New Cytotoxic Clerodane Diterpenoids from the Leaves and Twigs of *Casearia membranacea*

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Bioassay-guided fractionation of an EtOAc-soluble extract of *Casearia membranacea* has resulted in the isolation of six new clerodane diterpenes, caseamembrins A−F (**1**−**6**), and a known compound, *rel*-(2*S*,5*R*,6*R*,8*S*,9*S*,10*R*,18*S*,19*R*)-diacetoxy-18,19-epoxy-6-hydroxy-2-(2-methylbutanoyloxy)cleroda-3,13(16),14-triene (**7**). The structures of **1**−**6** were established on the basis of extensive 1D and 2D NMR spectroscopic analysis. In addition, the new derivatives, **8** and **9**, were prepared by acylation of **7** and **3**, respectively. The isolated diterpenoids and their derivatives were tested against human prostate (PC-3) and hepatoma (Hep3B) cancer cells. Compounds **1**, **3**−**5**, and **7** exhibited cytotoxicity against both tumor cells, with IC_{50} values below 3 μM, while compounds **2**, **6**, **8**, and **9** were less effective.

The genus *Casearia* contains about 180 species and occurs in the tropics. Only one species is distributed in Taiwan, namely, *Casearia membranacea* Hance (Flacourtiaceae), a tree found in the northern region of the island.[1] Previously, the genus has been reported to produce coumarins,[2] aryltetralin lignans,[3] and a series of clerodane diterpenoids.[4−14] In our continuing search for new antitumor agents from terrestrial plants,[15,16] an acetone extract of the leaves and twigs of *C. membranacea* showed activity when evaluated against human NUGC (gastric carcinoma), HONE-1 (mouth epidermoid carcinoma), and human prostate tumor cells. The preliminary biological activity prompted us to investigate *C. membranacea*, from which six new clerodane diterpenes (**1**−**6**) were isolated. The structure determination of these compounds was conducted primarily by one- and two-dimensional NMR data interpretation, with particular emphasis on HMBC and NOESY experiments.

Results and Discussion

The molecular formula of **1** was established as $C_{31}H_{46}O_8$ from HRFABMS and ^{13}C NMR spectra. The UV spectrum showed an absorption attributable to a monosubstituted conjugated diene at 223 nm. The IR spectrum displayed absorption bands diagnostic of a hydroxyl group (3450 cm^{-1}), ester carbonyl(s) (1728 br cm^{-1}), and olefinic bond(s) (1635 cm^{-1}). The ^1H NMR and ^{13}C NMR spectral values (Tables 1 and 2) revealed the presence of acetyloxy, butanoyloxy, and 2-methylbutanoyloxy substituents. These findings were further supported from the COSY and NOESY spectra as well as by the facile loss of acetic acid ($[M - 60]^+$), 2-methylbutyric acid ($[M - C_5H_{10}O_2]^+$), and butanoyl ($[M - C_4H_7O]^+$) moieties from the molecular ion in the EIMS.[3,6] Subtracting the ^{13}C NMR signals of carbon atoms (Table 2) assignable to these three esters afforded 20 carbon atoms, consistent with the product of a diterpenoid unit. The olefinic region of the ^1H NMR spectrum of **1** revealed proton signals with characteristic *trans*/*cis* coupling at δ 5.22 (d, J = 17.5 Hz, H-15a), 5.03 (d, J = 10.8 Hz, H-15b), and 6.47 (dd, J = 17.5, 10.8 Hz, H-14), indicating a terminal monosubstituted olefin. Conversely, another terminal methylene, identified by two proton signals at δ 4.97 and 5.02 (H-16), did not display any splitting, and thus it was connected to a quaternary carbon (C-13). An additional isolated olefinic singlet was detected at δ 5.39 (H-3) that was correlated to a methine carbon at δ 120.9 (C-3) in the HMQC spectrum. Two deshielded acetal proton singlets were observed at δ 6.68 (H-18) and 6.40 (H-19), which correlated to methine signals at δ 95.4 and 98.1, respectively.[3] In addition, the ^1H NMR spectrum revealed a methyl singlet at δ 0.98 (H-20), a methyl doublet at δ 0.93 (H-17), and two oxymethines at δ 5.39 (H-2) and 3.90 (H-6). Taking into account three olefinic bonds and three ester carbonyls, the extra degrees of unsaturation were presumed to be due to three rings to justify the existence of nine degrees of unsaturation. The spectral data of **1** are in agreement with the basic skeleton of clerodane diterpenoids previously isolated from *Casearia* species.[4−14] The presence of a six-carbon diene side chain at C-9 was confirmed by the long-range correlations between H-11/C-9, H-11, H-14/C-12, and H-12, H-15, H-16/C-13. The position of the acylated acetal at C-18/C-19 was proved by long-range correlations between H-18/C-5, C-19 and H-19/C-5, C-6, C-18. The proton signal at δ 5.97 (H-3) exhibited a long-range correlation with carbon signals at δ 26.6 (C-1), 54.0 (C-5), and 95.4 (C-18), indicating that a double bond is located between C-3 and C-4. The oxymethine signal at δ 3.90 (H-6), correlating to a carbon signal at δ 72.2 (C-6), had long-range correlations to carbon signals at δ 54.0 (C-5), 37.1 (C-8), and 36.4 (C-10), indicating that the hydroxyl group is located at C-6. The relative downfield shift of δ 5.39 (H-2), as well as its long-range correlations to carbon signals at δ 146.8 (C-4) and 175.0 (C-1′), established the point of attachment of the 2-methylbutanoyl ester to C-2. Likewise, the attachment of the acetate ester to C-19 (δ 98.1) and butanoyl ester to C-18 (δ 95.4) was determined by long-range correlations between H-18 (δ 6.68) and H-19 (δ 6.40) with the corresponding carbonyl signal of the attached ester in each case.

The relative stereochemistry of the ester side chain at C-2 was deduced to be β, as indicated by the broad singlet

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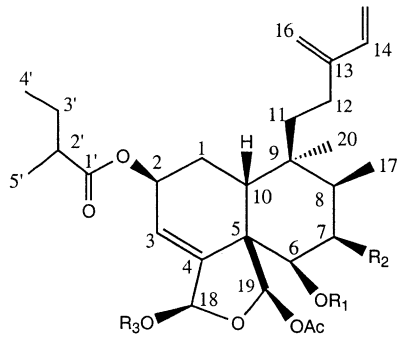
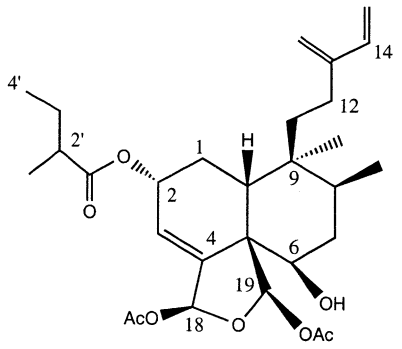


1 R_1 = H, R_2 = H, R_3 = $CO(CH_2)_2CH_3$ (1" / 4")
2 R_1 = H, R_2 = H, R_3 = Me
3 R_1 = H, R_2 = OH, R_3 = $CO(CH_2)_2CH_3$
4 R_1 = H, R_2 = OAc, R_3 = $CO(CH_2)_2CH_3$
7 R_1 = H, R_2 = H, R_3 = Ac
8 R_1 = nicotinyl, R_2 = H, R_3 = Ac
9 R_1 = Ac, R_2 = OAc, R_3 = $CO(CH_2)_2CH_3$



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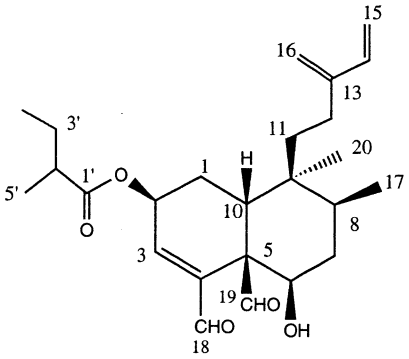

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at δ 5.39 and the chemical shift of C-2 at δ 66.4, while the chemical shift of C-10 at δ 36.4 suggested the β-axial orientation of H-10 (dd, J = 12, 5 Hz, *cis*-fused A/B rings).[14] The strong NOESY correlations (Figure 1) between H-2α/H-1α; H-1α/H-6α, H-8α, H-20α; H-6α/H-8α; H-18α/H-19α; and H-1β/H-10β established the relative stereochemistry of **1**. On the basis of the above observations, the structure of **1** was assigned as *rel*-(2*S*,5*R*,6*R*,8*S*,9*S*,10*R*,18*S*,19*R*)-19-acetoxy-18-butanoyloxy-18,19-epoxy-6-hydroxy-2-(2-methylbutanoyloxy)cleroda-3,13(16),14-triene. Compound **1** was named caseamembrin A.

Compound **2** was assigned a molecular formula of $C_{28}H_{42}O_7$, as deduced from its HRFABMS and ^{13}C NMR data. Its ^1H NMR spectrum displayed a characteristic singlet for a methoxyl group at δ 3.43, which showed a ^1J correlation to a carbon signal at δ 56.2. The HMBC spectrum showed that this methoxyl signal was correlated to the acetal carbon signal at δ 104.6 (C-18), which, in turn, was correlated to the proton signals at δ 6.44 (H-19) and 6.06 (H-3). Detailed inspection of the spectral data of **2** revealed values similar to those of **1** except for the absence of ^{13}C NMR and ^1H NMR signals assignable to the butanoyl ester (Tables 1 and 2) in addition to a significant upfield shift of H-18 (δ 5.50).[17] The NOESY spectrum displayed the same correlations as in the case of **1**. These findings led to the conclusion that **2** has the structure *rel*-(2*S*,5*R*,6*R*,8*S*,9*S*,10*R*,18*S*,19*R*)-19-acetoxy-18,19-epoxy-6-hydroxy-18-methoxy-2-(2-methylbutanoyloxy)cleroda-3,13-(16),14-triene (caseamembrin B).

The HRFABMS analysis of **3** indicated that it has a molecular formula $C_{31}H_{46}O_9$ with one extra oxygen atom more than **1**. On comparison with spectral data of **1**, the NMR spectrum of **3** was shown to lack the methylene protons and upfield carbon signal attributable to C-7 (δ_H 1.68, 1.88, and δ_C 36.9), and they were replaced by downfield oxymethine signals at δ_H 3.66 (1H, dd, J = 10.5, 8.7 Hz) and δ_C 72.5. This was associated with relative downfield shifts of both C-6 (4.5 ppm) and C-8 (6.1 ppm). It was suggested that **3** has the same structure as **1** but with a hydroxyl substitution at C-7. The HMBC spectrum confirmed the proposed structure through revealing cross-peaks between the oxymethine signals at δ_H 3.66 and δ_C 53.1 (C-5) and δ_C 11.0 (C-17). The coupling constant of H-7 (10.5 Hz) suggested that it is axial, and its NOE correlation with H-20 indicated its α-orientation. Other NOE correlations were similar to those of **1**. Consequently, **3** was assigned the structure *rel*-(2*S*,5*R*,6*S*,7*R*,8*S*,9*S*,10*R*,18*S*,19*R*)-19-acetoxy-18-butanoyloxy-18,19-epoxy-6,7-dihydroxy-2-(2-methylbutanoyloxy)cleroda-3,13(16),14-triene (caseamembrin C).

Compound **4** was found to possess a molecular weight of 604, corresponding to a molecular composition of $C_{33}H_{48}O_{10}$ and 10 units of unsaturation, as suggested by its FABMS and HRFABMS. Its spectral data (Tables 1 and 2) were almost identical to those of **3** except for the presence of an additional acetyl moiety (δ_C 171.7, 21.3; δ_H 1.87) in addition to a significant deshielding of H-7 (1.41 ppm) and C-7 (2.8 ppm). This clearly indicated the presence of an acetyloxyl moiety at C-7, which was substantiated by long-range correlations between H-7/C-6, C-8, C-17 as well as the ketone carbon at δ 171.7. All the spectral data of **4** led to the assignment of its structure as *rel*-(2*S*,5*R*,6*S*,7*R*,8*S*,9*S*,10*R*,18*S*,19*R*)-7,19-diacetoxy-18-butanoyloxy-18,19-epoxy-6-hydroxy-2-(2-methylbutanoyloxy)cleroda-3,13(16),14-triene (caseamembrin D).

The NMR data of **5** were closely related to those of **1** with the absence of signals assignable to the butanoyl ester and the presence of two acetate esters instead of one (δ_H 2.06, 1.86 and δ_C 170.1, 169.8, 21.2, 21.0). This was substantiated by the HRFABMS data, which revealed the molecular formula, $C_{29}H_{42}O_8$. The HMBC spectrum showed long-range correlations between H-18 (δ 6.69) and the carbonyl carbon of one acetyl at δ 170.1, while H-19 (δ 6.42) displayed correlations with the carbonyl carbon of the other acetyl at δ 169.8. It was obvious that **5** contained two acetoxyl moieties located at C-18 and C-19 of the acetal ring.[14] This was supported by the long-range correlations of H-18/C-3, C-5, C-19 and H-19/C-5, C-18. In contrast to compounds **1**−**4** and **6**, compound **5** possessed a negative sign of optical rotation, suggesting a different configuration at one of its chiral centers. Thorough inspection of the NMR data (Tables 1 and 2) of **5** in comparison with the previously discussed compounds revealed a relative deshielding of C-2

Table 1. ^{1}H NMR Data ($CDCl_3$, 300 MHz) of Compounds **1**−**6**[a,b]

position	**1**[c]	**2**	**3**	**4**	**5**	**6**
1	2.04 m	2.02 m	2.07 m	2.04 m	2.01 m	2.02 m
	1.92 m	1.88 m	1.82 m	1.95 m	1.75 m	1.88 m
2	5.39 brs	5.47 brd (2)	5.40 brs	5.44 m	5.58 dd (8.4,7.2)	5.54 brs
3	5.97 brd (4.2)	6.06 dd (3,1)	6.01 d (4.2)	6.03 d (4.3)	5.86 s	7.03 d (4.9)
6	3.90 d (7.6)	3.78 dd (8,4)	3.56 d (8.7)	3.69 d (6.5)	3.99 dd (11.4, 4.5)	3.86 t (7.5)
7	1.68 m	1.75 m	3.66 dd	5.07 m	1.75 m	1.88 m
	1.88 m	1.68 m	(10.5, 8.7)			1.95 m
8	1.81 m	1.72 m	1.65 m	1.85 m	1.80 m	1.78 m
10	2.41 dd (12,5)	2.35 m	2.29 brs	2.40 m	2.40 m	2.40 m
11	1.34 m	1.24 m	1.65 m	1.30 m	1.55 m	1.22 m
	1.55 m	1.51 m	1.22 m	1.72 m	1.58 m	1.52 m
12	2.19 m	2.07 m	2.05 m	1.87 m	2.12 m	1.95 m
14	6.47 dd (17.5, 10.8)	6.44 dd (17.5, 11)	6.42 dd (17.6, 11.1)	6.43 dd (17.5, 10.8)	6.45 dd (17.6, 7.5)	6.31 dd (17.6, 10.7)
15	5.22 d (17.5)	5.15 d (17.5)	5.17 d (17.6)	5.16 d (17.5)	5.21 d (17.6)	5.16 d (17.6)
	5.03 d (10.8)	5.02 d (11.0)	5.01 d (11.1)	5.06 d (10.8)	5.04 d (7.5)	5.01 d (10.7)
16	4.97 s	5.05 s	5.01 s	4.97 s	5.00s	5.00 s
	5.02 s	4.94 s	5.02 s	5.02 s	4.92 s	5.03 s
17	0.93 d (7.0)	0.93 d (7.0)	0.92 d (7.4)	0.93 d (6.5)	0.94 d (6.7)	0.90 d (6.7)
18	6.68 s	5.50 d (1)	6.69 s	6.74 s	6.69 s	9.41 s
19	6.40 s	6.44 s	6.50 s	6.52 s	6.42 s	10.3 s
20	0.98 s	1.92 s	0.92 s	1.01 s	0.92 s	0.99 s
2′	2.29 m	2.47 m	2.27 m	2.40 m	2.41 m	2.47 m
3′	1.83 m	1.68 m	2.07 m	1.73 m	2.23 m	1.72 m
	1.65 m	1.56 m		1.61 m		1.51 m
4′	0.93 t (overlap)	0.94 t (6.0)	0.95 t (6.5)	0.93 t (6.5)	0.96 t (6.4)	0.93 t (8.1)
5′	1.14 d (6.6)	1.15 d (7.0)	0.96 d (overlap)	1.17 d (7.0)	1.18 d (6.1)	1.20 d (6.9)
2″	2.29 t (7.4)		2.30 m	2.40 m		
				2.25 m		
3″	1.61 m		1.64 m	1.64 m		
4″	0.90 t (7.0)		0.97 t (overlap)	0.90 t (overlap)		
OAc	1.80 s	1.91 s		2.11 s	1.86 s	
				1.87 s	2.06 s	
OMe		3.43 s				
CHO						9.41 s
						10.30 s

[a] Chemical shifts in ppm, *J* values in Hz are in parentheses. [b] Assignments were made using HMQC and HMBC techniques. [c] Measured in acetone-d_6.

(δ 70.5) and the increase of the coupling constant of H-2 (dd, J = 8.4, 7.2 Hz) compared to the case of the other analogues with H-2α, where the coupling constants were much smaller (less than 2 Hz).[14] The β-orientation of H-2 was confirmed through the NOESY correlations between H-2β/H-1β and H-1β/H-10 (Figure 2). The structure of **5** was established as *rel*-(2*R*,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 (caseamembrin E).

The molecular formula of **6** was concluded to be $C_{25}H_{36}O_5$ as determined by the HRFABMS and ^{13}C NMR data. The NMR data (Tables 1 and 2) revealed signals attributable to the previously described basic clerodane skeleton with a 2-methylbutanoyl ester at C-2 and a hydroxyl group at C-6. The ^{1}H NMR spectrum revealed two characteristic aldehyde protons at δ 9.41 (H-18) and 10.30 (H-19), which were correlated by HMQC to the ketone carbon signals at δ 196.2 and 202.1, respectively. Due to the absence of the downfield acetal protons and to justify the presence of eight degrees of unsaturation, it was deduced that the C-18/C-19 acetal ring was opened, thus forming the two aldehyde units. The proposed structure of **6** was supported by the HMBC spectrum, which revealed correlations between H-19/C-5, H-18/C-4, H-3/C-18, H-2/C-1′, and H-10/C-6. NOE correlations (Figure 3) were consistent with those of **1**. The structure of **6** was established as *rel*-(2*S*,5*R*,6*R*,8*S*,9*S*,10*R*,18*S*,19*R*)-6-hydroxy-2-(2-methylbutanoyloxy)cleroda-3,13-(16),14-triene-18,19-dicarboxaldehyde (caseamembrin F).

Compound **7** was identified as *rel*-(2*S*,5*R*,6*R*,8*S*,9*S*,10*R*,18*S*,19*R*)-18,19-epoxy-18,19-diacetoxy-6-hydroxy-2-(2-methylbutanoyloxy)cleroda-3,13(16),14-triene through comparison with the data of the previous compounds as well as the literature values.[14] The 6-*O*-nicotinyl ester of **7** (compound **8**) as well as the 6,7-*O*-diacetyl ester of **3** and 6-*O*-acetyl ester of **4** were prepared and analyzed to confirm the proposed structures. The acetate esters of the latter two compounds were shown to be identical (compound **9**).

The cytotoxic activity of the isolated clerodanes **1**−**7** and derivatives **8** and **9** was evaluated in vitro against human prostate and hepatoma tumor cell lines. As shown in Table 3, compounds **1**, **3**−**5**, and **7** exhibited significant cytotoxicity against both tumor cells with IC_{50} below 3 μM, while compounds **2**, **8**, and **9** were less effective. Compound **6** showed more selective activity against PC-3 tumor cells. On the basis of the biological data we propose the following points from studying the structure and activity relationships. First, the acetoxyl group at C-18 is required for activity since replacement of it by the methoxyl group (**2**) resulted in loss of activity.[17] Second, the presence of a dialdehyde function in **6** results in a partial loss of activity. Third, the free hydroxyl group at C-6 is important because acylation of this position (**8** and **9**) leads to a reduction of

Table 2. ^{13}C NMR Data ($CDCl_3$, 75 MHz) of Compounds **1**−**6**[a,b]

carbon	**1**[c]	**2**	**3**	**4**	**5**	**6**
1	26.6 t	27.1 t	26.8 t	26.8 t	26.3 t	25.9 t
2	66.4 d	66.2 d	66.4 d	66.1 d	70.5 d	64.8 d
3	120.9 d	121.8 d	121.8 d	122.2 d	124.3 d	148.4 d
4	146.8 s	145.2 s	144.8 s	144.5 s	144.4 s	148.6 s
5	54.0 s	53.7 s	53.1 s	53.9 s	53.8 s	56.0 s
6	72.2 d	73.3 d	76.7 d	75.2 d	74.3 d	71.9 d
7	36.9 t	37.3 t	72.5 d	75.3 d	37.4 t	36.7 t
8	37.1 d	37.5 d	43.2 d	41.9 d	37.6 d	35.6 d
9	37.3 s	37.5 s	38.6 s	39.1 s	38.2 s	37.8 s
10	36.4 d	36.3 d	36.2 d	36.3 d	41.2 d	40.4 d
11	28.0 t	27.9 t	28.9 t	29.2 t	27.7 t	32.3 t
12	23.9 t	23.6 t	23.8 t	23.8 t	23.8 t	23.4 t
13	145.7 s	145.2 s	144.9 s	144.8 s	145.2 s	146.4 s
14	140.6 d	140.4 d	140.6 d	140.4 d	140.3 d	139.0 d
15	111.8 t	112.2 t	112.0 t	112.3 t	112.6 t	112.9 t
16	115.0 t	115.3 t	115.8 t	116.0 t	115.4 t	116.1 t
17	15.3 q	15.7 q	11.0 q	11.7 q	15.7 q	15.3 q
18	95.4 d	104.6 d	95.5 d	95.3 d	95.1 d	196.2 d
19	98.1 d	97.4 d	98.6 d	98.0 d	97.6 d	202.1 d
20	24.9 q	25.5 q	25.8 q	22.5 q	25.5 q	26.0 q
1′	175.0 s	176.2 s	173.3 s	175.8 s	176.6 s	175.9 s
2′	41.1 d	40.9 d	43.6 d	41.2 d	41.2 d	41.0 d
3′	26.9 t	27.0 t	26.1 t	27.0 t	26.8 t	27.0 t
4′	11.2 q	11.7 q	22.2 q	11.2 q	11.7 q	11.7 q
5′	16.3 q	16.4 q	22.3 q	16.7 q	16.6 q	16.5 q
1″	172.3 s		172.4 s	172.5 s		
2″	35.9 t		36.2 t	35.9 t		
3″	18.1 t		18.2 t	18.3 t		
4″	13.0 q		13.4 q	13.6 q		
OAc-7				171.7 s		
				21.3 q		
OAc-18					170.1 s	
					21.2 q	
OAc-19	168.9 s	170.3 s	169.3 s	169.3 s	169.8 s	
	20.9 q	21.5 q	21.3 q	21.0 q	21.7 q	
OMe		56.2 q				

[a] Multiplicity determined by DEPT (C = s, CH = d, CH_2 = t, CH_3 = q). [b] Assignments were made using HMQC and HMBC techniques. [c] Measured in acetone-d_6.

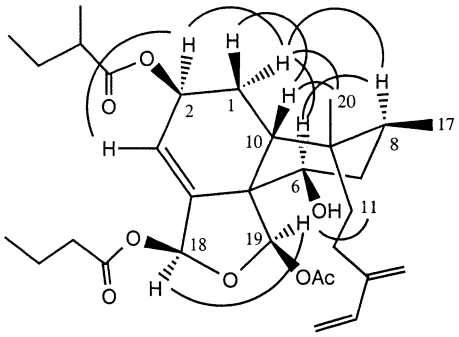

Figure 1. Key NOESY correlations for **1**.

activity. These results agree with previous reports that bulkiness of the substituent at C-6 has the greatest influence on activity.[8,18]

Experimental Section

General Experimental Procedures. Optical rotations were recorded on a JASCO DIP-1000 polarimeter. IR and UV spectra were measured on Hitachi T-2001 and Hitachi U-3210 spectrophotometers, respectively. Low-resolution EIMS and FABMS were recorded on a VG Quattro 5022 mass spectrometer. The ^1H, ^{13}C NMR, COSY, HMQC, HMBC, and NOESY spectra were recorded on a Bruker FT-300 or a Varian FT-500 spectrometer. The chemical shifts are given in δ (ppm) and coupling constants in Hz. Silica gel 60 (Merck) was used for column chromatography (CC), and precoated silica gel plates (Merck, Kieselgel 60 F-254, 1 mm) were used for

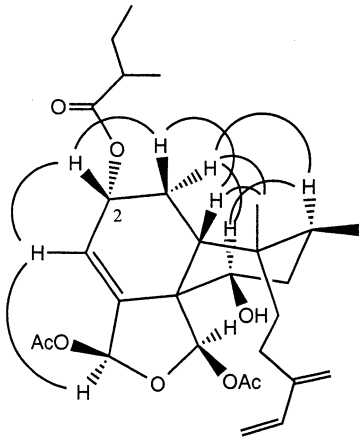

Figure 2. Key NOESY correlations for **5**.

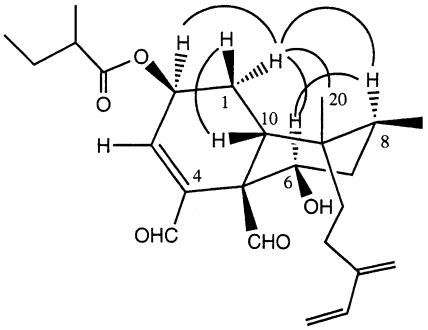

Figure 3. Selected NOESY correlations for **6**.

Table 3. Cytotoxicity of Clerodanes **1**−**9** against Human Prostate Cancer (PC) and Hepatoma (Hep3B) Cells (IC_{50}, μM)[a]

compound	PC-3	Hep3B
1	1.5	2.3
2	22.2	18.0
3	0.6	0.8
4	2.4	1.9
5	2.9	2.6
6	3.0	14.7
7	1.8	2.4
8	13	10.8
9	12.8	16.8
paclitaxel	0.016	0.031

[a] The concentration of compound that inhibits 50% (IC_{50}) of the growth of human tumor cell lines PC-3 (prostate cancer) and Hep3B (hepatoma) after a 48 h exposure according to the method described in the Experimental Section.

preparative TLC. Sephadex LH-20 (Amersham Pharmacia Biotech AB) was used for either purification or separation.

Plant Material. Leaves and twigs of *Casearia membranacea* were collected during May 2002 in Wu-Lai, Taipei County, Taiwan. The plant was authenticated by one of the authors (Y.C.S.). A voucher specimen (TP207-1) was deposited in the Institute of Marine Resources, National Sun Yat-sen University, Kaohsiung, Taiwan.

Extraction and Isolation. Leaves and twigs were dried and reduced into a coarse powder (2 kg), then extracted three times with acetone. The combined extract was evaporated in vacuo to yield 270 g of crude extract. The extract was partitioned between EtOAc−H_2O (1:1), then the EtOAc layer (125 g) was extracted with *n*-hexane−MeOH−H_2O (4:3:1), and the resulting hydro-alcoholic extract was evaporated to dryness in vacuo to yield a residue (35 g). The latter residue was applied to the top of a silica gel column and eluted with *n*-hexane−EtOAc (20:1 to 1:1) to produce 26 fractions. Elution with *n*-hexane−EtOAc (3:1) afforded fraction 10 (1.4 g), which was rechromatographed on a silica gel column using gradient mixtures of *n*-hexane−EtOAc to give **5** (35 mg, yield 0.0018%).

Fraction 11 [*n*-hexane−EtOAc (2:1), 5.1 g] was chromatographed on a silica gel column, using *n*-hexane−EtOAc (100:1 to 1:1) then *n*-hexane−CH_2Cl_2−MeOH (80:80:1), and finally on Sephadex LH-20, using MeOH for elution, to afford **1** (42.4 mg, yield 0.0021%) and **7** (247 mg, yield 0.012%). Fraction 12 [*n*-hexane−EtOAc (1:1), 4.2 g] was chromatographed on a silica gel column and eluted with a gradient mixture of CH_2Cl_2−EtOAc to afford fractions 12-A and 12-B. Fraction 12-A was purified on Sephadex LH-20 using CH_2Cl_2−MeOH (1:1) as solvent, then further chromatographed on a silica gel column, using *n*-hexane−EtOAc (4:1) for elution, to yield **6** (45 mg, yield 0.0022%). Fraction 12-B was repeatedly separated on Sephadex LH-20 columns using CH_2Cl_2−MeOH (1:1) then MeOH for elution, which was followed by silica gel column chromatography using *n*-hexane−acetone mixtures by increasing polarity for elution to give **4** (26 mg, yield 0.0013%), **7** (234 mg, yield 0.011%), and **2** (8 mg, yield 0.0004%). Fraction 16 [*n*-hexane−EtOAc (1:5), 980 mg] was repeatedly separated on Sephadex LH-20 using MeOH as solvent, then a silica gel column using CH_2Cl_2−MeOH mixtures by gradient elution to yield **3** (7 mg, yield 0.00035%).

Caseamembrin A (1): yellowish oil; $[\alpha]^{25}_D$ +24.6° (*c* 0.07, MeOH); UV (MeOH) (log ϵ) λ_{max} 223 (4.25) nm; IR ($CHCl_3$) ν_{max} 3450, 2962, 2873, 1728, 1635 cm^{-1}; ^1H and ^{13}C NMR, see Tables 1 and 2; FABMS *m/z* 547 $[M + H]^+$, $C_{31}H_{46}O_8$; EIMS *m/z* 546 $[M]^+$, 486 ($[M - 60]^+$), 466, 444 ($[M - 102]^+$), 475 ($[M - 71]^+$), 320, 212, 136; HRFABMS *m/z* 570.3155 ($[M + Na + H]^+$, calcd for $C_{31}H_{47}O_8Na$, 570.3154).

Caseamembrin B (2): yellowish oil; $[\alpha]^{25}_D$ +62° (*c* 3.82, MeOH); UV (MeOH) (log ϵ) λ_{max} 223 (4.18) nm; IR ($CHCl_3$) ν_{max} 3434, 2970, 1734, 1637 cm^{-1}; ^1H and ^{13}C NMR, see Tables 1 and 2; FABMS *m/z* 491 $[M + H]^+$, $C_{28}H_{42}O_7$; EIMS *m/z* 430, 416, 396, 368, 456, 187, 135, 121, 107, 93, 79, 69, 57; HRFABMS *m/z* 490.2924 ($[M]^+$, calcd for $C_{28}H_{42}O_7$, 490.2932).

Caseamembrin C (3): yellowish oil; $[\alpha]^{25}_D$ +196° (*c* 6.6, MeOH); UV (MeOH) (log ϵ) λ_{max} 224 (4.21) nm; IR ($CHCl_3$) ν_{max} 3433, 2980, 1727, 1630 cm^{-1}; ^1H and ^{13}C NMR, see Tables 1 and 2; FABMS *m/z* 563 $[M + H]^+$, $C_{31}H_{46}O_9$; EIMS *m/z* 411, 209, 202, 186, 171, 153, 141, 128, 115, 105, 91, 87, 60; HRFABMS *m/z* 586.3117 ($[M + Na + H]^+$, calcd for $C_{31}H_{47}O_9Na$, 586.3103).

Caseamembrin D (4): yellowish oil; $[\alpha]^{25}_D$ +268.5° (*c* 0.07, MeOH); UV (MeOH) (log ϵ) λ_{max} 222 (4.12) nm; IR ($CHCl_3$) ν_{max} 3438, 2975, 1742, 1632 cm^{-1}; ^1H and ^{13}C NMR, see Tables 1 and 2; FABMS *m/z* 627 $[M + Na]^+$, $C_{33}H_{48}O_{10}$; EIMS *m/z* 474, 457, 442, 432, 372, 185, 173, 161, 157, 135, 91, 81, 71, 60, 57, 43; HRFABMS *m/z* 627.3133 ($[M + Na]^+$, calcd for $C_{33}H_{48}O_{10}Na$, 627.3146).

Caseamembrin E (5): yellowish oil; $[\alpha]^{25}_D$ −131.3° (*c* 3.5, MeOH); UV (MeOH) (log ϵ) λ_{max} 223 (4.28) nm; IR ($CHCl_3$) ν_{max} 3517, 2097, 2971, 1738, 1627 cm^{-1}; ^1H and ^{13}C NMR, see Tables 1 and 2; FABMS *m/z* 541 $[M + Na]^+$, $C_{29}H_{42}O_8$; EIMS *m/z* 430, 388, 370, 328, 311, 299, 282, 239, 221, 210, 149, 135, 85, 81, 57, 43; HRFABMS *m/z* 541.2786 ($[M + Na]^+$, calcd for $C_{29}H_{42}O_8Na$, 541.2779).

Caseamembrin F (6): yellowish oil; $[\alpha]^{25}_D$ +29.1° (*c* 0.17, MeOH); UV (MeOH) (log ϵ) λ_{max} 224 (4.27), 220 (3.63) nm; IR ($CHCl_3$) ν_{max} 3456, 3088, 2962, 2878, 1733, 1639 cm^{-1}; ^1H and ^{13}C NMR, see Tables 1 and 2; FABMS *m/z* 417 $[M + H]^+$, 439 $[M + Na]^+$; $C_{25}H_{36}O_5$; EIMS *m/z* 187, 169, 135, 129, 107, 93, 91, 79, 57; HRFABMS *m/z* 417.2635 ($[M + H]^+$, calcd for $C_{25}H_{37}O_5$, 417.2642).

Acetylation of 3. Compound **3** (30 mg) was treated with Ac_2O−pyridine at room temperature. After the usual purification, this yielded **9** (7 mg): $[\alpha]^{25}_D$ +19.4° (*c* 1.4, MeOH); ^1H NMR (300 MHz, $CDCl_3$) δ 2.08 (1H, m, H-1), 5.39 (1H, brs, H-2), 5.98 (1H, d, J = 2.7 Hz, H-3), 3.88 (1H, m, H-6), 1.69 (1H, m, H-7), 1.85 (1H, m, H-7), 1.86 (1H, m, H-8), 2.41 (1H, m, H-10), 1.56 (1H, m, H-11) 1.35 (1H, m, H-11), 2.13 (2H, m, H-12), 6.48 (1H, dd, J = 17.5, 10.8 Hz, H-14), 5.04 (1H, d, J = 10.8 Hz, H-15) 5.23 (1H, d, J = 17.5 Hz, H-15), 4.98 (1H, s, H-16), 5.02 (1H, s, H-16), 0.88 (1H, d, J = 6.23 Hz, H-17), 6.68 (1H, s, H-18), 6.41 (1H, s, H-19), 0.95 (1H, s, H-20), 2.40 (1H, m, H-2′), 1.89 (1H, m, H-3′), 1.63 (1H, m, H-3′), 0.93 (1H, t, J = 6.2 Hz, H-4′), 1.18 (1H, overlap, H-5′), 2.03 (3H, s, Ac); ^{13}C NMR (75 MHz, $CDCl_3$) δ 26.8 (t, C-1), 65.9 (d, C-2), 124.1 (d, C-3), 143.3 (s, C-4), 52.9 (s, C-5), 73.8 (d, C-6), 72.9 (t, C-7), 41.7 (d, C-8), 39.0 (s, C-9), 36.8 (d, C-10), 29.0 (t, C-11), 23.9 (t, C-12), 144.5 (s, C-13), 140.4(d, C-14), 112.3 (t, C-15), 116.1 (t, C-16), 11.0 (q, C-17), 94.4 (d, C-18), 98.2 (d, C-19), 25.8 (q, C-20), 172.5 (s, C-1′, 43.7 (d, C-2′, 26.1 (t, C-3′), 22.4 (q, C-4′), 22.5 (q, C-5′), 170.2 (s, C-1″), 36.3 (t, C-2″), 18.2 (t, C-3″), 13.4 (q, C-4″), 21.0 (q, C-6Ac), 172.0 (s, C-6Ac), 169.1 (s, C-7Ac), 20.7 (q, C-7Ac), 170.0 (s, C-19Ac), 21.4 (q, C-19Ac); FABMS *m/z* 607 $[M + H]^+$.

Acetylation of 4. Compound **4** (10 mg) was treated with Ac_2O−pyridine at room temperature. The reaction mixture yielded a compound (6 mg) identical to **9** (^1H NMR).

Nicotinylation of 7. Compound **7** (20 mg) was treated with nicotinyl chloride (30 mg) in pyridine (1 mL) for 24 h and on the usual workup gave compound **8** (7 mg): $[\alpha]^{25}_D$ +144.9° (*c* 0.6, MeOH); ^1H NMR (300 MHz, $CDCl_3$) δ 2.07 (1H, m, H-1), 5.47 (1H, brs, H-2), 6.04 (1H, d, J = 4.2 Hz, H-3), 3.66 (1H, m, H-6), 1.65 (1H, m, H-8), 2.28 (1H, brs, H-10), 1.66 (1H, m, H-11), 1.26 (1H, m, H-11), 2.08 (2H, m, H-12), 6.43 (1H, dd, J = 17.0, 10.8 Hz, H-14), 5.05 (1H, d, J = 10.8 Hz, H-15), 5.18 (1H, d, J = 17.5 Hz, H-15), 5.00 (1H, s, H-16), 5.03 (1H, s, H-16), 0.91 (1H, d, J = 7.6 Hz, H-17), 6.56 (1H, s, H-18), 6.50 (1H, s, H-19), 0.95 (1H, s, H-20), 2.27 (1H, m, H-2′), 2.07 (1H, m, H-3′), 0.95 (1H, t, J = 6.5 Hz, H-4′), 0.96 (1H, overlap, H-5′), 2.30 (1H, m, H-2″), 1.65 (1H, m, H-3″), 0.96 (1H, t, H-4″), 1.88 (3H, s, Ac), 1.87 (3H, s, Ac), 1.72 (3H, s, Ac); ^{13}C NMR (75 MHz, $CDCl_3$) δ 26.6 (t, C-1), 65.8 (d, C-2), 123.4 (d, C-3), 144.9 (s, C-4), 52.1 (s, C-5), 75.1 (d, C-6), 36.8 (t, C-7), 37.0 (d, C-8), 37.4 (s, C-9), 36.2 (d, C-10), 27.8 (t, C-11), 23.7 (t, C-12), 144.0 (s, C-13), 140.4 (d, C-14), 112.2 (t, C-15), 115.5 (t, C-16), 15.5 (q, C-17), 94.7 (d, C-18), 98.1 (d, C-19), 25.4 (q, C-20), 175.6 (s, C-1), 41.1 (d, C-2), 26.9 (t, C-3), 11.6 (q, C-4), 16.6 (q, C-5), 164.3 (s, C-1), 123.7 (s, C-2), 151.1 (d, C-3), 153.7 (d, C-5), 125.5 (d, C-6), 127.4 (d, C-7), 172.5 (s, C-18Ac), 18.1 (q, C-18Ac), 169.7 (s, C-19Ac), 21.4 (q, C-19Ac); EIMS *m/z* 522, 419, 405, 388, 276, 187, 135, 124, 106, 71, 57, 43; HRFABMS *m/z* 624.3184 ($[M + H]^+$, calcd for $C_{35}H_{46}O_9N$, 624.3174).

Cytotoxicity Assay. The bioassay used against PC-3 (human androgen-independent prostate carcinoma) and Hep3B (human hepatocellular carcinoma) tumor cells was based on a sulforhodamine B (SRB) assay method.[19] Cells were inoculated into 96-well microtiter plates in RPMI 1640 medium containing 5% fetal bovine serum and incubated at 37° C, in 5% CO_2 and 95% air. After 24 h, two plates of each cell line were fixed in situ with trichloracetic acid (TCA). Following drug addition, the plates were incubated for an additional 48 h at 37° C, in 5% CO_2, 95% air, and 100% relative humidity. The assay was terminated by the addition of cold TCA. The supernatant was discarded, and the plates were washed five times with tap water and air-dried. Sulforhodamine B (SRB) solution (100 μL) at 0.4% w/v in 1% acetic acid was added to each well, and plates were incubated for 10 min at room temperature. After staining, unbound dye was removed by washing three times with 1% acetic acid, and the plates were air-dried. Bound stain was subsequently solubilized with 10 mM trizma base, and the absorbance read on an automated plate reader at a wavelength of 515 nm. The IC_{50} value was defined, by a comparison with the untreated cells, as the concentration of test sample resulting in a 50% reduction of absorbance. Paclitaxel was used as a standard compound and gave IC_{50} values of 0.16 and 0.031 μM, respectively, under the above conditions.

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