



SULFUR-CONTAINING COMPOUNDS FROM *SCORODOCARPUS BORNEENSIS* AND THEIR ANTIMICROBIAL ACTIVITY

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Key Word Index—*Scorodocarpus borneensis*; Olaceae; wood garlic; polysulfides; 2,4,5,7-tetra-thiaoctane 4,4-dioxide; 5-thioxo-2,4,6-trithiaheptane 2,2-dioxide; antimicrobial activity.

Abstract—Three novel sulfur-containing compounds were isolated from *Scorodocarpus borneensis*. Their chemical structures were determined to be 2,4,5,7-tetrathiaoctane 4,4-dioxide ($\text{CH}_3\text{SCH}_2\text{SO}_2\text{SCH}_2\text{SCH}_3$, **1**) and 5-thioxo-2,4,6-trithiaheptane 2,2-dioxide ($\text{CH}_3\text{SO}_2\text{CH}_2\text{SCSSCH}_3$, **2**), while the other compound was assumed to be *O*-ethyl *S*-methylthiomethyl thiosulfite ($\text{CH}_3\text{SCH}_2\text{SS(O)OCH}_2\text{CH}_3$, **3**) on the basis of spectroscopic data. This first isolation of compound **1** strongly suggests that *S. borneensis* possesses a polysulfide formation pathway similar to that of other *Allium* species. Compounds **1** and **2** exhibited antimicrobial activity against some bacteria and fungi. © 1998 Published by Elsevier Science Ltd. All rights reserved

INTRODUCTION

Scorodocarpus borneensis Becc., a species of the Olaceae family, is a tree which grows naturally on the island of Borneo and the Malay Peninsula. It has the native name of “wood garlic” due to its strong garlic-like smell, and sliced or powdered pulp of the fruit is sometimes used as seasoning by local people, for cooking fish and other food materials [1].

There has been extensive research performed on the *Allium* species, garlic and onion, and the mechanism for the enzymatic formation of their characteristic smell and physiological activities have been reported [2].

Since both the smell and the use of wood garlic for cooking are very similar to those of garlic, it can be expected that the fruit would also have physiological activity comparable to garlic [3].

Kubota *et al.* have already reported the isolation and identification of the volatile flavour compounds from the fruit of wood garlic. The main components were sulfur-containing compounds similar to those of garlic, while 2,4,5,7-tetrathiaoctane ($\text{CH}_3\text{SCH}_2\text{SSCH}_2\text{SCH}_3$), and 2,4,5,7-tetrathiaoctane 2,2-dioxide ($\text{CH}_3\text{SO}_2\text{CH}_2\text{SSCH}_2\text{SCH}_3$) showed fairly strong antimicrobial activity, especially against fungi [3, 4].

In this paper, we describe the isolation and structural determination of some more novel sulfur-con-

taining compounds in wood garlic and their antimicrobial activity against selected microorganisms.

RESULTS AND DISCUSSION

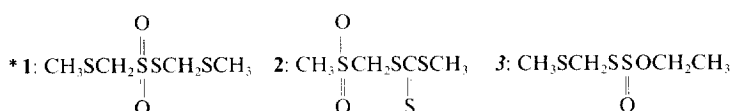
The ethyl acetate-soluble fraction of the ethanol extract from wood garlic fruit was fractionated by silica gel column chromatography. After monitoring for antimicrobial activity using the paper disc method, some fractions exhibited strong activity. These active fractions were further purified by normal-phase HPLC and three unknown compounds (**1–3**) were isolated. The chemical structures of these isolated compounds were determined spectroscopically and the data are summarized in Table 1.

Compound **1** was obtained as a colorless oil with a weak garlic-like odor (35 mg, 0.014% yield). The empirical molecular formula analyzed as $\text{C}_4\text{H}_{10}\text{O}_2\text{S}_4$ by HRFAB mass spectrometry (m/z [M]⁺: Calcd: 217.9564; Found: 218.9635). The IR spectrum in a thin film showed strong absorption bands at 1115 cm^{-1} and 1310 cm^{-1} , which could be assigned to a sulfonyl group. In CDCl_3 solution, the ^1H NMR spectrum exhibited four sharp singlet signals. By comparing the data with those of 2,4,5,7-tetrathiaoctane [5], two proton singlets at δ_{H} 2.28 and 2.43 could be assigned to two kinds of CH_2S . Two singlet signals at δ_{H} 4.31 and 4.36 indicated that each methylene proton was surrounded by different kinds of sulfur-containing groups. An assignment for four carbons from ^{13}C NMR data was made by the results of ^{13}C -off

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Table 1. Spectral data for compounds 1–3*

	1	2	3
IR (cm ⁻¹)	2980, 2920, 1415, 1310, 1190, 1115, 970, 810	2967, 2878, 1464, 1305, 1295, 1214, 1153, 1106, 1072, 969, 844, 805	2931, 1442, 1383, 1128, 1017, 888, 821 186 (M ⁺ , 1), 172 (4), 125 (CH ₃ CH ₂ OS(O) 5, 2)
MS <i>m/z</i> (%)	218.9635 [M + H] ⁺ †	216 ([M] ⁺ , 68), 149 (38), 137 (CH ₃ SCSSCH ₂ , 14), 119 (45), 91 (CH ₃ SCS, 100), 78 (CH ₂ SO ₂ , 26), 45 (CHS, 33)	106 (28), 93 (CH ₃ SCH ₂ S, 58), 61 (CH ₃ SCH ₂ , 100), 45 (CHS, 64)
¹ H NMR (δ, CDCl ₃)	2.28 (3H, <i>s</i> , CH ₃ S), 2.43 (3H, <i>s</i> , CH ₃ S), 4.31 (2H, <i>s</i> , SCH ₂ SSO ₂), 4.36 (2H, <i>s</i> , SCH ₂ SO ₂ S)	2.85 (3H, <i>s</i> , CH ₃ SCSS), 2.90 (3H, <i>s</i> , CH ₃ SO ₂), 5.02 (2H, <i>s</i> , SO ₂ CH ₂ SCSS)	1.38 (3H, <i>t</i> , <i>J</i> = 7.0 Hz, CH ₃), 2.30 (3H, <i>s</i> , CH ₃ S), 3.62 (2H, <i>dd</i> , <i>J</i> = 13.9 Hz, SCH ₂ SS(O)O), 4.10–4.22 (2H, <i>m</i> , CH ₂ OS(O)S)
¹³ C NMR (δ, CDCl ₃)	15.6 (CH ₃ S), 17.4 (CH ₃ S), 42.8 (SCH ₂ SSO ₂), 67.3 (SCH ₂ SO ₂ S)	21.1 (CH ₃ S), 39.3 (CH ₃ SO ₂), 56.1 (SO ₂ CH ₂ S), 220.3 (SCSS)	15.9 (CH ₃), 16.9 (CH ₃ S), 59.5 (SCH ₂ SS(O)O), 65.3 (CH ₂ OS(O)S)



† HR-FABMS data.

resonance and CH-COSY NMR, together with reference to the data for 2,4,5,7-tetrathiaoctane 2,2-dioxide [4]. The methylene carbon signal with a considerably low-field shift at δ_{C} 67.25 indicated the presence of a sulfonyl group with a strong deshielding effect bonded to the methylene group. From these results, the structure of compound **1** was determined to be 2,4,5,7-tetrathiaoctane 4,4-dioxide, CH₃S-CH₂SO₂SCH₂SCH₃. 2,4,5,7-tetrathiaoctane and **1** have been identified as odorous compounds in *Marasmius* species by Gmelin *et al.* [6]. They proposed a two-step enzymatic cleavage of γ -glutamyl transpeptidase and C-S lyase from the dipeptide precursor, γ -glutamyl-methylthiomethyl-cysteine sulfoxide (γ -glutamyl-marasmin), led to the formation of these compounds. However, there has been no previous report on the isolation of **1** from natural sources, suggesting that wood garlic possesses a precursor like γ -glutamyl-marasmin and that the polysulfide formation pathway is similar to that of other *Allium* genus.

Compound **2** was obtained as crystals and recrystallization from hexane-ethanol afforded yellow-colored needles (14.4 mg, 0.005% yield; m.p. 73°). The empirical molecular formula analyzed for C₄H₈O₂S₄ by HREI mass spectrometry (*m/z* [M]⁺: Calcd: 215.9407; Found: 215.9405). The IR spectrum in a KBr pellet (Table 1) had strong absorption bands at 1305 and 1153 cm⁻¹, and a medium-strength absorption band at 1214 cm⁻¹; the first two bands could be assigned to a sulfonyl group, the third to a thiocarbonyl group. The mass spectral fragment at *m/z*

45 (33%) in **2** suggested the presence of CHS in the molecule. Furthermore, fragment ions at *m/z* 91 (CH₃SC=S, 100%) and *m/z* 137 (CH₃SC=SSCH₂, 14%) suggested the existence of a thiocarbonyl group bonded to a thiomethyl group. In CDCl₃ solution, the ¹³C NMR spectrum exhibited four carbon signals, with the signal at δ_{C} 220.3 indicating the presence of a C=S group with a strong deshielding effect. The ¹H NMR spectrum exhibited three sharp singlet signals at δ_{H} 2.85 and 2.90 for methyl protons and δ_{H} 5.02 for a methylene proton. By comparing the data for 2,4,5,7-tetrathiaoctane 2,2-dioxide with those for bis[(methylsulfonyl)methyl] disulfide [4, 9], the signal at δ_{H} 2.90 could be ascribed to a methyl group coupled to a sulfonyl group (CH₃SO₂). In the HMQC spectrum, the other methyl proton signal at δ_{H} 2.85 was assigned to a methyl carbon signal at δ_{C} 21.1, and in the HMBC spectrum, this signal showed a cross-peak with the thiocarbonyl group signal at δ_{C} 220.3. The spectral data, together with reference to the literature data [10], enabled the signal to be assigned to CH₃SC=S. On the other hand, the singlet signal at δ_{H} 5.02 in the ¹H NMR spectrum indicated that the methylene proton was surrounded by different kinds of sulfur-containing groups. The methylene proton showing a cross-peak with the thiocarbonyl carbon at δ_{C} 220.3 in the HMBC spectrum, enabled the signal to be ascribed to SO₂CH₂SCS. These results determined the chemical structure of **2** to be CH₃SCSCH₂SO₂CH₃, 2,4,6-trithiaheptane-5-thione 2,2-dioxide. This is the first isolation of **2** from a natural plant source.

Compound **3** was obtained as a colorless oil with a

Table 2. Antimicrobial activity of compounds 1–3

Microorganism	MIC ($\mu\text{g ml}^{-1}$)			
	1	2	3	Control*
Bacteria				
<i>Staphylococcus aureus</i> FDA 209P	> 100	> 100	> 100	6.25
<i>Micrococcus luteus</i> PCI 1002	> 100	> 100	> 100	1.56
<i>Bacillus subtilis</i> PCI 219	50	50	> 100	0.39
<i>Microbacterium smegmatis</i> ATCC 607	> 100	50	> 100	0.39
<i>Escherichia coli</i> NIHJ	50	50	> 100	6.25
<i>Pseudomonas aeruginosa</i> IFO 3080	50	> 100	> 100	> 100
Fungi				
<i>Candida albicans</i> KF 1	50	50	> 100	1.56
<i>Saccharomyces cerevisiae</i> ATCC 9763	50	50	> 100	1.56
<i>Mucor racemosus</i> IFO 4581	50	25	> 100	0.39
<i>Aspergillus niger</i> KF 105	50	50	> 100	> 100

* Penicillin for bacteria and nystatin for fungi were used as control substances.

garlic-like odour (50.2 mg, 0.02% yield). The M_r was 186, as determined by EI mass spectrometry, although fragment ions at m/z 61 ($\text{CH}_3\text{SCH}_2^+$, 100%) and 45 (CHS^+ , 64%) suggested the presence of a thioether group in the molecule. The IR spectrum showed a strong absorption band at 1128 cm^{-1} , which could be assigned to a sulfinyl group. By comparing with the ^1H NMR data for ethyl *t*-butanesulfinate [7], the triplet signals at δ_{H} 1.35 and multiplet signals at δ_{H} 4.01 could be assigned to an ethyl group bonded to an $\text{S}(\text{O})\text{O}$ group, while the double-doublet signals at δ_{H} 3.5 could be assigned to CH_2SSO , with reference to the data for 2,2-dimethyl-3,5,6-trithiaheptane 3-oxide [8]. It is considered that each methylene group proton was observable as a double-doublet or multiplet, respectively, due to the stereostructure of the SO group; this was supported by the ^{13}C NMR, HMQC and HMBC data. These results and the chemical shift of the analogue reported in the literature [5, 7, 8] enabled **3** to be assigned as *O*-ethyl *S*-methylthiomethyl thiosulfite, $\text{CH}_3\text{SCH}_2\text{SS}(\text{O})\text{OCH}_2\text{CH}_3$, although the empirical molecular formula could not be determined because the $[\text{M}]^+$ was not apparent in the high-resolution MS spectra.

The antimicrobial activity and minimum inhibitory concentration (MIC) values are shown in Table 2. We have drawn the curve of the normal growth condition of bacteria and fungi, then the antimicrobial activity was examined using these in its best period of growth. Compound **1** exhibited antimicrobial activity with an MIC value of $50\text{ }\mu\text{g ml}^{-1}$ for most of the microorganisms tested, except for *Staphylococcus aureus*, *Micrococcus luteus* and *Microbacterium smegmatis*. Compound **2** had similar activity to that of compound **1**, while compound **3** did not show activity against any of the microorganisms tested. However, they all had lower activity than penicillin and nystatin. Compounds **1** and **2** showed weaker activity than 2,4,5,7-tetrathiaoctane 2,2-dioxide and $\text{CH}_3\text{SO}_2\text{CH}_2$

$\text{SSCH}_2\text{SO}_2\text{CH}_3$ that had been isolated from shiitake mushrooms by Takazawa *et al.* [9]. Compound **1**, **2**, 2,4,5,7-tetrathiaoctane 2,2-dioxide and $\text{CH}_3\text{SO}_2\text{CH}_2\text{SSCH}_2\text{SO}_2\text{CH}_3$ each possess one or two sulfonyl groups in their molecule, which can be considered in relationships between structure and antimicrobial activity of these four compounds.

EXPERIMENTAL

Isolation of sulfur-containing compounds

Fruits of wood garlic (248 g) collected in Sabah, Malaysia, were peeled, grated and then extracted with EtOH (300 ml $\times$ 3). Relatively strong antimicrobial activity against *S. cerevisiae* was found in the EtOAc-sol. fr. of the EtOH extract using the paper disc method. This fr. was fractionated by silica gel CC (hexane–Et₂O) and further purified by normal-phase HPLC, using hexane–2-PrOH as eluent. As a result, compounds **1**–**3** were isolated in a pure state with eluent ratios of 49:1, 95.5:4.5 and 97:3, respectively. Using a flow-rate of 7 ml min^{-1} , the R_s of **1**, **2** and **3** were 15.6, 36 and 22.3 min, respectively. The antimicrobial activity of each compound was subsequently examined.

Analysis

HPLC was carried out using a normal-phase column (Pegasil silica, 20 mm ϕ $\times$ 250 mm; Senshu Scientific, Tokyo) with a UV detector operated at 210 nm. IR spectra were measured by a Jasco IRA-1 diffraction grating spectrometer (**1**) and Perkin Elmer 1720 FT-IR spectrometer (**2** and **3**). ^1H and ^{13}C NMR were recorded using a JEOL JNM-GX-270 FT NMR (270 MHz) spectrometer, while COSY, HMQC and HMBC spectra were recorded with a JEOL JNM-A-400 FT NMR (400 MHz) spectrometer. Samples were

dissolved in CDCl_3 and TMS was used as int. standard. HR-FABMS data were taken with a JEOL JMS-AX505WA spectrometer, and LR- and NR-EIMS data were obtained with a JEOL JMS-700 spectrometer at an ionization voltage of 70 eV.

Antimicrobial assays

Activity against six bacteria and four fungi was examined by determining the minimum inhibiting concentration (MIC). Bioassays were performed using the two-fold agar dilution method. Test compounds (10 mg) were first dissolved in 1 ml of DMSO and 0.2 ml of sample soln added to 20 ml of the appropriate medium including agar.

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