51 (20), 39 (43); HRMS m/z exact mass calcd for $C_{15}H_{18}O_2$ 230.1307, found 230.1306.

[12-$^2H_3$]-Isovelleral (24) and [12-$^2H_3$]-2 (25). A mixture of 1 (300 mg, 1.29 mmol), D_2O (1.11 g, 55.4 mmol), and toluene (9 mL) was heated at 200 °C for 1 h. The organic phase was separated, dried over molecular sieves (4A), and concentrated. Chromatography (SiO_2, 1:4 EtOAc–heptane) gave **24** (96.3 mg, 32%) and **25** (82.5 mg, 27%) as white crystals. For **24**: mp 95–97 °C; $[\alpha]^{20}_D$ 237° (c 1.0, CCl_4); IR (CCl_4) as for **1** but with C–D absorption at 2220 cm^{-1} (weak), diminished absorption at 2940 cm^{-1}, and peak at 1080 cm^{-1} shifted to 1100 cm^{-1}; ^1H NMR ($CDCl_3$) as for **1** but not peak at δ 1.12 ppm; ^{13}C NMR ($CDCl_3$) as for **1** but very weak multiplet at δ 18.67 ppm and weakened singlets at δ 34.17 and 34.59 ppm; MS (EI) m/z 235 (M^+, 4.2), 217 ($M^+ - CD_3$, 0.7), 207 (5.8), 189 (5.6), 175 (9.1), 161 (5.6), 147 (11.1), 133 (12.2), 122 (17.6), 105 (28.0), 91 (42.2), 79 (28.9), 69 (28.4), 55 (43.6), 41. (100).

For **25**: mp 67–69 °C $[\alpha]^{20}_D$ −88.3° (c 1.0, CCl_4); IR (CCl_4) as for **2** but with C–D absorption at 2210 cm^{-1} (weak); ^1H NMR ($CDCl_3$) as for **2** but no peak at δ 1.16 ppm; ^{13}C NMR ($CDCl_3$) as for **2** but very weak multiplet at δ 19.77 ppm and weakened singlets at δ 37.30 and 38.37 ppm; MS (EI) m/z 235 (M^+, 4.9), 217 ($M^+ - CD_3$, 1.5), 106 (5.8), 189 (4.9), 175 (7.1), 165 (6.6), 147 (9.3), 133 (13.3), 122 (19.0), 105 (27.9), 91 (39.4), 79 (28.8), 69 (26.1), 55 (46.9), 41 (100).

[1-$^2H_2$,13-$^2H_3$]-Merulidial (26). A mixture of **7** (19 mg, 0.077 mmol), D_2O (0.22 g, 12.2 mmol), and toluene (1.80 mL) was heated at 185 °C for 1.5 h. The organic phase was separated, dried over molecular sieves (4A), and concentrated. The residue was purified by HPLC using LiChrosorb Si 60 (10 μm, mobile phase EtOAc/heptane (25/75), flow rate 1.0 mL/min, detection at 254 nm) affording **21** (0.8 mg, 4%; the deuterium content of this fraction could not be determined), **26** (2.6 mg), and a 1:2 mixture (3.0 mg) of **26** and [1-$^2H_2$, 13-$^2H_3$]-**8**. Although the mixture in the later fraction was not separated further, ^1H NMR indicated deuteration at C_1 and C_{13} of **8**, since the peaks at δ 2.86, 2.60, and 1.28 were greatly diminished.

Relevant data for **26**: ^1H NMR ($CDCl_3$) as for **7**[3] but diminished peaks at δ 2.75, 2.66, and 1.17 ppm; ^{13}C NMR ($CDCl_3$) as for **7** but weakened singlets at δ 164.65, 130.73, and 15.86 ppm. Peaks absent at δ 44.27 and 34.22 ppm; MS (EI) m/z 254 ($M^+ + 1$, 6), 253 (M^+, 26), 252 (38), 235 ($M^+ - CD_3$, 31), 223 (88), 205 (100), 192 (61), 177 (59), 163 (56), 149 (57), 137 (39), 121 (44), 107 (48), 93 (60), 79 (43), 69 (23), 55 (25), 41 (31).

Kinetic Determinations. 0.045 M solutions of **1** and **24** in [$^2H_8$] toluene were introduced in thoroughly cleaned and oven-dried NMR tubes. The samples were degassed and sealed under vacuum. At each of four temperatures duplicate samples were immersed in a thermostatic and stirred oil bath. Temperatures of the oil bath were measured with a Siebert & Kühn scientific mercury thermometer calibrated within ±0.2 °C and were constant to ±0.5 °C as checked with a Chromel-Alumel thermocouple in conjunction with a potentiometer. The samples were withdrawn periodically and cooled rapidly, and the progress of the reaction was monitored by integration of the well-resolved NMR signals from H-5 and H-13. The NMR shifts of the H-5 and H-13 aldehyde shifts in [$^2H_8$]-toluene with $SiMe_4$ as internal standard were, respectively, δ **1** (9.70, 9.17); **2** (9.79, 9.08); **14** (9.20, 9.03); **15** (9.60, 8.98).

The activation parameters were obtained by a least-squares treatment of the experimental data (36 points). The results are given with a 95% confidence interval in Table II.

The measurements of k_2 and k_{-3} were performed by introducing 0.045 M solutions of **1** and **2** in [$^2H_8$]-toluene into washed and oven-dried NMR tubes. *N,O*-Bis(trimethylsilyl)trifluoroacetamide (70 μL, 0.26 mmol) was added to each tube. The solutions were degassed, and the tubes were sealed under vacuum. Duplicate samples were immersed at 407.8 K in the same oil bath as was used for the kinetic experiments described above. The samples were withdrawn periodically and cooled rapidly, and the reaction was monitored by integration of the well-resolved NMR signals from H-5.

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Registry No. **1**, 37841-91-1; **2**, 109956-89-0; **3**, 96910-70-2; **3** (α-hemiacetal), 141315-78-8; **3** (β-hemiacetal), 141315-79-9; **4**, 141315-80-2; **4** (α-hemiacetal), 141315-81-3; **4** (β-hemiacetal), 141315-82-4; **7**, 68053-32-7; **8**, 121843-89-8; [1-$^2H_2$,13-$^2H_2$]-**8**, 141315-85-7; **9**, 108893-54-5; **10**, 121843-90-1; **11**, 2212-99-9; **12**, 69905-56-2; **13**, 131367-57-2; **14**, 131367-58-3; **15**, 131434-67-8; **16**, 141315-83-5; **17**, 131367-60-7; **18**, 131434-69-0; **19**, 141223-42-9; **20**, 141223-43-0; **21** (isomer 1), 141223-44-1; **21** (isomer 2), 141315-84-6; **22**, 141223-45-2; **23**, 141223-46-3; **24**, 131434-68-9; **25**, 131367-59-4; **26**, 141223-47-4.

Supplementary Material Available: ^{13}C and ^1H NMR spectra of **2**, **4**, **14**, **15**, **17**, **18**, and **21–26** (24 pages). This material is contained in many libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.

Theopederins A–E, Potent Antitumor Metabolites from a Marine Sponge, *Theonella* sp.[1]

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Theopederins A–E (**1–5**) has been isolated from a marine sponge *Theonella* sp. and their structures established mainly by extensive 2D NMR analyses as well as by comparison with spectral data of mycalamides A and B. Theopederins A–E are highly cytotoxic against P388 murine leukemia cells. Theopederins A and B (**1** and **2**) showed promising antitumor activity.

Marine sponges of the genus *Theonella* have proved to be a rich source of bioactive secondary metabolites possessing novel structural features; e.g., cytotoxic macrolides (swinholide A[2] and bistheonellide A[3]), cyclic peptides (theonellamide F,[4] keramamide A,[5] and theonellapeptolides[6]), and alkenyl pyridines (theonelladins[7]).

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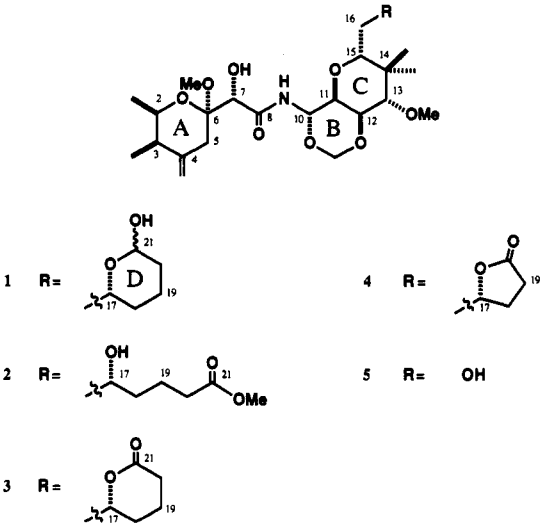
Table I. ^{13}C and ^{1}H NMR Data of Theopederin A (1) in $CDCl_3$

no.	major		minor	
	^{13}C	^{1}H	^{13}C	^{1}H
2	69.7 d	4.03 dq, 2.8, 6.6	69.8 d	4.01 dq, 2.9, 6.5
2-Me	18.0 q	1.19 d, 6.6	18.0 q	1.19 d, 6.5
3	41.3 d	2.25 dq, 2.8, 7.1	41.4 d	2.24 dq, 2.9, 7.1
3-Me	12.2 q	1.02 d, 7.1	12.1 q	1.01 d, 7.1
4	145.4 s		146.1 s	
4-CH_2	111.0 t	4.86 d, 1.9	110.6 t	4.84 d, 1.9
		4.74 t, 2.0		4.74 t, 2.0
5	34.1 t	2.36 d, 14.1	33.8 t	2.43 d, 14.3
		2.26 ddd, 14.1, 2.0, 1.9		2.34 ddd, 14.3, 2.0, 1.9
6	99.9 s		99.9 s	
6-OMe	48.6 q	3.30 s	48.8 q	3.29 s
7	71.7 d	4.26 s	72.3 d	4.27 s
7-OH		3.80 b		3.75 bs
8	172.0 s		171.6 s	
9-NH		7.52 d, 9.8		7.57 d, 10.0
10	73.7 d	5.80 dd, 9.8, 9.8	73.5 d	5.82 dd, 10.0, 10.0
10-OCH_2	86.4 t	5.12 d, 6.8	86.4 t	5.11 d, 7.0
		4.84 d, 6.8		4.83 d, 7.0
11	70.5 bd	3.78 dd, 9.8, 6.9	70.5 bd	3.80 dd, 10.0, 6.5
12	74.1 d	4.19 dd, 10.3, 6.9	74.4 d	4.18 dd, 10.4, 6.5
13	79.5 d	3.40 d, 10.3	79.6 d	3.42 d, 10.4
13-OMe	61.7 q	3.53 s	61.8 q	3.54 s
14	41.3 s		41.4 s	
14-Me_{eq}	23.2 q	0.98 s	23.4 q	0.96 s
14-Me_{ax}	13.5 bq	0.84 s	13.5 bq	0.83 s
15	76.3 d	3.40 m	77.0 d	3.37 dd, 6.9, 3.4
16	36.2 t	1.61 m	36.4 t	1.53 m
		1.53 m		1.43 m
17	74.0 d	3.33 m	67.3 d	3.82 m
18	30.0 t	1.64 m	31.0 t	1.67 m
		0.95 m		1.09 m
19	21.8 t	1.80 m	17.3 t	1.80 m
		1.41 m		1.53 m
20	32.6 t	1.80 m	29.6 t	1.61 m
		1.22 m		1.53 m
21	96.4 d	4.56 dd, 9.6, 2.0	91.7 d	5.16 bs
21-OH		3.18 b		2.92 b

A sponge of the genus *Theonella* with yellowish interior, collected off Hachijo-jima Island, 300 km southeast of Tokyo, contained a variety of bioactive metabolites with unusual structural features: antithrombin-active cyclic peptides cyclotheonamides A and B,[8] a cytotoxic cyclic peptide orbiculamide A,[9] an antithrombin-active linear peptide nazumamide A,[10] and cytotoxic orange pigments aurantosides A and B.[11] The same sponge also contained potent cytotoxic metabolites closely related to the mycalamides[12] and onnamide A.[13] In this paper, we describe the isolation and structure elucidation of these substances.

The lipophilic portion of EtOH extracts of the frozen specimens (15 kg) was partitioned between hexane and MeOH/H_2O (9:1) and subsequently between CCl_4 and MeOH/H_2O (8:2). The CCl_4 layer was fractionated by ODS flash chromatography. The active fraction eluted with MeOH/H_2O (7:3) was purified by gel filtration on Toyopearl HW40, followed by repeated reversed-phase HPLC to yield theopederins A–E[14] (1, 3.2×10^{-5}%; 2, 1.4×10^{-5}%; 3, 3.3×10^{-6}%; 4, 5.3×10^{-6}%; 5, 4.0×10^{-6}% based on wet weight).



Theopederin A (1) had a molecular formula of $C_{27}H_{45}NO_{10}$, established by high-resolution FAB mass and NMR

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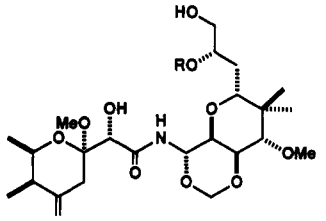
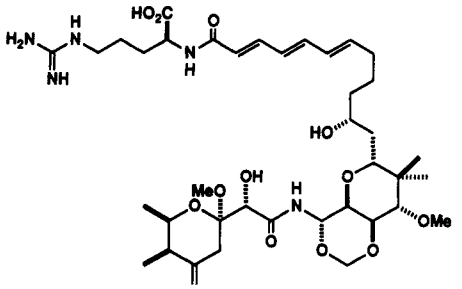
spectral data, indicating six degrees of unsaturation. Both ^1H and ^{13}C NMR spectra exhibited many doubled signals in a ratio of 4:3, indicating the existence of two inseparable conformers or isomers. The ^{13}C NMR spectrum showed two sets of exomethylene signals at δ 146.1 s, 145.4 s, 110.6 t, and 111.0 t and a pair of a carbonyl signal at δ 171.6 s and 172.0 s. The NMR spectra, including a COSY spectrum, also displayed doubled signals which indicated the presence of an $-OCH(CH_3)CH(CH_3)-$ unit, a methylene located between an exomethylene and a ketal carbon, an isolated oxymethine, four contiguous oxygenated methines, a dioxymethylene, a geminal dimethyl, two *O*-methyls, and an amide. These spectral features were reminiscent of the mycalamides A (6) and B (7) and onnamide A (8), antiviral



6 R=H
7 R=Me



8

and cytotoxic heterocyclic compounds from marine sponges. Comparison of the NMR data (Table I) with those reported in the literature readily revealed that theopederin A had the O1–C16 portion of the mycalamide skeleton. The remaining portion consisted of three methylenes, one oxymethine, and an acetal for both isomers. Due to heavily overlapping ^1H NMR signals in the aliphatic region arising from two sets of four nonequivalent methylenes, it was impossible to interpret the COSY spectrum. Therefore, we decided to try simultaneous analyses of HOHAHA,[16] HMQC,[17] and HMQC–HOHAHA[18] spectra. Particularly, the HMQC–HOHAHA experiment was helpful to assign methylene protons in signal cluster; H21 correlated with C18, C19, C20, and C21 in both isomers, thereby constructing the structure of the remaining portion. Thus, unambiguous assignments for all ^1H and ^{13}C NMR signals were made, defining a 6-substituted 2-hydroxytetrahydropyran in both isomers; the two isomers differed only in the stereochemistry of the acetal. Coupling constants of the acetal proton indicated that the major isomer had an equatorial while the minor had an axial hydroxyl group. This was supported both by a NOESY cross peak between H17 and H21 in the major isomer and by an HMBC[19] cross peak between C17 and H21 in the minor one.

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Scheme I

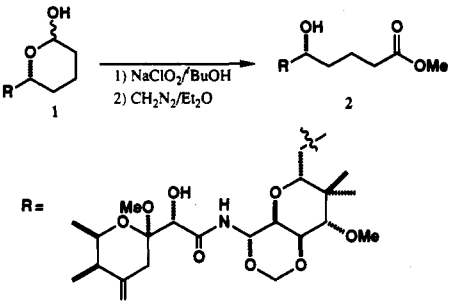

Table II. ^{13}C NMR Data of Theopederins B–E (2–5) in $CDCl_3$

no.	2	3	4	5
2	69.6 d	69.6 d	69.4 d	69.3 d
2-Me	17.9 q	18.0 q	17.4 q	18.3 q
3	41.3 d	41.2 d	41.1 d	41.2 d
3-Me	12.1 q	12.0 q	11.5 q	12.3 q
4	145.5 s	144.5 s	144.6 s	144.8 s
4=CH_2	110.6 t	111.1 t	111.0 t	110.9 d
5	33.7 t	33.6 t	32.9 t	33.0 t
6	99.8 s	99.3 s	99.7 s	99.7 s
6-OMe	48.9 q	48.8 q	48.2 q	48.8 q
7	72.7 d	71.6 d	71.5 d	71.0 d
8	171.8 s	172.0 s	171.7 s	171.7 s
10	73.8 d	73.7 d	73.4 d	74.6 d
10-OCH_2	86.9 t	86.3 t	86.4 t	86.7 t
11	71.3 d	70.3 d	70.0 d	71.0 d
12	74.4 d	74.0 d	73.9 d	74.2 d
13	79.1 d	79.3 d	79.2 d	79.4 d
13-OMe	61.8 q	61.6 q	61.4 q	61.3 q
14	41.7 s	40.8 s	41.1 s	39.6 s
14-Me_{eq}	23.1 q	23.5 q	23.0 q	23.4 q
14-Me_{ax}	13.6 q	14.0 q	13.5 q	14.6 q
15	80.3 d	75.7 d	75.8 d	80.2 d
16	35.5 t	35.8 t	34.6 t	61.5 t
17	71.0 d	78.3 d	79.1 d	
18	36.5 t	27.2 t	27.6 t	
19	20.8 t	18.2 t	28.1 t	
20	33.8 t	29.2 t	176.7 s	
21	174.2 s	171.9 s		
21-OMe	51.5 q			

The NOESY spectrum of theopederin A revealed cross peaks which secured the relative stereochemistry of the O1–C16 portion to be identical with that of the mycalamides. A NOESY cross peak between H5 and H17 in both isomers indicated that rings A and D in theopederin A were spatially proximate to each other. Chemical shifts of the C5 methylene protons were also affected by the orientation of the hydroxyl group on C21. Among the negative cross peaks in the NOESY spectrum, mostly due to the exchange of hydroxy protons, exchange peaks were observed between H21 of the major and minor isomers,[20] thereby revealing that the two isomers interconverted as fast as the exchange of hydroxyl with water protons; the amide proton signals did not give exchange cross peaks. In sharp contrast to other theopederins, 1 gave a very broad HPLC peak, 10 times wider than those of other congeners, probably due to a fast conversion between two isomers.

The structure of theopederin A (1) was further confirmed by chemical transformation to the second compound of this series, theopederin B (2). Upon oxidation with $NaClO_2$, followed by esterification, 1 afforded 2 (Scheme I), whose spectral data and HPLC retention time

(20) Another exchange cross peak between H17 of the two isomers was also observed in the NOESY spectrum.

Table III. ^{1}H NMR Data of Theopederins B–E (2–5) in $CDCl_3$

no.	2	3	4	5
2	4.01 dq, 2.8, 6.6	4.03 dq, 2.8, 6.6	4.03 dq, 2.7, 6.5	4.06 dq, 2.9, 6.6
2-Me	1.21 d, 6.6	1.19 d, 6.6	1.20 d, 6.5	1.21 d, 6.6
3	2.25 dq, 2.8, 7.1	2.25 dq, 2.8, 6.5	2.26 dq, 2.7, 7.1	2.27 dq, 2.9, 7.1
3-Me	1.01 d, 7.1	0.99 d, 6.5	1.01 d, 7.1	1.03 d, 7.1
4=CH_2	4.85 bs	4.86 bs	4.86 bs	4.86 dd, 2.0, 2.0
	4.75 bs	4.73 bs	4.74 bs	4.76 dd, 2.0, 2.0
5	2.41 d, 14.4	2.32 d, 13.8	2.35 d, 14.0	2.37 d, 14.1
	2.38 bd, 14.4	2.17 bd, 13.8	2.21 bd, 14.0	2.21 ddd, 14.1, 2.0, 2.0
6-OMe	3.31 s	3.30 s	3.30 s	3.33 s
7	4.30 d, 2.3	4.24 d, 2.9	4.27 d, 3.1	4.28 d, 2.8
7-OH	3.93 d, 2.3	3.96 d, 2.9	4.08 d, 3.1	3.77 d, 2.8
9-NH	7.46 d, 9.7	7.52 d, 9.5	7.52 d, 9.4	7.44 d, 9.1
10	5.89 dd, 9.7, 9.7	5.77 dd, 9.5, 9.5	5.81 dd, 9.4, 9.4	5.82 dd, 9.1, 9.1
10-OCH_2	5.15 d, 6.9	5.10 d, 7.1	5.13 d, 7.0	5.13 d, 6.9
	4.88 d, 6.9	4.85 d, 7.1	4.87 d, 7.0	4.91 d, 6.9
11	3.86 dd, 9.7, 6.7	3.78 dd, 9.5, 6.6	3.82 dd, 9.4, 6.4	3.85 dd, 9.1, 6.5
12	4.23 dd, 10.3, 6.7	4.19 dd, 9.4, 6.6	4.21 dd, 10.2, 6.4	4.21 dd, 9.8, 6.5
13	3.47 d, 10.3	3.40 d, 9.4	3.44 d, 10.2	3.42 d, 9.8
13-OMe	3.57 s	3.54 s	3.56 s	3.57 s
14-Me_{eq}	0.98 s	1.00 s	1.02 s	1.09 s
14-Me_{ax}	0.88 s	0.86 s	0.88 s	0.87 s
15	3.65 dd, 9.3, 2.6	3.40 d, 9.4	3.42 d, 10.2	3.59 dd, 7.7, 2.4
16	1.54 dt, 14.7, 2.6	1.88 m	1.94 m	3.64 ddd, 11.6, 7.4 2.4
	1.46 dt, 14.7, 9.3	1.59 m	1.59 dd, 14.2, 8.3	3.51 ddd, 11.6, 7.7, 4.5
16-OH				1.84 dd, 7.4, 4.5
17	3.65 ddt, 9.3, 2.6, 7.6	4.22 m	4.45 ddd, 14.2, 8.4, 6.0	
18	1.40 m	2.04 m	2.40 m	
	1.40 m	1.35 m	1.75 m	
19	1.73 dtt, 14.1, 7.6, 7.5	1.86 m	2.51 ddd, 17.6, 10.0, 3.8	
	1.63 dtt, 14.1, 7.6, 7.5	1.78 m	2.45 dd, 17.6, 11.1	
20	2.32 t, 7.5	2.48 ddd, 17.5, 7.3, 5.9		
		2.40 ddt, 17.5, 0.9, 7.8		
21-OMe	3.66 s			

on an ODS column were identical to the corresponding natural product.

The least polar compound, theopederin B (2), had a molecular formula of $C_{28}H_{47}NO_{11}$, which was established by high-resolution FAB mass and ^{13}C NMR data (Tables II and III). The ^{1}H and ^{13}C NMR spectra were very similar to those of 1 except for the presence of a third methoxyl group (δ_C 51.5 and δ_H 3.66) and an ester carbon (δ 174.2), which was supported by an IR absorption at 1730 cm^{-1}. In spite of obscure ^{1}H NMR signals of H15 and H17, the connectivities from H15 to H20 were established by analysis of the COSY spectrum. The HMBC spectrum showed cross peaks between the C21 carbon at δ 174.2 and $H_2$20 (δ 2.32) and *O*-methyl protons (δ 3.66), which revealed the presence of a methyl ester terminus in 2.

Theopederin C (3), $C_{27}H_{43}NO_{10}$, showed NMR spectra (Tables II and III) very similar to those of 2, except for the chemical shift of H17 and for the absence of the *O*-methyl signal on C21. A downfield shift of H17 (δ 3.65 in 2 to δ 4.45 in 3) indicated that C17 was esterified. An HMBC correlation between H17 and C21 revealed the presence of a δ-lactone in 3, in accordance with an IR band at 1730 cm^{-1}.

Theopederin D (4), $C_{26}H_{41}NO_{10}$, also had the O1–C16 substructure in common with theopederins A–C, secured by COSY, HMQC, and HMBC spectra (Tables II and III). The connectivities from H16 to $H_2$19 were straightforward by the COSY spectrum. The HMBC cross peaks between C19 methylene protons (δ 2.51 and 2.45) and an ester carbon at δ 176.7 (C20), together with a C17 oxymethine proton signal at δ 4.45, indicated the presence of a γ-lactone, which was also supported by an IR absorption at 1765 cm^{-1}.

Theopederin E (5), the most polar of all, had a molecular formula of $C_{22}H_{37}NO_9$ as revealed by high-resolution FAB mass and NMR spectral data. The ^{1}H NMR spectrum did not have signals for aliphatic protons in the δ 1.25–1.80 region; the other signals were very similar to those of compounds 1–4. In the COSY spectrum, H15 (δ 3.59) was coupled to oxygenated methylene protons on C16 (δ 3.64 and 3.51), which were in turn coupled to a hydroxyl proton at δ 1.84. Therefore, theopederin E was a simple derivative of the other congeners truncated at the bond between C16 and C17.

Theopederins A–E were markedly cytotoxic against P388 murine leukemia cells with IC_{50}'s of 0.05, 0.1, 0.7, 1.0, and 9.0 ng/mL, respectively. Theopederins A and B also showed promising antitumor activity against P388 (i.p.): T/C = 205% (0.1 mg/kg/day, treated on days 1, 2, and 4–6, i.p.) and T/C = 173% (0.4 mg/kg/day, treated on days 1, 2, and 4–6, i.p.), respectively. It is noteworthy that the structure of the moiety linked to C16 methylene affects cytotoxicity.

Theopederins A, B, and C might be generated by oxidative cleavage of a double bond of onnamide A, the most abundant cytotoxic component of the water-soluble portion of the EtOH extract of the same sponge.[21]

Experimental Section

NMR spectra were recorded on a Bruker AM600 NMR spectrometer operating at 600 MHz for ^{1}H and 150 MHz for ^{13}C. ^{1}H and ^{13}C NMR chemical shifts are referenced to solvent peaks: δ_H 7.24 (residual $CHCl_3$) and δ_C 77.0 for $CDCl_3$. Optical rotations were determined by a JASCO DIP-371 digital polarimeter. FAB mass spectra were measured by using glycerol as a matrix on a JEOL SX102 mass spectrometer. Infrared spectra were recorded on a JASCO IR-G infrared spectrometer.

Collection and Isolation. Specimens of the sponge were collected using SCUBA off Hachijo-jima Island (−15 to −20 m). The frozen samples (15 kg) were extracted with EtOH, concentrated, and partitioned between Et_2O and H_2O. The Et_2O-soluble portion was partitioned between *n*-hexane and MeOH/H_2O (9:1);

(21) Isolation and structure elucidation of congeners of onnamide A will be reported elsewhere.

the latter was subsequently partitioned between CCl_4 and $MeOH/H_2O$ (8:2). The CCl_4 layer (2.1 g) was fractionated by ODS flash chromatography with increasing amounts of MeOH in water. The fraction eluted with $MeOH/H_2O$ (7:3) was successively chromatographed on Toyopearl HW40 with $CHCl_3$/MeOH (1:1) and then with *n*-hexane/$CHCl_3$/MeOH (8:7:1) to yield two active fractions. The less polar fraction was purified by HPLC on Capcell Pak C_{18} with $MeOH/H_2O$ (6:4) to give **2** (2.1 mg). The other fraction was purified by HPLC on Senshupak ODS-H-4251 with $MeOH/H_2O$ (7:3) to afford **1** (4.8 mg) along with three active peaks. The first and second peaks were further purified by HPLC on Capcell Pak C_{18} with $MeOH/H_2O$ (6:4) to yield **5** (0.6 mg) and **4** (0.8 mg). The third peak was separated on silica gel with $CHCl_3$/MeOH (200:1), followed by HPLC on Capcell Pak C_{18} with $MeOH/H_2O$ (6:4) to yield **3** (0.5 mg).

Caution! Theopedern-rich samples cause adverse reactions. **1**: $[\alpha]_D$ +88.1° (*c* 0.14, $CHCl_3$); IR (film) 3350, 2950, 1680, 1520, 1455, 1435, 1375, 1345, 1320, 1295, 1265, 1220, 1185, 1165, 1100, 1065, 1025, 1005, 960, 915, 890, 870, 840, 795, 755, 660 cm^{-1}; HRFABMS *m/z* 494.2764 ($M^+ - CH_3O - H_2O$; $C_{26}H_{40}NO_8$, Δ 1.0 mmu); ^1H and ^{13}C NMR, see Table I.

2: $[\alpha]_D$ +49.1° (*c* 0.06, $CHCl_3$); IR (film) 3400, 2975, 1730, 1680, 1510, 1110, 1080, 1030 cm^{-1}; HRFABMS *m/z* 542.2982 ($M^+ - CH_3O$; $C_{27}H_{44}NO_{10}$, Δ 1.6 mmu); ^1H and ^{13}C NMR, see Tables II and III.

3: $[\alpha]_D$ +172.0° (*c* 0.03, $CHCl_3$); IR (film) 3350, 2950, 1730, 1685, 1520, 1460, 1375, 1240, 1185, 1120, 1100, 1070, 1030, 1010, 920, 875, 755 cm^{-1}; HRFABMS *m/z* 542.2963 (MH^+; $C_{27}H_{44}NO_{10}$, Δ −0.3 mmu); ^1H and ^{13}C NMR, see Tables II and III.

4: $[\alpha]_D$ +80.0° (*c* 0.04, $CHCl_3$); IR (film) 3300, 2900, 1765, 1675, 1515, 1175, 1100, 1065, 1025, 910, 870, 750 cm^{-1}; HRFABMS *m/z* 496.2552 ($M^+ - CH_3O$; $C_{25}H_{38}NO_9$, Δ 0.6 mmu); ^1H and ^{13}C NMR, see Tables II and III.

5: $[\alpha]_D$ +136.7° (*c* 0.03, $CHCl_3$); IR (film) 3350, 2950, 1680, 1525, 1460, 1380, 1220, 1190, 1170, 1130, 1110, 1070, 1035, 910, 875, 845, 795, 760 cm^{-1}; HRFABMS *m/z* 428.2306 ($M^+ - CH_3O$; $C_{21}H_{34}NO_8$, Δ 2.1 mmu); for ^1H and ^{13}C NMR, see Tables II and III.

Preparation of 2 from 1. To a mixture of **1** (2 mg), 2-methyl-2-butene (0.1 mL), NaH_2PO_4 (25 mg) in ^tBuOH (1.5 mL), and H_2O (0.4 mL) was added $NaClO_2$ (65 mg) and the mixture stirred at room temperature for 1 h. The reaction mixture was treated with excess CH_2N_2 and extracted with Et_2O, whose extract was separated by HPLC on Capcell Pak C_{18} with $MeOH/H_2O$ (6:4) to give **2** (1.1 mg); $[\alpha]_D$ +61.2° (*c* 0.05, $CHCl_3$); IR (film) 3400, 1730, 1680 cm^{-1}; FABMS *m/z* 542 ($M^+ - CH_3O$).

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Supplementary Material Available: ^1H NMR spectra of **1–5**, HMBC spectra of **1–5**, ^{13}C NMR spectra of **1** and **2**, and a NOESY spectrum of **1** (13 pages). This material is contained in many libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.

Phloeodictines A and B: New Antibacterial and Cytotoxic Bicyclic Amidinium Salts from the New Caledonian Sponge, *Phloeodictyon* sp.[1]

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Two new alkaloids, phloeodictine A (**1**) and phloeodictine B (**2**), possessing an unprecedented 6-hydroxy-1,2,3,4-tetrahydropyrrolo[1,2-*a*]pyrimidinium skeleton have been isolated from an undescribed species of the deep sponge *Phloeodictyon*. The structures were determined by extensive spectroscopic analysis particularly two-dimensional NMR experiments. Both compounds exhibited in vitro antibacterial activity against Gram-positive and Gram-negative bacteria and were moderately cytotoxic against KB cells.

As an outgrowth of our search for biologically active compounds from marine organisms, we report here that extracts from an undescribed species of the deep water sponge *Phloeodictyon*[2] (family Nepheliospongia, order Nepheliospongidae), collected in the south of the New Caledonian lagoon, strongly inhibit the growth of bacteria and are moderately cytotoxic. Bioassay-guided purification of the crude extract resulted in the purification of two novel bicyclic amidinium salts with a unique 6-hydroxy-1,2,3,4-tetrahydropyrrolo[1,2-*a*]pyrimidinium skeleton which we have named phloeodictine A (**1**) and phloeodictine B (**2**). This paper describes the isolation and structure elucidation of **1** and **2**.

Phloeodictyon sp. was kept frozen until workup. The lyophilized sponge was homogenized and consecutively extracted with heptane and methanol. The antimicrobial methanolic extract was desalted over Amberlite XAD-7 and subsequently subjected to medium-pressure reversed-phase liquid chromatography (H_2O–MeOH step gradient). Final purification of **1** and **2** was accomplished by repetitive preparative and semipreparative RP-HPLC [Delta-Pak C18, MeOH–NaCl (0.2 M)–THF (56:43:1 for

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(2) The sponge was identified by Prof. C. Levi, Museum National d'Histoire Naturelle, 75005 Paris. To our knowledge, specimens of this genus have not been chemically studied previously.

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