Current Topics in Phytochemistry
Vol. 17, 2021

Original Article

Novel acyclic sulfide, garlicnin L-5, from garlic

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ABSTRACT

As part of our continuing studies on the *Allium* sulfides exhibiting antitumor effects, we isolated one new sulfide and characterized its structure as a novel dimer compound combined with the partial structures of allyl, popenyl and 2-formylbutane moieties thorough the sulfinyl and thiosulfenyl functions.

KEYWORDS: *Allium sativum*, acyclic sulfide, sulfinyl function, thiosulfenyl function, dimer, 2-formyl butane moiety.

INTRODUCTION

Unexpectedly, we found a few clarified sulfides from garlic; very few cyclic sulfides from garlic (*Allium sativum*) [1-10], onion (*A. cepa*) [6, 11-15], and Welsh onion (*A. fistulosum*) had been found before our studies on them [12]. Therefore, we started research aiming at the isolation, structural characterization and pharmacological analysis of the cyclic sulfides from garlic that show antitumor activity. So far, we obtained four acyclic-type sulfides (garlicnins L-1, L-2, L-3, and L-4 [4]), nine 3,4-dimethylthiolane-type sulfides (garlicnins A [1], B_1, B_2, B_3, B_4, C_1, C_2, C_3 [2, 3], and M [7]), four 2-methylthiolane (and thiane)-type sulfides (garlicnins I_1 [5], I_2 [7], J_1 [5], and J_2 [8]), two 1,2-dithiolane-type sulfides (garlicnins G [5], and P [8]), and two 2-oxothiolane-type sulfides (onionins B_1, and B_2 [13]), together with the known sulfides, (*E*)-ajoene [16], and kujounin A_1 derivative [17] from the acetone extract of garlic. Furthermore, we recently isolated a new bis-thiolane-type compound, garlicnin IB [18]. The structures of these compounds are summarized in Table 1 in which the corrected structures of garlicnins A and B are represented according to the references [19, 20].

Onionin A_1 obtained from onion, which is the isomer of a major component garlicnin B_1 from garlic, showed potential in inhibiting the polarization of M2-activated macrophages that were capable of suppressing tumor-cell proliferation [21, 22]. The effects of onionin A_1 on tumor progression and metastasis in both osteosarcoma and ovarian cancer-bearing mouse models were examined. Tumor proliferation was depressed, and tumor metastasis was controlled by regulating macrophage activation. These results showed that the 3,4-dimethylthiolane-type sulfide, onionin A_1 was an effective agent for controlling tumors in both *in vitro* and *in vivo* models, and that the antitumor effects observed *in vivo* are likely caused by reversing the antitumor immune system. Activation of the antitumor immune system by onionin A_1 might be an effective adjuvant therapy for patients with osteosarcoma, ovarian cancer and other malignant tumors [14, 15]. Considering this fact, we continue further the experiment to obtain new sulfides. In this study, we deal with the structural characterization of new sulfide **1** from the acetone extract of garlic.

MATERIALS AND METHODS

General experimental procedures

The ^{1}H- and ^{13}C-NMR spectra were measured in $CDCl_3$ using a JEOL alpha 500 spectrometer at

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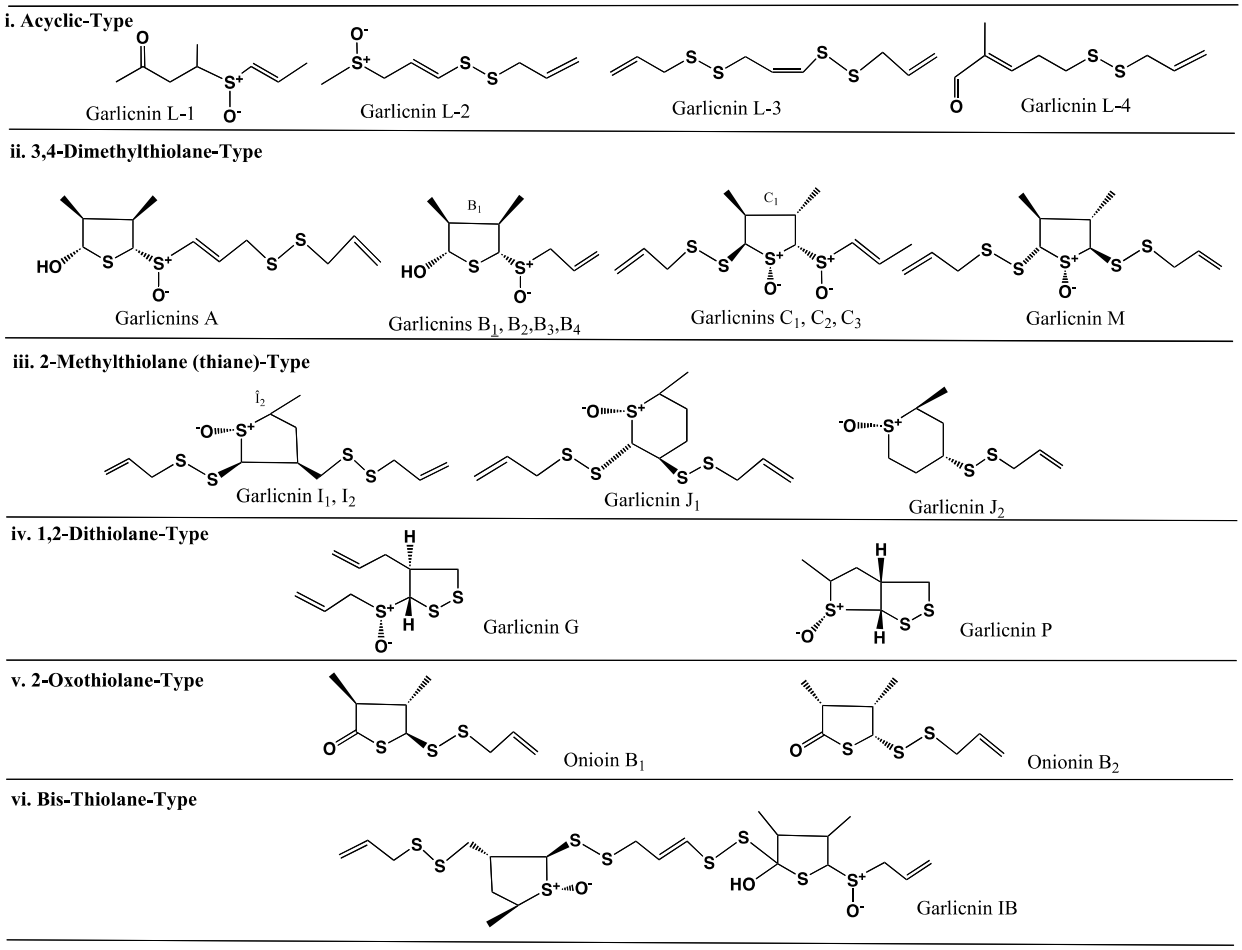
Table 1. Garlicnins and onionins isolated from garlic.



500 and 125 MHz, respectively, and the chemical shifts were found to be on the δ (ppm) scale. Column chromatography was carried out on silica gel 60 (230-400 mesh, Merck). Thin layer chromatography (TLC) was performed on silica gel plates (Kieselgel 60 F254; Merck). TLC spots were visualized under UV light (254/366 nm), sprayed with 10% H_2SO_4, and then heated.

Plant material

We used the Chinese garlic bulbs (*A. sativum* L., family Liliaceae) imported by Shinko Co., Ltd. A voucher specimen (GAR-20-10-36) was deposited in the Herbarium of the Botanical Garden at Sojo University, Kumamoto, Japan.

Extraction and isolation

Peeled Chinese garlic bulbs (Shinko Company, 2268 g) purchased at Kumamoto city were roughly chopped and homogenized in a mixer along with acetone (4.5 l). The mixture was subsequently soaked in acetone for 3 days at room temperature. The filtrate was concentrated at 40 °C *in vacuo* to give a suspension, which was extracted using ethyl acetate and water. The organic layer was taken and evaporated in reduced pressure at 40 °C to afford the residue (14.85 g), which was then column chromatographed on silica gel (width: 4.8 × length: 35 cm with *n*-hexane:acetone = 5:1 → 4:1 → 3:1 → 2:1) to provide fractions 1 (4.4673 g), 2 (1.1488 g), 3 (0.2607 g), 4 (1.2804 g), 5 (0.2470 g), 6 (0.5459 g), and 7 (1.6801 g). Fraction 2 was mostly composed of garlicnin B_1 and fraction 4 was mostly composed of *E*-ajoene [16]. The fraction 6 was then column chromatographed on silica gel with *n*-hexane:acetone = 2:1 to give three fractions:

fractions 0, 1 (78.4 mg), and 2 (279.3 mg). The fraction 2 was further silica gel chromatographed with $CHCl_3$:MeOH = 150:1 → 100:1 → 50:1 → 25:1 to afford one compound, named garlicnin L-5 (**1**, 34.8 mg).

Garlicnin L-5 (1)

Colorless resinous syrup, R*f* value: 0.36 ($CHCl_3$: MeOH = 20 : 1); 0.30 (*n*-hexane:acetone = 2:1) on TLC; ^{1}H- and ^{13}C-NMR spectra; allyl sulfinyl group (**1-a**): olefinic methylene at δ_H 5.48 (1H, d, *J* = 15.2 Hz, H-1a), and 5.53 (1H, d, *J*=10.3 Hz, H-1b); δ_C 125.1 (C-1), olefinic methine at δ_H 5.45 (1H, m, H-2); δ_C 124.7 (C-2), methylene at δ_H 3.76 (2H, d, *J*=7.5 Hz, H_2-3); δ_C 58.6 (C-3), 2-formyl-butane thiosulfenyl group (**1-b**): methylene at δ_H 3.30 (2H, d, *J*=7.5 Hz, H_2-5); δ_C 47.4 (C-5), methine at δ_H 3.07 (1H, t, *J*=7.5 Hz, H-6); δ_C 35.6 (C-6), formyl function at δ_H 9.80 (1H, d, *J*=10.3 Hz, 6-CH=O); δ_C 197.2 (6-CH=O), methine at δ_H 2.99 (1H, t, *J*=7.5 Hz, H-7); δ_C 36.4 (C-7), methylene at δ_H 3.30 (2H, d, *J*=7.5 Hz, H_2-8); δ_C 44.1 (C-8), propenyl group (**1-c**): methyl function at δ_H 1.98 (3H, d, *J*=1.75 Hz, H_3-13); δ_C 17.5 (C-13), olefinic methine at δ_H 6.96 (1H, q, *J*=6.85 Hz, H-12); δ_C 145.9 (C-12), olefinic methine at δ_H 6.32 (1H, d, *J*=16.6 Hz, H-11); δ_C 129.2 (C-11), as shown in Figure 1.

RESULTS AND DISCUSSION

Garlicnin L-5 (**1**) was obtained as a resinous syrup. The $[M+H]^+$ peak on the positive high-resolution fast-atom bombardment mass spectroscopy (HR-FAB-MS) was not obtained, but it showed unity on the TLC [R*f* value: 0.36 ($CHCl_3$:MeOH = 20:1); 0.30 (*n*-hexane:acetone = 2:1)]. The spectral analysis by the ^{1}H-^{1}H correlation spectroscopy (COSY), ^{1}H-detected heteronuclear correlation through multiple quantum coherence (HMQC), and heteronuclear multiple bond correlation (HMBC) revealed the presence of the 3 partial structures; however, these did not connect the 3 substructures. The sulfinyl function or the thiosulfenyl function would make the three substructures separated partial structures. Here, we assigned the NMR chemical shifts by taking the following points into consideration. The methylene carbon adjacent to the sulfinyl function appeared at about δ 47-60; on the other hand, the methylene carbon adjacent to the thiosulfenyl function appeared at around δ 40-45 [2, 3, 7, 18].

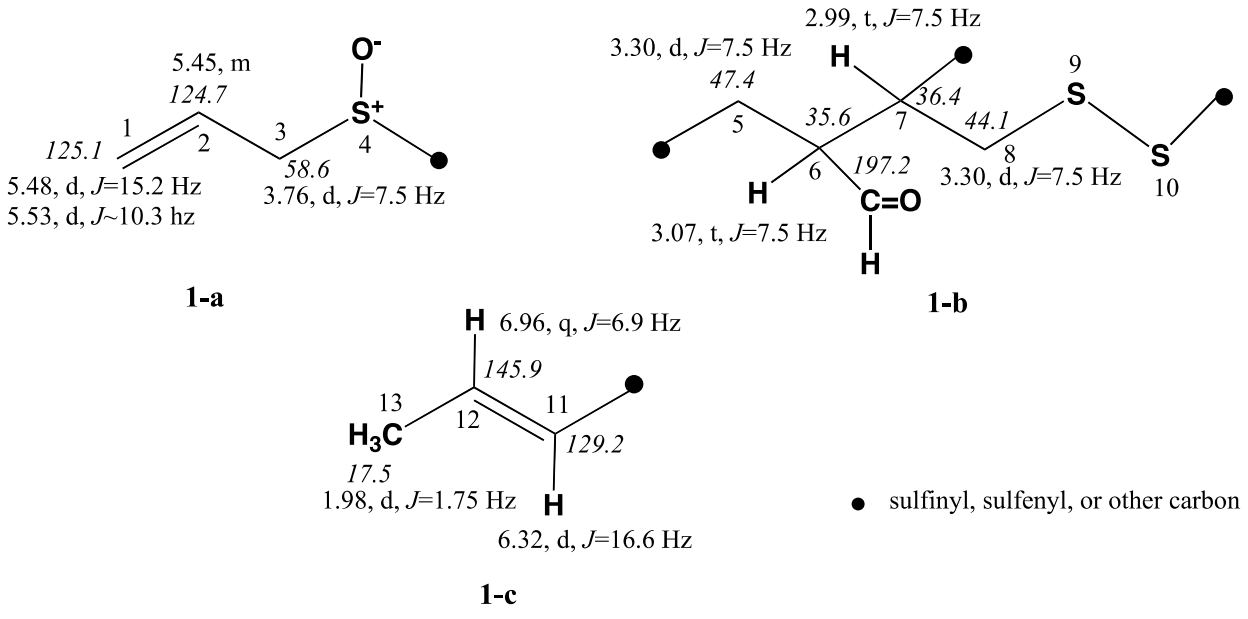


Figure 1. Partial structures of garlicnin L-5 (**1**) with NMR data.

The assignments of NMR data of three portions were made as follows: the first (**1-a**) was allyl sulfinyl group: from olefinic methylene at δ_H 5.48 (1H, d, *J*=15.2 Hz), and 5.53 (1H, d, *J*=10.3 Hz); δ_C 125.1, next to the olefinic methine at δ_H 5.45 (1H, m); δ_C 124.7, further to the methylene at δ_H 3.76 (2H, d, *J*=7.5 Hz); δ_C 58.6, second (**1-b**) was 2-formyl-butane thiosulfenyl group: from the methylene at δ_H 3.30 (2H, d, *J*=7.5 Hz); δ_C 47. 4, next to both the methine at δ_H 3.07 (1H, t, *J*=7.5 Hz); δ_C 35.6, and the formyl function at δ_H 9,80 (1H,d, *J*=10, 3 Hz); δ_C 197.2, further to the methine at δ_H 2.99 (1H, t, *J*=7.5 Hz); δ_C 36.4, next to the methylene at δ_H 3.30 (2H, d, *J*=7.5 Hz); δ_C 44.1, and third (**1-c**) was propenyl group: from the methyl at δ_H 1.98 (3H, d, *J*=1.75 Hz); δ_C 17.5,

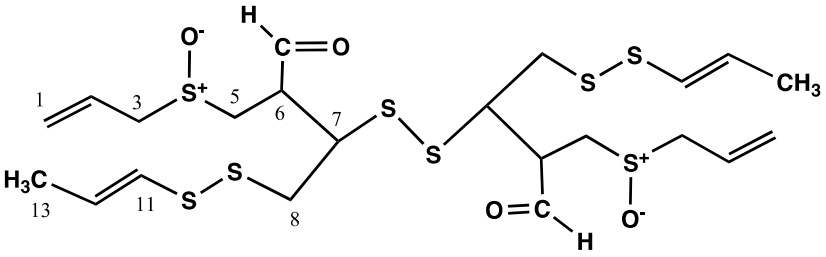


Figure 2. Structure of garlicnin L-5 (**1**).

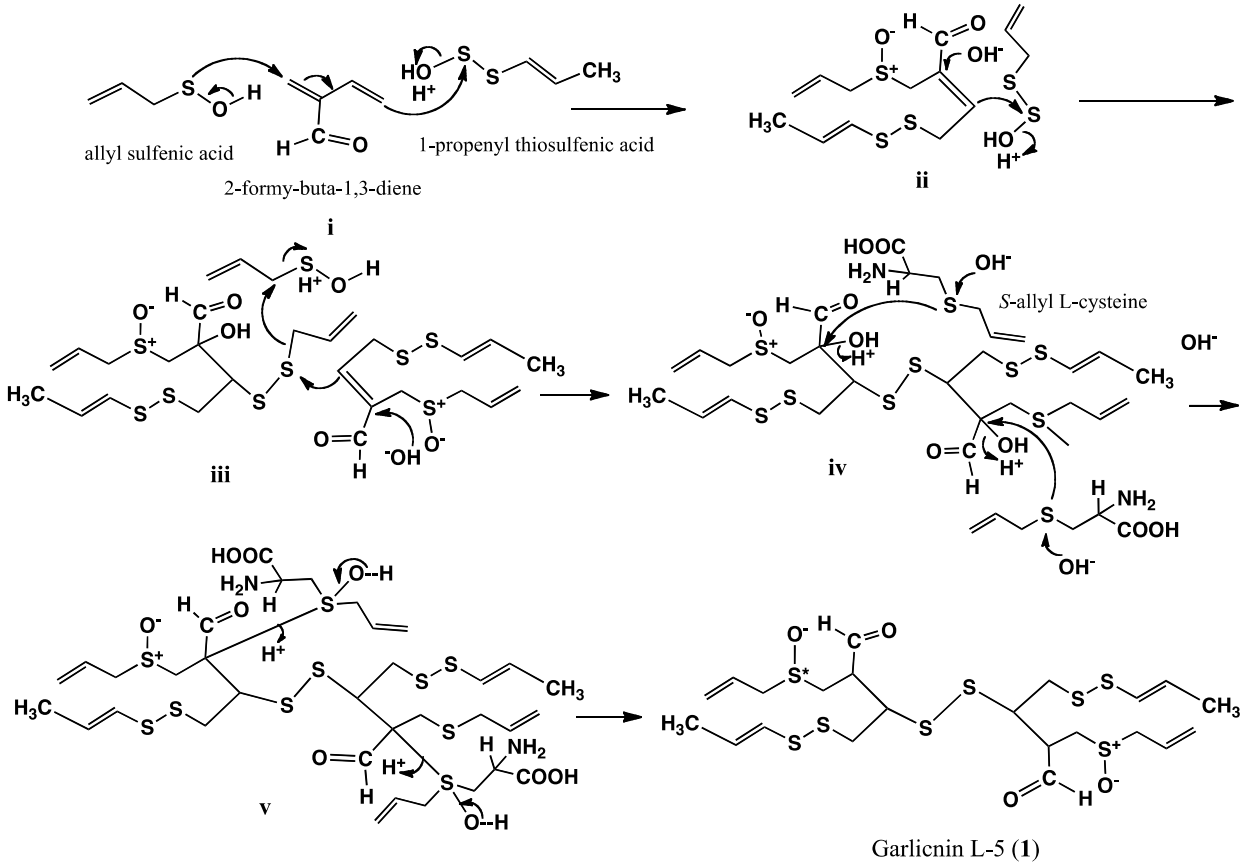


Figure 3. Hypothetical biogenesis of garlicnin L-5 (**1**).

next to the olefinic methine at δ_H 6.96 (1H, q, *J*=6.85 Hz); δ_C 145.9, further to the olefinic methine at δ_H 6.32 (1H, d, *J*=16.6 Hz); δ_C 129.2, as shown in Figure 1. Thus, the combinations between the sulfinyl group in **1-a** and the methylene group at C-5 in **1-b**, and between the thiosulfenyl group at S-9, 10 in **1-b** and the olefinic group at C-11 in **1-c**, were revealed. The remaining C-7 was considered to combine with the same molecule through the thiosulfenyl function because it appeared to form a dimer at δ_C 36.4; δ_H 2.99, t, *J*=7.8 Hz. Therefore, the structure of **1** was represented as shown in Figure 2. The generation of garlicnin L-5 (**1**) was hypothesized as shown in Figure 3, that is, **i** Combination of allyl sufenic acid and 1-propenyl thiosulfenic acid, which were derived from allicin, and 2-formyl-buta-1,3-diene to form **ii**, which then react with allyl thiosulfenic acid to give compound **iii**. Next, compound **iii** reacts with another sulfide (**ii**) by the withdrawal of allyl sufenic acid to form a dimer **iv**. Next, in the reaction processes in **iv** and **v**, two hydroxyl functions at C-6, C-6' would be reduced with the aid of *S*-allyl L-cysteine to afford garlicnin L-5 (**1**). 2-Formyl-butane may be derived from 2-(hydroxymethyl)butane-1,4-diol.

CONCLUSION

We isolated one acyclic sulfide, garlicnin L-5, from garlic and characterized its structure. This compound was a novel dimeric sulfide which consisted of three substructures of allyl moiety, 2-formyl-butane moiety, and propenyl moiety through the sulfinyl and thiosulfenyl bonds.

CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

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