Available online at www.sciencedirect.com
SCIENCE DIRECT®
PERGAMON
Phytochemistry 63 (2003) 37–40
PHYTOCHEMISTRY
www.elsevier.com/locate/phytochem

The lachrymatory principle of *Petiveria alliacea*

Roman Kubec[1], Seokwon Kim, Rabi A. Musah*

Department of Chemistry, State University of New York at Albany, Albany, NY 12222, USA

Received 24 September 2002; received in revised form 17 December 2002

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.

Keywords: *Petiveria alliacea*; Phytolaccaceae; Lachrymator; Sulfine; Thiobenzaldehyde *S*-oxide

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 thiosulfinates that are enzymatically formed from these amino acids upon disruption of the tissue of *P. alliacea*. As we proposed, these thiosulfinates 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 thiosulfinates 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 Et_2O. The extract was carefully concentrated and analyzed by C-8 HPLC. Along with the three benzyl-containing thiosulfinates 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 ^{1}H 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). ^{13}C NMR spectroscopy showed the presence of five magnetically non-equivalent carbon

* Corresponding author. Tel.: +1-518-437-3740; fax: +1-518-437-3741.
E-mail address: musah@csc.albany.edu (R.A. Musah).
[1] Current address: Department of Food Chemistry and Analysis, Institute of Chemical Technology, Technická 1905, Prague 6, 166 29, Czech Republic.

0031-9422/03/$ - see front matter © 2003 Elsevier Science Ltd. All rights reserved.
PII: S0031-9422(02)00759-8

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_7H_6OS (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 ^1H NMR spectroscopy. As shown by Barbaro et al. (1990), the ^1H 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 ^1H 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 $\sim\delta$ 9.65 ppm, indicating that the (*E*)-stereisomer 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 $\sim$99.95/0.05 according to ^1H NMR spectroscopic analysis. To the best of our knowledge, **1** is only the 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 petiveriin (*S*-benzylcysteine sulfoxide) serves as its precursor. The lachrymatory factor of onion, thiopropanethial *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 (isoalliin). Unlike isoalliin, petiveriin cannot degrade into an α,β-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 $E/Z \sim 20$–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_2O 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-tetrathiane dioxide.

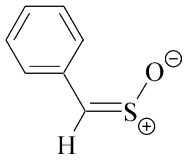

Fig. 1. The structure of **1**, (*Z*)-thiobenzaldehyde *S*-oxide.

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

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 discs containing the tested compound (20, 40, 100 or 200 μg) were evenly placed on agar plates that had been seeded with selected microorganisms. After 24 and 48 h incubation at 35 °C, the zones of inhibition surrounding the discs were measured. Discs with several common antibiotics were used as a control (Becton Dickinson Microbiology Systems). The following microorganisms were used: *Escherichia coli* (ATCC 25922), *Staphylococcus aureus aureus* (ATCC 25923), *Streptococcus agalactiae* (ATCC 13813), *Pseudomonas aeruginosa* (ATCC 27853), *Klebsiella pneumoniae pneumoniae* (ATCC 13883), *Stenotrophomonas maltophila* (ATCC 13637), and *Candida albicans* (ATCC 10231).

3.4. Isolation of the lachrymatory principle

The roots of *P. alliacea* (65 g) were mixed with H_2O (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 Et_2O (2×200 ml). Additional Et_2O (200 ml) was used for washing the filter cake. The Et_2O fractions were combined, evaporated (20 °C) and the yellow, odiferous residue was redissolved in 10 ml of MeCN. The extract was subjected to prep. C-8 HPLC using the following conditions: H_2O (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 $CHCl_3$ (2×100 ml). The chloroform fractions were combined, dried over $MgSO_4$ 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 **1**.

3.4.1. (Z)-Thiobenzaldehyde S-oxide (*1*)

A yellow, pungent oil; mp (uncorr.) 9–10 °C; UV λ_{max} (EtOH) nm (log ε): 206 (4.00), 230 (3.92), 310 (4.32); IR (neat) ν_{max} cm^{-1}: 3067 (*w*), 2999 (*m*), 1701 (*w*), 1489 (*w*), 1455 (*m*), 1175 (*m*), 1114 (*vs*), 940 (*m*), 793 (*m*), 751 (*m*), 687 (*s*); ^{1}H NMR (300 MHz; $CDCl_3$): δ 7.40–7.52 (3H, *m*, H-*meta*, H-*para*), 8.06–8.11 (2H, *dd*, J = 1.8, 8.1, H-*ortho*), 8.34 (1H, *s*, CH=S); ^{13}C NMR (75 MHz;

Table 1
Antimicrobial activity of thiobenzaldehyde *S*-oxide in the disk zone of inhibition assay[a,c]

Tested compound	Dose (μg/disc)	*Escherichia coli*[b]	*Staphylococcus aureus*[b]	*Streptococcus agalactiae*[b]	*Pseudomonas aeruginosa*[b]	*Klebsiella pneumoniae*[b]	*Stenotrophomonas maltophilia*[b]	*Candida albicans*[b]
1	20	–	–	–	–	–	–	10
	40	10	–	18	–	–	–	16
	100	12	12	20	–	12	–	np
	200	16	16	25	–	12	–	np
Gentamicin	10	26	22	20	16	14	20	np
Chloramphenicol	30	21	22	30	–	20	22	np
Tetracycline	30	25	24	32	14	22	14	np
Ampicillin	10	16	44	36	–	16	–	np
Amphotericin B	20	np	np	np	np	np	np	32

[a] –, No inhibition.
[b] Numbers represent diameter of zone of inhibition in mm.
[c] np, Not performed.

$CDCl_3$): 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 (49), 110 (35), 109 (52), 105 (20), 94 (26), 89 (31), 77 (36), 66 (42); MALDI-HRMS [MH^+] 139.0210 (C_7H_6OS req. 139.0212).

3.5. Reference compound

(*Z*)-Thiobenzaldehyde *S*-oxide (**1**) 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 propanethial *S*-oxide. Pure **1** was obtained by prep. C-8 HPLC employing the conditions described above.

Acknowledgements

The authors would like to thank Dr. Eric Block for helpful discussions and critical reading of the manuscript. The authors are also grateful to Dr. Gary Siuzdak at the Scripps Research Institute Center for Mass Spectrometry (La Jolla, CA) for technical support. Financial support for this work was provided by the Research Foundation of the State University of New York at Albany.

References

Barbaro, G., Battaglia, A., Giorgianni, P., Bonini, B.F., Maccagnani, G., Zani, P., 1990. Thioaldehyde *S*-oxide (monosubstituted sulfines) from thioacylsilane *S*-oxides. Synthesis and fluorine- and acid-induced *Z*/*E* isomerization. J. Org. Chem. 55, 3744–3748.

Block, E., 1981. New sulfine chemistry. In: Freidlina, R.K., Skorova, A.E. (Eds.), Organic Sulfur Chemistry. Pergamon Press, Oxford, pp. 15–34.

Block, E., Bayer, T., 1990. (*Z*,*Z*)-d,l-2,3-Dimethyl-1,4-butanedithial *S*,*S*′-dioxide: a novel biologically active organosulfur compound from onion. Formation of *vic*-disulfoxides in onion extracts. J. Am. Chem. Soc. 112, 4584–4585.

Block, E., Gillies, J.Z., Gillies, C.W., Bazzi, A.A., Putman, D., Revelle, L.K., Wang, D., Zhang, X., 1996. *Allium* chemistry: microwave spectroscopic identification, mechanism of formation, synthesis, and reactions of (*E*,*Z*)-propanethial *S*-oxide, the lachrymatory factor of the onion (*Allium cepa*). J. Am. Chem. Soc. 118, 7492–7501.

Bonini, B.F., Mazzanti, G., Zani, P., Maccagnani, G., Barbaro, G., Battaglia, A., Giorgianni, P., 1986. Synthesis and isolation of aryl thiobenzaldehyde *S*-oxides. J. Chem. Soc., Chem. Commun 964–965.

Hamid, A.M., Trippett, S., 1968. Some reactions of phenylsulphine and phenylsulphene. J. Chem. Soc. C 1612–1615.

Kubec, R., Musah, R.A., 2001. Cysteine sulfoxide derivatives in *Petiveria alliacea*. Phytochemistry 58, 981–985.

Kubec, R., Kim, S., Musah, R.A., 2002. S-Substituted cysteine derivatives and thiosulfinate formation in *Petiveria alliacea*—part II. Phytochemistry 61, 675–680.

National Committee for Clinical Laboratory Standards, 1990. Approved standard M2-A4. Performance Standards for Antimicrobial Disk Susceptibility Tests, fourth ed. Villanova, PA.

National Committee for Clinical Laboratory Standards, 2002. Performance Standards for Antimicrobial Disk Susceptibility Tests, seventh ed. Villanova, PA.

Ruiz, A., 1972. Clinical, Morphological, Histochemical and Clinical Pathological Studies of Anamú (*Petiveria alliacea*) Poisoning in Cattle. PhD thesis, Iowa State University, USA.

Youn, J.-H., Herrmann, R., 1986. A simple and efficient preparation of sulfinyl chlorides from disulfides and sulfuryl chloride. Tetrahedron Lett. 27, 1493–1494.

Zwanenburg, B., 1989. Sulfine chemistry. Phosphorus, Sulfur Silicon Relat. Elem 43, 1–24.

Zwanenburg, B., Damen, T.J.G., Philipse, H.J.F., De Laet, R.C., Lucassen, A.C.B., 1999. Advances in sulfine chemistry. Phosphorus, Sulfur Silicon Relat. Elem. 153–154, 119–136.