of sodium borohydride. The reaction mixture was stirred at room temperature for 17 h and then at 80 °C for 108 h. Aqueous workup gave back starting material **4c**. No evidence of any reaction product was detected.

Borane–Tetrahydrofuran Complex. To a stirred suspension of 500 mg (1.3 mmol) of **4c** in 16 mL of dry tetrahydrofuran, maintained at 1–5 °C, was added 1.3 mL (1.3 mmol) of a 1.0 M solution of borane–tetrahydrofuran in tetrahydrofuran. After 4 h of stirring at room temperature, no reaction products were detected by TLC (chloroform–methanol–ammonium hydroxide [75:24:1]). Addition of another 1.3 mL (1.3 mmol) of 1.0 M BH_3–THF and refluxing for 3.5 h similarly failed to effect any detectable reaction. Following the addition of a further 3.9 mL (3.9 mmol) of 1.0 M BH_3–THF and another 10 h of reflux, TLC revealed the presence of a multicomponent mixture containing spots corresponding to both starting material **4c** and desired product **5c** in addition to unidentified materials.

Lithium Aluminum Hydride. (a) In Dimethoxyethane. Substrate **4c** (500 mg, 1.26 mmol) was silylated by the general procedure. A solution of the silylated substrate in 10 mL of dimethoxyethane (dried over 4A sieves) was treated with 45 mg (1.1 mmol) of lithium aluminum hydride, and the resultant mixture was allowed to reflux under a nitrogen atmosphere for 21 h. An aliquot of the reaction mixture was quenched with 3 M HCl and subjected to aqueous workup. TLC and ^1H NMR analyses of the solid thus isolated showed only unchanged starting material.

(b) In Dioxane. Substrate **4c** (500 mg, 1.26 mmol) was silylated by the general procedure. A solution of the silylated substrate in 15 mL of dioxane (dried over 4A sieves) was treated with a total of 560 mg (14.8 mmol) of lithium aluminum hydride, which was added in four portions over a period of 6 days, during which time the reaction mixture was maintained at 90 °C. TLC analysis of an aliquot (aqueous workup) showed only unchanged starting material. Another 140 mg (3.65 mmol) of lithium aluminum hydride was added, and heating at 90 °C was continued for another 3 days. Aqueous workup of the reaction mixture gave 240 mg (~50% recovery) of a gum which TLC revealed to be a complex mixture of materials. The complexity of the mixture was further confirmed by ^1H NMR ($CDCl_3$) and mass spectra, neither of which contained evidence of the presence of desired product **5c**.

Calculation of Heats of Formation. The calculations were carried out on an IBM 3081 Model K computer operating at 15 Mips using the MNDO molecular orbital approximation.[28] The MNDO program was obtained through QCPE,[29] converted to VS FORTRAN 77, and adapted to run on the IBM 3081 computer. The structural input was generated by using the MOPAC/SYBYL[30] interface, and the geometries were found by minimizing the total energy using the standard Davidon–Fletcher–Powell[31] optimization procedure. All geometric variables were allowed to optimize.

X-ray Crystal Structure Analysis of 5c. Crystal data: $C_{20}H_{23}N_5O_3$, M_r 381.44, monoclinic, a = 13.559 (3) Å, b = 19.366 (2) Å, c = 20.328 (4) Å, β = 132.94 (2)°, V = 3907.6 Å^3, Z = 8, D_{calcd} = 1.297 g cm^{-3}, μ(Cu Kα radiation, λ = 1.5418 Å) = 7.0 cm^{-1}. Space group $P2_1/c(C_{2h}^5)$ uniquely from the systematic absences: $0k0$ when $k \neq 2n$, $h0l$ when $l \neq 2n$. Sample dimensions: 0.12 × 0.26 × 0.60 mm.

Preliminary unit-cell parameters and space group information were provided by oscillation and Weissenberg photographs. Intensity data ($h,k,\pm l$) were recorded on an Enraf-Nonius CAD-4 diffractometer (Cu Kα radiation, incident-beam graphite monochromator; ω–2θ scans, θ_{max} = 67°). From a total of 7036 independent measurements after averaging equivalent ($0,k,\pm l$) forms, only those 3543 reflections with $I > 3.0\sigma(I)$ were retained for the structure analysis and corrected for the usual Lorentz and polarization effects. Refined unit-cell parameters were derived from the diffractometer setting angles for 25 reflections ($40° < \theta < 49°$) widely separated in reciprocal space.

The crystal structure was solved by direct methods. Nonhydrogen atom coordinates were derived in part from an initial E map and from subsequent F_o Fourier syntheses. Hydrogen atoms, save those on the CH_2CH_3 moieties of the n-propyl substituents, were all located in difference Fourier syntheses evaluated following several rounds of full-matrix least-squares adjustment of non-hydrogen atom positional and anisotropic thermal parameters. Continuation of the least-squares iterations, with all hydrogen atoms included at their calculated positions, led to convergence at R = 0.065 (R_w = 0.095).[23]

Neutral atom scattering factors used in the structure-factor calculations were taken from ref 32. In the least-squares iterations, $\sum w\Delta^2$ ($w = 1/\sigma^2(|F_o|)$, $\Delta = (|F_o| - |F_c|)$ was minimized. Crystallographic calculations were performed on a PDP11/44 computer by use of the Enraf-Nonius SDP suite of programs incorporating the direct methods program MULTAN11/82.

Supplementary Material Available: Tables of fractional atomic coordinates, thermal parameters, bond lengths, bond angles, torsion angles, and displacements of atoms from least-squares planes (13 pages). Ordering information is given on any current masthead page.

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Asperketals A–F, New Diterpenoids of the Dilophol Class from the Caribbean Gorgonian *Eunicea asperula*

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Six new diterpenoids, asperketals A–F (**1–6**), have been isolated from the Caribbean sea whip *Eunicea asperula*. Compounds **1–5** are ketals and hemiketals possessing 10-membered-ring skeletons analogous to the recently reported marine metabolites dilophol and obscuronatin. Similarly, asperketal **6** is a new diterpenoid ketal related to the *Eunicea*-derived diterpenoid fuscol. The structures of these new compounds were assigned on the basis of chemical and spectral studies and particularly upon spectral analyses involving nuclear Overhauser enhancement difference spectroscopy (NOEDS).

Marine octocorals of the order Gorgonacea, the sea whips and sea fans (phylum Cnidaria), are recognized as a rich source of biologically active and structurally unique secondary metabolites.[1] In the Caribbean Sea, sea whips of

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

C	1[b]	2[c]	3[c]	4[c]	5[c]	6[c]
1	73.7 CH	74.4 CH	73.7 CH	72.9 CH	75.7 CH	81.2 CH
2	128.2 CH	128.2 CH	128.0 CH	127.8 CH	130.8 CH	41.2 CH[d]
3	133.5 C	133.4 C	133.1 C[e]	132.8 C	131.2 C	149.8 C
4	40.5 CH_2	40.4 CH_2	40.3 CH_2	40.4 CH_2	40.4 CH_2	113.6 CH_2
5	26.2 CH_2	26.0 CH_2	26.0 CH_2	26.0 CH_2	25.9 CH_2	109.8 CH_2
6	125.2 CH	125.4 CH	125.3 CH	125.3 CH	126.3 CH	144.8 CH
7	140.2 C	140.2 C	140.2 C	140.3 C	139.2 C	40.8 C
8	38.7 CH_2	38.4 CH_2	38.7 CH_2	38.8 CH_2	38.4 CH_2	35.8 CH_2
9	33.3 CH_2	33.1 CH_2	33.1 CH_2	33.3 CH_2[f]	32.9 CH_2[f]	20.7 CH_2
10	50.1 CH	49.6 CH[g]	50.6 CH[g]	50.1 CH[g]	51.5 CH[g]	42.7 CH[d]
11	50.7 CH	49.3 CH[g]	51.0 CH[g]	49.3 CH[g]	51.0 CH[g]	55.2 CH
12	104.4 C	118.2 C	107.0 C	114.1 C	116.8 C	114.9 C
13	37.7 CH_2	140.8 CH	31.8 CH_2	37.7 CH_2	37.3 CH_2	37.7 CH_2
14	119.7 CH	126.8 CH	120.4 CH	33.7 CH_2[f]	34.5 CH_2[f]	34.4 CH_2
15	135.2 C	86.8 C	132.8 C[e]	81.5 C	80.8 C	77.2 C
16	17.1 CH_3	16.9 CH_3	16.9 CH_3	16.9 CH_3	17.1 CH_3[h]	25.4 CH_3
17	20.7 CH_3	20.5 CH_3	20.6 CH_3	20.6 CH_3	21.1 CH_3	18.1 CH_3
18	13.8 CH_3	13.8 CH_3	13.5 CH_3	13.3 CH_3	16.5 CH_3[h]	11.9 CH_3
19	18.0 CH_3	29.3 CH_3[i]	17.9 CH_3	30.3 CH_3[i]	30.7 CH_3[i]	30.3 CH_3[i]
20	26.0 CH_3	27.7 CH_3[i]	25.9 CH_3	28.9 CH_3[i]	29.1 CH_3[i]	28.5 CH_3[i]
OCH_3			47.6 CH_3			

[a] ^{13}C NMR spectra were recorded at 50 MHz in C_6D_6 solution. Numbers of attached protons were determined from DEPT experiments. The δ values are in ppm downfield from Me_4Si. [b] Assignments were made from XHCORR and COLOC experiments. [c] Assignments were made by comparison with 1. [d–i] Signals within a column may be reversed.

the genus *Eunicea* (family Plexauridae) are particularly abundant, and they have been found to produce secondary metabolites, some of which appear to possess defensive properties.[2]

Several chemical investigations of *Eunicea* species have been reported, and four classes of diterpenoid molecules have been described. Cembrane derivatives are by far the most commonly reported class; in total some 9 cembranoids have been reported from 6 of the 15 varieties of *Eunicea* described from the Caribbean Sea.[3] Other classes of metabolites are the dolabellanes,[4] cubitanes,[5] and fuscol.[6]

In this paper we report the structures of six new diterpenoids, asperketals A–F (**1–6**, Chart I), isolated from the Caribbean gorgonian *Eunicea asperula* Milne Edwards and Haime. Asperketals A–E (**1–5**) are ketals and hemiketals related to the dilophol class of 10-membered-ring diterpenoids, while asperketal F (**6**) belongs to the fuscol class. In addition, we report the isolation of the known diterpenoid obscuronatin (**7**) as a minor metabolite. Obscuronatin was originally isolated from the Red Sea soft coral *Xenia obscuronata*.[7] Similar diterpenoids of the dilophol class were first observed from several brown marine algae of the family Dictyotacea.[8] Prior investigations of gorgonians identified as *E. asperula* resulted in the isolation of asperdiol (**8**), a cytotoxic diterpenoid of the cembrane class.[3g]

Chart I

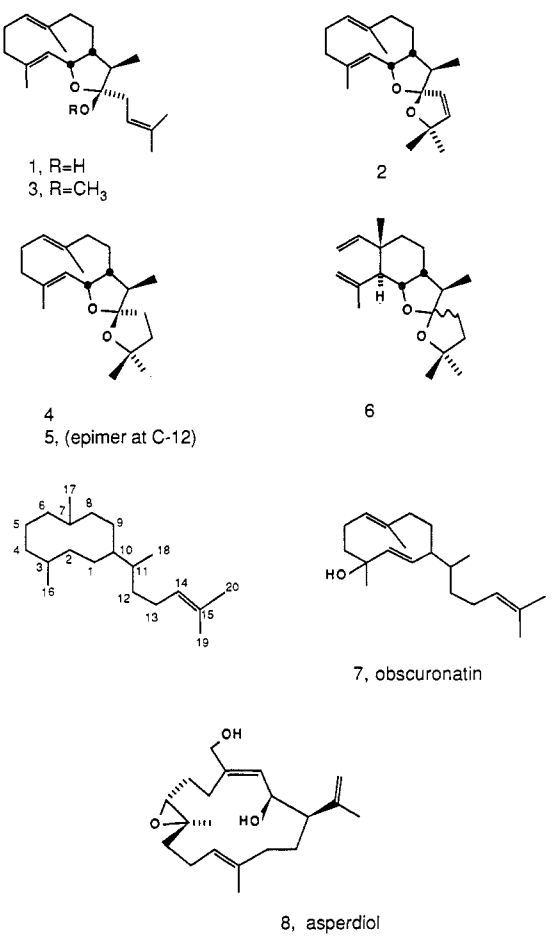

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Table II. ^{1}H NMR Assignments for Compounds 1–6[a]

C	1	2	3	4	5	6
1	5.08 (1 H, dd, 10.9, 8.3)	5.11 (1 H, dd, 10.8, 8.3)	4.81 (1 H, dd, 10.9, 8.3)	4.99 (1 H, dd, 10.9, 7.8)	4.87 (1 H, dd, 10.4, 6.9)	4.31 (1 H, dd, 10.8, 7.8)
2	4.93 (1 H, dd, 10.9, 0.3)	4.92 (1 H, dd, 10.9, 0.8)	4.93 (1 H, br d, 10.9)	4.93 (1 H, br d, 10.9)	5.21 (1 H, br d, 10.4)	1.90 (1 H, m)[c]
3						
4	2.05 (1 H, m)[c]	2.01 (1 H, m)[c]	1.99 (1 H, m)[c]	2.05 (1 H, m)[c]	*b*	5.08 (1 H, dd, 1.6, 1.6)
	1.74 (1 H, m)[c]	1.70 (1 H, m)[c]	1.75 (1 H, m)[c]	1.96 (1 H, m)[c]	*b*	4.80 (1 H, br d, 0.8)
5	2.00 (1 H, m)[c]	1.95 (1 H, m)[c]	2.00 (1 H, m)[c]	2.00 (1 H, m)[c]	*b*	4.90 (1 H, dd, 10.8, 1.4)
	1.95 (1 H, m)[c]	1.92 (1 H, m)[c]	1.94 (1 H, m)[c]	1.73 (1 H, m)[c]	*b*	4.87 (1 H, dd, 17.5 1.4)
6	4.82 (1 H, br dd, 8.1, 8.1)	4.81 (1 H, br dd, 7.7, 8.2)	4.80 (1 H, ddq, 8.1, 8.1, 1.5)	4.82 (1 H, ddq, 8.1, 8.1, 1.5)	4.86 (1 H, m)[c]	5.78 (1 H, dd, 17.5 10.8)
7						
8	2.44 (1 H, br ddd, 13.4, 3.4, 3.4)	2.44 (1 H, br ddd, 13.7, 3.2, 3.2)	2.42 (1 H, br ddd, 13.3, 3.8, 3.8)	2.43 (1 H, br ddd, 13.3, 3.9, 3.9)	2.05 (1 H, m)[c]	1.40 (1 H, m)[c]
	1.60 (1 H, m)[c]	1.55 (1 H, m)[c]	1.59 (1 H, m)[c]	1.61 (1 H, m)[c]	1.61 (1 H, m)[c]	1.06 (1 H, ddd, 13.1, 4.3, 4.3)
9	1.80 (1 H, m)[c]	1.80 (1 H, m)[c]	1.78 (1 H, m)[c]	1.80 (1 H, m)[c]	1.82 (1 H, m)[c]	1.58 (1 H, m)[c]
	1.41 (1 H, ddd, 14.0, 4.1, 4.1)	1.45 (1 H, m)[c]	1.40 (1 H, dddd, 14.1, 3.8, 3.8, 1.0)	1.42 (1 H, dddd, 13.7, 4.1, 4.1, 1.1)	1.45 (1 H, m)[c]	1.47 (1 H, m)[c]
10	2.09 (1 H, m)[c]	2.10 (1 H, m)[c]	2.05 (1 H, m)[c]	2.00 (1 H, m)[c]	1.85 (1 H, m)[c]	2.27 (1 H, m)[c]
11	1.54 (1 H, m)[c]	1.60 (1 H, m)[c]	1.70 (1 H, m)[c]	1.55 (1 H, m)[c]	2.08 (1 H, m)[c]	1.82 (1 H, m)[c]
12						
13	2.53 (1 H, br dd, 14.0, 8.7)	5.71 (1 H, d, 5.7)	2.62 (1 H, br dd, 15.2, 6.9)	2.05–1.95 (2 H, m)[c]	*b*	2.00
	2.35 (1 H, br dd, 14.0, 6.6)		2.56 (1 H, br dd, 15.2, 7.9)		*b*	−1.78 (3 H, m)[c]
14	5.53 (1 H, br dd, 8.7, 6.6)	5.46 (1 H, d, 5.7)	5.44 (1 H, br dd, 7.9, 6.9)	1.50 (1 H, m)[c]	*b*	1.47 (1 H, m)[c]
				1.85 (1 H, m)[c]		
15						
16	1.59 (3 H, br s)	1.54 (3 H, d, 1.3)	1.59 (3 H, d, 1.4)	1.59 (3 H, d, 1.4)	1.50 (3 H, d, 1.4)	1.85 (3 H, br s)
17	1.25 (3 H, br s)	1.21 (3 H, br s)	1.23 (3 H, d, 1.5)	1.21 (3 H, d, 1.5)	1.33 (3 H, br s)	0.83 (3 H, s)
18	1.19 (3 H, d, 6.8)	1.06 (3 H, d, 6.8)	1.23 (3 H, d, 6.8)	1.07 (3 H, d, 6.8)	0.99 (3 H, d, 7.2)	0.97 (3 H, d, 6.6)
19	1.53 (3 H, br s)	1.44 (3 H, s)[d]	1.57 (3 H, br s)	1.46 (3 H, s)[d]	1.47 (3 H, s)[d]	1.41 (3 H, s)[d]
20	1.59 (3 H, br s)	1.25 (3 H, s)[d]	1.68 (3 H, d, 1.1)	1.16 (3 H, s)[d]	1.15 (3 H, s)[d]	1.09 (3 H, s)[d]
others	2.29 (1 H, br s, OH)		3.30 (3 H, s, OCH_3)			

[a] ^{1}H NMR spectra were recorded in C_6D_6 solution at 360 MHz. Assignments were aided by spin decoupling, COSY, and NOEDS experiments. *J* values are reported in hertz and chemical shifts are given in δ units (ppm downfield from Me_4Si). [b] Nonassignable proton resonances. [c] Coupling constants were not determined. [d] Signals within a column may be reversed.

Eunicea asperula was collected in 1986, as part of an expedition to the Tobago Cays in the eastern Caribbean Sea. Freshly collected animals were stored frozen and subsequently exhaustively extracted with dichloromethane. Compounds 1–7 were purified by vacuum flash silica gel chromatography of the crude extract followed by high-performance liquid chromatography (HPLC) of several of the relatively nonpolar fractions. Although these metabolites were not isolated in large amounts, we believe the actual concentrations of these compounds in the living organism are much higher. Compounds **1** and **2**, in particular, were highly unstable and decomposed under the methods of isolation.

Asperketal A (**1**) crystallized from dichloromethane after HPLC purification and was found to be highly unstable in $CDCl_3$ solution. More than 90% of this compound decomposed within a few hours during NMR experiments. The compound analyzed for $C_{20}H_{32}O_2$ by high-resolution mass and ^{13}C NMR spectrometry. Six ^{13}C NMR bands (Table I) at δ 140.2 (s), 135.2 (s), 133.5 (s), 128.2 (d), 125.2 (d), and 119.7 (d) illustrated asperketal A to possess three double bonds and, thus, two rings. The ^{1}H NMR spectrum of **1** (Table II) showed low-field resonances at δ 5.53 (br dd, 8.7, 6.6), 5.08 (dd, 10.9, 8.3), 4.93 (dd, 10.9, 0.3), and 4.82 (br dd, 8.1, 8.1). Since there were only three olefinic protons, one of these four resonances was concluded to originate from a proton attached to an oxygen-bearing carbon. A direct carbon–proton NMR correlation experiment (XHCORR)[9] confirmed this assumption by illustrating that the proton at δ 5.08 corresponded with a carbon at δ 73.7 (d). This proton was coupled (J = 10.9 Hz) with an adjacent olefinic proton (δ 4.93), which showed asperketal A to bear an allylic oxygen bond (C-1). Proton decoupling and COSY[10] NMR experiments showed that protons at δ 5.53 (1 H, br dd, 8.7, 6.6), 2.53 (1 H, br dd, 14.0, 8.7), 2.35 (1 H, br dd, 14.0, 6.6), 1.59 (one of two 3 H, br s), and 1.53 (3 H, br s) were part of a terminal isoprene unit in **1**. This assignment was supported by mass spectral fragments at m/z 235 (M^+ − 69, relative intensity 39), 217 ($M^+ - H_2O$ − 69, 9), and 69 (18), which illustrate a characteristic loss of the C_5H_9 fragment.

Further consideration of ^{1}H NMR single-frequency decoupling data and COSY data showed that all of the protons in **1** belong to four isolated spin systems suggesting four partial structures. With the help of XHCORR data (all of the 16 proton-bearing carbons and their protons were precisely matched), three of these four units were confidently identified as a–c.

The infrared spectrum of asperketal A showed strong hydroxyl absorption at 3450 cm^{-1}. This observation, taken with the presence of a D_2O-exchangeable broad singlet at δ 2.22 in the ^{1}H NMR spectrum, showed **1** to possess a

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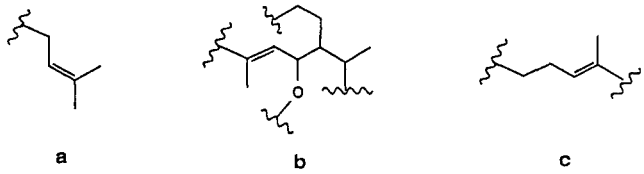

hydroxyl group. Since the only unassigned carbon in the ^{13}C NMR spectrum of **1** was a quaternary carbon resonance at δ 104.4, the oxygenation in asperketal A was concluded to be a cyclic hemiketal.

A long-range carbon–proton correlation experiment (COLOC;[11] Table III) allowed partial structures a–c and the hemiketal to be combined. The hemiketal carbon at δ 104.4 (C-12) showed couplings with two protons at δ 2.53 and 2.35 (C-13) and with a methyl group at δ 1.19 (C-18). Also, couplings between the carbon at δ 125.2 (C-6) and the proton at δ 1.60 (C-8) and between the carbon at δ 38.7 (C-8) and the methyl protons at δ 1.25 (C-17) were observed. Thus, the structure of asperketal A could be unambiguously assigned as a cyclic hemiketal of the dilophol class.[8] Another datum supporting this conclusion was a ^1H NMR resonance of a vinyl methyl (C-17) which was unusually high field shifted (δ 1.25 in C_6D_6, 1.37 in $CDCl_3$ solution). The phenomenon is due to transannular shielding by the C-2,3 double bond in germacrene rings[8,12] and requires an *E* configuration of both sites of unsaturation. The high-field ^{13}C NMR resonance assigned to C-16 (δ 17.1) is also characteristic of the 10-membered-ring system. Determination of the relative stereochemistry at four asymmetric carbons (C-1, C-10, C-11, C-12) was accomplished by NOE studies (discussed in the final section).

Asperketal B (**2**) analyzed for $C_{20}H_{30}O_2$ by high-resolution mass and ^{13}C NMR spectrometry. Comparison of ^1H NMR, ^{13}C NMR, and COSY spectra showed similarities between **2** and compound **1** and showed that partial structures b and c were also present. However, there were several significant differences in the spectral data. The high-resolution mass spectrum and ^{13}C NMR data for asperketal B indicated the presence of one additional ring. The infrared spectrum of **2** lacked hydroxyl absorption, and instead of one low-field proton at δ 5.53, the ^1H NMR spectrum of **2** showed two protons at δ 5.71 (d, J = 5.7) and 5.46 (d, J = 5.7). Also, the methyl resonances at δ 1.59 and 1.53 in **1** were shifted to δ 1.44 and 1.25 in **2** and were much sharper. Finally, ^{13}C NMR data revealed significant changes in the chemical shifts and multiplicities of the four carbons (C-12–C-15) associated with the terminal isoprene unit in asperketal A. These changes were accommodated in asperketal B by the construction of a bicyclic ketal constellation between C-12 and C-15. A small coupling constant (5.7 Hz) between two adjacent olefinic protons (δ 5.71 and 5.46) showed that the double bond had migrated to the C-13–C-14 position. The high-field resonances of the C-17 methyl (δ 1.21) in the ^1H NMR spectrum and the C-16 methyl (δ 16.9) in ^{13}C NMR spectrum of **2** indicated that asperketal B also possesses two *E* double bonds in identical transannular positions.

Asperketal C (**3**) analyzed for $C_{21}H_{34}O_2$ by high-resolution mass and ^{13}C NMR spectrometry. The spectral data for **3** were very similar to those for **1**, with the only apparent difference a change of hydroxyl group in **1** to methoxy in **3**. The presence of methoxy was indicated by new methyl resonances in the ^{13}C NMR spectrum (δ 47.6, q) and in the ^1H NMR spectrum at δ 3.30 (s). A change of fragmentation pattern in the mass spectrum from $M^+ - H_2O$, $M^+ - 69 - H_2O$ in **1** to $M^+ - OCH_3$, $M^+ - 69 - HOCH_3$ further supported this assignment. Therefore, asperketal C was identified as the methyl ketal analogue of **1**. Transannular shielding effects and ^{13}C NMR data again led to the conclusion that both the Δ^2 and Δ^6 double bonds have *E* configurations.

Table III. Results of the Two-Dimensional Carbon-Proton Long-Range Correlation Experiment (COLOC)[a] with Asperketal A (1)

C	protons	C	protons
1	9 (1.80)[b]	11	18
2	16	12	13 (2.53),[b] 13 (2.35),[b] 18
3	16	14	19, 20
6	8 (1.60),[b] 17	15	19, 20
7	17	16	2
8	9 (1.80),[b] 17	17	8 (2.44)[b]
9	8 (1.60)[b]	19	20
10	8 (2.44),[b] 18	20	19

[a] Experiments were performed at 50 MHz in C_6D_6 solution. Parameters were optimized for couplings of 6 Hz. [b] The numbers in parentheses are the ^1H NMR chemical shifts of the protons that correlate.

Asperketal D (**4**) analyzed for $C_{20}H_{32}O_2$ by high-resolution mass and ^{13}C NMR spectrometry. Examination of ^1H, ^{13}C, and COSY NMR data revealed that the 10-membered ring is also present in **4**. The absence of the two low-field olefinic resonances (δ 140.8 and 126.8) in the ^{13}C NMR spectrum of **4** and the presence of two new methylene carbons at δ 37.7 and 33.7 led to the conclusion that asperketal D is the C-13,C-14-dihydro derivative of **2**. Complete ^1H NMR analysis fully supported this assignment.

An obviously related diterpenoid, asperketal E (**5**), also analyzed for $C_{20}H_{32}O_2$ by combined spectral methods. This compound was highly comparable with **4** in that there were similar NMR bands and coupling constants observed. There were, however, several significant differences in the chemical shifts of protons at C-1, -2, -10, -18, and -19. In particular, the C-11 methine proton was shifted 0.53 ppm to low field in relation to its chemical shift in asperketal D (**4**). Therefore, **5** was assigned as the ketal epimer (C-12) of **4**. Asperketal **5**, like the other metabolites in this series, showed spectral features consistent with the assignment as a 10-membered-ring-containing diterpenoid.

The last metabolite, asperketal F (**6**), analyzed for $C_{20}H_{32}O_2$ by high-resolution mass and ^{13}C NMR spectrometry. The presence of two double bonds [δ 149.8 (s), 144.8 (d), 113.6 (t), and 109.8 (t)] in the ^{13}C NMR spectrum showed that **6** was also tricyclic. ^1H, ^{13}C, and COSY NMR data revealed that **6** also possessed the bicyclic ketal system formed with two 5-membered rings. Analysis of ^1H and ^{13}C NMR data showed, however, that asperketal F possessed an entirely different carbon skeleton. Proton resonances at δ 5.08 (1 H, dd, J = 1.6, 1.6), 4.80 (1 H, br d, J = 0.8), and 1.85 (3 H, br s), which were mutually coupled, along with carbon resonances at δ 149.8 (s), 113.6 (t), and 25.4 (q), were interpreted to indicate an isopropenyl group. Another cluster of olefin protons at δ 5.78 (1 H, dd, J = 17.5, 10.8), 4.90 (1 H, dd, J = 10.8, 1.4), and 4.87 (1 H, dd, J = 17.5, 1.4), which correlated with carbons at δ 144.8 (d) and 109.8 (t), were readily assigned to a terminal vinyl group. A proton at δ 4.87 showed long-range coupling with a high-field singlet methyl at δ 0.83 (C-17). Since these protons do not couple with other protons, both vinyl and methyl groups must be connected to a quaternary carbon (δ 40.8) assigned as C-7. The remaining

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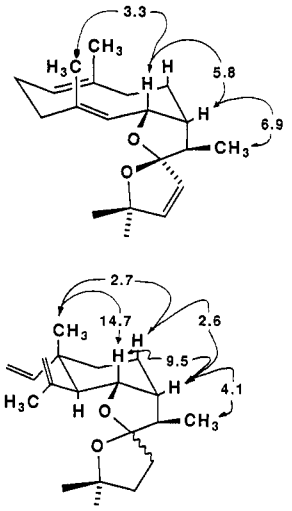

2

6

Figure 1. NOE enhancements (in percent) recorded by NOEDS methods for diterpenoids **2** and **6** (360 MHz).

component of asperketal F was assessed as a 6-membered ring on the basis of typical ^1H NMR features. Thus, ketal **6** was identified as a cyclic ketal possessing an "elemane" divinylcyclohexane ring. This skeleton might arise via a Cope rearrangement of either of the ketals **4** or **5**. Thermolysis of **4** and **5**, in separate experiments at 180 °C under N_2, failed to generate asperketal E and resulted instead in rapid decomposition. No data could be obtained to conclusively define the ketal stereochemistry of asperketal F at C-12.

Obscuronatin (**7**) was isolated as an oil in very small amounts. ^1H, ^{13}C, and COSY NMR data showed a very close correlation with published data for this compound. Obscuronatin, previously isolated from *Xenia obscuronata*, is the only diterpene of the dilophol class to be isolated from marine animals. Further studies to determine the stereochemistry of this compound have not been pursued.

Asperketals A–E (**1–5**) possess asymmetric centers at C-1, C-10, C-11, and C-12. Compound **6** has two additional centers at C-2 and C-7. Application of the nuclear Overhauser enhancement difference spectroscopy method (NOEDS)[13] in the analysis of ^1H NMR data for these compounds allowed the configurations at these centers to be determined. However, because of the overlapping of many proton resonances, this method was only partially successful. Irradiation of the C-1 proton of asperketal B (Figure 1) enhanced the C-16 methyl protons by 3.3% and confirmed the *E* configuration of the Δ^2 olefin. At the same time, the C-10 proton was enhanced by 5.8%, which implied that the C-1 and C-10 protons were oriented cis on the 10-membered ring. The C-10 proton was also enhanced (6.9%) by irradiation of the C-18 methyl protons. Thus, the configurations at three contiguous asymmetric centers were found to be 1*S**, 10*R**, and 11*R**. Irradiation of the C-18 methyl protons failed to produce significant enhancements of either the C-13 or C-14 protons.

The stereochemistry of asperketal F (**6**) (Figure 1) was pursued by using the same methods. Irradiation of the C-17 methyl enhanced the C-1 (14.7%) and C9-β (2.7%) protons (1,3-diaxial substituents), while the C-2 proton showed no enhancement. Thus the ring juncture of **6** was assigned as the expected trans–diaxial arrangement at C-2 and C-7. NOEDS methods were also used to probe the stereochemistry of the bicyclic ketal constellation in asperketal F. Irradiation of the C-1 proton enhanced the C-10 proton by 10.5%, while irradiation of the C-10 proton enhanced the proton resonances for the C-1, C-9β, and C-18 methyl protons by 9.5, 2.6, and 4.1%, respectively. Therefore, the configurations of the three adjacent asymmetric centers are 1*S**, 10*R**, and 11*R**, identical with that of **2**.

The configurations at C-12 in metabolites **1–5** were not confidently assignable by NOEDS methods (irradiation of C-18 methyl protons showed no enhancement of either the C-13 or the C-14 protons). This problem could be approached, however, by analysis of the chemical shifts of the C-11 protons. Consideration of a three-dimensional model of the ketal containing tetrahydrofuran rings in the asperketals showed that the C-11 proton chemical shift could be an effective predictor of the relative stereochemistry at C-12. When oriented cis on the 5-membered ring, the ketal oxygen eclipses the C-11 proton, thus deshielding it. When trans, the C-11 proton is distant from oxygen and would not be deshielded. Using this approach and comparing the chemical shifts of the C-11 protons in various asperketals, we propose the oxygen and C-11 protons to be trans oriented in diterpenoids **1–4** and cis in the epimeric asperketal E (**5**). Using the *R***S** notation, compounds **1**, **3**, and **5** possess the C-12 = *S** configurations, while **2** and **4** possess the C-12 = *R** configurations. This translates to overall relative stereochemistries of 1*S**, 10*R**, 11*R**, and 12*S** for asperketals A, C, and E (**1**, **3**, and **5**), and relative configurations of 1*S**, 10*R**, 11*R**, and 12*R** for asperketals B and D (**2** and **4**).

Experimental Section

General. Infrared spectra were recorded on a Perkin-Elmer 783 spectrophotometer. ^1H NMR and COSY spectra were recorded in $CDCl_3$ and C_6D_6 solutions on a 360-MHz ^1H NMR spectrometer constructed from an Oxford narrow-bore magnet and a Nicolet Fourier transform data system by Dr. John M. Wright of the UCSD NMR Facility. All chemical shifts are reported with respect to internal Me_4Si. NOE difference spectroscopy (NOEDS) experiments were performed in general as outlined by Hall and Sanders.[14] ^{13}C NMR, direct (XHCORR), and long-range (COLOC) carbon–proton correlation spectra[9,11] were recorded in C_6D_6 solution on an IBM WP-200 SY (50 MHz) spectrometer: all chemical shifts are reported with respect to Me_4Si. Both high- and low-resolution mass measurements were supplied by Dr. Richard W. Kondrat, University of California, Riverside. Optical rotations were measured on a Perkin-Elmer Model 141 polarimeter with a 10-cm microcell. Melting points were determined on a Fisher-Johns apparatus and are reported uncorrected. All solvents used either were spectral grade or were distilled from glass prior to use.

Collection and Extraction. *Eunicea asperula* (specimen CI86-194)[15] was collected by hand using SCUBA at 20–25-m depth in July 1986 along the offshore islands of the Tobago Cays, eastern Caribbean Sea. The collection was surface air-dried in the shade and immediately frozen. The gorgonian was next repeatedly extracted with CH_2Cl_2, and the combined extracts were evaporated to yield 36 g of crude organic materials (from 1 kg of dry weight of the gorgonian). Asperketals A–F (**1–6**) and obscuronatin (**7**) were eluted from a "vacuum flash" silica gel column with 10–20% EtOAc in isooctane and further purified with the same solvents by HPLC using preparative silica gel columns.

Asperketal A (1). The hemiketal **1** was isolated as a white solid after final purification by HPLC (Partisil 10 silica with 10% EtOAc in isooctane). Recrystallization gave 190 mg (0.5% of the crude extract) of **1**, mp 75–77 °C. Asperketal A showed $[\alpha]^{20}_D$ +75° (*c* 0.97, C_6H_6) and displayed the following spectral features:

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(15) Specimens of *Eunicea asperula* under the code Fenical CI86-194 are on deposit in the octocoral collection, Smithsonian Institution, Washington, DC, under the curatorship of Dr. Frederick M. Bayer. We thank Dr. Bayer for identifying this octocoral.

IR (film) 3450, 2920, 1660, 1465, 1380, 1160, 1100, 1050, 970, 960 cm^{-1}; HRMS: M^+, m/z obsd 304.2396, $C_{20}H_{32}O_2$ required 304.2402; low-resolution MS: m/z (relative intensity) 304 (6), 286 (47), 235 (39), 217 (9), 161 (100), 125 (40), 81 (44), 69 (18).

Asperketal B (2). The ketal **2** was obtained as a white solid after final purification by HPLC (Partisil 10 silica with 5% EtOAc in isooctane). The extract yielded 47 mg (0.14% of the crude extract) of **2**, mp 62–63 °C. Ketal **2** showed $[\alpha]^{20}_D$ +88° (*c* 0.65, C_6H_6) and exhibited the following features: IR (film) 2940, 1660, 1450, 1360, 1100, 1040, 990, 965 cm^{-1}; HRMS: M^+, m/z obsd 302.2237, $C_{20}H_{30}O_2$ required 302.2245; low-resolution MS: m/z (relative intensity) 302 (55), 273 (100), 163 (53), 161 (18), 151 (44), 109 (64), 81 (23).

Asperketal C (3). The ketal **3** was isolated as a white solid after final purification by HPLC (Partisil 10 silica with 5% EtOAc in isooctane). The extract yielded 23 mg (0.06% of the crude extract) of **3**, mp 72–73 °C. Asperketal C showed $[\alpha]^{20}_D$ +190° (*c* 1.88, C_6H_6) and exhibited the following spectral features: IR (film) 2920, 1660, 1460, 1370, 1080, 1050, 940, 920, 890, 840 cm^{-1}; HRMS: M^+, m/z obsd 318.2562, $C_{21}H_{34}O_2$ required 318.2558; low-resolution MS: m/z (relative intensity) 318 (1), 287 (6), 249 (50), 217 (14), 161 (100), 81 (89), 69 (28).

Asperketal D (4). The ketal **4** was obtained as a white solid after final purification by HPLC (Partisil 10 silica with 5% EtOAc in isooctane). The extract yielded 10 mg (0.03% of the crude extract) of **4**, mp 75–76 °C. Asperketal D exhibited $[\alpha]^{20}_D$ +126° (*c* 0.60, C_6H_6) and displayed the following spectral features: IR (film) 2940, 1660, 1450, 1360, 1140, 1010, 970, 880 cm^{-1}; HRMS: M^+, m/z obsd 304.2388, $C_{20}H_{32}O_2$ required 304.2402; low-resolution MS: m/z (relative intensity) 304 (44), 275 (45), 165 (46), 161 (69), 153 (59), 126 (76), 81 (100), 69 (87).

Asperketal E (5). The ketal **5** was obtained as an oil after final purification by HPLC (Partisil 10 silica with 5% EtOAc in isooctane). The extract yielded 9 mg (0.03% of the crude extract) of **5**, which showed $[\alpha]^{20}_D$ +54° (*c* 0.57, C_6H_6) and displayed the following features: IR (film) 2940, 1665, 1450, 1360, 1140, 1030, 960, 880 cm^{-1}; HRMS: M^+, m/z obsd 304.2406, $C_{20}H_{32}O_2$ required 304.2402; low-resolution MS: m/z (relative intensity) 304 (100), 275 (63), 165 (27), 161 (67), 152 (23), 126 (43), 81 (47), 69 (37).

Asperketal F (6). The ketal **6** was isolated as a white solid after final purification by HPLC (Partisil 10 silica with 5% EtOAc in isooctane). The extract yielded 14 mg (0.04% of the crude extract) of **6**, mp 54–55 °C. Asperketal F showed $[\alpha]^{20}_D$ +71° (*c* 0.77, C_6H_6) and displayed the following spectral features: IR (film) 2940, 1640, 1455, 1360, 1010, 980, 905, 885 cm^{-1}; HRMS: M^+, m/z obsd 304.2394, $C_{20}H_{32}O_2$ required 304.2402; low-resolution MS: m/z (relative intensity) 304 (34), 275 (100), 165 (24), 161 (63), 153 (1), 134 (40), 126 (51), 81 (84), 69 (99).

Obscuronatin (7). Obscuronatin (**7**) was obtained as an oil after purification by HPLC (Partisil 10 silica with 5% EtOAc in isooctane). The extract yielded 9 mg (0.03% of the crude extract) of **7**. The ^1H NMR spectrum of **7** (in C_6D_6) showed peaks at 5.31 (1 H, dd, 15.7, 10.1 Hz), 5.25 (1 H, br t, 7.0), 5.04 (1 H, br d, 15.7), 4.92 (1 H, br d, 11.0), 2.62 (1 H, m), 2.27–1.85 (7 H, m), 1.69 (3 H, br s), 1.60 (3 H, br s), 1.55–1.10 (6 H, m), 1.54 (3 H, br s), 1.06 (3 H, s), 0.92 (3 H, d, 6.7) ppm, in full accord with published data.[7] ^{13}C NMR resonances were also within ±0.04 ppm of the reported data for this compound.[7]

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Fascaplysin, an Unusual Antimicrobial Pigment from the Marine Sponge *Fascaplysinopsis* sp.

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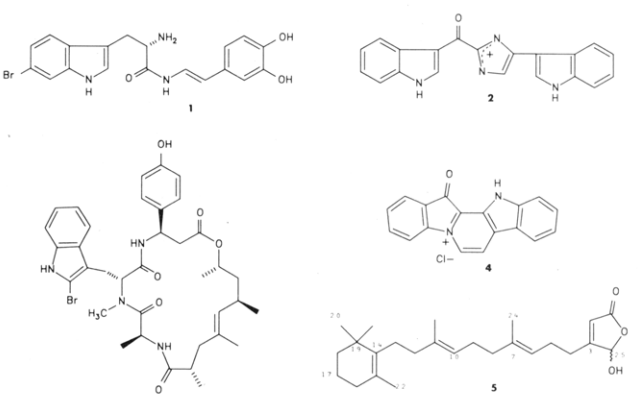
The structure of fascaplysin (**4**), a novel nitrogenous pigment exhibiting antimicrobial and cytotoxic properties, has been determined by spectral and X-ray analyses. The known compound luffariellolide (**5**) was also isolated.

A variety of biologically active metabolites containing an indole ring have been identified from marine sponges.[2,3] Among these are the tryptophan/tryptamine derivatives clionamide (**1**),[4,5] topsentin-A (**2**),[6] and jaspamide (**3**).[7] We now wish to report the isolation and structure determination of fascaplysin (**4**), a novel pentacyclic quaternary salt from the Fijian sponge *Fascaplysinopsis* Bergquist sp.[8] Fascaplysin inhibits the growth of several microbes, including *Staphylococcus aureus* (15-mm zone at 0.1 μg/disk), *Escherichia coli* (8-mm zone at 5 μg/disk), *Candida albicans* (11-mm zone at 1 μg/disk), and *Saccharomyces*

Chart I



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