

On the constituents of garlic sprouts grown in water

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ABSTRACT

Garlic sprouts were prepared using the water cultivation method and the constituents of their organ parts were examined. From the cloves, we obtained garlicnin B₁, which is believed to show an antitumor effect, together with *E*-ajoene and the γ -hydroxybutenolide derivative. The last one was also obtained from the aerial parts and roots, and this was not so far obtained from the native garlics. Garlic sprouts can be considered as useful vegetables as they contain garlicnin B₁ which is presumed to have antitumour effects.

KEYWORDS: *Allium sativum*, water cultivation, garlic sprout, garlicnin B₁, antitumor effect, γ -hydroxybutenolide.

INTRODUCTION

The biological activities of onion (*Allium cepa* L.), garlic (*A. sativum* L.), and Welsh onion (*A. fistulosum* L.), can generally be classified into two categories: activities associated with the prevention of cardiovascular disease and those associated with the prevention of cancer [1]. Regarding sulfides in garlic, a few sulfides have been elucidated to date [2-5]. Therefore, we isolated and structurally and pharmacologically

characterized the *Allium* sulfides (including the sulfoxides) that show antitumor activity.

We have previously isolated three new 3,4-dimethylthiolane-type sulfoxides namely onionins A₁–A₃ [6, 7] from acetone extracts of onion and Welsh onion [7], and six new acyclic-type sulfides [8], nine new 3,4-dimethylthiolane-type sulfoxides [9-12] including garlicnins B₁–B₄, which are the transferring isomers in the double bond of onionin A₁, four new 2-methylthiolane (and thiane)-type sulfoxides [13-15], and two new 1,2-dithiolane-type sulfoxides [14, 15] from the acetone extracts of garlic cultivated in China and Japan. The effect of onionin A₁, a representative 3,4-dimethylthiolane-type sulfoxide, on tumor progression and metastasis in both osteosarcoma and ovarian cancer-bearing mouse models was then examined. Regression of tumor growth and metastasis was controlled through the regulation of macrophage activation. These results showed that onionin A₁ is an effective agent for controlling tumor growth in both *in vitro* and *in vivo* models, and that the antitumor effects observed *in vivo* are likely caused by reversal of the antitumor immune system. Activation of the antitumor immune system by onionin A₁ might be an effective adjuvant therapy for patients with osteosarcoma, ovarian cancer, and other malignant tumors [9, 16, 17].

However, the bioactive constituents of the garlic sprouts propagated by the water cultivation

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are not yet elucidated. If the pharmacologically valuable effective compounds could be produced by simple cultivation method using water in shorter period, it would be regarded as a useful and effective method. Hence, this time, we examined whether garlic sprouts cultivated in water contain the sulfoxides found in the above *Allium* species. We attempted to cultivate garlic in water to obtain the garlic sprouts.

MATERIALS AND METHODS

General experimental procedures

Optical rotation was measured using a JASCO P-1020 ($l = 0.5$) automatic digital polarimeter (JASCO, Easton, MD). The ^1H - and ^{13}C -NMR spectra were measured in CDCl_3 using a JEOL alpha 500 spectrometer at 500 and 125 MHz, respectively (JEOL Ltd., Tokyo, Japan), and the chemical shifts were found to be on the δ (ppm) scale. The positive mode high-resolution fast atom bombardment mass spectroscopy (HR-FAB-MS) was measured using a JEOL JMS-DX303HF mass spectrometer. Column chromatography was carried out on silica gel 60 (230-400 mesh; Merck & Co., Kenilworth, NJ). Thin-layer chromatography (TLC) was performed on silica gel plates (Kieselgel 60 F254; Merck & Co.), and TLC spots were visualized under UV light (254/366 nm), sprayed with 10% H_2SO_4 , and then heated.

Plant material

The garlic bulbs (*A. sativum* L. family Liliaceae) cultivated in China were identified by Prof. Toshihiro Nohara. A voucher specimen (G-25-9-501) was deposited in the Herbarium of the Botanical Garden at Sojo University, Kumamoto, Japan.

Culture method

We used Chinese garlic (18 numbers of tubes; total weight 921.59 g) with a diameter of 5-5.5 cm diameter and 3 cm long produced at Shandong province in China and divided them into 216 species, peeled their rinds, and separated their crusts attached to the bottom parts (total 867.98 g). The bottoms were soaked in water and they were grown for 18 days at room temperature (6.9-17.6 °C). The aerial parts (stems) grew to an average length of 10.3 cm, and the roots were

av. 2.7 cm long with *av.* length of 2.83 cm for the cloves (mother fruits). They were separated into three organs: the cloves, 794.9 g; the aerial parts, 150.54 g; roots, 102.29 g.

Extraction and isolation

First the cloves (251.28 g) were sliced and homogenized with acetone (600 ml) and left to stand for 72 hours. The filtrate was concentrated to a smaller volume and then added with water and ethyl acetate. The organic layer was evaporated to dryness in reduced pressure at 40 °C to give the residue (1075.7 mg), which was then subjected to silica gel column chromatography and eluted with *n*-hexane-acetone = 6:1 \rightarrow 5:1 \rightarrow 4:1, gradually, to provide seven fractions, F-1 (255.2 mg), F-2 (28.0 mg), F-3 (76.0 mg), F-4 (40.3 mg), F-5 (112.5 mg), F-6 (94.1 mg), and F-7 (173.8 mg). The F-4 constituted of compound **1** in a pure state. F-5 was further purified with CHCl_3 -MeOH = 200:1 to afford two compounds **2** (42 mg) and **3** (12 mg).

The roots (102.29 g) and the aerial parts (150.54 g) were extracted with acetone and separated using silica gel column chromatography similarly as in the case with the fruits to give compound **3** (35.4 mg and 16.6 mg, respectively) as the sole product.

Compound 1: Colorless oil, $[\alpha]_{\text{D}}^{24} -10.2^\circ$ (c 0.5, CHCl_3). Positive HR-FAB-MS (m/z): 221.0671 $[\text{M} + \text{H}]^+$ (Calcd for $\text{C}_9\text{H}_{17}\text{O}_2\text{S}_2$: 221.0670). ^1H -NMR spectrum (CDCl_3): δ 1.04 (3H, d, $J=2.3$, 6.9 Hz, 3- CH_3), 1.23 (3H, d, $J=6.8$ Hz, 4- CH_3), 1.99 (1H, m, H-3), 2.15 (1H, m, H-4), 3.33 (1H, dd, $J=8.6$, 12.6 Hz, Ha-1'), 3.59 (1H, dd, $J=6.3$, 12.6 Hz, Hb-1'), 4.08 (1H, d, $J=5.7$ Hz, H-5), 5.04 (1H, d, $J=4.0$ Hz, H-2), 5.36 (1H, d, $J=6.9$ Hz, Ha-3'), 5.39 (1H, s, Hb-3'), 5.68 (1H, m, H-2'). ^{13}C -NMR spectrum (CDCl_3): δ 13.9 (CH_3 -3), 18.3 (CH_3 -4), 42.8 (C-4), 54.9 (C-3), 55.6 (C-1'), 74.9 (C-5), 84.0 (C-2), 124.5 (C-3'), 125.1 (C-2'). This was identical with 2-sulfenic acid-5-(prop-2-en-1-yl)-3,4-dimethylthiolan-1-ium-1-olate, garlicnin B₁.

Compound 2: Colorless oil, Positive HR-FAB-MS (m/z): 235.0288 $[\text{M} + \text{H}]^+$ (Calcd for $\text{C}_9\text{H}_{15}\text{OS}_3$: 235.0285). ^1H -NMR spectrum (CDCl_3): δ 3.36 (2H, d, $J=7.5$ Hz, H₂-3), 3.40 (2H, m, H₂-10), 3.55 (2H, m, H₂-8), 5.18 (1H, d, $J=11.5$ Hz, Ha-1), 5.19 (1H, d, $J=14.4$ Hz, Hb-1), 5.40 (1H, d,

$J=14.7$ Hz, Ha-12), 5.47 (1H, d, $J=10.5$ Hz, Hb-12), 5.87 (3H, m, H-2, 7, 11), 6.38 (1H, d, $J=14.9$ Hz, H-6). $^{13}\text{C-NMR}$ spectrum (CDCl_3): δ 41.3 (C-3), 52.8 (C-8), 54.2 (C-10), 116.5 (C-7), 119.4 (C-1), 124.1 (C-12), 125.5 (C-11), 132.6 (C-2), 135.0 (C-6). This was identical with (*E*)-4,5,9-trithiadodeca-1,6,11-triene 9-oxide, ajoene.

Compound **3** was obtained as a colorless crystal showing mp 103 °C and $[\alpha]_{\text{D}}^{25} +4.8^\circ$ (c 1.4, CHCl_3), and the molecular formula of $\text{C}_6\text{H}_8\text{O}_3$ in the HR-FAB-MS (m/z): $[\text{M}+\text{H}]^+$ 129.0554 (Calcd for $\text{C}_6\text{H}_9\text{O}_3$ 129.0552). $^1\text{H-NMR}$ spectrum (CDCl_3): δ 1.68 (3H, s, CH_3 -5), 1.91 (3H, s, CH_3 -3), 6.86 (1H, s, H-4). $^{13}\text{C-NMR}$ (CDCl_3): δ 10.4 (CH_3 at C-5), 24.8 (CH_3 at C-3), 131.5 (C-3), 148.1 (C-4), 104.6 (C-5), 172.3 (C-2).

X-ray analysis

All measurements were made on a Rigaku R-AXIS RAPID diffractometer using graphite monochromated Mo-K α radiation. All diagrams and calculations were performed using CrystalStructure [CrystalStructure 4.0: Crystal Structure Analysis Package, Rigaku Corporation (2000-2010). Tokyo 196-8666, Japan]. Crystal data: crystal dimension = 0.49 x 0.4 x 0.4 mm, orthorhombic, space group $\text{P}2_12_12_1$, $a = 5.9698(7)$ Å, $b = 8.574(1)$ Å, $c = 12.952(2)$ Å, $V = 663.0(2)$ Å 3 , $Z = 4$, $D_{\text{calc}} = 1.284$ g/cm 3 , 1519 unique data collected, all 1519 reflection data were used, $R = 0.0415$, $R_w = 0.0607$, CCDC reference number 990802 [18].

RESULTS AND DISCUSSION

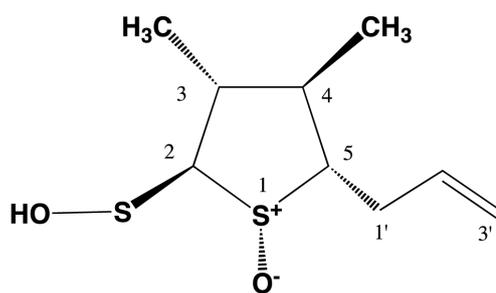
Chinese garlics were separated, their rinds were peeled, and the crusts attached to the bottom parts were taken off. The bottoms were soaked in water and they were grown for 18 days at room temperature. The sprouts were separated into three organs: the cloves, the aerial parts, and roots. Next, they were independently sliced and homogenized with acetone. After extraction, which proceeded for 3 days, the respective filtrates were concentrated and then added with water and ethyl acetate. The organic layer was evaporated under reduced pressure at 40 °C to give the respective extracts. In the case of the cloves, it gave the residue (1075.7 mg), which was then subjected to silica gel column chromatography and gradually eluted

with *n*-hexane-acetone = 6:1 \rightarrow 5:1 \rightarrow 4:1 to provide seven fractions, F-1 (255.2 mg), F-2 (28.0 mg), F-3 (76.0 mg), F-4 (40.3 mg), F-5 (112.5 mg), F-6 (94.1 mg), and F-7 (173.8 mg). F-1–F-3, F-6 and F-7 were recognized to contain many fatty acids, whose determination is out of scope of our experimental aim. The F-4 comprised of compound **1** in a pure state. The F-5 was further purified with CHCl_3 -MeOH = 200:1 to afford two compounds **2** (42 mg) and **3** (12 mg).

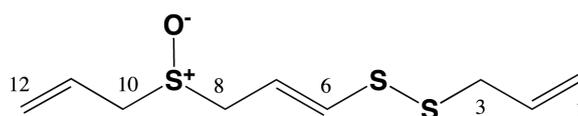
Compound **1** was identified as 2-sulfenic acid-5-(prop-2-en-1-yl)-3,4-dimethylthiolan-1-ium-1-olate, garlicnin B $_1$ [11] as shown in Figure 1. The 3,4-dimethylthiolane-type sulfoxides such as onionins A $_1$ –A $_3$ from onion and Welsh onion, and garlicnins A, B $_1$ –B $_4$, C $_1$ –C $_3$, from garlic are common compounds among these three *Allium* species and are included as major sulfoxides. Thereupon, we revealed the effects of macrophage activation and the antitumor activity of representative onionin A $_1$, which is the isomer of garlicnin B $_1$ substituted with 2-propenyl group instead of 1-propenyl group at C-5 in onionin A $_1$ having the core 3,4-dimethylthiolane-1-ium-1-olate framework. Thus, since onionin A $_1$ has been shown to exhibit antitumor effect, garlicnin B $_1$ is also expected to have antitumor effect. The occurrence of garlicnin B $_1$ (**1**) is significant because **1** is also regarded as effective against tumor.

Compound **2** was identical to (*E*)-4,5,9-trithiadodeca-1,6,11-triene 9-oxide, ajoene [5] as shown in Figure 1.

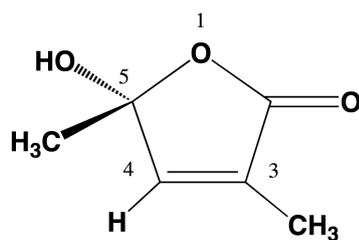
Compound **3** was obtained as a colorless crystal showing mp 103 °C, $[\alpha]_{\text{D}} +4.8^\circ$ (CHCl_3), and the quasi molecular formula of $[\text{C}_6\text{H}_8\text{O}_3 + \text{H}]^+$ at m/z 129.0554 in the HR-FAB-MS. The ^1H - and ^{13}C -NMR spectra showed the signals due to six protons of two methyl groups at δ 1.68 (3H, s), 1.91 (3H, s), and one olefinic proton at δ 6.86 (1H, s), and due to two methyl carbons at δ 10.4 and 24.8, two olefinic carbons at δ 131.5 and 148.1, one ketal carbon at δ 104.6, and one carbonyl carbon at δ 172.3. The heteronuclear multiple-bond correlation (HMBC) spectrum showed correlations from the methyl protons at δ 1.91 to the carbons at δ 131.5, 148.1 and 172.3, from the methyl protons at δ 1.68 to the carbons at δ 104.6 and 148.1, and from the proton at δ 6.86 to the carbons at δ 104.6.



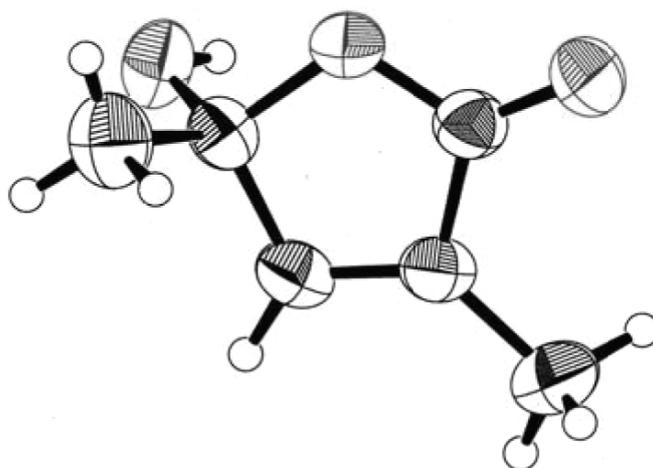
Compound 1 (1)



Compound 2 (2)



Compound 3 (3)

Figure 1. Structures of compounds 1–3 obtained by water cultivation of garlic.**Figure 2.** Perspective structure of compound 3 by X-ray analysis.

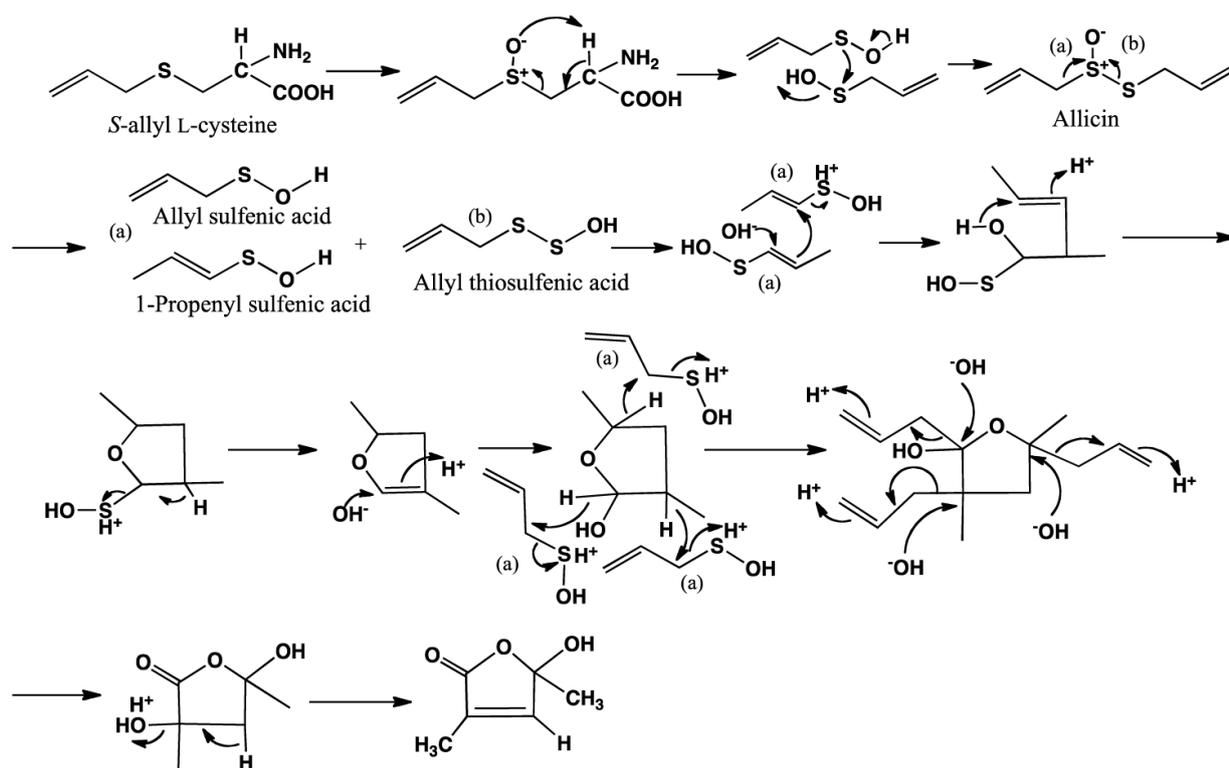


Figure 3. Hypothetical pathway of the production of compound 3.

Therefore, the structure of **3** was regarded as a γ -hydroxybutenolide derivative (Figure 1). In order to verify the steric configuration of the carbon attached to the γ -hydroxy group, X-ray analysis was performed to give a perspective structure, (*R*)-5-hydroxy-3,5-dimethylfuran-2(5*H*)-one, as shown in Figure 2.

The roots (102.29 g) and aerial parts (150.54 g) were independently extracted with acetone and separated using silica gel column chromatography similarly as in the case with the cloves to give compound **3** (35.4 mg and 16.6 mg, respectively) as the sole product.

The hypothetical pathway of the production of γ -hydroxybutenolide derivative was proposed as shown in Figure 3. First, two moles of 1-propenyl sulfenic acid derived from allicin formed one mole of tetrahydrofuran sulfinate, and then allyl (or 1-propenyl) sulfenic acid is presumed to be involved in the hydroxylation (for oxidation) on the pathway. This final compound did not contain sulfur atom; however 1-propenyl sulfenic acid was involved in the production of compound **3**.

This compound might be identical with that obtained from the toxins produced by a kind of mushroom, *Coprinus comatus* [19], however, in which the steric configuration at C-5 was not characterized.

CONCLUSION

Garlic sprouts were prepared using the water cultivation method, and the constituents of the three separated organ parts were examined. The cloves contained three compounds **1–3**; **1** was garlicnin B₁, the isomer of onionin A₁ showing antitumor effect. Therefore, the water cultivation method is an excellent method for obtaining chemopreventive agents, such as onionin A₁. Compound **2** was acyclic sulfide, ajoene, and **3** was characterized as the γ -hydroxybutenolide derivative. Compound **3** was also obtained from the roots and aerial parts. The presence of **3** along with **1** is worthy of note. This compound could also be obtained from fresh garlic using a quick separation procedure; however, we used only dried Chinese garlic thus far.

CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

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