Novel Cytotoxic Diterpenes from *Casearia arborea*

John A. Beutler,[†] Kate L. McCall,[§] Kathryn Herbert,[§] Delbert L. Herald,[‡] George R. Pettit,[‡] Tanya Johnson,[§] Robert H. Shoemaker,[§] and Michael R. Boyd*[,§]

Laboratory of Drug Discovery Research & Development, Developmental Therapeutics Program, Division of Cancer Treatment & Diagnosis, National Cancer Institute−FCRDC, Bldg. 1052, Rm. 121, Frederick, Maryland 21702-1201

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Cytotoxicity-guided fractionation of the dichloromethane−methanol extract of the roots of *Casearia arborea* yielded five novel clerodane diterpenes, casearborins A−E (**1**−**5**), as well as cucurbitacin B. The presence of cucurbitacins glycosides was also detected. The absolute configuration of casearborin E was determined by X-ray crystallography.

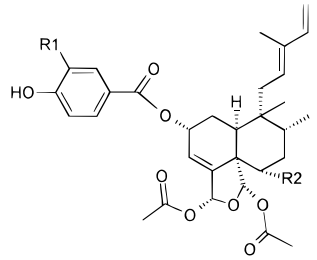
An extract of the roots of *Casearia arborea* (L. C. Rich.) Urban (Flacourtiaceae) was selected for fractionation based on a comparison with 19 other extracts from the Flacourtiaceae that showed cytotoxicity in the NCI 60-cell human tumor screen. The selected extract was generally more potent (mean GI_{50} 0.38 μg/mL) than the other extracts and showed a broader differential cytotoxicity (2.9 log units at GI_{50}). In addition, no phytochemistry has been reported on the roots of any species of *Casearia*. Plants of this genus and others in the Flacourtiaceae (*Laetia*, *Zuelania*) have recently been found to contain clerodane or kolovane diterpene esters, some of which have displayed cytotoxic, insect antifeedant, and LFA-1/ICAM binding inhibitory activity.[1−12]

Results and Discussion

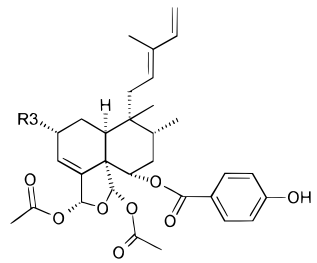
Fractionation of the extract by diol batch elution, Sephadex LH-20 permeation, and normal-phase cyano HPLC yielded five novel diterpenes, casearborins A−E (Scheme 1), as well as the known cucurbitacin B as major cytotoxic constituents. Cucurbitacin B was identified by comparison with literature NMR data.[13−15] The presence of other cucurbitacins, also potently cytotoxic, was detected; these were not further purified but were presumed to be acetylated cucurbitacin glycosides by their NMR spectra.

Casearborin A (**1**) was isolated as an optically active solid with UV maxima at 259 and 231 nm. FABMS analysis yielded an [M + Cs] ion corresponding to the molecular formula $C_{31}H_{38}O_8$. The unsaturation number of 13 could be accounted for by one benzoate ring, three other rings, three carbonyls, and three additional sites of unsaturation. That the benzoate was *p*-hydroxy-substituted was clearly evident by the pair of 8.6 Hz doublets in the ^{1}H NMR spectrum at δ 7.97 and δ 6.89, and by the shift in UV maximum to 298 nm with NaOH addition. Two acetal methine carbon signals at δ 94.5 and δ 98.8 were detected, consistent with the clerodane diterpenes previously isolated from the Flacourtiaceae. COSY, TOCSY, HSQC, and HMBC analyses of the NMR signals enabled complete assignment of the planar structure of diterpene **1**. The NMR data are recorded in Tables 1 and 2. Although NOE experiments were not sufficiently conclusive to permit assignment of relative stereochemistry, the configuration of the C-12,13 double bond was easily determined as *E*. All five diterpenes shared this double bond configuration.

Scheme 1. Structures of Casearborins



	R1	R2
1	H	H
2	MeO	H
4	H	OH



	R3
3	OH
5	OAc

The NMR spectrum of casearborin B (**2**, $[\alpha]_D$ +30°) differed from **1** principally in the aromatic region. A molecular formula of $C_{32}H_{40}O_9$ indicated addition of CH_2O. Analysis of the NMR spectra indicated that diterpene **2** was the *m*-methoxy-derivative of **1**, as seen by the HMBC correlation from the MeO protons at δ 3.96 to the aromatic singlet carbon at δ 146.3.

Casearborin C (**3**, $[\alpha]_D$ +35°) corresponded to a molecular formula of $C_{31}H_{38}O_9$, differing from diterpene **1** by one additional oxygen. HMBC analysis placed the hydroxybenzoate ester at C-6, based on a correlation between the oxymethine proton at δ 5.06 and the benzoate carbonyl at δ 166.1. The C-2 alcohol was similarly located by correlation of the oxymethine proton at δ 4.42 to the unsaturated carbons at δ 126.8 (C-3) and δ 142.2 (C-4). Both oxygenated carbons (C-2, C-6) were correlated to the H-10 proton at δ 2.44.

Casearborin D (**4**, $[\delta]_D$ +38°) was isomeric with diterpene **3**. The position of the hydroxybenzoate was changed to C-2, as demonstrated by the large shifts in H-2, H-6, C-2, and C-6 resonances (see Tables 1 and 2).

* To whom correspondence should be addressed. Tel.: (301) 846-5391. Fax: (301) 846-6919. E-mail: boyd@dtpax2.ncifcrf.gov.
[†] SAIC−Frederick.
[‡] Cancer Research Institute and Department of Chemistry and Biochemistry, Arizona State University, P.O. Box 872404, Tempe, AZ 85287-2404.
[§] Laboratory of Drug Discovery Research & Development, NCI.

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

proton	**1**	**2**	**3**	**4**	**5**	**6**	**7**
1a	2.02 m	2.02 m	2.02 m	2.03 m	2.09 m	∼2.0	2.24 m
1b	1.98 m	1.98 m	1.97 m	obsc	1.92 m	∼1.8	1.99 m
2	5.58 m	5.58 m	4.42 s	5.65 br s	5.45 t 4.3	4.43 br t 3.3	5.74 t 4.2
3	6.00 d 3.7	6.00 d 3.7	5.99 d 2.5	6.13 d 3.3	5.95 d 3.2	6.72 d 3.3	6.80 d 3.6
6	1.46 m	1.45 m	5.06 m	3.85 dd 3.4, 11.7	5.09 m	3.78 dd 8.0, 8.0	3.86 dd 7.3, 9.0
7a	1.73 m	1.72 m	1.84 m	1.74 m	1.90 m	∼1.6	1.64 m
7b	1.52 m	1.52 m	1.67 m	1.63 m	1.66 m	∼1.6	1.64 m
8	1.60 m	1.62 m	1.88 m	1.81 m	1.92 m	∼1.8	1.89 dd 7.2,6.9
10	2.34 dd 2.8, 12.4	2.33 dd 3.3, 12.3	2.44 dd 2.8, 13.0	2.47 dd 7.4, 9.1	2.37 dd 3.3, 13.7	∼2.0	2.07 m
11a	1.77 m	1.74 m	1.74 d 1.8	1.68 m	1.73 br d 17.7	2.18 dd 6.0, 16.4	2.20 m
11b	2.23 m	2.23 dd 8.2, 16.8	2.25 dd 8.0, 17.0	2.26 dd 8.2, 16.9	2.26 dd 8.4, 17.0	∼2.0	2.06 m
12	5.45 br s	5.44 dd 3.6, 6.9	5.42 br s	5.43 d 5.3	5.41 d 6.3	5.58 t 6.6	5.58 t 6.2
14	6.28 dd 10.1, 17.0	6.24 dd 10.7, 17.1	6.27 dd 10.7, 16.9	6.31 dd 10.7, 17.3	6.31 dd 16.9, 10.8	6.41 dd 10.6, 17.4	6.43 dd 11.0, 17.4
15a	5.08 d 17.2	5.08 d 17.2	5.06 d 17.2	5.09 d 17.3	5.09 d 16.9	5.10 d 17.4	5.13 d 17.4
15b	4.92 d 10.6	4.92 d 10.7	4.90 d 10.8	4.95 d 10.7	4.93 d 10.8	4.91 d 10.6	4.95 d 11.0
16	1.66 s 3H	1.66 s 3H	1.64 s 3H	1.67 s 3H	1.65 s 3H	1.77 s 3H	1.79 s 3H
17	0.88 d 6.7 3H	0.88 d 6.7 3H	0.90 d 6.5 3H	0.94 d 6.6 3H	0.90 d 6.8 3H	0.93 d 6.9 3H	0.95 d 6.9 3H
18	6.67 t 1.4	6.67 t 1.5	6.47 s	6.75 s	6.50 t 1.5		
19	6.37 s	6.37 s	6.73 s	6.56 s	6.76 s	4.81d 8.6, 3.87 d 8.6	4.87 d 8.7, 3.95 d 8.7
20	0.85 s 3H	0.85 s 3H	0.89 s 3H	0.85 s 3H	0.84 s 3H	0.98 s 3H	0.96 s 3H
3′	6.89 d 8.6	7.58 d 1.7	6.83 d 8.2	6.92 d 8.6	6.82 d 8.6		6.83 d 8.9
4′	7.97 d 8.7		7.97 d 7.9	7.97 d 8.6	7.98 d 8.6		7.87 d 8.7
6′	7.97 d 8.7	6.96 d 8.1	7.97 d 7.9	7.97 d 8.6	7.98 d 8.6		7.87 d 8.7
7′	6.89 d 8.6	7.65 dd 1.9, 8.1	6.83 d 8.2	6.92 d 8.6	6.82 d 8.6		6.83 d 8.9
18-OAc	2.06 s 3H	2.06 s 3H	2.01 s 3H	2.09 s 3H	2.05 s 3H		
19-OAc	1.94 s 3H	1.93 s 3H	1.93 s 3H	1.97 s 3H	1.99 s 3H		
2-OAc					2.12 s 3H		
MeO		3.96 s 3H					

Table 2. ^{13}C NMR of **1**−**7** (125 MHz, $CDCl_3$)

carbon	**1**	**2**	**3**	**4**	**5**	**6**	**7**
1	26.3	26.3	29.6	27.2	27.0	30.3	28.0
2	67.0	67.1	64.0	67.1	66.5	64.4	67.7
3	120.4	120.4	126.8	122.2	123.3	135.6	131.6
4	147.0	147.1	142.2	145.4	144.2	137.4	140.5
5	49.2	49.2	52.1	53.9	52.0	48.9	49.0
6	27.3	27.4	74.6	73.0	74.4	73.1	73.0
7	29.0	29.1	33.4	37.5	33.3	37.0	37.0
8	36.5	36.5	36.3	36.9	36.1	37.3	37.3
9	37.4	37.5	37.9	37.9	37.9	39.1	39.2
10	34.9	35.0	36.7	37.3	37.5	41.3	42.6
11	30.4	30.5	30.6	30.6	30.6	32.4	32.4
12	129.2	129.2	129.3	129.3	129.2	129.8	129.5
13	135.6	135.6	125.9	135.9	136.0	137.3	137.6
14	141.3	141.2	141.4	141.5	141.4	142.6	142.6
15	110.8	110.9	111.3	111.3	111.4	111.1	111.3
16	12.0	12.0	12.2	12.2	12.2	12.3	12.4
17	15.6	15.6	15.7	15.8	15.7	15.9	15.9
18	94.5	94.5	95.8	96.0	95.8	173.2	167.1
19	98.8	98.8	98.0	97.5	98.0	74.9	74.8
20	25.7	25.7	25.2	25.3	25.2	25.3	25.3
MeO		56.0					
1′	165.6	165.6	166.1	166.2	165.9		163.8
2′	123.0	122.7	121.5	122.4	121.6		122.0
3′	115.2	111.9	115.8	115.6	115.8		116.3
4′	131.9	146.3	132.5	132.2	132.5		132.9
5′	160.0	150.2	161.6	161.3	161.3		164.6
6′	131.9	114.0	132.5	132.2	132.5		132.9
7′	115.2	124.0	115.8	115.6	115.8		116.3
18-C=O	170.4	170.4	171.0	170.8	170.8		
19-C=O	169.8	169.7	170.4	170.1	170.3		
18-Me	21.2	21.2	21.5	21.5	21.5		
19-Me	21.4	21.4	22.0	21.9	22.1		
2-C=O					171.0		
2-Me					21.7		

Casearborin E (**5**, $[\delta]_D$ +100°) was characterized as having an extra acetate compared to diterpenes **3** and **4** ($C_{33}H_{40}O_{10}$). The position of the benzoate was determined as C-6 based on HMBC correlation of H-6 (δ 5.09) to the benzoate carbonyl at δ 165.9. The H-2 proton (δ 5.45) was correlated to an acetate carbonyl (δ 170.1), establishing this as the site of acetylation) (see Scheme 1).

In an attempt to elucidate the relative stereochemistry of the casearborins, the most abundant compound, casearborin D (**4**), was subjected to room-temperature hydrolysis with 15% ethanolic KOH for 2 h (see Scheme 2). In addition to *p*-hydroxybenzoic acid and its ethyl ester, two diterpenes were isolated and characterized. The major product $C_{20}H_{28}O_4$, **6**, $[\alpha]_D$ +48°, showed a single carbonyl resonance, indicating that the *p*-hydroxybenzoate and both acetates had been cleaved. Furthermore, the acetal functionality had been converted to a lactone by reduction of C-19 and oxidation of C-18. NOE studies of this somewhat simpler compound tentatively suggested relative stereochemistry of (rel)2*R*,5*R*,6*S*,8*R*,9*R*,10*S* for its parent. The second saponification product, **7**, $[\alpha]_D$ +48°, had a formula of $C_{27}H_{32}O_6$ by FABMS. NMR analysis indicated that it had not lost the *p*-hydroxybenzoate but had formed the lactone unit from the acetal in fashion similar to **6**.

The casearborins were relatively stable in $CDCl_3$ solution; however, degradation was observed on prolonged storage in this solvent, making extensive NOE studies difficult. NOE studies of casearborin E were carried out in benzene-d_6−MeOH-d_4 after assignment of NMR resonances in that solvent (Table 3). These studies were consistent with the relative stereochemistry provided by the following X-ray crystal structure analysis.

The relative and absolute stereochemical configurations of the series were conclusively established by X-ray crystallography. Casearborin E (**5**) recrystallized from benzene−methanol to yield colorless rosettes. Because diterpene **5** contained a relatively high percentage of oxygen, it was believed that this component should exhibit enough anomalous dispersion to allow the determination of absolute structure. As a consequence, a careful, ambient-temperature (24 °C) X-ray-data collection was conducted, in which Friedel-pair reflection intensities were recorded (whenever possible) immediately after each primary reflection measurement. The subsequent structure solution and refinement (R_1 = 0.0494) resulted in an unambiguous absolute structure determination, with the Flack absolute structure parameter $\chi^{16} = -0.05^{18}$ for the model of casearborin E shown in Figure 1. The Flack parameter value should be

Scheme 2. Hydrolysis of Casearborin D

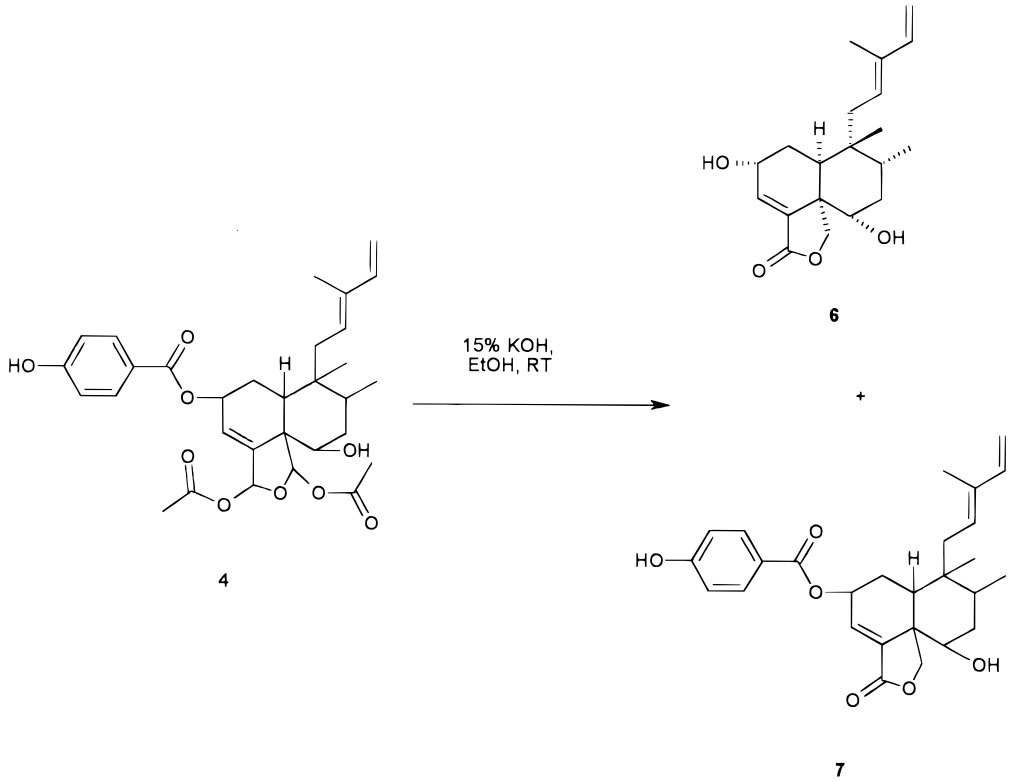

Table 3. NMR Shifts for Casearborin E (**5**), Benzene-d_6−MeOH-d_4 (8:1 v/v)

carbon	^{13}C shift (mult)	^{1}H shift (HSQC)	HMBC correlations
C-1	27.1 t	1.80 m	H-3, H-10
C-2	66.9 d	5.49 br t	H-1, H-10
C-3	123.2 d	6.03 dd 1.9, 4.0	H-16
C-4	144.6 s		H-2, H-3, H-6, H-19
C-5	52.4 s		H-3, H-7, H-10, H-19
C-6	74.7 d	5.26 dd 4.1, 12.1	H-7b, H-10, H-19
C-7	33.2 t	1.80 m, 1.30 m	
C-8	36.0 d	1.64 m	H-7, H-10, H-17, H-20
C-9	38.1 s		H-1, H-11, H-17, H-20
C-10	38.0 d	2.59 dd 9.4, 8.1	H-1, H-11, H-20
C-11	30.9 t	1.8 m, 2.21 dd 8.2, 17.2	
C-12	129.9 d	5.54 br d 6.5	H-11a, H-11b, H-16
C-13	136.2 s		H-15a, H-15b, H-16
C-14	141.8 d	6.25 dd 10.7, 17.3	H-12, H-15a,
C-15	111.1 t	4.96 d 10.8, 5.14 d 17.3	
C-16	12.1 q	1.73 s 3H	H-11, H-12, H-14
C-17	15.5 q	0.72 d 6.8 3H	
C-18	96.3 d	6.81 t	H-19
C-19	98.5 d	7.07 s	H-6, H-18
C-20′	25.1 q	0.77 s 3H	H-10
C-1′	166.4 s		H-6
C-2′	121.6 s		H-3′
C-3′,7′	116.4 d	7.22 d 8.7	H-3′, H-4′
C-4′,6′	133.0 d	8.54 d 9.0	H-4′
C-5′	163.5 s		H-4′
2-OAc Me	20.9 q	1.64 s 3H	
18-OAc Me	20.8 q	1.70 s 3H	
19-OAc Me	21.9 q	1.83 s 3H	
2-OAc C=O	170.7 s		2-OAc Me
18-OAc C=O	170.6 s		18-OAc Me
19-OAc C=O	169.8 s		H-19, 19-OAc Me

0.0 for the correct absolute structure and +1.0 for the inverted enantiomer. The tricyclic benzoate skeleton was consistent with the previously deduced unsaturation calculations. In addition to the four carbonyl groups, which could readily be deduced from bond-distance calculations (C1′−O1′, 1.203 Å; C21−O21, 1.173 Å; C23−O23, 1.184 Å; C25−O25, 1.206 Å), three additional olefinic bonds could also be assigned at C3−C4, 1.323 Å; C12−C13, 1.316 Å; and C14−C15, 1.319 Å. Finally, the absolute stereochemical assignments were made for all 8 chiral positions, as follows: 2*R*, 5*S*, 6*S*, 8*R*, 9*R*, 10*S*, 18*R*, 19*S*.

Evaluation of diterpenes **1**−**5** against the LOX and SF539 cell lines using a 2-day cytotoxicity assay, gave IC_{50} values in the range of 0.29 to 9.7 μM. In comparison, the casearins have been reported by others[1,3,7] to have IC_{50} values in the 0.17−29 μM range using the V-79 cell line.

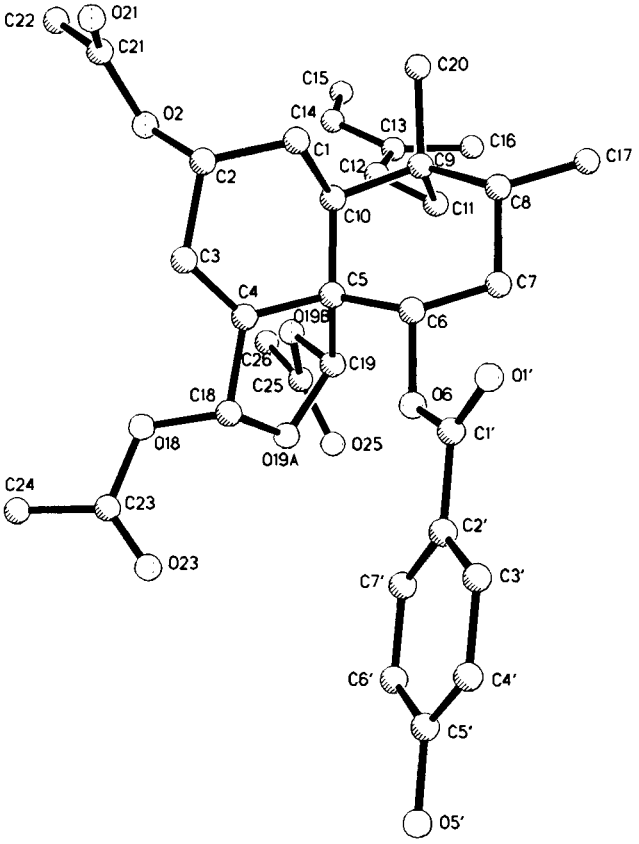

Figure 1. Computer-generated perspective (hydrogen atoms excluded) of casearborin E (**5**).

In summary, two distinct classes of cytotoxins have been isolated from *Casearia arborea*: cucurbitacins and clerodane diterpene esters. This is the first report of cucurbitacins from the Flacourtiaceae. Interestingly, these two classes of compounds do not show the same differential cytotoxicity pattern in the NCI 60-cell line cancer screen,[19] and the crude extract containing the mixture of the compounds presented a cytotoxicity profile that was a composite of the two types, that is, the extract did not compare[20,21] to cucurbitacin standards (data not shown).

The absolute stereochemistry obtained for casearborin E is identical to that obtained by Itokawa et al.[3] for casearin B. The casearborins are novel compounds that share common structural features with previously reported diterpenes from the Flacourtiaceae, with the structural novelty residing in the aromatic esters. In contrast to the cytotoxic casearins reported by Itokawa,[1,3,7] the casearborins lack C-7 oxygenation, but have esters at either C-2 or C-6.

Experimental Section

General Experimental Procedures. NMR spectra were obtained on a Varian VXR-500 spectrometer equipped with an inverse detection probe.

Plant Material. Roots of *C. arborea* (L.C.Rich.) Urban were collected by Douglas C. Daly in November 1988, near Jenaro Herrera, Peru (voucher in New York Botanical Garden, sample number Q65T1823).

Isolation. Ground, dry roots (308 g) were extracted with CH_2Cl_2−MeOH (1:1 v/v), and the solvent was evaporated to yield 15.9 g of extract. A 5.0-g aliquot was coated on 20 g of diol media (YMC) and eluted with 300 mL each of hexane, CH_2Cl_2, EtOAc, acetone, and MeOH in sequence. The CH_2Cl_2, EtOAc, and acetone eluates were cytotoxic. Fractionation of the CH_2Cl_2 eluate (916 mg) by permeation on Sephadex LH-20 in CH_2Cl_2−MeOH (1:1 v/v) was followed by cyano HPLC using a hexane-*i*PrOH gradient (20% *i*PrOH to 40% *i*PrOH over 40 min), which yielded casearborin A (12 mg), casearborin B (11 mg), and cucurbitacin B (14 mg). Fractionation of the EtOAc eluate in identical fashion resulted in isolation of casearborins C (44 mg), D (79 mg), and E (48 mg), along with a mixture of cucurbitacins, which included cucurbitacin B. Cucurbitacin B was identified by comparison of its NMR spectra with literature values.[13−15] The other cucurbitacins were not further purified and identified; however, ^{1}H NMR spectra suggested that they were acetylated cucurbitacin glycosides.

Casearborin A (1) NSC# 703075: clear glass; $[\alpha]_D$ +35° (*c* 0.59, MeOH); UV (MeOH) λ_{max} 231 nm (log ϵ 4.33), 259 nm (4.05); UV (MeOH + NaOH) λ_{max} 298 nm (log ϵ 4.26); ^{1}H NMR, see Table 1; ^{13}C NMR, see Table 2; FABMS (mb + CsI, positive mode) 671, 652, 546, 392, 287, HRFABMS (mb, CsI, positive mode) $[M + Cs]^+$ 671.1652 (calcd for $C_{31}H_{38}O_8Cs$ 671.1621).

Casearborin B (2) NSC# 703076: clear glass; $[\alpha]_D$ +30° (*c* 0.56, MeOH); UV (MeOH) λ_{max} 263 nm (log ϵ 3.95), 291 nm (3.74); UV (MeOH + NaOH) λ_{max} 232 nm (log ϵ 4.46), 315 nm (4.22); ^{1}H NMR, see Table 1; ^{13}C NMR, see Table 2; FABMS (mb + CsI, positive mode) 701, 652, 439, 546, 392, 287; HRFABMS (mb, CsI, positive mode) $[M + Cs]^+$ 701.1719 (calcd for $C_{32}H_{40}O_9Cs$ 701.1727).

Casearborin C (3) NSC# 703077: clear glass; $[\alpha]_D$ +35° (*c* 2.16, MeOH); UV (MeOH) λ_{max} 232 nm (log ϵ 4.20), 259 nm (4.00), UV (MeOH + NaOH) λ_{max} 300 nm (log ϵ 4.19); ^{1}H NMR, see Table 1; ^{13}C NMR, see Table 2; FABMS (mb + CsI, positive mode) 687, 652, 546, 418, 392, 297, 287; HRFABMS (mb, CsI, positive mode) $[M + Cs]^+$ 687.1581 (calcd for $C_{31}H_{38}O_9Cs$ 687.1570).

Casearborin D (4) NSC# 703078: clear glass; $[\alpha]_D$ +38° (*c* 0.98, MeOH); UV (MeOH) λ_{max} 232 nm (log ϵ 4.29), 259 nm (4.04)(MeOH + NaOH), λ_{max} 298 nm (log ϵ 4.25); ^{1}H NMR, see Table 1; ^{13}C NMR, see Table 2; FABMS (mb + CsI, positive mode) 687, 652, 546, 439, 392, 287; HRFABMS (mb, CsI, positive mode) $[M + Cs]^+$ 687.1588 (calcd for $C_{31}H_{38}O_9Cs$ 687.1570).

Casearborin E (5) NSC# 703079: clear glass; $[\alpha]_D$ +100° (*c* 1.21, MeOH); UV (MeOH) λ_{max} 231 nm (log ϵ 4.25), 259 nm (4.08); ^{1}H NMR, see Tables 1 and 3; ^{13}C NMR, see Tables 2 and 3; FABMS (mb + CsI, positive mode) 729, 652, 392, 287; HRFABMS (mb, CsI, positive mode) $[M + Cs]^+$ 729.1709 (calcd for $C_{33}H_{40}O_{10}Cs$ 729.1676).

Hydrolysis of Diterpene 4. A solution prepared from 20 mg of **4** in 15% KOH in EtOH (5 mL) was stirred at room temperature for 2 h. The reaction mixture was neutralized with 6 mL of 2 N HCl and extracted (x 3) with 10 mL of methyl *tert*-butyl ether. The organic phase was evaporated and purified by HPLC on a C_{18} column (21 mm × 250 mm, Rainin Dynamax), using a gradient of CH_3CN−H_2O from 60% organic to 100%. In order of elution were separated *p*-hydroxy-benzoic acid (1.9 mg, 38% yield); *p*-hydroxy-benzoic acid ethyl ester (3.5 mg, 59%); **6** (5.6 mg, 47%); and **7** (1.8 mg, 11%).

Product 6 (NSC#705905): clear glass; $[\alpha]_D$ +35° (*c* 0.5, MeOH); ^{1}H NMR, see Table 1; ^{13}C NMR, see Table 2; FABMS (mb, positive mode) 333, 309, 155, 135, 119; HRFABMS (glycerol, positive mode) $[M + H]^+$ 333.2074 (calcd for $C_{20}H_{29}O_4$ 333.2066).

Product 7 (NSC#705904): clear glass; $[\alpha]_D$ +35° (*c* 0.1, MeOH); ^{1}H NMR, see Table 1; ^{13}C NMR, see Table 2; FABMS (noba, positive mode) 460, 307, 289, 154, 137, 107 HRFABMS (glycerol, positive mode) $[M + H]^+$ 453.2276 (calcd for $C_{27}H_{33}O_6$ 453.2277).

X-ray Crystal Structure Determination. Casearborin E, **5**: A thick, plate-shaped X-ray sample (ca. 0.62 × 0.47 × 0.40 mm) was obtained by cleavage from a still larger, colorless rosette crystal grown from benzene−methanol solution. X-ray data collection was performed at 297 ± 1 K. Accurate cell dimensions were determined by least-squares fitting of 25 carefully centered reflections in the range of 35° < 0 < 40° using Cu Kα radiation.

Crystal Data: $C_{33}H_{40}O_{10}$, FW = 596.65, orthorhombic, $P2_12_12_1$, a = 10.494(2) Å, b = 17.693(4) Å, c = 17.745(4) Å, V = 3294.7(11) Å^3, Z = 4, ρ_c = 1.203 Mg/m^3, μ(Cu Kα) = 0.732 mm^{-1}, λ = 1.54180 Å.

All reflections corresponding to a complete octant ($0 \leq h \leq 12$, $0 \leq k \leq 20$, $0 \leq l \leq 20$) were collected over the range of $0 < 2\theta < 130°$ using the $\omega/2\theta$ scan technique. Friedel reflections were also collected whenever possible immediately after each reflection. Three intensity control reflections were also measured for every 60 min of X-ray exposure time and showed a maximum variation of −1.1% over the course of the collection. In all, 6255 reflections were collected. Subsequent statistical analysis of the complete reflection data set using the XPREP[17] program, verified the space group as $P2_12_12_1$. After Lorentz and polarization corrections, merging of equivalent reflections, and rejection of systematic absences, 5559 unique reflections [R(int) = 0.0267] remained, of which 5403 were considered observed [$I_o > 2\sigma(I_o)$] and were used in the subsequent structure determination and refinement. Linear and anisotropic decay corrections were applied to the intensity data as well as an empirical absorption correction based on a series of ψ-scans.[18] Structure determination was readily accomplished with the direct-methods program SHELXS.[19] All nonhydrogen atom coordinates were located in a routine run using default values in that program. The remaining H atom coordinates were calculated at optimal positions. All nonhydrogen atoms were refined anisotropically in a full-matrix least-squares refinement using SHELXL.[19] The H atoms were included, their Uiso thermal parameters fixed at 1.2 the Uiso of the atom to which they were attached and forced to ride that atom. The final standard residual R_1 value for **5** was 0.0494 for observed data and 0.0507 for all data. The goodness-of-fit on F^2 was 1.076. The corresponding Sheldrick R values were wR_2 of 0.1363 and 0.1379, respectively. A final difference Fourier map showed minimal residual electron density; the largest difference peak and hole being 0.235 and −0.220 e/Å^3, respectively. Final bond distances and angles were all within expected and acceptable limits. The absolute structure of casearborin E, as shown in Figure 1, could be assigned with certainty based upon the value of the Flack absolute structure parameter[16] (−0.05, esd 18). Consequently, using the numbering shown in Figure 1, the chiral centers of casearborin E can be assigned as follows: 2*R*, 5*S*, 6*S*, 8*R*, 9*R*, 10*S*, 18*R*, 19*S*.

Cytotoxicity Assay. Routine cytotoxicity determinations on fractions and pure compounds were performed using a 2-day assay with the SF539 and LOX IMVI cell lines, essentially as described.[22]

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Supporting Information Available: X-ray crystallographic tables of atomic coordinates, bond lengths and angles, and anisotropic thermal parameters for casearborin E (**5**). This material is available free of charge via the Internet at http://pubs.acs.org.

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(23) X-ray data for casearborin E (**5**) have been deposited in the Cambridge Crystallographic Data Centre. Copies of the data can be obtained free of charge on application to the Director, CCDC, 12 Union Road, Cambridge CB2 1EZ, UK (fax: +44-(0)1223−336033; or e-mail: deposit@ccde.cam.ac.uk).

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