Method for the Determination of Veratridine and Cevadine, Major Components of the Natural Insecticide Sabadilla, in Lettuce and Cucumbers

Xuejun Zang,[†] Elaine K. Fukuda,[‡] and Joseph D. Rosen*,[†]

Department of Food Science and Center for Advanced Food Technology, Cook College, Rutgers University, New Brunswick, New Jersey 08903

Sabadilla, an insecticide widely used by organic farmers, can be separated into five of its components (veratridine, cevadine, cevine, cevacine, and sabadine) by high-performance liquid chromatography (HPLC) and detected by atmospheric pressure chemical ionization liquid chromatography mass spectrometry (APCI/LCMS). Using APCI/LCMS in the selected ion monitoring mode as the determinative step, an analytical method has been developed for the detection and quantitation of the two largest sabadilla components, veratridine and cevadine, in lettuce and cucumbers spiked with veratrine. The insecticides are extracted with acetonitrile/water, and the extract is cleaned up by solid phase extraction (SPE) on a C-18 cartridge. The limits of detection (calculated at a signal to noise ratio of 10 to 1) of veratridine and cevadine in cucumbers and lettuce are 1–2 ppb, and the recoveries range from 74% to 101%.

Keywords: *Organic farming; sabadilla; veratridine; cevadine; cevine; cevacine; sabadine; high-performance liquid chromatography (HPLC); atmospheric pressure chemical ionization liquid chromatography mass spectrometry (APCI/LCMS)*

INTRODUCTION

Sabadilla is a powder made by grinding seeds of *Schoenocaulon officinale*, or sabadilla lily, three species of which grow wild in the Andes Mountains in Mexico, Guatemala, and Venezuela. It has been used by the Incas and Aztecs to control lice, and it has been utilized by Europeans since the 18th century (Pleasant, 1991). In the United Sates, sabadilla insecticide is used to control insects on a variety of crops including, but not limited to, cucumbers, squash, beans, melons, potatoes, and cabbage. Three to six percent of sabadilla is a mixture of over thirty alkaloids which are the active ingredients of this pesticide. Its chief constitutents (shown in Figure 1) are cevadine, cevine, sabadine, veratridine (Mandava, 1985), and cevacine. These materials have attracted considerable clinical interest as possible hypotensive agents (Ellenhorn, 1988). Veratridine acts upon the sodium ion channels that mediate the electrical excitability of nerve, heart, and skeletal muscles (Catteral, 1980). If inhaled or ingested by mammals, the sabadilla alkaloids act on the cardiovascular system and affect respiration, nerve fibers, and skeletal muscles. The principal signs of intoxication are gastrointestinal symptoms and hypotension (Catterla, 1980). The LD_{50} (i.p.) values in mice for veratridine and cevadine are 1.35 and 3.5 mg/kg, respectively (*Merck Index*, 1989). Cevadine is known to be irritating to the mucous membranes, and serious poisoning has resulted from local application (*Merck Index*, 1989).

Partially as a result of a number of well-publicized scares about pesticide residues in fruits and vegetables in recent years, the organic food business has grown substantially. It appears to be almost an article of faith among consumer activist organizations that eating (the

Figure 1. Structures and molecular weights of the sabadilla alkaloids discussed in text.
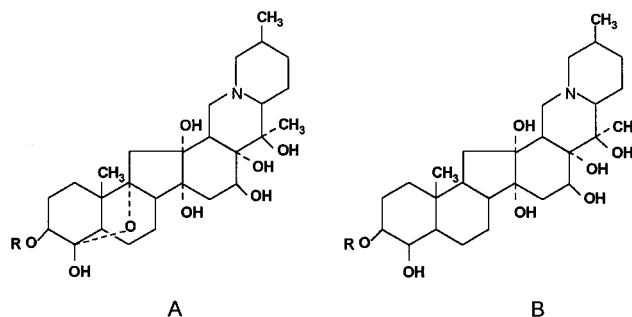
composition	structure	R	MW
veratridine	A	(3,4-dimethoxybenzoyl)	673
cevadine	A	(2-methylbut-2-enoyl)	591
cevine	A	H	509
cevacine	A	CH_3CO	551
sabadine	B	CH_3CO	537

two to three times more expensive) organic fruits and vegetables is safer than eating produce grown with synthetic pesticides. The public is for the most part unaware, however, that organic farmers do, in fact, use insecticides. Since these materials are of natural origin, it is automatically assumed that they are safe. Sabadilla, for one, has been used since before World War II (Crosby, 1971) with (to the best of our knowledge) no reported ill effects. However, it has never been adequately tested for chronic toxicity using currently

* Corresponding author [telephone (908) 932-9611, extension 229; fax (908) 932-6776].
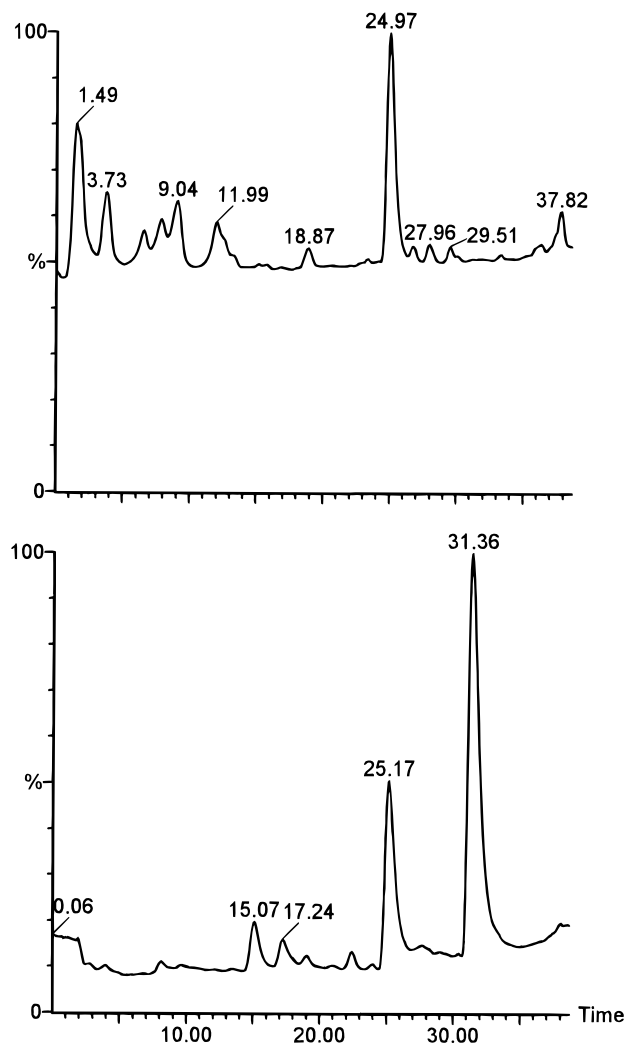† Department of Food Science.
‡ Center for Advanced Food Technology.

Determination of Veratridine and Cevadine

J. Agric. Food Chem., Vol. 45, No. 5, 1997 **1759**

Figure 2. HPLC chromatogram of commerical sabadilla pesticide extract with UV detection at 268 nm (upper trace) and detection by APCI (lower trace). Cevadine, veratridine, sabadine, cevacine, and cevine are at 31.4, 25.2, 17.2, 15.1, and 7.6 min, respectively.

Figure 3. APCI mass spectra of (from top to bottom) cevadine, veratridine, sabadine, cevacine, and cevine.

approved toxicological methods. No data are available on its carcinogenicity, teratogenicity, mutagenicity, or its possibly more deleterious effects on children. Furthermore, the chemical structure of the sabadilla alkaloids suggests that they may possess estrogenic activity. To make matters worse, there is no specified legal limit for its use, and even if there was, no adequate analytical method for the determination of sabadilla is available. This report describes newly developed methodology for the determination of the two main sabadilla alkaloids in lettuce and cucumbers spiked with veratrine.

MATERIALS AND METHODS

Chemicals. Veratrine, a mixture of alkaloids consisting of 38% veratridine, 59% cevadine, and 3% other alkaloids, was purchased from Sigma Chemical Company, St. Louis, MO. Sabadilla pesticide (containing 0.8% sabadilla alkaloids) was purchased from Necessary Trading Co., New Castle, VA. Water, acetonitrile, and methanol (Optima Grade) were purchased from Fisher Scientific, Springfield, NJ, as were caffeine and ammonium acetate.

Instrumentation. *APCI-LCMS Conditions.* Chromatography was carried out using a Varian 9012 Solvent Delivery System equipped with a Varian 9050 UV/vis detector (set at 268 nm) and interfaced to a Micromass Platform II mass spectrometer. The HPLC column was a Supelco Rx-C 18 (4.6
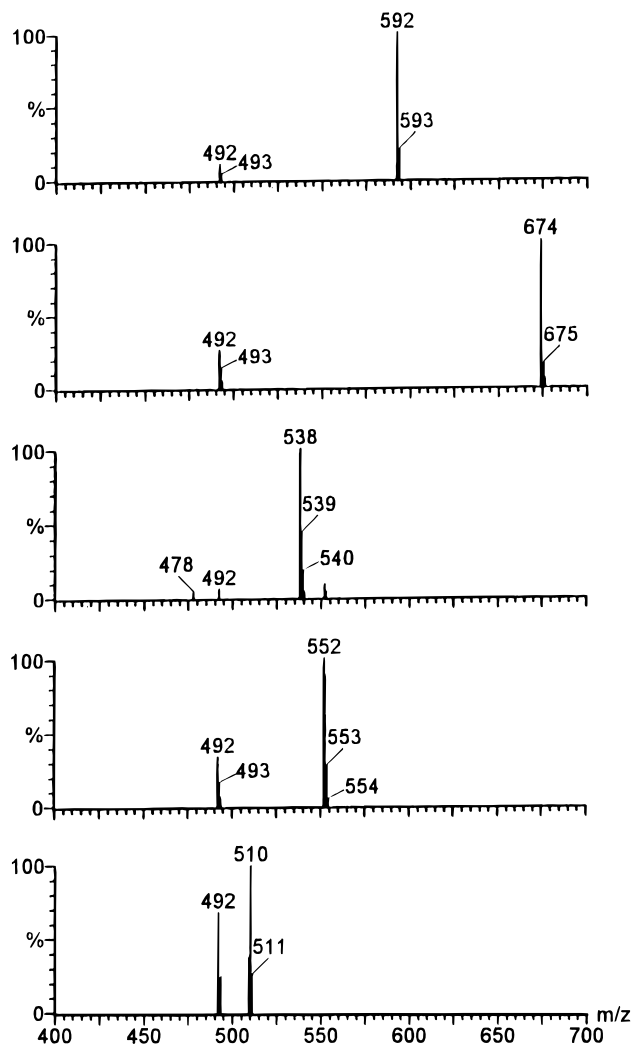
mm × 250 mm). Source and nebulizer temperature were 150 and 400 °C, respectively. The flow rates of the nitrogen drying gas and the nitrogen APCI sheath gas were 240 and 100 L/h, respectively. The mass spectrometer was operated in the APCI positive ion mode, with ion sampling cone voltage at 25 V. The scan range was 170−700 amu for determination of sabadilla components; for quantification in vegetables, selected ion monitoring at m/z 195, 674, and 592 was used. For separation of sabadilla pesticide, the initial mobile phase composition was methanol, acetonitrile, and 0.01 M ammonium acetate (20:20:60), programmed linearly to 20% methanol, 50% acetonitrile, and 30% 0.01 M ammonium acetate in 30 min and to 15% methanol, 80% acetonitrile, and 5% 0.01 M ammonium acetate in 40 min. For vegetable extracts the initial mobile phase composition of methanol/0.01 M ammonium acetate (10:90) was programmed linearly to 40% methanol and 60% 0.01 M ammonium acetate in 10 min, to 60:40 in 20 min, and to 95:5 in 50 min. The flow rate was 0.9 mL/min for both systems. A different elution profile was employed for the vegetable extracts in order to obtain a symmetrical peak shape for the internal standard, caffeine.

HPLC/MS Determination of Sabadilla Pesticide. Three grams of commercial pesticide was dissolved in 250 mL of acetonitrile and sonicated for 15 min. The solution was filtered and concentrated under reduced pressure to 50 mL. A 500 μL aliquot was transferred to a vial and evaporated to dryness under a gentle stream of nitrogen gas at room temperature before being redissolved in 500 μL of methanol. 100 μL of the extract was injected into the HPLC/MS system.

Determination of Percent Recovery. Recoveries were performed three separate times for each vegetable. To 50 g
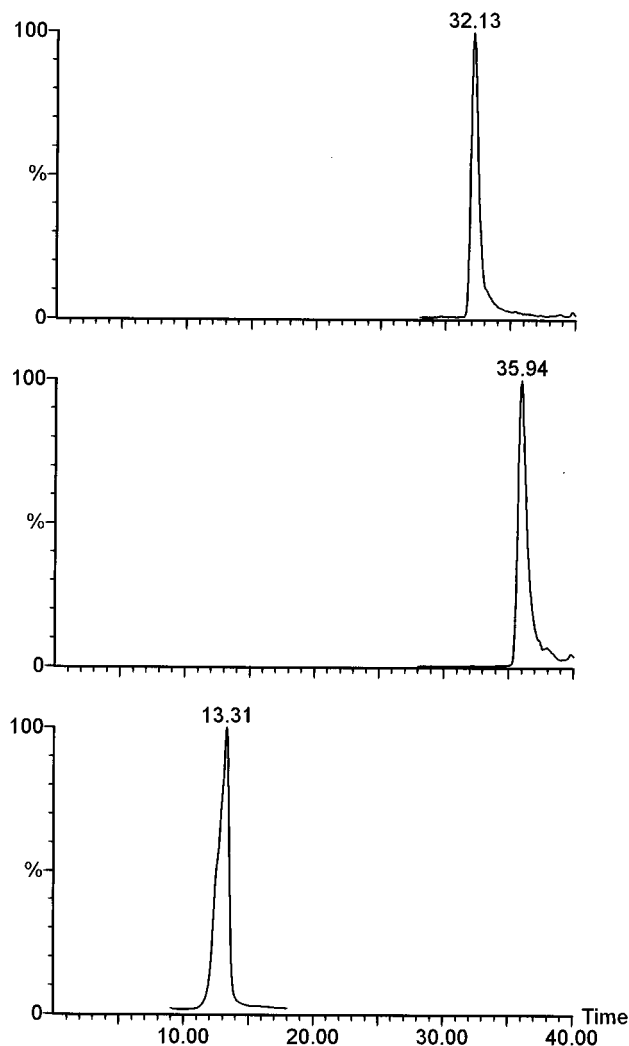
Figure 4. Detection of cevadine (upper trace) and veratridine (middle trace) in lettuce spiked at the 59 and 38 ppb levels, respectively. The lower trace is from the internal standard, caffeine.
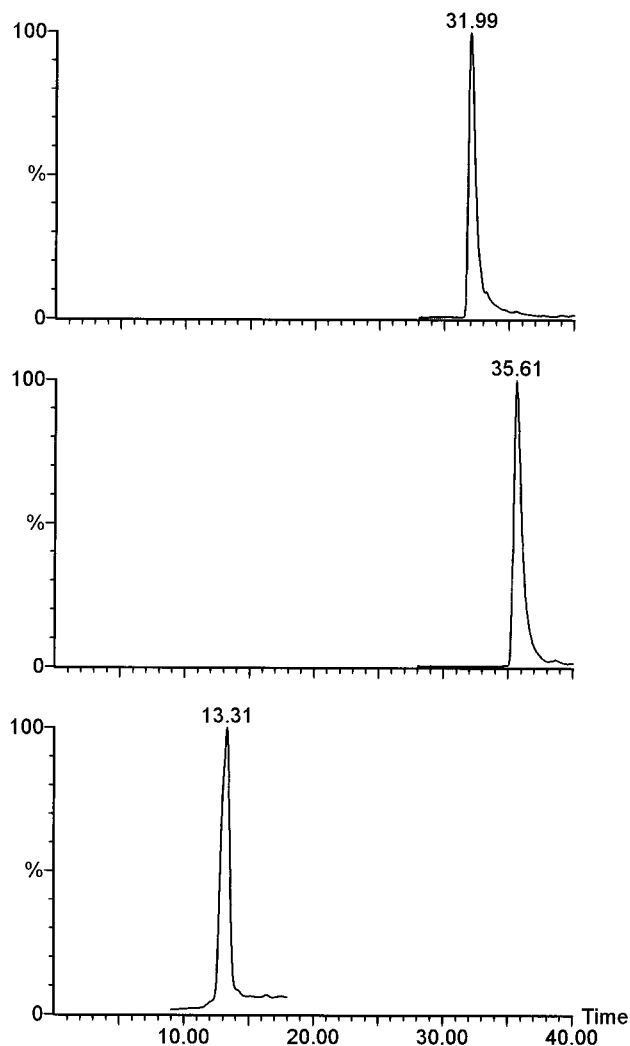

Figure 5. Detection of cevadine (upper trace) and veratridine (middle trace) in cucumbers spiked at 59 and 38 ppb, respectively. The lower trace is from the internal standard, caffeine.

of chopped vegetable (lettuce or cucumber) was added 50 μL of a 0.1 mg/mL solution of veratrine in methanol, covering the vegetable as equally as possible. The syringe was rinsed with methanol, and the rinsings were added to the vegetable. The spiked vegetable was allowed to stand for 30 min and then homogenized with 10 mL of water and 90 mL of acetonitrile in an explosion-proof blender for 1 min at low speed. The mixture was filtered, and the filtrate volume was measured. One-fifth of the filtrate (by volume) was evaporated under reduced pressure to remove all of the acetonitrile. A Supelco Envi-18 SPE 6 mL cartridge was placed on a vacuum manifold and conditioned first with 6 mL of methanol and then with 6 mL of water. The vegetable extract was loaded onto the cartridge and passed through at a flow rate of 1−2 mL/min. The cartridge was washed with 6 mL of water and dried under vacuum for 5 min. Afterward, the cartridge was eluted with 3 mL of methanol, and the eluate was dried under a gentle stream of nitrogen. 4 μL of internal standard stock solution (250 ng/μL of caffeine) was added to the final concentrate, and the volume was adjusted to 1 mL with methanol. 100 μL of the methanol solution was injected into the HPLC.

Determination of Limit of Detection. The above procedure was followed except that 50 g of vegetable was spiked with 5 μL of standard veratrine solution to fortify at the 10 ppb level (6 ppb cevadine and 4 ppb veratridine). Limits of detection were calculated using a signal to noise ratio of 10 obtained by comparison of the intensities of the selected ion monitoring peaks of cevadine and veratridine to the highest intensity of the noise either just before or just after the peak.

RESULTS AND DISCUSSION

The alkaloids in sabadilla are difficult to quantify by usual methods. They do not lend themselves to gas chromatography (GC) because of multiple hydroxyl groups and high molecular weight. In addition, lack of a strong ultraviolet chromophores (except for veratridine) makes it difficult to determine them with UV detectors. Zeitler *et al.* (1965) and Rucker (1976) developed several thin-layer chromatographic systems to isolate and analyze the alkaloids. Unfortunately, some alkaloids are somewhat unstable under the conditions required to achieve separation, thus making quantification difficult. Svoboda (1962) used countercurrent distribution in the quantitative determination of veratridine and other alkaloids in commercial veratrine. The percent veratridine found ranged from 28.4 to 31.0%. Ristic *et al.* (1986) used fluorometric methods (305 nm excitation wavelength and 365 nm emission wavelength) and determined that the average content of veratridine was 35.9%. Holan *et al.* (1984) demonstrated that the HPLC technique is applicable to some of the sabadilla alkaloids. The content of veratridine in commercial veratrine was found to be manufacturer-dependent and ranged from 23% to 41%. Methods for the detection of veratridine and cevadine by HPLC/UV have been published (Holan, 1984; Reed, 1986; Hare, 1996). However, because of the poor specificity of UV
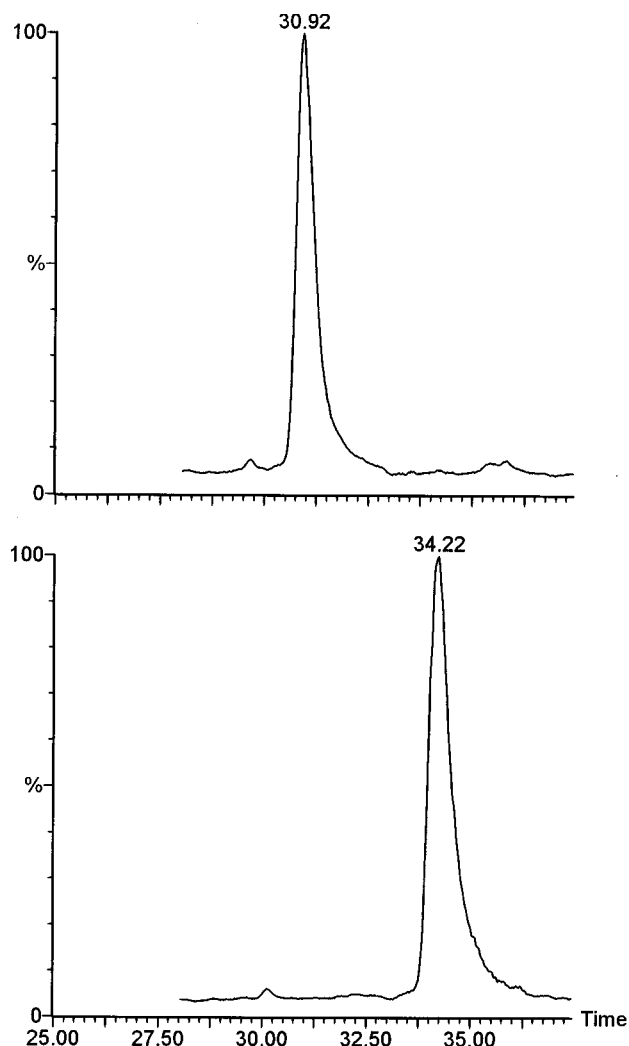
Determination of Veratridine and Cevadine

J. Agric. Food Chem., Vol. 45, No. 5, 1997 **1761**

Figure 6. Detection of cevadine (upper trace) and veratridine (lower trace) in lettuce spiked at 6 and 4 ppb, respectively.

absorption, these methods cannot be used to determine residues in food. In our study, we investigated the use of APCI/LCMS for the determination of these alkaloids.

Figure 2 (upper trace) is the chromatogram of the extract from the commercial sabadilla pesticide with UV detection at 268 nm. Veratridine is the only material observed because the other sabadilla components have very low absorption at this wavelength. Figure 2 (lower trace) shows the same chromatogram with total ion current detection. The mixture is separable into at leave five components, cevadine, veratridine, sabadine, cevacine, and cevine, at 31.4, 25.2, 17.2, 15.1, and 7.6 min, respectively. The cevine component is present at too low a concentration to be seen by total ion current detection but can be detected by mass chromatography. Figure 3 shows the mass spectra of cevadine, M + 1 592; veratridine, M + 1 674; sabadine, M + 1 538; cevacine, M + 1 552; and cevine, M + 1 510. All of the alkaloids except sabadine exhibit m/z 492, an ion which arises from the loss of the ester group. Sabadine exhibits a corresponding ion at m/z 478. (Note: The ion at m/z 492 in the sabadine mass spectrum is from background.) Figures 4 and 5 show the selected ion

monitoring determinations of veratridine and cevadine in lettuce and cucumbers spiked with veratrine at the 100 ppb level, respectively. Since veratrine is a mixture of alkaloids containing, according to the supplier, 38% veratridine and 59% cevadine, these recovery studies were performed at 38 and 59 ppb, respectively, for the two alkaloids. In three replicated determinations, veratridine was recovered at 81% ± 1% from lettuce and 74% ± 3% from cucumbers while cevadine was recovered at 96% ± 2% from lettuce and 101% ± 4% from cucumbers. Figure 6 shows the selected ion monitoring determinations for veratridine (4 ppb) and cevadine (6 ppb) in lettuce. The selected ion chromatograms in cucumbers (not shown) were essentially the same. Calculated limits of detection at a signal to noise ratio of 10:1 were 1−2 ppb.

Since the methodology for the detection of the sabadilla alkaloids in food is no longer such a formidable task, perhaps residue information might be provided to organic produce customers.

LITERATURE CITED

Catteral, W. A. Neurotoxins that act on voltage sensitive sodium channels in excitable membranes. *Annu. Rev. Pharmacol. Toxicol.* **1980**, *20*, 15−43.

Crosby, D. G. Minor Insecticides of Plant Origin. In *Naturally Occurring Insecticides*; Jacobson, M., Crosby, D. G., Eds.; Marcel Decker: New York, 1971; Chapter 5.

Ellenhorn, M. J.; Barceloux, D. G. *Medical Toxicology: Diagnosis and Treatment of Human Poisoning*; Elsevier: New York, 1988; p 1281.

Hare, J. D. Purification and quantitative analysis of veratridine and cevadine by HPLC. *J. Agric. Food Chem.* **1996**, *44*, 149−152.

Holan, G.; Johnson, W. P. M.; Rihs, K. Separation of veratridine using high-performance liquid chromatography or droplet countercurrent chromatography. *J. Chromatogr.* **1984**, *288*, 479−483.

Mandava, N. B. *CRC Handbook of Natural Pesticides*; CRC Press: Boca Raton, FL, 1985; Vol. 2, p 10.

Merck Index, 11th ed.; Merck & Co., Inc.: Rahway, NJ, 1989.

Pleasant, B. The return of an old insect killer. *Org. Gardening* **1991**, *39*, 52−53.

Reed, J. K.; Gerrie, J.; Reed, K. L. Purification of veratridine from veratrine using high-performance liquid chromatography. *J. Chromatogr.* **1986**, *356*, 450−454.

Ristic, L. Z.; Buric, I. D.; Draskovic, B. J.; Milovanovic, G. A. Photoluminescence of veratridine and its fluorometric determination. *Microchem. J.* **1987**, *35*, 196−200.

Rucker, G.; Taha, A. The use of p-acceptor for determination of alkaloids on thin layers. *J. Chromatogr.* **1977**, *132*, 165−167.

Svoboda, G. R. Use of countercurrent distribution in the quantitative determination of the alkaloids of commerical veratrine. *Anal. Chem.* **1962**, *34*, 1559−1562.

Zeitler, H. J. Separation of veratrum alkaloids by thin-layer chromatography. *J. Chromatogr.* **1965**, *18*, 180−183.