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Solenolides, New Antiinflammatory and Antiviral Diterpenoids from a Marine Octocoral of the Genus *Solenopodium*

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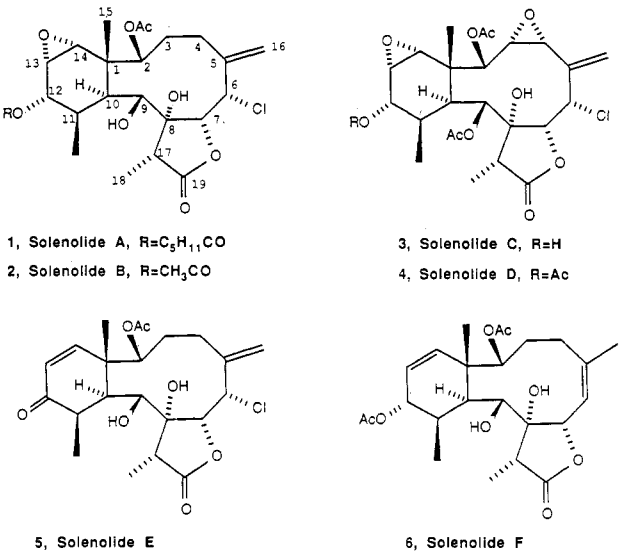
Six new diterpenoid lactones, solenolides A–F (1–6), have been isolated from a new species in Indopacific gorgonian of the genus *Solenopodium*. The solenolides are modifications of the well-known briarein class of marine metabolites, and several possess potent antiinflammatory and antiviral properties. The structures of solenolides A–F were assigned on the basis of extensive spectral analyses aided by selective chemical modifications.

In connection with our continuing studies of the chemical adaptations and biomedical potential of marine invertebrates, we have focused considerable attention on the marine sea whips and sea fans (order Gorgonacea, phylum Cnidaria), known to be a rich source of structurally novel secondary metabolites.[1] In this paper, we report the isolation of six new diterpenoids, solenolides A–F (1–6; Chart I) from *Solenopodium* sp., an apparently undescribed[2] Indopacific gorgonian collected in the shallow waters of Palau. The solenolides are closely related to other marine diterpenoids of the briarein class, typified by briarein-A, the first compound in this series, isolated from the Caribbean gorgonian *Briareum asbestinum*.[3] Related diterpenoids have subsequently been isolated from the gorgonian corals *B. polyanthes*,[4-6] *Erythropodium caribaeorum*,[7] and *Plexaureides praelonga*.[8] Briarein class diterpenoids are also commonly encountered in related orders of octocorals such as the true soft coral (Alcyonacea) of the genus *Minabea*,[9] the sea pansy *Renilla*,[10] and several sea pens (Pennatulacea) of the genera *Ptilosarcus*,[11,12] *Stylatula*,[13,14] *Scytalium*,[15] *Pteroides*,[16] and *Cavernulina*.[17]

Chart I



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(2) *Solenopodium* sp. was identified as an undescribed new member of this genus by Dr. Frederick M. Bayer, Department of Invertebrate Zoology, Smithsonian Institution, Washington, DC. Voucher specimens labeled PG-115 have been retained by Dr. Bayer for subsequent comparison.

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Table I. ^{13}C NMR Assignments for Solenolides A–F (1–6)[a]

C	1	2[b]	3[c]	4	5	6[b]
1	40.3 (s)	40.3	39.4	38.3 (s)	45.1 (s)	44.2 (s)
2	76.7 (d)	76.7	77.3	69.3 (d)[d]	77.0 (d)[d]	77.6 (d)
3	26.4 (t)	26.5[d]	59.3[d]	57.0 (d)[e]	26.7 (t)[e]	23.4 (t)[d]
4	26.4 (t)	27.2[d]	61.3[e]	61.4 (d)[f]	27.4 (t)[e]	24.0 (t)[d]
5	140.2 (s)	141.0	137.6	133.5 (s)	142.6 (s)	142.9 (s)
6	65.3 (d)	65.4	63.3[e]	60.0 (d)	66.4 (d)	120.8 (d)[e]
7	81.0 (d)	80.9	80.0	76.6 (d)[d]	81.2 (d)[d]	82.3 (d)
8	86.0 (s)	86.0	84.6	83.9 (s)	85.7 (s)	85.0 (s)
9	72.0 (d)	72.4	70.8[f]	74.9 (d)[d]	72.1 (d)	71.2 (d)[f]
10	33.6 (d)	36.6	38.7	35.9 (d)[g]	39.4 (d)	35.2 (d)[g]
11	37.3 (d)	37.4	39.4	37.3 (d)[g]	43.3 (d)	35.8 (d)[g]
12	70.3 (d)	71.8	70.6[f]	71.7 (d)[d]	203.4 (s)	70.2 (d)[f]
13	50.4 (d)	54.8	58.9[d]	56.6 (d)[e]	125.6 (d)	122.7 (d)[e]
14	62.4 (d)	61.9	61.3[e]	61.0 (d)[f]	155.0 (d)	141.5 (d)
15	19.5 (q)	20.2	17.1	16.5 (q)	20.2 (q)	20.3 (q)[h]
16	119.2 (t)	119.2	118.8	120.8 (t)	118.8 (t)	24.7 (q)
17	44.7 (d)	44.4	45.9	45.5 (d)	45.6 (d)	42.1 (d)
18	7.1 (q)	7.3	6.2	6.2 (q)	8.2 (q)	6.5 (q)
19	176.8 (s)	176.7	177.0	174.2 (s)	177.3 (s)	178.6 (s)
20	14.1 (q)	10.6	9.6	9.7 (q)	14.6 (q)	13.8 (q)
21	168.9 (s)[i]	168.5[i]	172.1[i]	169.0 (s)[i]	168.6 (s)[i]	168.0 (s)[i]
22	21.0 (q)[i]	170.7[i]	171.3[i]	169.6 (s)[i]	20.9 (q)[i]	170.8 (s)[i]
23	173.4 (s)[j]	20.9[i]	22.0[i]	169.9 (s)[i]		21.2 (q)[i]
24	34.0 (t)[j]	20.9[i]	20.9[i]	21.0 (q)[i]		20.8 (q)[h,i]
25	24.7 (t)[j]			21.0 (q)[i]		
26	31.4 (t)[j]			21.9 (q)[i]		
27	22.4 (t)[j]					
28	14.0 (q)[j]					

[a] The ^{13}C NMR spectra were recorded in $CDCl_3$ at 50 MHz unless otherwise specified. Multiplicities were obtained by single-frequency off-resonance decoupling and assignments were made based on J_R values and/or comparison to models. [b] Spectra were recorded in $CDCl_3$ at 25 MHz. [c] Spectra were recorded in MeOH-d_4 at 50 MHz. [d–h] Signals within a column may be reversed. [i] Resonance associated with an acetate ester. [j] Resonances associated with a hexanoate ester.

Solenopodium sp. was first encountered in 1979 as part of an expedition on the research vessel *Alpha Helix*.[18] Although currently classified as a gorgonian, *Solenopodium* resembles its Caribbean relatives *Erythropodium* and *Briareum* in possessing encrusting grow forms and in lacking the proteinaceous endoskeleton typically characteristic of the gorgonians.

Freshly collected animals were stored frozen and subsequently extracted with chloroform and ethyl acetate. Flash silica gel chromatography of the lipid–soluble extract from *Solenopodium*, followed by high-performance liquid chromatography, yielded the six solenolides. The three major metabolites, solenolides A (**1**), E (**5**), and D (**4**), each represented 6–9% of the organic extract. Together, the three minor metabolites, solenolides B (**2**), C (**3**), and F (**6**), comprised approximately 1% of the lipid-soluble material.

Solenolide A (**1**) crystallized from ether (mp 132–133 °C) following purification by silica HPLC. Data from chemical ionization mass (NH_3) and ^{13}C NMR spectrometry (Table I) established a molecular formula of $C_{28}H_{41}O_9Cl$ for this compound. Absorptions in the infrared spectrum indicated the presence of hydroxyl (3550–3500 cm^{-1}) and lactone (1775 cm^{-1}) functionalities. Carbonyl resonances in the ^{13}C NMR spectrum of **1** at δ 168.9, 173.4, and 176.8 confirmed the presence of a lactone and two other esters. In the ^1H NMR spectrum (Table II) of solenolide A, only one acetate methyl resonance was observed at δ 2.18 (3 H, s). The additional ester was determined to be a hexanoate based on ^1H NMR studies including 2D COSY and spin decoupling of a series of 11 contiguous protons [δ 0.91 (3 H, s, J = 7.0), 1.33 (4 H, m), 1.66 (2 H, m), and 2.35 (2 H, m)]. Two D_2O-exchangeable protons [δ 2.89 (1 H, s) and 4.16 (1 H, br m)] observed in the ^1H NMR spectrum suggested the presence of two hydroxyl groups in solenolide A. Thus, eight of the nine oxygen atoms in the molecular formula of compound **1** were accounted for by γ-lactone, hydroxyl, and ester functionalities. The remaining oxygen atom was lastly assigned as an epoxide based on ^{13}C NMR evidence [δ 50.4 (d) and 62.4 (d)] and ^1H NMR data [δ 3.46 (1 H, dd, J = 5.0, 3.0) and 2.89 (1 H, d, J = 3.0)]. Additional signals in the ^{13}C NMR spectrum of **1** at δ 140.2 (s) and 119.2 (t) suggested the presence of a terminal double bond, and a chlorine-bearing methine carbon was assigned to a resonance at δ 65.3 (d). The remaining ^{13}C NMR signals confirmed the presence of two additional methylenes [δ 26.5 (t) and 27.2 (t)], a quaternary bridgehead carbon [δ 40.3 (s)], three methine carbons that did not bear heteroatoms [δ 37.4 (d), 44.4 (d), and 36.6 (d)], and three methyl groups [δ 7.3 (q), 10.6 (q), and 20.2 (q)].

Consideration of the spectral data obtained for solenolide A suggested the molecule belonged to the briarein class of marine diterpenoids. On the basis of favorable NMR comparison to several briarein diterpenoids and considering results from spin-decoupling experiments, we assigned the gross structure **1** to solenolide A. A major problem confronted in assigning the structure of **1**, however, was to ascertain the location of the acetate and hexanoate esters. A detailed analysis of the ^1H NMR data showed that C-2 and C-12 both were esterified. From NMR data alone, however, we could not determine which carbon bore the acetate and which the hexanoate functionality.

Evidence to assign the locations of the acetate and hexanoate esters was unexpectedly obtained during the treatment of solenolide A with Zn/Cu couple in refluxing methanol. This latter reaction was conceived to open the lactone with reductive elimination of chloride. Three products were obtained and were assigned as **7–9** on the

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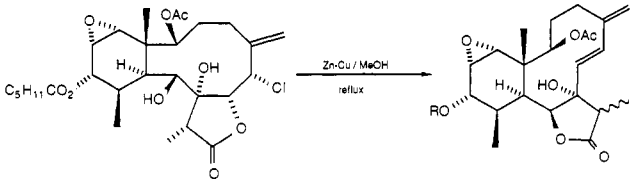
Table II. ^{1}H NMR Assignments for Solenolides A–F (1–6)[a]

C	solenolide A (1)	solenolide B (2)	solenolide C (3)[c]	solenolide D (4)	solenolide E (5)	solenolide F (6)
1	—	—	—	—	—	—
2	5.17 (1 H, bd, J = 5.7)	5.17 (1 H, bd, J = 6.0)	5.14 (1 H, d, J = 9.4)	5.13 (1 H, d, J = 9.3)	4.88 (1 H, d, J = 9.3)	4.76 (1 H, bd, J = 9.5)
3	2.47 (1 H, m)	[b]	3.51 (1 H, dd, J = 9.4, 3.9)	3.40 (1 H, dd, J = 3.8, 9.3)	2.44 (2 H, m)	[b]
	2.05 (1 H, m)					
4	[b]	[b]	3.94 (1 H, d, J = 3.9)	3.65 (1 H, d, J = 3.8)	1.69 (1 H, m)	[b]
					2.00 (1 H, m)	
5	—	—	—	—	—	—
6	5.34 (1 H, d, J = 3.2)	5.32 (1 H, d, J = 3.6)	5.51 (1 H, m)	5.38 (1 H, m)	5.17 (1 H, d, J = 2.9)	5.46 (1 H, bd, J = 9.0)
7	5.03 (1 H, m)	4.99 (1 H, m)	5.38 (1 H, d, J = 3.7)	5.07 (1 H, d, J = 3.5)	4.89 (1 H, bs)	5.32 (1 H, d, J = 9.0)
8	—	—	—	—	—	—
9	3.58 (1 H, t, J = 7.0)	3.57 (1 H, t, J = 7.5)	5.40 (1 H, d, J = 8.5)	5.34 (1 H, d, J = 8.5)	3.57 (1 H, dd, J = 9.8, 6.6)	3.64 (1 H, t, J = 8.5)
10	2.58 (1 H, m)	2.45 (1 H, bm)	1.78 (1 H, dd, J = 8.5, 2.3)	1.73 (1 H, dd, J = 8.5, 2.3)	3.10 (1 H, dd, J = 6.6, 4.4)	2.78 (1 H, dd, J = 8.0, 3.0)
11	2.10 (1 H, m)	2.32 (1 H, m)	2.25 (1 H, m)	2.24 (1 H, m)	2.78 (1 H, dd, J = 7.6, 4.4)	2.54 (1 H, m)
12	4.73 (1 H, dd, J = 5.0, 1.7)	4.57 (1 H, d, J = 4.9)	3.59 (1 H, ddd, J = 4.3, 3.5, 1)	4.60 (1 H, d, J = 4.7)	—	4.86 (1 H, dd, J = 5.8, 1.4)
13	3.46 (1 H, dd, J = 5.0, 3.0)	3.08 (1 H, d, J = 3.0)	3.04 (1 H, bd, J = 3.5)	3.12 (1 H, d, J = 3.0)	6.00 (1 H, d, J = 10.3)	5.82 (1 H, dd, J = 10.0, 5.8)
14	2.89 (1 H, d, J = 3.0)	2.89 (1 H, d, J = 3.0)	2.92 (1 H, d, J = 3.5)	2.89 (1 H, d, J = 3.0)	6.30 (1 H, d, J = 10.3)	5.48 (1 H, d, J = 10.0)
15	1.31 (3 H, s)	1.35 (3 H, s)	1.20 (3 H, s)	1.22 (3 H, s)	1.43 (3 H, s)	1.26 (3 H, s)
16	5.88 (1 H, bs)	5.88 (1 H, bs)	5.91 (1 H, d, J = 2.5)	6.11 (1 H, d, J = 2.5)	5.76 (1 H, d, J = 1.9)	1.86 (3 H, bs)
	5.72 (1 H, bs)	5.70 (1 H, bs)	5.89 (1 H, d, J = 2.5)	6.02 (1 H, d, J = 2.5)	5.30 (1 H, bs)	
17	3.10 (1 H, q, J = 7.3)	3.13 (1 H, q, J = 7.5)	2.70 (1 H, q, J = 7.2)	2.47 (1 H, q, J = 7.0)	3.23 (1 H, q, J = 7.8)	3.39 (1 H, q, J = 7.2)
18	1.13 (3 H, d, J = 7.3)	1.13 (3 H, d, J = 7.5)	1.09 (3 H, d, J = 7.2)	1.17 (3 H, d, J = 7.0)	1.20 (3 H, d, J = 7.8)	1.18 (3 H, d, J = 7.2)
19	—	—	—	—	—	—
20	1.04 (3 H, d, J = 7.4)	1.01 (3 H, d, J = 7.2)	1.03 (3 H, d, J = 7.2)	1.04 (3 H, d, J = 7.0)	1.23 (3 H, d, J = 7.6)	1.03 (3 H, d, J = 7.3)
8-OH	2.89 (1 H, s)	3.16 (1 H, bs)	4.38 (1 H, s)	3.60 (1 H, s)	3.24 (1 H, s)	—
9-OH	4.16 (1 H, bm)	4.14 (1 H, bd, J = 7.5)	—	—	4.04 (1 H, d, J = 9.8)	4.48 (1 H, d, J = 8.5)
	2.18 (3 H, s)	2.17 (3 H, s)	2.07 (3 H, s)	2.11 (3 H, s)	2.27 (3 H, s)	2.05 (3 H, s)
	0.91 (3 H, q, J = 7.0)	2.11 (3 H, s)	2.33 (3 H, s)	2.15 (3 H, s)		2.26 (3 H, s)
	1.33 (4 H, m)			2.23 (3 H, s)		
	1.66 (2 H, m)					
	2.35 (2 H, m)					

[a] Spectra were recorded in $CDCl_3$ solution at 360 MHz unless otherwise specified. Assignments were aided by spin-decoupling and 2D COSY experiments. J values are reported in hertz and the chemical shifts are reported in δ units (ppm relative to internal TMS). [b] Resonances were not assigned. [c] Spectrum was recorded in acetone-d_6.



1, Solenolide A

7,8, R=$C_5H_{11}CO$

9, R=H

basis of spectral analyses. In derivatives **7** and **8**, both the hexanoate and acetate esters were intact. The expected reaction had occurred at the chlorine atom, leading to opening of the γ-lactone. Subsequent closure of the resulting carboxylic acid with the C-9 hydroxyl had, however, also formed a γ-lactone between carbons 8 and 9. The resulting Δ^6 double bond was assigned an E configuration in **8** based on the observed 16.4-Hz coupling constant between the protons at C-6 and C-7. The proposed structural difference between compounds **7** and **8** is epimerization at C-17, which occurs under the conditions of the Zn/Cu reduction.

Derivative **9** was identical with **7** and **8** with the exception that the hexanoate ester had been eliminated. The C-12 methine proton (α to hexanoate) at δ 4.73 in **1** had moved upfield to δ 3.87 in derivative **9**. The acetate methyl in compound **9** was observed at δ 2.11 (3 H, s) and the acetoxyl methine proton (C-2) was identified at δ 5.02 (1 H, m). Selective hydrolysis of the C-12 ester in solenolide A suggests that the acetate at C-2 is buried within the 10-membered ring, inaccessible to the metal surface or to basic reactants. Treatment of **1** with Zn/Cu couple, for only 4 h, resulted only in reductive elimination to produce compounds **7** and **8**.

The relative stereochemistry of the substituents in **1** was determined by 2D NOESY and NOE difference experiments. The C-9 D_2O-exchangeable OH proton (δ 4.16) was shown to be within NOE proximity to the C-6 proton at δ 5.34. In the converse experiment, irradiation of the C-6 methine proton resulted in enhancement of the lactone proton at δ 5.03 and the C-9 OH proton. Thus, these three protons were all placed on the β face of the molecule. Similarly, the C-11, C-10, and C-9 methine protons were all found to be within NOE proximity on the opposite, α face of the molecule. Assignment of the C-2 acetate as a β substituent was based upon an observed NOE enhancement between the C-2 methine proton and the C-10 bridgehead proton.

In addition to NOE results, coupling constant analysis was useful in assigning the stereochemistry of the six-membered ring. The methine proton at C-11, already

determined to be α, was assigned as an equatorial proton based on its 1.7-Hz coupling to the equatorial C-12 proton. An observed 5.3-Hz coupling constant between the C-12 and C-13 methine protons suggested the C-13 epoxide proton was pseudoaxial. These stereochemical assignments were further confirmed by the observed NOE enhancement in the C-13 proton signal at δ 3.46 following irradiation of the C-12 methine proton (δ 4.73). In addition, an NOE enhancement was observed between the acetoxyl methine proton at C-2 (δ 5.17) and the C-14 epoxide proton at δ 2.89; these observations further supported the assignment of a β-acetoxyl substituent at C-2.

Once the structure of solenolide A was fully defined, the structure of a closely related minor metabolite, solenolide B (**2**), could be assigned based on spectral comparison to **1**. Compound **2** showed similar infrared absorptions that clearly defined the presence of the hydroxyl, ester, and γ-lactone functionalities. The NMR (Tables I and II) features of solenolide B were also analogous. Metabolite **2**, however, possessed an acetoxyl group in place of the hexanoate at C-12. The stereochemistry of the substituents in solenolide B was confirmed in the same manner as **1** using NOE measurements and coupling constant analysis.

Solenolide D (**4**), another major metabolite, also shared many spectral features in common with solenolides A and B. A molecular formula of $C_{26}H_{33}O_{11}Cl$ was established for solenolide D from chemical ionization (NH_3) mass and ^{13}C NMR spectrometry (Table I). The presence of hydroxyl, γ-lactone, and ester functionalities was confirmed by examination of the infrared spectrum of this metabolite. In comparison to solenolide B (**2**), one extra ester carbonyl resonance was observed in the ^{13}C NMR spectrum of **4**. Three acetate methyls were accounted for by close inspection of the proton NMR spectrum of solenolide D [δ 2.11 (3 H, s), 2.15 (3 H, s), and 2.23 (3 H, s)]. In addition to the acetate esters at carbons 2 and 12, compound **4** was also acetylated at C-9. The ^1H NMR spectrum of **4** lacked the C-9 D_2O-exchangeable OH proton seen in the spectrum of **2**, and the C-9 methine proton was shifted considerably downfield in solenolide D to δ 5.34 (1 H, d, J = 8.5).

The most obvious difference between solenolides B and D, however, was the presence of an additional epoxide in **4**. Resonances in the ^{13}C NMR spectrum of solenolide D at δ 69.3 (d), 61.3 (d), 58.9 (d), and 57.0 (d) were assigned to four epoxide carbons. The C-13,14 epoxide was clearly intact in solenolide D. Coupling of the C-13 epoxide methine to the C-12 proton and to the other epoxide methine proton (C-14) was recorded. The absence of two methylene carbons in the ^{13}C NMR spectrum suggested the location of the second epoxide to be at carbons 3 and 4. In the ^1H NMR spectrum of **4**, the C-3 and C-4 epoxide methine protons were observed at δ 3.40 (1 H, dd, J = 3.8, 9.3) and 3.65 (1 H, d, J = 3.8), respectively. The chemical shift of the C-4 methine proton suggested the epoxide was allylic; the observed coupling of the C-3 proton to the C-2 acetoxyl methine proton confirmed the location of this epoxide. Solenolide D is the 3,4-epoxy derivative of a known briarein metabolite, brianthein Z.[4] Extensive spectral comparison to brianthein Z further aided in the characterization of the structure of **4**.

Solenolide C (**3**), a minor metabolite, was isolated from more polar fractions of the crude extract. Comparison of the spectral features of solenolides C and D demonstrated the close structural relationship of these two natural products and defined the major differences between them. Only two acetate methyl resonances were apparent in the ^1H NMR spectrum of **3**. The C-12 acetate methine proton, observed in solenoide D at δ 4.60, was observed at higher field in the spectrum of **3** at δ 3.59. Thus, solenolide C was perceived to be the C-12 hydroxy derivative of solenolide D. This relationship was demonstrated by chemical transformation. Selective hydrolysis of the C-12 acetate ester in **4** was also achieved by treatment of solenolide D with Zn/Cu couple. Solenolide C was produced in 90% yield by this latter reaction.

As in the case of solenolides A and B, the stereochemistry of substituents on the γ-lactone and 6-membered ring was established by coupling constant analysis and NOE studies. The stereochemistries at C-2 and of the C-3,4 epoxide were also determined by NOE measurements. Irradiation of the proton at C-3 (δ 3.51) in solenolide C resulted in enhancement of the C-4 epoxide methine proton (δ 3.94) and the C-15 β-methyl group (δ 1.20). In another experiment, irradiation of the C-2 proton showed that the C-10 bridgehead methine proton (δ 1.78) and the syn terminal methylene (δ 5.91) were within NOE proximity on the opposite, α face of the molecule. No NOE enhancement was observed in the C-3 or C-4 proton when the C-2 acetoxyl methine proton was irradiated. The acetate at C-2 was, therefore, assigned as a β substituent and the epoxide was positioned as an α epoxide on the face opposite to the bridgehead methyl group. Based on the interconversion of **3** and **4**, assignment of the stereochemistry of substituents in solenolide C allowed us to also define the relative configuration of functionalities in solenolide D.

Another major component of the lipid extract, solenolide E (**5**), differed from solenolide A only in functionalities on the 6-membered ring. Absorptions for the ester, γ-lactone, and hydroxyl functionalities were apparent in the infrared spectrum of **5**. In addition, infrared absorption at 1680 cm^{-1}, in conjunction with absorption in the UV spectrum of solenolide E at λ_{max} 224 nm (ϵ 6900), suggested that **5** was an α,β-unsaturated ketone. ^{13}C NMR resonances at δ 203.4 (s), 125.6 (d), and 155.0 (d) was well as ^1H NMR signals at δ 6.00 (1 H, d, J = 10.3) and 6.30 (1 H, d, J = 10.3) further confirmed the presence of an α,β-unsaturated ketone. Spectral comparison showed **5** was closely related to ptilosarcenone, a compound possessing the same UV chromophore and identical substitution pattern on the 6-membered ring.[11,12] Spectral comparison of **5** to solenolide A showed that the substituents on the 10-membered ring were identical with those of **1**, including their relative stereochemistries. ^1H NOE measurements again confirmed this assignment.

Solenolide F (**6**) compared favorably to solenolide E. Compound **6**, however, did not possess the UV chromophore, the chlorine atom was absent, and an additional double bond was present in the molecule. An additional acetate methyl resonance was also observed in the ^1H NMR spectrum of solenolide F. COSY and spin-decoupling analysis led to the assignment of an allylic acetate ester at C-12. The C-12 acetoxyl methine proton, observed at δ 4.86 (1 H, dd, J = 5.8, 1.4) in **6**, was coupled to one proton of the Δ^{13}-cis double bond at δ 5.82 (1 H, dd, J = 10.0, 5.8) and to the methine proton at C-11 [δ 2.54 (1 H, m)]. Coupling constant analysis and NOE studies analogous to measurements made with metabolites **1**–**5** allowed assignment of the stereochemistry of the 6-membered ring in **6**.

Further consideration of the NMR data for compound **6** suggested the presence of a Δ^5 double bond. In place of the carbon bearing chlorine and the exocyclic methylene, resonances assigned to a trisubstituted olefin were observed in the ^{13}C NMR spectrum of solenolide F[δ 142.9 (s) and

120.8 (d)]. In the ^1H NMR spectrum, the terminal methylene protons, often seen as broad singlets between δ 5.3 and 6.2 in solenolides A–E, were replaced by a vinyl methyl resonance [δ 1.86 (3 H, s)]. The C-6 olefinic proton found at δ 5.46 showed a 9.0-Hz coupling to the lactone proton at δ 5.32. As in the case of solenolides A–E, coupling constant analysis, spectral comparison, and NOE measurements allowed the relative configurations of all substituents in solenolide F to be determined.

Solenolides A, D, E, and F were found to be potent antiinflammatory agents with efficacies comparable to that of indomethacin in topical assays.[19] Potencies in excess of 70% reduction of edema were observed at concentrations in the range of 15 μg (mouse ear assay). In in vitro enzyme testing, solenolide A was found to inhibit the arachidonic acid pathway enzyme 5-lipoxygenase. On the other hand, solenolide E was found to be an inhibitor of the related enzyme cyclooxygenase.[20]

The solenolides were also found to possess antiviral properties, with particular sensitivities toward influenza viruses.[20] Solenolides A and E inhibit Rhinovirus (plaque reduction assay) with IC_{50} values of 0.39 and 12.5 μg/mL, respectively. Solenolide A also inhibits Polio III, Herpes, Ann Arbor, and Maryland viruses. Solenolide D inhibits Semiliki Forest and Ann Arbor viruses, while solenolide E inhibits Herpes and Ann Arbor viruses.

In agrichemical screening, we can add to the earlier observations of insecticidal activity associated with briarein diterpenoids.[5,12] The solenolides were uniformly active as larvacides against Blowfly larvae with ED_{100} values at 30–35 ppm.

These data and early findings show that the briarein marine diterpenoids represent unique structure leads in several areas of pharmaceutical and agrichemical development.

Experimental Section

General Procedure. The instrumentation and general experimental procedures used in this study have been described elsewhere.[21]

Collection and Extraction. *Solenopodium* sp. (voucher number PG-115) was collected by hand using SCUBA at depths of 10–30 m in Sept 1979 in the Western Caroline Islands of Palau. Animals were stored frozen. Upon workup, the gorgonians were homogenized and repeatedly extracted with chloroform, followed by ethyl acetate. The extracts were then combined, filtered, and evaporated to give a residue that was partitioned between saturated brine and chloroform. This organic extract was subsequently dried over anhydrous magnesium sulfate, filtered, and evaporated to give 26.5 g of crude extract (from 300 g, dry weight of animal). The lipid extract was then chromatographed on silica gel by vacuum flash chromatography. Fractions were eluted with mixtures of isooctane and ethyl acetate. Solenolide F (6) was the least polar of the six metabolites, eluting with solenolide A (1) with 60% ethyl acetate. Next, solenolide E (5) eluted from the column with 70% ethyl acetate and was followed by solenolide D (4) in fractions eluting with 75% ethyl acetate. Fractions eluted with 85% ethyl acetate contained the most polar compounds, solenolides B and C.

Solenolide A (1). Solenolide A crystallized from diethyl ether following purification by HPLC (μ-Porasil, 60% ethyl acetate in isooctane). Repeated recrystallization yielded approximately 1.9 g (7.5% of the crude extract) of compound 1. Solenolide A, mp 132–133 °C, showed $[\alpha]^{20}_D$ −56° (*c* 0.63, $CHCl_3$) and exhibited the following spectral characteristics: IR (KBr) 3550–3500 br, 1775, 1725, 1375, 1250, 1170, 1022 cm^{-1}; CIMS (NH_3), *m/z* (relative intensity) 558 and 556 (100) for $C_{28}H_{41}O_9Cl$; HRMS (30 eV), *m/e* (formula and/or relative intensity) obsd 422.1571 ($C_{21}H_{26}O_9$, 3), 363.1346 ($C_{20}H_{24}O_4Cl$, 6.5), 231.0995 ($C_{14}H_{15}O_3$, 13), 133.0648 (36), 109.0668 (52), 99.0455 (100).

Solenolide B (2). Purification by HPLC (μ-Porasil, 85% ethyl acetate in isooctane) yielded 45 mg (0.20% of the crude extract) of solenolide B as a viscous oil. Compound 2 showed $[\alpha]^{20}_D$ −5° (*c* 1.02, $CHCl_3$) and exhibited the following spectral characteristics: IR (CH_2Cl_2) 3550, 3500, 3060, 1780, 1740, 1455, 1370, 1245, 1040, 1025, 960 cm^{-1}; CIMS (NH_3), *m/z* (relative intensity) 483.7 ($M^+ - CH_4$) (21) for $C_{23}H_{29}O_9Cl$, 472.0 (49), 467.9 (43), 338 (100); HRMS (20eV), *m/z* (formula and/or relative intensity) obsd 344.1597 (M^+ − 2AcOH − HCl, $C_{20}H_{24}O_5$, 3), 326.1478 ($C_{20}H_{22}O_4$, 3), 237.1118 ($C_{13}H_{17}O_4$, 15), 221.0813 (11), 195.0840 (17), 43.0079 (100).

Solenolide C (3). Purification by HPLC (μ-Porasil, 85% ethyl acetate in isooctane) yielded 62 mg (0.24% of the crude extract) of diacetate 3. An amorphous solid, mp 196–198 °C (dec), solenolide C showed $[\alpha]^{20}_D$ −25° (*c* 0.76, MeOH) and had the following spectral properties: IR (KBr) 3500–3540 br, 1770 br, 1735 br, 1735 br, 1375, 1220 br, 1020, 900, 750 cm^{-1}; CIMS (CH_4), *m/z* (formula and/or relative intensity) 514.5 (8) for $C_{24}H_{31}O_{10}Cl$, 478.1 ($C_{24}H_{30}O_{10}$, 46), 418.1 ($C_{22}H_{26}O_8$, 100); HRMS (20eV), *m/z* (formula and/or relative intensity) obsd 418.1593 (M^+ − AcOH − HCl, $C_{22}H_{26}O_8$, 1), 358.1466 ($C_{20}H_{22}O_6$, 1), 177.0927 (5), 123.0818 (10), 60.0226 (61), 43.0063 (100).

Solenolide D (4). Purification by HPLC (μ-Porasil, 60% ethyl acetate in isooctane) gave 1.73 g (6.5% of the crude extract) of triacetate 4 as an amorphous solid, mp 183–185 °C. Solenolide D showed $[\alpha]^{20}_D$ −16° (*c* 0.89, $CHCl_3$) and exhibited the following spectral characteristics: IR (CH_2Cl_2) 3450, 1780, 1740, 1370, 1240, 1225, 1215, 1020, 910 cm^{-1}; CIMS (NH_3), *m/z* (relative intensity) 556.1 (28) for $C_{26}H_{33}O_{11}Cl$.

Solenolide E (5). Purification by HPLC (μ-Porasil, 60% ethyl acetate in isooctane) yielded 2.39 g (9% of the crude extract) of monoester 5 as a viscous oil. Solenolide E showed $[\alpha]^{20}_D$ +11° (*c* 0.5, $CHCl_3$) and exhibited the following spectral properties: UV (MeOH) λ_{max} 224 nm (ϵ 6900); IR (neat) 3500 br, 1770, 1760 br, 1680, 1380, 1365, 1240, 1190, 1030, 960, 910 cm^{-1}; CIMS (NH_3), *m/z* 442 and 440 for $C_{22}H_{29}O_7Cl$; HRMS (30 eV), *m/z* (formula and/or relative intensity) obsd 344.1640 (M^+ − AcOH − HCl, $C_{20}H_{24}O_5$, 3), 326.1532 ($C_{20}H_{22}O_4$, 3), 263.1263 ($C_{15}H_{19}O_4$, 6), 165.0515 (20), 149.0604 (31), 135.0435 (51), 123.0446 (100).

Solenolide F (6). Purification by HPLC (μ-Porasil, 60% ethyl acetate in isooctane) yielded 140 mg (0.54% of the crude extract) of compound 6 as a viscous oil. Solenolide F showed $[\alpha]^{20}_D$ −45° (*c* 1.52, $CHCl_3$) and exhibited the following spectral characteristics: IR (CH_2Cl_2) 3500, 1775, 1755, 1730, 1370, 1245, 1215, 1120, 1045, 1018, 990 cm^{-1}; CIMS (NH_3), *m/z* (relative intensity) 449.7 (100) for $C_{24}H_{34}O_8$; HRMS (20 eV), *m/z* (formula and/or relative intensity) 330.1837 (M^+ − 2HOAc, $C_{20}H_{26}O_4$, 23), 315.1552 ($C_{19}H_{23}O_4$, 9), 312.1676 ($C_{20}H_{24}O_3$, 9), 212.1040 (23), 169.0897 (29), 107.1637 (100).

Reaction of Solenolide A (1) with Zn/Cu Couple. To a solution of solenolide A (1) (85 mg, 0.15 mmol) dissolved in MeOH (15 mL) 0.6 g of freshly prepared Zn/Cu alloy was used. The reaction mixture was refluxed for 38 h. Upon workup, the solution was filtered and the solvent was evaporated. Filtration through a short column of silica gel removed the inorganic material. Purification by HPLC yielded three products, 14.4 mg of derivative 7 (19% from natural product 1), 30.6 mg of compound 8 (40% from metabolite 1), and 12 mg of product 9 (20% from compound 1). Derivative 7 showed the following spectral characteristics: UV (MeOH) λ_{max} 234 nm (ϵ 5400); IR (CH_2Cl_2) 3540, 3050, 1780, 1730, 1375, 1240, 1185, 1020 cm^{-1}; CIMS (NH_3), *m/z* (relative intensity) 522 ($M + NH_4^+$, 100), 504 (25) for $C_{28}H_{40}O_6$; HRMS (30 eV), *m/z* (relative intensity) 476.2731 (M^+ − CO, $C_{27}H_{40}O_7$, 8), 444.2492 (M^+ − AcOH, $C_{26}H_{36}O_6$, 2.5), 416.2604 ($C_{25}H_{36}O_5$, 5), 360.1963 ($C_{21}H_{28}O_5$, 20), 166.0994 (52); ^1H NMR (360 MHz, $CDCl_3$, 58 °C) δ 6.36 (1 H, d, *J* = 13.1 Hz, H-6), 5.89 (1 H, d, *J* = 13.1 Hz, H-7), 5.12 (1 H, bs, H-16), 5.10 (1 H, m, H-12), 5.09 (1 H, bs, H-16), 4.84 (1 H, d, *J* = 9.7 Hz, H-2), 4.51 (1 H, s, H-9), 3.32 (1 H, dd, *J* = 3.9, 0.9 Hz, H-13), 2.90 (1 H, dd, *J* = 4.1, 1.1 Hz, H-14), 2.76 (1 H, q, *J* = 7.1 Hz, H-17), 2.73 (1 H, s, OH), 2.64 (1 H, m, H-3), 2.48 (1 H, m, H-11), 2.35 (2 H, t, *J* =

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7.5 Hz), 2.12 (1 H, m, H-3), 2.05 (3 H, s, OAc-Me), 1.64 (4 H, m), 1.40 (3 H, s, C-15 Me), 1.33 (3 H, d, J = 7.1 Hz, C-18 Me), 1.31 (2 H, m), 0.90 (3 H, d, J = 6.9 Hz, C-20 Me), 0.89 (3 H, t, J = 7.0 Hz). Compound 8 showed the following spectral characteristics: UV (MeOH) λ_{max} 237 nm (ϵ 6400); IR (CH_2Cl_2) 3580, 3050, 1780, 1730, 1370, 1240, 1170, 1015, 1000, 910 cm^{-1}; CIMS (NH_3), m/z (relative intensity) 503.5 (100) for $C_{28}H_{40}O_8$; HRMS (30 eV), m/z (relative intensity) 476.2810 (M^+ - CO, $C_{27}H_{40}O_7$, 11), 434.2681 (3), 360.1951 ($C_{21}H_{28}O_5$, 16), 176.0874 ($C_{11}H_{12}O_2$, 23), 166.0997 ($C_{10}H_{14}O_2$, 71), 124.0892 (81), 99.0812 (100); ^1H NMR (360 MHz, $CDCl_3$, 51 °C) δ 6.26 (1 H, d, J = 16.4 Hz, H-6), 5.76 (1 H, d, J = 16.4 Hz, H-7), 5.08 (1 H, dd, J = 7.6, 4.6 Hz, H-2), 5.00 (1 H, bs, H-16), 4.94 (1 H, bs, H-16), 4.87 (1 H, br t, J = 3.8 Hz, H-12), 4.26 (1 H, bs, H-9), 3.34 (1 H, t, J = 3.8 Hz, H-13), 2.81 (1 H, d, J = 3.8 Hz, H-14), 2.81 (1 H, q, J = 7.1 Hz, H-17), 2.32 (2 H, t, J = 7.3 Hz), 2.25 (1 H, m, H-3), 2.10 (3 H, s, OAc-Me), 1.95 (1 H, m, H-11), 1.84 (1 H, m, H-3), 1.63 (4 H, m), 1.32 (2 H, m), 1.31 (3 H, s, C-15 Me), 1.20 (3 H, d, J = 7.1 Hz, C-18 Me), 1.08 (3 H, d, J = 7.3 Hz, C-20 Me), 0.90 (3 H, t, J = 6.8 Hz). Product 9 showed the following spectral properties: UV (MeOH) λ_{max} 230 nm (ϵ 10500); IR (CH_2Cl_2) 3680, 3450-3400 br, 3050, 1780, 1735, 1370, 1185, 1070, 1035, 1000, 905 cm^{-1}; CIMS (NH_3), m/z (relative intensity), 424.2 ($M + NH_4^+$, 54), 406.5 (<1) for $C_{22}H_{30}O_7$; HRMS (20 eV), m/z (relative intensity) 378.2059 (M^+ - CO, $C_{21}H_{30}O_6$, 9), 336.1963 (5), 306.1472 ($C_{17}H_{28}O_5$, 32), 124.0890 (59), 109.0649 (59), 43.0194 (100); ^1H NMR (360 MHz, $CDCl_3$, 58 °C) δ 6.14 (1 H, d, J = 16.2 Hz, H-6), 5.83 (1 H, d, J = 16.2 Hz, H-7), 5.02 (1 H, m, H-2), 5.00 (1 H, bs, H-16), 4.97 (1 H, bs, H-16), 4.14 (1 H, d, J = 2.5 Hz, H-9), 3.87 (1 H, bs, H-12), 3.33 (1 H, dd, J = 4.2, 3.7 Hz, H-13), 2.95 (1 H, d, J = 3.7 Hz, H-14), 2.81 (1 H, ddd, J = 7.0 Hz, H-17), 2.41 (1 H, m, H-10), 2.11 (3 H, s, OAc-Me), 1.78 (1 H, m, H-11), 1.32 (3 H, s, C-15 Me), 1.18 (3 H, d, J = 7.0 Hz, C-18 Me), 1.03 (3 H, d, J = 7.5 Hz, C-20 Me). Following the identical procedure outlined above, solenolide A was refluxed for only 4 h. Upon workup, the product mixture was determined to be comprised of derivatives 7 (15.1 mg, 20% from 1) and 8 (32.5 mg, 43% from 1), with 31.0 mg (37%) of unreacted starting material remaining.

Conversion of Solenolide D (4) to Solenolide C (3). Following the same procedure described above, 15 mg (0.027 mmol) of compound 4 was refluxed in MeOH with 0.5 g of Zn/Cu couple for 4.5 h. Upon workup and HPLC purification, 12.5 mg (90% yield from 4) of a product, identical in all respects with solenolide C (3), was obtained.

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Synthesis of 2-Deoxy-2,2-difluoro-D-ribose and 2-Deoxy-2,2-difluoro-D-ribofuranosyl Nucleosides

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A program to synthesize fluorinated D-ribose and fluorinated nucleosides was initiated with hopes of finding compounds of potential value as anticancer and/or antiviral agents. Our approach is illustrated by a simple and stereocontrolled synthesis of 2-deoxy-2,2-difluoro-D-ribose. This was followed with the synthesis of a series of 1-(2-deoxy-2,2-difluororibofuranosyl)pyrimidine nucleosides. (*R*)-2,3-*O*-Isopropylideneglyceraldehyde was coupled with ethyl bromodifluoroacetate by using Reformatskii conditions to yield the carbon skeleton for the desired carbohydrate. Hydrolytic removal of the blocking groups with concomitant closure gave the γ-lactone 3. Reduction to the γ-lactol ultimately yielded 2-deoxy-2,2-difluoro-D-ribose (6). Functionalization of the difluoro carbohydrate with a leaving group at the anomeric position followed by displacement of the group with various pyrimidine bases yielded 1-(2-deoxy-2,2-difluororibofuranosyl)pyrimidine nucleosides.

The introduction of fluorine into a metabolite, such as a carbohydrate or a nucleoside, is a unique way of achieving distinctive modification with minimal disturbance of the overall stereochemistry. Replacing a hydrogen atom of the natural substrate with fluorine may alter the biochemical activity, producing a molecule that may inhibit one or more enzymes or be partly metabolized into an even more active substance. It has been argued that the van der Waals radii of the elements hydrogen (1.20 Å) and fluorine (1.35 Å) are sufficiently close to account for pseudosubstrate activity.[1]

A program was initiated to synthesize fluorinated D-ribose and fluorinated nucleosides with hopes of finding some unique biological activity. In recent years a renaissance of interest in the chemical synthesis of carbohydrates and functionalized nucleosides has occurred.[2] Our approach is illustrated by a simple and stereocontrolled synthesis of 2-deoxy-2,2-difluoro-D-ribose.[3] This was

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