New Clerodane Diterpenoids from *Casearia membranacea*

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Bioassay-guided fractionation of an AcOEt extract of *Casearia membranacea* resulted in the isolation of six new clerodane diterpenes, caseamembrins G–L (**1**–**6**). The structures of the new compounds, including their relative configurations, were established by an extensive study of their spectral data, especially 2D NMR. The cytotoxic activities of the isolated diterpenes against human oral epidermoid (KB), cervical epitheloid (Hela), and liver (Hep59T/VGH) carcinoma cell lines were investigated.

1. Introduction. – The Genus *Casearia* is a rich source of clerodane-type diterpenes [1–5], many of them with established cytotoxic [6–8] or immunomodulatory [9] activities. *Casearia membranacea* HANCE (Flacourtiaceae) is a tree growing wildly in the northern region of Taiwan [10]. Recently, we reported the isolation of several diterpenes from the same species [11][12]. In continuation of a search for new cytotoxic diterpenes from local flora, we carried out a phytochemical investigation of an AcOEt extract of *C. membranacea*. In this communication, we wish to report the isolation of six new clerodane diterpenes, caseamembrins G–L (**1**–**6**). The structures of the new compounds, including their relative configurations, were established by an extensive study of their spectral data, especially 2D NMR. The cytotoxic activities of the isolated diterpenes against human-cancer cell lines were assessed.

2. Results and Discussion. – The molecular formula of **1** was established as $C_{28}H_{40}O_8$ from FAB-MS and ^{13}C-NMR spectra. The UV spectrum showed absorption attributable to a monosubstituted conjugated diene at λ 223 nm. The IR spectrum displayed absorption bands diagnostic of an OH group (3447 cm^{-1}), ester carbonyl(s) (1745 and 1738 cm^{-1}) and olefinic bond(s) (1645 cm^{-1}). ^{1}H- and ^{13}C-NMR Data (*Tables 1* and *2*) revealed the presence of two acetyloxy and butanoyloxy substituents. The spectral data of **1** were in good agreement with the basic skeleton of clerodane diterpenes previously isolated from *Casearia* [7][8][13] and established the structure as *rel*-(2*S*,5*R*,6*R*,8*S*,9*S*,10*R*,18*R*,19*S*)-18,19-bis(acetyloxy)-18,19-epoxy-2-hydroxy cleroda-3,13(16),14-trien-6-yl butanoate.

The ^{1}H-NMR (*Table 1*) of **1** revealed the presence of two acetate groups at δ 2.02 and 1.86. The ^{13}C-NMR (*Table 2*) showed two ester carbonyl absorptions and δ 170.2 and 170.0 together with two acetate Me groups at δ 21.7 and 21.3 and a butanoyl ester at δ 173.1 (C=O), 36.6 (CH_2), 18.3 (CH_2), and 13.7 (Me). Subtracting the

Table 1. *^{1}H-NMR Data* (δ in ppm, J in Hz) *of* **1**–**6** *in $CDCl_3$*. Trivial numbering.

	1	**2**	**3**	**4**	**5**	**6**
H_β–C(1)	1.98 (*m*)	2.03 (*m*)	2.06 (*m*)	2.02 (*m*)	2.02 (*m*)	1.95 (*m*)
H_α–C(1)	1.93 (*m*)	1.92 (*m*)	1.92 (*m*)[a])	1.88 (*m*)	1.93 (*m*)[a])	
H–C(2)	4.39 (br. *s*)	4.38 (br. *s*)	5.39 (br. *s*)	5.66 (*m*)	5.66 (br. *s*)	5.44 (br. *s*)
H–C(3)	5.96 (*d*, *J* = 3)	5.94 (*d*, *J* = 3)	6.16 (*d*, *J* = 3.5)	6.92 (br. *s*)	6.96 (*d*, *J* = 3)	6.02 (*d*, *J* = 4.2)
H–C(6)	4.92 (*m*)	4.97 (*m*)	3.93 (*dd*, *J* = 10.8, 4.8)	4.04 (*t*, *J* = 7.5)	5.06 (*d*, *J* = 10.5)	3.57 (*d*, *J* = 9.9)
CH_2(7) or H–C(7)	1.67 (*m*)	1.69 (*m*)	1.78 (*m*)	1.84 (*m*)	3.89 (*t*, *J* = 10.5)	3.65 (*dd*, *J* = 10.2, 9.9)
H–C(8)	1.88 (*m*)[a])	1.85 (*m*)	1.72 (*m*)	1.75 (*m*)[a])	1.67 (*m*)	1.69 (*m*)
H–C(10)	2.31 (*m*)	2.30 (*m*)	2.24 (*m*)	2.33 (*m*)	2.43 (*dd*, *J* = 10.3)	2.29 (*m*)
CH_2(11)	1.50 (*m*), 1.22 (*m*)	1.52 (*m*), 1.24 (*m*)	1.58 (*m*)[a]), 1.28 (*m*)	1.39 (*m*), 1.27 (*m*)	1.42 (*m*), 1.28 (*m*)[a])	1.65 (*m*)[a]), 1.26 (*m*)
CH_2(12)	2.11 (*m*)	2.10 (*m*)	2.09 (*m*)	1.90 (*m*)	1.94 (*m*)	2.08 (*m*)
H–C(14)	6.39 (*dd*, *J* = 17.5, 9.9)	6.38 (*dd*, *J* = 17.5, 9.9)	6.44 (*dd*, *J* = 17.7, 10.8)	6.31 (*dd*, *J* = 17.6, 10.7)	6.34 (*dd*, *J* = 17.5, 10.6)	6.43 (*dd*, *J* = 17.4, 10.8)
CH_2(15)	5.19 (*d*, *J* = 17.5), 4.99 (*d*, *J* = 9.9)	5.18 (*d*, *J* = 17.5), 4.97 (*d*, *J* = 9.9)	5.15 (*d*, *J* = 17.7), 5.02 (*d*, *J* = 11.1)	5.17 (*d*, *J* = 17.6), 5.03 (*d*, *J* = 10.7)	5.20 (*d*, *J* = 17.6), 5.05 (*d*, *J* = 10.5)	5.16 (*d*, *J* = 17.7), 5.03 (*d*, *J* = 10.8)
CH_2(16)	5.01 (*s*) 4.90 (*s*)	4.99 (*s*) 4.88 (*s*)	5.06 (*s*) 4.95 (*s*)	5.02 (*s*) 4.98 (*s*)	5.02 (*s*) 4.98 (*s*)	5.02 (*s*) 5.00 (*s*)
Me(17)	0.89 (*d*, *J* = 7.5)	0.90 (*d*, *J* = 7.5)	0.94 (*d*, *J* = 7.5)	0.91 (*d*, *J* = 7.4)	0.97 (*d*, *J* = 7.3)	1.03 (*d*, *J* = 7.2)
H–C(18)	6.50 (*s*)	6.48 (*s*)	6.55 (*s*)	10.33 (*s*)	10.43 (*s*)	6.50 (*s*)
H–C(19)	6.47 (*s*)	6.50 (*s*)	5.28 (*s*)	9.37 (*s*)	9.22 (*s*)	6.24 (*s*)
Me(20)	0.95 (*s*)	0.94 (*s*)	0.91 (*s*)	0.96 (*s*)	1.03 (*s*)	0.92 (*s*)
MeO	–	–	3.50 (*s*)	–	–	–
CH_2(2′) or H–C(2′)	1.69 (*m*)	1.70 (*m*)	2.44 (*m*)	2.42 (*m*)	–	2.46 (*m*)
CH_2(3′)	1.62 (*m*)[a]) 1.57 (*m*)	1.63 (*m*)[a]) 1.56 (*m*)	1.71 (*m*) 1.53 (*m*)	1.72 (*m*)[a]) 1.54 (*m*)	–	1.69 (*m*)[a]) 1.61 (*m*)
Me(4′)	0.92 (*t*, *J* = 7.5)	0.91 (*t*, *J* = 7.5)	0.98 (*t*, *J* = 7.5)	0.96 (*t*, *J* = 7.5)	–	0.97 (*t*, *J* = 7.4)
Me(5′)	–	–	1.19 (*d*, *J* = 7.5)	1.19 (*d*, *J* = 7.5)	–	1.18 (*d*, *J* = 7.4)
CH_2 (2″)	–	2.21 (*m*)	–	–	–	2.32 (*m*)
CH_2 (3″)	–	1.61 (*m*)[a])	–	–	–	1.67 (*m*)[a])
Me (4″)	–	0.92 (*t*, *J* = 7)	–	–	–	0.95 (*m*)
AcO–C(2)	–	–	–	–	2.10 (*s*)	–
AcO–C(7)	–	–	–	–	2.03 (*s*)	–
AcO–C(18)	1.86 (*s*)	1.88 (*s*)	1.85 (*s*)	–	–	1.85 (*s*)
AcO–C(19)	2.02 (*s*)	–	–	–	–	–

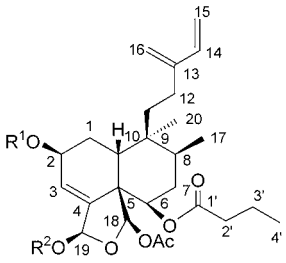
[a]) Overlapped signals in the same column.

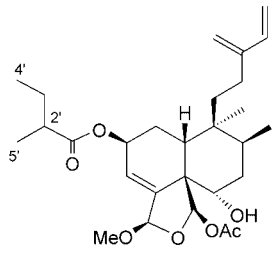
signals of the C-atoms assignable to the three ester moieties afforded a diterpene with twenty C-atoms. The olefinic region of the ^{1}H-NMR spectrum revealed proton signals with characteristic *cis*/*trans* coupling at δ 5.19 (*d*, *J* = 17.5 Hz, H_a–C(15)), 4.99 (*d*, *J* = 9.9 Hz, H_b–C(15)) and 6.39 (*dd*, *J* = 17.5, 9.9 Hz, H–C(14)) indicating a terminal monosubstituted olefin. Another terminal methylene, identified by 2 *s* at δ 4.90 and 5.01 (CH_2(16)), did not display any splitting, apparently due to its connection to a quaternary C-atom (C(13)). An additional isolated olefinic signal was detected at δ 5.96 (H–C(3)) correlated to a methine C-atom at δ 126.7 (C(3)) in the HMQC spectrum. Two deshielded acetal proton *s* were observed at δ 6.47 (H–C(19)) and 6.50 (H–C(18))

Table 2. *^{13}C-NMR Data* (δ in ppm) *of* **1**–**6** *in $CDCl_3$*. Trivial numbering.

	1	**2**	**3**	**4**	**5**	**6**
C(1)	29.5	29.5	27.2	25.9	26.0	27.0
C(2)	63.7	63.7	66.4	69.9	64.8	66.2
C(3)	126.7	126.6	120.9	153.4	148.0	122.2
C(4)	142.2	142.3	145.2	147.5	149.1	144.9
C(5)	52.2	52.2	52.1	55.8	54.1	53.2
C(6)	73.8	73.8	71.6	72.7	71.8	77.1
C(7)	33.1	33.1	35.7	37.0	77.2	72.9
C(8)	36.2	36.2	36.9	35.6	34.0	43.4
C(9)	37.4	37.4	37.4	38.5	38.9	38.7
C(10)	36.7	36.7	36.3	44.9	40.2	36.3
C(11)	28.0	27.9	27.6	29.7	29.7	29.1
C(12)	23.9	23.8	23.9	23.4	23.1	23.9
C(13)	145.3	145.3	145.9	146.3	146.0	144.9
C(14)	140.3	140.3	140.6	139.0	138.9	140.6
C(15)	112.7	112.7	112.1	113.1	113.0	112.1
C(16)	115.3	115.4	115.7	116.1	116.3	115.8
C(17)	15.6	15.6	15.7	15.3	15.2	11.7
C(18)	98.4	98.3	98.5	202.1	199.3	98.4
C(19)	95.4	95.1	104.0	196.0	192.7	95.5
C(20)	25.5*	25.5	25.6	26.0	25.9	25.9
MeO	–	–	50.3	–	–	–
C(1′)	173.1	173.2	175.9	176.0	–	175.8
C(2′)	36.6	36.6	41.2	41.1	–	41.3
C(3′)	18.3	18.3	27.0	26.8	–	26.9
C(4′)	13.7	13.7	11.8	11.7	–	11.7
C(5′)	–	–	16.6	16.6	–	16.7
C(1″)	–	172.9	–	–	–	173.2
C(2″)	–	36.3	–	–	–	36.3
C(3″)	–	18.3	–	–	–	18.3
C(4″)	–	13.6	–	–	–	13.5
Me*C*OO–C(2)	–	–	–	–	171.5	
*Me*COO–C(2)	–	–	–	–	21.2	–
Me*C*OO–C(7)	–	–	–	–	172.3	–
*Me*COO–C(7)	–	–	–	–	22.4	–
Me*C*OO–C(18)	170.2	170.0	170.0	–	–	169.4
*Me*COO–C(18)	21.7	21.7	21.6	–	–	21.3
Me*C*OO–C(19)	170.0	–	–	–	–	–
*Me*COO–C(19)	21.3	–	–	–	–	–

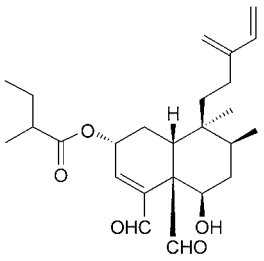
corresponding to methine signals at δ 95.4 and 98.4, respectively [13]. In addition, the ^{1}H-NMR spectrum revealed a Me *s* at δ 0.95 (Me(20)), a Me *d* at δ 0.89 (Me(17)), and 2 OCH groups at δ 4.39 (H–C(2)) and 4.92 (H–C(6)). Taking into account 3 C=C bonds and 3 ester C=O, the extra degrees of unsaturation were presumed to be due to 3 rings to justify the existence of 9 degrees of unsaturation. The presence of a C_6-diene side chain at C(9) was confirmed by the long-range correlations between CH_2(12)/C(9), H–C(14)/C(16), and CH_2(15)/C(13). In addition, the COSY plot revealed connectivities between CH_2(11)/CH_2(12) of the side chain as well as between CH_2(2′)/CH_2(3′)/Me(4′) of the butanoyl moiety. The position of the acylated acetal at C(18)/C(19) was established by long-range correlations H–C(19)/C(3) and C(18), and H–C(18)/C(4), C(5), C(6), and C(19). The signal at δ 5.96 (H–C(3)) exhibited a long-range correlation with signals at δ 28.0 (C(1)), 52.2 (C(5)), and 95.4 (C(19)) establishing that a C=C bond is located between C(3) and C(4). The OCH signal at δ 4.92 (H–C(6)), corresponding to a C-atom at δ 73.8, revealed long-range correlations to C(4), C(18), and C(1′) indicating that the butanoyloxy group is located at C(6). The attachment of the two acetate ester to C(18)

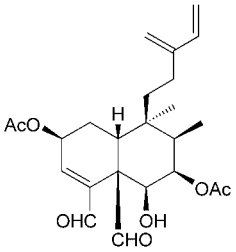


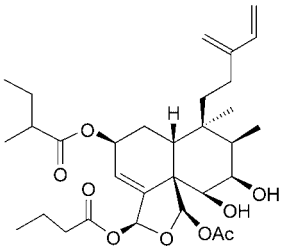


1 R^1= H, R^2 = Ac
2 R^1= H, R^2 = $CO(CH_2)_2CH_3$
7 R^1=R^2= Ac
8 R^1=Ac, R^2 = $CO(CH_2)_2CH_3$

3







4 **5** **6**

and C(19) was confirmed by long-range correlations between H–C(18) and H–C(19) with the corresponding carbonyl signal of the attached ester in each case.

The relative configuration of OH–C(2) of **1** was deduced to be β as indicated by the splitting pattern of H–C(2) (br. *s*) and the chemical shift of C(2) (δ 63.7) [8]. The NOESY correlations (*Fig. 1*) H–C(2)/H_α–C(1), H_α–C(1)/H–C(6), H_α–C(8), and H_α–C(20), H–C(19)/H–C(6), and H–C(18)/H–C(19) established the relative configuration of **1**.

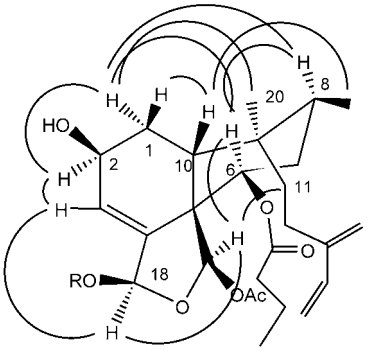


Fig. 1. *Selected NOESY correlations in* **1** *and* **2**

The ^{1}H-NMR of the acetate derivative **7** of **1** revealed a Me *s* at δ 2.07 and a significant downfield shift of the signal assigned to H–C(2), from δ 4.39 to 5.46, confirming the presence of a OH group at C(2) (see *Exper. Part*).

The molecular formula of **2** was established as $C_{30}H_{44}O_8$ from FAB-MS and ^{13}C-NMR spectra, with an additional C_2H_4 unit as compared to **1**. The UV and IR spectra revealed similar functionalities to those of **1**. The ^{1}H- and ^{13}C-NMR data (*Tables 1* and *2*) revealed the presence of one acetyloxy and two butanoyloxy substituents attached to the clerodane diterpene skeleton. The structure of **2** was established as *rel*-(2*S*,5*R*,6*R*,8*S*,9*S*,10*R*,18*R*,19*S*)-18-(acetyloxy)-18,19-epoxy-2-hydroxy-cleroda-3,13(16),14-triene-6,19-diyl dibutanoate (=caseamembrin H).

The NMR data (*Tables 1* and *2*) of **2** revealed the presence of one acetate group: Me *s* at δ(H) 1.88 and MeC=O at δ(C) 170.0 and 21.7. The ^{13}C-NMR showed two sets of butanoyl signals: δ(C) 173.2 and 172.9 (2 C=O), 33.1(2 CH_2), 18.3 (2 CH_2), and 13.7 and 13.6 (2 Me). This was confirmed by the ^{1}H-NMR spectrum (*Table 1*) as well as COSY, NOESY (*Fig. 1*), and HMBC experiments. The OCH signal at δ 4.97 was assigned to H–C(6) and revealed HMBC correlations to C-atoms at δ(C) 36.2 (C(8)), 98.3 (C(18)), and 173.2 (C(1′)) indicating that one butanoyloxy group is located at C(6). On the other hand, the HMBC correlations of the proton at δ 6.47 (H–C(19)) with C-atoms at δ 126.6 (C(3)), 98.3 (C(18)) and 172.9 (C(1″)) indicated the attachment of the other butanoyloxy group at C(19). The only acetate group was positioned at C(18) as established by HMBC correlation between H–C(18) and the C=O at δ 170.0. The relative configuration of **2** was identical to that of **1** as indicated by comparison of NMR data as well as the NOESY (*Fig. 1*) correlations.

The downfield shift ($\Delta\delta$ 1.06) observed for H–C(2) in the acetate derivative of **2** (compound **8**) provided an additional support for the hydroxylation at C(2).

Compound **3** has a molecular formula $C_{28}H_{42}O_7$ as deduced from its FAB-MS and ^{13}C-NMR. Its ^{1}H- and ^{13}C-NMR (*Tables 1* and *2*) indicated the presence of a clerodane diterpene with one acetyloxy and one 2-methylbutanoyl moiety as well as a MeO group. Based on the spectral data, **3** was characterized as *rel*-(2*S*,5*R*,6*S*,8*S*,9*S*,10*R*,18*R*,19*S*)-18-(acetyloxy)-18,19-epoxy-6-hydroxy-19-methoxy-cleroda-3,13(16),14-trien-2-yl 2-methylbutanoate (=caseamembrin I).

The low-field shift of H–C(2) (δ 5.39, br. *s*) of **3** and its HMBC correlation to the C=O at δ 175.9 (C(1′)) established that the 2-methylbutanoate group was attached at C(2). The ^{1}H-NMR spectrum displayed a characteristic *s* for a MeO group at δ 3.50 corresponding to a δ(C) 50.3. This was associated with a relatively high-field shift of H–C(19) (δ 5.28) along with a low-field shift of C(19) (δ 104.0) as compared to their counterparts in **1** (δ(H) 6.50, δ(C) 95.4). In the HMBC spectrum, MeO was correlated to δ(C) 104.0 (C(19)), which, in turn, was correlated to protons at δ 6.55 (H–C(18)) and 6.16 (H–C(3)). On the other hand, HMBC correlations H–C(18)/Me*C*=O (δ 170.0) and C(4) (δ 145.2) located the acetoxy group at C(18). The OCH proton at δ 3.93 (H–C(6)) exhibited HMBC correlations to C(8) (δ 36.9) and C(18) (δ 98.5), implying that an OH group is positioned at C(6). The data of **3** were almost identical to those of caseamembrin B [11] with β-oriented OH–C(6). However, H–C(6) of **3** resonated at δ(H) 3.93 (*dd*, J = 10.8, 4.8 Hz) and C(6) δ(C) 71.6, while the corresponding values in caseamembrin B were δ(H) 3.78 (*dd*, J = 8.0, 4.0 Hz) and δ(C) 73.3. The differences in coupling constants and J values were ascribed to a reversal of configuration at C(6). This was further suggested by an opposite sign of the optical rotation as well as NOESY cross-peaks H–C(6)/H_β–C(10) and H_β–C(17) (*Fig. 2*).

The molecular formula of **4** was determined to be $C_{25}H_{36}O_5$ by analysis of the FAB-MS and ^{13}C-NMR data. The NMR data (*Tables 1* and *2*) revealed signals attributable to the previously described basic clerodane skeleton with just one 2-methylbutanoyl ester at C(2) that was substantiated by the HMBC experiment. The structure of **4** was

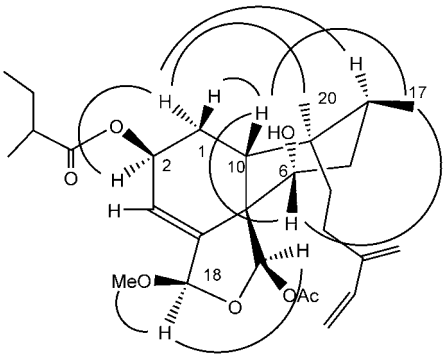


Fig. 2. *Key NOESY correlations of* **3**

established as *rel*-(2*R*,5*R*,6*R*,8*S*,9*S*,10*R*)-6-hydroxy-18,19-dioxocleroda-3,13(16),14-trien-2-yl 2-methylbutanoate (=caseamembrin J).

The ^{1}H-NMR of **4** revealed two characteristic aldehydic protons at δ 9.37 (H–C(19)) and 10.33 (H–C(18)) that were attached to the C=O at δ 196.0 and 202.1, respectively. Due to the absence of the characteristic 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, giving rise to the two aldehydic units. A OH group was located at C(6) as evidenced by the OCH proton at δ 4.04 (t, J = 7.7 Hz, H–C(6)) that exhibited HMBC correlation with δ(C) 202.1 (C(18)). The C(2) signal of **4** resonated at relatively lowfield (δ 69.9) when compared with related analogues with a β-oriented acyloxy group at C(2) [8][12], especially with caseamembrin F [11], suggesting an α-oriented acyloxy group at C(2). This was verified by the NOESY correlation H–C(2)/H_β–C(10) as well as by the sign of the optical rotation of **4** opposite to that of its isomer caseamembrin F [11].

The NMR data of **5** (*Tables 1* and *2*) were closely related to those of **4**, with respect to the clerodane skeleton, having two CHO at C(4) and C(5). No signals assignable to the 2-methylbutanoyloxy group were present. Based on the spectral data, **5** was assigned the structure of *rel*-(2*S*,5*R*,6*R*,7*R*,8*S*,9*S*,10*R*)-2,7-bis(acetyloxy)-6-hydroxycleroda-3,13(16),14-triene-18,19-dicarboxaldehyde (=caseamembrin K).

Two acetate moieties were observed for **5** at δ(H) 2.03 and 2.10 and δ(C) 171.5, 172.3, 21.2, and 21.4. This was ascertained by the FAB-MS data, which revealed a molecular formula $C_{24}H_{32}O_6$. In addition, in the NMR spectra of **5**, the CH_2(7) signals of **4** were replaced by downfield OCH signals at δ(H) 3.89 and δ(C) 77.2. HMBC Correlations H–C(7)/C(5) (δ(C) 54.1) and C=O of the second acetate at (δ(C) 172.3) as well as the COSY correlation H–C(7)/H–C(6) confirmed the acetyloxy substitution at C(7). On the other hand, the strong NOESY correlations H–C(7)/H–C(6), H_α–C(8), and Me_α(20) were in good agreement with the α-orientation of both H–C(6) and H–C(7), while the NMR data of H–C(2) and C(2) established the β-acetyloxy substitution at C(2).

The molecular formula of **6** was determined as $C_{31}H_{46}O_9$ as concluded from its mass spectral and ^{13}C-NMR data. A clerodane diterpene similar to the previous compounds with a 2-methylbutanoyloxy, a butanoyloxy, and an acetyloxy group was evident from its spectral data. The structure of **6** was assigned as *rel*-(2*S*,5*R*,6*S*,7*R*,8*R*,9*R*,10*R*,18*R*,19*S*)-18-(acetyloxy)-19-(butanoyloxy)-18,19-epoxy-6,7-dihydroxycleroda-3,13(16),14-trien-2-yl 2-methylbutanoate (=caseamembrin L).

Detailed inspection of the COSY and HMBC data established the existence of the three ester moieties of **6**. The OCH proton at δ 5.44 (H–C(2)) revealed a HMBC correlation to the C=O of the 2-methylbutanoyl

moiety at δ(C) 175.8, whereas the proton at δ 6.24 (H–C(19)) had a correlation to the C=O at δ(C) 173.2. The C=O of the acetyl moiety at δ(C) 169.4 showed a correlation with the acetal proton at δ 6.50 (H–C(18)). The remaining 2 OCH at δ 3.57 and 3.65 were assigned to H–C(6) and H–C(7), respectively, as a result of HMQC correlations to methine C-atoms at δ(C) 77.1 and 72.9. This was supported by the observation of the COSY correlation H–C(6)/H–C(7) as well as HMBC correlations H–C(6)/C(4) and H–C(7)/C(9). On the other hand, the 2-methylbutanoyloxy group at C(2), the butanoyloxy group at C(19), and the acetyloxy group at C(18) were determined to be β-oriented by using the same argument as in **1**. In addition, The NOESY (*Fig. 3*) showed the correlations H–C(18)/H–C(19), H–C(18)/H–C(6), H–C(6)/H–C(7), and H–C(7)/H–C(8) thus indicating the β-orientation of OH–C(6) and OH–C(7) (*Fig. 3*).

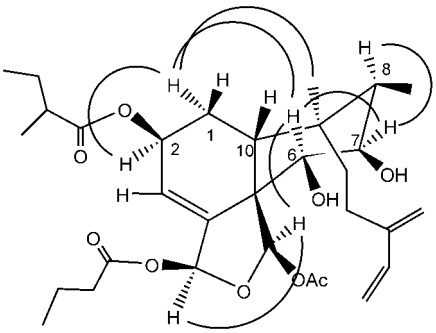


Fig. 3. *Key NOESY correlations of* **6**

All the isolated compounds and acetate derivatives were evaluated for their cytotoxic activity against KB, Hela, and Hepa. As shown in *Table 3*, compounds **1**, **2**, **6**, and **7** showed mild activity against three tumor cell lines, while **3**–**5** were inactive.

Table 3. *Cytotoxicity of Compounds* **1**–**8** *against Human-Tumor Cells* (ED_{50} in μg/ml)[a])

	KB[b])	Hela[c])	Hepa59T/VGH[d]
1	3.74	4.35	3.93
2	5.88	6.50	5.82
3	> 20	> 20	> 20
4	> 20	> 20	> 20
5	> 20	> 20	> 20
6	5.69	7.74	5.72
7	4.86	6.29	4.69
8	14.44	14.16	10.1
Doxorubicin	0.15	0.14	0.19

[a]) The concentration that inhibits 50% of the growth of human tumor cell lines after 72 h exposure according to the method described in the *Exper. Part.* [b]) Oral epidermoid carcinoma. [c]) Human cervical epitheloid carcinoma. [d]) Human liver carcinoma.

Experimental Part

General. CC = column chromatography. Optical rotations: *Jasco DIP-1000* polarimeter. IR Spectra: *Hitachi T-2001* spectrophotometer; $\tilde{\nu}$ in cm^{-1}. UV Spectra: *Hitachi U-3210* spectrophotometer; in nm. ^{1}H- and ^{13}C-NMR Spectra: *Bruker FT-300* at 300 and 75 MHz, resp.; δ in ppm, J in Hz. MS: *VG Quattro-5022* mass spectrometer; m/z (rel. %).

Plant Material. Leaves and twigs of *Casearia membranacea* were collected during May 2002 in Taipei County, Taiwan. A voucher specimen (TP207-1) has been deposited at the Institute of Marine Resources, National Sun Yat-sen University, Kaohsiung, Taiwan.

Extraction and Isolation. The dried and ground leaves and twigs of *Casearia membranacea* (6 kg) were extracted thrice with acetone, then the acetone extract was evaporated. The resulting residue was partitioned between AcOEt/H_2O to produce an AcOEt extract (270 g) that was mixed with hexane/MeOH/H_2O 4:3:1 to yield a hexane and a MeOH extract. The latter (110 g) was subjected to CC (silica gel (230 mesh), and hexane/AcOEt 100:1 → 1:5): *Fractions F1–F25*. *F15* (1.76 g) was subjected to CC (*Sephadex LH-20*): *F15.1–F15.6*. *F15.3* (518 g) was subjected to CC (silica gel, hexane/AcOEt 200:1 → 5:1): *F15.3.1–F15.3.15*. *F15.4* (681 mg) was separated by CC (*Sephadex LH-20*, MeOH) to give a mixture (392 mg) that was further fractionated by CC (*Sephadex LH-20*, CH_2Cl_2/MeOH 1:1), followed by flash column chromatography (silica gel, hexane/CH_2Cl_2/AcOEt 20:1:0 → 0:40:1) to give a mixture (40 mg). The latter mixture was finally purified on prep. TLC (hexane/AcOEt 4:1): **3** (27 mg). *F15.5* (251 mg) was further fractionated by CC (silica gel, hexane/AcOEt gradient): **4** (40 mg). *F18* (6.9 g) was subjected to CC (silica gel, hexane/AcOEt/acetone 5:1:1): *F18.1–F18.15*). *F18.5* (1.4 g) was separated by CC (*Sephadex LH-20*, MeOH): **2** (172 mg). *F21* (8.1 g) was fractionated by CC (silica gel, hexane/CH_2Cl_2/AcOEt 100:2:3 → 1:2:3): *F21.1–F21.13*. *F21.4* (442 mg) was separated by CC (*Sephadex LH-20*, MeOH) to give a mixture (*F21.4.4*) that was purified by CC (silica gel, hexane/CH_2Cl_2/acetone 5:3:1): **1** (55 mg). *F21.6* (807 mg) was separated by CC (*Sephadex LH-20*, MeOH) to yield the fraction *F21.6.1*, which was further purified by CC (silica gel, hexane/CH_2Cl_2/AcOEt 100:2:3): **5** (*F21.6.1.5*; 30 mg) and another fraction *F21.6.1.10*. The latter was separated by CC (*Sephadex LH-20*, CH_2Cl_2/MeOH 1:1): **6** (13 mg).

Caseamembrin G (= rel-*(2*S*,5*R*,6*R*,8*S*,9*S*,10*R*,18*R*,19*S*)-18,19-Bis(acetyloxy)-18,19-epoxy-2-hydroxycleroda-3,13(16),14-trien-6-yl Butanoate* = *Butanoic Acid* rel-*(1*R*,3*S*,5*S*,6a*R*,7*S*,8*S*,10*R*,10a*R*)-1,3-Bis(acetyloxy)-3,5,6,6a,7,8,9,10-octahydro-5-hydroxy-7,8-dimethyl-7-(3-methylenepent-4-enyl)-1*H*-naphtho[1,8a-c]furan-10-yl Ester*; **1**): Colorless powder. $[\alpha]_D^{25} = +8.6$ ($c = 0.2$, $CHCl_3$). UV (MeOH) 223 (4.08). IR (KBr): 3447, 2965, 2932, 1745, 1738, 1645, 1595, 1227, 1102. ^{1}H-NMR ($CDCl_3$, 300 MHz): *Table 1*. ^{13}C-NMR ($CDCl_3$, 75 MHz): *Table 2*. FAB-MS: 527 ($[M+Na]^+$). EI-MS (70 eV): 504 (1, M^+), 486 (7, $[M-H_2O]^+$), 444 (9, $[M-AcOH]^+$), 416 (1, $[M-C_4H_8O_2]^+$), 384 (11, $[M-2AcOH]^+$), 81 (35, $C_6H_9^+$), 71 (100, $C_4H_7O^+$). HR-ESI-MS: 527.2622 ($C_{28}H_{40}NaO_8^+$; calc. 527.2621).

Acetylation of **1**: At r.t., **1** (10 mg) was treated with Ac_2O/pyridine 1:1 for 10 h. Usual workup yielded 7 mg of caseamembrin G2-acetate **7**). ^{1}H-NMR ($CDCl_3$, 300 MHz): 2.10 (*m*, H_β–C(1)); 1.93 (*m*, H_α–C(1)); 5.46 (br. *s*, H–C(2)); 5.94 (*d*, *J* = 3, H–C(3)); 4.96 (*m*, H–C(6)); 1.67 (*m*, CH_2(7)); 1.64 (*m*, H–C(8)); 2.26 (*t*, *J* = 7.0, H–C(10)); 1.50 (*m*, H_a–C(11)); 1.22 (*m*, H_b–C(11)); 2.11 (*m*, CH_2(12)); 6.44 (*dd*, *J* = 17.5, 9.9, H–C(14)); 5.23 (*d*, *J* = 17.5, H_a–C(15)); 5.02 (*d*, *J* = 9.9, H_b–C(15)); 5.05 (*s*, H_a–C(16)); 4.93 (*s*, H_b–C(16)); 0.91 (*d*, *J* = 7.5, Me(17)); 6.51 (*s*, H–C(18)); 6.54 (*s*, H–C(19)); 0.95 (*s*, Me(20)); 1.69 (*m*, CH_2(2′)); 1.62 (*m*, H_a–C(3′)); 1.57 (*m*, H_b–C(3′)); 0.92 (*t*, *J* = 7.5, Me(4′)); 2.07 (*s*, AcO–C(2)); 1.86 (*s*, AcO–C(18)); 2.02 (*s*, AcO–C(19)). ^{13}C-NMR ($CDCl_3$, 75 MHz): 29.8 (C(1)); 66.2 (C(2)); 123.1 (C(3)); 144.3 (C(4)); 52.1 (C(5)); 73.6 (C(6)); 36.6 (C(7)); 36.5 (C(8)); 37.4 (C(9)); 37.2 (C(10)); 28.1 (C(11)); 23.9 (C(12)); 145.2 (C(13)); 140.4 (C(14)); 112.5 (C(15)); 115.5 (C(16)); 15.6 (C(17)); 98.3 (C(18)); 95.3 (C(19)); 25.4 (C(20)); 173.0 (C(1′)); 33.1 (C(2′)); 18.3 (C(3′)); 13.7 (C(4′)); 170.5 (Me*C*OO–C(2)); 21.4 (*Me*COO–C(2)); 170.1 (Me*C*OO–CC(18)); 21.7 (*Me*COO–C(18)); 170.0 (Me*C*OO–C(19)); 21.3 (*Me*COO–C(19)). COSY: H–C(2)/H_β–C(1) and H–C(3). NOESY: H–C(2)/H_α–C(1), H–C(10)/H–C(17) and H_α–C(11). FAB-MS: 427 ($[M-2\,HOAc+H]^+$). EI-MS (70 eV): 486 (0.1, $[M-HOAc]^+$), 427 (0.2, $[M-2AcOH+H]^+$), 384 (0.3), 297 (1), 253 (1), 225 (2), 135 (37), 107 (39), 93 (67), 71 (93).

Caseamembrin H (= rel-*(2*S*,5*R*,6*R*,8*S*,9*S*,10*R*,18*R*,19*S*)-18-(Acetyloxy)-18,19-epoxy-2-hydroxycleroda-3,13(16),14-triene-6,19-diyl Dibutanoate* = *Butanoic Acid* rel-*(1*R*,3*S*,5*S*,6a*R*,7*S*,8*S*,10*R*,10a*R*)-1-(Acetyloxy)-3,5,6,6a,7,8,9,10-octahydro-5-hydroxy-7,8-dimethyl-7-(3-methylenepent-4-enyl)-1*H*-naphtho[1,8a-c]furan-3,10-diyl Ester* **2**): Colorless powder. $[\alpha]_D^{25} = +7.6$ ($c = 0.2$, $CHCl_3$). UV (MeOH) 224 (4.02). IR (KBr): 3498, 2967, 1729, 1632. ^{1}H-NMR ($CDCl_3$, 300 MHz): *Table 1*. ^{13}C-NMR ($CDCl_3$, 75 MHz): *Table 2*. FAB-MS: 555 ($[M+Na]^+$). EI-MS (70 eV): 532 (1, M^+), 514 (2, $[M-H_2O]^+$), 472 (1, $[M-AcOH]^+$), 444 (7, $[M-C_4H_8O_2]^+$), 356 (1, $[M-2C_4H_8O_2]^+$), 81 (10, $C_6H_9^+$), 71 (100, $C_4H_7O^+$). HR-ESI-MS: 555.2930 ($C_{30}H_{44}NaO_8^+$; calc. 555.2935).

Acetylation of **2**: As described above for **7**: 8 mg of caseamembrin H2-acetate (**8**). ^{1}H-NMR ($CDCl_3$, 300 MHz): 2.06 (*m*, H_β–C(1)); 1.90 (*m*, H_α–C(1)); 5.44 (br. *s*, H–C(2)); 5.92 (*d*, *J* = 3, H–C(3)); 4.98 (*m*, H–C(6)); 1.69 (*m*, CH_2(7)); 1.49 (*m*, H–C(8)); 2.30 (*t*, *J* = 7.1, H–C(10)); 1.56 (*m*, H_α–C(11)); 1.24 (*m*, H_β–C(11)); 2.10 (*m*, CH_2(12)); 6.43 (*dd*, *J* = 17.5, 9.9, H–C(14)); 5.21 (*d*, *J* = 17.5, H_a–C(15)); 4.96 (*d*, *J* = 9.9, H_b–C(15)); 5.04 (*s*, H_a–C(16)); 4.92 (*s*, H_b–C(16)); 0.91 (*d*, *J* = 7.5, Me(17)); 6.53 (*s*, H–C(18)); 6.51

(*s*, H–C(19)); 0.92 (*s*, Me(20)); 1.72 (*m*, CH_2(2′)); 1.63 (*m*, H_a–C(3′)); 1.60 (*m*, H_b–C(3′)); 0.93 (*t*, *J* = 7.5, Me(4′)); 2.27 (*m*, CH_2(2″)); 1.64 (*m*, CH_2(3″)); 0.93 (*t*, *J* = 7.0, Me(4″)); 2.11 (*s*, AcO–C(2)); 1.90 (*s*, AcO–C(18)). ^{13}C-NMR ($CDCl_3$, 75 MHz): 29.7 (C(1)); 66.3 (C(2)); 123.0 (C(3)); 144.5 (C(4)); 52.1 (C(5)); 73.7 (C(6)); 36.6 (C(7)); 36.3 (C(8)); 37.4 (C(9)); 37.2 (C(10)); 28.1 (C(11)); 23.9 (C(12)); 145.2 (C(13)); 140.4 (C(14)); 112.5 (C(15)); 115.6 (C(16)); 15.6 (C(17)); 98.3 (C(18)); 95.1 (C(19)); 25.5 (C(20)); 173.0 (C(1′)); 33.1 (C(2′)); 18.3 (C(3′)); 13.7 (C(4′)); 172.7 (C(1″)); 36.5 (C(2″)); 18.2 (C(3″)); 13.6 (C(4″)); 170.5 (Me*C*OO–C(2)); 21.4 (*Me*COO–C(2)); 169.9 (Me*C*OO–C(18)); 21.7 (*Me*COO–C(18)).

Caseamembrin I (= rel-*(2*S*,5*R*,6*S*,8*S*,9*S*,10*R*,18*R*,19*R*)-18-(Acetyloxy)-18,19-epoxy-6-hydroxy-19-methoxycleroda-3,13(16),14-trien-2-yl 2-Methylbutanoate* = *2-Methylbutanoic Acid* rel-*(1*R*,3*R*,5*S*,6*aR*,7*S*,8*S*,10*S*,10*aR*)-1-(Acetyloxy)-3,5,6,6a,7,8,9,10-octahydro-10-hydroxy-3-methoxy-7,8-dimethyl-7-(3-methylenepent-4-enyl)-1*H*-naphtho[1,8a–c]furan-5-yl Ester*; **3**): Colorless powder. $[\alpha]_D^{25} = -10.2$ (*c* = 0.2, CH_2Cl_2). UV (MeOH) 222 (5.04). IR (KBr): 3441, 2930, 2932, 1742, 1726, 1640, 1227, 1275. ^{1}H-NMR ($CDCl_3$, 300 MHz): *Table 1*. ^{13}C-NMR ($CDCl_3$, 75 MHz): *Table 2*. FAB-MS: 513 ($[M + Na]^+$). EI-MS (70 eV): 490 (4, M^+), 458 (4, $[M - MeOH]^+$), 430 (6, $[M - AcOH]^+$), 388 (1, $[M - C_5H_{10}O_2]^+$), 297 (3.5, $[M - MeO - C_5H_{10}O_2]^+$), 279 (2, $[M - MeO - C_5H_{10}O_2 - H_2O]^+$), 81 (85, $C_6H_9^+$). HR-ESI-MS: 513.2824 ($C_{28}H_{42}NaO_7^+$; calc. 513.2828).

Caseamembrin J (= rel-*(2*R*,5*R*,6*R*,8*S*,9*S*,10*R*)-6-Hydroxy-18,19-dioxocleroda-3,13(16),14-trien-2-yl 2-Methylbutanoate* = *2-Methylbutanoic Acid* rel-*(2*R*,4a*R*,5*R*,7*S*,8*S*,8a*R*)-4,4a-Diformyl-1,2,4a,5,6,7,8,8a-octahydro-5-hydroxy-7,8-dimethyl-8-(3-methylenepent-4-enyl)naphthalen-2-yl Ester*; **4**): Colorless powder. $[\alpha]_D^{25} = -10.3$ (*c* = 0.2, CH_2Cl_2). UV: 224 (1.99). IR (KBr): 3435, 2927, 1724, 1635, 1265, 739. ^{1}H-NMR ($CDCl_3$, 300 MHz): *Table 1*. ^{13}C-NMR ($CDCl_3$, 75 MHz): *Table 2*. FAB-MS: 439 ($[M + Na]^+$). EI-MS (70 eV): 416 (1, M^+), 398 (2, $[M - H_2O]^+$), 286 (3, $[M - C_5H_{10}O_2 - CO]^+$), 81 (100, $C_6H_9^+$). HR-ESI-MS: 416.2564 ($C_{25}H_{36}NaO_5^+$; calc. 416.2563).

Caseamembrin K (= rel-*(2*S*,5*R*,6*R*,7*R*,8*S*,9*S*,10*R*)-2,7-Bis(acetyloxy)-6-hydroxycleroda-3,13(16),14-triene-18,19-dial* = rel-*(2*R*,4a*S*,5*R*,6*S*,7*S*,8*S*,8a*S*)-2,6-Bis(acetyloxy)-1,2,4a,5,6,7,8,8a-octahydro-5-hydroxy-7,8-dimethyl-8-(3-methylenepent-4-enyl)naphthalene-4,4a-dicarboxaldehyde*; **5**): Colorless powder. $[\alpha]_D^{25} = 72.4$ (*c* = 0.2, CH_2Cl_2). UV: 221 (3.57). IR (KBr): 3460, 3054, 2981, 1728, 1264, 896, 738. ^{1}H-NMR ($CDCl_3$, 300 MHz): *Table 1*. ^{13}C-NMR ($CDCl_3$, 75 MHz): *Table 2*. FAB-MS: 455 ($[M + Na]^+$). EI-MS (70 eV): 432 (2, M^+), 414 (5, $[M - H_2O]^+$), 372 (4, $[M - AcOH]^+$), 351 (1, $[M - C_6H_9]^+$), 312 (7, $[M - 2AcOH]^+$), 231 (27, $[M - C_6H_9 - 2AcOH]^+$), 81 (47, $C_6H_9^+$). HR-ESI-MS: 455.2045 ($C_{24}H_{32}NaO_7^+$; calc. 455.2047).

Caseamembrin L (= rel-*(2*S*,5*R*,6*S*,7*R*,8*R*,9*R*,10*R*,18*R*,19*S*)-18-(Acetyloxy)-19-(butanoyloxy)-18,19-epoxy-6,7-dihydroxycleroda-3,13(16),14-trien-2-yl 2-Methylbutanoate* = *2-Methylbutanoic Acid* rel-*(1*R*,3*S*,5*S*,6*aR*,7*R*,8*R*,9*R*,10*S*,10*aR*)-1-(Acetyloxy)-3,5,6,6a,7,8,9,10-octahydro-9,10-dihydroxy-7,8-dimethyl-7-(3-methylenepent-4-enyl)-3-(1-oxobutyl)-1*H*-naphtho[1,8a–c]furan-5-yl Ester*; **6**): Colorless powder. $[\alpha]_D^{25} = +45.5$ (*c* = 0.2, $CHCl_3$). UV (MeOH) 224 (4.02). IR (KBr): 3405, 2964, 1735, 1223, 1063, 734. ^{1}H-NMR ($CDCl_3$, 300 MHz): *Table 1*. ^{13}C-NMR ($CDCl_3$, 75 MHz): *Table 2*. FAB-MS: 585 ($[M + Na]^+$). EI-MS (70 eV): 562 (1, M^+), 460 (3, $[M - C_5H_{10}O_2]^+$), 414 (8, $[M - AcOH - C_4H_8O_2]^+$), 60 (100, $AcOH^+$). HR-ESI-MS: 585.3039 ($C_{31}H_{46}NaO_9^+$; calc. 585.3035).

Cytotoxicity Assay. The cells for assay were cultured in RPMI-1640 medium supplemented with a 5% CO_2 incubator at 37°. The cytotoxicity assay depends on the binding of methylene blue to fixed monolayers of cells at pH 8.5, washing the monolayer, and releasing the dye by lowering the pH value. Samples and control standard drugs were prepared at concentrations of 1, 10, 40, and 100 μg/ml. After seeding 2880 cells/well in a 96-well microplate for 3 h, the sample or standard agent (20 μl) was placed in each well and incubated at 37° for 3 days. After removing the medium from the microplates, the cells were fixed with 10% formaldehyde in 0.9% saline for 30 min, then dyed with 1% (*w*/*v*) methylene blue in 0.01M borate buffer (100 μl/well) for 30 min. The 96-well plate was dipped 4 × into 0.01M borate buffer to remove the dye. Then EtOH/0.1M HCl 1:1 (100 μl/well) was added as a dye-eluting solvent, and the absorbance was measured on a microtiter plate reader (*Dynatech*, *MR 7000*) at a wavelength of 650 nm. The ED_{50} value was defined by a comparison with the untreated cells as the concentration of test sample resulting in 50% reduction of absorbance.

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