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Full Papers

Novel Bioactive Clerodane Diterpenoids from the Leaves and Twigs of *Casearia sylvestris*

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Fractionation of a methanol extract of the leaves and twigs of *Casearia sylvestris*, as directed by activity against KB cell cytotoxicity, led to the isolation of three novel clerodane diterpenoids, casearvestrins A−C (**1**−**3**). The structures of **1**−**3** were deduced from one- and two-dimensional NMR experiments, including relative stereochemical assignments based on ROESY correlations and COSY coupling constants. All three compounds displayed promising bioactivity, both in cytotoxicity assays against a panel of tumor cell lines and in antifungal assays via the growth inhibition of *Aspergillus niger* in a disk diffusion assay.

In our collaborative program to search for novel antineoplastic agents from the plant kingdom, a methanol extract of the leaves and twigs and *Casearia sylvestris* Sw. (Flacourtiaceae) showed promising activity, as evaluated against the KB cytotoxicity assay. The leaves of this plant were examined previously by Itokawa and colleagues,[1−4] who characterized 18 novel clerodane diterpenoids, which they termed alphabetically casearins A through R. de Carvalho et al. extended the set to 20 novel clerodane diterpenoids from the leaves with the description of casearins S and T,[5] and similar compounds have been characterized, both from other species of *Casearia*[6−11] and from other genera of the Flacourtiaceae.[12−14] In two cases, as reported by Etse et al.[15] from *Monodora brevipes* (Annonaceae) and by our group[16] from *Licania intrapetiolaris* (Chrysobalanaceae), a few members of this class have been discovered from species outside of the Flacourtiaceae. Herein, the isolation and structure elucidation of three new clerodane diterpenoids, casearvestrins A−C (**1**−**3**), respectively, are described. Although the diterpene core of these molecules is similar to the one observed in the score of compounds described earlier from the leaf, a new trivial name was assigned because of differences that were found in both the stereochemistry and optical rotation data between these two subclasses.[17] Using bioactivity-directed fractionation procedures, none of the previously described clerodane diterpenoids were isolated; however, **1**−**3** were active in cytotoxicity assays and were inhibitors of the growth of *Aspergillus niger* in an antifungal assay.

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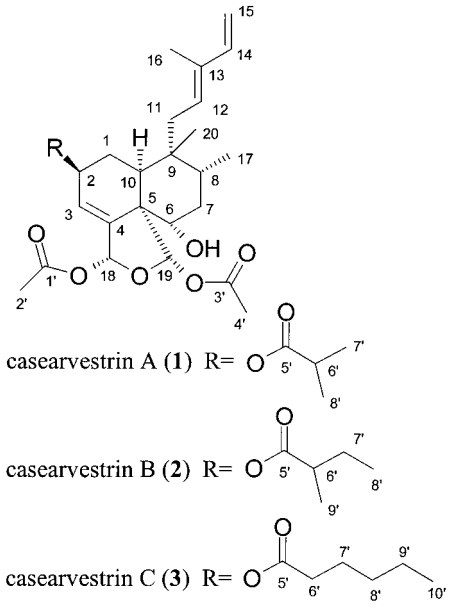
Table 1. ^{1}H, ^{13}C, and DEPT-135 NMR Data for Compounds **1**−**3**

	casearvestrin A (**1**)			casearvestrin B (**2**)			casearvestrin C (**3**)		
position	δ_C	DEPT	δ_H, mult (*J* in Hz)	δ_C	DEPT	δ_H, mult (*J* in Hz)	δ_C	DEPT	δ_H, mult (*J* in Hz)
1	26.1	CH_2	2.13 m/1.70[a] m	26.1	CH_2	2.15 m/1.70[a] m	26.1	CH_2	2.13 m/1.67[a] m
2	70.4	CH	5.60 m	70.4	CH	5.62 m	70.5	CH	5.62 m
3	124.1	CH	5.91 brs	124.2	CH	5.90 brs	124.1	CH	5.91 brs
4	144.3	C		144.2	C		144.3	C	
5	53.5	C		53.5	C		53.4	C	
6	74.0	CH	4.01 dd (12.2, 3.6)	74.0	CH	4.01 dd (12.2, 3.6)	74.0	CH	4.00 dd (12.2, 3.7)
7	37.6	CH_2	1.79[a] m/1.61[a] m	37.6	CH_2	1.75[a] m/1.60[a] m	37.6	CH_2	1.76[a] m/1.63[a] m
8	36.8	CH	1.86[a] m	36.8	CH	1.85 m[a]	36.8	CH	1.85 m[a]
9	38.5	C		38.5	C		38.5	C	
10	41.3	CH	2.41 dd (13.9, 2.3)	41.3	CH	2.42 dd (14.2, 2.4)	41.3	CH	2.41 dd (14.0, 2.6)
11	28.7	CH_2	2.36 dd (16.3, 9.3)/1.58[a] m	28.7	CH_2	2.36 m/1.56[a] m	28.7	CH_2	2.36 m/1.58[a] m
12	126.3	CH	5.27 d (7.7)	126.3	CH	5.27 d (7.2)	126.3	CH	5.26 d (7.4)
13	133.7	C		133.8	C		133.7	C	
14	133.3	CH	6.62 dd (17.2, 10.8)	133.3	CH	6.62 dd (17.3, 10.8)	133.3	CH	6.62 dd (17.3, 10.8)
15	114.4	CH_2	5.20 d (17.2)/5.11 d(10.8)	114.4	CH_2	5.20 d (17.2)/5.11 d (10.8)	114.4	CH_2	5.20 d (17.2)/5.11 d (10.8)
16	20.3	CH_3	1.82 s	20.3	CH_3	1.81 s	20.3	CH_3	1.81 s
17	15.6	CH_3	0.93 d (6.8)	15.6	CH_3	0.93 d (7.2)	15.6	CH_3	0.91 m
18	95.2	CH	6.71 s	95.2	CH	6.71 s	95.2	CH	6.71 s
19	96.9	CH	6.47 s	96.9	CH	6.47 s	96.9	CH	6.47 s
20	25.0	CH_3	0.82 s	25.0	CH_3	0.82 s	25.0	CH_3	0.81 s
1′	170.1	C		170.0	C		170.0	C	
2′	21.3[b]	CH_3	2.10 s	21.3[b]	CH_3	2.11 s	21.3[b]	CH_3	2.10 s
3′	169.2	C		169.2	C		169.2	C	
4′	21.2[b]	CH_3	2.02[a] s	21.2[b]	CH_3	2.01 s	21.2[b]	CH_3	2.01 s
5′	176.9	C		176.5	C		173.6	C	
6′	34.1	CH	2.57 septet (7.0)	41.1	CH	2.37 m	34.4	CH_2	2.32 t (7.5)
7′	18.9	CH_3	1.19 d (7.0)	26.8	CH_2	1.70 m/1.50 septet (7.0)	24.9	CH_2	1.61 m
8′	18.9	CH_3	1.19 d (7.0)	11.6	CH_3	0.93 m	31.3	CH_2	1.33 m[a]
9′				16.5	CH_3	1.16 d (7.0)	22.2	CH_2	1.33 m[a]
10′							13.9	CH_3	0.91 m
OH			not observed			not observed			not observed

[a] Assigned on the basis of HMQC data. [b]Interchangeable.



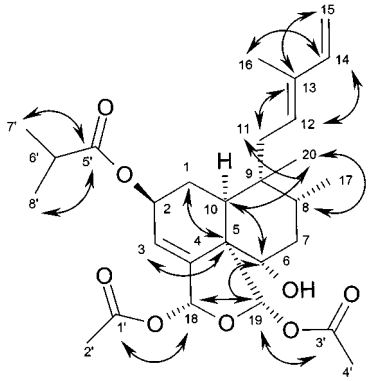


Figure 1. Selected HMBC correlations for casearvestrin A (**1**).

Results and Discussion

Casearvestrin A (**1**) was isolated as an amorphous solid. Although it was UV active (λ_{max} 234 nm), an absorbance near 254 nm, indicative of a benzene chromophore as noted in our earlier study with similar compounds,[16] was not observed. Diagnostic peaks for carbonyl moieties were detected in the IR spectrum at 1748, 1731, and 1714 cm^{-1}. From the low-resolution ESIMS data, the molecular ion was observed via a Na^+ adduct at *m*/*z* 527 $[M + Na]^+$, and two fragments were noted that accounted for the sequential loss of 60 amu at *m*/*z* 467 $[M + Na - AcOH]^+$ and *m*/*z* 407 $[M + Na - 2(AcOH)]^+$. The exact mass of the Na^+ adduct of the molecular ion of **1**, determined by HRFABMS, corresponded to a molecular formula of $C_{28}H_{40}O_8Na$ and an index of unsaturation of 9.

The complete ^{1}H, ^{13}C, and DEPT-135 NMR data for **1** are shown in Table 1, and the major HMBC correlations are illustrated in Figure 1. Three ester moieties were suggested from the IR data (vide supra) and by analysis of the ^{13}C and DEPT-135 NMR data. Two of these were assigned as acetate esters (C-1′ and C-2′ at δ_C 170.05 and δ_H/δ_C 2.10/21.34, respectively, and C-3′ and C-4′ at δ_C 169.21 and δ_H/δ_C 2.02/21.18, respectively). From the COSY spectrum, a single-proton septet at δ_H 2.57 (H-6′) was coupled to a six-proton doublet at δ_H 1.19 (H_3-7′ and H_3-8′), and together with HMBC correlations from this doublet to δ_C 176.89 (C-5′), the third ester was construed as being an isobutyrate unit. A six-carbon, diene side chain was noted also (C-11 to C-16). One double bond was terminal based on classic *cis* and *trans* coupling,[18] of 10.8 and 17.2 Hz, respectively, from the methylene signals at δ_H 5.11, 5.20 (H_2-15) to the methine resonance at δ_H 6.62

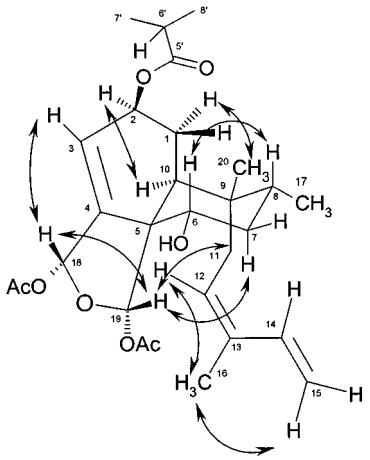


Figure 2. Selected ROESY correlations for casearvestrin A (**1**).

(H-14). The other double bond was trisubstituted, and the methine δ_H 5.27 (H-12) had a broad doublet splitting pattern that was correlated in the COSY spectrum to the methylene at δ_H 2.36, 1.58 (H_2-11). HMBC correlations from H-12 to C-14, H-14 to C-12 and C-16, H_2-15 to C-13, and H_2-11 to C-13 finalized the assembly of this side chain. The configuration of the C-12 to C-13 double bond was found to be *Z* based both on the chemical shift of the C-16 resonance[18] and from the ROESY correlations from H-12 to H_3-16 and from H_3-16 to H-15$_{trans}$ (δ_H 5.20) (Figure 2). In the casearvestrin and casearin series of clerodane diterpenoids, all of which have been isolated from *C. sylvestris*, the C-12 to C-13 double bond has been *Z* consistently.[1−5] In contrast, in similar diterpenoids isolated from other species of *Casearia* and other genera of the Flacourtiaceae, the C-12 to C-13 double bond has been either *E*, or, alternatively, the double bond has been translocated to between C-13 and C-16 such that the molecule has two adjacent terminal ethylene units.[6−14]

To account for the remaining four degrees of unsaturation in the molecular structure of **1**, its core was configured with one double bond and three rings. A double bond was assigned between the methine C-3 and the quaternary C-4, and through COSY correlations, H-3 was coupled to both the adjacent H-2 and the allylic H-18. One of the aforementioned acetate side chains (C-1′ and C-2′) was attached to this allylic position (δ_H/δ_C 6.71/95.19) from HMBC correlations observed between H-18 and C-1′. A cyclic hemiacetal moiety accounted for the downfield chemical shift of both C-18 and C-19 (δ_C 96.87), and this was supported by HMBC cross correlations from H-18 to C-19 and H-19 (δ_H 6.47) to C-18. The second acetate side chain (C-3′ and C-4′) was connected to C-19 from HMBC correlations between H-19 and C-3′. An additional ring was construed by COSY correlations from H-2 to H_2-1 and from H_2-1 to H-10. The quaternary bridge carbon (δ_C 53.45, C-5) was determined by HMBC correlations to C-5 from both H_2-1 and H-3; the C-5 resonance is one of the distinguishing aspects of these molecules.[5,8,10,16] The final ring was assigned from HMBC correlations from H-10 to C-6, H-6 to C-19, and H-19 to C-6 and COSY correlations observed from H-6 to H_2-7; the chemical shift values for position C-6 (δ_H/δ_C 4.01/73.99) were consistent for a hydroxyl-substituted methine group.[16,18] The NMR data supported two methyl groups in this molecule. One of these (C-17) was attached to C-8 from the doublet splitting pattern of H_3-17 and the COSY correlation between H-8 and H_3-17. The other methyl group (C-20) was one of the substituents on the quaternary C-9, and the additional substituent was the aforementioned diene side chain (C-11 to C-16). These assignments were supported by HMBC correlations from H_3-20 to C-11, C-10, and C-8. The final task was to place the isobutyrate-ester side chain (C-5′ to C-8′), and this was connected to C-2; the chemical shift value for the C-2 resonance (δ_C 70.43) was nearly identical to that observed in the intrapetacin series of clerodane diterpenoids, which also had an ester moiety at this position.[16]

Since we were unable to crystallize **1** in a form suitable for X-ray analysis, ^{1}H NMR coupling constants and ROESY data were used to assign the relative stereochemistry of the eight chiral centers. An axial position was assigned for H-10 based on the *J* value of 13.9 Hz for the coupling between H-10 and H_2-1.[18] A 1,3-diaxial ROESY correlation was observed between H-10 and H-2, and subsequently, the isobutyrate-ester side chain was placed in a pseudoequatorial position. The hydroxyl moiety was positioned in an equatorial position due to the characteristic 1,2-diaxial coupling between H-6 and H_2-7 of 12.2 Hz.[18] A 1,3-diaxial ROESY correlation was observed between H-6 and H-8, which enables the H_3-17 methyl group to be placed in an equatorial orientation. The diene side chain (C-11 to C-16) was found to be axial due to ROESY correlations between H_2-11 and H-19, and this was supported by the methyl substituent, C-20, being in an equatorial position from ROESY correlations between H_2-1 and H_3-20. The hemiacetal protons, H-18 and H-19, were found to be *syn* via weak ROESY cross correlations, and H-19 was construed as β due to ROESY correlations from H-19 to both H_2-11 and H_2-7. The relative stereochemistry of **1** is inconsistent at position C-2 with those of the other clerodane diterpenoids isolated previously from this genus,[1−5] and all of those compounds have been reported to have a positive value for the $[\alpha]_D$, whereas the $[\alpha]_D$ of **1** was found to be negative. In previous work on structurally related compounds that had the identical relative stereochemistry, similar ^{1}H−^{1}H coupling constants, ROESY (or NOESY) correlations, and optical rotation data were observed.[16]

The spectroscopic data for casearvestrin B (**2**) were nearly identical to those of **1**. The low-resolution ESIMS displayed two fragments for the sequential loss of acetate moieties, and the exact mass of the Na^+ adduct of the molecular ion, determined by HRFABMS, corresponded to a molecular formula of $C_{29}H_{42}O_8Na$ and an index of unsaturation of 9. The complete ^{1}H, ^{13}C, and DEPT-135 NMR data for **2** are shown in Table 1, and the COSY, HMQC, and HMBC experiments supported the same connectivities in **2** as in **1**. The major difference between these two compounds was the addition of 14 amu in the ESIMS of **2** compared to that of **1**. The ester side chain attached to C-2 was construed as 2-methylbutyrate, and this conclusion was supported by HMBC cross correlations (see Experimental Section). The C-7′ methylene accounted for the molecular weight difference, and the H_3-9′ methyl group was evident from the doublet splitting pattern in the ^{1}H NMR spectrum. The COSY and ROESY correlations used to assign the relative stereochemistry of **1** were observed also for **2**, and the $[\alpha]_D$ values for **1** and **2** are of the same magnitude and in the same direction (see Experimental Section). There is an additional chiral center in **2** at C-6′, which we were unable to assign.

The complete ^{1}H, ^{13}C, and DEPT-135 NMR data for casearvestrin C (**3**) are shown in Table 1, and the COSY, HMQC, and HMBC experiments supported the same connectivities in **3** as was observed in both **1** and **2**. Using HRFABMS, the exact mass of the Na^+ adduct of the molecular ion corresponded to a molecular formula of

Table 2. Cytotoxicity and Antifungal Activity of Compounds **1**−**3** and the Positive Controls, Camptothecin and Amphotericin B

compound	EC_{50} value (μg/mL) vs KB cells ± SEM	zone of inhibition of *A. niger* (mm^2) ± SEM
casearvestrin A (**1**)	1.4[a] ± 0.7	348[a] ± 38
casearvestrin B (**2**)	1.4[b] ± 0.5	296[a] ± 36
casearvestrin C (**3**)	0.34[b] ± 0.03	177[a] ± 49
camptothecin[c]	0.01[c]	not tested
amphotericin B[c]	not tested	254[c]

[a] $n = 3$. [b] $n = 4$. [c] Typical average value.

Table 3. Cytotoxicity of Compounds **1**−**3** Against a Panel of Tumor Cell Lines[a]

	cell line[b]		
compound	LX-1	HCT116	A2780
casearvestrin A (**1**)	0.54	0.71	0.82
casearvestrin B (**2**)	0.20	0.25	0.32
casearvestrin C (**3**)	0.29	0.26	0.42
camptothecin[c]	0.12	0.009	0.004

[a] Results are expressed as IC_{50} values (μM). [b] Key: LX-1 = human lung cancer; HCT116 = human colon cancer; A2780 = human ovarian cancer. [c] Typical average value.

$C_{30}H_{44}O_8Na$ and an index of unsaturation of 9. Similar to our previous observation for **2**, an additional methylene unit in the ester side chain attached to C-2 accounted for the molecular weight difference. This hexanoate side chain (C-5′ to C-10′) was characterized by the triplet splitting pattern of H_2-6′ and by having only one terminal methyl group (C-10′); HMBC correlations among this side chain illustrated clearly the connectivity (see Experimental Section). The COSY and ROESY correlations used to assign the relative stereochemistry of **1** and **2** were observed for **3** as well, and the specific rotations for all three of these molecules are of the same magnitude and in the same direction (see Experimental Section).

The bioactivity data of compounds **1**−**3** are shown in Tables 2 and 3, and compound **3** was the most potently cytotoxic in the KB assay. These compounds were examined also against an antifungal assay, as we observed antifungal activity in an earlier study with structurally similar compounds.[16] Indeed, **1**−**3** induced significant zones of inhibition of *Aspergillus niger*, with **1** being the most potent. Furthermore, **1**−**3** were evaluated at Bristol-Myers Squibb, Princeton, NJ, against a panel of tumor cell lines consisting of a variety of tissue types, including lung (LX-1), colon (HCT116), and ovary (A2780). All three compounds had comparable IC_{50} values for each of the cell lines tested that ranged between 0.2 and 0.8 μM (Table 3). Itokawa and Takeya have reviewed their extensive cytotoxicity results for casearins A−R against cloned Chinese hamster lung fibroblast cells (V-79).[3] By their convention compounds **1**−**3** would fall into the second tier of bioactive clerodane diterpenoids (group II); those which lack an oxygenated moiety at C-6 seem to be more cytotoxic (group I), whereas those with a hydroxyl group at C-7 seem to be less potent (group III).

Experimental Section

General Experimental Procedures. IR, UV, and specific rotations were recorded on a Nicolet Avatar 360 FT-IR, a Varian Cary 3 UV−vis spectrophotometer, and a Rudolph Autopol IV polarimeter, respectively. All NMR experiments were performed in $CDCl_3$ with TMS as an internal standard; gs-COSY, ROESY, gs-HMQC, gs-HMBC, and ^{1}H NMR spectra were run on a Bruker AMX-500 instrument using a Bruker 5 mm broad-band inverse probe with z-gradient or a Nalorac 3 mm microinverse broad-band probe with z-gradient, while a Bruker DPX-300 instrument was utilized for the DEPT-135 and ^{13}C NMR spectra using a Bruker 5 mm QNP probe. Low-resolution ESIMS were determined on a Finnigan LCQ instrument with an electrospray interface; high-resolution FABMS were measured with a Micromass Autospec mass spectrometer (Manchester, UK). Column chromatography was carried out on Si gel 60 (70−230 mesh, Merck, Darmstadt, Germany), and fractions were monitored via TLC (Si gel 60 F_{254} plates, 0.25 mm thickness) visualized with 5% phosphomolybdic acid in EtOH. A Waters Delta Prep 3000 was utilized for preparative-scale HPLC in the reversed phase on an 8 μm Inertsil ODS-3 column (10 mm i.d. × 50 mm guard and 20 mm i.d. × 250 mm column; Metachem Technologies, Torrance, CA).

Plant Material. The leaves and twigs of *Casearia sylvestris* were collected in December 1995, from a large tree of 25 m in height, 30 cm in diameter at breast height, growing in a tropical rain forest of Ecuador, within the Experimental Station of Escuela Superior Politecnica de Chimborazo (ESPOCH) in Los Vencedores (77° 56′ W longitude, 1° 30′ S latitude), Municipality of Puyo, Pastaza, at an altitude of about 900 m above sea level. Voucher herbarium specimens (Soejarto et al. 9403) have been deposited at the herbaria of ESPOCH (Riobamba, Ecuador) and the Field Museum of Natural History (Chicago, IL) under accession number 2161544.

Extraction and Isolation. The dried leaves and twigs (540 g) were percolated initially with cold MeOH overnight (2 × 2 L), and the MeOH extract was concentrated under reduced pressure. This extract was partitioned first between 10% (aqueous) MeOH and hexane, and then the aqueous MeOH fraction was partitioned further between $CHCl_3$−MeOH (4:1) and water. The organic layer was washed with 1% saline until there was a reduced presence of tannins.[19] This organic fraction (ca. 11.0 g) was mixed with Celite and separated further on a flash Si gel column that was developed using a gradient of 100% hexane to 100% $CHCl_3$ to 20% MeOH. The fractions were combined into 15 pools based on TLC properties and examined against the KB assay. Pools 3 through 5 had promising activity and were combined (ca. 1.9 g), adsorbed onto Celite, and separated on a second flash Si gel column using a gradient of 100% hexane to 25% acetone. Again, fractions were combined into 14 pools based on TLC properties and tested vs the KB assay. Pool 11 (ca. 120 mg) was purified via preparative RP-HPLC using an isocratic solvent system of 85:15 MeOH−H_2O to yield **1**. Compounds **2** and **3** came from a similar HPLC separation of a portion of pool 10 (ca. 180 mg) using an isocratic solvent system of 80:20 MeOH−H_2O.

Casearvestrin A (1): opaque amorphous solid (18.6 mg), yield 0.0034% w/w; $[\alpha]^{21}_D$ −57.0° (*c* 0.30, $CHCl_3$); UV (MeOH) λ_{max} (log ϵ) 202 (4.21), 234 (4.37) nm; IR ($CHCl_3$) ν_{max} 3029, 2970, 2930, 2875, 1748, 1731, 1714, 1647, 1234, 1157 cm^{-1}; ^{1}H NMR ($CDCl_3$, 500 MHz) and ^{13}C NMR ($CDCl_3$, 75 MHz) data, see Table 1; HMBC ($CDCl_3$) data, see Figure 1; LRESIMS *m/z* 1031 $[2M + Na]^+$ (9), 527 $[M + Na]^+$ (99), 467 $[M + Na - AcOH]^+$ (22), 407 $[M + Na - 2(AcOH)]^+$ (6); HRFABMS *m/z* 527.2606 (calcd for $C_{28}H_{40}O_8Na$, 527.2621).

Casearvestrin B (2): opaque amorphous solid (11.6 mg), yield 0.0021% w/w; $[\alpha]^{21}_D$ −50.5° (*c* 0.29, $CHCl_3$); UV (MeOH) λ_{max} (log ϵ) 202 (4.24), 234 (4.38) nm; IR ($CHCl_3$) ν_{max} 3029, 2965, 2935, 2875, 1748, 1733, 1725, 1229, 1214, 1177 cm^{-1}; ^{1}H NMR ($CDCl_3$, 500 MHz) and ^{13}C NMR ($CDCl_3$, 75 MHz) data, see Table 1; HMBC ($CDCl_3$) H-1 → C-5, H-3 → C-5, H-6 → C-10, C-19, H-11 → C-13, C-20, H-12 → C-14, H-14 → C-12, H-15 → C-13, H-16 → C-14, H-20 → C-8, C-10, H-18 → C-19, C-1′, H-19 → C-18, C-3′, H-6′ → C-8′, H-7′ → C-5′, C-9′, H-8′ → C-6′, H-9′ → C-5′, C-7′; LRESIMS *m/z* 1059 $[2M + Na]^+$ (28), 541 $[M + Na]^+$ (99), 481 $[M + Na - AcOH]^+$ (8), 421 $[M + Na - 2(AcOH)]^+$ (3); HRFABMS *m/z* 541.2759 (calcd for $C_{29}H_{42}O_8Na$, 541.2777).

Casearvestrin C (3): opaque amorphous solid (23.3 mg), yield 0.0043% w/w; $[\alpha]^{21}_D$ −61.2° (*c* 0.42, $CHCl_3$); UV (MeOH) λ_{max} (log ϵ) 202 (4.25), 234 (4.35) nm; IR ($CHCl_3$) ν_{max} 3029, 2955, 2940, 2870, 1748, 1733, 1224, 1169 cm^{-1}; ^{1}H NMR ($CDCl_3$, 500 MHz) and ^{13}C NMR ($CDCl_3$, 75 MHz) data, see

Table 1; HMBC ($CDCl_3$) H-1 → C-5, H-3 → C-5, H-6 → C-10, C-19, H-11 → C-13, C-20, H-12 → C-14, H-14 → C-12, H-15 → C-13, H-16 → C-14, H-20 → C-8, C-10, H-18 → C-19, C-1′, H-19 → C-18, C-3′, H-6′ → C-8′, H-7′ → C-5′; LRESIMS *m/z* 1087 $[2M + Na]^+$ (36), 555 $[M + Na]^+$ (99), 495 $[M + Na - AcOH]^+$ (22), 435 $[M + Na - 2(AcOH)]^+$ (8); HRFABMS *m/z* 555.2945 (calcd for $C_{30}H_{44}O_8Na$, 555.2934).

Cytotoxicity Assays. The KB (human oral epidermoid carcinoma) cytotoxicity assay was carried out as described previously to both monitor the fractionation process and measure the EC_{50} values for pure compounds (Table 2).[16] For the tumor cell panel, the cell lines were maintained in McCoy's 5A medium (GIBCO) and 10% heat-inactivated fetal bovine serum (GIBCO). The in vitro cytotoxicity of **1**−**3** was assessed in tumor cells by a tetrazolium-based colorimetric assay, which takes advantage of the metabolic conversion of MTS [3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfenyl)-2*H*-tetrazolium, inner salt] (Promega) to a reduced form that absorbs light at 492 nm.[20] Cells were seeded 24 h prior to drug addition. Following a 72 h incubation at 37 °C with serially diluted test compound, MTS, in combination with the electron coupling agent phenazine methosulfate, was added to the cells. The incubation was continued for 3 h, then the absorbance of the medium at 492 nm was measured with a spectrophotometer to obtain the number of surviving cells relative to control populations. The results are expressed as the median cytotoxic concentrations (IC_{50} values; Table 3). These values were calculated from six-point dose response curves using 5-fold serial dilutions, and each point on the curve was tested in duplicate.

Antifungal Assay. An *Aspergillus niger* antifungal assay was run as previously described,[16] except that the zone of inhibition (ZoI) is reported as an area and not as a diameter. This refinement was implemented because the ZoI area is a better measure of antifungal activity since a doubling of the diameter translates into a 4-fold increase in area. Thus, our positive control (0.01% solution of amphotericin B) typically produced an 18 mm diameter ZoI or a ZoI with an area of 254 mm^2. Compounds **1**−**3** were each tested by spotting onto the plates 10 μL of a 1.0% solution dissolved in DMSO.

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