Cytotoxic Clerodane Diterpenoids from Fruits of *Casearia grewiifolia*†

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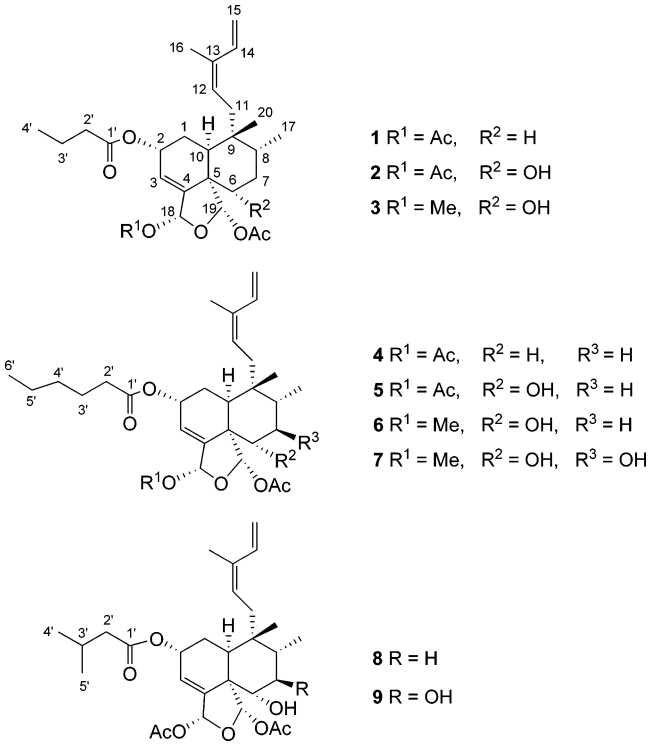
Bioactivity-guided fractionation of the ethyl acetate-soluble fraction of a methanol extract of the fruits of *Casearia grewiifolia* afforded eight new clerodane diterpenes, caseargrewiins E−L (**1**−**8**), and a known clerodane diterpene, esculentin B (**9**). The structures of **1**−**8** were established on the basis of 1D and 2D NMR spectroscopic data. Most of these compounds exhibited cytotoxicity against three cancer cell lines with IC_{50} values in the range 0.15−6.00 μg/mL.

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] The genus *Casearia* is a rich source of diterpenoids of the clerodane type.[3−7] Many of these diterpenoids have been reported to possess cytotoxic, immunomodulatory, trypanocidal, antimalarial, and antimycobacterial activities.[7−20] In a previous paper we reported the isolation and characterization of four new clerodane diterpenes, caseargrewiins A−D, and two known clerodane diterpenes from the bark of *C. grewiifolia*.[18] In our continuing search for bioactive constituents from Thai plants, the EtOAc partition from the crude MeOH extract of the fresh fruits of *C. grewiifolia* was shown to be active against three cancer cell lines (IC_{50} range 0.15−1.38 μg/mL). Further investigation of the fresh fruits of *C. grewiifolia* by bioactivity guidance led to the isolation of eight new clerodane diterpenes, caseargrewiins E−L (**1**−**8**), together with the known clerodane diterpene esculentin B (**9**).



1 R^1 = Ac, R^2 = H
2 R^1 = Ac, R^2 = OH
3 R^1 = Me, R^2 = OH

4 R^1 = Ac, R^2 = H, R^3 = H
5 R^1 = Ac, R^2 = OH, R^3 = H
6 R^1 = Me, R^2 = OH, R^3 = H
7 R^1 = Me, R^2 = OH, R^3 = OH

8 R = H
9 R = OH

Results and Discussion

Clerodane diterpenes **1**−**9** were isolated as colorless, amorphous solids from the EtOAc partition from the MeOH extract of the fresh fruits of *C. grewiifolia* using a combination of silica gel medium-pressure column chromatography and semipreparative C18 reversed-phase column HPLC. Esculentin B (**9**) was identified by comparison with NMR data from the literature.[14]

Compound **1** was assigned the molecular formula $C_{28}H_{40}O_7$, as deduced from the HRESITOFMS mass spectrum (observed *m*/*z* 511.2874 $[M + Na]^+$). The IR spectrum of compound **1** showed the presence of ester carbonyl groups (1735, 1727 cm^{-1}). The ^1H and ^{13}C NMR and DEPT data (Tables 1 and 3) indicated 28 carbons attributable to seven quaternary (including three carbonyl carbons), eight methine, seven methylene, and six methyl carbons. The ^1H NMR spectrum showed the presence of a methyl doublet at δ 0.89 (J = 7.0 Hz, H-17), a methyl singlet at δ 0.81 (H-20), one oxymethine at δ 5.40 (brs, H-2), two acetal-acyloxy methine protons at δ 6.67 (s, H-18) and 6.36 (s, H-19), and a trisubstituted olefinic proton at δ 5.90 (brd, J = 4.1 Hz, H-3), consistent with the basic skeleton of clerodane diterpenes isolated from the genus *Casearia*.[3−7] 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 protons of the 17-methyl group. The six-carbon diene side chain C-11−C-16 showed the presence of a terminal unsaturated methylene group, supported by the observation of resonances at δ 5.19 (d, J = 17.2 Hz, H-15a) and 5.10 (d, J = 10.8 Hz, H-15b), which were coupled to the methine proton at δ 6.64 (dd, J = 10.8, 17.2 Hz, H-14). The other double bond was trisubstituted with a broad doublet methine proton signal observed at δ 5.33 (J = 7.9 Hz, H-12). This was also confirmed by COSY correlations of H-11 to H-12, and H-14 to H-15. The HMBC spectrum exhibited correlations of H-12 to C-9 and C-14; H-11 to C-13; H-14 to C-12 and C-16; and H-15 to C-13, revealing that this side chain was located at the C-9 position. 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 butanoyloxy group was deduced from the resonances at δ 2.36 (2H, t, J = 7.4 Hz, H-2′), 1.71 (2H, m, H-3′), and 1.01 (3H, t, J = 7.4 Hz, H-4′). The HMBC spectrum showed correlation of the oxymethine proton, H-2, to the carbonyl carbon (δ_C 173.2) of the butanoyloxy group, which indicated that the butanoyloxy was located at C-2 (δ_C 66.4).

The relative stereochemistry of **1** was assigned from the coupling constants and the NOESY correlations of those protons. The NOESY spectrum showed correlations between H-10 and H-12, and H-10 and C-9 methyl protons, indicating that H-10 was equatorial and the A/B ring junction was *cis*. The lack of correlation between H-2 and H-10 suggested that H-2 was equatorial. NOESY correlations between H-11 and H-19, H-11, and 17-methyl protons revealed the α-orientation of an acetate group at C-19 and that the

† Dedicated to Professor Apichart Suksamrarn in honor of his 60th birthday.

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Table 1. ^{1}H NMR Data (δ, ppm) for Compounds **1**−**4** in $CDCl_3$[a]

position	**1**	**2**	**3**	**4**
1	1.91 m	1.90 m	1.89 m	1.91 m
2	5.40 brs	5.44 brs	5.47 brs	5.40 brs
3	5.90 brd (4.1)	5.98 brd (3.9)	6.06 brd (3.7)	5.87 brd (4.2)
6	1.42 m	3.78 dd (3.9, 12.2)	3.75 dd (4.1, 12.3)	1.40 m
7	1.48 m, 1.64 m	1.64 m, 1.60 m	1.61 m	1.44 m, 1.64 m
8	1.62 m	1.74 m	1.72 m	1.61 m
10	2.23 dd (6.6, 10.3)	2.35 m	2.33 m	2.18 dd (4.2, 12.3)
11	1.65 m, 2.35 m	1.57 m, 2.37 m	1.58 m, 2.37 m	1.67 m, 2.37 m
12	5.33 brd (7.9)	5.27 brd (7.7)	5.30 brd (8.2)	5.32 brd (7.5)
14	6.64 dd (10.8, 17.2)	6.62 dd (10.7, 17.0)	6.63 dd (10.7, 17.4)	6.64 dd (10.8, 17.2)
15	5.19 d (17.2), 5.10 d (10.8)	5.18 d (17.0), 5.10 d (10.7)	5.18 d (17.4), 5.09 d (10.7)	5.18 d (17.2), 5.09 d (10.8)
16	1.82 s	1.80 s	1.80 s	1.80 s
17	0.89 d (7.0)	0.91 d (6.8)	0.92 d (6.4)	0.87 d (6.9)
18	6.67 s	6.72 s	5.50 s	6.67 s
19	6.36 s	6.50 s	6.48 s	6.36 s
20	0.81 s	0.77 s	0.76 s	0.79 s
*Me*COO-18	2.10 s	2.09 s		2.09 s
*Me*O-18			3.44 s	
*Me*COO-19	2.00 s	2.00 s	2.00 s	2.00 s
2′	2.36 t (7.4)	2.36 t (7.0)	2.33 t (7.4)	2.36 t (7.3)
3′	1.71 m	1.64 m	1.63 m	1.34 m[b]
4′	1.01 t (7.4)	0.99 t (7.3)	0.97 t (7.0)	1.64 m
5′				1.34 m[b]
6′				0.90 t (6.6)

[a] Figures in parentheses are coupling constants in Hz. [b] Signals were in overlapping regions of the spectrum.

Table 2. ^{1}H NMR Data (δ, ppm) for Compounds **5**−**8** in $CDCl_3$[a]

position	**5**	**6**	**7**	**8**
1	1.93 m	1.92 m	1.92 m	1.88 m
2	5.45 brs	5.42 brs	5.43 brs	5.43 brs
3	5.97 brd (3.7)	5.98 brd (3.7)	6.19 brd (6.2)	6.01 brd (3.9)
6	3.78 dd (3.9, 12.1)	3.78 dd (4.0, 12.2)	3.64 d (9.8)	3.79 m
7	1.64 m	1.60 m	3.56 dd (9.8, 11.3)	1.61 m, 1.78 m
8	2.34 m	1.82 m	1.70 m	1.74 m
10	2.30 m	2.35 m[b]	2.24 m	2.35 m
11	1.60 m, 2.37 m	1.62 m, 2.40 m	1.52 brd (16.3), 2.54 dd (9.2, 16.0)	1.59 m, 2.37 m
12	5.27 brd (8.9)	5.35 brd (8.8)	5.24 brd (8.8)	5.27 brd (8.2)
14	6.62 dd (10.7, 17.2)	6.68 dd (10.9, 17.3)	6.65 dd (10.8, 17.3)	6.62 dd (10.9, 17.2)
15	5.19 d (17.2), 5.10 d (10.7)	5.20 d (17.3), 5.09 d (10.9)	5.20 d (17.3), 5.12 d (10.8)	5.20 d (17.2), 5.10 d (10.9)
16	1.80 s	1.82 s	1.80 s	1.80 s
17	0.90 d (6.6)	0.94 m	0.90 m	0.92 d (6.6)
18	6.73 s	5.50 s	5.30 s	6.72 s
19	6.50 s	6.46 s	6.56 s	6.50 s
20	0.78 s	0.78 s	0.80 s	0.78 s
*Me*COO-18	2.10 s			2.08 s
*Me*O-18		3.38 s	3.51 s	
*Me*COO-19	2.00 s	1.96 s	1.98 s	1.98 s
2′	2.40 t (7.5)	2.35 m[b]	2.36 t (7.3)	2.27 d (6.6)
3′	1.25 m	1.28 m	1.34 m	2.13 m
4′	1.63 m	1.65 m	1.68 m	1.01 d (6.6)
5′	1.31 m	1.35 m	1.32 m	1.01 d (6.6)
6′	0.90 t (6.6)	0.90 m	1.04 m	

[a] Figures in parentheses are coupling constants in Hz. [b] Signals were in overlapping regions of the spectrum.

17-methyl group was equatorial. The acetal-acyloxy protons H-18 and H-19 were assigned as β-oriented 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, and comparison of the specific rotation (+24.2) with the analogue casearborin A [+35.0 (*c* 0.59, MeOH],[10] the structure of **1** was determined to be a new clerodane diterpenoid, *rel*-(2*R*,5*S*,8*R*,9*R*,10*S*,18*R*,19*S*)-18,19-diacetoxy-18,19-epoxy-2-butanoyloxyclero-3,12(*Z*),14-triene, which we named caseargrewiin E.

Compound **2** was assigned the molecular formula $C_{28}H_{40}O_8$, as deduced from the HRESITOFMS mass spectrum (observed *m*/*z* 527.2621 $[M + Na]^+$). The IR spectrum indicated the presence of hydroxyl (3465 cm^{-1}) and carbonyl ester groups (1742 cm^{-1}). The ^{1}H and ^{13}C NMR spectra of **2** (Tables 1 and 3) indicated that it has one more oxymethine than **1**, and MS confirmed this extra oxygen. Since the remaining oxymethine appeared as a doublet of doublets (δ_H 3.78, J = 3.9, 12.2 Hz), it must be adjacent to two protons, and therefore **2** appeared to be the C-6 hydroxy analogue of compound **1**. The ^{1}H and ^{13}C NMR assignments were confirmed by analysis of the 2D NMR spectra. The relative stereochemistry of **2** was assigned from the coupling constants together with the NOESY correlation of those protons and was supported by comparison of its optical rotation to the most closely related compound, casearborin D.[10] The coupling constants observed for H-6 (J = 3.9, 12.2 Hz) in the ^{1}H NMR spectrum indicated that H-6 is in an axial position. The 1,3-diaxial NOESY correlation was also observed for H-6 and H-8, which enabled the H-17 methyl group to be equatorial. On the basis of the above data, the structure of **2** was elucidated as *rel*-(2*R*,5*S*,6*S*,8*R*,9*R*,10*S*,18*R*,19*S*)-18,19-diacetoxy-18,19-epoxy-2-bu-

Table 3. ^{13}C NMR Data (δ, ppm) for Compounds **1**−**8** in $CDCl_3$[a]

position	1	2	3	4	5	6	7	8
1	26.2 t[a]	26.8 t	27.0 t	27.4 t	26.8 t	26.4 t	27.0 t	26.7 t
2	66.4 d	66.2 d	66.2 d	66.3 d	66.2 d	66.8 d	66.0 d	66.3 d
3	120.5 d	121.7 d	121.7 d	120.4 d	121.6 d	121.3 d	125.0 d	121.8 d
4	147.0 s	145.3 s	146.4 s	146.9 s	145.3 s	146.8 s	144.9 s	145.5 s
5	49.1 s	53.5 s	53.5 s	49.0 s	53.5 s	53.4 s	51.0 s	53.5 s
6	29.3 t	72.8 d	73.2 d	29.2 t	72.9 d	72.1 d	75.9 d	72.9 d
7	27.4 t	37.2 t	37.4 t	27.3 t	37.1 t	36.6 t	73.2 d	37.3 t
8	36.6 d	36.6 d	36.7 d	36.4 d	36.5 d	36.2 d	40.8 d	36.8 d
9	37.6 s	37.7 s	37.8 s	37.6 s	37.7 s	37.5 s	38.7 s	37.8 s
10	34.7 d	36.7 d	36.5 d	34.7 d	36.7 d	36.7 d	36.6 d	36.7 d
11	29.2 t	29.1 t	29.2 t	29.3 t	29.1 t	28.8 t	30.0 t	29.1 t
12	126.8 d	126.6 d	127.0 d	126.8 d	126.8 d	126.8 d	126.6 d	126.6 d
13	133.5 s	133.5 s	133.3 s	133.5 s	133.4 s	133.3 s	133.6 s	133.5 s
14	133.4 d	133.4 d	133.4 d	133.4 d	133.4 d	133.1 d	133.4 d	133.4 d
15	114.1 t	114.3 t	114.3 t	114.1 t	114.2 t	113.5 t	114.5 t	114.3 t
16	20.4 q	20.4 q	20.4 q	20.4 q	20.3 q	19.3 q	20.4 q	20.4 q
17	15.7 q	15.6 q	15.6 q	15.6 q	15.6 q	14.7 q	11.0 q	15.6 q
18	96.4 d	95.7 d	104.8 d	94.6 d	95.8 d	104.6 d	104.1 d	95.7 d
19	99.1 d	97.3 d	97.1 d	99.1 d	97.5 d	98.0 d	98.0 d	97.3 d
20	25.6 q	24.9 q	25.6 q	25.6 q	24.9 q	24.2 q	24.7 q	24.9 q
*Me*COO-18	21.2 q	21.3 q		21.2 q	21.4 q			21.2 q
*Me*O-18			56.2 q			54.3 q	56.3 q	
Me*C*OO-18	170.3 s	170.2 s		170.3 s	170.2 s			170.1 s
*Me*COO-19	21.2 q	21.2 q	21.5 q	21.2 q	21.2 q	20.5 q	21.4 q	21.3 q
Me*C*OO-19	169.5 s	169.3 s	170.1 s	169.5 s	169.3 s	170.1 s	169.5 s	169.2 s
1′	173.2 s	173.1 s	173.4 s	173.3 s	173.3 s	173.4 s	173.1 s	172.5 s
2′	36.5 t	36.5 t	36.5 t	34.6 t	34.5 t	34.0 t	34.5 t	43.6 t
3′	18.7 t	18.6 t	18.6 t	31.1 t	31.1 t	31.0 t	31.3 t	26.1 d
4′	13.6 q	13.6 q	13.6 q	24.8 t	24.8 t	24.6 t	25.6 t	22.3 q
5′				22.3 t	22.2 t	22.0 t	22.4 t	22.4 q
6′				13.9 q	13.9 q	13.0 q	13.8 q	

[a] Multiplicities were determined by analyses of the DEPT and/or HSQC spectra.

tanoyloxy-6-hydroxycleroda-3,12(*Z*),14-triene and was named caseargrewiin F.

Compound **3** had the molecular formula $C_{27}H_{40}O_7$, as deduced from the HRESITOFMS mass spectrum (observed *m*/*z* 499.2672 $[M + Na]^+$). The IR spectrum showed the presence of hydroxyl (3494 cm^{-1}) and carbonyl ester groups (1730 cm^{-1}). The ^1H and ^{13}C NMR spectra of **3** (Tables 1 and 3) were similar to those of **2** except that an acetoxy group at C-18 was displaced by a methoxy group (δ_H 3.44, δ_C 56.2), which was indicated by the HMBC correlation of methoxy protons to C-18. The relative stereochemistry of **3** was identical to that of **2** on the basis of the same characteristics of NOESY correlations and the coupling constants of those protons, as well as the same sign of specific optical rotation. On the above evidence, **3** was assigned as *rel*-(2*R*,5*S*,6*S*,8*R*,9*R*,10*S*,18*S*,19*S*)-18-methoxy,19-acetoxy-18,19-epoxy-2-butanoyloxy-6-hydroxycleroda-3,12(*Z*),14-triene and was named caseargrewiin G.

Compound **4** had the molecular formula $C_{30}H_{44}O_7$, as deduced from the HRESITOFMS mass spectrum (observed *m*/*z* 539.3100 $[M + Na]^+$). The IR spectrum indicated the presence of carbonyl ester groups (1738 cm^{-1}). The ^1H and ^{13}C NMR spectra of **4** (Tables 1 and 3) were similar to those of **1** except for a butanoyloxy group at C-2, which was displaced by a hexanoyloxy group [δ_H 2.36 (2H, t, J = 7.3 Hz), 1.64 (2H, m), 1.34 (4H, m), 0.90 (3H, t, J = 6.6 Hz)]. The ^1H and ^{13}C NMR assignments were confirmed by analysis of the DEPT and 2D NMR spectra. Compound **4** was thus identified as *rel*-(2*R*,5*S*,8*R*,9*R*,10*S*,18*R*,19*S*)-18,19-diacetoxy-18,19-epoxy-2-hexanoyloxycleroda-3,12(*Z*),14-triene, which has been named caseargrewiin H.

Compound **5** had the molecular formula $C_{30}H_{44}O_8$, as deduced from the HRESITOFMS mass spectrum (observed *m*/*z* 555.2924 $[M + Na]^+$). The IR spectrum showed the presence of hydroxyl (3460 cm^{-1}) and carbonyl ester groups (1726 cm^{-1}). The ^1H and ^{13}C NMR spectra of **5** (Tables 2 and 3) indicated that it has one more oxymethine than **4**, which agrees with the MS data. Because this oxymethine appeared as a doublet of doublets (δ_H 3.78, dd, J = 3.9, 12.1 Hz), it must be adjacent to two protons; this indicated that **5** was the C-6 hydroxy analogue of compound **4**. The assignment was confirmed by analysis of the 2D NMR spectra of **5**. The structure of **5** is very close to a known compound, casearvestrin C,[12] accept that the configurations of the C-12,13 double bond and C-2 in **5** were *Z* and *R*, respectively. Differences in chemical shifts in the ^1H and ^{13}C NMR spectra between compound **5** and casearvestrin C were noted at δ_H 1.93 and 2.13 (for H-1), and 5.45 and 5.62 (for H-2), whereas the carbon signals differed at δ_C 66.2 and 70.5 (for C-2), respectively. The sign of specific rotation of **5** (+49.8) was opposite of that of casearvestrin C [−61.2 (*c* 0.42, $CHCl_3$)], which had the relative configuration *S* at C-2.[12] Thus, compound **5** was established as a new epimer, *rel*-(2*R*,5*S*,6*S*,8*R*,9*R*,10*S*,18*R*,19*S*)-18,19-diacetoxy-18,19-epoxy-2-hexanoyloxy-6-hydroxycleroda-3,12(*Z*),14-triene, which has been named caseargrewiin I.

Compound **6** had the molecular formula $C_{29}H_{44}O_7$, as deduced from the HRESITOFMS mass spectrum (observed *m*/*z* 527.2987 $[M + Na]^+$). The IR spectrum showed the presence of hydroxyl (3546 cm^{-1}) and carbonyl ester groups (1734 cm^{-1}). The ^1H and ^{13}C NMR spectra of **6** (Tables 2 and 3) were similar to those of **5** except for an acetoxy group at C-18, which was displaced by a methoxy group (δ_H 3.38, δ_C 54.3). This was confirmed by the HMBC correlation of methoxy protons to C-18. The structural assignment was also confirmed by analysis of the 2D NMR spectra. Thus, **6** was established as *rel*-(2*R*,5*S*,6*S*,8*R*,9*R*,10*S*,18*S*,19*S*)-18-methoxy,19-cetoxy-18,19-epoxy-2-hexanoyloxy-6-hydroxycleroda-3,12(*Z*),14-triene, which has been named caseargrewiin J.

Compound **7** had the molecular formula $C_{29}H_{44}O_8$, as deduced from the HRESITOFMS mass spectrum (observed *m*/*z* 543.2989 $[M + Na]^+$). The IR spectrum indicated hydroxyl (3494 cm^{-1}) and ester carbonyl groups (1741 cm^{-1}). The ^1H and ^{13}C NMR spectra of **7** (Tables 2 and 3) indicated that it had one more oxymethine group than **6**, consistent with the MS data. Because this oxymethine, δ_H 3.44 (dd, J = 9.8, 11.3 Hz), appeared as a doublet of doublets, it must be adjacent to two protons, indicating that **7** is the C-7 hydroxy analogue of compound **6**. The coupling constants observed

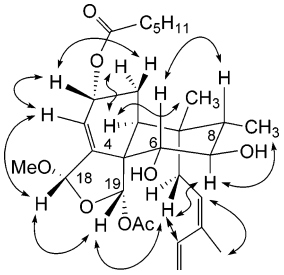

Figure 1. Selected NOESY correlations of **7**.

between H-6 and H-7 (J = 9.8 Hz) and the lack of correlation between these two protons in the NOESY spectrum indicated that H-6 and H-7 are axial. A 1,3-diaxial NOESY correlation was also observed for H-6 and H-8, which enabled the H-17 methyl group to be assigned as equatorial. The NOESY correlations between protons on **7** (Figure 1) led to the assignment of its stereochemistry. On the basis of the above evidence, and comparison of the specific optical rotation (+12.4) with those of known compounds,[4,11,18,20] the structure of **7** was elucidated as *rel*-(2*R*,5*S*,6*R*,7*R*,8*S*,9*S*,10*S*,-18*S*,19*S*)-18-methoxy,19-acetoxy-18,19-epoxy-2-hexanoyloxy-6,7-dihydroxycleroda-3,12(*Z*),14-triene, which has been named caseargrewiin K.

Compound **8** had the molecular formula $C_{29}H_{42}O_8$, deduced from the HRESITOFMS mass spectrum (observed *m*/*z* 541.2768 [M + Na]$^+$). The ^{1}H and ^{13}C NMR spectra of **8** (Tables 2 and 3) were similar to those of **2** except for the butanoyloxy group, which was displaced by a 3-methylbutanoyloxy group [δ_H 2.27 (2H, d, J = 6.6 Hz), 2.13 (2H, m), 1.01 (6H, d, J = 6.6 Hz)] at the C-2 position. The structural assignment was confirmed by analysis of the DEPT and 2D NMR spectra. Thus, compound **8** was determined to be *rel*-(2*R*,5*S*,6*S*,8*R*,9*R*,10*S*,18*R*,19*S*)-18,19-diacetoxy-18,19-epoxy-2-(3-methylbutanoyloxy)-6-hydroxycleroda-3,12(*Z*),14-triene and was named caseargrewiin L.

The biological test results of the isolated compounds are shown in Table 4. Caseargrewiins E−L (**1**−**8**) and the known esculentin B (**9**) showed significant cytotoxicity against three cancer cell lines (KB, BC1, and NCI-H187) with IC_{50} values ranging from 0.15 to 6.00 μg/mL. Among these, **1** and **3** have respective IC_{50} values against KB of 0.66 and 0.67 μg/mL, while **2**, **5**, and **9** have respective IC_{50} values against BC1 of 0.20, 0.21, and 0.17 μg/mL, which are close to the control drug, ellipticine (Table 4).

Experimental Section

General Experimental Procedures. 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 taken using 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 spectra were obtained using a Micromass LCT mass spectrometer, and the lock mass calibration was applied for the determination of accurate masses. Preparative medium-pressure liquid chromatography (MPLC) was carried out on a Büchi apparatus, and HPLC was carried out on an Agilent 1100 series instrument with a semipreparative C18 column (Zorbax SB-C18, 5 μm, 9.4 × 250 mm).

Table 4. Biological Activities of Compounds **1**−**9**

	cytotoxicity (IC_{50}, μg/mL)		
compound	KB[a]	BC1[b]	NCl-H187[c]
1	0.66	0.91	0.15
2	inactive	0.20	4.72
3	0.67	3.97	5.57
4	0.88	0.21	0.54
5	3.04	0.36	0.33
6	inactive	0.99	1.22
7	inactive	3.40	5.11
8	6.0	inactive	0.32
9	inactive	0.17	0.68
ellipticine	0.67	0.13	
doxorubicin	0.23	0.13	0.048

[a] Human epidermoid carcinoma in the mouth. [b] Human breast cancer cell. [c] Human small cell lung cancer.

Plant Material. Fruits of *Casearia grewiifolia* Vent. were collected on the Khon Kaen University campus in June 2005 and 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, Thailand.

Extraction and Isolation. The fresh fruits of *C. grewiifolia* (1.5 kg) were ground and extracted with MeOH (1.5 L ×3). The concentrated MeOH solution (1 L) was partitioned successively between hexane−H_2O and then EtOAc−H_2O to give a crude hexane extract (17.5 g) and a crude EtOAc extract (29.3 g), respectively. The EtOAc extract (8.5 g) was subjected to silica gel MPLC and eluted with increasing concentrations of EtOAc in hexane and MeOH in EtOAc. Each fraction was collected on the basis of the eluent composition to give five major fractions (EF_1−EF_5). Fraction EF_1 (3.0 g, 0−30% EtOAc−hexane) was again subjected to MPLC to give five fractions ($EF_{1.1}$−$EF_{1.5}$). Fraction $EF_{1.1}$ (300.0 mg, 0−20% EtOAc−hexane) was then purified by HPLC (20% H_2O−MeOH, flow rate 2.5 mL/min) to give compound **1** (34.7 mg). Fraction $EF_{1.2}$ (850.0 mg, 21−30% EtOAc−hexane) was purified by HPLC (25% H_2O−MeOH, flow rate 3.0 mL/min) to afford compounds **4** (29.4 mg), **2** (110.3 mg), **3** (164.0 mg), **9** (53.3 mg), **7** (25.8 mg), **5** (125.8 mg), and **6** (14.8 mg). Fraction $EF_{1.3}$ (400.0 mg, 31−35% EtOAc−hexane) was purified by HPLC (32% H_2O−CH_3CN, flow rate 2.5 mL/min) to give a subfraction designated as $EF_{1.3}H_1$ and additional amounts of compounds **5** (61.0 mg) and **2** (31.0 mg). Subfraction $EF_{1.3}H_1$ was further purified by HPLC (38% H_2O−MeOH, flow rate 2.5 mL/min), affording compound **8** (15.0 mg).

Cytotoxicity Assay. Cytotoxic assays against 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.[21] The reference compounds were ellipticine and doxorubicin (Table 4).

Caseargrewiin E (1): colorless, amorphous solid; $[\alpha]^{28}_D$ +24.2 (*c* 0.290, MeOH); UV (MeOH) λ_{max} (log ϵ) 202 (4.47), 234 (4.67) nm; IR (KBr) ν_{max} 3020, 2962, 2932, 1735, 1727, 1500, 1460, 1375, 1215, 758 cm^{-1}; ^{1}H and ^{13}C NMR data, see Tables 1 and 3; HRESITOFMS *m*/*z* 511.2874 [M + Na]$^+$ (calcd for $C_{28}H_{40}O_7$ + Na, 511.2774).

Caseargrewiin F (2): colorless, amorphous solid; $[\alpha]^{26}_D$ +68.3 (*c* 0.300, MeOH); UV (MeOH) λ_{max} (log ϵ) 203 (4.08), 234 (3.91) nm; IR (KBr) ν_{max} 3465, 2968, 1742, 1633, 1463, 1376, 1220 cm^{-1}; ^{1}H and ^{13}C NMR data, see Tables 1 and 3; HRESITOFMS *m*/*z* 527.2621 [M + Na]$^+$ (calcd for $C_{28}H_{40}O_8$ + Na, 527.2723).

Caseargrewiin G (3): colorless, amorphous solid; $[\alpha]^{28}_D$ +23.6 (*c* 0.200, MeOH); UV (MeOH) λ_{max} (log ϵ) 202 (3.42), 230 (3.02) nm; IR (KBr) ν_{max} 3494, 2970, 1730, 1633, 1460, 1375, 1177 cm^{-1}; ^{1}H and ^{13}C NMR data, see Tables 1 and 3; HRESITOFMS *m*/*z* 499.2672 [M + Na]$^+$ (calcd for $C_{27}H_{40}O_7$ + Na, 499.2774).

Caseargrewiin H (4): colorless, amorphous solid; $[\alpha]^{29}_D$ +42.4 (*c* 0.200, MeOH); UV (MeOH) λ_{max} (log ϵ) 202 (4.59), 234 (4.46) nm; IR (KBr) ν_{max} 3024, 2972, 1738, 1462, 1374, 1221, 769 cm^{-1}; ^{1}H and ^{13}C NMR data, see Tables 1 and 3; HRESITOFMS *m*/*z* 539.3100 [M + Na]$^+$ (calcd for $C_{30}H_{44}O_7$ + Na, 539.3087).

Caseargrewiin I (5): colorless, amorphous solid; $[\alpha]^{26}_D$ +49.8 (*c* 0.200, MeOH); UV (MeOH) λ_{max} (log ϵ) 202 (4.12), 234 (4.10) nm; IR (KBr) ν_{max} 3460, 3019, 2963, 1726, 1455, 1376, 866 cm^{-1}; ^{1}H and ^{13}C NMR data, see Tables 2 and 3; HRESITOFMS *m*/*z* 555.2924 [M + Na]$^+$ (calcd for $C_{30}H_{44}O_8$ + Na, 555.3036).

Caseargrewiin J (6): colorless, amorphous solid; $[\alpha]^{26}_D$ +22.8 (*c* 0.200, MeOH); UV (MeOH) λ_{max} (log ϵ) 202 (3.69), 233 (3.58) nm; IR (KBr) ν_{max} 3546, 2974, 1734, 1469, 1384, 1261 cm^{-1}; ^{1}H and ^{13}C NMR data, see Tables 2 and 3; HRESITOFMS *m*/*z* 527.2987 [M + Na]$^+$ (calcd for $C_{29}H_{44}O_7$ + Na, 527.3087).

Caseargrewiin K (7): colorless, amorphous solid; $[\alpha]^{26}_D$ +12.4 (*c* 0.300, MeOH); UV (MeOH) λ_{max} (log ϵ) 203 (4.12), 234 (4.67) nm; IR (KBr) ν_{max} 3494, 2972, 1741, 1463, 1376, 1223 cm^{-1}; ^{1}H and ^{13}C NMR data, see Tables 2 and 3; HRESITOFMS *m*/*z* 543.2989 [M + Na]$^+$ (calcd for $C_{29}H_{44}O_8$ + Na, 543.3036).

Caseargrewiin L (8): colorless, amorphous solid; $[\alpha]^{28}_D$ +108.1 (*c* 0.300, MeOH); UV (MeOH) λ_{max} (log ϵ) 203 (3.96), 234 (4.67) nm;

IR (KBr) ν_{max} 3523, 2964, 1756, 1728, 1467, 1373, 1223 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 2 and 3; HRESITOFMS *m*/*z* 541.2768 $[M + Na]^+$ (calcd for $C_{29}H_{42}O_8$ + Na, 541.2880).

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