Onionin A from *Allium cepa* Inhibits Macrophage Activation

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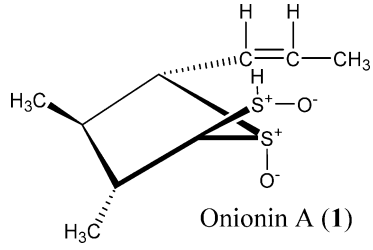
Onionin A (**1**), a new, stable, sulfur-containing compound, was isolated from acetone extracts of bulbs of onion (*Allium cepa*), and its structure was characterized as 3,4-dimethyl-5-(1*E*-propenyl)-tetrahydrothiophen-2-sulfoxide-*S*-oxide, on the basis of the results of spectroscopic analysis. This compound showed the potential to suppress tumor-cell proliferation by inhibiting the polarization of M2 alternatively activated macrophages.

A blend of onion (*Allium cepa* L.; Liliaceae) mixed with honey and vinegar is sometimes used as an antidiabetic agent and to control blood pressure. Moreover, *A. cepa* is known to exhibit anticarcinogenic activities via enzymatic inhibition, enzymatic induction, and apoptosis. In addition, it possesses anti-inflammatory, antioxidant, antimicrobial, antifungal, antiparasitic, and antispasmodic properties. Further, ingestion of onions may prevent certain cardiovascular diseases.[1−5]

Wagner et al. isolated thiosulfinates and α-sulfinyldisulfides from chloroform extracts of onion.[6] However, these compounds were not genuine constituents and were found to be volatile and unstable. This same group also isolated a new biologically active compound, 2,3-dimethyl-5,6-dithiabicyclo[2.1.1]hexane 5-oxide.[7] In order to develop natural, healthy foods that potentially can prevent and combat disease, a sulfur-containing substance from an acetone-soluble extract of *A. cepa* has been isolated and characterized.

Onions were roughly chopped and blended in a mixer along with acetone; subsequently, the mixture was soaked in acetone for three days at room temperature. The filtrate was evaporated at 40 °C in vacuo to obtain a residue, which was subjected to polystyrene gel (Diaion HP-20) column chromatography and then repeatedly chromatographed on silica gel to yield a new compound named onionin A (**1**). The results of a qualitative analysis using the sodium nitroprusside test confirmed the presence of sulfur in this compound.



Onionin A (**1**)

The positive HRFABMS of **1** showed a peak corresponding to $[M + Na]^+$ at *m/z* 243.0489 (calcd for $C_9H_{16}O_2S_2Na$, 243.0489) and a base peak corresponding to $[C_6H_{11}OS]^+$ at *m/z* 131.0525 (calcd for $C_6H_{11}OS$, 131.0531). The IR spectrum of **1** showed absorption bands at 1027 and 2366 cm^{-1}, which corresponded to sulfoxide and SH groups, respectively. In the ^1H NMR spectrum of **1**, three secondary methyl groups appeared at δ 1.05 (3H, d, *J* = 6.3 Hz), 1.28 (3H, d, *J* = 6.9 Hz), and 1.90 (3H, dd, *J* = 1.7, 6.9 Hz), along with signals from two olefinic protons at δ 6.03 (1H, dd, *J* = 1.7, 13.8 Hz) and 6.47 (1H, dq, *J* = 6.9, 13.8 Hz) and four methine protons at δ 1.97 (1H, m), 2.16 (1H, m), 4.01 (1H, d, *J* = 5.8 Hz), and 4.99 (1H, dd, *J* = 3.4, 10.9 Hz). The ^{13}C NMR spectrum exhibited three methyl signals at δ 13.9, 18.1, and 18.3, four methine carbon signals at δ 42.9, 55.0, 79.2, and 83.5, and two olefinic carbon signals at δ 131.7 and 139.6 (Figure 1). The $^1H-^1H$ COSY spectrum showed the presence of a sequential correlation from the S−H at δ 4.31 to the methine proton at δ 4.99, to the methine proton at δ 1.97, to the methine proton at δ 2.16, to the methine proton at δ 4.01, to the olefinic proton at δ 6.03, to the olefinic proton at δ 6.47, and to the methyl protons at δ 1.90. Also observed were vicinal correlations between the methine proton at δ 1.97 and the methyl protons at δ 1.05 and between the methine proton at δ 2.16 and the methyl protons at δ 1.28 (Figure 1). The HMBC spectrum also exhibited correlations from the methine proton at δ 4.99 to the carbon at δ 79.2, from the methyl protons at δ 1.05 to the three methine carbons at δ 42.9, 55.0, and 83.5, from the methyl protons at δ 1.28 to the three methine carbons at δ 42.9, 55.0, and 79.2, from the methine proton at δ 4.01 to the methine carbon at 83.5, from the olefinic proton at δ 6.03 to the olefinic carbon at δ 139.6, from the olefinic proton at δ 6.47 to the olefinic carbon at δ 131.7, and from the methyl protons at δ 1.90 to two olefinic carbons at δ 131.7 and 139.6 (Figure 1). The configuration at C-1′ was determined to be *E* from the ^1H NMR signal of H-1′ at δ 6.03 (1H, dd, *J* = 1.7, 13.8 Hz) and from the NOESY correlation between H-1′ and H-2′. The $^1H-^1H$ COSY

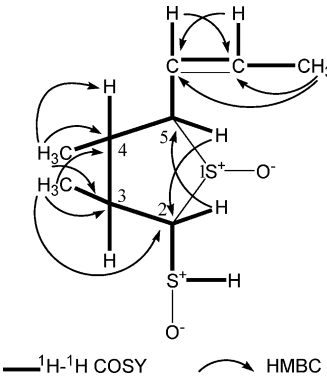


Figure 1. Key $^1H-^1H$ COSY and HMBC interactions of onionin A (**1**).

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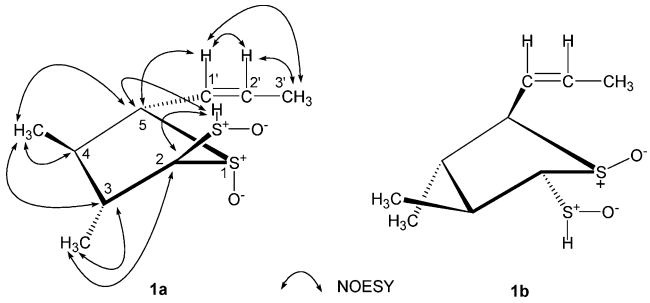
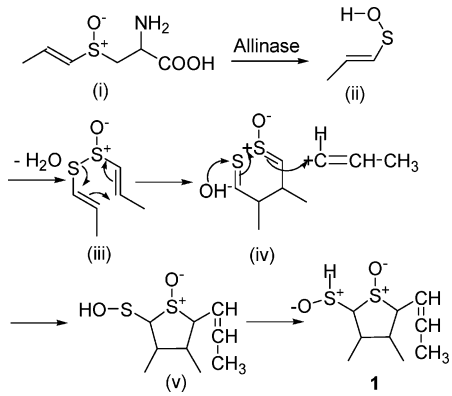


Figure 2. Key NOESY interactions in structure of **1a**.

Chart 1. Plausible Biosynthetic Pathway for Production of **1**



and HMBC spectra revealed the planar structure of **1** to be 3,4-dimethyl-5-(1*E*-propenyl) tetrahydrothiophen-2-sulfoxide-*S*-oxide, as shown in Figure 1. Furthermore, the NOESY spectrum showed the following $^1H-^1H$ correlations: SH and H-2; CH_3-3 and H-2; CH_3-3 and H-3; CH_3-4 and H-4; CH_3-4 and H-3; CH_3-4 and H-5; H-5 and SH; H-5 and H-1′; and H-1′ and H-2′ (Figure 2).

On the basis of the above-mentioned NOESY results, two relative structures of **1** are possible (**1a** and **1b**) (Figure 2). Moreover, to determine the conformation of **1**, an aromatic-solvent-induced NMR shift was used initially. Comparison of the ^1H NMR spectrum of **1** in $CDCl_3$ with that in C_6D_6 (Table S1, Supporting Information) showed that most of the signals in the latter case were shifted upfield by approximately 0.45 ppm. The only signals that remained relatively unchanged were those of CH_3-3, H-2, and H-4. These induced shifts indicated the formation of a collision complex between the aromatic solvent and the axial conformer of **1** (**1a**), which has an S^+-O^- axial configuration.[8,9]

Second, greater changes were observed in the ^1H NMR chemical signals by $\Delta\delta$ 0.17, 0.51, and 0.35 owing to C$\underline{H}_3$-3, H-4, and H-2, respectively, after sequential addition of the Eu(fod)$_3$ shift reagent in $CDCl_3$ than in the signals of C$\underline{H}_3$-4, H-3, and H-5 by $\Delta\delta$ 0.06, 0.18, and 0.16, respectively (Table S2, Supporting Information). Thus, it was considered that the 1-oxide group on the tetrahydrothiophene skeleton is in an axial arrangement,[8] and therefore, **1** may be proposed as having the structure **1a**. However, the absolute configuration of **1** remains to be elucidated.

The formation of **1** can be proposed as shown in Chart 1: 1-propenesulfenic acid (ii) derived by analogy from (+)-*S*-propenyl-L-cysteine-*S*-oxide (i) present in garlic would yield 1-propenyl-1-propenethiosulfinate (iii), which would then get converted to 2,3-dimethylbutanedithial 1-oxide (iv) by [3,3]-sigmatropic rearrangement.[7] Next, compound iv could undergo substitution to yield v, which would get converted to **1**.

Macrophages that infiltrate cancer tissues are referred to as tumor-associated macrophages (TAMs) and are closely involved in the development of the tumor microenvironment.[10−12] Since TAMs have anti-inflammatory functions, they are considered to be a type of alternatively activated macrophages (M2).[13,14] In the case of certain types of tumors, the presence of TAMs is associated with poor prognosis in patients.[15−17] Therefore, inhibition of M2-macrophage polarization is known to suppress tumor-cell proliferation.

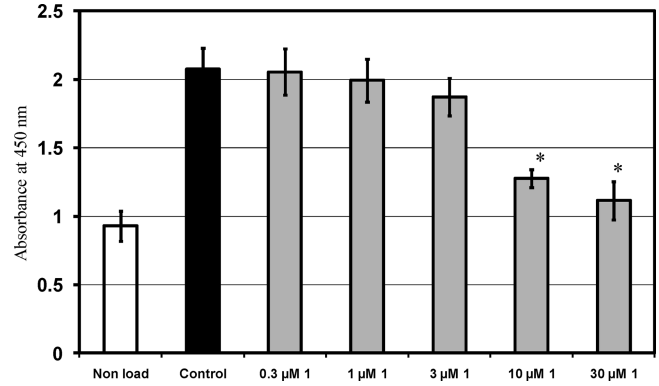


Figure 3. Effect of onionin A (**1**) on CD163 expression. Human monocyte-derived macrophages (5×10^4 cells per well of a 96-well plate) were incubated with IL-10 (20 nM) in the presence of the indicated concentration of **1** for two days, followed by determination of CD163 expression by cell-ELISA as described in the Experimental Section. (Data are presented as the mean ± SD; $^*p < 0.001$ vs control.)

Incubation of human monocyte-derived macrophages with interleukin (IL)-10 for two days increased CD163 expression. Under the same conditions, the effects of **1** on IL-10-induced CD163 expression were measured. It was found that **1** inhibited CD163 expression; this finding suggests that onionin A suppresses polarization of M2 macrophages.

Experimental Section

General Experimental Procedures. The optical rotation was measured with a JASCO P-1020 ($l = 0.5$) automatic digital polarimeter. The IR spectrum was measured with a Fourier transform FT/IR-4200 spectrometer (JASCO). The ^1H and ^{13}C NMR spectra were measured in $CDCl_3$ and C_6D_6 with a JEOL alpha 500 spectrometer at 500 and 125 MHz, respectively, and chemical shifts are on the δ (ppm) scale. The HRFABMS were measured with a JEOL JMS-DX303HF mass spectrometer and taken in a glycerol matrix containing NaI. Column chromatography was carried out on Diaion HP-20 (Mitsubishi Chemical Industries) and silica gel 60 (230−400 mesh, Merck). TLC was performed on silica gel plates (Kieselgel 60 F254, Merck). TLC spots were visualized by UV light (254/366 nm) and sprayed with 10% H_2SO_4 and anisaldehyde sprays followed by heating.

Plant Material. Onion bulbs (*Allium cepa* L., family Liliaceae) (yellow variety) were cultivated and collected at Kikuchi, Kumamoto Prefecture, Japan. Onions were purchased from CGC Japan Co. Ltd. at Kumamoto City in October 2009 and were identified by Prof. Kotaro Murakami. A voucher specimen (SBGH 09-10-16-121) has been deposited in the Herbarium of the Botanical Garden at Sojo University, Kumamoto, Japan.

Extraction and Isolation. The fresh peeled bulbs (10.74 kg) of onions were roughly chopped and blended in a mixer along with acetone. Subsequently, the mixture was soaked in acetone for three days at room temperature. The filtrate was concentrated at 40 °C in vacuo to obtain a syrup residue (959.9 g), which was then subjected to passage over a polystyrene gel (Diaion HP-20), eluting with H_2O and MeOH. The eluted fraction with MeOH (12.4 g) was subjected to silica gel column chromatography ($CHCl_3$−MeOH, 100:1 → 50:1 → $CHCl_3$−MeOH−H_2O, 7:3:0.5 → 6:4:1) to provide nine fractions (fr. 1−9). Fr. 4 [1.37 g, $CHCl_3$−MeOH, 100:1, eluate] was subjected to silica gel column chromatography ($CHCl_3$−MeOH, 50:1) to afford five subfractions (fr. 4-1 to fr.4-5). Fr. 4-2 (527.8 mg) was then repeatedly chromatographed on silica gel (*n*-hexane−acetone, 4:1) to afford a new compound named onionin A **1** (42.2 mg).

Onionin A (1): pale yellow, amorphous powder; $[\alpha]^{24}_D$ +16.7 (*c* 0.2, $CHCl_3$); IR ν_{max} (KBr) 1027 and 2366 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) δ 1.05 (3H, d, $J = 6.3$ Hz, CH_3-3), 1.28 (3H, d, $J = 6.9$ Hz, CH_3-4), 1.90 (3H, dd, $J = 1.7$, 6.9 Hz, H_3-3′), 1.97 (1H, m, H-3), 2.16

(1H, m, H-4), 4.01 (1H, d, $J = 5.8$ Hz, H-5), 4.31 (1H, d, $J = 10.9$ Hz, SH), 4.99 (1H, dd, $J = 3.4$, 10.9 Hz, H-2), 6.03 (1H, dd, $J = 1.7$, 13.8 Hz, H-1′), 6.47 (1H, dq, $J = 6.9$, 13.8 Hz, H-2′); ^{1}H NMR (C_6D_6, 500 MHz) δ 0.84 (3H, d, $J = 6.9$ Hz, CH_3-3), 0.93 (3H, d, $J = 6.9$ Hz, CH_3-4), 1.30 (3H, dd, $J = 1.7$, 6.9 Hz, H_3-3′), 1.63 (1H, m, H-3), 2.12 (1H, m, H-4), 3.52 (1H, d, $J = 5.8$ Hz, H-5), 4.91 (1H, dd, $J = 3.2$, 10.7 Hz, H-2), 5.01 (1H, d, $J = 10.9$ Hz, SH), 5.45 (1H, dd, $J = 1.5$, 15.1 Hz, H-1′), 6.18 (1H, dq, $J = 6.9$, 13.8 Hz, H-2′); ^{1}H NMR ($CDCl_3$ and 0.01 equiv Eu(fod)$_3$, 500 MHz) δ 1.07 (3H, d, $J = 6.9$ Hz, CH_3-3), 1.27 (3H, d, $J = 6.9$ Hz, CH_3-4), 1.86 (3H, d, 6.9 Hz, H_3-3′), 2.06 (1H, m, H-3), 2.52 (1H, m, H-4), 4.04 (1H, d, $J = 5.8$ Hz, H-5), 5.09 (1H, brs, H-2), 6.05 (1H, d, $J = 14.8$ Hz, H-1′), 6.52 (1H, m, H-2′); ^{1}H NMR ($CDCl_3$ and 0.02 equiv Eu(fod)$_3$, 500 MHz) δ 1.12 (3H, d, $J = 6.9$ Hz, CH_3-3), 1.30 (3H, d, $J = 6.9$ Hz, CH_3-4), 1.86 (3H, dd, $J = 1.4$, 6.6 Hz, H_3-3′), 2.09 (1H, m, H-3), 2.59 (1H, m, H-4), 4.09 (1H, d, $J = 5.7$ Hz, H-5), 5.20 (1H, brs, H-2), 6.10 (1H, dd, $J = 14.8$ Hz, H-1′), 6.58 (1H, m, H-2′); ^{1}H NMR spectrum ($CDCl_3$ and 0.03 equiv Eu(fod)$_3$, 500 MHz) δ 1.22 (3H, d, $J = 6.3$ Hz, CH_3-3), 1.34 (3H, d, $J = 6.9$ Hz, CH_3-4), 1.87 (3H, d, $J = 6.9$ Hz, H_3-3′), 2.15 (1H, m, H-3), 2.66 (1H, m, H-4), 4.17 (1H, d, $J = 5.8$ Hz, H-5), 5.34 (1H, brs, H-2), 6.18 (1H, d, $J = 14.9$ Hz, H-1′), 6.60 (1H, brs, H-2′); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 13.9 (CH3-3), 18.1 (CH3-4), 18.3 (C-3′), 42.9 (C-4), 55.0 (C-3),79.2 (C-5), 83.5 (C-2), 131.7 (C-1′), and 139.6 (C-2′); positive HRFABMS *m/z* 243.0489 $[M + Na]^+$ (calcd for $C_9H_{16}O_2S_2Na$, 243.0489) (70%); and base peak at *m/z* 131.0525 $[C_6H_{11}OS]^+$ (calcd for $C_6H_{11}OS$, 131.0531).

Determination of the Inhibitory Effect of Compound 1 on CD163 Expression. Human monocyte-derived macrophages (5×10^4 cells per well of a 96-well plate) were incubated with onionin A (**1**, 30 μM) for 24 h after treatment with IL-10 (20 nM) for two days, followed by the determination of CD163 expression by cell-ELISA.

Cell Enzyme-Linked Immunosorbent Assay (Cell-ELISA). Expression of CD163 on human monocyte-derived macrophages was evaluated using a cell-ELISA procedure, as described previously.[18] Briefly, each well of a 96-well plate was blocked with Block Ace and washed three times with PBS containing 0.05% Tween 20 (washing buffer). The wells were incubated with anti-CD163 antibody and AM3K (2 μg/mL) and dissolved in washing buffer for 1 h. The wells were then washed with washing buffer three times and reacted with HRP-conjugated anti-mouse IgG antibody, followed by reaction with Ultrasensitive TMB (Moss, Inc., Pasadena, MD). The reaction was terminated by the addition of 1 M sulfuric acid, and the absorbance at 450 nm was read on a micro-ELISA plate reader.

Statistics. All data are representative of two or three independent experiments. Data are expressed as means ± SD. Mann−Whitney's *U*-test was used for two-group comparison. A value of $p < 0.05$ was considered statistically significant.

Supporting Information Available: Tables of NMR chemical shifts, NMR spectra of onionin A (**1**), and MS spectra of onionin A (**1**). This material is available free of charge via the Internet at http://pubs.acs.org.

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