A New C-20 Polyacetylene from the Sponge *Callyspongia pseudoreticulata*

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The Indonesian marine sponge *Callyspongia pseudoreticulata* was found to contain (3*S*,18*S*,4*E*,16*E*)-eicosa-1,19-diyne-3,18-diol-4,16-diene (**1**), the structure of which was determined by detailed spectroscopic analysis. Its absolute configuration was established using the modified Mosher's method after esterification of the secondary alcohols with Mosher's reagent.

Straight-chain acetylenic compounds have been isolated frequently from sponges of the order Haplosclerida and are considered as good chemotaxonomical markers for the order.[1,2] Distinct types of straight-chain acetylenic compounds are recognized on the basis of the length of the chain, the number and position of the double and triple bonds, and the nature and position of the functional groups. These various types may be markers for genera and families. For example, *Petrosia* species characteristically contain hydroxylated compounds, whereas brominated compounds are found in *Xestospongia* species. Besides their interest as chemical markers, several straight-chain compounds possess notable biological activities including antifungal and antimicrobial activities, HIV reverse transcriptase inhibition, and cytotoxicity.[3]

In our ongoing search for biologically active and significant metabolites from sponges,[4] the CH_2Cl_2-soluble fraction of the MeOH extract of the Indonesian sponge *Callyspongia pseudoreticulata* (Haplosclerida, Callyspongiidae) was found to be toxic against nauplii of the brine shrimp *Artemia salina* (LD_{50} = 5 μg/mL). In this paper, we report the structure determination, including the absolute configuration, of the major constituent [**1**, (3*S*,18*S*,4*E*,16*E*)-eicosa-1,19-diyne-3,18-diol-4,16-diene] of this toxic fraction.

Compound **1** was isolated as a white solid after chromatography of the CH_2Cl_2-soluble fraction on a Si gel column. HRCIMS (NH_3) of compound **1** gave a pseudomolecular ion $(M+ NH_4)^+$ at *m/z* 320.2593 appropriate for $C_{20}H_{34}NO_2$ (calcd 320.2590). This indicated the molecular formula $C_{20}H_{30}O_2$ (6 unsaturations) for compound **1**. The IR spectrum indicated the presence of triple bonds (2110 cm^{-1}) and hydroxyl groups (3294 cm^{-1}). The ^{13}C NMR spectrum obtained by the procedure of broadband proton noise decoupling contained only 10 signals, suggesting that **1** possessed a 2-fold element of symmetry. Five different methylenes (δ_C 28.8, 29.1, 29.4, 29.5, and 31.9), terminal alkyne (δ_H 2.57; δ_C 74.0 and 83.4), disubstituted double bond (δ_H 5.61 and 5.92; δ_C 128.4 and 134.6), and secondary alcohol (δ_H 4.84; δ_C 62.8) functionalities were readily identified from the ^1H and ^{13}C NMR data of compound **1** (Table 1). The presence of two hydroxyl groups was ascertained by the formation of the diacetyl derivative **2** upon treatment of **1** with the mixture pyridine/acetic anhydride, 1:1. The $^1H-^1H$ COSY spectrum of **1** showed correlations that linked the secondary alcohol to the terminal alkyne and to the disubstituted olefin (δ_H 4.84 to 2.57 and 5.61). In addition, the HMBC spectrum indicated that the two vinylic protons were coupled to the methylene at δ_C 31.9. This implied the presence of substructure **3**. The latter was confirmed by comparison with the NMR data reported for natural compounds bearing the same substructure.[5,6] The *E* configuration was assigned to the double bond on the basis of the coupling constant ($J_{4,5}$ and $J_{16,17}$ = 15 Hz). Because **1** was symmetrical, it had to be the dimer of the $C_{10}H_{17}O$ fragment identified from the NMR spectra. All these data coupled to the complete analysis of the 1D and 2D NMR spectra ($^1H-^1H$ COSY, HMQC, HMBC) indicated that compound **1** is (4*E*,16*E*)-eicosa-1,19-diyne-3,18-diol-4,16-diene. This compound has not been previously reported from either natural sources or synthesis.

Table 1. NMR Data of Compound **1** ($CDCl_3$, 600 and 150.87 MHz, *J* in Hz)

position	δ_C	δ_H (multiplicity, *J*)
HC-1 and -20	74.0	2.57, d, 2
C-2 and -19	83.4	
HC-3 and -18	62.8	4.84, br d, 6
HC-4 and -17	128.4	5.61, ddt, 15, 6, 1
HC-5 and -16	134.6	5.92, dtd, 15, 7, 1
H_2C-6 and -15	31.9	2.07, dt, 7, 7
H_2C-7 and -14	28.8[a]	1.61 to 1.26, m
H_2C-8 and -13	29.1[a]	
H_2C-9 and -12	29.4[a]	
H_2C-10 and -11	29.5[a]	

[a] Assignments may be interchanged.

The absolute configuration of compound **1** was determined by preparing both Mosher's diesters, **4** and **5**, of **1**.[7] Positive $\Delta\delta$'s ($\delta S - \delta R$) were observed for H-1 and H-20 (+0.04), while negative $\Delta\delta$'s were observed for H-4 and H-17 (−0.1) indicating that the absolute configuration of compound **1** is 3*S*, 18*S*. It has to be mentioned that the NMR spectrum of both **4** and **5** revealed the presence of traces of the other diastereoisomer (ca. 10:1 ratio). This can be attributed to the fact that in 10% of the molecules one or both of the secondary alcohol groups possess the *R* configuration, indicating that **1** is a mixture of stereoisomers (3*S*, 18*S* + 3*R*, 18*R* and/or 3*S*, 18*R*).

Several polyacetylene derivatives containing the 1,4-enyne-3-ol fragment have already been isolated from Haplosclerida sponges.[1] Although the absolute configuration of all of them is not known, it appears that the *S* configuration is the most frequently encountered.

Compound **1** is toxic for nauplii of the brine shrimp *Artemia salina* (LD_{50} = 2 μg/mL). This indicated that **1** is

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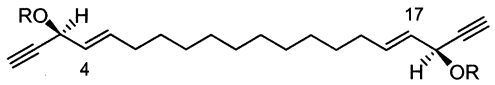
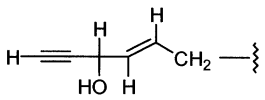


1 R = H

2 R = $COCH_3$

4 R = (*R*) - MTPA

5 R = (*S*) - MTPA



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responsible at least in part for the toxicity of the CH_2Cl_2-soluble fraction of the MeOH extract of the sponge.

Experimental Section

General Experimental Procedures. The IR spectra were obtained on a Bruker IFS 25 instrument as a film on a NaCl disk or as a KBr pellet. The ^1H and ^{13}C NMR spectra were recorded in $CDCl_3$ at 600 and 150.87 MHz, respectively, using a Varian Unity 600 instrument, or at 300 and 75.4 MHz on a Bruker DA 300 instrument using TMS as internal standard. HREIMS measurements were performed on a Micromass Autospec 3F instrument. Thin-layer chromatography analyses (TLC) were performed on 0.25 mm Polygram silica gel SILG/UV_{254} precoated plates (Macherey Nagel) and column chromatographies on Si gel (MN Kieselgel 0.04−0.063 mm), using the flash technique.

Biological Material. The sponges were collected by scuba diving in South Sulawesi, Spermonde Archipelago, off Ujung Pandang in May 1998. The sponge consists of a dense mass of black-colored branches, which may reach an overall length of more than 1 m. The branches are 0.5−1 cm in diameter and have large numbers of evenly distributed small oscules with raised rims. The surface is smooth, and the ectosome is obscured by black-pigmented grains. The ectosomal skeleton is made from the usual double meshed reticulation of primary and secondary fibers (10−25 μm of diameter), making larger meshes of up to 400 μm subdivided into smaller meshes of 80−150 μm. All fibers are cored by a single spicule. The interior skeleton is irregularly rectangular. Primary fibers are 30−50 μm in diameter and have a core of 2−5 spicules. Secondary fibers are 12−20 μm and are singly cored. Spicules are composed of straight equidiametrical strongyles of 55−65 × 0.5−1.5 μm. These characters match those of the subgenus *Callyspongia* and the species *C. pseudoreticulata*.

Extraction and Isolation. Specimens of *Callyspongia pseudoreticulata* stored in MeOH (9.7 g dry weight) were repeatedly extracted with MeOH/CH_2Cl_2, 50:50, and the combined extracts were concentrated. The water content of the extract was adjusted to 100 mL before partitioning against CH_2Cl_2. The organic layer was evaporated to dryness in vacuo, leading to a solid residue (0.65 g), a part of which (430 mg) was flash chromatographed on a Si gel column using as eluent the mixture hexane/acetone, 100:0 to 80:20. The chromatography was monitored by TLC and the compounds visualized by spraying with an ethanolic solution of phosphomolybdic acid. This led to the isolation of the major compound **1** as a white solid (33 mg).

Compound 1: $[\alpha]^{20}_D$ +26° (*c* 1, MeOH); IR broad intense OH band at 3294 cm^{-1} and sharp band of low intensity at 2110 cm^{-1}; ^1H and ^{13}C NMR, see Table 1; HRCIMS (NH_3) *m/z* 320.2593 [5, $(M + NH_4)^+$, calcd for $C_{20}H_{34}NO_2$ 320.2590], 302.2489 [23, $(M - H_2O + NH_4)^+$], 285.2228 [27, $(M - H_2O + H)^+$], 284.2383 [22, $(M - 2H_2O + NH_4)^+$], 267.2116 [15, $(M - 2H_2O + H)^+$], 247.2062 (6, $M^{+\bullet} - C_3H_3O^\bullet$), 229.1956 (6, $M^{+\bullet} - C_3H_3O^\bullet - H_2O$), 185 (13), 171 (20).

Acetylation of 1. Treatment of compound **1** (5 mg) with the mixture pyridine/acetic anhydride, 1:1 (1 mL), for 24 h at room temperature led to the diacetyl derivative **2**, which was purified by flash chromatography on a Si gel column using as eluent the mixture hexane/acetone, 100:0 to 80:20.

Compound 2: IR low-intensity bands at 3285 and 2120 cm^{-1} and intense bands at 1732 and 1226 cm^{-1}; ^1H NMR (300 MHz) δ 2.09 (s, $COCH_3$), 2.56 (d, J = 2; H-1 + H-20), 5.53 (ddt, J = 15,6,1; H-4 + H-17), 5.84 (m; H-3 + H-18), 6.00 (dtd, J = 15,7,1; H-5 + H-16); CIMS (NH_3) *m/z* 404 [61, $(M+ NH_4)^+$], 344 (30), 327 (43), 285 (92), 267 (100).

Preparation of the MTPA Esters 4 and 5. Freshly distilled oxalyl chloride (55 μL) was added to a solution of (*R*)-MTPA (28 mg) and DMF (8.5 μL) in dry hexane (2.5 mL) at room temperature. After 90 min the solution was concentrated in vacuo and IR analysis showed that formation of (*S*)-MTPACl was complete. The residue was dissolved in THF (1 mL) and added to a solution of **1** (5 mg) and DMAP (25 mg) in 1 mL of THF. The resulting solution was stirred at room temperature for 24 h. Addition of water (5 mL) and extraction with CH_2Cl_2 (3 × 10 mL) led to an organic phase that afforded ester **4** (5 mg) after evaporation and flash chromatography on a Si gel column using as eluent the mixture hexane/acetone, 90:10. The same procedure starting from (*S*)-MTPA afforded ester **5** (6 mg).

Compound 4: ^1H NMR (300 MHz) δ 1.32 (m, H_2-7 to H_2-14), 2.06 (m, H_2-6 + H_2-15), 2.59 (d, J = 2; H-1 + H-20), 3.55 (s, OCH_3), 5.60 (ddt, J = 15,6,1; H-4 + H-17), 6.04 (m; H-3 + H-5 + H-16 + H-18), 7.40 (6H arom), and 7.53 (4H arom).

Compound 5: ^1H NMR (300 MHz) δ 1.32 (m, H_2-7 to H_2-14), 2.06 (m, H_2-6 + H_2-15), 2.63 (d, J = 2; H-1 + H-20), 3.59 (s, OCH_3), 5.50 (ddt, J = 15,6,1; H-4 + H-17), 6.04 (m; H-3 + H-5 + H-16 + H-18), 7.40 (6H arom) and 7.53 (4H arom).

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