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BIS(SULFATO)-CYCLOSIPHONODICTYOL A, A NEW DISULFATED SESQUITERPENE-HYDROQUINONE FROM A DEEP WATER COLLECTION OF THE MARINE SPONGE *SIPHONODICTYON CORALLIPHAGUM*

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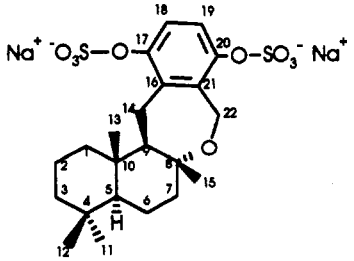
ABSTRACT.—A new compound, *bis*(sulfato)-cyclosiphonodictyol A [**1**], which inhibits the binding of [^{3}H]-LTB$_4$ to intact human neutrophils with an IC$_{50}$ value of 44 μM, was isolated from the sponge *Siphonodictyon coralliphagum*. The sponge was collected using the Johnson-Sea-Link manned submersible at a depth of 195 feet in the Bahamas. The compound was isolated via reversed-phase chromatography and its structure determined spectroscopically. To the best of our knowledge, **1** is the first marine-derived compound with two aromatic sulfate ester functionalities, and is also the first in the siphonodictyal series to contain an oxepane functionality.

Many marine sponges are known to yield sesquiterpene-hydroquinone compounds, some examples of which are chromazonarol from *Disidea pallescens* (1), avarol from *Disidea avara* (2), aureol and 8-epichromazonarol from *Smenospongia aurea* (3), and strongylin A from *Strongylophora hartmani* (4). *Siphonodictyon coralliphagum* Rützler (Haplosclerida, Niphatidae) is a boring sponge that occurs throughout the Caribbean. In shallow water habitats, it is most commonly observed as a series of bright yellow chimneys protruding from coral heads. In deeper water habitats (>150 feet), it occurs as a thick encrusting yellow mat. A series of sesquiterpene phenolic aldehydes, siphonodictyals A, B, C, D, and E, the monosulfated siphonodictyols G and H, and siphonodictyoic acid have been reported from *Siphonodictyon* species (5,6). In this paper we describe the bioassay-guided isolation and structure elucidation of *bis*(sulfato)-cyclosiphonodictyol A [**1**].

Compound **1** was isolated using a bioassay-guided approach from an EtOH extract of *Siphonodictyon coralliphagum* via solvent partitioning, ultrafiltration, and reversed-phase chromatography on a C$_{18}$ column. ^{1}H-Nmr and homonuclear decoupling experiments indicated the presence of two ortho-coupled aromatic protons [δ 7.31 (d, J=9.0 Hz), 7.16 (d, J=9.0 Hz)], four methyl singlets [δ 0.83 (3H, s), 0.86 (3H, s), 0.89 (3H, s), 1.42 (3H, s)], a benzylic methylene group [δ 3.44 (d, J=16.0 Hz), 2.65 (dd, J=16.0 and 9.5 Hz)], and an isolated oxygenated methylene group [δ 4.98 (d, J=15.5



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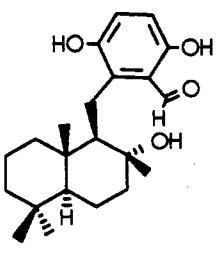

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Hz), 4.84 (d, J=15.5 Hz)]. Comparison of the ^{13}C-nmr data with those of siphonodictyal A [**2**] (5) suggested a structure in which a cyclization/reduction has taken place between the C-8 hydroxyl and the C-22 aldehyde to form a seven-membered cyclic ether. The structure of **1** was confirmed and all chemical shift assignments made by proton-detected one- and multiple-bond ^{1}H-^{13}C correlation experiments (Table 1). The presence of sulfate on one or both of the phenolic hydroxyls was suggested by strong ir bands at 1230 and 1055 cm^{-1}. Fabms confirmed that **1** was a disulfated compound with the formula $C_{22}H_{30}O_9S_2Na_2$. The ^{13}C-nmr chemical shift of the C-15 methyl group in **1** (δ 22.1) was consistent with an axial configuration as found in chromazonarol (δ 20.6) rather than the equatorial configuration found in epichromazonarol (δ 27.0) (3). This relative stereochemistry was confirmed by a series of difference nOe experiments. The following enhancements which support the assigned stereochemistry were observed: irradiation of the Me-13 protons enhanced the resonances observed for the Me-12 and Me-15 protons suggesting that all three are axial. Irradiation of the Me-12 protons enhanced the Me-13 proton resonance. Irradiation of the Me-15 protons enhanced the resonances observed for the Me-13 protons and the H-14a proton indicating the assigned stereochemistry at C-9.

The leukotrienes are 5-lipoxygenase metabolites of arichidonic acid. In the lipoxygenase pathway, the enzyme 5-lipoxygenase catalyzes the oxidation of arachidonic acid to 5-HPETE. Leukotriene A synthase converts 5-HPETE to

TABLE 1. ^{1}H- and ^{13}C-Nmr Data for **1** (CD_3OD).

Position	^{13}C δ (mult.)	^{1}H δ (mult., J in Hz)	Observed long-range ^{1}H-^{13}C correlations
1	40.7 t	a 0.90 m	
		b 2.13 m	C-3, C-5
2	19.7 t	a 1.50 m	
		b 1.67 m	
3	43.3 t	a 1.15 m	C-2, C-11, C-12
		b 1.36 m	
4	34.4 s		
5	57.4 d	0.94 dd (2.1, 12.0)	
6	21.4 t	a 1.39 m	C-8, C-10
		b 1.75 m	
7	41.2 t	a 1.52 m	
		b 1.68 m	
8	81.2 s		
9	59.2 d	1.53 d (9.5)	
10	40.1 s		
11	33.8 q	0.86 s	C-3, C-5, C-12
12	21.8 q	0.83 s	C-3, C-5, C-11
13	16.2 q	0.89 s	C-5, C-9, C-10
14	23.7 t	a 2.65 dd (16.0, 9.5)	C-8, C-9, C-16, C-17
		b 3.44 d (16.0)	C-8, C-9, C-10, C-16, C-17, C-21
15	22.1 q	1.42 s	C-7, C-8, C-9
16	138.2 s		
17	148.3 s		
18	122.0 d	7.31 d (9.0)	C-16, C-20
19	121.1 d	7.16 d (9.0)	C-17, C-21
20	147.6 s		
21	136.0 s		
22	59.3 t	a 4.84 d (15.5)	C-8, C-16, C-20, C-21
		b 4.98 d (15.5)	C-8, C-16, C-20, C-21

the unstable epoxide LTA_4 which is in turn converted to LTB_4 through the action of LTA hydrolase. LTB_4 has been implicated in aggregation, chemotaxis, and degranulation (7). A specific antagonist of LTB_4 receptor binding may have potential in inflammatory and allergic diseases. *bis*(Sulfato)-cyclosiphonodictyol A inhibits binding of [^{3}H]-LTB_4 to human neutrophils with an IC_{50} value of 44.5 μM (n=3).

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Spectral data were measured on the following instruments: ir, Perkin-Elmer 1310; uv, Perkin-Elmer Lambda 3B; nmr, Bruker AM-360 with an Aspect 3000 computer and Bruker AMX-500 with a X-32 computer; ms, Kratos MS-80RFA, FAB-NOBA (Chemical Instrumentation Center, Yale University); optical rotation, Jasco DIP-360 Digital polarimeter. ^{1}H-Nmr chemical shifts are reported as δ values in ppm relative to CD_3OD (3.30 ppm). ^{13}C-Nmr chemical shifts are reported as δ values in ppm relative to CD_3OD (49.0 ppm). ^{13}C-Nmr multiplicities were measured using the DEPT sequence, and one- and multiple-bond ^{1}H-^{13}C connectivities were determined via the 2D proton-detected HMQC and HMBC experiments, respectively.

ANIMAL MATERIAL.—The sample (DBMR number: 27-IX-88-1-015) was collected in September 1988, off Cockburn Town, San Salvador, Bahamas, on a rock wall at a depth of 195 feet using the Johnson-Sea-Link I manned submersible. The sponge was encrusting, aproximately 50 cm in diameter, yellow externally and internally. The sample corresponds most closely to *Siphonodictyon coralliphagum* (9). A voucher specimen is on deposit at the Harbor Branch Oceanographic Museum, Fort Pierce, Florida (catalog number 003:00907).

BIOASSAY PROTOCOL.—Binding of [^{3}H]LTB_4 to receptors in human neutrophils was measured as described by Gorman and Lin (8). Intact human neutrophils were suspended in Hank's Balanced Solution (HBSS) at a concentration of 3×10^6 cells/assay tube. An aliquot of the cell suspension (300 μl) was added to triplicate tubes containing 50 μl [^{3}H]LTB_4 (specific activity 32 Ci/mmol, Dupont NEN, Boston, MA) at a final concentration of 0.5 nM, 100 μl buffer, and 50 μl drug or buffer. Nonspecific binding was determined in the presence of 300 nM LTB_4. The reaction was initiated by addition of cell suspension and continued at 4° for 20 min. Bound radioactivity was isolated by vacuum filtration through Whatman GF/C glass fiber filters using a Brandel Cell Harvester and unbound radioactivity removed with 2×5 ml washes with ice-cold saline. Filters were placed in polyethylene scintillation mini-vials to which were added 3.5 ml of Formula-989 scintillation cocktail (NEN). After equilibration, radioactivity determinations and data calculations were performed using non-linear regression analysis on RS-1.

EXTRACTION AND ISOLATION.—The diced sponge (500 g) was extracted by blending with EtOH (3×2000 ml). This extract was dried under vacuum to obtain a yellow hygroscopic solid (43.8 g) which was partitioned between *n*-BuOH and H_2O. The *n*-BuOH fraction was then partitioned between EtOAc and H_2O and the H_2O partition subjected to ultrafiltration on 100, 5, and 1 kDa filters. The <1 kDa fraction was separated by reversed-phase hplc (Vydac protein & peptide C_{18}, H_2O-MeOH, 70:30) to yield **1** (3.4 mg).

bis(*Sulfato*)-*cyclosiphonodictyol A* [**1**].—Colorless amorphous solid; $[\alpha]^{24}D$ +12.0° (c=0.2 MeOH); uv (MeOH) λ max 266 (410), 262 (410), 217 (4200), 203 (9400) nm; ir (film on KBr) ν max 3500 br, 2930, 1463, 1381, 1260, 1230, 1055, 1030, 995, 928, 830 cm^{-1}; ^{1}H- and ^{13}C-nmr data, see Table 1; hrfabms *m/z* observed 571.1116 $[M+Na]^+$ ($C_{22}H_{30}O_9S_2Na_3$ requires 571.1026).

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