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Bioactivity-guided fractionation of the ethyl acetate extract of a marine sponge, *Xestospongia* sp., led to the isolation of a new thiophene-*S*-oxide acyclic sesterterpene (**1**). The chemical structure was extensively analyzed using NMR and mass spectral data. Compound **1** showed weak cytotoxicity against Vero cells.

Keywords: acyclic sesterterpene; thiophene; *Xestospongia* sp.

1. Introduction

Sulfur is often found in marine natural products in forms of the isothiocyanate, thiocyanate, thioacetate, thiol, sulfone, and sulfate functional groups, whereas the thiazole is a common natural heterocyclic moiety [1]. Acyclic sesterterpenes were mainly obtained from sponges of the family Thorectidae [2]. Some sesterterpenes are substituted with the sulfate(s) on a five-membered lactone ring at one end of the linear skeleton and exhibited some biological activities, particularly brine shrimp toxicity [2]. However, thiophene-containing acyclic sesterterpenes produced by marine sponges have not yet been reported. Although sponges of the genus *Xestospongia* have been known to produce a wide range of secondary metabolite classes such as alkaloids, polycyclic quinone and hydroquinones, polyacetylenic derivatives, aminoalcohols, and sterols [3], acyclic sesterterpenes have never been found from *Xestospongia* spp. As part of our ongoing survey for biologically active secondary metabolites from sponges, we collected 18 sponge species within 32,000 m^2 area of the Sikao Bay, the Andaman Sea, Thailand. Herein, we describe the first discovery of an acyclic sesterterpene containing a thiophene-*S*-oxide moiety (**1**) isolated from a sponge, *Xestospongia* sp., collected from the Sikao Bay.

2. Results and discussion

Compound **1** (Figure 1) was isolated from the EtOAc extract of a sponge *Xestospongia* sp. as pale yellowish oil by repeat chromatography on silica gel columns and finally by preparative silica gel thin layer chromatography. The HREIMS of **1** gave a molecular ion peak at *m/z* 386.2643 compatible with a formula of $C_{25}H_{38}OS$, with seven degrees of unsaturation. Its ^1H NMR spectral data (Table 1) showed five
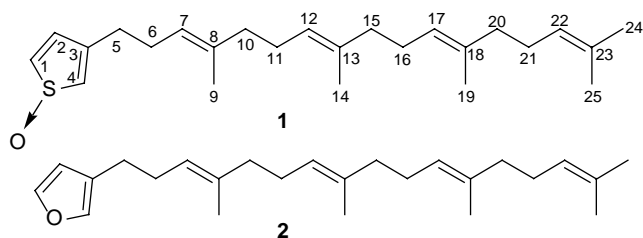
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Figure 1.   Structures of a new isolated compound (**1**) and a known furospinusolin-1 (**2**).

groups of proton signals, including five methyls (δ_H 1.52, 1.55, 1.59, 1.60, and 1.67), eight aliphatic methylenes (δ_H 2.34 and overlapping signals at δ_H 2.05–2.11 and δ_H 2.17–2.20), four olefinics (overlapping signals at δ_H 5.22–5.28), and three aromatics (δ_H 7.12, 6.15, and 7.07). Its ^{13}C NMR and DEPT-135 spectral data

revealed 25 signals which were assigned as 7 sp^2 methine carbons (δ_C 111.9, 124.3, 124.7, 124.8, 124.9, 139.2, and 142.8), 5 sp^2 quaternary carbons (δ_C 125.1, 134.9, 135.6, 135.0, and 131.1), 8 sp^3 methylene carbons (δ_C 25.3, 26.9, 27.0, 27.2, 28.8, 40.1, 40.2, and 40.6), and 5 methyl carbons (δ_C 17.7, 25.8 and overlapping signals at δ_C 16.1 accounting for 3 methyl groups).

Construction of the linear skeleton of **1** was achieved by careful analysis of ^{1}H–^{1}H COSY and HMBC cross peaks. The COSY spectrum showed continual cross peaks among the overlapping proton signals (δ_H 2.05–2.11, 2.17–2.20, and 5.22–5.28) at H$_2$-5 (δ_H 2.34)/H$_2$-6 (δ_H 2.19)/H-7 (δ_H 5.28), H$_2$-10 (δ_H 2.11)/H$_2$-11(δ_H 2.17)/H-12 (δ_H 5.22), H$_2$-15 (δ_H 2.05)/H$_2$-16 (δ_H 2.18)/H-17 (δ_H 5.24), and H$_2$-20 (δ_H 2.20)/H$_2$-21 (δ_H 2.10)/H-22 (δ_H 5.26), indicating the presence of four sets of –CH$_2$CH$_2$CH= spin systems. The connectivity of these spin systems was based on H–C long-range correlations in the HMBC spectrum at H-7/C-10 (δ_C 40.1), H-12/C-15 (δ_C 40.6), H-17/C-20 (δ_C 40.2), and H$_2$-20/C-22 (δ_C 124.8). The overlapping carbon signals at δ_C 16.1 represented three methyl carbons because it showed cross peaks with three methyl protons at δ_H 1.60, 1.52, and 1.59 in the HMQC spectrum. These carbon signals were assigned as C-9, C-14, and C-19 through the HMBC cross peaks at H$_3$-9 (δ_H 1.60)/C-7 (δ_C 124.9), C-8, C-10; H$_3$-14 (δ_H 1.52)/C-12 (δ_C 124.3), C-13, C-15; and H$_3$-19 (δ_H 1.59)/C-17 (δ_C 124.7), C-18, C-20, respectively. The methyl protons

Table 1. ^{1}H NMR (500 MHz) and ^{13}C NMR (125 MHz) spectral data of **1** (CDCl$_3$).

Position	**1**	
	^{1}H (mult, *J*, Hz)	^{13}C
1	7.12 (br s)	142.8
2	6.15 (br s)	111.9
3	–	125.1
4	7.07 (br s)	139.2
5	2.34 (t, *7.5, 15.0*)	25.3
6$^\Delta$	2.19 (m)	28.8
7*	5.28 (m)	124.9
8	–	134.9
9**	1.60 (s)	16.1
10	2.11 (m)	40.1
11$^\Delta$	2.17 (m)	27.2
12*	5.22 (t, *7.0,13.5*)	124.3
13	–	135.6
14**	1.52 (s)	16.1
15	2.05 (m)	40.6
16$^\Delta$	2.18 (m)	26.9
17*	5.24 (m)	124.7
18	–	135.0
19**	1.59 (s)	16.1
20	2.20 (m)	40.2
21$^\Delta$	2.10 (m)	27.0
22*	5.26 (m)	124.8
23	–	131.1
24	1.67 (s)	25.8
25	1.55 (s)	17.7

Note: *, Δ, ** may be interchanged.

at δ_H 1.67 (H$_3$-24) and 1.55 (H$_3$-25) showing ^2J-correlations with C-23 (δ_C 131.1) and a ^3J-correlation with C-22 revealed a geminal dimethyl at one end of the linear skeleton. The above-mentioned NMR spectral data were accounted for C$_{21}$H$_{35}$ and four degrees of unsaturation. The remaining C$_4$H$_3$SO and three degrees of unsaturation were confidentially deduced as a thiophene-*S*-oxide ring by the appearance of a fragment ion at *m/z* 272 due to the loss of 114 mass unit in the EIMS spectrum and the typical broad singlets resonating at δ_H 7.12 (H-1), 6.15 (H-2) and 7.07 (H-4) in the ^{1}H NMR spectrum. The thiophene-*S*-oxide ring and its connectivity at the other end of the linear skeleton were finally confirmed by the following HMBC cross peaks at H-1/C-3 (δ_C 125.1), C-4 (δ_C 139.2); H-2/C-4; H-4/C-1 (δ_C 142.8), C-2 (δ_C 111.9); and H$_2$-5/C-4 and C-2.

The geometry of the double bonds in acyclic chain was assigned by comparison of the ^{13}C NMR chemical shifts with the typical values of methyls on trisubstituted double bonds in isoprenoid chains. The substituted methyl carbon of the *E-oriented* isoprenoid moiety is shifted more upfield than that of the *Z*-orientation [4]. In the case of *E* double bonds, the methyl chemical shifts in the range of δ_C 15.8–16.3 are typical, whereas the methyl chemical shifts in the range of 23.3–23.7 ppm are typical for the *Z* double bonds, depending on the NMR solvent used [5,6]. Using this information, all double bonds in the chain of **1** were in *E*-orientation. Compound **1** was then proposed as a new acyclic sesterterpene and differs from the well-known furospinusolin-1 (**2**), isolated from *Spongia idia* [7–10], by the presence of a thiophene-*S*-oxide ring instead of a furan ring. Compound **1** exhibited weak cytotoxicity against Vero cells with an IC$_{50}$ of 31 μM.

3. Experimental

3.1 General experimental procedures

^{1}H and ^{13}C NMR spectra were obtained using a 500 MHz FT-NMR spectrometer, Varian Unity Inova 500 (CA, USA), and TMS was used as an internal standard. The HREIMS spectral data were obtained on a MAT 95 XL Mass Spectrometer, Thermo finnigan (Egelsbach, Germany) with methane as a reagent gas. The IR spectrum was recorded on a PerkinElmer FT-IR Spectrometer (Massachusetts, USA). The UV spectrum was determined by a Milton Roy Spectronic 3000 Diode Array Spectrometer (New York, USA). Analytical and preparative TLC were performed on precoated aluminum sheets (DC Kieselgel F$_{254}$, No. 1.05554.0001) and on precoated glass plates (DC Kieselgel 60 F$_{254}$, No. 1.13895.0001), respectively, Merck (Darmstadt, Germany).

3.2 Animal material

Sponge samples were collected at a depth of 10 m by a SCUBA diver (April 2009) in the Sikao Sea, Trang Province, Thailand. The samples were kept in sealed plastic packs in an ice box during transfer to the laboratory and immediately frozen before extraction. The sponge was identified to be *Xestospongia* sp. by Dr. Udomsak Darumas (School of Sciences, Walailak University, Nakhonsrithammarat, Thailand). The sponge is a black massive shape with 3 mm in diameter, scattered on one face of the lamellae, connected with shallow subdermal cavities with numerous ramifications. The non-ocular side is pierced by numerous ramifications of 10 μm in diameter of ostia. It has rough surface and hard texture. Choanosomal skeletons appear as a highly dense network of short longitudinal undivided, irregularly parallel tracts. Megascleres are oxeas with some strongly and stylote forms. Spicules are only one size category showing variability only in thickness.

3.3 Extraction and isolation

The sponge (2.0 kg, wet weight) was homogenized and macerated in MeOH (3 × 5 liter, 72 h). After filtration and concentration, the aqueous methanol fraction was repeatedly partitioned with EtOAc until complete extraction (confirmed by routine TLC analysis). The EtOAc solution was concentrated under reduced pressure to give a residue (3.5 g), which was further fractionated by silica gel vacuum liquid chromatography with step gradient elution of C_6H_{14}, C_6H_{14}:CH_2Cl_2 (8:2, 1:1, 0:1, v/v) and CH_2Cl_2:MeOH (8:2, 1:1, 3:7, v/v), to give six fractions (70, 300, 300, 200, 150, and 800 mg, respectively). All fractions were examined for cytotoxicity against Vero cells before further isolation. The first fraction (70 mg) showed a positive result and was chosen for further purification by silica gel column chromatography using stepwise gradient elution with C_6H_{14}, C_6H_{14}−CH_2Cl_2 (8:2, 6:4, 1:1, 4:6, 2:8, 0:1, v/v) and CH_2Cl_2−MeOH (9:1, 8:2, 7:3, v/v). Five fractions (5, 25, 15, 10, and 12 mg, respectively) were collected. The second fraction (25 mg) was purified further by silica gel preparative TLC (20 × 20 cm) using a mixture of C_6H_{14}:CH_2Cl_2 (7:3, v/v) as the mobile phase (triple developments). The developed preparative TLC was detected under UV light at 254 nm and the quenching band was scraped. After washing the scraped powder with CH_2Cl_2, compound **1** (10 mg) was obtained.

3.3.1 3-(4,8,12,16-Tetramethyl-heptadeca-3,7,11,15-tetraenyl)-thiophene-1-oxide (1)

Pale yellowish oil. UV (MeOH) λ_{max} (log ε) 218 (4.42) nm: IR (Nujol) ν_{max} cm^{-1}: 2925,1642, 1230, 1025; ^1H (CDCl₃, 500 MHz); and ^{13}C NMR (CDCl₃ 125 MHz) spectral data, see Table 1. EIMS m/z (%):386 (M⁺·, 10), 371 (17), 315 (18), 293 (7), 285 (15), 272 (5), 217

(8), 204 (15), 175 (10), 161 (12), 149 (17), 147 (10), 135 (27), 123 (22), 95 (27), 81 (94), 69 (100); HREIMS m/z: 386.2643 (calcd for $C_{25}H_{38}OS$, 386.2645).

3.4 Biological activity determination

Cytotoxicity against Vero cells (African green monkey kidney cell, ATCC CCL-81) was determined by the green fluorescent protein detection method [11]. The assay was carried out by adding 45 μl of cell suspension (3.3×10^4 cell ml⁻¹) to 384-well plates which contained 5 μl of the test compound. The compound was dissolved in 0.5% DMSO (maximum concentration 50 μg ml⁻¹) and further twofold serially diluted until a minimum concentration of 1.56 μg ml⁻¹ was prepared. After 4 days incubation at 37°C with 5% CO_2, the fluorescence signals were measured by using a Spectra Max M5 microplate reader (Molecular Devices, USA) in bottom reading mode, with excitation and emission wavelengths of 485 and 535 nm. The IC_{50} values were derived from dose–response curves by the SoftMax Pro software (Molecular Devices, CA, USA). Ellipticine ($IC_{50} = 1.03$ μM) and 0.5% DMSO were used as positive and negative controls, respectively.

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