Preparative Enzymatic Peptide Synthesis. In a typical synthesis, the ester donor Bz-Tyr-OEt (125 mg, 0.4 mmol) and the nucleophile acceptor (1.6 mmol) were incubated at 37 °C in 1,1,1-trichloroethane containing 0.8% MeOH and DIPEA and 0.09% or 1% water depending on whether the enzymatic catalyst was PEG-Chti or Chti-carbonate. The enzyme/substrate ratio was comparable to that used for small-scale experiments. The reaction was monitored by HPLC and stopped when all the ester substrate was used.

In the cases of Bz-Tyr-Phe-NH$_2$ and Bz-Lys-Phe-NH$_2$, the precipitated peptides were filtered, washed with benzene, and dried. Bz-Tyr-Phe-NH$_2$ was further washed with water and dried under vacuum. Bz-Lys-Phe-NH$_2$ was recrystallized from methanol/ether (3/1, v/v). Bz-Tyr-Tyr-NH$_2$ and Bz-Thr-Thr-NH$_2$ were washed with 1 mM HCl and crystallized from methanol/ether. Crystallization of Bz-Tyr-Ser-NH$_2$ under the same conditions was preceded by acetone precipitation of the excess of Ser-NH$_2$. In the cases of Bz-Tyr-Gly-NH$_2$ and Bz-Tyr-Lys-NH$_2$, the reaction mixture was dried and redissolved in MeOH. The excess nucleophile was discarded by chloroform precipitation, and the product was further purified by flash chromatography (CHCl$_3$/MeOH (3/1) for Bz-Tyr-Gly-NH$_2$ and CHCl$_3$/MeOH/CH$_3$COOH (32%) (10/3.5/1) for Bz-Tyr-Lys-NH$_2$).

Reutilization of the Biocatalyst. The reaction mixture in benzene (4 mL) consisted of 5 mM Bz-Tyr-OEt, 20 mM Phe-NH$_2$, about 0.1% (v/v) water, 1 μM PEG-Chti, 0.8% (v/v) MeOH, and DIPEA to solubilize the starting material and was incubated at 37 °C. The reaction was monitored by HPLC. When a plateau was reached, the peptide was separated by filtration and benzene, water, and reagents were added so that the volume, water content, and the ester/nucleophile ratio were maintained constant throughout the experiment.

Acknowledgment. J.V.S. thanks the Ministerio de Educacion y Ciencia for a grant (FAR 88-0276-C3).

Registry No. Chti, 9004-07-3; PEG, 25322-68-3; Bz-Tyr-OEt, 3483-82-7; Phe-NH$_2$, 5241-58-7; Tyr-NH$_2$, 4985-46-0; Thr-NH$_2$, 49705-99-9; Ser-NH$_2$, 6791-49-7; Gly-NH$_2$, 598-41-4; Arg-NH$_2$, 2788-83-2; Lys-NH$_2$, 32388-19-5; Glu-NH$_2$, 636-65-7; Bz-Tyr-Phe-NH$_2$, 119153-83-2; Bz-Tyr-Tyr-NH$_2$, 119153-84-3; Bz-Tyr-Thr-NH$_2$, 129678-28-0; Bz-Tyr-Ser-NH$_2$, 129678-29-1; Bz-Tyr-Gly-NH$_2$, 7369-86-0; Bz-Tyr-Arg-NH$_2$, 131684-49-6; Bz-Tyr-Lys-NH$_2$, 131684-50-9; Bz-Tyr-Glu-NH$_2$, 131704-13-7; Ac-Phe-OEt, 2361-96-8; Ac-Trp-OEt, 2382-80-1; Bz-Arg-OEt, 971-21-1; Bz-Lys-OMe, 17039-40-6; Bz-Ala-OMe, 7244-67-9; Z-Ser-OMe, 1676-81-9; Z-Glu-OBzl, 3705-42-8; Bz-Asp-OMe, 82933-21-9; Ac-Phe-Phe-NH$_2$, 24809-25-4; Ac-Trp-Phe-NH$_2$, 119206-29-0; Bz-Arg-Phe-NH$_2$, 66127-60-4; Bz-Lys-Phe-NH$_2$, 119237-54-6; Bz-Ala-Phe-NH$_2$, 71448-22-1; Z-Ser-Phe-NH$_2$, 25529-46-8; Z-Glu-Phe-NH$_2$, 130970-34-2; Bz-Asp-Phe-NH$_2$, 131684-51-0; agarose, 9012-36-6.

Fuscosides A–D: Antiinflammatory Diterpenoid Glycosides of New Structural Classes from the Caribbean Gorgonian *Eunicea fusca*

Jongheon Shin and William Fenical*

Scripps Institution of Oceanography, University of California, San Diego, La Jolla, California 92093-0228

Received November 5, 1990

Four new diterpenoid arabinose glycosides, fuscosides A–D (**1**, **3**, **5**, and **6**), have been isolated from the Caribbean gorgonian *Eunicea fusca* Duchassaing and Michelotti. Fuscoside A (**1**) is a diterpenoid 2′-*O*-acetylarabinose glycoside possessing a new bicyclic carbon skeleton related in biosynthetic origin to the eremophilane class of sesquiterpenoids. The structures of these new compounds were assigned on the basis of chemical and spectral studies. Fuscosides A and B (**1**, **3**) are effective topical antiinflammatory agents with potencies equivalent to indomethacin and manoalide. Fuscoside B (**3**) selectively inhibits the synthesis of leukotrienes LTB$_4$ and LTC$_4$, but not PGE$_2$, in the mouse peritoneal macrophage, suggesting it is a selective inhibitor of leukotriene synthesis.

Marine octocorals of the order Gorgonacea, the sea whips and sea fans (phylum Cnidaria) are abundant, chemically-rich invertebrates found in the reef and and shallow-water habitats of the Caribbean Sea.[1,2] As part of our interest in this interesting group of invertebrates,[3,4] we have focused our attention on the antiinflammatory metabolites produced by Caribbean gorgonians of the genera *Pseudopterogorgia* and *Eunicea*.[5–7] We recently described the pseudopterosins and secopseudopterosins, novel antiinflammatory and analgesic glycosides isolated from several species of *Pseudopterogorgia*.[8] Other gorgonian-derived glycosides include the muricins, aminogalactose glycosides possessing truncated steroidal aglycones, isolated from the pacific gorgonian *Muricea fruticosa*,[9] and moritoside, an aromatic glycoside isolated from a Japanese gorgonian of the genus *Euplexaura*.[10]

In this paper, we report the structures of four new diterpenoid arabinose glycosides, fuscosides A–D (**1**, **3**, **5**, and **6**), isolated from the Caribbean gorgonian *Eunicea fusca* Duchassaing and Michelotti. Fuscoside A (**1**) possesses a novel carbon skeleton that has a bicyclic component related to the eremophilane class of sesquiterpenoids. To the best of our knowledge, fuscoside A (**1**) is the first metabolite possessing this rearranged diterpenoid carbon

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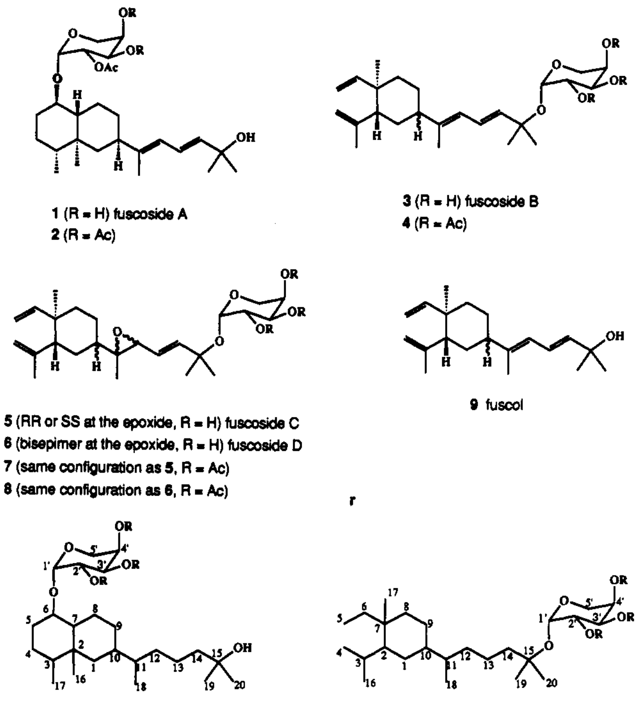
Table I. ^{13}C NMR Assignments for Compounds 1–4 and 7–9

C	1[a]	2[b]	3[a]	4[a]	7[a]	8[a]	9[a]
1	44.1 CH_2	44.5 CH_2	32.6 CH_2	32.5 CH_2	28.9 CH_2	29.8 CH_2	32.5 CH_2
2	37.7 C	37.7 C	52.7 CH	52.6 CH	52.1 CH	52.3 CH	52.6 CH
3	42.5 CH	42.6 CH	147.5 C	147.6 C	147.4 C	147.4 C	147.5 C
4	29.3 CH_2	29.5 CH_2	112.1 CH_2	112.1 CH_2	112.2 CH_2	112.3 CH_2	112.0 CH_2
5	34.3 CH_2	34.6 CH_2	109.9 CH_2	109.9 CH_2	110.0 CH_2	110.1 CH_2	109.6 CH_2
6	80.5 CH	80.9 CH	150.2 CH	150.2 CH	149.9 CH	149.9 CH	150.1 CH
7	51.7 CH	51.6 CH	39.7 C	39.8 C	39.8 C	39.9 C	39.6 C
8	22.7 CH_2	22.9 CH_2	39.8 CH_2	39.8 CH_2	39.3 CH_2	39.2 CH_2	39.7 CH_2
9	31.1 CH_2	31.3 CH_2	26.5 CH_2	26.5 CH_2	23.7 CH_2	23.0 CH_2	26.4 CH_2
10	41.9 CH	42.4 CH	47.7 CH	47.6 CH	46.3 CH	46.5 CH	47.5 CH
11	143.7 C	142.5 C	144.3 C	144.3 C	65.4 C	65.4 C	143.2 C
12	122.5 CH	123.2 CH	122.3 CH	122.2 CH	61.9 CH	62.2 CH	122.9 CH[c]
13	123.1 CH	123.7 CH	126.5 CH	126.2 CH	125.6 CH	124.9 CH	123.0 CH[c]
14	139.2 CH	140.1 CH	135.5 CH	135.5 CH	139.9 CH	140.4 CH	139.1 CH
15	70.9 C	70.4 C	77.5 C	77.5 C	76.8 C	77.0 C	70.9 C
16	12.3 CH_3	12.2 CH_3	24.7 CH_3	24.8 CH_3	24.8 CH_3	24.8 CH_3	24.7 CH_3
17	14.7 CH_3	14.8 CH_3	16.6 CH_3	16.6 CH_3	16.5 CH_3	16.5 CH_3	16.4 CH_3
18	14.9 CH_3	14.8 CH_3	15.4 CH_3	15.4 CH_3	14.0 CH_3	13.7 CH_3	15.3 CH_3
19	29.9 CH_3	30.3 CH_3	28.5 CH_3[d]	27.5 CH_3[d]	27.7 CH_3[d]	27.1 CH_3[d]	29.8 CH_3
20	29.9 CH_3	30.3 CH_3	26.1 CH_3[d]	26.4 CH_3[d]	25.8 CH_3[d]	26.3 CH_3[d]	29.8 CH_3
1′	98.4 CH	98.9 CH	93.9 CH	91.5 CH	91.5 CH	91.7 CH	
2′	72.5 CH	69.7 CH	69.6 CH	69.4 CH[e]	69.3 CH[e]	69.3 CH[e]	
3′	69.6 CH	69.5 CH	70.9 CH[e]	68.5 CH[e]	68.5 CH[e]	68.4 CH[e]	
4′	67.7 CH	67.9 CH	69.0 CH[e]	67.4 CH[e]	67.3 CH[e]	67.4 CH[e]	
5′	62.3 CH_2	60.8 CH_2	62.1 CH_2	60.2 CH_2	60.3 CH_2	60.3 CH_2	
OAc	171.4 C	169.9 C		170.5 C	170.4 C	170.4 C	
		169.9 C		170.3 C	170.3 C	170.3 C	
		169.7 C		170.2 C	170.2 C	170.2 C	
	20.9 CH_3	20.4 CH_3		21.0 CH_3	20.9 CH_3	21.0 CH_3	
		20.4 CH_3		20.9 CH_3	20.9 CH_3	20.9 CH_3	
		20.4 CH_3		20.8 CH_3	20.8 CH_3	20.8 CH_3	

[a] ^{13}C NMR spectra were recorded at 50 MHz in $CDCl_3$ and C_6D_6 solution. Numbers of attached protons were determined by DEPT experiments. The δ values are in ppm downfield from internal Me_4Si. Assignments for 1–3 were aided by XHCORR and COLOC experiments. Assignments for others were aided by comparison with **3**. [b] In C_6D_6 solution. [c–e] Carbons in the same column may be reversed.

skeleton. Fuscosides B–D (**3**, **5** and **6**) are glycosides possessing fuscol (**9**) aglycons. Fuscol was first isolated in 1978 as part of a prior investigation of this organism.[11] Fuscol derivatives have also been isolated from an Australian soft coral of the genus *Lobophytum*.[12]

Scheme I



1 (R = H) fuscoside A
2 (R = Ac)

3 (R = H) fuscoside B
4 (R = Ac)

5 (RR or SS at the epoxide, R = H) fuscoside C
6 (bisepimer at the epoxide, R = H) fuscoside D
7 (same configuration as 5, R = Ac)
8 (same configuration as 6, R = Ac)

9 fuscol

We collected *E. fusca* for the first time in 1986 as part of an expedition to Martinque and the Tobago Cays, in the eastern Caribbean Sea. Freshly collected animals were stored frozen and subsequently exhaustively extracted with dichloromethane. Silica vacuum flash chromatography followed by HPLC separation of the nonpolar fractions gave fuscol (**9**) as a major metabolite. In addition to fuscol, ^1H NMR analysis of the polar flash chromatographic fractions showed the presence of major quantities of several diterpene glycosides. Subsequently, purification by C_{18} reversed-phase flash chromatography followed by C_{18} reversed-phase HPLC separation yielded pure samples of fuscosides A–D (**1**, **3**, **5**, and **6**).

Fuscoside A (**1**) was isolated as a viscous oil that was analyzed for $C_{27}H_{44}O_7$ by high-resolution mass and ^{13}C NMR spectrometry. A carbon signal at δ 171.4 (s) in the ^{13}C NMR spectrum (Table I) and a strong absorption band at 1740 cm^{-1} in the IR spectrum indicated the presence of an ester. A sharp methyl singlet at δ 2.10 in the ^1H NMR spectrum revealed that the ester was an acetate (Table II). A ^{13}C NMR band at δ 98.4 (d) assigned to a sugar anomeric carbon and four carbons in the region between δ 60–75 confirmed that **1** was a pentose glycoside. A ^1H NMR COSY experiment revealed an isolated spin system of six protons in the region of δ 5.0–3.7. Through the use of XHCORR experiments, the sugar was identified as arabinose in a α-pyranoside configuration. The low-field shift of the C-2′ proton (δ 4.95) indicated that the acetate ester was at the sugar C-2′ position. The presence of an arabinose pyranoside in the compound was confirmed by acetylation. Treatment of **1** with acetic anhydride in pyridine gave a triacetate **2** as the major product. The ^1H NMR spectrum of **2** was very similar to **1**, with the

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Table II. ^{1}H NMR Assignments for Compounds 1–4, 7, and 8

no.	1^a	2^a	2^b	3^a	4^a	7^a	8^a
1	1.64 (1 H, br d, 12.7)	*c*	*c*	1.61 (1 H)^d	1.65–1.45 (2 H)^d	1.65–1.45 (2 H)^d	1.65–1.45 (2 H)^d
	0.94 (1 H, dd, 12.6, 12.6)	0.96 (1 H)^d		1.46 (1 H)^d			
2				2.01 (1 H, dd, 12.1, 3.8)	1.99 (1 H)^d	2.05–1.95 (1 H)^d	2.05–1.95 (1 H)^d
3	1.24–1.19 (1 H)^d	*c*	*c*				
4	1.49–1.39 (2 H)^d	*c*	*c*	4.82 (1 H, br s)	4.81 (1 H, br s)	4.85 (1 H, br s)	4.83 (1 H, br s)
		2.05 (1 H)^d	*c*	4.59 (1 H, br s)	4.58 (1 H, br s)	4.61 (1 H, br s)	4.59 (1 H, br s)
5	2.08 (1 H)^d	*c*	*c*	4.91 (1 H, br d, 15.6)	4.91 (1 H, br d, 16.2)	4.91 (1 H, br d, 16.5)	4.90 (1 H, br d, 16.4)
	1.45 (1 H)^d	*c*	*c*	4.90 (1 H, br d, 12.0)	4.90 (1 H, br d, 12.0)	4.91 (1 H, br d, 11.8)	4.89 (1 H, br d, 11.7)
6	3.20 (1 H, m)	3.18 (1 H, ddd, 10.1, 10.1, 4.5)	3.04 (1 H, m)	5.82 (1 H, dd, 17.8, 10.5)	5.82 (1 H, dd, 17.9, 10.5)	5.81 (1 H, dd, 17.7, 10.7)	5.79 (1 H, dd, 17.8, 10.5)
7	1.15 (1 H)^d	1.15 (1 H)^d	*c*				
8	2.01 (1 H, m)	1.97 (1 H)^d	*c*	1.58–1.44 (2 H)^d	1.65–1.45 (2 H)^d	1.65–1.45 (2 H)^d	1.65–1.45 (2 H)^d
	1.15–1.09 (1 H)^d	*c*	*c*				
9	1.72 (1 H, m)	*c*	*c*	1.58–1.44 (2 H)^d	1.65–1.45 (2 H)^d	1.65–1.45 (2 H)^d	1.65–1.45 (2 H)^d
	1.24–1.19 (1 H)^d						
10	2.13 (1 H, m)	2.15 (1 H)^d	*c*	1.98 (1 H)^d	1.99 (1 H)^d	2.05–1.95 (1 H)^d	2.05–1.95 (1 H)^d
12	5.83 (1 H, br d, 10.8)	5.84 (1 H, br d, 10.8)	6.02 (1 H, br d, 10.4)	5.88 (1 H, br d, 10.6)	5.83 (1 H, br d, 10.5)	3.22 (1 H, d, 6.6)	3.20 (1 H, d, 6.6)
13	6.47 (1 H, dd, 15.3, 10.8)	6.47 (1 H, dd, 15.3, 10.8)	6.66 (1 H, dd, 15.2, 10.8)	6.44 (1 H, dd, 15.5, 10.7)	6.39 (1 H, dd, 15.5, 10.7)	5.57 (1 H, dd, 15.9, 6.5)	5.53 (1 H, dd, 15.9, 6.6)
14	5.75 (1 H, d, 15.3)	5.75 (1 H, d, 15.3)	5.73 (1 H)^d	5.60 (1 H, d, 15.4)	5.54 (1 H, d, 15.5)	5.79 (1 H, d, 15.9)	5.84 (1 H, d, 15.9)
16	0.73 (3 H, s)	0.73 (3 H, s)	0.51 (3 H, s)	1.71 (3 H, br s)	1.70 (3 H, br s)	1.71 (3 H, br s)	1.71 (3 H, br s)
17	0.76 (3 H, d, 6.6)	0.76 (3 H, d, 6.4)	0.69 (3 H, d, 6.2)	1.01 (3 H, s)	1.00 (3 H, s)	1.00 (3 H, s)	1.00 (3 H, s)
18	1.74 (3 H, d, 0.9)	1.74 (3 H, br s)	1.70 (3 H, br s)	1.78 (3 H, br s)	1.77 (3 H, br s)	1.22 (3 H, s)	1.21 (3 H, s)
19	1.35 (3 H, s)	1.35 (3 H, s)	1.22 (3 H, s)	1.35 (3 H, s)^e	1.31 (3 H, s)^e	1.31 (3 H, s)^e	1.30 (3 H, s)^e
20	1.35 (3 H, s)	1.35 (3 H, s)	1.22 (3 H, s)	1.42 (3 H, s)^e	1.35 (3 H, s)^e	1.33 (3 H, s)^e	1.33 (3 H, s)^e
1′	5.00 (1 H, d, 3.7)	5.08 (1 H, d, 3.6)	5.30 (1 H, d, 3.1)	5.09 (1 H, d, 2.2)	5.21 (1 H, d, 3.6)	5.23 (1 H, d, 3.5)	5.21 (1 H, d, 3.5)
2′	4.95 (1 H, dd, 9.6, 3.7)	5.12 (1 H, ddd, 10.4, 3.5, 1.4)	5.63 (1 H, dd, 11.2, 3.2)	3.76 (1 H)^d	5.11 (1 H, dd, 10.1, 3.7)	5.10 (1 H, dd, 10.3, 3.5)	5.13 (1 H, dd, 10.3, 3.5)
3′	4.02 (1 H, dd, 9.9, 3.6)	5.34 (1 H)^d	5.74 (1 H)^d	3.76 (1 H)^d	5.36 (1 H, dd, 10.4, 3.6)	5.37 (1 H)^d	5.35 (1 H)^d
4′	4.00 (1 H, br s)	5.33 (1 H, br s)	5.56 (1 H, br s)	3.98 (1 H, br s)	5.33 (1 H, br s)	5.33 (1 H, br s)	5.33 (1 H, br s)
5′	3.98 (1 H)^d	4.06 (1 H, br d, 12.9)	3.85 (1 H, br d, 13.0)	3.95 (1 H)^d	4.14 (1 H, dd, 13.0, 1.0)	4.14 (1 H, br d, 12.9)	4.12 (1 H, br d, 13.0)
	3.70 (1 H, dd, 10.9, 1.8)	3.63 (1 H, dd, 12.9, 1.3)	3.46 (1 H, dd, 13.0, 1.2)	3.68 (1 H, dd, 12.6, 1.8)	3.64 (1 H, dd, 13.0, 1.9)	3.65 (1 H, dd, 12.9, 1.3)	3.63 (1 H, br d, 13.0)
OAc	2.10 (3 H, s)	2.14 (3 H, s)	1.78 (3 H, s)		2.12 (3 H, s)	2.13 (3 H, s)	2.13 (3 H, s)
		2.04 (3 H, s)	1.77 (3 H, s)		2.07 (3 H, s)	2.08 (3 H, s)	2.08 (3 H, s)
		2.01 (3 H, s)	1.65 (3 H, s)		2.01 (3 H, s)	2.00 (3 H, s)	2.01 (3 H, s)

[a] ^{1}H NMR spectra were measured at 360 MHz in $CDCl_3$ solution. Assignments were aided by spin decoupling and COSY experiments. *J* values are reported in Hz and chemical shifts are given in δ units (ppm downfield from Me_4Si). [b] In C_6D_6 solution. [c] Unassignable proton resonances. [d] Coupling constants were not measured. [e] Protons in the same column may be reversed.

downfield shifts of the sugar methine protons as the only differences. A ^{1}H NMR COSY experiment confidently assigned all of the sugar protons (Table II). Unfortunately, attempts to isolate the sugar by hydrolysis were unsuccessful and resulted in overall decomposition.

A combination of ^{1}H NMR COSY and XHCORR data revealed the aglycon component of fucoside A to possess the same C_8 side chain as found in fuscol (9). The chemical shift of the C-15 carbon (δ 70.9) was identical with the carbon shift from the free hydroxyl bearing carbon (C-15) in fuscol. Thus, compound 1 was proposed to possess a tertiary alcohol at C-15. This assignment was supported by trichloroacetylation of 2 with trichloroacetyl isocyanate and measurement of the chemical shifts of relevant protons. The chemical shifts of most protons (measured in benzene-d_6 solution) were unchanged by the esterification, while the C-19 and -20 methyl protons were shifted from δ 1.22 (6 H, s) to 1.66 (6 H, s). Also, the C-14 olefinic proton was shifted from δ 5.73 to 6.42. Thus, compound 1 was confirmed to possess a free hydroxyl group at C-15.

The remaining components of the diterpenoid aglycon were also identified by interpretation of combined spectral data. Consideration of the molecular formula revealed that the remaining diterpenoid component had a formula of $C_{12}H_{20}$. Since there were no olefinic carbons in the ^{13}C NMR spectrum except those from the side-chain diene, the C_{12} component must be a saturated bicyclic system. The combination of ^{13}C NMR, COSY NMR, and XHCORR data revealed four partial structures (Figure 1).

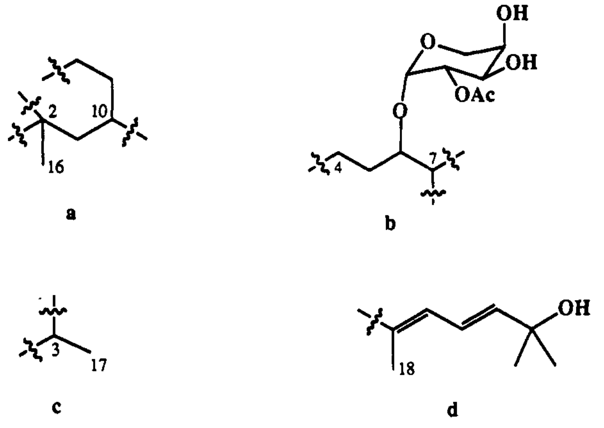


Figure 1. Partial structures a–d for fucoside A (1).

A methyl singlet at δ 0.73 was readily assigned as attached to the carbon at δ 37.7, which was the only quaternary carbon of the bicyclic component. COSY NMR data revealed a spin system of seven protons. XHCORR data showed that these protons were attached to four carbons at δ 44.1 (t), 41.9 (d), 31.1 (t), and 22.7 (t). Consideration of the coupling pattern revealed that one end of the spin system (methylene protons at δ 1.64 (1 H, br d, 12.7) and 0.94 (1 H, dd, 12.6, 12.6) attached to the carbon at δ 44.1) was connected to the quaternary carbon at δ 37.7 yielding partial structure a. A downfield band at δ 3.20 was readily assigned to the methine proton on the carbon bearing the

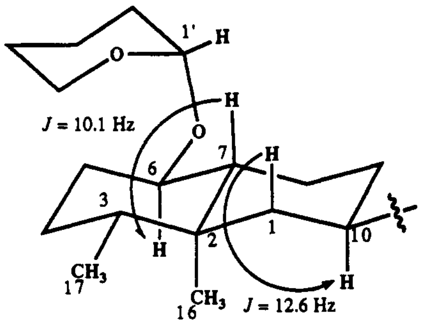
Table III. Results of Two-Dimensional Carbon–Proton Long-Range Correlation Experiments (COLOC) with Compounds 1–3[a]

C	1[b,d]	2[b–e]	3[b]
1	10, 16 (17)[f]	16	
2	16 (17)[f]	16, 17	
3	16 (17)[f]	16, 17	16
4		17	
5			
6			17
7	16 (17)[f]	16	5 (4.91),[g] 17
8			
9			
10	18	18	12, 18
11	18	18	18
12	18	14, 18	13, 18
13		12, 14	14
14	12, 19 (20)[f]	12, 19 (20)[f]	12, 13, 19 (20)[f]
15	19 (20)[f]	13, 14, 19 (20)[f]	13, 19 (20),[f] 1
16			
17			
18		10, 12	12
19			
20			
1′	5′ (3.70)[g]	5′ (3.46)[g]	
2′			
3′		1′, 5′ (3.46)[g]	
4′	5′ (3.70)[g]	5′ (3.85),[g] 5′ (3.46)[g]	
5′		1′	1′

[a] Experiments for 1 and 3 were performed at 50 MHz in $CDCl_3$ solution. Experiments for 2 were performed at 50 MHz in C_6D_6 solution. [b–e] Parameters were optimized for 6-, 8-, 10-, and 12-Hz coupling constants, respectively. [f] Signals not well resolved. [g] Chemical shifts of protons that correlate.

α-arabinopyranoside. This proton was coupled by three protons at δ 2.08, 1.45, and 1.15. XHCORR data showed that these protons were attached to the carbons at δ 34.3 (t, the former two protons) and 51.7 (d, proton at δ 1.15). The proton at δ 2.08 was further coupled to two proton resonances at δ 1.49–1.39, which correlated to a common methylene carbon at δ 29.3, thus yielding partial structure b. A doublet methyl group at δ 0.76 (J = 6.6 Hz) was attached to a methine carbon (partial structure c). Due to overlapping of high-field proton signals, the methine proton could not be assigned precisely (δ 1.24–1.19). The C_8 side chain as found in fuscol (9) was recognized as partial structure d. The geometries of the double bonds were assigned on the basis of NMR data, the $\Delta^{11,12}$ olefin as E (on the basis of the shielded methyl carbon resonance at δ 14.9) and the $\Delta^{13,14}$ olefin as E (on the basis of the clear trans olefin coupling constant of 15.3 Hz).

Linking these partial structures was accomplished by COLOC NMR experiments (Table III). The long-range coupling between the C-10 carbon and the C-18 protons allowed connection of partial structures a and d. COLOC data showed several carbon–proton correlations relating methyl protons. However, due to the similar chemical shifts of the C-16 and -17 methyl protons (δ 0.73 and 0.76, respectively), further interpretation was impossible. Finally, COLOC experiments were performed with the triacetate 2 in benzene-d_6 solution (in benzene-d_6, the C-16 and -17 protons were observed at δ 0.51 and 0.69, respectively (Table II). Long-range correlations of the C-1, C-2, and C-3 carbons to the C-17 and C-16 protons allowed partial structures a and c to be connected. Also, coupling between the carbon at δ 29.5 (C-4, structure d) and the C-17 protons allowed connection of b to c. Connection of a and b was also achieved by the observation of coupling between the C-7 carbon (δ 51.6) and the C-16 methyl protons.



Results of a ^1H NMR NOEDS experiment with compound 2

proton (s) irradiated	proton (s) enhanced	measured enhancements (%)
6	16, 1′	4.1, 8.5
16	6, 10, 17	5.8, 3.2, 8.8
17	16	4.2

Figure 2. Configurations of the bicyclic components of 1 and 2 based on proton NMR coupling constants and the results of an NOEDS experiment.

Fuscoside A (1) has five asymmetric carbon centers (C-2, -3, -6, -7, and -10), the relative stereochemistries of which were determined by a combination of ^1H NMR, ^{13}C NMR, and ^1H NMR NOEDS data (Figure 2). The high-field chemical shift of the C-16 methyl carbon (δ 12.3) indicated a trans diaxial ring junction. Large vicinal coupling (J = 12.6 Hz) between the C-1 (δ 0.94) and C-10 protons revealed that the C-10 proton was axial on the cyclohexane ring. By the same method, the large coupling (J = 10.1 Hz observed for compound 2) between the C-6 and C-7 protons illustrated the trans diaxial orientation for these protons. These assignments were confirmed by ^1H NMR NOEDS experiments. Irradiation of the C-16 methyl protons of 2 enhanced the C-6 and C-10 protons by 5.8 and 3.2%, respectively, supporting their axial orientations. At the same time, the C-17 methyl protons were enhanced by 8.8%, indicating the syn orientation between the C-16 and C-17 methyl groups. Irradiation of the C-6 proton enhanced both the C-16 and C-1′ protons by 4.1 and 8.5%. Finally, the C-16 protons were enhanced by the irradiation of the C-17 protons (4.2%). Thus, the overall structure of fucoside A (1) could be assigned with confidence, with relative stereochemistry as $2S^*$, $3R^*$, $6R^*$, $7R^*$, and $10R^*$.

Fuscoside B (3) was isolated as an oil that was analyzed for $C_{25}H_{40}O_5$ by high-resolution mass and ^{13}C NMR spectrometry. The chemical shifts of many signals in the ^1H and ^{13}C NMR spectra of 3 were very similar to those from fuscol (Tables I and II). However, five additional carbon signals at δ 93.9 (d), 70.9 (d), 69.6 (d), 69.0 (d), and 62.1 (t) indicated the presence of a pentose pyranoside. Corresponding sugar resonances were also observed in the ^1H NMR spectrum δ 5.09 (1 H, d, J = 2.2 Hz) and 4.0–3.6 (5 H). Due to overlapping of the proton signals, the structure of the sugar was determined by spectral analysis of the corresponding triacetate derivative. Acetylation of 3 with acetic anhydride in pyridine gave the triacetate 4 in high yield. A ^1H NMR COSY experiment with this derivative allowed the confident assignment of the sugar protons and indicated an α-arabinopyranoside component as in 1 (Table II). The complete structure of 3 was confirmed by XHCORR and COLOC NMR experiments (Table III). All of the proton-bearing carbons and their protons were precisely matched. The long-range coupling between the C-15 carbon and the C-1′ sugar proton confirmed the position of the arabinoside on the side chain. Also, several carbon–proton couplings confirmed the diterpenoid aglycon component of **3**. Thus, the structure of fuscoside B (**3**) was unambiguously determined as fuscol C-15 α-arabinopyranoside. As in previous studies,[11] spectral data could not be used to determine the relative stereochemistry at C-10.

Two closely related metabolites, fuscosides C and D (**5** and **6**), were isolated as a mixture. Isolation of the pure metabolites was unsuccessful with both silica and C_{18} reversed-phase HPLC. Finally, separation was achieved for the mixture of triacetate derivatives produced by treatment of the natural mixture with acetic anhydride in pyridine. Separation by HPLC (30% EtOAc in isooctane) gave roughly equal amounts of the two acetates **7** and **8** (12.1 and 12.9 mg, respectively).

Fuscosides C and D triacetate (**7**, **8**) were analyzed for $C_{31}H_{46}O_9$ by high-resolution mass and ^{13}C NMR spectrometry. Spectral data from these compounds were highly comparable with that from the triacetate **4**. The only differences in the ^{13}C NMR spectra were the replacement of two olefinic carbons in **4** by epoxide signals (δ 60–66) in compounds **7** and **8** (Table I). Corresponding differences were also found in the ^{1}H NMR spectra (Table II). The C-12 olefinic proton (δ 5.83) of **4** was significantly shifted upfield in **7** (δ 3.22) and **8** (δ 3.20). In addition, the C-18 vinyl methyl protons were shifted upfield (from δ 1.77 to 1.22 in **7** and 1.21 in **8**). Therefore, **7** and **8** were assigned as the epimeric pair of 11,12-epoxide derivatives of triacetate **4**. Other data supporting this assignment were the UV properties of these derivatives showing the disappearance of the strong absorption (241 nm, ε 44 000) characteristic of the fuscol side chain. The structures of the epoxides were confirmed by synthesis. Epoxidation of fuscoside B (**3**) with *m*-chloroperbenzoic acid (*m*-CPBA) in buffered dichloromethane gave **5** and **6** as the major products. On the basis of the insertion mechanism of the *m*-CPBA epoxidation, the absolute configurations of the epoxides must be 11*R*, 12*R*, and 11*S*, 12*S*. The respective assignments and configurations for each epoxide isomer could not be determined, however, by spectral methods.

The absolute stereochemistry of arabinose in the fuscosides was also not determined with confidence. Several attempts to isolate the free sugar by hydrochloric acid hydrolysis of **3** resulted in unexpected decomposition of both the sugar and aglycon. Hence, because limited sample size precluded continued attempts to hydrolyze the fuscosides, the absolute stereochemistry of arabinose was predicted by comparison of the optical rotations of fuscol and fuscoside B (**3**). By use of Hudson's Rules of Isorotation,[13] the molecular rotation [M] of **3** was defined as sum of the $[M]_{fuscol} + [M]_{sugar}$. From the measured optical rotations of fuscol[11] and **3**, their molecular rotations were calculated as +51° and −378°, respectively. Therefore, the molecular rotation of the sugar was expected to be −429°. The measured molecular rotations of D- and L-arabinose were −156° and +155°, respectively (after 22.5 h).[14] Although there was substantial difference between the calculated and actual values of the molecular rotations of the sugar, the large negative value seems to indicate that arabinose occurs in the less common D configuration.

Fuscol (**9**) was isolated as an oil. The ^{1}H NMR spectrum of **9** was in full accord with reported data for this metabolite. Moreover, the optical rotation of **9** was very similar ($[\alpha]_D$ +17.6°, fuscol +16.3°) to the reported value.[11]

Samples of *E. fusca* were also collected in the Florida Keys (July 1987) and at St. Croix Island (November 1987). The results of comprehensive TLC analysis, using authentic standards, showed that the chemical compositions of these animals were identical with earlier Martinique and the Tobago Cays collections.

Fuscosides A and B (**1**, **3**) are potent topical antiinflammatory agents.[17] At testing doses of 50 μg, fuscoside A reduces phorbol ester (PMA)-induced mouse ear edema by 85%. In the same experiment, fuscoside B (**3**) reduced edema by 52%. For calibration, indomethacin and manoalide reduced edema by 80 and 38%, respectively, at the same concentration. In experiments using murine macrophage cells as a model for prostaglandin and leukotriene synthesis (calcium ionophore activated arachidonic acid metabolism), fuscoside B (**3**) selectively blocked the production of LTC_4, but had no effect upon the production of PGE_2. Thus, fuscoside B appears to be a selective inhibitor of leukotriene synthesis, an important target for the design of new selective antiinflammatory agents.[17]

Experimental Section

General Procedures. ^{1}H NMR and COSY spectra were recorded in $CDCl_3$ and C_6D_6 solutions. NOE difference spectroscopy (NOEDS) experiments were performed in general as outlined by Hall and Sanders.[15] ^{13}C NMR spectra, direct (XHCORR), and long-range (COLOC) carbon–proton correlation spectra were recorded in $CDCl_3$ and C_6D_6 solutions at 50 MHz. All chemical shifts are reported with respect to internal Me_4Si. Both high- and low-resolution mass measurements were supplied by the Mass Spectrometry Service Laboratory at the University of Minnesota. Optical rotations were measured with use of a 10-cm microcell. All solvents used were spectral grade or distilled from glass prior to use.

Collection and Extraction. *E. fusca* (specimen number CI 86-195)[16] was collected by hand using SCUBA at 20–25-m depth in July 1986 at Martinique and the Tobago Cays in the eastern Caribbean Sea. The collection was surface air-dried in the shade and immediately frozen. The gorgonians were next exhaustively extracted with CH_2Cl_2, and the combined extracts were evaporated to yield 40 g of crude organic materials (from 1.5-kg dry weight of the gorgonian). Fuscol (**9**), the major metabolite, was isolated by silica vacuum flash chromatography followed by silica HPLC (15% EtOAc in isooctane) of the fraction eluted with 20% EtOAc in isooctane. Polar flash chromatographic fractions (70–100% EtOAc in isooctane) were combined and refractionated by C_{18} reversed-phase (RP) flash vacuum chromatography. Final purifications by C_{18} RP-HPLC (100–90% MeOH/H_2O) gave fuscosides A–D (**1**, **3**, **5**, and **6**). *E. fusca* (specimen number F 87-23)[16] was also collected at the Florida Keys in July 1987. TLC analysis using authentic standards of **1**, **3**, **5**, and **6** showed that the chemical components of the animals were identical with those collected from the eastern Caribbean Sea. Several specimens were also collected from St. Croix, U. S. Virgin Islands, in November 1987. The results of TLC analysis were again identical with the other collections.

Fuscoside A (1). Fuscoside A was isolated as an oil by C_{18} RP-HPLC (90% MeOH/H_2O). The extract yielded 95 mg (0.2% of the extract) of **1**, which showed $[\alpha]_D$ −64° (*c* 0.6, $CHCl_3$) and exhibited the following spectral features: HRMS *m/z* M^+ obsd 480.3113, $C_{27}H_{44}O_7$ required 480.3088, $(M - C_7H_{11}O_6 - H_2O)^+$ obsd 271.2429, $C_{20}H_{31}$ required 271.2427; low-resolution MS *m/z* (relative intensity) 480 (4), 462 (7), 288 (7), 271 (14), 217 (9), 189 (14), 175 (53), 161 (17), 157 (40), 135 (13), 125 (22), 119 (16), 107 (71), 95 (31), 81 (39), 43 (100); IR (film) 3420, 2975, 2930, 1740,

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[13] (a) Ripperger, H.; Preiss, A.; Schmidt, J. *Phytochemistry* **1981**, *20*, 2434. (b) Lemieux, R. U.; Koto, S. *Tetrahedron* **1974**, *30*, 1933.

[14] Arabinose shows mutarotation. L-Arabinose shows $[\alpha]_D$ +173° (6 min), +105.1° (22.5 h). The Merck Index, 9th edition.

[15] Hall, L. D.; Sanders, J. K. M. *J. Am. Chem. Soc.* **1980**, *102*, 5703.

[16] Specimens of *E. fusca*, under the code names Fenical CI86-195 and F87-23, are on deposit in the octocoral collection, Smithsonian Institution, Washington, DC under the curatorship of Dr. Frederick M. Bayer.

[17] Jacobson, P. B.; Jacobs, R. S. *Fed. Proc.*, **1991**, *805*, A510.

1650, 1450, 1375, 1240, 1145, 1055, 1010, 760 cm^{-1}; UV (MeOH) 239 nm (ϵ 28 000).

Acetylation of Fuscoside A (1). To a stirred solution of 55 mg (0.12 mmol) of **1** in 3 mL of dry pyridine was added 2 mL of dry acetic anhydride. After the mixture was stirred for 3 h, pyridine and excess acetic anhydride were removed under vacuum. Purification by HPLC (45% EtOAc in isooctane) gave 42 mg (0.07 mmol, 65% yield) of **2** as the major product. The acetate **2** was characterized by NMR data (Tables I–III).

Reaction of 2 with Trichloroacetyl Isocyanate. The acylation reaction was performed in an NMR tube in C_6D_6 solution. After the proton chemical shifts of **2** (Table II) were measured, one drop of neat trichloroacetyl isocyanate was added. After the tube was shaken for 5 min, the ^{1}H NMR spectrum showed that ca. 70% of the reactant (by integration of downfield protons), had been converted to product. The ^{1}H NMR data of the product were as follows: δ 7.98 (1 H, s, –NH), 6.76 (1 H, dd, 15.4, 11.1, H-13), 6.42 (1 H, d, 15.1, H-14), 5.98 (1 H, br d, 11.5, H-12), 5.74 (1 H, dd, 11.2, 3.4, H-3′), 5.61 (1 H, dd, 11.3, 3.6, H-2′), 5.54 (1 H, br s, H-4′), 5.30 (1 H, d, 3.3, H-1′), 3.86 (1 H, br d, 13.0, H-5′), 3.47 (1 H, dd, 13.0, 1.3, H-5′), 3.05 (1 H, m, H-6), 1.79 (3 H, s, –OAc), 1.77 (6 H, s, –OAc and H-18), 1.69 (3 H, s, –OAc), 1.66 (6 H, s, H-19 and -20), 0.68 (3 H, d, 6.1, H-17), 0.51 (3 H, s, H-16).

Fuscoside B (3). Fuscoside B was isolated as an oil by C_{18} RP-HPLC (90% MeOH/H_2O). The extract yielded 1.0 g (2.5% of the extract) of fuscoside A, which showed $[\alpha]_D$ −90° (*c* 1.0, $CHCl_3$) and displayed the following spectral features: HRFABMS *m/z* (M + Na)$^+$ obsd 443.2773, $C_{25}H_{40}O_5Na$ required 443.2775; HREIMS *m/z* (M − $C_5H_8O_4$)$^+$ obsd 288.2452, $C_{20}H_{32}O$ required 288.2455; low-resolution MS *m/z* (relative intensity) 288 (2), 271 (35), 189 (14), 161 (18), 147 (21), 135 (22), 119 (51), 109 (63), 93 (50), 81 (49), 73 (100), 69 (43), 43 (50); IR (film) 3400, 2980, 2930, 1635, 1440, 1260, 1135, 1080, 990 cm^{-1}; UV (MeOH) 241 nm (ϵ 44 000).

Acetylation of Fuscoside B (3). To a stirred solution of 24 mg (0.05 mmol) of **3** in 2 mL of dry pyridine was added 1.5 mL of dry acetic anydride. After the mixture was stirred for 3 h at 80 °C, pyridine and excess acetic anhydride were removed under vacuum. The triacetate **4** was obtained quantitatively. ^{1}H and ^{13}C NMR data for **4** are shown in Tables I and II, respectively.

Isolation of Fuscosides C and D (5, 6). A mixture (~1:1 by integration of peaks in the ^{1}H NMR spectrum) of the epoxides **5** and **6** was isolated as an oil by C_{18} RP-HPLC (90% MeOH/H_2O). The extract gave 25 mg (0.06% of the extract) of the mixture of **5** and **6**. Several attempts to separate the mixture by C_{18} RP-HPLC failed.

Acetylation of Fuscosides C and D (5, 6). To a stirred solution of 25 mg of the 1:1 mixture of **5** and **6** in 2 mL of dry pyridine was added 1 mL of dry acetic anhydride. After the mixture was stirred for 2 h, pyridine and excess acetic anhydride were removed under vacuum. Separation by HPLC (30% EtOAc in isooctane) gave the triacetates **7** (12.1 mg) and **8** (12.9 mg) as the major products. Compound **7**, an oil, showed $[\alpha]_D$ −62° (*c* 0.7, $CHCl_3$) and displayed the following spectral features: HRFABMS *m/z* (M + H)$^+$ obsd 563.3199, $C_{31}H_{47}O_9$ required 563.3220; IR (film) 2980, 2940, 1750, 1640, 1440, 1375, 1250, 1230, 1135, 1070, 1015, 895, 750 cm^{-1}; UV (MeOH) no λ_{max}. Compound **8**, an oil, exhibited $[\alpha]_D$ −87° (*c* 0.5, $CHCl_3$) and displayed the following spectral features: HRFABMS *m/z* (M + H)$^+$ obsd 563.3168, $C_{31}H_{47}O_9$ required 563.3220; IR (film) 2980, 2940, 1750, 1640, 1440, 1375, 1250, 1230, 1135, 1075, 1020, 895, 760 cm^{-1}; UV (MeOH) no λ_{max}.

Epoxidation of Fuscoside B (3). To a stirred solution of 30 mg (0.07 mmol) of **3** in 4 mL of CH_2Cl_2 buffered with anhydrous Na_2HPO_4 was added 1.3 equiv of mCPBA. After the mixture was stirred for 2 h at room temperature, the CH_2Cl_2 layer was washed with 10% Na_2SO_3 (2 × 20 mL), 5% $NaHCO_3$ (2 × 10 mL), and finally with brine. After the solvent was evaporated and the mixture was dried under vacuum, purification by C_{18} RP-HPLC (95% MeOH/H_2O) gave 13.4 mg (0.03 mmol, 43% combined yield) of a 1:1 mixture of **5** and **6** and 14.6 mg (49%) of unreacted **3**. The ^{1}H NMR spectrum of the mixture was identical with that derived from the natural mixture.

Fuscol (9). Fuscol was isolated as an oil by silica HPLC (15% EtOAc in isooctane). The extract gave 2.5 g (6% of the extract) of fuscol, which showed $[\alpha]_D$ +17.6° (*c* 0.9, $CHCl_3$): ^{1}H NMR ($CDCl_3$) δ 6.49 (1 H, dd, 15.3, 10.8, H-13), 5.87 (1 H, br d, 10.7, H-12), 5.82 (1 H, dd, 17.8, 10.4, H-6), 5.77 (1 H, d, 15.2, H-14), 4.90 (1 H, d, 16.5, H-5), 4.90 (1 H, d, 10.6, H-5), 4.81 (1 H, d, 1.4, H-4), 4.58 (1 H, br s, H-4), 2.03 (1 H, dd, 12.1, 3.9, H-2), 1.99 (1 H, m, H-10), 1.79 (3 H, s, H-18), 1.70 (3 H, s, H-16), 1.36 (6 H, s, H-19 and H-20), and 1.01 (3 H, s, H-17). ^{13}C NMR data for **9** are shown in Table I.

Acknowledgment. This research is a result of combined research and ship funding from the National Science Foundation, chemistry and Oceanography Divisions, under Grant CHE86-20217, and from the California Sea Grant Program, under Grant NA89AA-D-SG140 (project No. R/MP-39), and in part by the California State Resources Agency. We thank the captain and crew of the research vessel Columbus Iselin (University of Miami) for their aid and assistance. We gratefully acknowledge the governments of Martinique and the Tobago Cays for permission to perform research in their territorial waters and appreciate the taxonomic assignments provided by Dr. Frederick M. Bayer, Smithsonian Institution, Washington, DC. We particularly thank Professor R. S. Jacobs and Peer Jacobson for providing pharmacological data prior to publication.

Supplementary Material Available: Copies of the ^{13}C NMR spectra for fuscosides A, B, and C/D mixture and C and D triacetates (**1**, **3**, **5**–**8**) (5 pages). Ordering information is given on any current masthead page.