93.72 μM). The mixture was stirred under N_2 at room temperature for 18 h, and the solvent was removed in vacuo. The product was purified by preparative TLC (EtOAc−hexane, 40:60) to give the (*R*)-ester (**4R**, 11.2 mg, 82.8%): $[\alpha]^{29}_D$ +85.5° (*c* 0.86, MeOH); ^1H NMR ($CDCl_3$, 400 MHz) δ 1.98 (2H, m, H-1), 5.64 (1H, brs, H-2), 6.05 (1H, d, *J* = 3.6 Hz, H-3), 1.47 (2H, m, H-6), 1.74 (1H, m, H-7a), 1.65 (1H, m, H-7b), 1.64 (1H, m, H-8), 2.34 (1H, brd, *J* = 13.2 Hz, H-10), 2.34 (1H, dd, *J* = 16.4, 9.0 Hz, H-11a), 1.68 (1H, m, H-11b), 5.21 (1H, brd, *J* = 8.2 Hz, H-12), 6.61 (1H, dd, *J* = 17.5, 10.5 Hz, H-14), 5.16 (1H, d, *J* = 17.5 Hz, H-15a), 5.08 (1H, d, *J* = 10.5 Hz), 1.72 (3H, s, C*H*$_3$-16), 0. 90 (3H, d, *J* = 6.6 Hz, C*H*$_3$-17), 6.73 (1H, s, H-18), 6.37 (1H, s, H-19), 0.79 (3H, s, C*H*$_3$-20), 2.08 (3H, s, CO*CH*$_3$-18), 1.82 (3H, s, CO*CH*$_3$-19) [the α-methoxy-α-(trifluoromethyl)phenyl acetate portion exhibited δ 7.56 and 7.43 (5H, m, C_6H_5), 3.63 (3H, s, OCH_3)]; HRESITOFMS *m/z* 657.2648 $[M + Na]^+$ (calcd for $C_{34}H_{41}F_3O_8$ + Na, 657.2651).

Preparation of the (*S*)-α-Methoxy-α-(trifluoromethyl)-phenyl Acetate of 4. To a solution of the alcohol (−)-**4** (11.2 mg, 26.78 μM), in pyridine (1.0 mL), were added (dimethylamino)pyridine (2.0 mg) and (*R*)-MPTA-Cl (17.88 μL, 0.956 μM). The mixture was stirred under N_2 at room temperature for 18 h, and the solvent was removed in vacuo. The product was purified by preparative TLC (EtOAc−hexane, 40: 60) to give the (*S*)-ester (**4S**, 14.0 mg, 82.5%): $[\alpha]^{29}_D$ +86.6° (*c* 0.95, MeOH); ^1H NMR ($CDCl_3$, 400 MHz) δ 2.00 (2H, m, H-1), 5.62 (1H, brs, H-2), 6.03 (1H, d, *J* = 3.6 Hz, H-3), 1.46 (2H, m, H-6), 1.74 (1H, m, H-7a), 1.62 (1H, m, H-7b), 1.62 (1H, m, H-8), 2.35 (1H, brd, *J* = 12.1 Hz, H-10), 2.34 (1H, dd, *J* = 16.4, 9.0 Hz, H-11a), 1.68 (1H, m, H-11b), 5.23 (1H, brd, 8.2 Hz, H-12), 6.63 (1H, dd, *J* = 17.2, 10.7 Hz, H-14), 5.21 (1H, d, *J* = 17.2 Hz, H-15a), 5.09 (1H, d, *J* = 10.7 Hz), 1.73 (3H, s, C*H*$_3$-16), 0. 90 (3H, d, *J* = 6.6 Hz, C*H*$_3$-17), 6.69 (1H, s, H-18), 6.33 (1H, s, H-19), 0.80 (3H, s, C*H*$_3$-20), 2.06 (3H, s, CO*CH*$_3$-18), 1.86 (3H, s, CO*CH*$_3$-19) [the α-methoxy-α-(trifluoromethyl)phenyl acetate portion exhibited δ 7.60 and 7.40 (5H, m, C_6H_5), 3.57 (3H, s, O*CH*$_3$)]; HRESITOFMS *m/z* 657.2636 $[M + Na]^+$ (calcd for $C_{34}H_{41}F_3O_8$ + Na, 657.2651).

***rel*-(2*S*,5*R*,6*R*,8*S*,9*S*,10*R*,18*S*,19*R*)-18,19-Diacetoxy-18,-19-epoxy-6-methoxy-2-(2-methylbutanoyloxy)cleroda-3,-13(16),14-triene (5):** colorless crystals, yield 0.0009% w/w; R_f = 0.71 (EtOAc−hexane, 60:40); mp 190−192 °C; $[\alpha]^{25}_D$ +71.2° (*c* 0.48, MeOH); EIMS *m/z* 555 $[M + Na]^+$ (calcd for $C_{30}H_{44}O_8$ + Na, 555).

***rel*-(2*S*,5*R*,6*R*,8*S*,9*S*,10*R*,18*S*,19*R*)-18,19-Diacetoxy-18,-19-epoxy-6-hydroxy-2-(2-methylbutanoyloxy)cleroda-3,-13(16),14-triene (6):** colorless amorphous solid, yield 0.0019% w/w; R_f = 0.57 (EtOAc−hexane, 60:40); mp 165−167 °C; $[\alpha]^{22}_D$ +77.5° (*c* 0.40, MeOH); HRESITOFMS *m/z* 541.2771 $[M + Na]^+$ (calcd for $C_{29}H_{42}O_8$ + Na, 541.2777).

Antimalarial Assay. Antimalarial activity was evaluated against the parasite *Plasmodium falciparum* (K1, multidrug-resistant strain), using the method of Trager and Jensen.[23] Quantitative assessment of malarial activity in vitro was determined by means of the microculture radioisotope technique based upon the method described by Desjardins et al.[24] The inhibitory concentration (IC_{50}) represents the concentration that causes 50% reduction in parasite growth as indicated by the in vitro uptake of [^{3}H]-hypoxanthine by *P. falciparum*. The positive control compound used was artemisinin (Table 3).

Antimycobacterial Assay. Antimycobacterial activity was assessed against *Mycobacterium tuberculosis* H37Ra using the Microplate Alamar Blue Assay (MABA).[25] The standard drugs isoniazid and kanamycin sulfate were used as the positive controls (Table 3).

Cytotoxicity Assay. Cytotoxicity assays against the human epidermoid carcinoma (KB), human breast cancer (BC1), and human small cell lung cancer (NCI-H187) cell lines were performed employing the colorimetric method as described by Skehan and co-workers.[26] The reference substance used was ellipticine (Table 3).

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