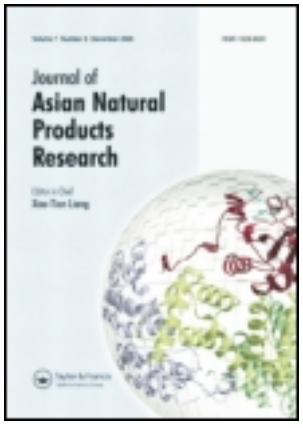

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A new dipeptide isolated from the bulb of garlic

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(*R*)-3-(allylthio)-2-((*R*)-3-(allylthio)-2-aminopropanamido)propanoic acid was isolated from the bulb of garlic, together with four known amino acids. Its structure was elucidated on the basis of 2D NMR and MS techniques. To the best of our knowledge, (*R*)-3-(allylthio)-2-((*R*)-3-(allylthio)-2-aminopropanamido)propanoic acid, which showed antibacterial activity against the *Staphylococcus aureus* antibiotic resistant strain, was the first example of dipeptide from garlic.

Keywords: dipepeptide; garlic; antibacterial activity

1. Introduction

Garlic (*Allium sativum* L.) is used worldwide as a spice, food, and folk medicine [1–3]. Many medicinal substances derived from garlic had been reported so far, most of which are organosulfur compounds [4–8]. The types of compounds isolated from garlic are intimately related to the methods of processing garlic [9–12], because alliin, the major sulfur-containing amino acid in garlic, is easily converted to diallyl thiosulfinate (allicin) *via* alliinase action when the raw garlic bulb is chopped or crushed. Furthermore, allicin, as the above reaction product, is considerably unstable and easily decomposed into other kinds of organosulfur compounds [10]. It is natural that different processing technologies of garlic could result in different medical effects. It is said that eating newly unearthed garlic, rather than any others, is good for our heath. However, to the best of our knowledge, the chemical investigation on the newly unearthed garlic has not been carried out till now, though there are many studies on the chemical components and bioactivities of garlic such as oil-macerated garlic [8,9]. As a part of our continuing studies on secondary metabolites from a medical plant or food, we isolated one new dipeptide **3** from newly unearthed garlic (Figure 1), together with four known amino acids L-deoxyalliin **1** [13,14], alliin **2** [15], tryptophan, and phenylalanine.

2. Results and discussion

(*R*)-3-(allylthio)-2-((*R*)-3-(allylthio)-2-aminopropanamido)propanoic acid, obtained as an amorphous, optically active powder, had the molecular formula $C_{12}H_{20}N_2O_3S_2$, as established by HR-ESI-MS analysis (*m/z* 305.0952 $[M + H]^+$) and confirmed by ^{13}C NMR spectroscopy. The IR spectrum of **3** showed absorption bands for carbonyl (1686, 1753) and C=C groups (1612). The ^{13}C NMR spectrum (Table 1) showed a total of 12 signals (4 CH, 6 CH_2, and 2 quaternary C atoms), in agreement with the molecular formula. The ^1H and ^{13}C NMR spectra were rather similar to those of **1** [16], one of the major sulfur-containing amino acids present in

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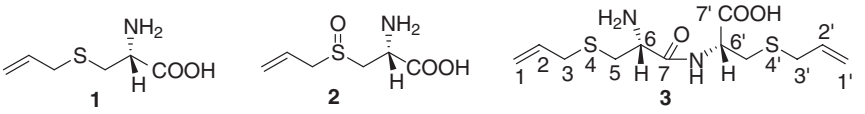

Figure 1. The structures of compounds **1–3**.

the garlic, but the number of carbon signals of **3** in the ^{13}C NMR spectrum was two times as those of **1**, which suggested that **3** might be a new dimeric of **1**. The structure of **3** was further revealed by detailed analysis of one- and two-dimensional NMR spectra, as follows. $^{1}H-^{1}H$ COSY and HMQC experiments indicated the presence of the following structures: $CH_2{=}CHCH_2$ and CH_2CH $(NH_2)C(O)$, which was further confirmed by a HMBC experiment (Figure 2). The above-mentioned two fragments were linked by an S atom based on the HMBC cross-peaks H-5/C-3. A signal at δ_C 53.7 was assigned to C(6′) on the basis of the HMBC cross-peaks H-6′/C-7, C-7′, and C-5′.

The configuration of **3** was revealed through acid hydrolysis of **3**. The obtained product of acid hydrolysis was found to be identical to **1** because it has the same optical rotation ($[\alpha]_D^{25}$ −13.1 ($c = 2$, H_2O)) and ^{1}H NMR spectrum as **1**. Therefore, the absolute configurations of **3** at C(6) and C(6′) were both elucidated to be (*R*).

On the basis of the above discussion and by comparison with the literature data of **1** [16], the structure of the new dipeptide, named (*R*)-3-(allylthio)-2-((*R*)-3-(allylthio)-2-aminopropanamido)propanoic acid, was established. To the best of our knowledge, **3** was the first example of dipeptide isolated and elucidated from garlic.

Compounds **1–3** showed inhibitory activities against *Staphylococcus aureus* (compound **1**, 370 μg/disk, zone diameter: 8 mm; compound **2**, 350 μg/disk, zone diameter: 12 mm; compound **3**, 90 μg/disk, zone diameter 9 mm).

3. Experimental

3.1 General experimental procedures

Optical rotations were measured with a Jasco DIP-370 digital polarimeter (Tokyo, Japan). IR spectra were obtained on an IR-450 infrared spectrophotometer (Tokyo, Japan) with KBr pellets. FAB-MS and HR-FAB-MS were carried out on an Autospe-3000 spectrometer (Manchester, UK). Nuclear magnetic resonance spectra were recorded on Bruker AM 500 MHz spectrometer (Bruker, Fallanden, Switzerland). δ in ppm rel. to Me_4Si as internal standard. Solvents were distilled before use. TLC and columm chromatography (CC) were carried out on plates precoated with gel F254 and silica gel H (SiO_2, Qingdao

Table 1. ^{1}H and ^{13}C NMR spectral data of compound **3** in MeOD (500 MHz for ^{1}H and 125 MHz for ^{13}C, res.; δ in ppm).

Position	δ_C	δ_H	Position	δ_C	δ_H
1	119.0	5.24–5.28 (m)	1′	118.3	5.09–5.13 (m)
2	134.5	5.83–5.91 (m)	2′	135.2	5.70–5.76 (m)
3	35.0	3.26–3.26 (m)	3′	35.7	3.10–3.18 (m)
5	33.1	2.99–3.04 (m)	5′	32.5	2.85–2.90 (m)
6	53.0	4.24–4.27 (m)	6′	53.7	4.60–4.65 (m)
7	168.1	–	7′	173.2	–
CONH		8.81	NH_2		4.60

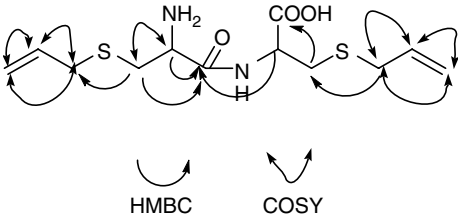

Figure 2. Key HMBCs and COSYs for **3**.

Haiyang Chemical Co. Ltd, Qingdao, China).

3.2 Plant material

The bulb of garlic was collected in Boluo city, Guangdong Province of China and identified by professor Shi-Yi Ou (Jinan University). A specimen of the plant has been deposited in the Department of Food Science and Engineering, Jinan University, Guangzhou, China.

3.3 Extraction and isolation

The garlic (8.0 kg) was frozen by liquid nitrogen of − 196°C, and then broken, extracted with 90% ethanol. After the removal of the solvent by evaporation, the residue was dissolved in water and chromatographed on a D-101 resin with H_2O and ethanol to give two fractions. The H_2O eluent as the main amino acid was concentrated to obtain 9.0 g crude amino acid, which was chromatographed on ion-cation exchange resin (Amberlite IR-120) with 5% NaOH solution to give four fractions. Fraction 2 was subjected to CC (silica gel; H_2O/MeOH; 25:75) to furnish **2** (150 mg), **1** (50 mg), tryptophan (20 mg), and phenylalanine (15 mg); fraction 4 was chromatographed on CC (silica gel; petroleum ether/AcOEt; 2:3) to yield **3** (15 mg).

3.3.1 (R)-3-(Allylthio)-2-((R)-3-(allylthio)-2-aminopropanamido) propanoic acid

Colorless amorphous powder. $[\alpha]_D^{19.2}$ − 10.1 ($c = 0.1$, CH_3OH). IR (KBr) ν_{max} (cm^{-1}): 3002, 2633, 1753, 1686, 1612, 87, 1440, 783. For ^1H and ^{13}C NMR spectral data, see Table 1. ESI-MS: *m/z* 305 (100) $[M + H]^+$. HR-ESI-MS: *m/z* 305.0952 $[M + H]^+$ (calcd for $C_{12}H_{21}N_2O_3S_2$, 305.0988).

3.4 Acid hydrolysis of 3

During the study, 1.0 ml of a 3.0 g/l aqueous solution of **3** was treated with 10.0 mol/l HCl (1.0 ml) at 80°C for 18 h. The mixture was immediately purified by acid cation exchange resin (Amberlite® IRP-69, Shanghai Aladdin Industrial Corporation, Shanghai, China) by using NH_4OH (30%) to afford **1** (2.0 mg), which was identified by ^1H NMR and TLC and optical rotations measurement.

3.5 Antibacterial assay

S. aureus was spread with an aseptic spreader on the surface of the agar medium dish. The samples were dissolved in CH_3OH and added to each paper disk with syringes, respectively. The disks were dried with flow air and put onto agar media inoculated with the testing organism for 10 h at 42°C. The antibacterial activity was calculated by the diameter of the inhibition zone.

Acknowledgements

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