J Korean Soc Appl Biol Chem (2013) 56, 457–459
DOI 10.1007/s13765-013-3069-x

SHORT COMMUNICATION

Characterization of Alkyl Thiosulfinate in *Allium hookeri* Root Using HPLC-ESI-MS

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Received: 25 March 2013 / Accepted: 17 May 2013 / Published Online: 31 August 2013

Abstract Allicin produced by alliinase system of *Allium hookeri* was evaluated via high performance liquid chromatography (HPLC). Allicin contents of *A. hookeri* were 56.6±3.5 μg per g of fresh root and 12.7±3.2 μg per g of fresh stem. These values were relatively low as compared with garlic. HPLC-electrospray ionization-mass spectrometry analyses showed *A. hookeri* root extract contained ten alkyl thiosulfinates, and the chemical structures were characterized by MS/MS analyses.

Keywords allicin · *Allium hookeri* · thiosulfinate · electrospray ionization-mass spectrometry

Allium hookeri Thwaites (Liliaceae family) is a wild herb distributed in India and Myanmar. The root of the plant has been used as food and medicine in Southeast Asia (Sangtam et al., 2012). Recently, this plant was introduced to South Korea and has been cultivated in the southern region. *A. hookeri* has pungent flavor like other allium species when the plant tissue is damaged. *Allium* species contain *S*-methyl cysteine sulfoxide (methiin), *S*-allyl cysteine sulfoxide (alliin), *S*-1-propenyl cysteine sulfoxide (isoalliin), and *S*-propyl cysteine sulfoxide (propiin) (Fig. 1). The major flavor component of garlic (*A. sativum*) is allicin. This compound is formed when the tissue is damaged due to hydrolysis product of alliin brought about by alliinase (Dewick, 2002). Other thiosulfinates are formed by alliinase and *S*-alkyl cysteine sulfoxide system. Allicin and other organic sulfur compounds act as beneficial agents to reduce cholesterol levels and reduce the risk of heart attacks. Anti-inflammatory effects of *A. hookeri* extract have been reported (Bae and Bae, 2012; Kim et al., 2012); however, the chemical compound of this plant is not known. We report the allicin content and characterize the alkyl thiosulfinate compounds in *A. hookeri* root using high performance liquid chromatography-electrospray ionization-mass spectrometry (HPLC-ESI-MS).

Allicin was isolated from store-purchased garlic. The homogenate of fresh garlic was extracted with dichloromethane, and the extract was purified using a preparative silica gel thin layer chromatography (TLC) plate, which was developed in hexane/ethyl acetate (60/40). Allicin, the main band under UV lamp, was extracted with water and stored at 4°C. The allicin content of an aqueous solution was determined on UV spectrophotometer using an extinction coefficient of 14.64 mL/mg·cm at 240 nm (Lawson et al., 1991). The diluted aqueous allicin solutions were used as external HPLC standards. The UV detector response at 240 nm to allicin was found to be linear over the range of 4.1 to 410 ng.

Fresh whole plant of *A. hookeri* was obtained from local market in November 2012 and stored in a deep freezer. The root and stem of *A. hookeri* were homogenized for 1 min using 5 mL of distilled water per gram of the sample. The homogenate was allowed to stand at room temperature for 10 min and centrifuged. The supernatant was mixed with equal volume of methanol, filtered, and injected into the HPLC. The extracts were separated through Gemini C-18 column (3×150 mm, 5 μm, Phenomenex, USA) with flow rate of 0.5 mL/min. The mobile phase was a binary gradient elution of (A) water and (B) acetonitrile under the following conditions: 0–40 min linear gradient from 90 to 50% A and maintained 50% A for 5 min. Liquid chromatography-mass spectrometry (LC-MS) analyses were performed with an Agilent 1100 HPLC (Agilent, USA) and a Bruker HCT 300 (Bruker, Germany) mass spectrometer equipped with an ESI interface. The inlet flow from HPLC into the ion source was reduced 20 μL/min by splitter. The ESI parameters were as follows: dry temperature was 300°C, nebulizer gas was 15 psi, and dry gas was 5 L/min. The mass spectrometer was operated in positive ion mode.

Allicin contents of *A. hookeri* were 56.6±3.5 μg per g of fresh

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Table 1 Allicin contents of *A. hookeri* and *A. sativum*

Plants	Allicin content (fresh weight)
A. hookeri root	56.6±3.5 μg/g[a]
A. hookeri stem	12.7±3.2 μg/g[a]
A. sativum clove	2.25±0.25 mg/g[a] (2.67–3.91 mg/g)[b]

[a]Means±SD of triplicate experiments.
[b]reported value in the reference (Khar et al., 2011)
Allicin content was calculated as the following standard curve: HPLC peak area of allicin=2771×ng of allicin+41 (r^2 >0.999)

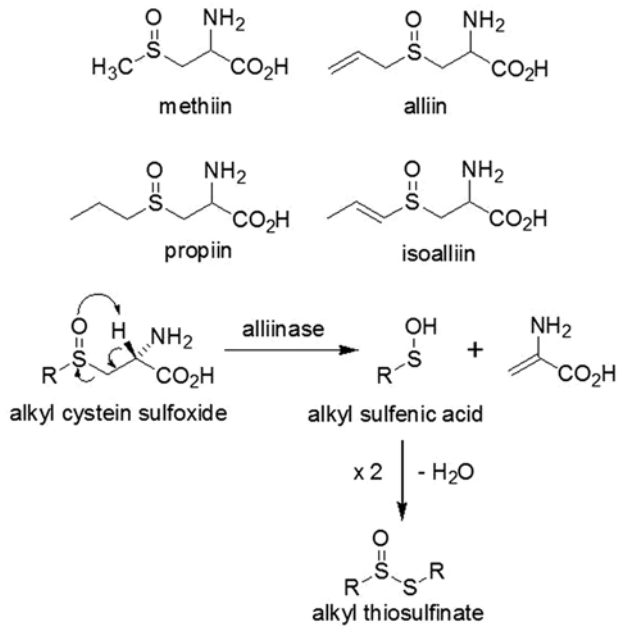


Fig. 1 Precursors of alkyl thiosulfinates (substrates of alliinase) reported in *Allium* species and alliinase reaction mechanism (Dewick, 2002)

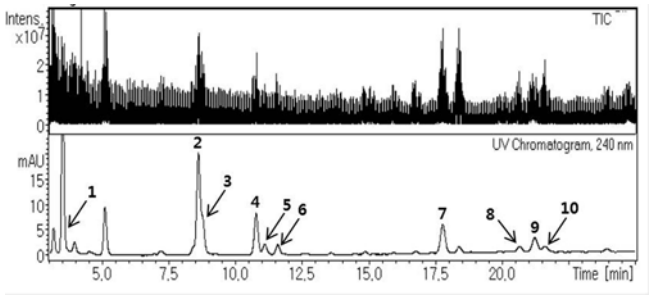


Fig. 2 TIC and HPLC chromatograms of *A. hookeri* root extract

root and 12.7±3.2 μg per g of fresh stem, respectively. These values were relatively low as compared with garlic (Table 1). HPLC chromatogram showed allicin (diallyl thiosulfinate, **7**) was not the major compound in *A. hookeri* root. Thus, other alkyl thiosulfinates were characterized by MS/MS analyses.

Ten alkyl thiosulfinates were characterized in *A. hookeri* root by HPLC-ESI-MS. Endogenous enzyme system predicted the production of dimethyl thiosulfinate (**1**) from two molecules of methiin (Fig. 1 and 2). The signal of *m/z* 111 $[M+H]^+$ was detected at 3.5 min, and its product ion was *m/z* 65 (1 of Fig. 3). This peak was characterized as dimethyl thiosulfinate (**1**). The peaks of 2, 3, and 4 were major components of *A. hookeri* root, and $[M+H]^+$ ions of these peaks were *m/z* 137. These compounds were formed from combination of methiin, alliin, and isoalliin using alliinase system (Fig. 1). Peaks 2 and 4 showed large product ions at *m/z* 73, and peak 3 showed large product ion at *m/z* 95 and small product ion at *m/z* 73 (2, 3, and 4 of Fig. 3). The intensity of the product ions in MS/MS depends on the relative stability of the product ions. The CH_2=CH-CH_2S^+ ion is more stable than the

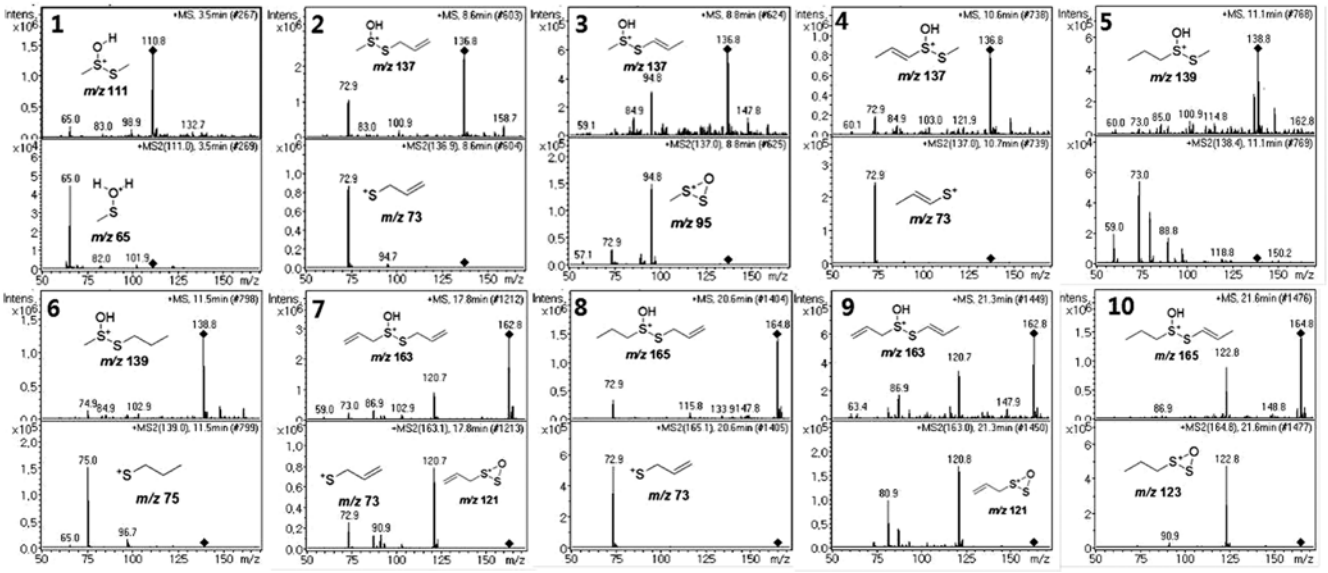
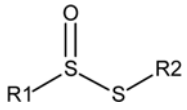


Fig. 3 Full scan mass spectra and product ions mass spectra of alkyl thiosulfinate from *A. hookeri* root extract

Table 2 Alkyl thiosulfinate founded in *A. hookeri* root extract



Compound name	R_1	R_2	$[M+H]^+$ (*m/z*)	Product ions (*m/z*)	RT (min)
Dimethyl thiosulfinate (**1**)	$-CH_3$	$-CH_3$	111	65	3.5
Allyl methyl thiosulfinate (**2**)	$-CH_3$	$-CH_2CH=CH_2$	137	73	8.6
1-Propenyl methyl thiosulfinate (**3**)	$-CH_2CH=CH_2$	$-CH_3$	137	95	8.8
Methyl 1-propenyl thiosulfinate (**4**)	$-CH=CHCH_3$	$-CH_3$	137	73	10.6
Methyl propyl thiosulfinate (**5**)	$-CH_2CH_2CH_3$	$-CH_3$	139	79,73,59	11.1
Propyl methyl thiosulfinate (**6**)	$-CH_3$	$-CH_2CH_2CH_3$	139	75	11.5
Diallyl thiosulfinate (**7**)	$-CH_2CH=CH_2$	$-CH_2CH=CH_2$	163	121	17.8
Allyl propyl thiosulfinate (**8**)	$-CH_2CH_2CH_3$	$-CH_2CH=CH_2$	165	73	20.6
1-Propenyl allyl thiosulfinate (**9**)	$-CH_2CH=CH_2$	$-CH=CHCH_3$	163	121,81	21.2
1-Propenyl propyl thiosulfinate (**10**)	$-CH_2CH_2CH_3$	$-CH=CHCH_3$	165	123	21.6

CH_3-CH=CHS^+ ion (Khar et al., 2011), thus allyl methyl sulfinate (**2**) shows stronger *m/z* 73 signal than 1-propenyl methyl sulfinate (**3**). Peak 4 also showed $[M+H]^+$ at *m/z* 137 and large product ion at 73; this peak was tentatively characterized as methyl 1-propenyl thiosulfinate (**4**). Peaks 5 and 6 had the same $[M+H]^+$ signals at *m/z* 139, but peak 6 showed a stable product ion at *m/z* 75, which corresponded to $CH_3CH_2CH_2S^+$ ion. Thus peaks 5 and 6 were characterized as methyl propyl thiosulfinate (**5**) and propyl methyl thiosulfinate (**6**), respectively. The $[M+H]^+$ of peak 7 was at *m/z* 163, from which major product ion at *m/z* 121 and 73 were formed. This fragment pattern was in good agreement with the reference standard of allicin (diallyl thiosulfinate, **7**). Peak 9 had also *m/z* 163 $[M+H]^+$, and a product ion *m/z* 121 instead of *m/z* 73. This peak was characterized as 1-propenyl allyl thiosulfinate (**9**). The peaks of 8 and 10 had the same $[M+H]^+$ signals at *m/z* 165, which indicates these two compounds are isomers. Peaks 8 had a product ion at *m/z* 73 (CH_2=CH-CH_2S^+ ion), and peak 10 had a product ion at *m/z* 123, thus peak 8 and 10 were characterized as allyl propyl thiosulfinate (**8**) and 1-propenyl propyl thiosulfinate (**10**), respectively (Table 2). This paper is the first report of characterization of sulfur containing compounds in *A. hookeri*, and will be useful to find alkyl thiosulfinate compounds with LC-MS in other plants.

Acknowledgment This work was supported by Institute of Korean Medicine Industry, Mokpo National University.

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