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Original Article

Determination of absolute configuration of the most abundant garlic sulfide, garlicnin B_1

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ABSTRACT

As part of our continuing studies on the *Allium* sulfides exhibiting antitumor effects, we identified several sulfides termed garlicnins and characterized their structures. They were classified into acyclic-, 3,4-dimethylthiolane-, 2-methylthiolane-, 1,2-dithiolane-, 2-oxothiolane-, and bis-thiolane-type sulfides. However, it was pointed out that some parts of 3,4-dimethylthiolane-type had structural inaccuracies. Therefore, we corrected them and determined the absolute configuration of the major sulfide, garlicnin B_1, which is also the most abundant *Allium* sulfide and expected to possess antitumor effect.

KEYWORDS: *Allium sativum*, absolute configuration, 3,4-dimethylthiolane-type, garlicnin B_1, onionin A_1.

INTRODUCTION

Unexpectedly, a small number of sulfides had been found in garlic (*Allium sativum*) prior to our studies on garlic [1-10], onion (*A. cepa*) [6, 11-15], and Welsh onion (*A. fistulosum*) [12]. Therefore, we started our research aiming at the isolation, structural characterization and pharmacological analysis of the cyclic sulfides from garlic that show antitumor activity. Firstly, we isolated onionin A_1 from the acetone extract of onion, and determined its structure as 3,4-dimethylthiolane *S*-oxide (**1'**), as shown in Fig. 1, based on the ^{1}H-^{1}H correlation spectroscopy (COSY) analysis that included the correlation between H-5 and H-1', and the proton assignments of $\underline{H}$-S^+-O^- and $\underline{H}$-2 at C-2, and the determination of the relative configuration by the aromatic solvent-induced NMR shifts [16, 17]. In relation to the structure of onionin A_1, we also deduced the structure of garlicnin B_1 (**2'**) isolated from garlic. However, Block *et al*. later denied its structure and corrected it as 3,4-dimethyl-5-allylsulfinylthiolane-2-ol (**2**) (Fig. 1) [18], because the continuity of 9 carbons was not observed in 2D ^{13}C-^{13}C NMR incredible natural abundance double quantum transfer experiments (INADEQUATE). Kubec *et al.* also corrected onionin A_1 as (*E*)-3,4-dimethyl-5-(1-propenylsulfinyl) thiolane-2-ol (**1**) (Fig. 1) [19]; he just made some structural corrections and retained the former names of onionin A and garlicnin. Here, we recognized the validity of their claims and corrected the structures of onionin A_1 and garlicnins B_1.

Regarding the biological activities of onionin A_1 (**1**) obtained from onion, which is the isomer of the major component garlicnin B_1 (**2**) from garlic, it showed the potential in inhibiting the polarization of M2-activated macrophages that were capable of suppressing tumor-cell proliferation [20, 21]. Then, 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

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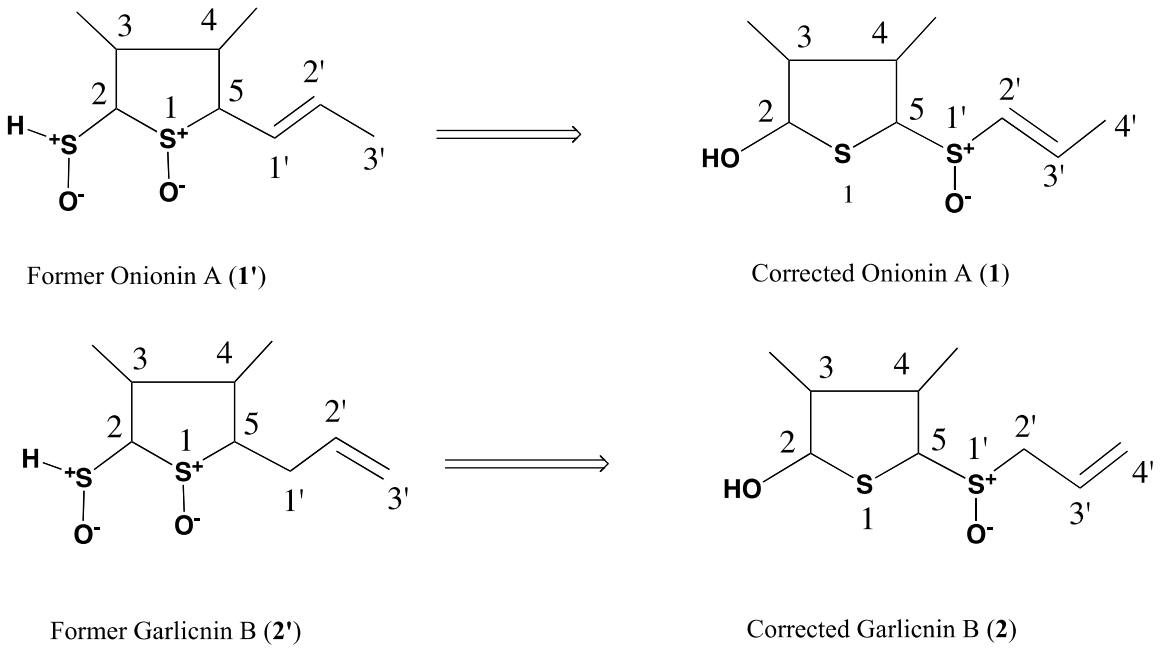


Fig. 1. Corrected structures of onionin A_1 (**1**) and garlicnin B_1 (**2**).

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]. Therefore, similar to onionin A_1, garlicnin B_1 is also expected to have sufficient antitumor effect. Since garlicnin B_1 is the most common sulfide in garlic, we determined its absolute structure by the Mosher method [22, 23] because it was difficult to crystallize garlicnin B_1.

MATERIALS AND METHODS

General experimental procedures

Optical rotation was measured using a JASCO P-1020 (l = 0.5) automatic digital polarimeter. The ^{1}H- and ^{13}C-NMR spectra were measured in $CDCl_3$ using a JEOL alpha 500 spectrometer at 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. cepa* L., family Liliaceae) imported by Shinko Co. Ltd. A voucher specimen (GAR-21-3-25) was deposited in the Herbarium of the Botanical Garden at Sojo University, Kumamoto, Japan.

Extraction and isolation of garlicnin B_1

Peeled Chinese garlic bulbs (Shinko Company, 2268 g) purchased from 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 partitioned between 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 x 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, yield 0.05065 %), 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 [24].

Garlicnin B_1 (2)

^{1}H-NMR spectrum: δ 1.04 (3H, dd, *J*=2.3, 6.9 Hz, C$\underline{H}_3$ at C-3), 1.23 (3H, d, *J*=6.8 Hz, C$\underline{H}_3$ at C-4), 1.98 (1H, m, H-3), 2.15 (1H, m, H-4), 3.33 (1H, dd, *J*=8.6, 12.6 Hz, H-2'a), 3.59 (1H, dd, *J*=6.3, 12.6 Hz, H-2'b), 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*=17.2 Hz, H-3'b), 5.37 (d, *J*=10.3 Hz, H-3'a), 5.68 (1H, m, H-2').

^{13}C-NMR spectrum: δ 13.9 (C$\underline{H}_3$ at C-3), 18.3 (C$\underline{H}_3$ at C-4), 42.8 (C-4), 54.9 (C-3), 55.6 (C-2'), 74.9 (C-5), 84.0 (C-2), 124.5 (C-4'), 125.1 (C-3') as shown in Fig. 2. The nuclear Overhauser effect spectroscopy (NOESY) spectrum showed the following ^{1}H-^{1}H correlations: C$\underline{H}_3$ at C-3 and H-2; C$\underline{H}_3$ at C-4 and H-5; and C$\underline{H}_3$ at C-3 and C$\underline{H}_3$ at C-4 as shown in Fig. 2.

Preparation of (*S*)- and (*R*)- esters of garlicnin B_1 (2)

After leaving the mixture of garlicnin B_1 (**2**, 38.7 mg), (*R*)-(-)-α-methoxy-α-(trifluoromethyl)phenylacetyl (MTPA) chloride and pyridine (1 ml) at room temperature for 2 hours, it was poured into ice-water. Then, the mixture was shaken with $CHCl_3$ and water. The organic layer was washed with sodium bicarbonate-saturated water, and water. The organic layer was evaporated under reduced pressure at 50 °C to give the residue, which was then subjected to preparative silica gel chromatography with *n*-hexane. Under UV light, the sensitive top portion was scrapped off and extracted with $CHCl_3$. Evaporation of the solvent gave garlicnin B_1 (*S*)-MTPA ester (6.2 mg), *Rf* value: 0.73 on TLC with

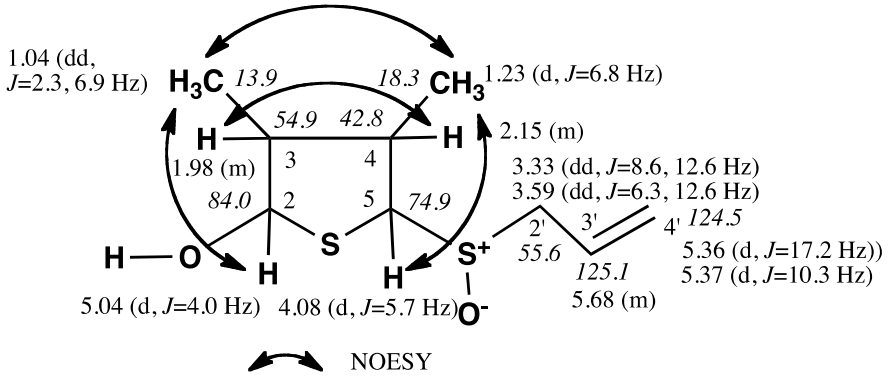


Fig. 2. ^{1}H- and ^{13}C-NMR spectra of garlicnin B_1 (**2**) with NOESY spectrum.

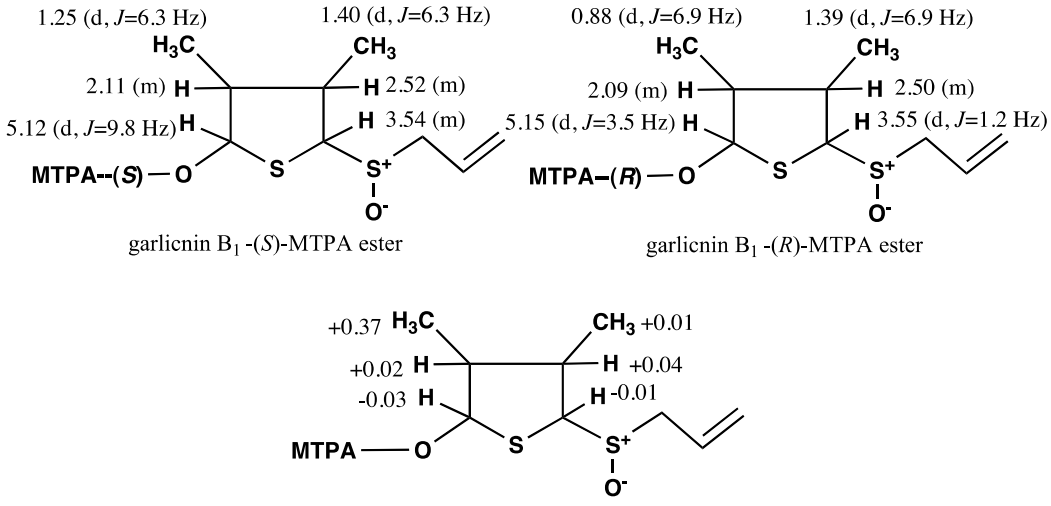


Fig. 3. Proton chemical shifts of garlicnin B_1-(*S*)- and -(*R*)-esters and their differences.

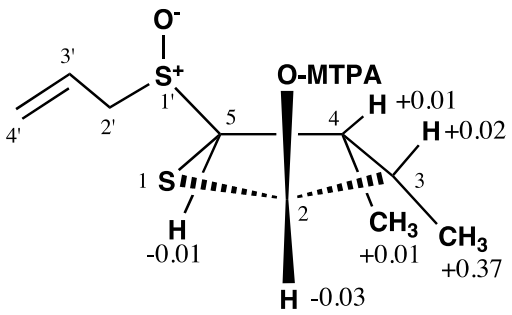


Fig. 4. Molecular model of garlicnin B_1 MTPA ester with MTPA ester on the upper side of the vertical axis wherein the configuration at C-2 is *S*.

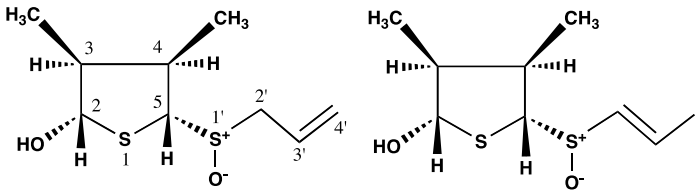
n-hexane and acetone = 2 : 1. Then, the ^{1}H-NMR spectrum was measured. Simultaneously, the garlicnin B_1 (*R*)-MTPA ester (4.3 mg) was also prepared.

^{1}H-NMR spectrum of garlicnin B_1 (*S*)-MTPA ester

^{1}H-NMR spectrum: δ 1.25 (3H, d, *J*=6.3 Hz, C$\underline{H_3}$ at C-3), 1.40 (3H, d, *J*=6.3 Hz, C$\underline{H_3}$ at C-4), 2.11 (1H, m, H-3), 2.52 (1H, m, H-4), 3.54 (1H, m, H-5), 5.12 (1H, d, *J*=9.8 Hz, H-2) as shown in Fig. 3.

^{1}H-NMR spectrum of garlicnin B_1 (*R*)-MTPA ester

^{1}H-NMR spectrum: δ 0.88 (3H, d, *J*=6.9 Hz, C$\underline{H_3}$ at C-3), 1.39 (3H, d, *J*=6.9 Hz, C$\underline{H_3}$ at C-4), 2.09



garlicnin B_1 (**2**) onionin A_1 (**1**)

Fig. 5. Structures of garlicnin B_1 (**2**) and onionin A_1 (**1**).

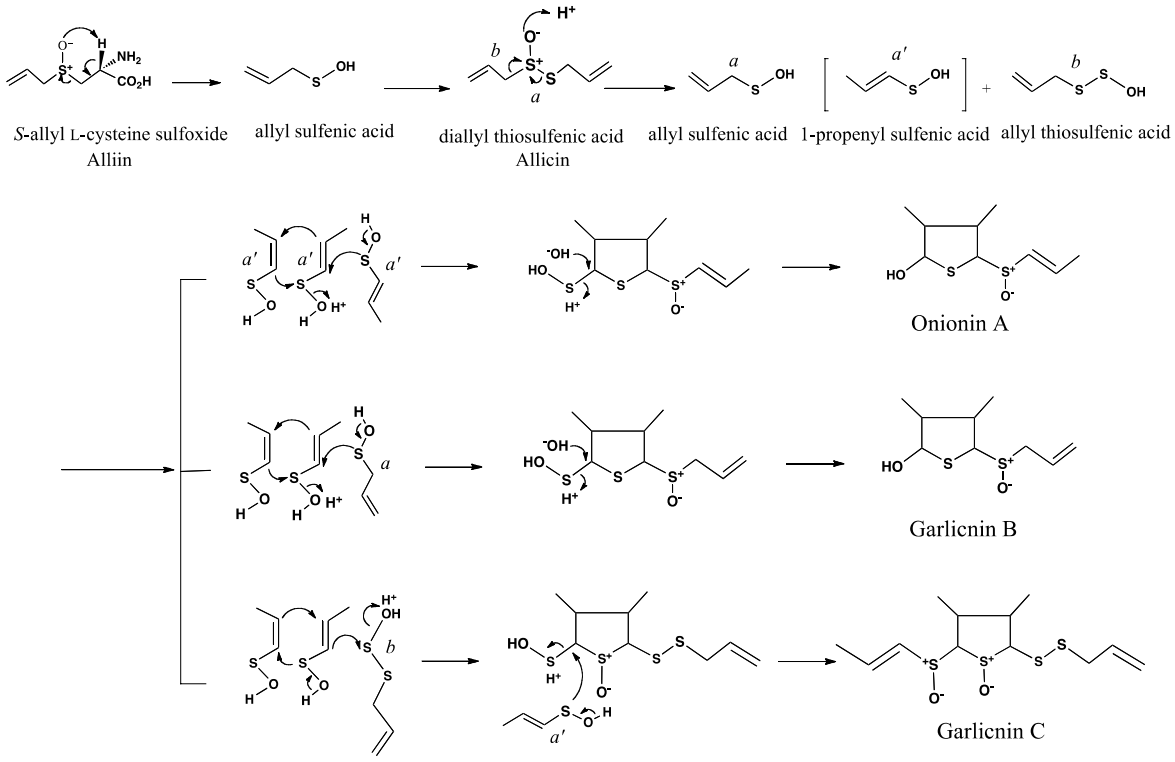


Fig. 6. Hypothetical pathways to onionin A, garlicnin B and garlicnin C.

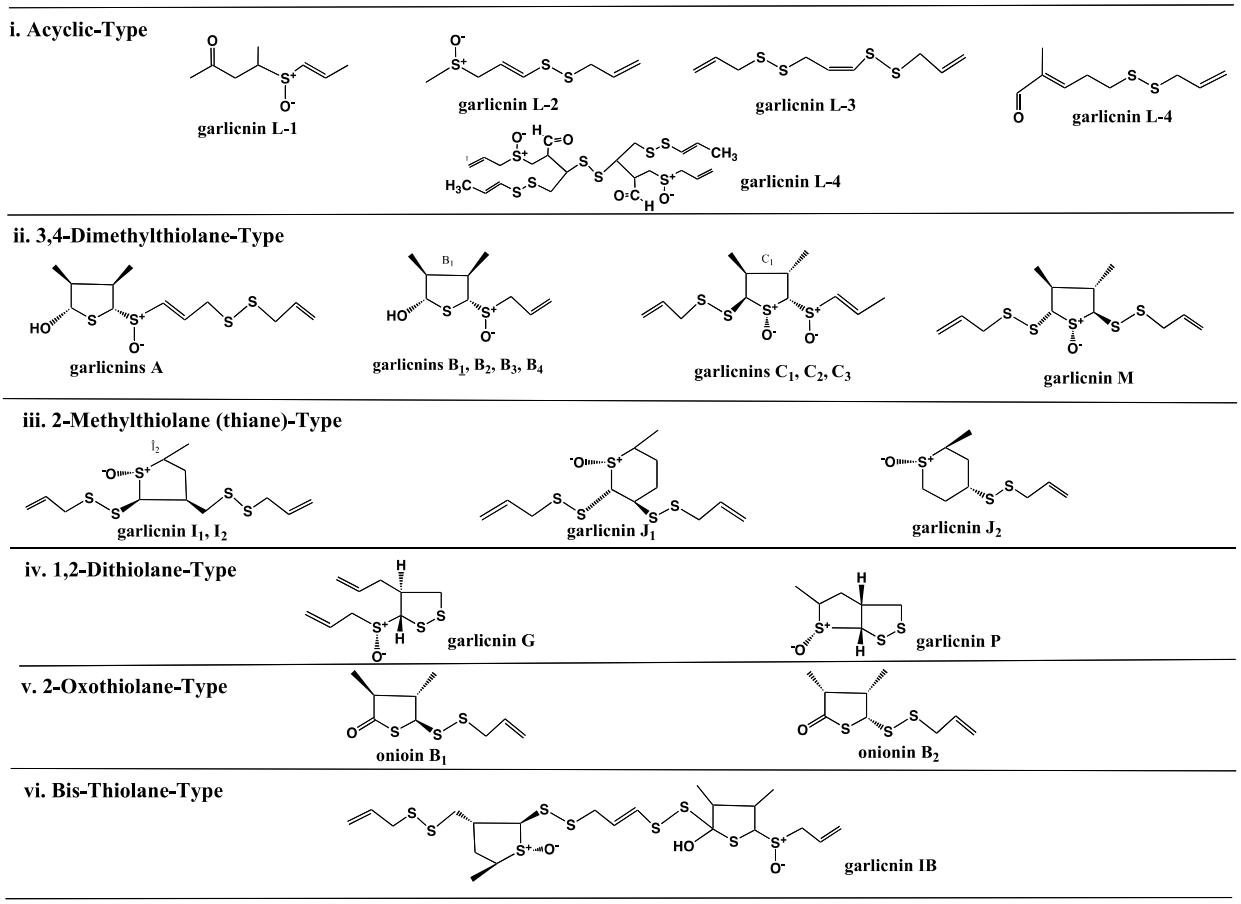
(1H, m, H-3), 2.50 (1H, m, H-4), 3.55 (1H, d, *J*=1.2 Hz, H-5), 5.15 (1H, d, *J*=3.5 Hz, H-2) as shown in Fig. 3.

RESULTS AND DISCUSSION

For preparing garlicnin B_1-(*S*)-MTPA ester and garlicnin B_1-(*R*)-MTPA ester, the starting material garlicnin B_1 (**2**) was obtained from the acetone extract of commercial garlic and its structure was confirmed by measurements of its ^{1}H- and ^{13}C-NMR spectra as shown in Fig. 2. Moreover, the NOESY spectrum gave the correlations between H-2, H-5, C$\underline{H_3}$ at C-3, and C$\underline{H_3}$ at C-4 as shown in Fig. 2.

Then, garlicnin B_1 (*S*)-MTPA ester and garlicnin B_1 (*R*) ester were derived by reaction of (*R*)-MTPA chloride and (*S*)-MTPA chloride in pyridine to give (*S*)-MTPA ester and (*R*)-MTPA ester, respectively. They were measured with ^{1}H-NMR spectra to give the respective proton's chemical shifts of H-2, C$\underline{H_3}$ at C-3, C$\underline{H_3}$ at C-4, and H-5, and their differences as shown in Fig. 3. Furthermore, the model of garlicnin B_1 MTPA ester with MTPA ester on the upper side of the vertical axis was drawn as shown in Fig. 4 wherein the configuration at C-2 is *S*. The differences of the chemical shifts between garlicnin B_1 (*S*)-MTPA ester and garlicnin B_1 (*R*)-MTPA ester were +0.37, +0.01, +0.02, and +0.01, at C$\underline{H_3}$ at C-3, C$\underline{H_3}$ at C-4, H-3, and H-4, respectively, in the right side, and -0.01 at H-5 in the left side. Here, it was confirmed that the absolute configuration at C-2 of garlicnin B_1 was *S*. Therefore, the structure of garlicnin B_1 (**2**) was expressed as 3β,4β-dimethyl-5α-allylsulfinylthiolane-2α-ol as shown in Fig. 5 by taking the result of NOESY into consideration. Since the chemical shifts of protons of H-2, H-3, H-4, C$\underline{H_3}$ at C-3, and C$\underline{H_3}$ at C-4, and carbons of C-2, C-3, C-4, $\underline{C}H_3$ at C-3, and $\underline{C}H_3$ at C-4 in both garlicnin B_1 (**2**) and onionin A_1 (**1**) were almost identical [11],

Table 1. Garlic sulfides (garlicnins and onionins) so far obtained from garlic by us.



the absolute configuration of onionin A_1 (**1**) was deduced to be the same as that of garlicnin B_1 (**2**), that is, (*E*)-3β,4β-dimethyl-5α-(1-propenylsulfinyl) thiolane-2α-ol.

We corrected the structures of garlicnin B_1 and onionin A_1; we hypothesized the biogenesis of their sulfides together with garlicnin C as shown in Fig. 6. Furthermore, we illustrated all the structures of sulfides obtained from garlic as listed in Table 1.

CONCLUSION

In addition to the partial structural corrections of garlicnin B_1 (**2**) and onionin A_1 (**1**) according to the claims by Block [18] and Kubec [19], we determined the absolute configuration of the most abundant *Allium* sulfide, garlicnin B_1, isolated from garlic as 3β,4β-dimethyl-5α-allylsulfinylthiolane-2α-ol having *S* configuration at C-2 using the Mosher method. In connection with the structure of garlicnin B_1, the absolute configuration of onionin A_1 (**1**) was also deduced as (*E*)-3β,4β-dimethyl-5α-(1-propenylsulfinyl)thiolane-2α-ol.

CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

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