Structure and Stereochemistry of New Cytotoxic Clerodane Diterpenoids from the Bark of *Casearia lucida* from the Madagascar Rainforest[1]

Chaturvedula V. Sai Prakash, Jeannine M. Hoch, and David G. I. Kingston*

Department of Chemistry, M/C 0212, Virginia Polytechnic Institute and State University, Blacksburg, Virginia 24061-0212

Received August 13, 2001

Bioassay-guided fractionation of a CH_2Cl_2/MeOH extract of the bark of *Casearia lucida* resulted in the isolation of 11 new clerodane diterpenes, namely, casearlucins A−K (**1**−**11**), and three known clerodane diterpenoids, *rel*-(2*S*,5*R*,6*R*,8*S*,9*S*,10*R*,18*S*,19*R*)-diacetoxy-18,19-epoxy-6-hydroxy-2-(2ξ-methylbutanoyloxy)cleroda-3,13(16),14-triene (**12**), *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-(2ξ-methylbutanoyloxy)cleroda-3,13(16),14-triene (**13**), and *rel*-(2*S*,5*R*,8*S*,9*S*,10*R*,18*S*,19*R*)-18,19-diacetoxy-18,19-epoxy-2-(2ξ-methylbutanoyloxy)cleroda-3,13(16),14-triene (**14**). The structures of compounds **1**−**11** were established on the basis of extensive 1D and 2D NMR spectroscopic data interpretation. All compounds exhibited cytotoxicity activity against the A2780 ovarian cancer cell line, but none of the six compounds selected for testing in multiple cell lines showed significant selectivity.

In our continuing search to discover bioactive compounds from the Suriname and Madagascar rainforests as part of an International Cooperative Biodiversity Group (ICBG) program,[2] we obtained a sample of a cytotoxic CH_2Cl_2/MeOH extract of the Malagasy plant *Casearia lucida* Hils. & Bojer ex Tul. (Flacourtiaceae) from the National Cancer Institute. The genus *Casearia* contains about 180 species, widely distributed in the tropics; there are five species found in Madagascar. *C. lucida* is endemic, but widespread within this country. The genus is a rich source of diterpenoids of the clerodane type, some of which exhibit cytotoxic, insect antifeedant, and DNA binding inhibitory activity.[3−12] Similar clerodane diterpenoids were recently isolated from the roots of *Licania intrapetiolaris*.[13] The extract of *C. lucida* was selected for bioassay-guided fractionation based on its cytotoxicity, since it had an IC_{50} value of 10.8 μg/mL against the A2780 ovarian cancer cell line. The crude extract after extensive chromatography followed by reversed-phase HPLC yielded 11 new bioactive clerodane diterpenes, casearlucins A−K (**1**−**11**), in addition to the three known clerodane diterpenes (**12**−**14**).

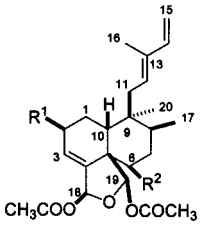
Results and Discussion

Initial liquid−liquid extraction of the crude extract indicated that the bioactivity was concentrated in the $CHCl_3$-soluble portion of a $CHCl_3$/aqueous MeOH partition. Chromatography of this fraction on a MCI gel column followed by column chromatography over RP C_{18} and then by reversed-phase HPLC furnished 11 new clerodane diterpenes, casearlucins A−K (**1**−**11**), as well as the three known compounds (**12**−**14**). The structures of the three known compounds were identified as *rel*-(2*S*,5*R*,6*R*,8*S*,-9*S*,10*R*,18*S*,19*R*)-diacetoxy-18,19-epoxy-6-hydroxy-2-(2ξ-methylbutanoyloxy)cleroda-3,13(16),14-triene (**12**), *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-(2ξ-methylbutanoyloxy)cleroda-3,13(16),14-triene (**13**), and *rel*-(2*S*,5*R*,8*S*,9*S*,10*R*,18*S*,19*R*)-18,19-diacetoxy-18,19-epoxy-2-(2ξ-methylbutanoyloxy)cleroda-3,13(16),14-triene (**14**), by comparison of their spectral data with values reported in the literature.[9]

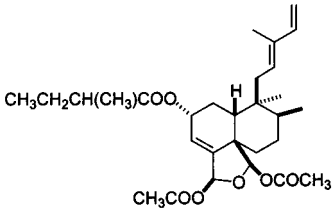
Casearlucin A (**1**) was isolated as an optically active colorless viscous liquid whose molecular formula was established as $C_{29}H_{42}O_8$ from HRFABMS and ^{13}C NMR spectral data. The IR spectrum showed the presence of hydroxyl (3455 cm^{-1}) and two ester carbonyl groups (1755 and 1735 cm^{-1}) in its molecular structure. The fragments observed in the positive mode of the CIMS at 501, 459, 399, and 297 indicated the successive loss of water, two acetic acid molecules, and a $C_5H_{10}O_2$ group from the molecular ion. The ^1H and ^{13}C NMR spectra (Tables 1 and 3) of **1** revealed the presence of a 2-methylbutanoyloxy substituent in its structure. The ^1H NMR spectrum of **1** showed the presence of a methyl singlet at δ 0.80, a methyl doublet at δ 0.92 (J = 7.1 Hz), two oxymethines at δ 5.43 (br s) and 3.78 (d, J = 6.9 Hz), two acetal-acyloxy methine protons at δ 6.72 (t, J = 1.6 Hz) and 6.50 (s), and a trisubstituted olefinic proton at δ 6.00 (d, J = 4.6 Hz), consistent with the basic skeleton of clerodane diterpenes isolated earlier from the genus of *Casearia*.[9−12] The basic skeleton of a clerodane diterpene was also supported by the COSY (H-1/H-2; H-2/H-3; H-6/H-7; H-7/H-8) and HMBC (H-1/C-2, C-10; H-3/C-2, C-4, C-5, H-18/C-4, C-5, H-19/C-5, C-6, H-6/C-5, C-7, C-8, H-8/C-7, C-9, C-10) correlations. The presence of the two acetate groups at C-18 and C-19 was supported by the HMBC correlations: H-18 and H-19/O*C*OCH$_3$ and C*H*$_3$COO/C-18, C-19. The olefinic region of **1** showed the presence of a terminal unsaturated methylene group, supported by the observation of resonances at δ 4.92 (d, J = 11.2 Hz) and 5.09 (d, J = 17.6 Hz), and of a trisubstituted double bond, indicated by a doublet of doublets (J = 17.1, 10.5 Hz) at δ 6.25. The COSY, HMQC, and HMBC spectral data indicated that the terminal unsaturated methylene group was correlated with the methyl group at δ 1.65, the trisubstituted double bond at δ 6.25, and the methylene protons at δ 1.58 and 2.23, representing C-11 of the six-carbon side chain located at C-9. This was further supported by the HMBC correlations H-11/C-12, C-9, C-10, C-8 and H-8/C-9, C-10, C-11. The 2-methylbutanoyloxy chain was located at C-2 based on the chemical shift of H-2[9] and from the HMBC correlations H-2′/C-1′, C-3′, C-5′, C-2 and H-2/C-1′, C-2′, C-1, C-3. The ^{13}C NMR values for all the carbons were assigned on the basis of HMQC and HMBC spectra and were in good agreement with the structure. A close comparison of the ^1H and ^{13}C NMR values of **1** and **12** (Tables 1−3) indicated that the two compounds were identical except for the side chain at C-9. The side chain

* To whom correspondence should be addressed. Tel: (540) 231-6570. Fax: (540) 231-7702. E-mail: dkingston@vt.edu.

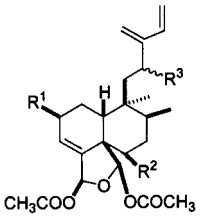
10.1021/np010405c CCC: $22.00
Published on Web 01/26/2002



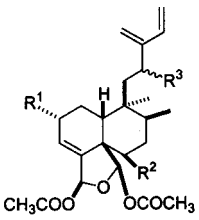
1 $R^1 = CH_3CH_2CH(CH_3)COO$, $R^2 = OH$
3 $R^1 = CH_3CH_2CH(CH_3)COO$, $R^2 = OCOCH_3$
6 $R^1 = CH_3CH_2CH(CH_3)COO$, $R^2 = H$



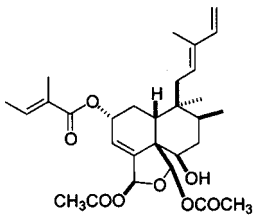
7



4 $R^1 = CH_3CH_2CH(CH_3)COO$, $R^2 = OCOCH_3$, $R^3 = H$
8 $R^1 = CH_3CH_2CH(CH_3)COO$, $R^2 = OH$, $R^3 = \cdots OH$
9 $R^1 = CH_3CH_2CH(CH_3)COO$, $R^2 = OH$, $R^3 = —OH$
12 $R^1 = CH_3CH_2CH(CH_3)COO$, $R^2 = OH$, $R^3 = H$
13 $R^1 = CH_3CH_2CH(CH_3)COO$, $R^2 = OCH_3$, $R^3 = H$
14 $R^1 = CH_3CH_2CH(CH_3)COO$, $R^2 = R^3 = H$



2 $R^1 = CH_3COO$, $R^2 = OCH_3$, $R^3 = H$
5 $R^1 = CH_3CH_2CH(CH_3)COO$, $R^2 = OCH_3$, $R^3 = H$
10 $R^1 = CH_3CH_2CH(CH_3)COO$, $R^2 = OH$, $R^3 = \cdots OH$
11 $R^1 = CH_3CH_2CH(CH_3)COO$, $R^2 = OH$, $R^3 = —OH$
16 $R^1 = CH_3CH_2CH_2CH_2CH_2COO$, $R^2 = R^3 = H$



15

of **1** was identical to that of casearinol A (**15**), isolated earlier from the leaves of *C. guianensis*,[11] and this was confirmed by their almost identical ^1H and ^{13}C NMR values. The relative stereochemistry at all eight chiral centers of **1** was assigned as being the same as that of **12**, on the basis of their almost identical coupling constants (Tables 1 and 2). This was further supported by the NOESY correlations of **1** (Figure 1) similar to the correlations of **12**.[9] The Δ^{12} double bond was assigned the *E* configuration on the basis of the NOE correlation observed between H-12 and H-14. An attempt to assign the stereochemistry of the 2-methylbutanoyl side chain by analysis of methyl 2-methylbutanoates on a chiral GC column failed when the racemic mixture failed to resolve on our column. On the basis of the above spectral information, casearlucin A (**1**) was assigned 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-hydroxy-2-(2ξ-methylbutanoyloxy)-cleroda-3,12,14-triene.

Casearlucin B (**2**) was also obtained as a colorless viscous liquid, and its molecular formula was assigned as $C_{27}H_{38}O_8$ by HRFABMS. Its IR spectrum showed the absence of a hydroxyl group and the presence of three ester carbonyl groups (1758, 1728, and 1723 cm^{-1}). The ^1H NMR spectrum of **2** was very similar to that of **13** (Tables 1 and 2), except for the replacement of the signals corresponding to the 2-methylbutanoyloxy side chain at C-2 with those for an additional acetate group and changes in the coupling constants of the C-2 protons. In the absence of any other assignable oxymethine protons in the ^1H NMR spectrum of **2**, the acetate group was placed at C-2. This was supported by the HMBC correlations H-1/C-10, C-2 and H-2/C-3, $OCOCH_3$. The ^{13}C NMR spectral values were assigned to all the carbons on the basis of HMQC and HMBC data and are given in Table 3. From Table 3, it was found that the carbon value for C-2 was observed at δ 71.0, which was consistent with a C-2 substituent having an α-orientation.[9,14] The relative stereochemistry of the acetate at C-2 was confirmed as α by the broad triplet at δ 5.58 (J = 6.9 Hz) for the C-2 oxymethine proton, consistent with pseudoaxial-axial coupling with the axial proton of the C-1 methylene group. The axial orientation of H-2 was confirmed by the strong NOESY correlation between H-2 and the equatorial proton of H-1 and from the correlation observed between H-2 and the axial proton at H-10. Comparison of the ^1H and ^{13}C NMR values at C-2 for compound **2** with that of *rel*-(2*R*,5*R*,8*S*,9*S*,10*R*,18*S*,19*R*)-18,19-diacetoxy-18,19-epoxy-2-hexanoyloxycleroda-3,13-(16),14-triene (**16**)[9] with an α-orientation at C-2 supported the stereochemistry further. The relative stereochemistries of the remaining seven chiral centers were the same as those of **1** on the basis of their identical coupling constants, and this was confirmed by the NOESY correlations shown in Figure 2. Thus, casearlucin B (**2**) was established as *rel*-(2*R*,5*R*,6*R*,8*S*,9*S*,10*R*,18*S*,19*R*)-2,18,19-triacetoxy-18,19-epoxy-6-hydroxycleroda-3,13(16),14-triene.

Casearlucin C (**3**) was isolated as a colorless liquid and determined to have the molecular formula $C_{31}H_{44}O_9$ by HRFABMS. Its IR spectrum showed the absence of a hydroxy group and three acetate groups (ν_{max} 1750, 1735, and 1730 cm^{-1}). Its ^1H NMR spectrum was very similar to that of **1**, except for the presence of a signal for an additional acetate group at δ 2.07. The presence of additional carbon signals at δ 170.3 and 21.6 in the ^{13}C NMR spectrum of **3**, together with the appearance of the resonance of the C-6 oxymethine proton at δ 4.94, indicated the presence of the third acetate group at C-6. The mass spectrum of **3**, which contained a molecular ion peak 42 mass units greater than that of **1**, supported the structure further. Acetylation of compound **1** furnished a product whose spectral data were identical to those of **3**, confirming the structure and stereochemistry of **3**. Thus, casearlucin C (**3**) was assigned as *rel*-(2*S*,5*R*,6*R*,8*S*,9*S*,10*R*,18*S*,19*R*)-

Table 1. ^{1}H NMR Data for Compounds **1**−**7**[a] ($CDCl_3$, 500 MHz)

position	**1**	**2**	**3**	**4**	**5**	**6**	**7**
1	1.88 m	1.66 m	1.93 m	1.94 m	1.68 m	1.90 m	1.73 m
		2.14 m			2.04 m		2.06 m
2	5.43 br s	5.58 br t	5.42 br s	5.42 br s	5.58 br t	5.36 br s	5.56 br t
		6.9			6.9		7.2
3	6.00 d 4.6	5.82 br s	5.98 d 3.9	5.96 d 4.1	5.79 br s	5.88 d 3.0	5.74 br s
6	3.78 d 6.9	3.48 dd	4.94 dd	4.96 dd	3.49 dd	1.72 m	1.70 m
		3.9, 12.1	4.6, 12.8	4.5, 12.6	3.7, 12.4		
7	1.67 m	1.72 m	1.67 m	1.64 m	1.68 m	1.45 m	1.45 m
8	1.80 m	1.86 m	1.85 m	1.84 m	1.84 m	1.69 m	1.68 m
10	2.37 br t	2.34 dd	2.44 dd	2.37 dd	2.35 m	2.24 m	2.19 m
	8.9	13, 4.6	10.2, 3.8	9.9, 3.6			
11	1.58 m, 2.23 dd	1.26 m,1.46 m	1.66 m, 2.23 dd	1.25 m,1.44 m	1.24 m,1.47 m	1.68 m,2.22 m	1.67 m,2.22 m
	8.0, 16.7		8.2, 16.9				
12	5.37 br s	2.08 m	5.36 br s	2.06 m	2.13 m	5.36 br s	5.39 br s
14	6.25 dd	6.42 dd	6.25 dd	6.43 dd	6.40 dd	6.25 dd	6.30 dd
	17.1, 10.5	17.6, 11.0	17.4, 10.5	17.2, 10.8	17.6, 10.8	17.4, 10.6	17.8, 10.8
15	4.92 d 11.2	5.01 d 10.8	4.92 d 10.8	5.02 d 10.7	5.01 d 10.8	4.91 d 10.8	4.92 d 10.7
	5.09 d 17.6	5.18 d 17.6	5.09 d 17.4	5.16 d 17.6	5.18 d 17.6	5.09 d 17.4	5.07 d 17.2
16	1.65 s	4.90 s	1.66 s	4.93 s	4.90 s	1.65 s	1.64 s
		5.02 s		5.05 s	5.02 s		
17	0.92 d 7.1	0.92 d 6.8	0.90 d 7.4	0.92 d 6.6	0.93 d 6.8	0.88 d 7.4	0.87 d 6.6
18	6.72 t 1.6	6.62 t 1.6	6.49 t 1.6	6.51 t 1.6	6.62 br s	6.66 t 1.6	6.63 t 1.6
19	6.50 s	6.27 s	6.56 s	6.50 s	6.37 s	6.35 s	6.31 s
20	0.80 s	0.93 s	0.81 s	0.90 s	0.92 s	0.82 s	0.85 s
2′	2.44 m		2.44 m	2.42 m	2.37 m	2.45 m	2.36 m
3′	1.69 m		1.67 m	1.72 m	1.68 m	1.68 m	1.69 m
4′	0.96 t 7.6		0.95 t 7.8	0.94 t 7.6	0.94 t 7.6	0.96 t 7.6	0.92 t 7.5
5′	1.17 d 6.9		1.17 d 6.6	1.16 d 6.9	1.16 d 7.1	1.17 d 6.8	1.15 d 7.1
Ac Me	1.93 s	1.86 s	1.94 s	1.88 s	1.86 s	1.92 s	1.93 s
2.06 s	2.06 s	2.06 s	2.04 s	2.08 s	2.04 s	2.07 s	
			2.09 s	2.07 s	2.06 s		
OMe		3.30 s			3.32 s		

[a] Assignments made on the basis of COSY and HMQC spectral data and comparison with the literature values.[9]

Table 2. ^{1}H NMR Data for Compounds **8**−**14**[a] ($CDCl_3$, 500 MHz)

position	**8**	**9**	**10**	**11**	**12**	**13**	**14**
1	1.86 m	1.82 m	1.76 m	1.78 m	1.90 m	1.88 m	1.90 m
2	5.42 br s	5.40 br s	5.57 br t	5.53 br t	5.41 br s	5.40 br s	5.36 br s
			7.2	7.4			
3	5.99 d 4.3	5.98 d 4.0	5.85 br s	5.85 br s	5.98 d 4.1	5.89 d 4.2	5.87 d 4.1
6	3.76 br t	3.74 br t	3.96 dd	3.95 dd	3.79 br t	3.30 dd	1.72 m
	7.8	7.4	4.0, 12.2	4.2, 12.0	8.2	3.9, 12.2	
7	1.70 m	1.72 m	1.68 m	1.68 m	1.72 m	1.46 m	1.46 m1.80 m
8	1.82 m	1.80 m	1.76 m	1.75 m	1.78 m	1.67 m	1.64 m
10	2.20 dd	2.04 m	2.20 m	2.04 m	2.32 dd	2.30 br t	2.22 m
	10.6, 6.4				10.4, 6.1	9.2	
11	1.88 m	1.85 m	1.88 m	1.82 m	1.26 & 1.48 m	1.20 & 1.43 m	1.25 & 1.47 m
12	4.47 dd	4.73 dd	4.45 dd	4.72 dd	2.08 m	2.06 m	2.06 m
	9.0, 10.4	9.2, 10.8	9.2, 10.6	9.0, 11.0			
14	6.34 dd	6.29 dd	6.28 dd	6.28 dd	6.40 dd	6.38 dd	6.43 dd
	17.8, 11.0	17.8, 11.2	17.6, 10.8	17.6, 11.2	17.4, 10.8	17.8, 10.4	17.6, 10.8
15	5.13 d11.0	5.17 d 9.8	5.16 d 11.4	5.16 d 11.2	4.98 d 10.7	4.98 d 10.8	4.97 d 10.8
	5.46 d 17.6	5.47 d 17.4	5.45 d 17.6	5.47 d 17.8	5.14 d 17.7	5.12 d 17.4	5.15 d 17.6
16	5.13 s	5.15 s	5.12 s	5.17 s	4.93 s	4.89 s	4.93 s
	5.15 s	5.29 s	5.14 s	5.28 s	5.02 s	5.01 s	5.02 s
17	1.02 d 6.6	1.06 d 6.6	1.03 d 6.8	1.06 d 6.6	0.88 d 6.2	0.89 d 7.0	0.86 d 6.8
18	6.73 br s	6.73 t 1.6	6.69 t 1.6	6.70 t 1.6	6.73 br s	6.63 t 1.6	6.67 t 1.6
19	6.37 s	6.40 s	6.33 s	6.35 s	6.45 s	6.38 s	6.29 s
20	1.07 s	1.05 s	1.10 s	1.08 s	0.89 s	0.87 s	0.91 s
2′	2.43 m	2.42 m	2.36 m	2.36 m	2.43 m	2.42 m	2.42 m
3′	1.68 m	1.66 m	1.65 m	1.65 m	1.65 m	1.66 m	1.68 m
4′	0.94 t 7.5	0.92 t 7.4	0.91 t 7.6	0.92 t 7.5	0.94 t 7.2	0.92 t 7.1	0.92 t 7.2
5′	1.16 d 6.8	1.16 d 6.6	1.13 d 6.8	1.16 d 6.6	1.15 d 7.2	1.14 d 7.2	1.16 d 6.8
MeCO	1.92 s	1.99 s	1.94 s	1.97 s	1.86 s	1.82 s	1.88 s
	2.05 s	2.06 s	2.07 s	2.07 s	2.04 s	2.02 s	2.01 s
OMe						3.26 s	

[a] Assignments made on the basis of COSY and HMQC spectral data and comparison with the literature values.[9]

6,18,19-triacetoxy-18,19-epoxy-2-(2ξ-methylbutanoyloxy)-cleroda-3,12,14-triene.

The molecular formula of casearlucin D (**4**) was also determined to be $C_{31}H_{44}O_9$ by HRFABMS. Its ^{1}H NMR spectrum was almost identical to that of **12**, except for signals for an additional acetate group at δ 2.06, similar to that of **3**. This information together with the presence of signals for two additional carbons at δ 170.3 and 21.6 in the ^{13}C NMR spectrum suggested the presence of an acetate group at C-6 in place of the hydroxyl group. This

Table 3. ^{13}C NMR Data for Compounds **1**−**14**[a] ($CDCl_3$, 125 MHz)

carbon	1	2	3	4	5	6	7	8	9	10	11	12	13	14
1	26.8	26.7	26.7	26.8	26.7	25.8	25.8	26.7	26.7	26.3	26.3	26.8	27.0	26.2
2	66.2	71.0	66.0	66.0	70.6	66.4	71.1	66.2	66.2	70.6	70.5	66.3	66.2	66.4
3	121.9	123.6	123.3	123.3	123.8	120.5	123.2	121.9	121.9	124.5	124.3	121.9	121.4	120.5
4	145.2	145.4	144.2	144.4	145.2	147.1	145.7	145.4	145.2	144.2	143.9	145.50	145.1	145.4
5	53.6	53.2	52.1	52.3	53.2	49.1	49.2	54.0	53.9	53.9	53.9	53.9	53.2	49.4
6	73.0	83.2	73.8	73.9	83.2	29.2	30.6	73.7	73.6	74.6	74.6	73.1	81.9	29.4
7	36.9	31.4	33.2	33.1	31.4	27.5	27.8	37.5	37.4	37.5	37.3	37.3	31.2	27.3
8	37.7	37.1	37.7	37.4	37.1	34.7	36.7	37.5	37.5	37.9	37.9	37.6	37.1	37.3
9	37.5	41.3	37.4	37.1	41.3	36.7	38.3	39.2	38.6	39.2	39.3	37.5	37.5	37.4
10	36.9	38.4	36.2	36.9	38.3	37.5	39.1	40.2	41.0	44.8	44.8	36.5	36.5	34.3
11	30.4	27.6	30.3	27.8	27.6	30.4	30.2	38.4	36.9	38.8	36.9	28.0	27.8	28.2
12	129.0	23.8	129.0	23.8	23.8	129.3	128.9	67.5	80.8	67.4	80.9	23.8	23.8	23.7
13	135.8	145.9	135.8	145.0	145.3	135.7	135.8	149.7	145.9	149.4	147.6	145.1	146.3	147.3
14	141.3	140.4	141.3	140.5	140.4	141.4	141.4	137.1	135.5	136.6	135.2	140.5	140.5	140.6
15	111.2	112.6	111.3	112.3	112.6	110.9	111.0	115.1	116.2	115.4	116.2	112.3	112.2	112.2
16	12.1	115.6	12.1	115.6	115.5	12.1	12.1	115.1	117.3	115.4	116.2	115.6	115.6	115.4
17	15.7	15.9	15.5	15.8	15.9	15.7	15.7	16.1	15.9	16.1	16.0	15.8	15.9	15.8
18	95.8	95.8	95.3	95.2	95.7	94.6	94.2	95.1	95.5	95.1	95.1	95.6	96.2	94.5
19	97.1	98.0	97.3	98.2	98.0	98.9	98.5	97.9	97.7	97.5	97.3	97.9	98.4	99.6
20	25.0	25.7	25.1	25.6	25.7	25.7	25.7	24.6	26.8	24.5	26.8	25.5	25.6	26.2
1′	175.9	175.9	175.9	176.6	176.1	176.1	176.1	176.2	176.5	176.4	175.9	176.0	176.2	
2′	41.3	41.2	41.2	41.3	41.3	41.3	41.4	41.3	41.3	41.3	41.3	41.2	41.3	
3′	27.2	27.2	27.1	26.9	27.2	26.9	27.0	26.9	26.9	26.8	27.1	27.1	27.3	
4′	11.7	11.7	11.7	11.8	11.7	11.8	11.7	11.6	11.8	11.7	11.7	11.7	11.7	
5′	16.7		16.7	16.7	16.7	16.7	16.7	16.7	16.6	16.7	16.5	16.7	16.7	16.7
OCO	170.2[b]	170.3[b]	170.3[b]	170.3[b]	170.3[b]	170.4[b]	170.6[b]	170.2[b]	170.2[b]	170.0[b]	170.5[b]	170.1[b]	170.3[b]	170.3[b]
MeCO	21.5[c]	21.6[c]	21.6[c]	21.6[c]	21.7[c]	21.5[c]	21.6[c]	22.0[c]	21.6[c]	22.0[c]	21.9[c]	21.5[c]	21.6[c]	21.4[c]
OCO	169.5	170.2[b]	170.2[b]	170.1[b]	169.8[b]	169.8[b]	169.8[b]	170.2[b]	170.2[b]	170.0[b]	170.5[b]	169.8[b]	169.8[b]	170.0[b]
MeCO	21.3[c]	21.4[c]	21.6[c]	21.4[c]	21.5[c]	21.3[c]	21.4[c]	21.3[c]	21.2[c]	21.3[c]	21.3[c]	21.3[c]	21.5[c]	21.3[c]
OCO		169.8[b]	169.6[b]	169.8[b]										
MeCO		21.3[c]	21.3[c]	21.3[c]										
OMe			57.6			57.6								

[a] Assignments made on the basis of HMQC and HMBC spectra and comparison with the literature data.[9,11] [b,c] Values having the same superscript in their respective columns are interchangeable.

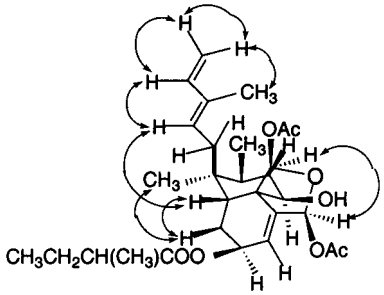


Figure 1. Selected NOESY correlations for **1**.

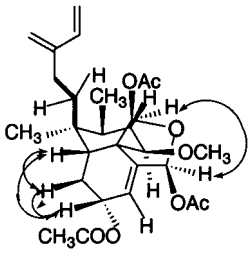


Figure 2. Selected NOESY correlations for **2**.

was supported by the observation of the ^1H NMR signal for the C-6 oxymethine proton at δ 4.96, similar to that observed for **3**. The ^{13}C NMR values for all the carbons were assigned on the basis of HMQC and HMBC and are given in Table 3. Acetylation of **12** with Ac_2O−pyridine furnished a product whose spectral data were identical to those of **4**, confirming the structure and stereochemistry unambiguously. Thus, the structure of casearlucin D (**4**) was established as *rel*-(2*S*,5*R*,6*R*,8*S*,9*S*,10*R*,18*S*,19*R*)-6,18,19-triacetoxy-18,19-epoxy-2-(2ξ-methylbutanoyloxy)cleroda-3,13(16),14-triene.

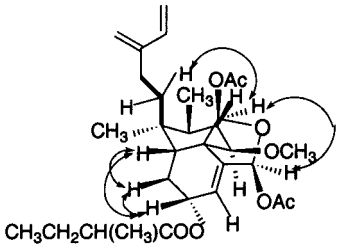


Figure 3. Selected NOESY correlations for **5**.

Casearlucin E (**5**) was isolated as a colorless oil, with a molecular formula of $C_{30}H_{44}O_8$ (HRFABMS). A close comparison of the ^1H NMR spectral data of **5** with that of **13** (Tables 1 and 2) indicated the nature of the two compounds, except for the orientation of the oxymethine at the C-2 position. The C-2 oxymethine signals at δ 5.58 (br t, J = 6.9 Hz,^1H) and 70.6 (^{13}C) were almost identical to those of C-2 in **2**, indicating their similar nature and α orientation. This was further supported by the NOESY correlations (Figure 3) observed between the H-2 proton to the axial proton at H-10 and to the equatorial proton of the H-1 methylene group. The ^{13}C NMR values were assigned on the basis of HMQC and HMBC spectral data, confirming the structure of casearlucin E (**5**) 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-methoxy-2-(2ξ-methylbutanoyloxy)cleroda-3,13(16),14-triene.

Casearlucin F (**6**) was established as $C_{29}H_{42}O_7$ by HR-FABMS data. The ^1H and ^{13}C NMR data of compound **6** (Tables 1−3) were very similar to those of **1**, except for the lack of signals for the oxymethine group at C-6. The presence of a methylene group in place of the oxymethine group at C-6 was supported by the presence of a multiplet centered at δ 1.72 and a peak at δ 29.2 in the ^1H and ^{13}C NMR spectra, respectively. The oxymethine group at C-2

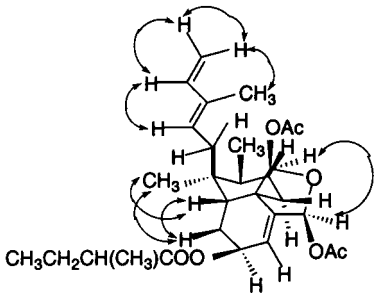


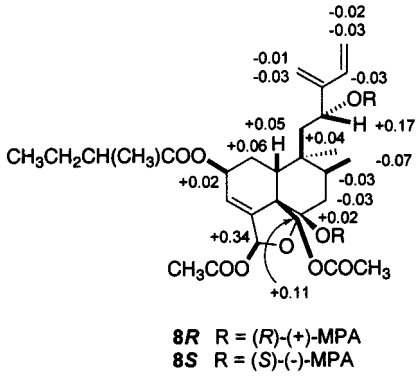
Figure 4. Selected NOESY correlations for **6**.

in **6** was assigned as β, as in **1**, because of the almost identical appearance of the C-2 proton signal in the ^{1}H NMR spectrum as well as the absence of a correlation between H-2 and H-10 in the NOESY spectrum (Figure 4). The structure of casearlucin F (**6**) was thus assigned as *rel*-(2S,5*R*,8*S*,9*S*,10*R*,18S,19*R*)-18,19-diacetoxy-18,19-epoxy-2-(2ξ-methylbutanoyloxy)cleroda-3,12,14-triene on the basis of the above spectral data.

The molecular formula of casearlucin G (**7**) was $C_{29}H_{42}O_7$ (HRFABMS), identical to that of **6**. The ^{1}H NMR data (Table 1) showed chemical shift values and coupling constants very similar to those of **6**, except for the signals for the C-2 methine proton. The ^{13}C NMR values for all the carbons were assigned on the basis of HMQC and HMBC spectra and are given in Table 3. HMBC correlations H-6/C-5, C-7, C-19, C-8, C-4; H-7/C-6, C-8, C-5; H-10/C-1, C-5, C-6 also served to confirm the assignment of **7** as a stereoisomer of **6**. The ^{1}H and ^{13}C NMR data of the oxymethine proton at C-2 indicated the α orientation of the acyloxy group, as in compounds **2** and **5**. The relative stereochemistry at the remaining six chiral carbons was assigned as that of **1** (or **6**) on the basis of their very similar coupling constants. The structure of casearlucin G (**7**) was thus assigned as *rel*-(2*R*,5*R*,8*S*,9*S*,10*R*,18*S*,19*R*)-18,19-diacetoxy-18,19-epoxy-2-(2ξ-methylbutanoyloxy)cleroda-3,12,14-triene.

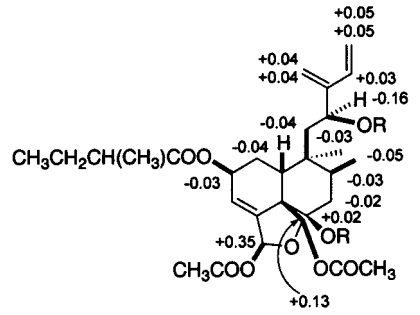
Casearlucin H (**8**) was isolated as a viscous liquid; its molecular formula was established as $C_{29}H_{42}O_9$ by HR-FABMS and ^{13}C NMR spectra. The ^{1}H and ^{13}C NMR data (Tables 2 and 3) were similar to those of **12**, except for the presence of signals for an additional secondary hydroxyl group in **8**. The signals for the oxymethine were observed at δ 4.47 (dd, J = 9.0, 10.4 Hz) and 67.5 in the ^{1}H and ^{13}C NMR spectrum, respectively. COSY (H-11/H-12) and HMBC (H-12/C-11, C-9, C-13; H-11/C-9, C-12, C-10, C-13) correlations confirmed the presence of the oxymethine proton at the C-12 position. The ^{1}H NMR spectra for the ring protons of **8** and **12** had almost identical coupling constants and NOESY correlations, indicating that the relative stereochemistry of the eight chiral carbons (C-2, C-5, C-6, C-8, C-9, C-10, C-18, C-19) in **8** was the same as that of **12**. The assignment of the stereochemistry of C-12 was carried out by the Mosher ester method as described by Latypov et al.[15] and used for a related labdane diterpenoid by Zhou et al.[2a] Acylation of **8** with (*R*)-(+) and (*S*)-(−) MPA yielded the (*R*)-diester (**8*R***) and the (*S*)-diester (**8*S***). The chemical shift differences ($\Delta\delta^{RS} = \delta^R - \delta^S$) of the individual protons of **8*R*** and **8*S*** are shown in Figure 5. The systematic arrangement of positive and negative $\Delta\delta^{RS}$ values indicated that the absolute configuration of C-12 is *R*, as indicated in structure **8**.[16]

Since previous authors have only assigned relative stereochemistries to some compounds in this series,[9] the $\Delta\delta^{RS}$ values of the protons around the C-6 position were



8*R* R = (*R*)-(+)-MPA
8*S* R = (*S*)-(-)-MPA

Figure 5. Difference in δ^{RS} ($\delta^R - \delta^S$) values for the (*R*)- and (*S*)-MPA esters **8*R*** and **8*S*** in $CDCl_3$.



9*R* R = (*R*)-(+)-MPA
9*S* R = (*S*)-(-)-MPA

Figure 6. Difference in δ^{RS} ($\delta^R - \delta^S$) values for the (*R*)- and (*S*)-MPA esters **9*R*** and **9*S*** in $CDCl_3$.

also analyzed to determine the absolute configuration at C-6 and, thus, of the rest of the molecule. This analysis indicated that the absolute configuration at C-6 is *R* (Figure 5), thus allowing the assignment of absolute stereochemistry to this molecule. On the basis of the above spectral and chemical information, the structure of casearlucin H (**8**) was established as (2*S*,5*R*,6*R*,8*S*,9*S*,10*R*,12*R*,18*S*,19*R*)-18,19-diacetoxy-18,19-epoxy-6,12-dihydroxy-2-(2ξ-methylbutanoyloxy)cleroda-3,13(16),14-triene.[16] It is probable that the other compounds reported here have the same absolute stereochemistry, but the lack of available sample prevented experimental verification of this assumption.

The molecular formula of casearlucin I (**9**) was deduced as $C_{29}H_{42}O_9$ by HRFABMS, identical to that of **8**. Its ^{1}H and ^{13}C NMR data were very similar to those of **8**, except for differences of the proton and carbon values for the C-12 position (Tables 2 and 3). The coupling constants and NOESY correlations of **9** were closely comparable with those of **8** and **12**, and the relative stereochemistries at the eight chiral carbons (C-2, C-5, C-6, C-8, C-9, C-10, C-18, C-19) of **9** were thus the same as those of **8** and **12**. These findings indicated that **9** is an isomer of **8** at the C-12 position. To confirm the stereochemistry at C-12, the Mosher ester method was used as described for **8**. The (*R*)-ester (**9*R***) and the (*S*)-ester (**9*S***) were prepared, and their ^{1}H NMR spectra determined. The chemical shift differences ($\Delta\delta^{RS} = \delta^R - \delta^S$) of the individual protons of **9*R*** and **9*S*** are shown in Figure 6. From the systematic arrangement of negative and positive $\Delta\delta^{RS}$ values, the absolute configuration at C-12 was established as *S*. The absolute stereochemistry at C-6 was shown to be *R* by the same experiment. Thus, casearlucin I (**9**) was established as (2*S*,5*R*,6R,8*S*,9*S*,10*R*,12*S*,18*S*,19*R*)-18,19-diacetoxy-18,19-epoxy-6,12-dihydroxy-2-(2ξ-methylbutanoyloxy)cleroda-3,13(16),14-triene.

Table 4. Cytotoxicity of Selected Clerodane Diterpenoids

	IC_{50} (μg/mL)									
compound	A2780	A2780/DP-S	A2780/DDP-R	SKBR3	PC3	HCT116	LX-1	K562	ABAE	HT-29
1	3.0	1.0	2.8	1.2	2.5	1.3	1.0	1.1	0.8	0.8
3	2.8	7.7	9.6	14	15	4.5	5.0	3.8	3.6	12
4	2.9	9.4	4.6	10	13	4.7	3.9	3.9	4.1	6.2
5	2.9	3.0	4.1	3.9	4.3	1.8	1.8	1.7	0.9	2.8
12	2.9	1.2	2.1	1.4	2.8	1.6	1.1	1.4	0.8	1.0
13	2.9	1.4	2.9	3.5	3.0	1.0	1.2	1.4	0.8	1.3

Compounds **10** and **11** exhibited the same molecular formula $C_{29}H_{42}O_9$, established on the basis of HRFABMS and ^{13}C NMR spectral data. The ^1H and ^{13}C NMR of **10** and **11** (Tables 1−3) were essentially identical to those of **8** and **9**, respectively, except for the signals for the proton at C-2. The relative configuration of the acyloxy group at C-2 was assigned as α in both compounds, since the proton and carbon signals were very similar to those of **2** and **5**. This assignment was further supported by the NOESY spectra of **10** and **11**, which showed correlations of the H-2 β proton to H-1 β and H-10, similar to those observed for **2**. Thus, the structures of casearlucins J (**10**) and K (**11**) were assigned as *rel*-(2*R*,5*R*,6*R*,8*S*,9*S*,10*R*,12*R*,18*S*,19*R*)-18,19-diacetoxy-18,19-epoxy-6,12-dihydroxy-2-(2ξ-methylbutanoyloxy)cleroda-3,13(16),14-triene and *rel*-(2*R*,5*R*,6*R*,8*S*,9*S*,10*R*,12*S*,18*S*,19*R*)-18,19-diacetoxy-18,19-epoxy-6,12-dihydroxy-2-(2ξ-methylbutanoyloxy)cleroda-3,13(16),14-triene, respectively.

The isolated compounds were tested for cytotoxicity against A2780 ovarian cancer cells. As shown in Table 4, all the isolated compounds (**1**−**14**) were found to be cytotoxic, with IC_{50} values ranging between 2.7 and 3.1 μg/mL. Those compounds available in adequate amounts were also tested in a panel of mammalian cell lines (Table 4). Although the range of activities was somewhat larger in this panel, none of the compounds showed significant selectivity for any cell line, indicating that the compounds most probably function as general cytotoxic agents, thus diminishing their potential as anticancer agents or lead compounds for such agents. The fact that all the compounds isolated had similar activities suggests that the activity mainly depends on the basic skeleton of the clerodane diterpenes, rather than on any specific substituents thereon.

Experimental Section

General Experimental Procedures. Optical rotations were recorded on a Perkin-Elmer 241 polarimeter. IR and UV spectra were measured on MIDAC M-series FTIR and Shimadzu UV-1201 spectrophotometers, respectively. NMR spectra were obtained on a JEOL Eclipse 500 spectrometer. Mass spectra were obtained on a JEOL HX-110 instrument. The chemical shifts are given in δ (ppm) with TMS as internal reference and coupling constants in Hz. Sephadex LH-20 and reversed-phase Si gel (LRP-2, 200 μm) were used for column chromatography. Reversed-phase HPLC was performed on a Shimadzu LC-10AT instrument with an ODS C_{18} column.

Cytotoxicity Bioassays. The A2780 ovarian cancer cell line assay was performed at Virginia Polytechnic Institute and State University as previously reported.[17] In brief, growth inhibition was determined using a microplate assay in which the A2780 cells were seeded in RPMI 1640 media plus L-glutamine (Gibco) and 10% fetal bovine serum (Gibco) at a cell density of 2.7×10^5 cells/mL. Samples were dissolved in 50% DMSO and transferred to the seeded microtiter wells at a 1:50 dilution, for a final testing concentration of 20 μg/mL. Microtiter plates were incubated at 37 °C and 5% CO_2 for 48 h. The old medium was then replaced with RPMI 1640 (as above) plus 1% Alamar Blue (Biosource International). After another 4 h of incubation, fluorescence of the Alamar Blue reagent was measured using a Cytofluor (Millipore) at an emission of 530 nm, an excitation of 590 nm, and a gain of 40. Percent fluorescence is directly proportional to percent inhibition, and growth inhibition was elucidated using a linear regression analysis of the dose response scheme. Activity was reported in terms of an IC_{50} value, which is the concentration (μg/mL) necessary to produce 50% inhibiton. Actinomycin D (IC_{50} 2 ng/mL) was used as a positive control. The remaining cytotoxicity assays were carried out at Bristol-Myers Squibb Pharmaceutical Research Institute, Princeton, NJ, using the indicated cell lines and similar conditions.

Plant Material. The bark of *Casearia lucida* Hils. & Bojer ex Tul. (Flacourtiaceae) was collected by James L. Zarucchi and Amy Pool of the Missouri Botanical Garden, Etienne Rakotobe of Centre National D'Application Recherches Pharmaceutiques (CNARP), and Armand Randrianasolo of Parc Botanique et Zoologique de Tsimbazaza in the Toamasina Province, 10 km north of Fenoarivo at the Station Forestiere de Tampolo, Madagascar, in 1991. The plant was a tree 14 m tall, and it was growing in low forest on very sandy soil. The collection was assigned collector number Zarucchi et al. 7379, sample number Q66V0274: a voucher specimen has been deposited at the Missouri Botanical Garden, St. Louis, MO, and at the Parc Botanique et Zoologique de Tsimbazaza in Madagascar. The plant was identified by James Zarucchi at Missouri Botanical Garden.

Extract Preparation. Field-dried plant was ground in a hammermill, then extracted by overnight percolation at room temperature with a 1:1 mixture of reagent-grade CH_2Cl_2/MeOH. The solvent was withdrawn quickly by suction, and the specimen was covered with 100% MeOH. After 30 min the MeOH wash was drained into the same flask as the CH_2Cl_2/MeOH extract, and the combined organic solvent extracts were evaporated on a rotary evaporator below 40 °C to give a thick concentrate, which was transferred into a glass bottle for storage. The bottle was dried overnight under high vacuum to give the dried CH_2Cl_2/MeOH extract N037509.

Extraction and Isolation. The crude extract (3.0 g) was suspended in aqueous MeOH (MeOH/H_2O, 9:1, 500 mL) and extracted with *n*-hexane (3 × 500 mL). The aqueous layer was then diluted to 60% MeOH (v/v) with H_2O and extracted with $CHCl_3$ (3 × 500 mL). The aqueous layer was concentrated, and the residue obtained was suspended in H_2O (25 mL) and extracted with *n*-BuOH (3 × 25 mL). The $CHCl_3$ extract was found to be the most cytotoxic and was fractionated over Sephadex LH-20 using MeOH/H_2O (100:0 to 40:60) to furnish eight fractions (A−H), of which fractions B−E were found to be active. Fraction B on column chromatography over RP C_{18} using MeOH/H_2O (80:20) followed by reversed-phase HPLC with the mobile phase CH_3CN/H_2O (60:40) yielded the two new clerodane diterpenes **1** (6.3 mg) and **2** (2.1 mg) and the known compound **12** (12.4 mg). Fraction C on preparative C_{18} reversed-phase TLC (MeOH/H_2O, 75:25) followed by reversed-phase HPLC with the mobile phase CH_3CN/H_2O (70:30) yielded the four new clerodane diterpenoids **8** (1.1 mg), **9** (0.76 mg), **10** (0.83 mg), and **11** (0.72 mg). Similarly fraction D on column chromatography over RP C_{18} using MeOH/H_2O (70:30) followed by reversed-phase HPLC with mobile phase CH_3CN/H_2O (70:30) furnished the two new clerodane diterpenes **3** (2.2 mg) and **4** (6.2 mg) and the known compound (**13**, 24.6 mg). Fraction E on column chromatography over RP C_{18} using MeOH/H_2O (90:10) furnished five fractions E-1 to E-5. Fraction E-1 on reversed-phase HPLC with the mobile phase CH_3CN/H_2O (80:

20) furnished the new clerodane diterpene **5** (4.2 mg). Fraction E-5 on reversed-phase HPLC with the mobile phase CH_3CN/H_2O (60:40) furnished the other two new clerodane diterpenes **7** (0.72 mg) and **6** (0.8 mg) as well as the known clerodane diterpene **14** (0.68 mg). The three known compounds **12**−**14** were identified by comparison of their spectral data with literature values.[9]

Casearlucin A (1): viscous liquid; $[\alpha]_D$ +10.6° (*c* 0.52, MeOH); UV (MeOH) λ_{max} 226 nm (ϵ 12 400); IR ν_{max} 3455, 2960, 1755, 1735, 1645, 1453, 1128, 1065, 753 cm^{-1}; ^1H NMR, see Table 1; ^{13}C NMR, see Table 3; CIMS (positive mode) *m/z* (rel int) 518 (M^+, 2), 501 (4), 459 (15), 399 (25), 297 (17), 205 (28), 135 (23), 103 (72), 61 (100); HRFABMS *m/z* 517.2789 [M − H]$^+$ (calcd for $C_{29}H_{41}O_8$, 517.2802).

Casearlucin B (2): viscous oil; $[\alpha]_D$ +31.0° (*c* 0.22, MeOH); UV (MeOH) λ_{max} 232 nm (ϵ 11 310); IR ν_{max} 2956, 1753, 1728, 1723, 1642, 1451, 1120, 1065, 748 cm^{-1}; ^1H NMR, see Table 1; ^{13}C NMR, see Table 3; EIMS *m/z* (rel int) 490 (M^+, 6), 431 (12), 430 (28), 388 (16), 371 (23), 370 (18), 297 (21), 241 (17), 135 (56), 85 (78), 59 (100); HRFABMS *m/z* 431.2444 [M − OAc]$^+$ (calcd for $C_{25}H_{35}O_6$, 431.2435).

Casearlucin C (3): colorless oil; $[\alpha]_D$ +23.2° (*c* 0.35, MeOH); UV (MeOH) λ_{max} 228 nm (ϵ 10 800); IR ν_{max} 2955, 1750, 1735, 1730, 1648, 1450, 1118, 1056, 750 cm^{-1}; ^1H NMR, see Table 1; ^{13}C NMR, see Table 3; EIMS *m/z* (rel int) 560 (M^+, 3), 500 (16), 459 (15), 458 (22), 440 (18), 314 (28), 297 (26), 187 (16), 159 (23), 135 (36), 85 (65), 57 (100); HRFABMS *m/z* 501.2852 [M − OAc]$^+$ (calcd for $C_{29}H_{41}O_7$, 501.2853).

Acetylation of Casearlucin A (1). Acetylation (Ac_2O/Py, 1:1, 0.3 mL; room temperature) of casearlucin A (**1**, 1.5 mg) and usual workup gave product (1.2 mg), which was identical (TLC and ^1H NMR) with casearlucin C (**3**).

Casearlucin D (4): colorless oil; $[\alpha]_D$ +18.6° (*c* 0.64, MeOH); UV (MeOH) λ_{max} 224 nm (ϵ 12 280); IR ν_{max} 3455, 1755, 1735, 1727, 1645, 1453, 1117, 1052, 737 cm^{-1}; ^1H NMR, see Table 1; ^{13}C NMR, see Table 3; EIMS *m/z* (rel int) 560 (M^+, 2), 501 (15), 500 (21), 458 (17), 441 (25), 440 (17), 314 (14), 297 (19), 159 (16), 135 (48), 85 (72), 57 (100); HRFABMS *m/z* 501.2856 [M − OAc]$^+$ (calcd for $C_{29}H_{41}O_7$, 501.2853).

Casearlucin E (5): colorless oil; $[\alpha]_D$ +6.8° (*c* 0.21, MeOH); UV (MeOH) λ_{max} 226 nm (ϵ 12 200); IR ν_{max} 2955, 1751, 1734, 1645, 1450, 1120, 1057, 753 cm^{-1}; ^1H NMR, see Table 1; ^{13}C NMR, see Table 3; EIMS *m/z* (rel int) 489 (5) 472 (14), 430 (21), 413 (18), 412 (16), 371 (19), 297 (23), 187 (11), 161 (25), 135 (45), 85 (100), 57 (81); HRFABMS *m/z* 531.2977 [M − H]$^+$ (calcd for $C_{30}H_{43}O_8$, 531.2958).

Casearlucin F (6): colorless oil; $[\alpha]_D$ +10.4° (*c* 0.16, MeOH); UV (MeOH) λ_{max} 232 nm (ϵ 11 200); IR ν_{max} 2955, 1750, 1738, 1732, 1638, 1453, 1126, 1063, 750 cm^{-1}; ^1H NMR, see Table 1; ^{13}C NMR, see Table 3; HRFABMS *m/z* 442.2713 [M − AcOH]$^+$ (calcd for $C_{27}H_{38}O_5$, 442.2720).

Casearlucin G (7): colorless oil; $[\alpha]_D$ +7.6° (*c* 0.18, MeOH); UV (MeOH) λ_{max} 234 nm (ϵ 10 960); IR ν_{max} 2948, 1758, 1735, 1727, 1642, 1446, 1128, 1054 cm^{-1}; ^1H NMR, see Table 1; ^{13}C NMR, see Table 3; HRFABMS *m/z* 442.2721 [M − AcOH]$^+$ (calcd for $C_{27}H_{38}O_5$, 442.2720).

Casearlucin H (8): viscous liquid; $[\alpha]_D$ +8.2° (*c* 0.12, MeOH); UV (MeOH) λ_{max} 224.2 nm (ϵ 12 400); IR λ_{max} 3435, 2955, 1750, 1732, 1640, 1453, 1123, 750 cm^{-1}; ^1H NMR, see Table 2; ^{13}C NMR, see Table 3; HRFABMS *m/z* 475.2711 [M − OAc] $^+$ (calcd for $C_{27}H_{39}O_7$, 475.2696).

Preparation of the (*R*)-(−)-α-Methoxyphenyl Acetate of 8. Compound **8** (0.4 mg, 0.00075 mmol) was treated with (*R*)-(−)-α-MPA (1.24 mg, 0.0075 mmol) and 1-[3-(dimethylamino)propyl]-3-ethylcarbodiimide hydrochloride (EDC) (1.44 mg, 0.0075 mmol) in the presence of a catalytic amount of 4-pyrrolidinopyridine in CH_2Cl_2 (0.5 mL), and the mixture was stirred for 18 h at room temperature. The di-[(*R*)-(−)-α-methoxyphenyl acetate] (**8*R***, 0.23 mg) was obtained after purification by preparative TLC (*n*-hexanes/EtOAc, 70:30): ^1H NMR ($CDCl_3$) δ 2.06 (2H, m, H-1), 5.32 (1H, brs, H-2), 5.99 (1H, d, J = 4.2 Hz, H-3), 4.48 (1H, dd, J = 4.2, 12.0 Hz, H-6), 1.70 (2H, m, H-7), 1.82 (1H, m, H-8), 2.22 (1H, dd, J = 10.8, 6.4 Hz, H-10), 1.92 (2H, m, H-11), 5.01 (1H, dd, J = 9.2, 10.4 Hz, H-12), 6.29 (1H, dd, J = 17.8, 10.8 Hz, H-14), 5.15 (1H, d, J = 11.2 Hz, H-15), 5.41 (1H, d, J = 17.8 Hz, H-15), 5.13 (2H, s, H-16), 0.95 (3H, d, J = 6.8 Hz, CH_3-17), 6.36 (1H, brs, H-18), 6.59 (1H, brt, J = 1.6 Hz, H-19), 1.05 (3H, s, CH_3-20), 1.92 (3H, s, AcMe), 2.06 (3H, s, AcMe). The α-methoxyphenylacetic acid part had δ 7.20−7.40 (10H, m, aromatic protons), 4.75 (2H, s), 3.39 (6H, s, 2 × $-OCH_3$); HRFABMS *m/z* 563.3021 [M − OAc − AcOH]$^+$ (calcd for $C_{34}H_{43}O_7$, 563.3009).

Preparation of the (*S*)-(+)-α-Methoxyphenyl Acetate of 8. Compound **8** (0.4 mg, 0.00075 mmol) was treated with (*S*)-(+)-α-MPA (1.24 mg, 0.0075 mmol) and EDC (1.44 mg, 0.0075 mmol) in the presence of a catalytic amount of 4-pyrrolidinopyridine in CH_2Cl_2 (0.5 mL), and the mixture was stirred for 18 h at room temperature. The di-[(*S*)-(+)-α-methoxyphenyl acetate] (**8*S***, 0.20 mg) was obtained after purification by preparative TLC (*n*-hexanes/EtOAc, 70:30): ^1H NMR ($CDCl_3$) δ 2.00 (2H, m, H-1), 5.30 (1H, brs, H-2), 5.82 (1H, d, J = 4.2 Hz, H-3), 4.46 (1H, dd, J = 4.0, 12.2 Hz, H-6), 1.73 (2H, m, H-7), 1.85 (1H, m, H-8), 2.17 (1H, dd, J = 10.8, 6.4 Hz, H-10), 1.88 (2H, m, H-11), 4.84 (1H, dd, J = 9.2, 10.4 Hz, H-12), 6.32 (1H, dd, J = 17.8, 10.8 Hz, H-14), 5.17 (1H, d, J = 11.2 Hz, H-15), 5.44 (1H, d, J = 17.8 Hz, H-15), 5.14 (1H, s, H-16), 5.16 (1H, s, H-16), 1.02 (3H, d, J = 6.8 Hz, CH_3-17), 6.02 (1H, brs, H-18), 6.48 (1H, brt, J = 1.6 Hz, H-19), 1.06 (3H, s, CH_3-20), 1.90 (3H, s, AcMe), 1.98 (3H, s, AcMe). The α-methoxyphenylacetic acid part had δ 7.22−7.40 (10H, m, aromatic protons), 4.72 (2H, s), 3.34 (6H, s, 2 × $-OCH_3$); HRFABMS *m/z* 563.3004 [M − OAc − AcOH]$^+$ (calcd for $C_{34}H_{43}O_7$, 563.3009).

Casearlucin I (9): viscous liquid; $[\alpha]_D$ +13.6° (*c* 0.16, MeOH); UV (MeOH) λ_{max} 224 nm (ϵ 12 400); IR ν_{max} 3450, 2960, 1748, 1735, 1640, 1450, 1122, 1055, 750 cm^{-1}; ^1H NMR, see Table 2; ^{13}C NMR, see Table 3; HRFABMS *m/z* 475.2702 [M − OAc]$^+$ (calcd for $C_{27}H_{39}O_7$, 475.2696).

Preparation of the (*R*)-(−)-α-Methoxyphenyl Acetate of 9. Compound **9** (0.3 mg, 0.00056 mmol) was treated with (*R*)-(−)-α-MPA (1.00 mg, 0.006 mmol) and EDC (1.20 mg, 0.0062 mmol) in the presence of a catalytic amount of 4-pyrrolidinopyridine in CH_2Cl_2 (0.5 mL), and the mixture was stirred for 18 h at room temperature. The di-[(*R*)-(−)-α-methoxyphenyl acetate] (**9*R***, 0.14 mg) was obtained after purification by preparative TLC (*n*-hexanes/EtOAc, 70:30): ^1H NMR ($CDCl_3$) δ 2.02 (2H, m, H-1), 5.28 (1H, brs, H-2), 5.98 (1H, d, J = 4.2 Hz, H-3), 4.46 (1H, dd, J = 4.2, 12.2 Hz, H-6), 1.72 (2H, m, H-7), 1.81 (1H, m, H-8), 2.18 (1H, dd, J = 10.8, 6.4 Hz, H-10), 1.88 (2H, m, H-11), 4.87 (1H, dd, J = 9.2, 10.4 Hz, H-12), 6.33 (1H, dd, J = 17.8, 10.8 Hz, H-14), 5.18 (1H, d, J = 11.2 Hz, H-15), 5.44 (1H, d, J = 17.8 Hz, H-15), 5.15 (1H, s, H-16), 5.17 (1H, s, H-16), 0.96 (3H, d, J = 6.6 Hz, CH_3-17), 6.38 (1H, brs, H-18), 6.59 (1H, brt, J = 1.6 Hz, H-19), 1.06 (3H, s, CH_3-20), 1.93 (3H, s, AcMe), 2.06 (3H, s, AcMe). The α-methoxyphenylacetic acid part had δ 7.20−7.42 (10H, m, aromatic protons), 4.75 (2H, s), 3.40 (6H, s, 2 × $-OCH_3$).

Preparation of the (*S*)-(+)-α-Methoxyphenyl Acetate of 9. Compound **9** (0.3 mg) was treated with (*S*)-(+)-α-MPA (1.24 mg) and EDC (1.44 mg) in the presence of a catalytic amount of 4-pyrrolidinopyridine in CH_2Cl_2 (0.5 mL), and the mixture was stirred for 18 h at room temperature. The di-[(*S*)-(+)-α-methoxyphenyl acetate] (**9*S***, 0.15 mg) was obtained after purification by preparative TLC (*n*-hexanes/EtOAc, 70:30): ^1H NMR ($CDCl_3$) δ 2.06 (2H, m, H-1), 5.31 (1H, brs, H-2), 6.01 (1H, d, J = 4.2 Hz, H-3), 4.44 (1H, dd, J = 4.2, 12.2 Hz, H-6), 1.74 (2H, m, H-7), 1.84 (1H, m, H-8), 2.22 (1H, dd, J = 10.8, 6.4 Hz, H-10), 1.91 (2H, m, H-11), 5.03 (1H, dd, J = 9.2, 10.4 Hz, H-12), 6.30 (1H, dd, J = 17.8, 10.8 Hz, H-14), 5.13 (1H, d, J = 11.2 Hz, H-15), 5.39 (1H, d, J = 17.8 Hz, H-15), 5.11 (1H, s, H-16), 5.13 (1H, s, H-16), 1.01 (3H, d, J = 6.6 Hz, CH_3-17), 6.03 (1H, brs, H-18), 6.46 (1H, brt, J = 1.6 Hz, H-19), 1.07 (3H, s, CH_3-20), 1.92 (3H, s, AcMe), 1.99 (3H, s, AcMe). The α-methoxyphenylacetic acid part had δ 7.21−7.42 (10H, m, aromatic protons), 4.71 (2H, s), 3.33 (6H, s, 2 × $-OCH_3$).

Casearlucin J (10): viscous liquid; $[\alpha]_D$ +11.2° (*c* 0.18, MeOH); UV (MeOH) λ_{max} 226.2 nm (ϵ 12 400); IR ν_{max} 3445, 2952, 1748, 1735, 1640, 1450, 1120, 1058, 745 cm^{-1}; ^1H NMR, see Table 2; ^{13}C NMR, see Table 3; HRFABMS *m/z* 475.2693 [M − OAc]$^+$ (calcd for $C_{27}H_{39}O_7$, 475.2696).

Casearlucin K (11): viscous liquid; $[\alpha]_D$ +10.2° (*c* 0.18, MeOH); UV (MeOH) λ_{max} 226 nm (ϵ 12 400); IR ν_{max} 3445, 2960, 1750, 1735, 1645, 1450, 1128, 1063, 745 cm^{-1}; ^1H NMR, see Table 2; ^{13}C NMR, see Table 3; HRFABMS *m*/*z* 475.2683 [M − OAc]$^+$ (calcd for $C_{27}H_{39}O_7$, 475.2696).

Acetylation of Compound 12. Acetylation (Ac_2O/pyridine, 1:1, 0.5 mL; room temperature) of compound **12** (2.5 mg) as previously described furnished a product (2.1 mg), which was identical by TLC and ^1H NMR to casearlucin C (**4**).

Acknowledgment. This work was supported by International Cooperative Biodiversity Grant Number U01 TW/CA-00313 from the Fogarty Center, National Institutes of Health, and this support is gratefully acknowledged. We thank Dr. C. R. Fairchild and Mrs. Kathy A. Johnston (Bristol-Myers Squibb Pharmaceutical Research Institute, Princeton, NJ) for the cytotoxicity measurements of Table 4. We also thank Dr. Armand Randrianasolo (Missouri Botanical Garden, formerly of Parc Botanique et Zoologique de Tsimbazaza), Dr. James Zarucchi, Ms. Amy Pool (Missouri Botanical Garden), and Mr. Etienne Rakotobe (Centre National d'Application et des Recherches Pharmaceutiques, CNARP) for the plant collection, and the staff of CNARP and the Parc Botanique et Zoologique de Tsimbazaza for their help in the collection, drying, and processing of the plant samples. We are grateful to Drs. Gordon Cragg and David Newman of the National Cancer Institute for making the extract available, to Dr. Tom McCloud and the Natural Products Support Group staff at Frederick Cancer Research and Development Center for extract preparation, to Dr. Rabodo Andriantsiferana and Dr. Maminirina Andriantsoa of CNARP for their assistance in obtaining approval for the use of this extract, and to Dr. Jim Miller (Missouri Botanical Garden) for information about the collection. We also thank Mr. Bill Bebout (Virginia Polytechnic Institute and State University, VPISU) for obtaining the mass spectra, Dr. Harold McNair and Ms. Laura Nakovich (VPISU) for attempted analysis of methyl 2-methylbutanoates by chiral GC, and Dr. Raouf Hussain (Bristol-Myers Squibb Pharmaceutical Research Institute, Wallingford, CT) for his assistance in sharing initial cytotoxicity information on the extract.

Supporting Information Available: ^1H NMR spectra for compounds **1**−**14**. This material is available free of charge via the Internet at http://pubs.acs.org.

References and Notes

(1) Biodiversity Conservation and Drug Discovery in Madagascar, Part 2. For part 1, see: Seo, Y.; Berger, J. M.; Hoch, J. M.; Glass, T. E.; Mamber, S. W.; Kingston, D. G. I. *J. Nat. Prod*. **2002**, *65*, in press.

(2) (a) Zhou, B.-N.; Baj, N. J.; Glass, T. E.; Malone, S.; Werkhoven, M. C. M.; van Troon, F.; David, M.; Wisse, J. H.; Kingston, D. G. I. *J. Nat. Prod*. **1997**, *60*, 1287−1293. (b) Abdel- Kader, M. S.; Wisse, J. H.; Evans, R.; van der Werff, H.; Kingston, D. G. I. *J. Nat. Prod*. **1997**, *60*, 1294−1297. (c) Abdel-Kader, M. S.; Bahler, B. D.; Malone, S.; Werkhoven, M. C. M.; van Troon, F.; David, M.; Wisse, J. H.; Bursuker, I.; Neddermann, K. M.; Mamber, S. W.; Kingston, D. G. I. *J. Nat. Prod*. **1998**, *61*, 1202−1208. (d) Yang, S.-W.; Zhou, B.-N.; Wisse, J. H.; Evans, R.; van der Werff, H.; Miller, J. S.; Kingston, D. G. I. *J. Nat. Prod*. **1998**, *61*, 901−906. (e) Yang, S.-W.; Abdel-Kader, M.; Malone, S.; Werkhoven, M. C. M.; Wisse, J. H.; Bursuker, I.; Neddermann, K.; Fairchild, C.; Raventos-Suarez, C.; Menendez, A. T.; Lane, K.; Kingston, D. G. I. *J. Nat. Prod*. **1999**, *62*, 976−983. (f) Yang, S.-W.; Zhou, B.-N.; Malone, S.; Werhoven, M. C. M.; van der Troon, F.; Wisse, J. H.; Kingston, D. G. I. *J. Nat. Prod*. **1999**, *62*, 1173−1174.

(3) Guittet, E.; Stoven, V.; Lallemend, J. Y.; Ramiandrasoa, F.; Kunesch, G.; Moretti, C. *Tetrahedron* **1988**, *44*, 2893−2901.

(4) Itokawa, H.; Totsuka, N.; Takeya, K.; Watanabe, K.; Obata, E. *Chem. Pharm. Bull.* **1988**, *36*, 1585−1588.

(5) Itokawa, H.; Totsuka, N.; Morita, H.; Takeya, K.; Iitaka, Y.; Schenkel, E. P.; Motidome, M. *Chem. Pharm. Bull.* **1990**, *38*, 3384−3388.

(6) Morita, H.; Nakayama, M.; Kojima, H.; Takeya, K.; Itokawa, H.; Schenkel, E. P.; Motidome, M. *Chem. Pharm. Bull.* **1991**, *39*, 693−697.

(7) Chen, T. B.; Weimer, D. F. *J. Nat. Prod*. **1991**, *54*, 1612−1618.

(8) Khan, M. R.; Gray, A. I.; Sadler, I. H.; Waterman, P. G. *Phytochemistry* **1990**, *29*, 3591−3595.

(9) Gibbons, S.; Gray, A. I.; Waterman, P. G. *Phytochemistry* **1996**, *41*, 565−570.

(10) De Carvalho, P. R. F.; Furlan, M.; Young, M. C. M.; Kingston, D. G. I.; Bolzani, V. S. *Phytochemistry* **1998**, *49*, 1659−1662.

(11) Hunter, M. S.; Corley, D. G.; Carron, C. P.; Rowold, E.; Kilpatrick, B. F.; Durley, R. C. *J. Nat. Prod*. **1997**, *60*, 894−899.

(12) Beutler, J. A.; McCall, K. L.; Herbert, K.; Herald, D. L.; Pettit, G. R.; Johnson, T.; Shoemaker, R. H.; Boyd, M. R. *J. Nat. Prod*. **2000**, *63*, 657−661.

(13) Oberlies, N. H.; Burgess, J. P.; Navarro, H. A.; Pinos, R. E.; Soejarto, D. D.; Farnsworth, N. R.; Kinghorn, A. D.; Wani, M. C.; Wall, M. E. *J. Nat. Prod*. **2001**, *64*, 497−501.

(14) In this paper the steroid convention is used for convenience in designating α,β relative stereochemistry; α is used to designate substituents oriented below the plane of the molecule as drawn, and β is used for substituents above the plane of the ring. The system adopted by the *Chemical Abstracts Index Guide* would have required the α,β designations to change with the stereochemistry at C-2.

(15) Latypov, Sh. K.; Seco, J. M.; Quiñoá, E.; Riguera, R. *J. Org. Chem*. **1996**, *61*, 8569−8577.

(16) One referee was concerned about the reliability of stereochemical determinations based on the analysis of Mosher diesters, since "one cannot make convincing arguments about the positions of the two aromatic moieties relative to each other". We accept this concern, but regrettably the preferred solution of preparing the relevant monoester was not available to us because of lack of available sample. Since the two aromatic moieties are relatively distant from each other, we believe that the assignments given are correct, but acknowledge that there is some uncertainty associated with them.

(17) McBrien, K. D.; Bery, R. L.; Lewes, S. E.; Nedderman, K. M.; Bursuker, I.; Huang, S.; Klehr, S. E.; Leet, J. E. *J. Antibiot.* **1995**, *48*, 1446−1452.

NP010405C