SYNTHESIS OF TRITIUM LABELED VERATRIDINE WITH HIGH SPECIFIC ACTIVITY

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SUMMARY

Synthesis of veratridine labeled with tritium on the aromatic residue at position 5' is described. First, a precursor bromoveratridine, 3-(5-bromoveratroyl) veracevine, was prepared which was converted into the labeled veratridine by catalytic dehalogenation employing tritium gas. Labeling was done in the last step of the synthesis so that handling of labeled intermediates could be avoided. Veratridine was obtained with 4.48 Ci/mmole specific activity and with 97% radiochemical purity.

Key Words: veratridine, veratrine, ^{3}H-labeled synthesis

INTRODUCTION

Veratridine is a naturally occurring plant alkaloid of steroidal structure, isolated from plants of suborder Melanthaceae of the family Liliaceae, and has been found to depolarize excitable membranes by increasing Na^{+} permeability (1). The sources, chemistry, pharmacology and therapeutic uses of veratrum alkaloids have been extensively reviewed (2,3,4). Extensive studies have been performed on the veratrum alkaloids for their hypotensive effects, and have shown veratridine to be a useful tool for studying the mechanism by which sodium ion permeability is controlled in excitable tissues. In order to study the binding activity with nerve preparations, tritium labeled varatridine of high specific activity is needed. Previous attempts of labeling using the Wilzbach method (5) have resulted in specific activities 500 mCi/mmole (6); however, materials of higher specific activity is necessary to achieve the degree of sensitivity required for such studies. In the present paper, we report 4.48 Ci/mmole specific activity which is about ten times higher than reported previously.

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MATERIALS AND METHODS

Veratrine and 5-bromovanillin were purchased from Aldrich Chemical Co. Melting points were determined on a Fischer-Johns melting point apparatus. IR spectra were recorded on Perkin-Elmer grating infrared spectrophotometer model 257 in chloroform solution. UV spectra were recorded on Beckman spectrophotometer. NMR spectra were obtained on a 60 MHz Varian spectrometer. Mass spectrum was taken on an AEl-MS-902 mass spectrometer. All radioactivity measurements were carried out on a Tri-carb Model 3375 (Packard) scintillation counter. Preparative tlc was done on alumina GF (500 micron) precoated plates using cyclohexane-chloroform-methanol-triethylamine (16:8:1:1) as solvent system.

(A) Preparation of Veracevine (5) from Commercial Veratrine.

A solution of 7 g of veratrine in 150 ml of methanol was chilled to 5° and then 6.2 ml of 5N sodium hydroxide was added drop by drop. The brownish solution was stirred at 0° for 24 hours and diluted with 250 ml of ice-cooled water and extracted quickly several times (5-6 times) with chloroform. The chloroform extract was dried with anhydrous sodium sulfate. The residue obtained from concentration of the chloroform extract was dissolved in 100 ml of boiling ether. Veracevine (3.7 g) crystalized out rapidly with m.p. 182° (Lit. 180-182°). The m.p., infrared spectra and chromatographic behavior of the prepared veracevine were identical with the authentic veracevine.

(B) Preparation of 5-bromoveratroyl Chloride. (4)

(i) 5-bromoveratraldehyde (2) was prepared by methylation of 5-bromovanillin (1) by the method of Jones and Robinson (7).

(ii) 5-bromoveratric acid (3). 5-bromoveratraldehyde 1.23 g in acetone (100 ml) was treated with potassium permanganate in aqueous acetone (1:1 v/v) with stirring at room temperature until tlc indicated complete reaction. 50 ml of water was added, acetone was removed under reduced pressure, and sulphur dioxide was bubbled through the solution to remove manganese dioxide. The white precipitate was filtered off and treated with aqueous sodium hydrogen carbonate. This solution was extracted with chloroform and these extracts were discarded. The aqueous solution was acidified with concentrated hydrochloric acid, precipitating the bromoveratric acid 1.3 g (99%) m.p. 192-193°.

(iii) 5-bromoveratroyl chloride (4). 2 g of 5-bromoveratric acid was refluxed with 6 ml of thionyl chloride for four hours. At this time the thionyl chloride was removed by evaporation on a rotary evaporator. Sublimation of the resulting solid (65°C, 0.1 mm Hg) gave 1.7 g (88.8%) of 5-bromoveratroyl chloride with m.p. 75-76° (Lit. 70-75°).

(C) Preparation of Bromoveratridine. (6).

Veracevine 255 mg (0.5 mmole) was dissolved in 0.5 ml of pyridine and this stirred solution was treated with 5-bromoveratroyl chloride 140 mg (0.5 mmole) in 0.5 ml of pyridine at room temperature. The mixture was stirred for sixteen hours, cooled with crushed ice, brought to pH 8 with 10% sodium carbonate solution and extracted thoroughly with chloroform. The chloroform extract was dried over anhydrous sodium sulfate and evaporated under reduced pressure. The residue was dissolved in benzene and the solution was evaporated to dryness; the procedure was repeated until the residue was free of pyridine. The residue obtained was subjected to preparative tlc yielding 75.8 mg (20.2%) of a pure compound, m.p. 157°. Mass spectra showed intense peaks at m/e 260 and 262 corresponding to the bromoveratrate radical and at m/e 491 corresponding to the remaining alkyl fragment, but the intensity of molecular ion peak (m/e 751, 753) was very weak.

The IR spectrum was very similar to that of veratridine in those regions of the spectrum corresponding to vibrational modes of the alkyl portion of the molecule and a carbonyl stretching band at 1710 cm^{-1} was observed. This, in addition to the formation of veratridine upon catalytic reduction, clearly establishes this product as bromoveratridine.

(D) Hydrogenolysis of Bromoveratridine. (6).

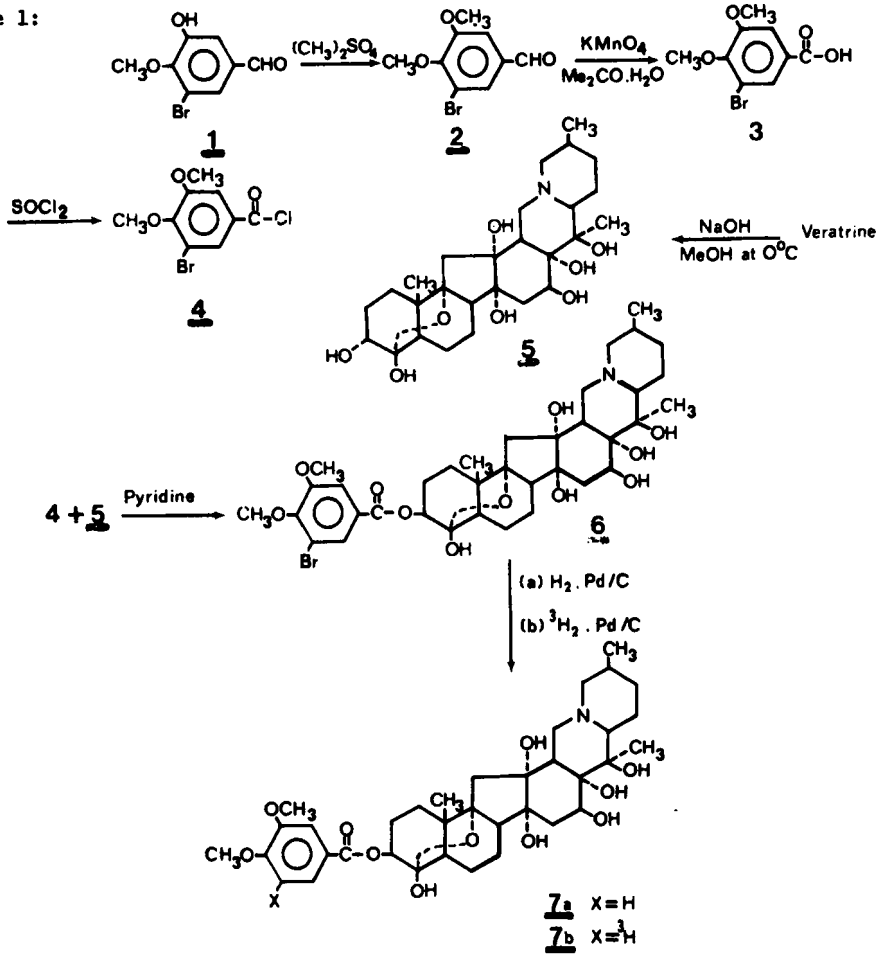
10 mg of bromoveratridine was dissolved in 1 ml of dioxane containing 10 µl of triethylamine and 10 mg of 10% Pd/C was added. The reaction mixture was stirred in an atmosphere of hydrogen for sixteen hours. Catalyst was removed by filtration and the filtrate was evaporated to dryness. Residue was dissolved in ethanol and evaporated. The compound was separated by preparative tlc which gives the melting point 182°. UV and IR spectra were the same as veratridine (7a).

(E) Preparation of ^{3}H Veratridine. (7b).

30 mg of bromoveratridine was dissolved in 3 ml of dioxane containing 30 µl of triethylamine and 30 mg of Pd/C. The mixture was stirred in an atmosphere of tritium gas (5 Ci, in 2.2-fold moles excess) for sixteen hours and then stirred in an atmosphere of hydrogen gas for five hours. Labile tritium was removed in vacuo using several ethanol additions and the solution was filtered to remove the catalyst and the remaining ethanol removed in vacuo. The above steps were carried out by New England Nuclear. The compound of interest was purified by preparative tlc. The final product was found to be 97% pure with a specific activity of 4.48 Ci/mmole.

RESULTS AND DISCUSSION

Bromoveratridine has been prepared and reduced to tritiated veratridine by Scheme 1:



In order to obtain clear separation on thin-layer chromatography, several solvent systems were tried. In many cases the veratridine was degraded during chromatography, but with cyclohexane-chloroform-methanol-triethylamine (16:8:1:1) as a solvent system, a satisfactory recovery was obtained.

The NMR spectrum of bromoveratridine was essentially that of veratridine except for the characteristic proton peak for the proton at position 5'. Infrared spectra were also found to be very similar except for a few peaks. Specifically, bromoveratridine shows a peak at 1570 cm^{-1} which disappears upon reduction. This change was found useful in establishing the end point of the catalytic reduction. The mass spectrum of the bromoveratridine showed intense peaks at m/e 260 and 262 corresponding to an aromatic radical, and at m/e 491 corresponding to veracevine radical, the absence of an intense molecular ion peak is typical of an ester of an aromatic acid and a high molecular weight alcohol. Conclusive evidence for the assigned structure of bromoveratridine rests in the fact that veratridine is obtained upon catalytic reduction.

The expected specific activity of the veratridine was not obtained. It is probable that there was tritium-hydrogen exchange during the subsequent hydrogenation (utilized to remove all traces of bromoveratridine).

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