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Cytotoxic Clerodane Diterpenoids from *Casearia obliqua*

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A crude bioactive EtOH extract of the twigs of *Casearia obliqua* afforded two new clerodane diterpenes, caseobliquins A (**1**) and B (**2**). Additionally, bioactivity-directed fractionation on a bioactive hexane extract of the leaves from this species led to the isolation of the known clerodane diterpenes *rel*-6*β*-hydroxyzuelanin-2*β*-benzoate and *rel*-2α-hydroxyzuelanin-6*β*-benzoate (**3** and **4**) as a mixture and 2*β*-hydroxyzuelanin-6*β*-cinnamate (**5**). The structures of the new clerodanes **1** and **2** were established on the basis of their 1D and 2D NMR spectroscopic data, and the new compound **1** and the known substance **5** had their absolute configurations determined by circular dichroism spectroscopy. The cytotoxicity of several of the compounds isolated was evaluated against a small panel of human tumor cell lines.

The plant genus *Casearia* (Salicaceae *sensu lato*) contains about 180 species distributed in the tropics[1] and is known as a rich source of clerodane-type diterpenes.[2−9] This genus is widely distributed in Brazil, where *Casearia sylvestris* is the most frequent species, occurring from the Amazon basin throughout the Cerrado to the Atlantic forest and the south of Brazil.[10]

In a continuing investigation on bioactive clerodane diterpenes from *Casearia* species, phytochemical studies were carried out for the first time on *Casearia obliqua* Spreng, a tree that grows commonly in the southern part of Brazil, where it is popularly known as "guaçatonga-vermelha".[11] Antimalarial, cytotoxic, immunomodulatory, and trypanocidal effects have been shown by several clerodane diterpenoids from plants of the genus *Casearia*.[2−9]

Results and Discussion

A bioactive ethanol extract from the twigs of *C. obliqua* was partitioned with hexane, Et_2O, EtOAc, and H_2O. The cytotoxic Et_2O fraction was chromatographed on Sephadex LH-20, followed by preparative RP-HPLC on a C_{18} column, affording the new clerodane diterpenes caseobliquin A (**1**) and caseobliquin B (**2**). The bioactive hexane extract from leaves of *C. obliqua* was chromatographed on silica gel, followed by preparative RP-HPLC on a C_{18} column, yielding three known compounds, *rel*-6*β*-hydroxyzuelanin-2*β*-benzoate (**3**) and *rel*-2α-hydroxyzuelanin-6*β*-benzoate (**4**) (which were obtained as a mixture) and 2*β*-hydroxyzuelanin-6*β*-cinnamate (**5**). The structures of compounds **1** and **2** were deduced on the basis of their spectroscopic data (UV, IR, CD, 1D and 2D NMR, MS) and by comparison with values reported in the literature. The cytotoxic activity of several of the diterpenes isolated was evaluated against a small panel of human cancer cell lines.

Caseobliquin A (**1**) was obtained as a yellow amorphous solid and showed a cationized ion at *m*/*z* 619.2513 $[M + Na]^+$ (calcd 619.2513), corresponding to a molecular formula of $C_{33}H_{40}O_{10}$, determined by HRTOFESIMS. The IR spectrum showed the presence of hydroxy group (3436 cm^{-1}) and carbonyl ester (1749, 1735, 1717 cm^{-1}) functionalities. The ^{13}C and DEPT NMR data (Table 2) revealed 33 signals attributable to nine quaternary carbons (including four carbonyls), 14 methines, four methylenes, and six methyls. The ^1H NMR spectrum (Table 1) showed the presence of two methyl groups at *δ* 0.97 (d, *J* = 7.0 Hz, H-17) and 0.92 (s, H-20), an oxymethine proton at *δ* 5.54 (m, H-2), two acetal-acyloxy methine protons at *δ* 6.39 (t, *J* = 1.5 Hz, H-18) and 6.67 (s, H-19), and a trisubstituted olefinic proton at *δ* 5.95 (sl, H-3), consistent with a casearin clerodane skeleton as isolated from *Casearia* species.[10,12−14] The six-carbon diene side chain (C-11−C-16) indicated the presence of a terminal unsaturated methylene group, supported by the resonances at *δ* 5.09 (d, *J* = 17.5 Hz, H-15) and

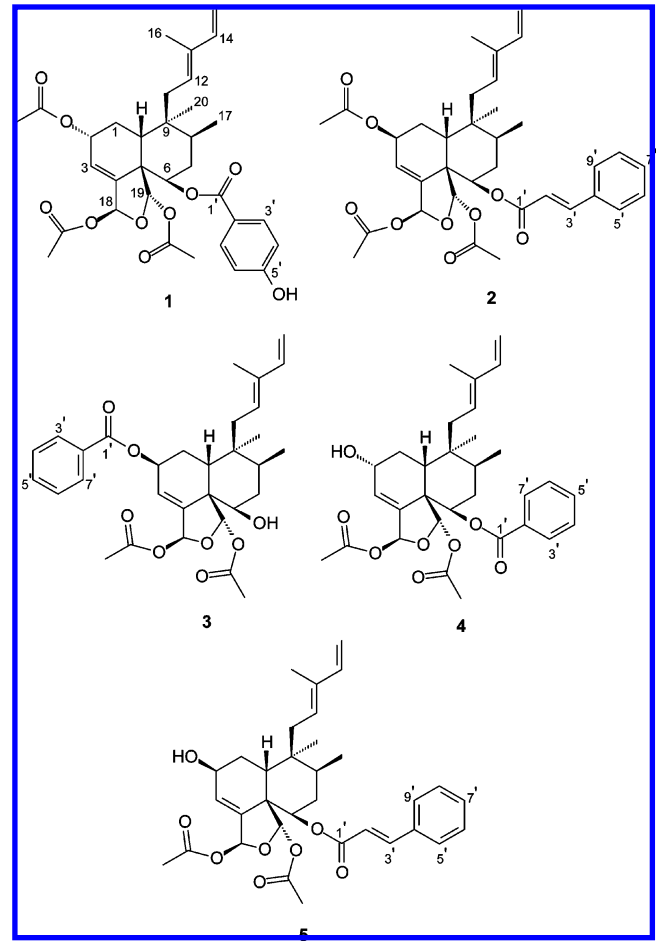

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Table 1. ^{1}H NMR Spectroscopic Data (500 MHz, CD_3OD) for Compounds **1** and **2**

	caseobliquin A (**1**)	caseobliquin B (**2**)		caseobliquin A (**1**)	caseobliquin B (**2**)
position	δ_H (J in Hz)	δ_H (J in Hz)	position	δ_H (J in Hz)	δ_H (J in Hz)
1	1.84 m	1.99 m	17	0.97 d (7.0)	0.97 d (7.0)
	1.89 m	2.09 m			
2	5.54 m	4.41 m	18	6.39 t (1.5)	6.49 t (1.5)
3	5.95 brs	6.06 d (4.0)	19	6.67 s	6.67 s
4			20	0.92 s	0.91 s
5			1′		
6	5.34 dd (4.5, 12.0)	5,11 dd (4.5, 12.0)	2′		6.48 d (16.0)
7	1.78 m	1.78 m	3′	6.83 d (9.0)	7.80 d (16.0)
	1.89 m	1.93 m			
8	2.04 m		4′	7.97 d (9.0)	
9			5′		7.63 m
10	2.58 dd (3.0, 14.0)	2.57 dd (3.5, 14.4)	6′	7.97 d (9.0)	7.40 m
11	1.80 m 2.29 dd (8.0, 17.0)	2.33 dd (8.8, 17.5)	7′	6.83 d (9.0)	7.40 m
12	5.54 m	5.58 m	8′		7.40 m
13			9′		7.63 m
14	6.43 dd (17.5, 11.0)	6.41 dd (10.5, 17.5)	O_2CCH_3-2	2.07 s	1.90 s
15	4.93 d (11.0)	4.92 d (10.5)	O_2CCH_3-18	2.03 s	2.05 s
	5.09 d (17.5)	5.10 d (17.5)		1.95 s	1.94 s
16	1.67 s	1.67 s	O_2CCH_3-19		

Table 2. ^{13}C NMR Spectroscopic Data (125 MHz, δ_C, mult.) for Compounds **1**−**4**

position	**1**[a]	**1**[b]	**2**[b]	**3**[a]	**4**[a]
1	29.6	27.2, CH_2	30.2	37.4, CH_2	29.4, CH_2
2	70.5	72.2, CH	64.4	67.1, CH	63.8, CH
3	125.3	126.5, CH	128.1	121.7, CH	126.5, CH
4	143.1	144.5, qC	142.5	145.6, qC	142.3, qC
5	52.6	53.3, qC	57.8	53.7, qC	51.9, qC
6	75.0	76.5, CH	75.5	72.8, CH	74.7, CH
7	34.9	34.5, CH_2	34.1	27.0, CH_2	33.1, CH_2
8	38.1	37.3, CH	37.6	37.1, CH	36.4, CH
9	39.2	39.5, qC	37.6	37.6, qC	37.7, qC
10	44.9	43.3, CH	38.0	36.7, CH	36.1, CH
11	31.0	31.1, CH_2	31.0	30.4, CH_2	30.4, CH_2
12	128.8	130.4, CH	130.8	128.9, CH	129.0, CH
13	137.0	136.9, CH	136.3	135.7, qC	135.7, qC
14	141.0	142.7, CH	142.5	141.2, CH	141.2, CH
15	111.3	111.1, CH_2	111.0	111.0, CH_2	111.0, CH_2
16	13.8	12.0, CH_3	12.2	11.9, CH_3	11.9, CH_3
17	15.4	15.8, CH_3	15.7	15.5, CH_3	15.4, CH_3
18	94.9	96.0, CH	94.0	95.6, CH	95.4, CH
19	97.2	98.4, CH	98.2	97.0, CH	97.7, CH
20	24.8	25.3, CH_3	25.4	25.0, CH_3	25.0, CH_3
1′	165.1	167.1, qC	167.7	165.8, qC	166.0, qC
2′	121.9	121.8, qC	118.2	130.5, qC	130.5, qC
3′	115.6	116.3, CH	147.1	129.6, CH	129.8, CH
4′	132.3	133.1, CH	135.0	128.4, CH	128.6, CH
5′	160.0	163.9, qC	129.1	133.1, CH	133.4, CH
6′	132.3	133.1, CH	130.0	128.4, CH	128.6, CH
7′	115.6	116.3, CH	130.5	129.6, CH	129.8, CH
8′			130.0		
9′			129.1		
O_2CCH_3-2	170.8	172.5, qC	179.0		
	21.0	21.0, CH_3	23.1		
O_2CCH_3-18	170.0	171.8, qC	171.9	170.0, qC	170.1, qC
	21.0	20.9, CH_3	21.0	21.2, CH_3	21.1, CH_3
O_2CCH_3-19	169.8	171.1, qC	171.1	169.4, qC	169.6, qC
	21.9	21.9, CH_3	21.8	21.5, CH_3	21.7, CH_3

[a] $CDCl_3$. [b] CD_3OD; multiplicities were determined by analyses of the DEPT 90° and 135°.

4.93 (d, J = 11.0 Hz, H-15), which were coupled to a methine proton signal at δ 6.43 (dd, J = 11.0, 17.5 Hz, H-14). The other methine proton signal was observed as a multiplet at δ 5.54 (H-12). This side chain was also confirmed by gCOSY correlations of H-11 to H-12 and H-14 to H-15. The gHMBC spectrum exhibited correlations of H-11 to C-9, C-10, C-12, and C-13; H-14 to C-12 and C-16; and H-15 to C-13, revealing that this side chain is located at the C-9 position. The configuration of the C-12,13 double bond was found to be *E* on the basis of the NOESY correlations between H-12 and H-14 (Figure 1). The *p*-hydroxybenzoate group was

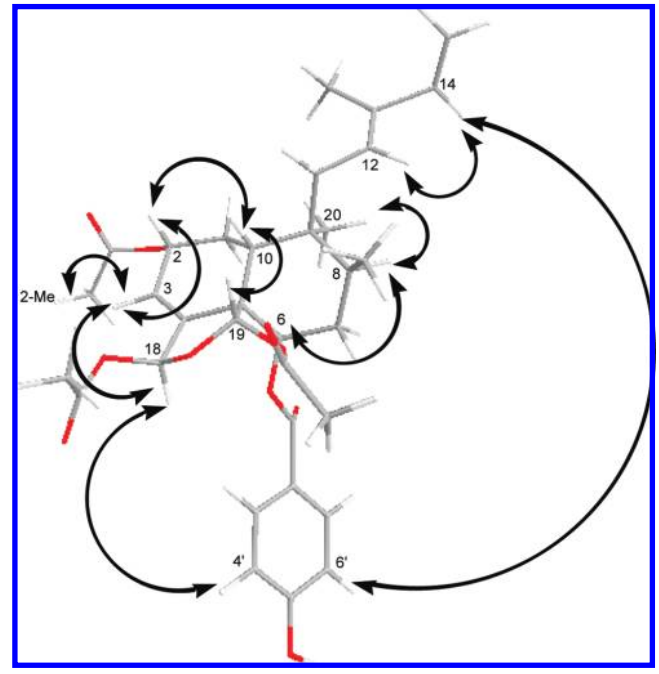

Figure 1. Selected NOE enhancements observed for **1**.

deduced from the resonances at δ 6.83 (2H, d, J = 9.0 Hz, H-3′ and H-7′) and 7.97 (2H, d, J = 9.0 Hz, H-4′ and H-6′). The gHMBC spectrum showed a correlation of the oxymethine proton, H-6, to the carbonyl carbon (δ 167.1, C-1′) of the *p*-hydroxybenzoate group, which indicated that the *p*-hydroxybenzoate is located at C-6 (δ 76.5). The H-18 (δ 6.39) and H-19 (δ 6.67) protons were correlated to carbonyl groups (δ 171.8 and 171.1), respectively, establishing these as sites of acetylation. By elimination, the third acetate group (δ 172.5 and 21.0) could be located at C-2.

On comparing compound **1** with other clerodane diterpenoids, this compound showed differences in the configurations of the carbons C-5, C-6, C-8, C-9, C-10, and C-18, as indicated by chemical shift data for compound **1** in relation to literature values.[15] The relative configuration of all eight chiral centers was assigned on the basis of their coupling constants and by NOESY correlations (Table 1 and Figure 2). A 1,3-diaxial NOESY correlation was observed for H-6 and H-8, which indicated that the C-8 methyl group (C-17) is in an equatorial orientation. The *trans* configuration between the C-17 and C-20 methyl groups was proposed because of the chemical shift at δ 25.3 for C-20, which also supported a

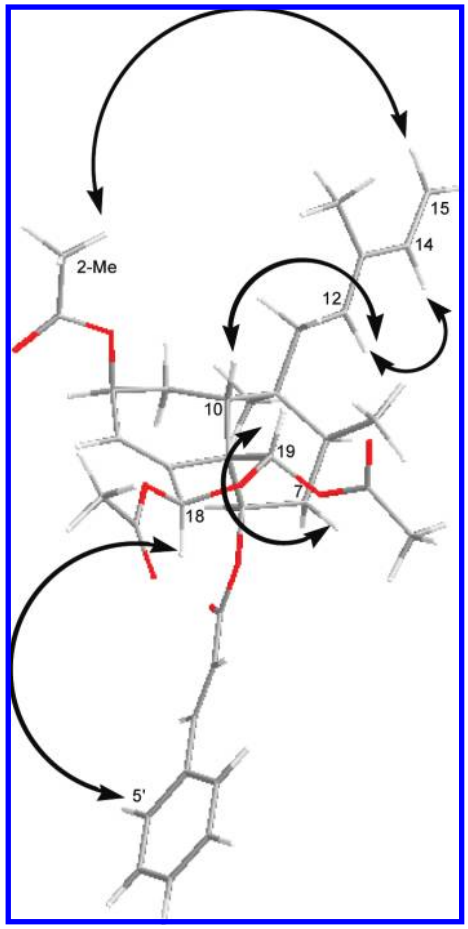

Figure 2. Selected NOE enhancements observed for **2**.

cis-A/B ring junction.[10,15,17] The *J* values of 3.0 and 14.0 Hz for the coupling of H-10 to H-1 protons revealed that H-10 is in an axial position, while the NOESY correlations between H-2/H_β-10 showed that H-2 is β-oriented. The chemical shift at δ_C 70.5 in $CDCl_3$ for the C-2 oxymethine carbon was consistent with the C-2 substituent having an α-orientation, as reported for related compounds.[1,18] The acetal proton, H-19, was also assigned with a β-orientation on the basis of the NOESY correlation of H-19 to H-10, and to the other acetal proton, H-18, an α-orientation was assigned because of the NOE enhancement observed for H-18 with the protons H-4′/H-6′ from the *p*-hydroxybenzoate moiety (δ 7.97). This could occur only if H-18 is on the opposite face of the diacetal furan ring in relation to H-19 (Figure 1).[16]

X-ray crystallographic data were used earlier to assign the absolute configuration for a series of clerodane diterpenes similar to **1**.[15] The absolute stereochemistry of **1** was determined by circular dichroism spectroscopy, in which a positive Cotton effect was exhibited at 241 nm. The C-9 carbon of the clerodane diterpenes occurs with a *R* orientation when a negative Cotton curve is evident,[12] so **1** was assigned with an *S* orientation at C-9. Following this argument, it was possible to determine the complete absolute configuration of compound **1**, as follows: 2*R*, 5*R*, 6*R*, 8*S*, 9*S*, 10*R*, 18*S*, 19*S*. This new compound, named caseobliquin A, is a diastereoisomer of casearborin E.[15]

Caseobliquin B (**2**) was isolated as a yellow amorphous solid, with a molecular formula of $C_{35}H_{41}O_9$ as determined by HRTOFES-IMS (observed *m*/*z* 629.2747 $[M + Na]^+$). The complete ^1H and ^{13}C NMR data for **2** are shown in Tables 1 and 2. The entire data set was similar to that for **1** except for the modification from the substituent at C-6, with that in **1** a *p*-hydroxybenzoate moiety and that for **2** a cinnamoyl moiety.

Table 3. Cytotoxic Activity of Compounds Isolated from *Casearia obliqua* against Human Tumor Cell Lines[a]

	cell line			
compound	HL-60 (leukemia)	HCT-8 (colon)	MDA/MB-435 (melanoma)	SF-295 (glioblastoma)
1	>5	>5	>5	>5
3 and **4**[b]	1.0	0.13	0.33	0.14
5	>5	>5	>5	>5
doxorubicin	0.07	1.5	0.12	0.75

[a] Data are presented as IC_{50} values given in μM obtained by nonlinear regression. [b] Tested as a mixture.

gCOSY, gHMQC, and gHMBC NMR experiments performed supported the same connectivities for **2** as in **1**. The cinnamoyl group was deduced from the resonances at δ 6.48 (1H, d, $J = 16.0$ Hz, H-2′), 7.80 (1H, d, $J = 16.0$ Hz, H-3′), 7.40 (3H, m, H-6′, H-7′ and H-8′), and 7.63 (2H, m, H-5′ and H-9′). The gHMBC spectrum showed a correlation of the oxymethine proton, H-19 (δ 6.67), linked to the acetate carbonyl at δ 171.1. The ester groups were located by comparison with appropriate values reported in the literature,[16] the cinnamoyl was placed at C-6 (δ 75.5), and the acetate groups were placed at carbons C-2 (δ 64.4) and C-18 (δ 94.0). These assignments were corroborated by NOE enhancements between H-12 and the methyl group (δ 1.90) of the acetate moiety at position 2 and between H-15 and the methyl group (δ 2.05) of the acetate moiety at position 18 (Figure 2).

Khan et al.[16] used NOESY data to assign the relative configuration for a series of clerodane diterpenoids that have a two-dimensional structure identical to that of **2**, differing only in the stereochemistry of C-2. The chemical shift at δ 64.4 for the C-2 oxymethine carbon was consistent with the C-2 substituent having a β-orientation, as reported for related compounds,[9,17] whereas from the literature this value is δ 70.2 (α-orientation).[16] On the basis of the evidence above, compound **2** could be determined to have the relative configuration 2*S*, 5*R*, 6*R*, 8*S*, 9*S*, 10*R*, 18*S*, 19*S*. This is a diastereoisomer of *rel*-2α-acetoxyzuelanin-6β-cinnamate[16] and has been named caseobliquin B.

The three known clerodane diterpenes (**3**−**5**) were identified on the basis of their spectroscopy data (UV, IR, and NMR) and comparison with appropriate values reported in the literature. The ^{13}C NMR data of compounds **3** and **4** and the absolute configuration of **5** are included in this paper, in order to complement the lack of this information in previous reports of these compounds.[16,19]

Four compounds were evaluated for their cytotoxic activity, using the MTT assay, corroborating the well-established cytotoxicity of this compound class. The cytotoxic activities of compound **1**, a mixture **3** and **4**, and **5** were evaluated in vitro against HL-60 (human myeloblastic leukemia), HCT-8 (human colon carcinoma), MDA/MB-435 (human melanoma), and SF-295 (human glioblastoma) cells. Inhibitory concentration (IC_{50}) values were determined, and the results are shown in Table 3. The mixture of compounds **3** and **4** showed significant cytotoxicity against four cancer cell lines (HL-60, HCT-8, MDA/MB-435, and SF-295), with IC_{50} values ranging from 0.13 to 1.0 μM, close to data obtained for the positive control, doxorubicin (Table 3).

Experimental Section

General Experimental Procedures. Optical rotations were measured on a Perkin-Elmer 341 LC polarimeter. UV spectra were measured on a Hewlett-Packard 8453 A spectrometer. The circular dichroism spectra were obtained on a JASCO PU-2089 HPLC system configurated with UV−vis (MD-2010) and circular dichroism (CD-2095) detectors. IR spectra were measured on a Nicolet Impact 400 spectrometer using KBr disks. The 1D (^1H, ^{13}C, DEPT 90, NOESY, and DEPT 135) and 2D ($^1H-^1H$ gCOSY, gHMQC, gHMBC, and NOESY) NMR experiments were recorded on a Varian INOVA 500 spectrometer (11.7 T) at 500 MHz (^1H) and 125 MHz (^{13}C) with a pulse field gradient. The samples were dissolved in $CDCl_3$ or CD_3OD, using residual $CHCl_3$ or

CH_3OH as internal standard. Positive-ion HRMS were recorded on a UltrOTOFq (Bruker Daltonics) ESI-qTOF mass spectrometer using an internal standard. Analytical HPLC was performed on a Varian Pro Star 230 with a UV−vis detector (model 330) using a Phenomenex C_{18} column (250 mm × 4.6 mm, 5 μm), in order to develop the reversed-phase conditions, which were scaled up for preparative work. Preparative HPLC was performed on a Varian Prep-Star 400 system using a Phenomenex C_{18} (250 mm × 21.2 mm, 10 μm) preparative column. Column chromatography was performed over silica gel 230−400 mesh (Merck) or on Sephadex LH-20 (Pharmacia Biotech). TLC was performed using Merck silica gel 60 (>230 mesh) and precoated silica gel 60 PF_{254} plates. Spots on TLC plates were visualized under UV light and by spraying with anisaldehyde-H_2SO_4 reagent followed by heating at 120 °C.

Plant Material. *Casearia obliqua* leaves and twigs were collected in May 2007 at Campinas municipality, São Paulo, Brazil. The plant material was identified by one of the authors (R.B.T.). A voucher specimen (IAC46529) has been deposited in the herbarium of the Instituto Agronômico de Campinas (São Paulo State, Brazil). Recent studies on the phylogeny of the Flacourtiaceae, based on plastid rbcL DNA sequences, have shown that the family is polyphyletic and recognized by two major clades, one with close affinities to the Salicaceae and the other with the Achariaceae. Among those genera of Flacourtiaceae now assigned to Salicaceae *sensu lato* is the genus *Casearia*.[20]

Extraction and Isolation. The twigs (534.0 g) of *C. obliqua* were extracted exhaustively by maceration with hexane, ethanol, and water (3.0 L × 3), successively, at room temperature. The ethanol extract was concentrated under reduced pressure (≤40 °C) to yield 9.3 g of a crude extract. This ethanol extract was then diluted with MeOH−H_2O (3:1) and partitioned successively with hexane (1.0 L × 3), Et_2O (1.0 L × 3), and EtOAc (1.0 L × 3). After removal of the solvent, each extract yielded 2.9, 1.0, 1.1, and 4.0 g of residue, respectively. Part of the cytotoxic Et_2O fraction (1.0 g) was subjected to gel permeation chromatography on Sephadex LH-20 (78.0 × 3.0 cm), eluted with methanol, to afford 95 fractions, which were combined on the basis of TLC visualized with anisaldehyde-H_2SO_4. Cytotoxic fraction 50 (54.0 mg) was submitted to preparative RP-HPLC on a C_{18} column (Phenomenex Luna 250 mm × 21.2 mm, 10 μm), eluted with MeOH−H_2O−AcOH (85:15:0.01) at a flow rate of 15 mL/min and UV detection at 254 nm, affording the clerodane diterpenes **1** (6.0 mg) and **2** (1.3 mg).

The leaves (94.7 g) of *C. obliqua* were extracted exhaustively by maceration successively with hexane, ethanol, and water (3.0 L × 3) at room temperature. The hexane extract was concentrated under reduced pressure (≤40 °C) to yield 1.5 g of a crude extract. This extract exhibited cytotoxicity against four human cancer cell lines and was subjected to column chromatography over silica gel (15.0 × 4.5 cm), eluted in a gradient step mode from hexane to EtOAc, yielding 15 fractions. Cytotoxic fraction 8 (114.0 mg) was subjected to preparative RP-HPLC on a C_{18} column, eluted with MeOH−H_2O (78:22) at a flow rate 14 mL/min and UV detection at 254 nm, affording the clerodane diterpenes **5** (6.2 mg) and a mixture of **3** and **4** (1.7:1.0; 10.0 mg).

Caseobliquin A (1): yellow, amorphous solid; $[\alpha]^{25}_D$ +52 (*c* 1.0, MeOH); UV ($CHCl_3$) λ_{max} (log ε) 241 (3.48), 280 (3.50) nm; CD (*c* 0.03, MeOH) $\Delta\varepsilon$ (nm) +4.5 (241), +2.4 (280); IR (KBr) ν_{max} 3436, 2950, 1735, 1250 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 1 and 2; HRTOFESIMS *m/z* 619.2513 $[M + Na]^+$ (calcd for $C_{33}H_{40}O_{10}Na$, 619.2513).

Caseobliquin B (2): yellow, amorphous solid; $[\alpha]^{29}_D$ +51 (*c* 0.5, $CHCl_3$); UV ($CHCl_3$) λ_{max} (log ε) 243 (4.58) nm; ^1H and ^{13}C NMR data, see Tables 1 and 2; HRTOFESIMS *m/z* 629.2747 $[M + Na]^+$ (calcd for $C_{35}H_{42}O_9Na$, 629.2721).

***rel*-6*β*-Hydroxyzuelanin-2*β*-benzoate and *rel*-2α-Hydroxyzuelanin-6*β*-benzoate (3 + 4, 1.7:1 mixture):** yellow, amorphous solid; $[\alpha]^{30}_D$ +70 (*c* 1.0, $CHCl_3$); UV ($CHCl_3$) λ_{max} (log ε) 243 (4.12) nm; IR (KBr) ν_{max} 3448, 2925, 1756, 1714, 1272, 715 cm^{-1}; ^{13}C NMR data, see Table 2; HRTOFESIMS *m/z* 577.2199 $[M + K]^+$ (calcd for $C_{31}H_{38}O_8K$, 577.2198).

2*β*-Hydroxyzuelanin-6*β*-cinnamate (5): yellow, amorphous solid; $[\alpha]^{30}_D$ +66 (*c* 1.0, $CHCl_3$); UV ($CHCl_3$) λ_{max} (log ε) 239 (3.80), 282 (4.00) nm; CD (*c* 0.03, MeOH) $\Delta\varepsilon$ (nm) +20.0 (239), +9.6 (282); IR (KBr) ν_{max} 3440, 1712, 1637 cm^{-1}; ^{13}C NMR data, see Table 2; HRTOFESIMS *m/z* 587.2616 $[M + Na]^+$ (calcd for $C_{33}H_{40}O_8Na$, 587.2615).

Cytotoxicity Assay. The cytotoxicity of the clerodane diterpenes (**1**, **3** and **4**, **5**) was tested against four tumor cell lines: SF-295 (human glioblastoma), HCT-8 (human colon carcinoma), HL-60 (human leukemia), and MDA/MB-435 (human melanoma). Cells were maintained in RPMI 1640 medium, supplemented with 10% fetal bovine serum, 2 mM glutamine, 100 mg/mL streptomycin, and 100 U/mL penicillin, at 37 °C with 5% CO_2. Cells were seeded in 96-well plates (10^5 cells/well for adherent cells or 0.3×10^6 cells/well for suspended cells in 100 μL of medium). After 24 h, the compounds (0.39−25 μg/mL) dissolved in DMSO were added to each well. Doxorubicin (0.01−0.58 μg/mL) was used as positive control. The proliferation of tumor cells was quantified by the ability of living cells to reduce the yellow dye 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2*H*-tetrazolium bromide (MTT) to a blue formazan product. At the end of 69 h incubation, the medium was replaced by fresh medium containing 0.5 mg/mL of MTT. Three hours later, the formazan product was dissolved in DMSO, and absorbance was measured using a multiplate reader at 595 nm (DTX-880, Beckman Coulter). The IC_{50} values were obtained by nonlinear regression using the Graphpad program (Institutive Software for Science, San Diego, CA).[21]

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Supporting Information Available: 1D and 2D NMR data for compounds **1**−**4** (Figures S1−S30). This information is available free of charge via the Internet at http://pubs.acs.org.

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