Isolation and Identification of Cyclooctasulfur From the Octocoral Sinularia humilis (van Ofwegen, 2008)

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Abstract

Chemical examination of the octocoral Sinularia humilis, collected in the waters of Taiwan, led to the isolation of S₈ (I). Its structure was determined by a single-crystal x-ray diffraction analysis and this is the first time that S₈ has been reported from a marine invertebrate.

Keywords

Sinularia, cyclooctasulfur, octocoral, x-ray, marine invertebrate

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Introduction

Zero-valence sulfur (S⁰), in particular S₈, was proven to be an intermediate in the marine sulfur cycle,¹ and S₈ were found in the culture medium of Ganoderma lucidum.² This component was claimed to be one of the active constituents from G. lucidum that inhibits histamine release.² Furthermore, this interesting molecule was also found to be produced by the Gram-positive bacterium Streptomyces albulus TO 447 to the plants Boscia senegalensis,⁴ Comptosorus sibiricus,⁵ Dictytera chinensis,⁶ Moringa oleifera,⁷ and Zostera marina.⁸ In previous studies, sulfur-containing terpenoids thioflaxibilolide A,⁹ sinulasulfoxide, siulasulfone,¹⁰ and sinulariaoid A¹¹ were isolated from soft corals of the genus Sinularia distributed widely in the tropical Indo-Pacific Ocean, and these compounds were found to display extensive bioactivities, including anti-inflammation,⁹,¹⁰ neuroprotective activity,⁹ and cytotoxicity.¹¹ In connection with our continuing studies of marine invertebrates and their constituents, we have focused considerable attention on invertebrates found in the marine habitat of the waters around Taiwan. In this research, we have completed the structural identification by x-ray analysis of cyclooctasulfur S₈ (I) from Sinularia humilis (van Ofwegen, 2008) (phylum Cnidaria, class Anthozoa, subclass Octocorallia, order Alcyonacea, suborder Alcyoniina, family Alcyoniidae).

Results and Discussion

In this study, we have identified cyclooctasulfur (I) by utilizing single-crystal diffraction analysis. Suitable colorless prisms were obtained from a solution of dichloromethane. The crystal (0.1199×0.172×0.010 mm³) belongs to the monoclinic system, space group P2₁/n (#13), with a = 8.1635(4) Å, b = 13.0540(6) Å, c = 8.3904(3) Å, V = 824.23(6) Å³, Z = 4, Dcalcd = 2.067 Mg/m³, λ (Mo Kα) = 0.71073 Å. Intensity data (θ range = 2.956–27.496°) were obtained using an x-ray diffractometer. All 10 418 reflections were collected. Using direct methods and a full-matrix least-squares refinement,⁹,¹⁰ the structure of I was obtained.

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The refined structural model converged to a final R1 = 0.0335; wR2 = 0.0773 for 1521 observed reflections (I > 2σ[I]) and 73 variable parameters. An Oak Ridge Thermal Ellipsoid Plot diagram is shown in Figure 1. Crystal data, data collection, structure refinement details, and structural parameters (primary bond distance and angle) for 1 are given in Tables 1 and 2, respectively.

In previous studies, a series of secondary metabolites, including steroids and cembrane- and capnosane-type diterpenoids, were isolated from the octocoral *Sinularia humilis*, and sulfur-containing natural products were obtained from various octocorals belonging to the genus *Sinularia*. Metabolism of the sulfur element was proven to be processed by the associated bacteria. To the best of our knowledge, this is the first occasion on which cyclooctasulfur was obtained from a marine invertebrate.

### Experimental

#### General

Column chromatography was carried out with silica gel (230-400 mesh, Merck). Thin layer chromatography was performed on plates precoated with Kieselgel 60 F254 (0.25-mm-thick, Merck), then sprayed with 10% H2SO4 solution followed by heating to visualize the compounds. Normal-phase high performance liquid chromatography (NP-HPLC) was performed using a system comprised of a Hitachi L-7100 pump and a Rheodyne 7725i injection port. A semipreparative normal-phase column (Luna, 5 µm, silica 250 mm × 10 mm) was used for NP-HPLC.

#### Animal Material

Specimens of *Sinularia humilis* (van Ofwegen, 2008) investigated in this study were collected by hand using self-contained underwater breathing apparatus equipment off the coast of Southern Taiwan in July 2020 and stored at −20 °C until extraction. A voucher specimen (NMMBA-TWSC-2020131) was deposited in the National Museum of Marine Biology and Aquarium, Taiwan.

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**Figure 1.** Structure of cyclooctasulfur (1) and the computer-generated oak ridge thermal ellipsoid plot (ORTEP) diagram of 1.

**Table 1.** Crystal Data and Structure Refinement Details for 1.

| Parameter                        | Value                      |
|----------------------------------|----------------------------|
| Empirical formula                | S₈                         |
| Formula weight                   | 256.48                     |
| Temperature                      | 200(2) K                   |
| Wavelength                       | 0.71073 Å                  |
| Crystal system                   | Monoclinic                 |
| Space group                      | P2/n (#13)                 |
| Unit cell dimensions             | a = 8.1635(4) Å, b = 13.0540(6) Å, c = 8.3904(3) Å |  
| Volume                           | 824.23(6) Å³              |
| Z                                | 4                          |
| Density (calculated)             | 2.067 Mg/m³                |
| Absorption coefficient           | 2.065 mm⁻¹                 |
| F(000)                           | 512                        |
| Crystal size                     | 0.199 × 0.172 × 0.010 mm³  |
| θ range for data collection      | 2.956-27.496°              |
| Index ranges                     | −10 ≤ h ≤ 10, −16 ≤ k ≤ 16, −10 ≤ l ≤ 10 |
| Reflections collected            | 10 418                     |
| Independent collections          | 1890 (Rint = 0.0578)       |
| Completeness to θ = 25.242°     | 99.9%                      |
| Absorption correction            | Semi-empirical from equivalents |
| Max. and min. transmission       | 0.9595 and 0.8015          |
| Refinement method                | Full-matrix least-squares on F² |
| Data/restraints/parameters       | 1890/0/73                  |
| Goodness-of-fit on F²            | 1.060                      |
| Final R indices (I > 2σ[I])      | R₁ = 0.0335, wR₂ = 0.0773  |
| R indices (all data)             | R₁ = 0.0494, wR₂ = 0.0883  |
| Extinction coefficient           | n/a                        |
| Largest diff. peak and hole      | 0.725 and −0.453 e.Å⁻³     |

Note: Symmetry transformations used to generate equivalent atoms: #1 -x + 3/2, y, -z + 3/2; #2 -x + 1/2, y, -z + 3/2.

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**Table 2.** Bond Lengths (Å) and Angles (°) for 1.

| Bond/Angle | Value                  |
|------------|------------------------|
| S(1)-S(1)#1 | 2.0434(15)             |
| S(1)#1-S(1)-S(2) | 108.55(5)             |
| S(2)-S(3) | 2.0466(11)             |
| S(3)-S(4) | 2.0493(12)             |
| S(4)-S(4)#1 | 2.0578(18)             |
| S(5)-S(5)#2 | 2.062(2)               |
| S(6)-S(7) | 2.0476(15)             |
| S(7)-S(8) | 2.0406(12)             |
| S(8)-S(8)#2 | 2.0483(17)             |

**Note:** Symmetry transformations used to generate equivalent atoms: #1 -x + 3/2, y, -z + 3/2; #2 -x + 1/2, y, -z + 3/2.
**Extraction and Isolation**

Freeze-dried *S. humilis* (wet/dry weight = 998/447 g) was minced and extracted at room temperature with MeOH/CH₂Cl₂ (1:1). The extract was concentrated under reduced pressure to produce 20.5 g crude extract, which was partitioned between EtOAc and H₂O. The EtOAc phase was subjected to column chromatography on silica gel and eluted with gradients of hexanes/EtOAc to yield 13 fractions A1–A13. Fractions A3 and A4 were then combined and purified by NP-HPLC using a mixture of hexanes/EtOAc (40:1, flow rate: 5.0 mL/min) to yield 4 sub-fractions A3A–A3D. Fraction A3B was chromatographed on silica gel and eluted with light petroleum to yield 4 sub-fractions A3B1–A3B4. Fraction A3B2 was chro-

**Single-Crystal x-ray Crystallography of Cyclooctasulfur (I)**

Suitable colorless prisms of 1 were obtained from a solution of dichloromethane. Diffraction data were obtained using an x-ray diffractometer (Bruker D8 Venture, Bremen, Germany) using Mo Kα radiation (λ = 0.71073 Å) at 200(2) K. The structure was solved by direct methods and refined by a full-matrix least-squares procedure. Crystallographic data for the structure of cyclooctasulfur (I) were deposited with the Cambridge Crystallographic Data Center (CCDC) as supplementary publication number CCDC 2082127.

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The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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**References**

1. Pjevac P, Kamyshny Jr, Dyksma S, Mußmann M. Microbial consumption of zero-valence sulfur in marine benthic habitats. *Environ Microb.* 2014;16(11):3416-3430.

2. Tasaka K, Mio M, Izushi K, Akagi M, Makino T. Anti-allergic constituents in the culture medium of *Ganoderma lucidum*. (II). The inhibitory effect of cyclooctasulfur on histamine release. *Agents Actions*. 1988;23(3/4):157-160.

3. Hayashi H, Tarui N, Murao S. Isolation and identification of cyclo-

4. Lognay G, Seck D, Marlier M, Haubruege E, Gaspar C, Severin M. Identification of elemental sulfur (S₈) in Bascia steurugleisii (Pers.) Lam ex poir. Leaves. *Bull Recb Agron Gembloux*. 1993;28(4):501-505.

5. Li N, Li X, Yang SL, Zhang P. Isolation and identification of heteroatom-containing and polyns from *Composorus sibiricus* rupr. *Chin J Med Chem*. 2004;14(6):368-369.

6. Gao YT, Yang XW, Ai TM. Studies on the chemical constituents in herbs of ethanolic extract from herbs of *Diospyros chinensis*. *China J Chin Mater Med*. 2006;31(12):985-987.

7. Faizi S, Sumbul S, Versiani MA, saleem R, Sana A, Siddiqui HI. GC/GCMS analysis of the petroleum ether and dichloromethane extracts of *Moringa oleifera* roots. *Asian J Trop Biomed*. 2014;4(8):650-654.

8. Holmer M, Frederiksen MS, Møllegaard H. Sulfur accumulation in seagrass (*Zostera marina*) and effect of sulfur on seagrass growth. *Aquat Bot*. 2005;81(4):367-379.

9. Chen BW, Chao CH, Su JH, et al. A novel symmetric sulfur-containing bisembranoid from the Formosan soft coral *Sinularia flexibilis*. *Tetrahedron Lett.* 2010;51(44):5764-5766.

10. Putra MY, Iantaro A, Panza E, et al. Sinulasulfoxide and sinulasul-

11. Lei LF, Chen MF, Wang T, et al. Novel cytotoxic nine-membered 9,11-secosteroidal glycosides from the South China Sea soft *Sinularia* sp. *J Org Chem*. 2012;77(30):3937-3939.

12. Sun P, Meng LY, Tang H, et al. Sinulariosides A and B, bioactive 9,11-secoesteroidal glycosides from the South China Sea soft coral *Sinularia humilis* ofwegen. *J Nat Prod*. 2012;75(9):1656-1659.

13. Sun LJ, Li WS, Li J, et al. Uncommon diterpenoids from the South China Sea soft coral *Sinularia humilis* and their stereochem-

14. Chen BW, Chao CH, Su JH, et al. A novel symmetric sulfur-containing bisembranoid from the Formosan soft coral *Sinularia flexibilis*. *Tetrahedron Lett.* 2010;51(44):5764-5766.

15. Putra MY, Iantaro A, Panza E, et al. Sinulasulfoxide and sinulasul-

16. Aboua S, Gonzalez-Rizzo S, Grimonprez A, Gros O. First description of sulphur-oxidizing bacterial symbiosis in a cnidarian (*Medusozoa*) living in sulphidic shallow-water environments. *PLOS ONE*. 2015;10(5):e0127625.

17. Sheldrick GM. *SHELXT*–Integrated space-group and crystal-

18. Sheldrick GM. *SHELXL*–Integrated space-group and crystal-

19. CCDC homepage. Available online: http://www.ccdc.cam.ac.uk/conts/retrieving.html.