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## Note

# Purification of veratridine from veratrine using high-performance liquid chromatography

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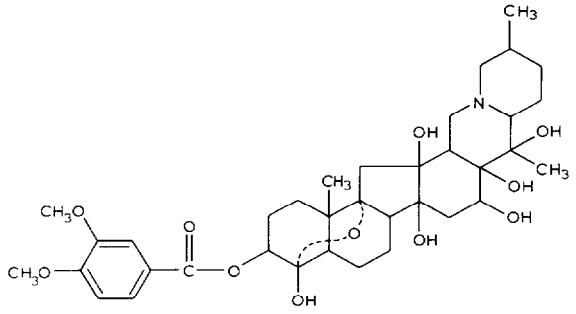
The veratrum alkaloids constitute an abundant group of steroid-like polycyclic nitrogen-containing ring structures found in liliaceous plants. The alkaloids in two veratrum species, *Veratrum album*, Linn, and *Veratrum viride*, Aiton, and in the species *Schoenocaulon officinale*, Gray, have been perhaps the best characterized although as many as 20 different alkaloids have been identified to date[1,2]. These molecules have a particularly rich pharmacology and have been the focus of considerable clinical interest as possible hypotensive agents[1–3]. Unfortunately, some of the effects have been difficult to interpret particularly where mixtures of the alkaloids have been used.

Veratridine (I), one of the major alkaloid components of veratrine which is extracted from the seeds of *Schoenocaulon officinale*, the "Sabadilla seeds", has been widely used as a neuropharmacological tool to study the electrical properties of nerve and muscle fibres[4–6]. Its ability to depolarize cells by altering the membrane-associated sodium channels is now well documented[4].




I

We have found commercial veratridine, when available, to vary substantially in purity. Veratrine, while readily available from a number of suppliers, is a mixture

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of alkaloids and without further purification is generally unsuitable for biological studies.

Recently, Holan *et al.*[7] have reported the separation of up to 1 g of veratrine by preparative droplet counter-current chromatography. Unfortunately this rather specialized procedure is not widely used and is therefore not a very practical method for routinely isolating veratridine for most biochemical, pharmacological or physiological studies.

In this paper, we describe a rapid purification of the major veratrum alkaloids in veratrine using a simple one-step semi-preparative reversed-phase high-performance liquid chromatographic (HPLC) procedure. The use of a volatile solvent system allows recovery by lyophilization of individual species, one of which is veratridine.

## EXPERIMENTAL

### *High-performance liquid chromatography*

Chromatography was carried out using a Varian Model LC8500 chromatographic system equipped with a Valco universal injector valve. The sample loop volume was 50 $\mu$l. The detector unit was a Varian Varichrom UV detector set at 220 nm. Separation of the veratrine alkaloids was performed at ambient temperature on a 50 × 0.94 cm I.D. Whatman Partisil M9/$C_8$ reversed-phase semi-preparative column (10 $\mu$m particle size). Analytical HPLC was performed on a 30 × 0.39 cm I.D. Waters $\mu$Bondapak $C_{18}$ reversed-phase analytical column. The mobile phase was methanol–0.1 *M* ammonium acetate, pH 5.5 (60:40). Prior to use the solution was filtered and then de-gassed by bubbling helium through the mixture. Absorption spectra were recorded on a Cary 210 spectrometer. Veratridine was identified by mass spectrometry with a Finnigan MAT-44/S gas chromatograph–mass spectrometer using a direct injection probe. The mass spectra were obtained by electron impact (EI) with an electron energy of 70 eV and an ionization current of 80 mA.

### *Sample preparation*

Veratrine (K and K Labs. or Sigma) was dissolved in water acidified with glacial acetic acid. The solution (500 mg/ml) was either filtered or centrifuged (5 min) at 2600 *g* to remove any insoluble material. The material was dried under nitrogen and reconstituted in mobile phase (1 g original weight/ml) prior to column application.

## RESULTS AND DISCUSSION

The separation of veratridine from the mixture of veratrine alkaloids was first described by Wright and Luff in 1878[8] and subsequently modified in 1935 by Blount[9]. These methods basically involved solubilizing the nitrate salt of veratridine and repeatedly precipitating the insoluble sulfate form. We found using HPLC analysis that the final "pure" veratridine was in fact highly contaminated. In addition, the procedure was tedius and yields were generally unsatisfactory.

Fig. 1 shows a typical chromatographic separation of the crude veratrine on the M9/$C_8$ reversed-phase column using the methanol–ammonium acetate mobile phase. As expected, the mixture is highly heterogeneous with three major peaks and numerous minor components. The second major peak (2) with a retention time of 21 min was subsequently identified as veratridine. This peak was collected, lyophilized,

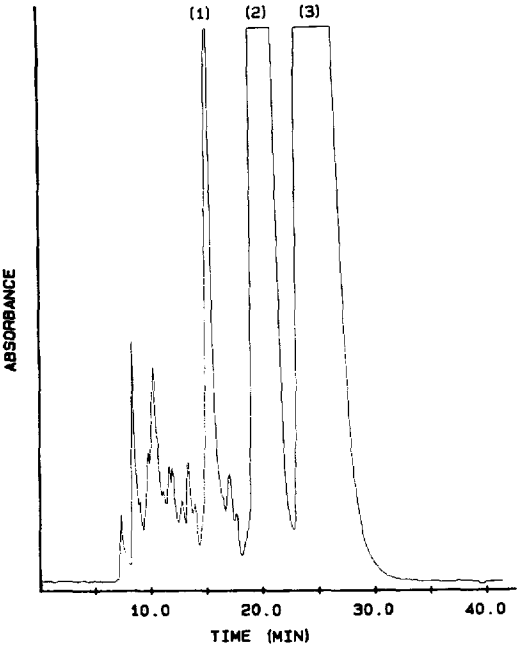


Fig. 1. HPLC chromatogram (50 × 0.94 cm I.D.) Whatman Partisil M9/$C_8$ column) showing a typical separation of the components of crude veratrine. Sample, 20 mg dissolved in 50 μl of methanol–0.1 *M* ammonium acetate, pH 5.5 (60:40); flow-rate, 3 ml/min; ambient temperature; detector, 220 nm. (1), (3) unknown compounds; (2) veratridine.

redissolved in mobile phase and subjected to analysis on the $C_{18}$ reversed-phase analytical column (Fig. 2). The material eluted as a major peak (98.4%) with minor impurities. The recovery from this single chromatographic separation on the $C_8$ column was approximately 71% of the expected yield. This is based on the absorbance

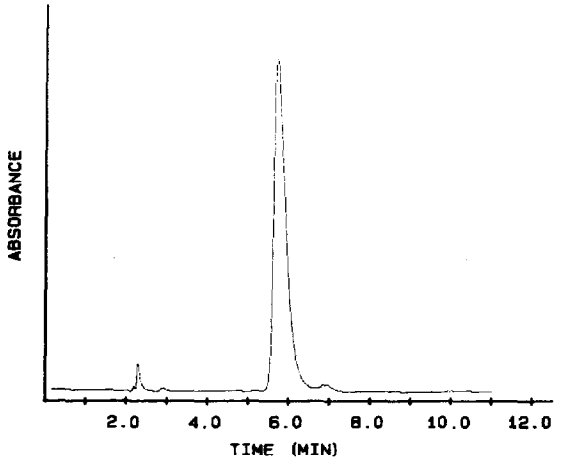


Fig. 2. Analytical HPLC profile (30 × 0.39 cm I.D. Waters $C_{18}$ μBondapak column) of recovered fraction (2) from Fig. 1. 50 μl sample; flow-rate, 2 ml/min; detector, 220 nm.

at 292 nm as described below. Rechromatography of the peak resulted in material that was >99% pure although the recovery was only about 48% of the expected yield.

The relative amount of veratridine in the crude veratrine mixture was best determined by absorbance. Fig. 3 shows the absorption spectrum of veratrine and the rechromatographed veratridine. The long wavelength band at 292 nm is a characteristic electronic transition of aromatic rings. None of the other components showed this band, an observation consistent with the fact that veratridine is unique among the veratrine alkaloids identified to date in that it is the only one which is an ester of the aromatic veratric acid.

The molar extinction coefficient at 292 nm for veratridine (acetate salt) was found to be $11.5 \cdot 10^3\ M^{-1}\ cm^{-1}$. Using this value, it is estimated that crude veratrine contains approximately 21% (w/w) veratridine although again, this varies with the lot. We have not identified the other species.

Mass spectral analysis of the second peak (Fig. 1) also confirms its identity as veratridine. Fig. 4 shows the high mass spectral scan with an apparent molecular ion at $m/z$ 673 which corresponds to the molecular weight of veratridine. The base peak at $m/z$ 491 corresponds to a mass fragment of the parent compound less the veratric acid. This is the dehydrated nitrogen containing base, cevine.

Veratridine, unlike the other components was also found to be highly fluores-

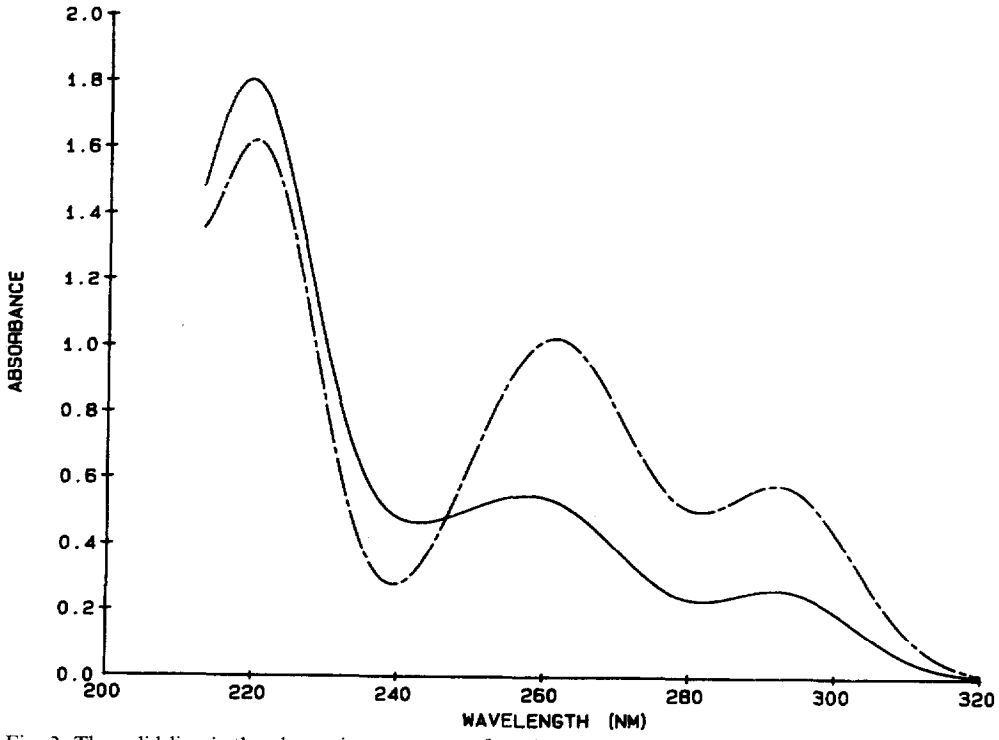


Fig. 3. The solid line is the absorption spectrum of crude veratrine (147 μg/ml). The broken line is the absorption spectrum of rechromatographed veratridine in dilute acetic acid (64 μg/ml).

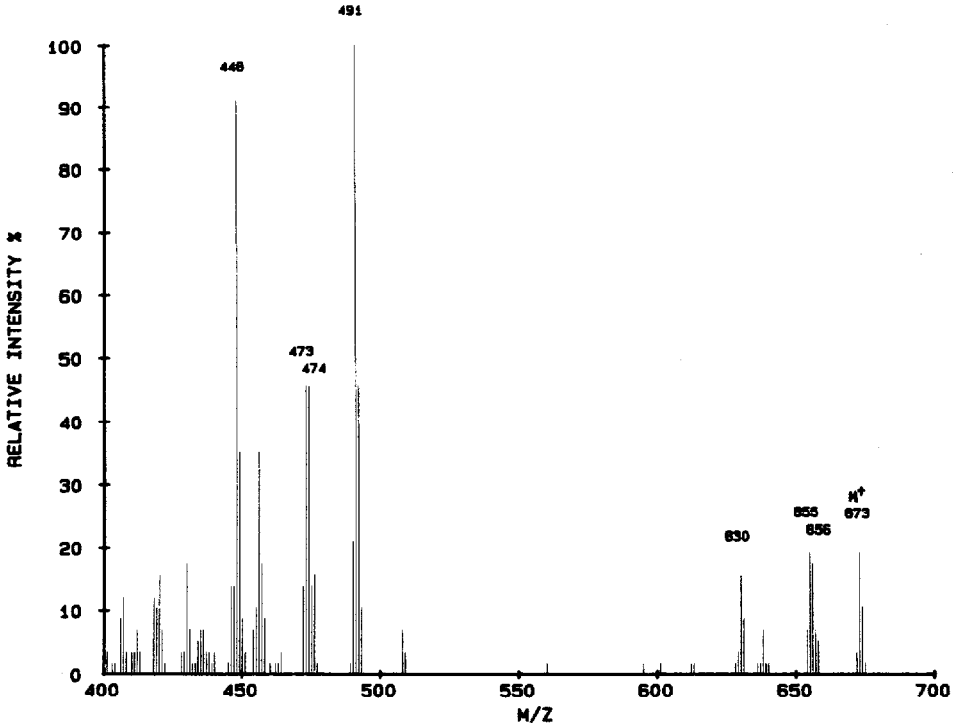


Fig. 4. High mass EI spectral scan of purified veratridine obtained using a direct injection probe.

cent, with a broad structureless emission band at 366 nm and a small shoulder at 393 nm.

The veratridine isolated by this procedure is pharmacologically active and has been shown to prolong the open state of the sodium channel in cultured pheochromocytoma (PC12) cells[10], a cell line of neuronal origin. The activation constant ($K_{0.5}$) is 14 $\mu M$ a value in the lower range of previously published values for veratridine[6].

The chromatographic procedure outlined here can readily accomodate up to 50 mg of the veratrine extract, thus yielding approximately 8 mg of veratridine in a single step. With multiple applications, the quantities of veratridine obtained are more than sufficient for most *in vitro* pharmacological studies.

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