Caucanolides A−F, Unusual Antiplasmodial Constituents from a Colombian Collection of the Gorgonian Coral *Pseudopterogorgia bipinnata*

Claudia A. Ospina,[†] Abimael D. Rodríguez,*[,†] Juan A. Sánchez,[‡] Eduardo Ortega-Barria,[§] Todd L. Capson,[⊥] and Alejandro M. S. Mayer[∥]

Department of Chemistry, University of Puerto Rico, P. O. Box 23346, U.P.R. Station, San Juan, Puerto Rico 00931-3346, Departamento de Ciencias Biológicas, Universidad de los Andes, P. O. Box 4976, Bogotá, Colombia, Instituto de Investigaciones Científicas Avanzadas y Servicios de Alta Tecnología, Centro de Estudios Biomédicos, Clayton, Building 175, P. O. Box 7250, Panama 5, Republic of Panama, Smithsonian Tropical Research Institute, P. O. Box 2072, Balboa, Ancón, Panama, and Department of Pharmacology, Chicago College of Osteopathic Medicine, Midwestern University, 555 31st Street, Downers Grove, Illinois 60515

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Six new diterpenoids, caucanolides A−F (**1**−**6**), have been isolated from extracts of the gorgonian octocoral *Pseudopterogorgia bipinnata* collected near the Colombian Southwestern Caribbean Sea. The structures of **1**−**6** were elucidated by comprehensive analysis of spectroscopic data. The caucanolides showed in vitro antiplasmodial activity against the malaria parasite, *Plasmodium falciparum*. In addition to possessing structures based on novel carbon skeletons, one of these metabolites, caucanolide B (**2**), constitutes the only example from nature of a secondary metabolite possessing the N^1,N^1-dimethyl-N^2-acylformamidine functionality.

The chemical diversity of gorgonian secondary metabolites reported to date has shown that these marine organisms represent an excellent resource for the discovery of novel pharmacologically active agents.[1] In particular, gorgonian octocorals of the genus *Pseudopterogorgia* are a rich source of unusual biologically active diterpenoids, sesquiterpenes, and polyhydroxylated steroids with diverse structures.[2] In a continuation of our search for biologically active compounds from the genus *Pseudopterogorgia*, we have examined the gorgonian coral *Pseudopterogorgia bipinnata* (Gorgoniidae) collected from Providencia (Old Providence) Island located in the Southwestern Caribbean Sea during March 2002.[3] A sample of the organic extract of this animal was included in an initial screening carried out as part of an effort in the discovery of new antimalarial agents through a collaborative agreement with the Panama International Cooperative Biodiversity Group (ICBG) program.[4] In the present study, this extract was found to be active in inhibiting the growth of *Plasmodium falciparum* and, thus, merited further chemical investigation.

Results and Discussion

The 1:1 $CHCl_3$/MeOH extract of the gorgonian coral *P. bipinnata* was subjected to our standard solvent partitioning scheme,[3b] and the hexane and $CHCl_3$ extracts were purified by a combination of gel filtration chromatography on Bio-Beads SX-3 (toluene), silica gel chromatography eluting with hexane/EtOAc mixtures, and HPLC to afford known compounds kallolide A acetate,[5] kallolide C,[5] bipinnapterolide A,[3d] gersemolide,[6] pinnatin B,[3c] bipinnatolide F[3d] (for their structures see the Supporting Information), and the new bilactone diterpenoids caucanolides A−F (**1**−**6**).[7] The structures were elucidated on the basis of spectroscopic data including HRMS measurements and long-range $^1H-^{13}C$ correlations. The relative stereochemistries were determined by a combination of analysis of NOESY data together with $^1H-^1H$ coupling constants of **1**−**6**. However, since the new metabolites are "open-chain" conformationally flexible systems, and no rigorous molecular mechanics/dynamics calculations were performed to establish the dominant conformations of **1**−**6**, the stereochemical assignments should be considered tentative, rather than definitive. Notwithstanding, in support of our proposed configurations, helpful stereochemical analogies were drawn to known relatives of the pseudopterane and cembrane classes of diterpenes that were co-isolated during this investigation.

Caucanolide A (**1**) (yield 0.11% on dry gorgonian weight basis) was obtained as a viscous optically active oil, $[\alpha]_D$ −42.0° (*c* 0.7, $CHCl_3$). While no pseudomolecular ion peak was detected in the HRFABMS, an intense $[M + H - H_2]^+$ fragment ion at *m/z* 373.1650 appropriate for a molecular formula of $C_{21}H_{26}O_6$ (calcd 373.1651 for $C_{21}H_{25}O_6$) was detected instead, requiring nine sites of unsaturation. Its IR spectrum showed prominent absorption bands at 3083, 1764, 1754, 1661, and 1626 cm^{-1}, indicative of olefin and α,β-unsaturated carbonyl functionalities, and the UV spectrum showed absorptions at λ_{max} (ε) 204 (26 200) and 246 (15 500) nm, suggesting the presence of α,β-butenolide moieties in compound **1**.

The ^1H NMR spectrum of compound **1** indicated the presence of two trisubstituted olefins [δ 7.20 (br d, 1H) and 6.77 (br q, 1H)]; an isopropenyl group [δ 5.05 (br s, 1H), 4.90 (br s, 1H), and 1.76 (s, 3H)]; a methoxyl group [δ 3.21 (s, 3H)]; and a vinyl methyl group [δ 1.99 (d, 3H)]. Furthermore, its ^1H NMR spectrum revealed the presence of an isolated pair of mutually coupled methylene groups [δ 2.47 (br t, 2H) and 2.25 (br t, 2H)]; two adjacent sp^3 methines [δ 5.25 (dd, 1H) and 2.78 (d, 1H)]; and an α-substituted-β,β-dimethyl-α,β-unsaturated aldehyde [δ 10.10 (s, 1H), 2.20 (s, 3H), and 2.02 (s, 3H)].

The ^{13}C NMR and DEPT NMR spectra indicated the presence of eight quaternary carbons, five methyls, three methylenes, and five methine carbons (Table 1). Further, the ^{13}C NMR spectrum of compound **1** indicated the

* To whom correspondence should be addressed. Tel: +787-764-0000, ext. 4799. Fax: +787-751-0625. E-mail: arodrig@cnnet.upr.edu.
[†] University of Puerto Rico.
[‡] Universidad de los Andes.
[§] Instituto de Investigaciones Científicas.
[⊥] Smithsonian Tropical Research Institute.
[∥] Midwestern University.

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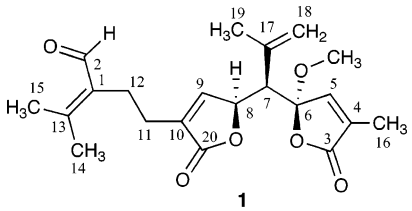

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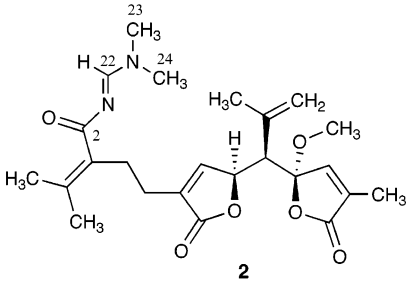

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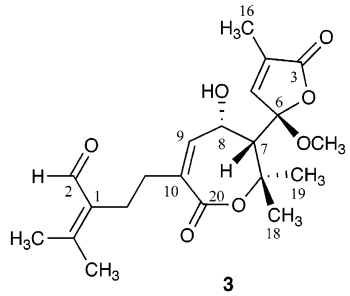

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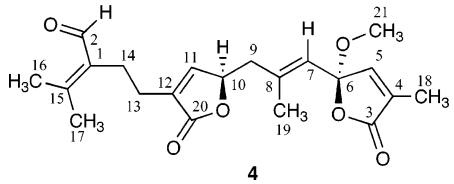

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presence of eight double-bond carbons between δ 160.0 and 115.0, in addition to an aldehyde carbonyl at δ 190.7 (d) and two lactone carbonyls at δ 173.3 (s) and 170.6 (s). Other features of the ^{13}C NMR spectrum included resonance lines for a ketal carbon (δ 108.4), a methoxyl carbon (δ 51.0), and an oxygenated (sp^3) methine carbon (δ 79.8). The overall NMR spectral data for caucanolide A (**1**), which included results from COSY and spin decoupling experiments, thus showed the presence of four carbon−carbon double bonds and three carbonyls, accounting for seven sites of unsaturation, and the lack of ^{13}C NMR evidence for further unsaturated functionality indicated the presence of two additional rings in **1**.

A pair of nearly coalescent triplets [δ 2.47, J = 7.9 Hz (H_2-12); 2.25, J = 7.9 Hz (H_2-11)] in the ^{1}H NMR spectrum showed COSY correlations to each other and HMBC correlations to the aldehyde carbonyl resonance at δ 190.7 (C-2) and the lactone carbonyl resonance at δ 173.3 (C-20), respectively. Additional HMBC correlations between the methylene carbon resonance at δ 23.6 (C-12) and the aldehyde proton H-2 [δ 10.10 (s)] and between the olefinic ^{1}H resonance at δ 7.20 (H-9) and the ^{13}C methylene resonance at δ 24.5 (C-11) were used to identify a $(CH_3)_2C$=C(CHO)−CH_2−CH_2−$C(CO_2-)$=CH−CH−O− fragment (C-1 to C-2, C-10 to C-17, and C-20) in **1**. Further support stemmed from a weak COSY correlation observed between the lactonic methine at δ 5.25 (H-8) and the olefinic doublet at 7.20 (J = 1.4 Hz; H-9) and a strong HMBC correlation between H-9 and C-20, which indicated that the lactone functionality in this major portion of the molecule is in fact a 2(5*H*)-furanone.[8]

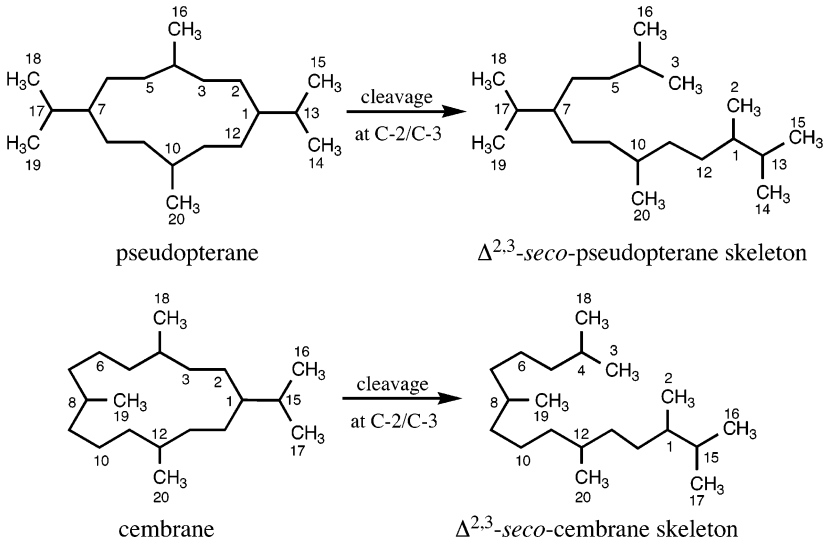
The resonances (δ 5.05/4.90; H_2-18a/18b) assigned to the methylene protons of an isopropylene fragment both showed weak COSY correlations, attributed to allylic coupling, to a methine resonance at δ 2.78 (H-7). The COSY data further showed that H-7 (J = 5.8 Hz) was coupled to the lactonic methine H-8, demonstrating that the isopropylene residue was part of an extended ^{1}H spin system. HMBC correlations between the nonprotonated olefinic carbon at δ 138.1 (C-17) and the ^{1}H resonances at δ 2.78 (H-7) and 5.25 (H-8) and between the olefinic ^{1}H resonance at δ 7.20 (H-9) and the ^{13}C methine resonance at δ 57.3 (C-7) demonstrated that the isopropylene group had to be bonded to C-7. Similarly, HMBC correlations between δ 108.4 (C-6) and 2.78 (H-7), 5.25 (H-8), 6.77 (H-5), and 3.21 (−OC*H*$_3$); between δ 134.7 (C-4) and 6.77 (H-5) and 1.99 (H_3-16); and between δ 170.6 (C-3) and 6.77 (H-5) and 1.99 (H_3-16)

Table 1. ^{1}H NMR (500 MHz), ^{13}C NMR (125 MHz), ^{1}H−^{1}H COSY, NOESY, and HMBC Spectroscopic Data for Caucanolide A (**1**) in $CDCl_3$[a]

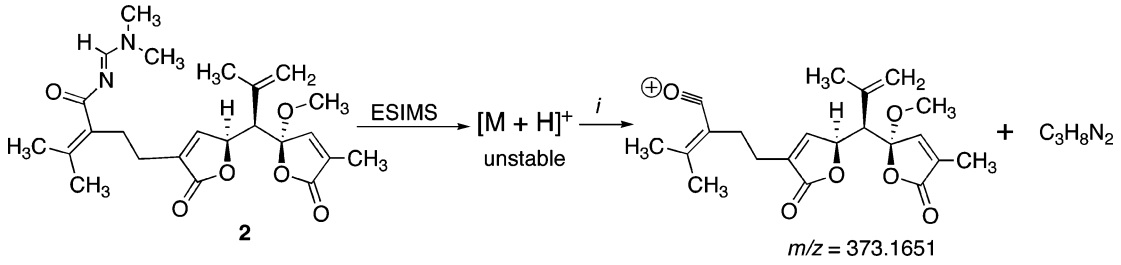
atom	δ_H, mult. (J in Hz)	δ_C (mult.)[b]	^{1}H−^{1}H COSY	NOESY	HMBC[c]
1		156.8 (C)			H_2-12, H_3-14, H_3-15
2	10.10, s	190.7 (CH)		H_2-12, H_3-15	H_2-12
3		170.6 (C)			H-5, H_3-16
4		134.7 (C)			H-5, H_3-16
5	6.77, br q (1.6)	145.1 (CH)	H_3-16	H-7, H_3-16, H_2-18, H_3-19	H_3-16
6		108.4 (C)			H-5, H-7, H-8, H_3-21
7	2.78, d (5.8)	57.3 (CH)	H-8	H-5, H-8, H-9, H_3-15, H_2-18, H_3-19	H-8, H-9, H_2-18, H_3-19
8	5.25, dd (5.8, 1.4)	79.8 (CH)	H-7, H-9	H-7, H-9	H-7, H-9
9	7.20, br d (1.4)	148.4 (CH)	H-8	H-7, H-8, H_2-11, H_2-12, H_2-18, H_3-19	H-7, H-8, H_2-11
10		133.7 (C)			H-8, H-9, H_2-11, H_2-12
11	2.25, br t (7.9)	24.5 (CH_2)	H_2-12	H-9, H_2-12	H-9, H_2-12
12	2.47, br t (7.9)	23.6 (CH_2)	H_2-11	H-2, H-9, H_2-11, H_3-14	H-2, H_2-11
13		135.5 (C)			H-2, H_2-12, H_3-14, H_3-15
14	2.02, s	23.4 (CH_3)		H_2-12	H_3-15
15	2.20, s	19.4 (CH_3)		H-2, H-7	H_3-14
16	1.99, d (1.6)	10.6 (CH_3)	H-5	H-5, H_3-19	H-5
17		138.1 (C)			H-7, H-8, H-18a, H_3-19
18a	4.90, br s	118.8 (CH_2)	H-18b, H_3-19	H-5, H-7, H-9, H-18b	H-7, H_3-19
18b	5.05, br s		H-18a, H_3-19	H-5, H-7, H-9, H-18a, H_3-19	
19	1.76, s	23.9 (CH_3)	H_2-18	H-5, H-7, H-9, H_3-16, H-18b	H-7, H_2-18
20		173.3 (C)			H-9, H_2-11
21	3.21, s	51.0 (CH_3)			

[a] Chemical shift values are in ppm relative to TMS. Spectra were recorded at 25 °C. [b] ^{13}C NMR multiplicities were obtained from a DEPT experiment. [c] Protons correlated to carbon resonances in the ^{13}C column.

Scheme 1



Scheme 2



indicated that C-7 had to be connected to a quaternary ketal carbon (C-6) that was in turn bonded to an adjacent olefinic methine carbon (C-5), to complete the final ring required in **1**, and also that C-4 had methyl (C-16) and carbonyl (C-3) substituents. The carbonyl substituent on C-4 had to be that of another 2(5*H*)-furanone group in order to account for the remaining atoms and unsaturation site in the molecular formula of caucanolide A (**1**). The complete planar structure of caucanolide A and the unambiguous assignment of all its ^{1}H and ^{13}C NMR signals were thus achieved by analysis of ^{1}H−^{1}H COSY, DEPT, HMQC, and HMBC spectroscopic data (Table 1).

The relative stereochemistry of **1** was determined on the basis of NOESY and scalar coupling constant data. However, because of the conformational flexibility of **1** and the effect of both electronegative substituents and dihedral angle on coupling constant values, our configuration assignments should be considered as being tentative. Nevertheless, some helpful conclusions could be drawn from these data. The small vicinal coupling constant between H-7 and H-8 (5.8 Hz) and the NOESY correlation observed between them required that both protons be on the same α face of the molecule. On the other hand, NOESY correlations between H_3-19 and the deshielded olefin protons H-9 and H-5 showed that the isopropenyl alkyl residue is on the opposite *β* face as drawn. These relative configurations are consistent with an anti relationship between the methoxyl and isopropenyl groups appended to C-6 and C-7, respectively, as indicated by strong NOESY correlations between H-5 and H-7 and between H-5 with H_3-19 and both H-18a/H-18b.[9] While these data do not establish the stereochemical assignment with sufficient rigor, it is interesting to note that the proposed configuration for **1**, namely, 6*R**, 7*R**, 8*S**, is analogous to that of several known pseudopterane diterpenes isolated during this investigation (kallolide A acetate, kallolide C, bipinnapterolide A, and gersemolide). This finding is consistent with the notion that a pseudopterane intermediate might serve as a precursor to caucanolide A (**1**) via oxidation/cleavage at C-2/C-3 (Scheme 1).

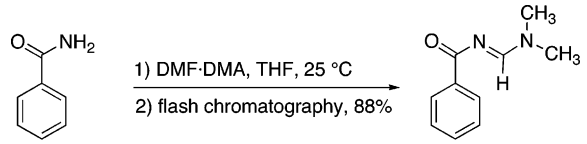
As was the case with the previous metabolite, caucanolide B (**2**) (yield 0.003%) was isolated as an optically active oil, $[\alpha]_D$ −20.8° (*c* 0.6, $CHCl_3$), which gave no pseudomolecular ion species in the HRESIMS. Instead, an intense fragment ion at *m/z* 373.1651 was observed, suggesting at first a molecular formula of $C_{21}H_{26}O_6$. However, further analysis of the combined spectroscopic data (namely, IR, ^{1}H, ^{13}C, and DEPT-135 NMR data) compelled us to identify this ion peak as a $[M + H - C_3H_8N_2]^+$ fragment appropriate for a molecular formula of $C_{24}H_{32}N_2O_6$, requiring 10 sites of unsaturation. Difficulties arose in confirming this conclusion through further mass spectrometric analysis, as FAB and FD mass spectrometry also failed to provide a clear-cut pseudomolecular ion peak under a variety of conditions. As seen earlier with caucanolide A (**1**), the HRESIMS of **2** provided a clear indication as to the susceptibility of the pseudomolecular ion species $[M + H]^+$ to break under ESIMS low-energy conditions, suggesting facile formation of a very stable acylium ion (Scheme 2).[10] On the other hand, evidence in support of the actual molecular formula for **2** was obtained from the ^{13}C NMR spectrum, which showed 24 resolved resonances (Table 2). HMQC and DEPT-135 data show that all of the 32 hydrogen atoms found in the molecular formula were attached to carbons (7 × CH_3, 3 × CH_2, 5 × CH). Eight olefinic [*δ* 149.0 (C), 148.5 (CH), 145.1 (CH), 138.2 (C), 134.8 (C), 133.7 (C), 125.0 (C), 118.8 (CH_2)], an imine [*δ* 162.6 (CH)], and three carbonyl [*δ* 173.3 (C), 172.0 (C), 170.6 (C)] resonances in the ^{13}C NMR spectrum accounted for eight sites of unsaturation. Therefore, the remaining

Table 2. ^{1}H NMR (300 MHz), ^{13}C NMR (75 MHz), ^{1}H−^{1}H COSY, NOESY, and HMBC Spectroscopic Data for Caucanolide B (**2**) in $CDCl_3$[a]

atom	δ_H, mult. (*J* in Hz)	δ_C (mult.)[b]	^{1}H−^{1}H COSY	NOESY	HMBC[c]
1		149.0 (C)			H_3-14, H_3-15
2		172.0 (C)			H_2-12
3		170.6 (C)			H-5, H_3-16
4		134.8 (C)			H_3-16
5	6.78, q (1.5)	145.1 (CH)	H_3-16	H-7, H-18a, H_3-19	H_3-16
6		108.4 (C)			H-5, H-7, H_3-21
7	2.79, d (5.8)	57.3 (CH)	H-8	H-5, H-8, H-9, H_2-18	H_2-18, H_3-19
8	5.25, dd (5.8, 1.4)	79.9 (CH)	H-7, H-9	H-7, H-9	H-7, H-9
9	7.18, br d (1.4)	148.5 (CH)	H-8	H-7, H-8, H_2-11, H_2-12, H-18a, H_3-19	H-7
10		133.7 (C)			
11	2.40, br t (7.5)	24.9 (CH_2)	H_2-12	H-9	
12	2.55, br t (7.5)	28.0 (CH_2)	H_2-11	H-9, H_3-14	
13		125.0 (C)			H_3-14, H_3-15
14	1.89, s	22.9 (CH_3)		H_2-12	H_3-15
15	2.09, s	23.5 (CH_3)			H_3-14
16	2.00, d (1.5)	10.6 (CH_3)	H-5		
17		138.2 (C)			H-7, H_3-19
18a	4.91, br s	118.8 (CH_2)	H-18b, H_3-19	H-5, H-7, H-9, H-18b	H-7, H_3-19
18b	5.06, br s		H-18a, H_3-19	H-7, H-18a, H_3-19	
19	1.77, s	23.9 (CH_3)	H_2-18	H-5, H-7, H-9, H-18b	H_2-18
20		173.3 (C)			H-9
21	3.21, s	51.0 (CH_3)			
22	8.02, br s	162.6 (CH)	H_3-23, H_3-24	H_3-23	H_3-23, H_3-24
23	2.96, br s	36.5 (CH_3)	H_3-22	H_3-22	H_3-24
24	2.89, br s	31.5 (CH_3)	H_3-22		H_3-23

[a] Chemical shift values are in ppm relative to TMS. Spectra were recorded at 25 °C. [b] ^{13}C NMR multiplicities were obtained from a DEPT experiment. [c] Protons correlated to carbon resonances in the ^{13}C column.

Scheme 3



two sites of unsaturation required by the molecular formula had to be accounted for by rings. Moreover, strong IR absorption bands at 2853 and 1681 cm^{-1} hinted at the presence of an unusual *N*-acyl-imine (−CO−N=CH−) moiety,[11] and a very strong band at 1757 cm^{-1} was indicative of ester carbonyl functionalities.

The molecular formula of caucanolide B (**2**) differed from that of caucanolide A (**1**) by the addition of $C_3H_6N_2$. Comparison of the ^{1}H and ^{13}C NMR data obtained for **2** with data obtained for **1** (Tables 1 and 2) showed that, otherwise, these molecules were closely related. The major differences in the ^{1}H NMR of **2** compared with **1** were the replacement of the aldehyde methine singlet with an imine methine singlet (δ 8.02; H-22) and the appearance of two additional *N*-methyl singlets at δ 2.96 (H_3-23) and 2.89 (H_3-24). The ^{13}C NMR spectrum of **2** was missing the aldehyde carbonyl present in the spectrum of **1**, but it contained a new amide carbonyl resonance at δ 172.0 (C-2), an imine methine carbon at δ 162.6 (C-22), and two *N*-methyl resonances at δ 36.5 (C-23) and 31.5 (C-24). Analysis of the COSY, HMQC, and HMBC data obtained for **2** showed that the differences in the NMR data described above (including strong upfield shifts observed for C-1, C-2, and C-13) could only be consistent with the presence of a N^1,N^1-dimethyl-N^2-acylformamidine moiety in **2** in place of the C-2 aldehyde in **1**.[12] In efforts to further define the molecular structure of **2**, we prepared, in 88% isolated yield, N^1,N^1-dimethyl-N^2-benzoylformamidine following a procedure previously disclosed (Scheme 3)[13] and compared its NMR spectra with those of **2**. After taking into account the small variations in chemical shifts due to the electronic properties of the benzene substituent, the ^{1}H and ^{13}C NMR resonances ascribable to the *N,N*-acylformamidine functionality in the model compound were similar to those present in caucanolide B (**2**). Inasmuch as caucanolide B exists as a single stereoisomer at 25 °C, the geometry about the acylformamidine moiety is drawn in the *E* form.

Examination of NOESY and scalar coupling constant data showed that the relative stereochemistry of **2** at C-6, C-7, and C-8 was identical with that found in **1**. Thus, caucanolide B (**2**) could be envisioned as a precursor for caucanolide A (**1**) (or vice versa) via partial reduction, in this case at C-2. Unfortunately, owing to the limited amounts of **2** available, we were unable to pursue a chemical interconversion study. As there was no possibility of exposure to DMF during this investigation, and there is no other apparent possible artifactual origin for such unit, the presence in **2** of the N^1,N^1-dimethyl-N^2-acylformamidine moiety is certainly a very intriguing feature. As far as we have been able to ascertain, caucanolide B (**2**) is the only example of a naturally occurring substance possessing such functionality. A plausible biogenetic route leading to the *N,N*-acylformamidine functionality in **2** might entail a reaction between the corresponding amide of **1** and a suitable formylating agent that is biochemically equivalent to a *N,N*-dimethylformamide dialkyl acetal such as DMF·DMA.

Caucanolide C (**3**) (yield 0.003%) was isolated as an optically active colorless oil, $[\alpha]_D$ −7.1° (*c* 1.1, $CHCl_3$), which had strong IR absorption bands at 3380 and 1759 cm^{-1} indicative of hydroxyl and ester carbonyl functional groups. HRFABMS data showed a pronounced fragment ion at *m/z* 397.1627 ($[C_{21}H_{26}O_6 + Na]^+$ requires 397.1627) produced through elimination of H_2O from the cationized molecular ion of **3**. These mass spectrometric data in combination with IR and ^{13}C NMR data allowed the molecular formula of **3** to be assigned as $C_{21}H_{28}O_7$, a molecular formula possessing an extra H_2O and one degree of unsaturation less in comparison to caucanolide A (**1**). The HMQC and the broadband-decoupled ^{13}C NMR spectra established that **3** possessed eight quaternary, five methine, two methylene,

Table 3. ^{1}H NMR (300 MHz) and ^{13}C NMR (75 MHz) Spectroscopic Data for Caucanolides C−F (**3**−**6**)[a]

	caucanolide C (**3**)		caucanolide D (**4**)		caucanolide E (**5**)		caucanolide F (**6**)	
atom	δ_H mult. (*J*)	δ_C (mult.)	δ_H mult. (*J*)	δ_C (mult.)	δ_H mult. (*J*)	δ_C (mult.)	δ_H mult. (*J*)	δ_C (mult.)
1		157.0 (C)[c]		156.9 (C)		156.7 (C)		156.8 (C)
2	10.10, s	190.7 (CH)[c]	10.11, s	190.6 (CH)	10.10, s	190.7 (CH)	10.10, s	190.7 (CH)
3		171.1 (C)		171.8 (C)		170.6 (C)		170.5 (C)
4		133.6 (C)		131.9 (C)[c]		129.6 (C)		129.7 (C)
5	6.75, br q (1.6)	146.3 (CH)[c]	6.80, br q (1.4)	145.3 (CH)[c]	7.05, br q (1.4)	138.8 (CH)	7.01, br q (1.3)	138.7 (CH)
6		107.1 (C)[c]		106.6 (C)		147.9 (C)		147.9 (C)
7	1.23, br s	25.9 (CH)	5.41, br q (1.0)	123.5 (CH)[c]	5.26, s	115.9 (CH)	5.11, s	116.8 (CH)
8	4.40, br d (1.4)	86.3 (CH)		139.9 (C)[c]		76.0 (C)		76.1 (C)
9a	7.03, d (1.4)	146.4 (CH)[c]	2.36, dd (3.3, 14.8)	43.8 (CH_2)[c]	2.08, br m	44.7 (CH_2)	2.05, br m	43.2 (CH_2)
9b			2.39, dd (2.8, 14.8)		2.08, br m		2.20, br m	
10		135.0 (C)	5.00, br m	79.4 (CH)	5.09, ddd (1.5, 5.5, 7.0)	78.0 (CH)	5.09, m	77.9 (CH)
11	2.29, t (7.9)	24.5 (CH_2)	7.05, br d (1.1)	147.6 (CH)[c]	7.13, br d (1.5)	149.7 (CH)	7.08, br d (1.2)	149.5 (CH)
12	2.52, t (7.9)	23.5 (CH_2)		134.3 (C)		132.9 (C)		133.1 (C)
13		135.5 (C)	2.26, t (7.8)	24.5 (CH_2)	2.25, t (7.9)	24.4 (CH_2)	2.25, t (7.9)	24.4 (CH_2)
14	2.04, s	23.4 (CH_3)[c]	2.51, t (7.8)	23.4 (CH_2)	2.50, t (7.9)	23.4 (CH_2)	2.50, t (7.9)	23.5 (CH_2)
15	2.20, s	19.4 (CH_3)		135.5 (C)		135.5 (C)		135.5 (C)
16	1.98, d (1.6)	10.5 (CH_3)[c]	2.21, s	19.4 (CH_3)	2.20, s	19.3 (CH_3)	2.20, s	19.4 (CH_3)
17		86.2 (C)	2.03, s	23.5 (CH_3)	2.01, s	23.4 (CH_3)	2.02, s	23.5 (CH_3)
18	1.20, s	12.9 (CH_3)[b]	1.94, d (1.4)	10.4 (CH_3)	2.01, d (1.4)	10.5 (CH_3)	2.01, d (1.3)	10.5 (CH_3)
19	1.10, s	12.7 (CH_3)[b]	1.91, br d (1.0)[c]	18.1 (CH_3)[c]	1.57, s	24.4 (CH_3)	1.61, s	23.3 (CH_3)
20		173.4 (C)		173.3 (C)		173.7 (C)		173.7 (C)
21	3.19, s	51.0 (CH_3)[c]	3.33, s	51.5 (CH_3)	3.26, s	50.7 (CH_3)	3.25, s	50.7 (CH_3)

[a] Spectra were recorded at 25 °C. Chemical shift values are in ppm relative to TMS. ^{13}C NMR multiplicities were obtained from NMR DEPT experiments. [b] Signal assignments could be reversed. [c] Signal appeared as two closely spaced lines of lower intensity.

and six methyl carbons, that is, a total of 27 hydrogens attached to 21 carbons. One more proton was inferred from the ^{1}H NMR and HMQC spectra that revealed a broad ^{1}H signal at δ 1.87, indicative of an exchangeable proton attributed to an alcohol proton on the basis of a 3380 cm^{-1} band in the IR spectrum. This brought the total proton count to 28. The alcohol group also accounted for one of the four oxygenated sp^3-carbons [δ 107.1 (C), 86.3 (CH), 86.2 (C), and 51.0 (CH_3)]. Furthermore, three oxygenated sp^2-carbons were ascribed to the carbonyl carbon signals of an aldehyde [δ 190.7 (CH)] and two ester carbonyls [δ 173.4 (C) and 171.1 (C)].

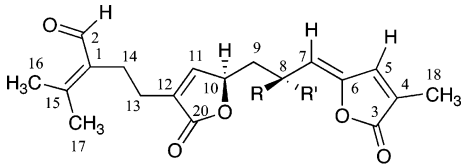
Many of the combined NMR spectroscopic features of **3** were similar to those of caucanolide A (**1**). For instance, close inspection of ^{1}H and ^{13}C NMR data (Table 3) showed that the α-substituted-β,β-dimethyl-α,β-unsaturated aldehyde and 3-methyl-2(5*H*)-furanone moieties that flanked the central ring portion of the molecule were intact. However, the NMR data further showed that the isopropenyl alkyl residue and the internal butenolide ring in **1** were absent. The carbon signals at δ 173.4 (C, C-20), 135.0 (C, C-10), 146.4 (CH, C-9), 86.3 (CH, C-8), 86.2 (C, C-17), 25.9 (CH, C-7), 12.9 (CH_3, C-18), and 12.7 (CH_3, C-19), the strong IR absorption ascribed to a lactone functional group, and a characteristic three-proton spin system from C-7 to C-9, showed that caucanolide C (**3**) possesses instead an α,β-unsaturated ϵ-lactone linking C-20 with C-17, with hydroxyl and *gem*-dimethyl groups at the γ and ϵ positions, respectively. The significant upfield shift of H-8, when compared to **1** and **2**, supported the proposed change in lactone ring size. These assumptions were subsequently corroborated by comprehensive analysis of COSY, NOESY, HMQC, and HMBC data, which allowed the complete assignment of all ^{1}H and ^{13}C NMR signals (Table 3), confirming the structure assignment as shown in **3**.

The relative stereochemistry about the large ring system in **3** was established by analysis of proton−proton coupling constants and NOESY correlations. A noticeably small vicinal coupling between H-7$_{ax}$ and H-8 ($^3J_{H-7ax/H-8} < 2$ Hz) indicated that this latter proton was in an equatorial position, thus requiring a dihedral angle between H-7 and H-8 close to 90°. NOESY correlations between H-8 (δ 4.40) and H-7 (δ 1.23) showed that they are both β as drawn. A NOESY correlation between H-9 (δ 7.03) and H-8 (δ 4.40) showed that the C-8 hydroxyl is α, and a NOESY correlation between H-5 (δ 6.75) and H$_3$-19 (δ 1.10) demonstrated that the 2(5*H*)-furanone functionality is α at C-7, as shown in **3**. Since caucanolide A (**1**) is a logical precursor of caucanolide C (**3**), upon regioselective hydration of the isopropenyl double bond and concomitant translactonization, it is presumed that **3** also has the same relative configuration at C-6, C-7, and C-8. Unfortunately, attempts to correlate chemically compounds **1** and **3** were unsuccessful, presumably due to decomposition of **1** under the strong hydrolytic conditions needed for these transformations.

The molecular formula of caucanolide D (**4**) (yield 0.006%) was determined to be $C_{21}H_{26}O_6$ on the basis of HRFABMS [(M + H)$^+$, *m/z* 375.1808 (0.0 mmu error)] in combination with ^{1}H and ^{13}C NMR data. The IR data suggested the presence of olefin (3085 and 1653 cm^{-1}), ester (1757 and 1754 cm^{-1}), and aldehyde (2763 and 1718 cm^{-1}) moieties. The ^{1}H and ^{13}C NMR data (Table 3) with DEPT, ^{1}H−^{1}H COSY, and HMQC data suggested the presence of $-CH_2-CH_2-C(CHO)=C(CH_3)_2$, $-CH_2-C(CH_3)=CH-$, $-OCH_3$, a 3,5-disubstituted-2(5*H*)-furanone, a 3,5,5-trisubstituted-2(5*H*)-furanone, an olefinic methyl, and a ketal carbon. In the HMBC spectrum, long-range couplings were observed from the olefinic proton at δ 7.05 (H-11) of the 3,5-disubstituted-2(5*H*)-furanone group to the methylene carbon at δ 24.5 (C-13) of the $-CH_2-CH_2-C(CHO)=C(CH_3)_2$ group and to the methylene carbon at δ 43.8 (C-9) of the $-CH_2-C(CH_3)=CH-$ group. The olefinic proton at δ 5.41 (H-7) of the latter moiety was long-range coupled to the ketal carbon at δ 106.6, which, in turn, was coupled with the olefinic proton at δ 6.80 (H-5) and the $-OCH_3$ protons at δ 3.33 (H$_3$-21) of the 3,5,5-trisubstituted-2(5*H*)-furanone group. In addition, the olefinic proton H-5 in the latter functionality was long-range coupled to the olefinic methyl

carbon at δ 10.4 (C-18). Thus, the planar structure of caucanolide D was determined as **4**, having a unique $\Delta^{2,3}$-*seco*-cembrane skeleton, as shown (Scheme 1).

The assignment of the relative stereochemistry of caucanolide D (**4**) was difficult because of the "open-chain" nature of relevant portions of the molecule, together with the remoteness of the stereocenters from one another. However, since isomers **1** and **4** possess similar functional group arrangements (except for the carbon skeletal framework about C-7), we surmised that there must exist a close biogenetic interrelationship between them. Insofar as pseudopterane diterpenes have been shown to originate from a cembrane precursor via a photochemically induced [1,3] sigmatropic rearrangement,[3b,c] one could safely assume that **1** and **4** must have the same relative configuration at all common chiral centers. Indeed, NOEs were observed among H-10 and H_3-19, indicating both are on the bottom face of the molecule, and since NOE cross-peaks were also detected between H-7 and both H-5 and H_3-21, the latter sets of protons were arranged spatially on the top face of the molecule, thus arguing for the dominance of one conformation of **4** in solution. The $\Delta^{7,8}$-trisubstituted olefin is shown with *E* geometry on the basis of the shielded methyl carbon resonance at δ 18.1. These data, combined with comprehensive analysis of molecular models of **4** and comparison with the measured vicinal coupling constant data, confirmed the relative stereochemistry of caucanolide D as shown in **4**, namely, 6*R**, 7*E*, 10*R**.



5 R = CH_3 R' = OCH_3
6 R = OCH_3 R' = CH_3

Caucanolides E (**5**) and F (**6**) (yields 0.006% and 0.004%, respectively) were found to have similar IR, MS, UV, and ^{1}H and ^{13}C NMR spectra, as well as comparable optical rotations. The HRMS of compounds **5** and **6** suggested the same molecular formula of $C_{21}H_{26}O_6$, thus indicating nine degrees of unsaturation in these metabolites. The IR spectra of caucanolides E and F indicated the presence of olefin, ester, and aldehyde functionalities, and the UV spectra (MeOH) showed maxima near λ_{max} 209 and 261 nm. The ^{13}C NMR spectra displayed distinct resonances for all 21 carbon atoms, and since they contained eight olefinic carbon resonances in addition to three carbonyls, these molecules were judged to be bicyclic. Interpretation of the ^{1}H and ^{13}C NMR, ^{1}H–^{1}H COSY, HMQC, and HMBC spectroscopic data revealed the presence in these compounds of the same interconnected $-CH_2-CH_2-C(CHO)=C(CH_3)_2$ and 3,5-disubstituted-2(5*H*)-furanone groups present in caucanolide D (**4**). On the other hand, these spectroscopic data also indicated the presence of a distinct $-CH_2-C(CH_3,OCH_3)-CH=C-$ fragment not present in **4** that further incorporates an α,β-unsaturated carbonyl group in its structure. The long-range correlation of the methylene protons near δ 2.08 (H_2-9) of the latter fragment with C-10 near δ 78.0 was observed in the HMBC spectra of these compounds, indicating that C-9 should be connected to C-10. Considering the higher λ_{max} in the UV spectra of **5** and **6** than **4**, this observation suggested that the remaining 2(5*H*)-furanone moiety in compounds **5** and **6** might be connected to the nonprotonated olefin carbon of the $-CH_2-C(CH_3,OCH_3)-CH=C-$ fragment, leading to a 5-ethylidenyl-3-methyl-2(5*H*)-furanone functionality. Indeed, the long-range correlation of the olefinic carbon C-6 at δ 147.9 of compounds **5** and **6** with the olefinic protons H-5 and H-7 indicated that the olefinic carbons C-5 and C-7 should be connected through C-6, thus effectively extending the conjugation of the terminal 2(5*H*)-furanone moiety. Thus, the planar structures of caucanolide E and F were determined as **5** and **6**, having the same $\Delta^{2,3}$-*seco*-cembrane skeleton as caucanolide D (**4**), but with a partially rearranged functionality. Indeed, caucanolides E (**5**) and F (**6**) could be envisioned as products arising from a 1,3-allylic transposition rearrangement of caucanolide D (**4**) and, thus, might be considered artifacts of the extraction procedure in MeOH.

The relative stereochemistry of compounds **5** and **6** was determined by NOESY experiments with the aid of computer-generated three-dimensional models, which very interestingly indicated that these compounds have a peculiar U-shaped stereostructure. In each case the NOESY data clearly argued for a selected conformation and thus enable our most compelling argument since both isomers were available for comparison. Thus, in each molecule NOEs were observed among H-5, H-7, and H_3-18, clearly indicating the *Z* geometry for the Δ^6 olefin. In the case of caucanolide E (**5**), however, NOE correlations were observed between the olefinic protons H-7 and the methyl protons attached to C-8 (H_3-19), and H-10 has a NOE correlation to the methoxyl proton H_3-21. On the other hand, in the NOESY spectrum of caucanolide F (**6**) NOE cross-peaks were observed between H-10 and H_3-19. From these spectroscopic data, the relative stereochemistries of caucanolide E (**5**) and caucanolide F (**6**) were determined to be 8*R**, 10*R** and 8*S**, 10*R**, respectively.

The co-occurrence of compounds **1**–**6** with several known skeletal classes, pseudopterane (kallolide A acetate, kallolide C, bipinnapterolide A, gersemolide), cembrane (bipinnatolide F), and gersolane (pinnatin B) within the same specimen of *P. bipinnata*, provides circumstantial support that the caucanolides might be synthesized in vivo by oxidation and concomitant ring cleavage of two of these families of compounds. Indeed, it is tempting to speculate that a pseudopterane intermediate might serve as a precursor to caucanolides A–C (**1**–**3**) and that a cembrane intermediate could likewise give rise to the caucanolides D–F (**4**–**6**) via oxidation/cleavage at the C-2/C-3 position (Scheme 1). The caucanolides are, therefore, exceptional in various respects. While several marine *seco*-cembrane terpenoids have been reported before, there are no other examples of such natural products arising through oxidative cleavage of the C-2/C-3 bond. The closest structural relatives to caucanolides D–F (**4**–**6**) are compounds likely stemming from the oxidation/cleavage of a cembrane precursor at the C-7/C-8,[3f] C-8/C-9,[14] or C-12/C-13[15] position. Additionally, $\Delta^{4,5}$-, $\Delta^{5,6}$-, and $\Delta^{11,12}$-*seco*-cembranes have been isolated from tobacco.[16] The structural core of caucanolides A–C (**1**–**3**) is also unprecedented in a natural product. Thus, compounds **1**–**3** represent the first examples of a new structural class of diterpenes, namely, the $\Delta^{2,3}$-*seco*-pseudopteranes. The presence of many unprecedented functionalities in compounds **1**–**6**, such as the N^1,N^1-dimethyl-N^2-acylformamidine moiety in caucanolide B (**2**), the 5-ethylidenyl-3-methyl-2(5*H*)-furanone group found in caucanolides E (**5**) and F (**6**), and the α,β-unsaturated ϵ-lactone functionality present in caucanolide C (**3**), makes this new family of compounds structurally intriguing.

Interestingly, caucanolide A (**1**) demonstrated significant in vitro antiplasmodial activity against chloroquine-

resistant *Plasmodium falciparum* W2 (IC_{50} 17 μg/mL), while caucanolide D (**4**) (IC_{50} 15 μg/mL) was equally potent to **1**, and caucanolides B (**2**), C (**3**), E (**5**), and F (**6**) showed very weak activity against this clone (IC_{50} values $\geq$ 50 μg/mL).[17] On the other hand, caucanolide A (**1**) inhibited growth of human cancer cells T-47D, CCRF-CEM, NCI-H460, and MCF-7 (IC_{50} values 25.8, 25.5, 12.5, and 7.6 μg/mL, respectively),[18] and when tested for in vitro antituberculosis activity against *Mycobacterium tuberculosis* $H_{37}Rv$, compound **1** marginally inhibited mycobacterial growth by 21% at a concentration of 6.25 μg/mL.[19] When subjected to in vitro antiviral testing against Herpes simplex viruses HSV-1 and HSV-2, both **1** and **4** were found to be inactive.[20] Caucanolides A (**1**) and E (**5**) were not active against the hepatitis B virus,[21] and **1** showed no toxicity against the flu A viruses H1N1 and H3N2.[22] Additionally, compounds **1**, **4**, **5**, and **6** demonstrated minimal effects on the release of TXB_2 and O_2^-, and lactate dehydrogenase (LDH), a marker for cell cytotoxicity, from *E. coli* lipopolysaccharide activated rat neonatal microglia in vitro.[23]

Experimental Section

General Experimental Procedures. Optical rotations were measured with a Perkin-Elmer polarimeter Model 243B. Infrared and UV spectra were recorded with a Nicolet Magna 750 FT-IR and a Hewlett-Packard diode array spectrophotometer Model 8452A, respectively. ^{1}H NMR spectroscopic data were generated with a 500 or 300 MHz FT-NMR spectrometer and the ^{13}C NMR spectroscopic data with a 125 or 75 MHz FT-NMR spectrometer. ^{1}H−^{1}H COSY, NOESY, DEPT, HMQC, and HMBC experiments were measured with a 300 MHz FT-NMR spectrometer. High-resolution ESI, FAB, EI, and FD mass spectra were obtained at the Mass Spectroscopy Laboratory of the University of Illinois at Urbana−Champaign. HPLC separations were carried out on a 10 mm × 25 cm Ultrasphere-Cyano Polar-Bonded column, 5 mm, eluted isocratically with hexane/2-propanol mixtures at 1.0 mL/min, with UV detection at 220 nm. Lowest energy conformers were searched using the MMFF force field implemented in the McSpartan Pro program (Wavefunction, Inc., Irvine, CA). Column chromatography was performed on silica gel (35−75 mesh). TLC analyses were carried out using glass silica gel plates, and spots were visualized by exposure to I_2 vapors or heating silica gel plates sprayed with 5% H_2SO_4 in EtOH. All solvents used were spectroscopic grade or were distilled from glass prior to use. Reagents from commercial suppliers were used as provided. The percentage yield of each compound is based on the weight of the dry gorgonian coral.

Animal Material. The specimens from this study corresponded to the deep-water morphotype of *Pseudopterogorgia bipinnata* (Verrill). It is the most abundant gorgonian species in the area of Providencia (Old Providence) Island and other reefs of the Southwestern Caribbean.[24] The deep morphotype is different from the typical shallow form, traditionally known *P. bipinnata*[25] because it has longer branches, up to 12 cm long (2.5−4.0 cm in the shallow morphotype) and separated by internodes of 1−2 cm (0.4−1.0 cm in the shallow morphotype). The voucher specimen presented the characteristic scaphoid sclerites with the diagnostic belts of fused tubercles on the convex side, which were nearly identical to sclerites of *P. bipinnata* samples from the Bahamas and Belize. Although there is an obvious cline in *P. bipinnata* morphotypes, at intermediate depths (ca. 15−20 m) they can be found in the same habitat as observed at Providencia Island. Whereas colonies of the shallow morphotype can be purple-violet, the deep morphotype analyzed in this study is beige-pale yellow, which made it easy to collect this particular morphotype specifically in the field. Despite the overlap in habitat distributions of the two morphotypes, in a study of *P. bipinnata* populations in Belize, both mitochondrial and nuclear DNA sequences have shown that they are the same species.[26] Nevertheless, it is intriguing that two morphotypes of *P. bipinnata* can be found in the same habitat. It is possible that the morphotype fate in the colony form of this species is decided very early in the colony development, which may result in qualitative differences in intermediate habitats. The natural products chemistry of this poorly known *P. bipinnata* morphotype may contribute to the understanding of phenotypic plasticity in this abundant and ecologically important octocoral species.

Collection, Extraction, and Isolation. The gorgonian coral *P. bipinnata* was collected by scuba from shallow reef waters off Providencia (Old Providence) Island, Colombia, in March 2002 and frozen shortly after collection. The specimens were partially sun-dried and kept frozen prior to extraction. The freeze-dried animal (0.11 kg) was cut into small pieces and blended with 1:1 MeOH/$CHCl_3$ (10 × 1 L). The combined organic extracts were filtered and then concentrated, and the brown residue obtained (25 g) was suspended in water and extracted with hexane, $CHCl_3$, and EtOAc. Rotoevaporation of the combined hexane extract followed by overnight storage under high vacuum produced 12 g of a dark brown oily residue that was purified subsequently by size-exclusion chromatography on a Bio-Beads SX-3 column eluted with toluene. Fractions were pooled on the basis of their TLC and NMR profiles to yield eight primary fractions, denoted as I−VIII. Fraction VII (949 mg) was flash chromatographed over silica gel (30 g) with mixtures of hexane/EtOAc of increasing polarity (10−50%) and then with mixtures of EtOAc/MeOH (0−100%) to yield 19 subfractions, denoted A−S. Subfraction H (27 mg, 0.025% yield) was identified as the known pseudopterane diterpene kallolide A acetate.[5] Subfraction P (160 mg) was further purified by consecutive column chromatography over silica gel using mixtures of $CHCl_3$/hexane/acetone and normal-phase HPLC with 8% 2-propanol in hexane as eluant, to afford caucanolide A (**1**) (52 mg) and caucanolide B (**2**) (3.0 mg) as colorless homogeneous oils. Subfraction Q (27 mg) was in turn purified by normal-phase HPLC with 8% 2-propanol in hexane as eluant, to yield caucanolide D (**4**) (4.8 mg), caucanolide E (**5**) (3.9 mg), and caucanolide F (**6**) (1.5 mg) as pure colorless oils. Purification of fraction VIII (349 mg) by normal-phase HPLC led to the isolation of the known compounds gersemolide[6] (1.0 mg, 0.0009%) and pinnatin B[3c] (5 mg, 0.005% yield). Rotoevaporation of the combined $CHCl_3$ extract produced, after storage under high vacuum, 4.1 g of a greenish oil, which was loaded onto a column of silica gel (150 g) and eluted with a 99:1 mixture of $CHCl_3$/MeOH. Fractions were pooled on the basis of their TLC and NMR profiles to yield 22 primary fractions, denoted I−XXII. Fractions IV (76 mg) and V (424 mg) were combined and subjected to successive column chromatography over silica gel using mixtures of 60:39:1 $CHCl_3$/hexane/acetone, 60:40 hexane/EtOAc, and CH_2Cl_2/acetone, and normal-phase HPLC, leading to the known compounds bipinnatolide F[3d] (1.0 mg, 0.0009% yield), kallolide C[5] (2.0 mg, 0.002% yield), and bipinnapterolide A[3d] (37.2 mg, 0.03% yield) as well as additional quantities of caucanolide A (**1**) (64 mg), caucanolide C (**3**) (3.3 mg), caucanolide D (**4**) (2.0 mg), caucanolide E (**5**) (3.0 mg), and caucanolide F (**6**) (2.5 mg).

Caucanolide A (1): colorless oil; $[\alpha]_D^{20}$ −42.0° (*c* 0.7, $CHCl_3$); IR (film) ν_{max} 3083, 2861, 2763, 1764, 1754, 1661, 1626, 876, 795 cm^{-1}; UV (MeOH) λ_{max} 204 (ϵ 26 200), 246 (ϵ 15 500) nm; ^{1}H NMR ($CDCl_3$, 500 MHz) and ^{13}C NMR ($CDCl_3$, 125 MHz) (see Table 1); HRFABMS (MB) *m/z* $[M + H - H_2]^+$ calcd for $C_{21}H_{25}O_6$ 373.1651, found 373.1650.

Caucanolide B (2): colorless oil; $[\alpha]_D^{20}$ −20.8° (*c* 0.6, $CHCl_3$); IR (film) ν_{max} 3082, 2853, 1757, 1681 cm^{-1}; UV (CH_3CN) λ_{max} 195 (ϵ 17 200), 205 (ϵ 16 200) nm; ^{1}H NMR ($CDCl_3$, 300 MHz) and ^{13}C NMR ($CDCl_3$, 75 MHz) (see Table 2); HRESIMS *m/z* $[M + H - C_3H_8N_2]^+$ calcd for $C_{21}H_{25}O_6$ 373.1651, found 373.1651.

Caucanolide C (3): colorless oil; $[\alpha]_D^{20}$ −7.1° (*c* 1.1, $CHCl_3$); IR (film) ν_{max} 3380, 3077, 2855, 2762, 1759, 1754, 1664, 1451 cm^{-1}; UV (MeOH) λ_{max} 210 (ϵ 16 100), 244 (ϵ 6000) nm; ^{1}H NMR ($CDCl_3$, 300 MHz) and ^{13}C NMR ($CDCl_3$, 75 MHz) (see

Table 3); HRFABMS (MB) *m/z* $[M + Na - H_2O]^+$ calcd for $C_{21}H_{26}O_6Na$ 397.1627, found 397.1627.

Caucanolide D (4): colorless oil; $[\alpha]_D^{20}$ −16.2° (*c* 1.4, $CHCl_3$); IR (film) ν_{max} 3085, 2855, 2763, 1757, 1754, 1718, 1653, 1630, 1449 cm^{-1}; UV (MeOH) λ_{max} 205 (ϵ 16 600), 240 (ϵ 12 800) nm; ^1H NMR ($CDCl_3$, 300 MHz) and ^{13}C NMR ($CDCl_3$, 75 MHz) (see Table 3); HRFABMS (MB) *m/z* $[M + H]^+$ calcd for $C_{21}H_{27}O_6$ 375.1808, found 375.1808.

Caucanolide E (5): colorless oil; $[\alpha]_D^{20}$ +72.2° (*c* 1.3, $CHCl_3$); IR (film) ν_{max} 3088, 2830, 2764, 1753, 1660, 1259, 1051 cm^{-1}; UV (MeOH) λ_{max} 206 (ϵ 16 500), 261 (ϵ 16 100) nm; ^1H NMR ($CDCl_3$, 300 MHz) and ^{13}C NMR ($CDCl_3$, 75 MHz) (see Table 3); HREIMS *m/z* $[M]^+$ calcd for $C_{21}H_{26}O_6$ 374.1729, found 374.1733.

Caucanolide F (6): colorless oil; $[\alpha]_D^{20}$ +34.5° (*c* 1.3, $CHCl_3$); IR (film) ν_{max} 3085, 2834, 2764, 1754, 1666, 1059 cm^{-1}; UV (MeOH) λ_{max} 209 (ϵ 19 900), 261 (ϵ 21 500) nm; ^1H NMR ($CDCl_3$, 300 MHz) and ^{13}C NMR ($CDCl_3$, 75 MHz) (see Table 3); HREIMS *m/z* $[M]^+$ calcd for $C_{21}H_{26}O_6$ 374.1729, found 374.1723.

Attempted Hydration and Translactonization of Caucanolide A (1). A solution of caucanolide A (**1**, 10 mg, 0.027 mmol) and aqueous 2.5% H_2SO_4 (5 drops) in THF (1.5 mL) was heated to 50 °C for 12 h. After quenching with 1 N NaOH (5 mL) the resulting suspension was extracted with $CHCl_3$ (2 × 6 mL). After concentration of the combined organic layers, TLC, GC-MS, and NMR (^1H and ^{13}C) analyses indicated the absence of caucanolide C (**3**) among the many reaction products obtained.

***N*¹,*N*¹-Dimethyl-*N*²-benzoylformamidine.**[13] To a solution of benzamide (896 mg, 7.4 mmol) in dry THF (5 mL) was added *N,N*-dimethylformamide dimethyl acetal (DMF·DMA) (2 mL, 15 mmol). After stirring the reaction mixture at 25 °C for 10 h, the solvent and excess reagent were removed in vacuo to give a solid residue that was purified by silica gel flash column chromatography using $CHCl_3$ as eluant (1.15 g, 88% yield): white solid; IR (neat) ν_{max} 3060, 3022, 2927, 2814, 1639, 1590, 1565, 1481, 1422, 1326, 1129, 1090, 1059, 1024, 913, 708 cm^{-1}; UV (CH_3CN) λ_{max} 201 (ϵ 13 500), 241 (ϵ 9200), 278 (ϵ 13 000), 288 (ϵ 12 400) nm; ^1H NMR ($CDCl_3$, 300 MHz) δ 8.61 (br s, 1H), 8.26 (dd, J = 1.5, 6.9 Hz, 2H), 7.47 (m, 1H), 7.39 (m, 2H), 3.16 (br s, 3H), 3.12 (br s, 3H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 177.6 (C), 160.6 (CH), 136.7 (C), 131.7 (CH), 129.6 (CH), 129.6 (CH), 127.8 (CH), 127.8 (CH), 41.2 (CH_3), 35.1 (CH_3); ESIMS *m/z* 177 $[M + H]^+$, 105 $[M + H - C_3H_8N_2]^+$; HRESIMS *m/z* $[M + H]^+$ calcd for $C_{10}H_{13}N_2O$ 177.1023, found 177.1028.

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Supporting Information Available: Molecular structures of all the known natural products isolated in the present study, ^1H and ^{13}C NMR spectra for compounds **1**, **2**, **4**, and **5**, and representative 2D NMR data ($^1H-^1H$ COSY, HMQC, HMBC, and NOESY) for compounds **1** and **5**. This material is available free of charge via the Internet at http://pubs.acs.org.

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