Toxicon 65 (2013) 5–8

Contents lists available at SciVerse ScienceDirect

Toxicon

journal homepage: www.elsevier.com/locate/toxicon

Short communication

A simple, rapid method for the extraction of whole fire ant venom (Insecta: Formicidae: *Solenopsis*)

Eduardo Gonçalves Paterson Fox [a,*], Daniel Russ Solis [b], Lucilene Delazari dos Santos [c], Jose Roberto Aparecido dos Santos Pinto [b], Anally Ribeiro da Silva Menegasso [b], Rafael Cardoso Maciel Costa Silva [a], Mario Sergio Palma [b], Odair Correa Bueno [b], Ednildo de Alcântara Machado [a]

[a] *Laboratório de Entomologia Médica e Molecular, Instituto de Biofísica Carlos Chagas Filho, Federal University of Rio de Janeiro (IBCCF/UFRJ), Rio de Janeiro, Brazil*
[b] *Instituto de Biociências, UNESP – Universidade Estadual Paulista, Campus de Rio Claro, Departamento de Biologia – Centro de Estudos de Insetos Sociais, SP, Brazil*
[c] *Center of Studies of Venoms and Venomous Animals (CEVAP), University of São Paulo State (UNESP), Botucatu, SP, Brazil*

ARTICLE INFO

Article history:
Received 12 June 2012
Received in revised form 19 October 2012
Accepted 13 December 2012
Available online 18 January 2013

Keywords:
Hymenoptera
Allergen
Toxin
Myrmecology
Chromatography

ABSTRACT

The invasive fire ant *Solenopsis invicta* is medically important because its venom is highly potent. However, almost nothing is known about fire ant venom proteins because obtaining even milligram-amounts of these proteins has been prohibitively challenging. We present a simple and fast method of obtaining whole venom compounds from large quantities of fire ants. For this, we separate the ants are from the nest soil, immerse them in dual-phase mixture of apolar organic solvent and water, and evaporate each solvent phase in separate. The remaining extract from the aqueous phase is largely made up of ant venom proteins. We confirmed this by using 2D gel electrophoresis while also demonstrating that our new approach yields the same proteins obtained by other authors using less efficient traditional methods.

Solenopsis fire ants are native to the Americas, with most of the species occurring in lower regions of South America (Tschinkel, 2006). The most notorious of these is *Solenopsis invicta* Buren which was introduced to Alabama, US in the early 20th century and has since then successfully invaded warm regions around the world including in the Galapagos, China, and Vietnam (Lofgren, 1986; Luo, 2005; Ascunce 2011) via commercial ships. This species is characterized by high population densities, aggressive behavior and a very potent sting. In the United States alone, more than 14 million people per year are stung by fire ants, as many as 100,000 of them seek medical attention (Apperson and Adams, 1983) and more than 80 people have died because of high sensitivity to compounds within the venom (deShazo et al., 1990; Stablein et al., 1985; Rhoades et al., 1989; Stafford, 1996; Prahlow and Barnard, 1989).

Fire ant venom includes a major (more than 95%) fraction of piperidinic alkaloids and a minor (less than 5%) aqueous fraction of allergenic proteins (e.g., Baer et al., 1979; Hoffman et al., 1988, 1990, 2005; Hoffman, 1993). The composition and toxic and antimicrobial properties of the piperidinic alkaloids are well described (Blum et al., 1958; Storey et al., 1991; Jouvenaz et al., 1972; Howell et al., 2005). However, almost nothing is known about the proteins. Indeed, only four proteins have been described in any detail (Hoffman, 1993; Tschinkel, 2006; King and Spangfort, 2000) out of an estimated total of over 40 fire ant venom proteins (Pinto et al., 2012). This is due to the difficulty of extracting amounts of proteins sufficient for proper purification and extensive characterization. Indeed,

* Corresponding author. Tel.: +55 21 2205 3314.
E-mail addresses: ofox@biof.ufrj.br, ofoxofox@gmail.com (E. Gonçalves Paterson Fox).

0041-0101
http://dx.doi.org/10.1016/j.toxicon.2012.12.009

methods of fire ant venom extraction described in the literature are extremely inefficient because they are based on "milking" the venom from individual ants (e.g., Padavattan et al., 2008). We propose here a novel venom protein extraction method that is simpler, faster and provides extraction yields orders of magnitude higher.

1. Ant collection & separation from nest soil

First, locate a fire ant nest in the field and shovel the upper portion of the mound into a bucket that was rimmed with Teflon paint. Following the methods described in Banks et al. (1981), separate the ants from the nest earth by slowly flooding the bucket with water (one drop every ~2 seconds). This takes several hours thus the extraction solution can be prepared in the mean time (below). Once completely flooded, the ants form a raft at the surface of the water (Banks et al., 1981).

2. Preparing the extraction solution

Obtain a clean glass recipient of appropriate size (e.g., 500 mL, depending on the amount of obtained ants). We recommend using a wide-mouth recipient (e.g., beaker or glass tumbler) rather than a narrow-mouth recipient (e.g., Erlenmeyer) because it is easier to put the ants inside in a single move. Add into the glass recipient a small quantity (ca. 1 mL per gram of ant) of distilled water or preferred buffer solution and a larger amount (ca. 5 mL per gram of ant) of a strong apolar solvent such as hexane (hexane was preferred because it is less volatile than ether or chloroform). The extraction mixture should clearly separate into two phases, and the volume of organic solvent should be enough to completely immerse the ants.

3. Venom extraction

Wearing protective rubber gloves, transfer the raft of floating ants into the extraction solution. Alternatively a cleaner extract can be obtained if the ants are first transferred into another recipient for several hours during which they dry and clean themselves. Transferring the ants requires utmost care, because accidents can result in escaped ants, stings and solvent spillage. When the ants enter the organic solvent, they instinctively discharge their venom while sinking – perhaps because of their aggressive nature – and rapidly die. These two phases are easily separated into individual tubes using pipettes or a separatory funnel (mind to use glass tubes for organic solvent). The tubes can be frozen at −20 °C for long-term storage. The upper, organic phase contains venom alkaloids and cuticular hydrocarbons. Venom alkaloids can be separated from the cuticular hydrocarbons by washing this organic phase with additional hexane through a silica column and then eluting the alkaloids with acetone (further described in Chen and Fadamiro 2009). The lower, aqueous phase contains water-soluble proteins. These proteins can be extracted by either precipitation, or lyophilizing this phase and resuspending it in a solution of preference. A video was produced illustrating the extraction procedure http://youtu.be/dWo-4uxpZK4; all steps are summarized in Fig. 1.

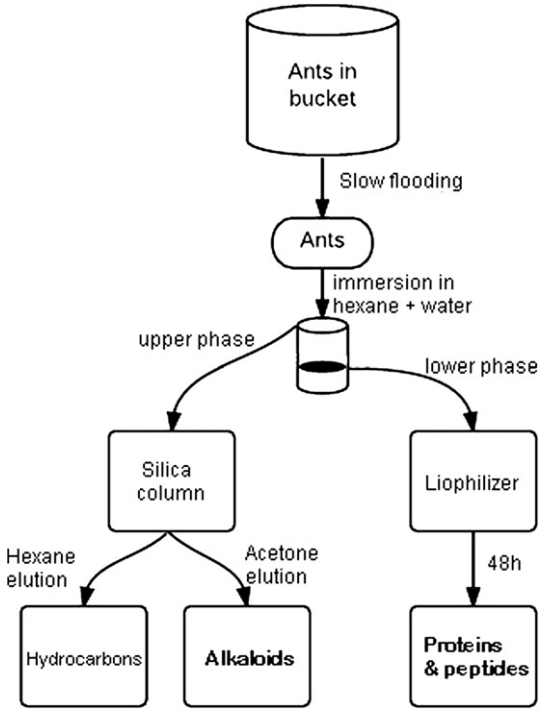

Fig. 1. Fluxogram illustrating the venom extraction steps described.

Supplementary video related to this article can be found at http://dx.doi.org/10.1016/j.toxicon.2012.12.009.

We performed the described extraction procedures on whole nests of *S. invicta* collected on the campus of the Federal University of Rio de Janeiro. Species identification followed Pitts et al. (2005) using the following diagnostic characters: absence of post-petiolar process, complete mandibular costulae, presence of a frontal medial streak, well developed median clypeal tooth, and males being distinctly black. Voucher specimens are deposited in the Adolph Hempel Entomological Collection of Instituto Biológico de Sao Paulo, SP, Brazil. Hexane was purchased from Merck. Protein quantification was made by the method of Bradford (1976), using bovine serum albumin as standard. The extracted venom alkaloids were air-dried and weighed using a digital precision scale (Bioprecisa FA – 2104N TDS Instrumental Tecnológico).

We estimated the number ants used based on their total wet weight (each fire ant weights on average 0.8 mg). We thus deduced that each ant yields approximately 10 μg of alkaloids and 50–100 ng of protein.

To compare the quality of extracted proteins with proteins obtained by other venom extraction methods, we prepared a bidimensional gel electrophoresis (2DE gel) using about 300 μg of putative protein from an aqueous phase extraction from *S. invicta*, and a 2DE gel of pure venom protein extract purchased from Vespa Labs Inc. (Spring Mills, PA, USA) (Fig. 2; also refer to Pinto et al., 2012). Gels were digitalized with a table scanner, and the software Adobe Photoshop CS was used to discard color information, normalize contrast between images, and number the obtained spots. The general patterns of the two

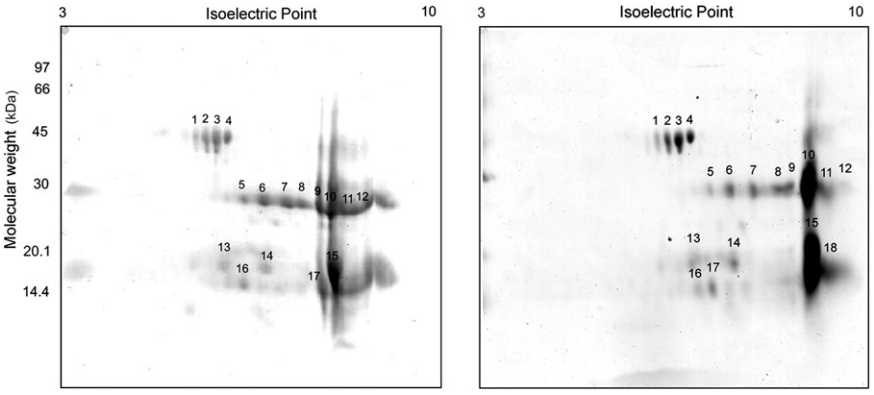

Fig. 2. Bidimensional gel electrophoresis of fire ant venom from *Solenopsis invicta*, as obtained by the methods herein described (left), and from the commercial extract of Vespa Labs, US (right).

2DE gels are clearly similar. Indeed, most proteins are found at similar isoelectric points vs. molecular size positions, and the number of obtained proteins was almost identical. Furthermore, preliminary results of mass spectrometry analysis of individual protein spots in similar positions showed no difference in protein content; the individual proteomic identifications of fire ant venom are dealt within a separate publication (Pinto et al., 2012).

The described method of venom extraction is rapid and inexpensive, and depends only on the ability of locating and handling fire ants and the necessary solvents. This method can likely be adapted for venom extraction from other aggressive hymenopterans (e.g., other ants, or cold-anesthetized bees and wasps). Furthermore, the protocol may be further revisited and optimized to increase the purity of each fraction and possibly replace the used solvents with environment-friendly alternatives (e.g., using ethanol or cold acetone). We hope that the presented method will encourage investigators to advance the study of venom proteins and peptides of fire ants and other venomous insects.

Acknowledgments

The present investigation was funded by grants from FAPESP, CNPq, and FAPERJ. We thank Miles Guralnik for technical information on the purchased venom sample, Sandra Fox Lloyd for assistance in obtaining and extracting fire ant colonies, and Daniela R. P. Fernandes, Diogo Gama dos Santos and Willy Jablonka for help making the accompanying video. We are indebted to Yannick Wurm for revising and proofreading the manuscript.

Conflict of interest

The authors declare that there are no conflicts of interest.

References

Apperson, C.S., Adams, C.T., 1983. Medical and agricultural importance of red imported fire ant. Florida Entomologist 66, 121–126.

Ascunce, M.S., Yang, C.-C., Oakey, J., Calcaterra, L., Wu, W.-J., Shih, C.-J., Goudet, J., Ross, K.J., Shoemaker, D.D., 2011. Global invasion history of the fire ant *Solenopsis invicta*. Science 331, 1066–1068.

Baer, H., Liu, T.Y., Anderson, M.C., Blum, M., Schmidt, W.H., James, F.J., 1979. Protein components of fire ant venom. Toxicon 17, 397–405.

Banks, W.A., Lofgren, C.S., Jouvenaz, D.P., Stringer, C.E., Bishop, P.M., Williams, D.F., Wojcik, D.P., Glancey, B.M., 1981. Techniques for Collecting, Rearing and Handling Imported Fire Ants. USDA. Scientific and Educational Administrative Advances in Agricultural Technology. AAT-S-21.

Blum, M.S., Walker, J.R., Callahan, P.S., Novak, A.F., 1958. Chemical, insecticidal, and antibiotic properties of fire ant venom. Science 128, 306–307.

Bradford, M.M., 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein–dye binding. Analytical Biochemistry 72, 248–254.

Chen, L., Fadamiro, H.Y., 2009. Re-investigation of venom chemistry of *Solenopsis* fire ants. I. Identification of novel alkaloids in *S. richteri*. Toxicon 53 (5), 469–478.

deShazo, R.D., Butcher, B.T., Banks, W.A., 1990. Reactions to stings of the red imported fire ant. New England Journal of Medicine 323, 462–466.

Hoffman, D.R., 1993. Allergens in Hymenoptera venom XXIV: the amino acid sequences of imported fire ant venom allergens Sol i II, Sol i III and Sol i IV. Journal of Allergy and Clinical Immunology 91, 71–78.

Hoffman, D.R., Dove, D.E., Jacobson, R.S., 1988. Allergens in Hymenoptera venom. XX. Isolation of four allergens from imported fire ant (*Solenopsis invicta*) venom. Journal of Allergy and Clinical Immunology 82, 818–827.

Hoffman, D.R., Smith, A.M., Schmidt, M., Moffitt, J.E., Guralnick, M., 1990. Allergens in Hymenoptera venom. XXII. Comparison of venoms from two species of imported fire ants, *Solenopsis invicta* and *richteri*. Journal of Allergy and Clinical Immunology 85, 988–996.

Hoffman, D.R., Sakell, R.H., Schmidt, M., 2005. Sol i 1, the phospholipase allergen of imported fire ant venom. Journal of Allergy and Clinical Immunology 115, 611–616.

Howell, G., Butler, J., DeShazo, R.D., Farley, J.M., Liu, H.L., Nanayakkara, N.P.D., Yates, A., Yi, G.B., Rockhold, R.W., 2005. Cardiodepressant and neurologic actions of *Solenopsis invicta* (imported fire ant) venom alkaloids. Annals of Allergy Asthma and Immunology 94, 380–386.

Jouvenaz, D.P., Blum, M.S., McConnell, J.G., 1972. Antibacterial activity of venom alkaloids from the imported fire ant, *Solenopsis invicta* Buren. Antimicrobial Agents Chemotherapy 2, 291–293.

King, T.P., Spangfort, M.D., 2000. Structure and biology of stinging insect venom allergens. International Archives of Allergy and Immunology 123 (2), 99–106.

Lofgren, C.S., 1986. The economic importance and control of imported fire ants in the United States. In: Vinson, S.B. (Ed.), Economic Impact and Control of Social Insects. Praeger, New York, pp. 227–256.

Luo, L.Z., 2005. Considerations and suggestions on managing the red imported fire ant, *Solenopsis invicta* Buren in China. Plant Protection 31, 25–28.

Padavattan, S., Schmidt, M., Hoffman, D.R., Markovit-Housley, Z., 2008. Crystal structure of the major allergen from fire ant venom, Sol i 3. Journal of Molecular Biology 383, 178–185.

Pinto, J.R.A.S., Fox, E.G.P., Santos, L.D., Menegasso, A.R.S., Costa-Manso, E., Machado, E.A., Bueno, O.C., Palma, M.S., 2012. Proteomic view of the venom from the fire ant *Solenopsis invicta* Buren. Journal of Proteome Research 11 (9), 4643–4653.

Pitts, J.P., McHugh, J.V., Ross, K.G., 2005. Cladistic analysis of the fire ants of the *Solenopsis saevissima* species-group (Hymenoptera: Formicidae). Zoologica Scripta 34 (5), 493–505.

Prahlow, J.A., Barnard, J.J., 1989. Fatal anaphylaxis due to fire ant stings. American Journal of Forensic Medicine and Pathology 19, 137–142.

Rhoades, R.B., Stafford, C.T., James, F.K., 1989. Survey of fatal anaphylactic reactions to imported fire ant stings. Journal of Allergy and Clinical Immunology 84, 159–162.

Stablein, J.J., Lockey, R.F., Hensel, A.E., 1985. Death from imported fire ant stings. Immunological Allergy Practice 7, 279–282.

Stafford, C.T., 1996. Hypersensitivity to fire ant venom. Annals of Allergy and Asthma Immunology 77, 87–95.

Storey, G.K., Vander Meer, R.K., Boucias, D.G., McCoy, C.W., 1991. Effect of the fire ant (*Solenopsis invicta*) venom alkaloids on the *in vitro* germination and development of selected entomogenous fungi. Journal of Invertebrate Pathology 58, 88–95.

Tschinkel, W.R., 2006. The Fire Ants. Belknap Press of Harvard University Press, Cambridge.