The lachrymatory principle of *Petiveria alliacea*

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Abstract

The lachrymatory principle of *Petiveria alliacea* has been isolated from a fresh homogenate of the root. Its structure and geometric configuration have been determined as (Z)-thiobenzaldehyde S-oxide by means of NMR, IR, MALDI-MS and by comparison with an authentic compound obtained by synthesis. This unique compound represents only the third naturally occurring sulfine (thiocarbonyl S-oxide) to be reported. Its formation and possible subsequent rearrangements are discussed. Its antibacterial and antifungal activities are also reported.

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1. Introduction

*Petiveria alliacea* L. (Phytolaccaceae) is a perennial shrub widely distributed in South and Central America, some areas of Africa and the southeastern United States. It is popularly used in folk medicine for treating a wide variety of disorders.

In our previous contributions, we have reported identification of several novel cysteine derivatives, including diastereoisomers of S-benzyl- and S-(2-hydroxyethyl)cysteine sulfoxides (petiveriin A/B and 6-hydroxyethiin A/B, respectively) (Kubec and Musah, 2001; Kubec et al., 2002). We have also isolated four novel thiosulfimates that are enzymatically formed from these amino acids upon disruption of the tissue of *P. alliacea*. As we proposed, these thiosulfimates can readily decompose to yield a variety of breakdown products, including those previously identified in this plant (e.g. dibenzyl sulfides, benzyl 2-hydroxyethyl sulfides, benzaldehyde and stilbenes).

The present study was stimulated by our sensory observations experienced while working with the plant. Upon disruption of the tissue, an intense alliaceous odor was immediately emitted, causing irritation to nasal and ocular mucosae, leading to a serious nasal discharge and lachrymation, respectively. However, none of the thiosulfimates we isolated in the previous study possesses such a strong odor and all of them are devoid of lachrymatory effects, indicating that another compound is responsible for these properties. Thus, the present paper describes our investigation and identification of the lachrymatory principle of *P. alliacea*.

2. Results and discussion

A fresh homogenate of the plant was extracted using Et₂O. The extract was carefully concentrated and analyzed by C-8 HPLC. Along with the three benzyl-containing thiosulfimates described in our previous study (Kubec et al., 2002), another component was abundantly present in the extract. The compound (1) was subsequently isolated by prep. C-8 HPLC as a yellow, pungent oil, exhibiting very strong lachrymatory properties.

The ¹H NMR spectrum of 1 was quite simple, containing only signals corresponding to five aromatic protons (3H, δ 7.40–7.52 ppm and 2H, δ 8.06–8.11 ppm) together with one singlet with a very downfield chemical shift (1H, δ 8.34 ppm). ¹³C NMR spectroscopy showed the presence of five magnetically non-equivalent carbon

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atoms, with one methine carbon shifted far downfield (δ 166.7 ppm). The GC/EIMS spectrum of 1 displayed a m/z [M+]+ of 138 and revealed the presence of one sulfur atom in the molecule ([M+ + 2]/[M+] of 4.3%). The IR spectrum contained a very strong absorption band at 1114 cm\(^{-1}\) and the MALDI-HRMS data showed a m/z [MH]+ of 139.0210, corresponding to a molecular formula of C\(_7\)H\(_6\)OS (req. 139.0212). Based on the above spectral data, the structure of 1 was determined to be thiobenzaldehyde S-oxide. The geometric configuration of 1 was determined by means of \(^1\)H NMR spectroscopy. As shown by Barbaro et al. (1990), the \(^1\)H chemical shifts of the methine protons of (E)- and (Z)-thiobenzaldehyde S-oxides differ significantly, being δ 9.65 and 8.30 ppm (in CDCl\(_3\)), respectively. Since the \(^1\)H NMR spectrum of the isolated lachrymator contained a singlet at δ 8.34 ppm, the configuration of 1 was assigned as (Z) (Fig. 1). No signal was observed at ~δ 9.65 ppm, indicating that the (E)-stereoisomer of 1 was not present in detectable quantities in the fraction isolated from the plant. The identity of 1 was subsequently confirmed by comparison of its spectral data with those of a synthetically prepared sample of thiobenzaldehyde S-oxide. The latter was obtained as a mixture of both isomers, with a Z/E ratio of ~99.95/0.05 according to \(^1\)H NMR spectroscopic analysis. To the best of our knowledge, 1 is the only third naturally occurring sulfine (thiocarbonyl S-oxide) to be reported, along with the lachrymatory principle of onion, (Z)-thiopropanal S-oxide, and (Z,Z)-(±)-2,3-dimethylbutanedithial S,S'-dioxide, also from onion (Block and Bayer, 1990).

Although the mechanism of the formation of 1 is unclear, it seems reasonable to assume that petiverin (S-benzyleysteine sulfoxide) serves as its precursor. The lachrymatory factor of onion, thiopropanal S-oxide, is formed via a [1,4]-sigmatropic rearrangement of 1-propenesulfenic acid which in turn is formed from allinase-induced cleavage of (E)-S-(1-propenyl)cysteine sulfoxide (isoallin). Unlike isoallin, petiverin cannot degrade into an \(\alpha,\beta\)-unsaturated sulfenic acid that can undergo a [1,4]-sigmatropic rearrangement. Therefore, a different mechanism must be operative in the formation of 1. However, the possibility that 1 is formed from a non-amino acid precursor cannot be ruled out, and thus, further research is needed to elucidate the mechanism by which 1 is formed.

Sulfines are relatively unstable compounds that undergo facile decomposition to a variety of products (Block, 1981; Zwanenburg, 1989; Zwanenburg et al., 1999). As shown by Block et al. (1996), they also readily dimerize, yielding the corresponding 1,2-dithietane 1,1-dioxide derivatives. In our hands, 1 was stable on storage below 0 °C, being a solid at these temperatures (mp 9–10 °C). We have also found that 1 can be analyzed by GC without significant decomposition, even when relatively high injection temperatures (i.e. 260 °C) are employed. The only decomposition products detected in the chromatograms were (E)- and (Z)-stilbenes (in ratios of E/Z~20–50/1) which accounted for <8% of the total sample. It was reported that CDCl\(_3\) solutions of 1 slowly decomposed in a few days at 25 °C, being converted into (E)-stilbene and elemental sulfur. The decomposition was complete in 10 min when the solution was heated at 110 °C (Bonini et al., 1986; Barbaro et al., 1990). On the other hand, Hamid and Trippett (1968) showed that an Et\(_2\)O solution of 1 slowly decomposed at room temperature to give (E)- and (Z)-stilbenes, together with (E)-4,5-diphenyl-1,2,3-trithiolane 1,1-dioxide and 5,6-diphenyl-1,2,3,4-tetrahthiane dioxide.

The sulfine 1 possesses a very intense alliaceous odor with benzaldehyde notes resembling the typical aroma of the plant when bruised or cut. Since the thiosulfinates described previously as well as their major decomposition products (sulfides, stilbenes) do not possess such potent organoleptic properties, we believe that 1 is primarily responsible for the aroma of freshly cut *P. alliacea*.

Aside from its potent lachrymatory properties, 1 also causes an intense burning sensation and irritation when applied to skin. Ruiz (1972) described similar observations in farmers exposed to *P. alliacea*. He reported that some of the workers, after repeated exposure to the plant, developed urticariform dermatitis on the hands and arms which disappeared approximately 10 min after washing with soap and water. It was noted that some 15 minutes after inhalation of the plant volatiles, many individuals developed a headache that persisted for about 3 h. Ruiz (1972) also reported that dietary exposure of *P. alliacea* to cows resulted in mild bloat and frequent eructation of gases possessing an alliaceous odor. This was followed by profuse lachrymal secretion that persisted for several hours after feeding. He postulated that the volatile substance of *P. alliacea* responsible for the lachrymatory effect probably entered the blood stream via intestinal and/or lung capillary absorption when the compound reached the lungs with the eructated ruminal gases. Ruiz (1972) also reported that the milk of cows that had been fed with *P. alliacea* possessed an undesirable garlic-like off flavor. It is likely that sulfine 1 was responsible for the observed physiological effects, as well as for the off-flavor characteristics of the milk.

We have also determined the antimicrobial activity of 1 against a panel of several bacteria (both gram negative and positive) and yeast. As shown in Table 1, the sulfine

![Fig. 1. The structure of 1, (Z)-thiobenzaldehyde S-oxide.](Image)
exhibits modest activity against most of the microorganisms tested.

3. Experimental

3.1. General experimental procedures and plant material

All apparatus used in the present study were identical with those described previously (Kubec and Musah, 2001). The roots of *P. alliacea* originated from the same batch that was analyzed in the previous study. They were stored in a freezer at −30 °C for several months prior to analysis.

3.2. GC analysis

The sulfine was analyzed using a Rtx-1 fused silica capillary column (30 m×0.25 mm; 25 µm; Restek Corp.), employing the following temperature program: 100 °C (held for 2 min), raised linearly to 260 °C at 6 °C min⁻¹. Helium (0.7 ml min⁻¹) was used as the carrier gas. Different injection temperatures were studied (100–260 °C).

3.3. Microbial assays

All experiments were strictly conducted using the standard experimental procedures established by the National Committee for Clinical Laboratory Standards (NCCLS, standard M2-A4) (NCCLS, 1990, 2002). Two EtOH solutions of the sulfine were prepared and a 20-µl aliquot of each solution was placed on a 6-mm disc (Becton Dickinson Microbiology Systems). The following microorganisms were used: *Escherichia coli* (ATCC 25922), *Staphylococcus aureus* (ATCC 25923), *Streptococcus agalactiae* (ATCC 13813), *Pseudomonas aeruginosa* (ATCC 27853), *Klebsiella pneumoniae* pneumoniae (ATCC 13883), *Stenotrophomonas maltophilia* (ATCC 13637), and *Candida albicans* (ATCC 10231).

3.4. Isolation of the lachrymatory principle

The roots of *P. alliacea* (65 g) were mixed with H₂O (150 ml) and homogenized using a Waring blender. The resulting slurry was allowed to stand at room temp., for 30 min, filtered and the filtrate was extracted with Et₂O (2×200 ml). Additional Et₂O (200 ml) was used for washing the filter cake. The Et₂O fractions were combined, evaporated (20 °C) and the yellow, odoriferous residue was redissolved in 10 ml of MeCN. The extract was subjected to prep. C-8 HPLC using the following conditions: H₂O (A), MeCN (B) as the mobile phase (18 ml min⁻¹), A/B 75/25 (0 min), 0/100 (in 30 min) held for 10 min. The fraction eluting at 13.4 min was collected. The collected fractions were combined, the MeCN evaporated using a vacuum rotary evaporator (20 °C) and the aqueous portion was extracted with CHCl₃ (2×100 ml). The chloroform fractions were combined, dried over MgSO₄ and carefully concentrated to ca. 20 ml using a rotary evaporator (20 °C). The remaining chloroform was stripped off by a gentle stream of air, affording 26 mg of I.

3.4.1. (Z)-Thiobenzaldehyde S-oxide (I)

A yellow, pungent oil; mp (uncorr.) 9–10 °C; UV λ <sub>max</sub> (EtOH) nm (log ε): 206 (4.00), 230 (3.92), 310 (4.32); IR (neat) ν <sub>max</sub> cm⁻¹: 3067 (w), 2999 (m), 1701 (w), 1489 (w), 1455 (m), 1175 (m), 1114 (vs), 940 (m), 793 (m), 751 (m), 687 (s); ¹H NMR (300 MHz; CDCl₃): δ 7.40–7.52 (3H, H-meta, H-para), 8.06–8.11 (2H, dd, J = 1.8, 8.1, H-ortho), 8.34 (1H, s, CH=S); ¹³C NMR (75 MHz; 

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<th>Pseudomonas aeruginosa&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Klebsiella pneumoniae&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Stenotrophomonas maltophilia&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Candida albicans&lt;sup&gt;a&lt;/sup&gt;</th>
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<sup>a</sup> –, No inhibition.

<sup>b</sup> Numbers represent diameter of zone of inhibition in mm.

<sup>c</sup> np, Not performed.
CDCl₃): δ 128.9, 129.3 (C-ortho, meta), 131.9 (C-para), 133.0 (C-quaternary), 166.7 (CH=S); GC/MS (EI, 70 eV) m/z (rel. int.): 140 [M++2] (4), 138 [M+] (100), 137 (82), 121 (35), 109 (52), 105 (20), 94 (26), 89 (31), 77 (36), 66 (42); MALDI-HRMS [MH+] 139.0210 (C₇H₆OS req. 139.0212).

3.5. Reference compound

(Z)-Thiobenzaldehyde S-oxide (I) has potent lachrymatory properties and appropriate precautions should be taken during its synthesis. Phenylmethanesulfinyl chloride was synthesized from dibenzyl disulfide (Aldrich) and thionyl chloride (Youn and Herrmann, 1986). Thionyl chloride and triethylamine were freshly distilled before use. Crude (Z)-thiobenzaldehyde S-oxide was prepared from phenylmethanesulfinyl chloride according to the method of Block et al. (1996) for synthesis of propionethial S-oxide. Pure I was obtained by prep. C-8 HPLC employing the conditions described above.

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References


