[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, HARVARD UNIVERSITY]

The Isolation of Crystalline Hypotensive Veratrum Ester Alkaloids by Chromatography[1]

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Neogermitrine and germitetrine B have been isolated from the amorphous alkaloid fraction from *Veratrum album* by chromatography on sulfuric acid-washed alumina. By the same procedure, protoveratrine A and protoveratrine B have been isolated from protoveratrine.

The past few years have witnessed a greatly increased interest in the pharmacology and in the clinical use of the veratrum alkaloids in the treatment of hypertension.[3] This development has, in turn, stimulated much active research on the isolation and characterization of the hypotensive tertiary alkamine ester alkaloids which occur in *Veratrum* and related plants, and nearly a dozen new members of the series have been reported during the past few years.[4-8]

Widespread clinical use and chemical study of the individual ester alkaloids have been retarded by the difficulties encountered in isolation of pure, crystalline compounds. The isolation of individual alkaloids has been accomplished only by methods involving use of excessively time-consuming countercurrent distribution procedures unsuitable for large-scale purification.[4-8]

Several groups have reported unsuccessful attempts to obtain crystalline ester alkaloids from veratrum mixtures by chromatography. Jacobs and Craig[9] chromatographed the amorphous fraction from *Veratrum viride* on alumina, and used benzene–2.5% methanol as eluant. The only alkaloid they crystallized from their fractions was the inactive alkamine, rubijervine. Fried, White and Wintersteiner[4a] chromatographed the amorphous fraction from *Veratrum viride* on sulfuric acid–washed alumina and showed that an enrichment in hypotensive activity was effected by the fractionation. However, those authors obtained no crystalline esters by chromatography alone, and they subjected their chromatographic fractions to 24-plate countercurrent distribution in order to obtain the crystalline esters. Finally, Grant and Jenkins[10] recently reported an attempt to purify a water-soluble depressor principle from *Veratrum viride* by chromatography. They obtained an amorphous fraction which resisted crystallization.

We have found that several of the active tertiary alkamine esters of the veratrum series can be isolated in a pure state by chromatography of a crude mixture of the alkaloids on sulfuric acid–washed alumina.[11] In our first experiments, we used the amorphous alkaloid fraction from *Veratrum album* obtained by the method of Craig and Jacobs[12] after removal of ether-insoluble alkaloids. When this fraction was dissolved in chloroform and passed through a column of alumina (25–30 g. per g. of alkaloids), the first alkaloid fractions eluted with chloroform contained neogermitrine, easily isolated by crystallization from ether. We believe that we are the first to report the presence of this ester in *Veratrum album*. In subsequent experiments, the crude amorphous alkaloid fraction was first freed of inactive alkamines, according to the procedure of Fried, White and Wintersteiner.[4a] Chromatography of the residual amorphous bases now gave a cleaner separation of the ester alkaloids. The first alkaloid fractions eluted with chloroform yielded neogermitrine, as before. The next few fractions gave a mixture upon crystallization from ether. The alkaloidal material recovered from the subsequent fractions eluted with chloroform afforded crystalline germitetrine B. The identity of this material was confirmed by mixed melting point, infrared spectrum and X-ray diffraction pattern comparisons with an authentic specimen of germitetrine B.[13,14]

Protoveratrine obtained by the method of Craig and Jacobs[12] has been shown to be inhomogeneous.[5c,7,8] By countercurrent distribution procedures, the material has been shown to have two chief components, protoveratrine A and protoveratrine B.[8] (The latter compound has also been named neoprotoveratrine[5c] and veratetrine.[7b])

We have found that the chief components of the protoveratrine mixture can be isolated in a pure state by chromatography of the mixture on alumina. Protoveratrine was dissolved in chloroform and chromatographed as above. The first alkaloid fractions eluted with chloroform readily yielded protoveratrine A on crystallization. The homogeneity of the material was demonstrated by its behavior upon 14-plate countercurrent distribution using chloroform–2% acetic acid solution.[8] Its identity was confirmed by direct comparison with an authentic specimen of protoveratrine A.[13]

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(11) We wish to thank Dr. Max Tishler of Merck & Co., Rahway, N. J., for supplying us with Merck sulfuric acid–washed alumina. In the sequel, the term "alumina" refers to this material.

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(13) We thank Dr. H. A. Nash, Pitman–Moore Co., Indianapolis, Ind., for authentic specimens of germitetrine B, protoveratrine A and protoveratrine B.

(14) We thank Dr. S. F. Kern, Eli Lilly and Co., Indianapolis, Ind., for the X-ray diffraction pattern measurements and their interpretation.

The alkaloidal material recovered from the next few fractions eluted from the column with chloroform proved to be mixtures when analyzed by countercurrent distribution. The alkaloid fractions eluted with chloroform–1% methanol yielded protoveratrine B on crystallization from ether. The homogeneity of the product was demonstrated by countercurrent distribution,[8] and its identity was confirmed by direct comparison with an authentic specimen of protoveratrine B.[13]

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Experimental

Isolation of Neogermitrine and Germitetrine B by Chromatography of *Veratrum album* Amorphous Bases.—The total alkaloids obtained from *Veratrum album* were separated into ether-insoluble and ether-soluble fractions by the procedure described by Craig and Jacobs.[12] The ether-soluble fraction was treated for the removal of inactive alkamines according to the procedure of Fried, White and Wintersteiner.[4a] The residual ether-soluble mixture was designated as the "amorphous bases."

A solution of the amorphous bases (10 g.) in chloroform (100 ml., Merck reagent, containing 0.75% alcohol) was chromatographed on alumina (250 g., in a column of 30 mm. diameter).[11] After a forerun of 150 ml. containing no solid material, the next 100-ml. fraction yielded 150 mg. of yellow oil. The following three 100-ml. fractions eluted with chloroform were combined and evaporated to dryness *in vacuo*. The colorless amorphous residue on crystallization from ether yielded neogermitrine as colorless needles, m.p. 237–239° dec. Recrystallization from acetone–water gave elongated rods, 660 mg., m.p. 236–237° dec., $[\alpha]^{23}D$ −78° (*c* 2.00, pyr.). The mixed melting point with an authentic specimen of neogermitrine[6b] was not depressed, and the infrared spectra of the two samples were identical.

The amorphous material recovered from the following two 100-ml. fractions yielded a crystalline mixture from ether. These were set aside for rechromatography, which afforded additional neogermitrine.

The next three 100-ml. fractions of the chloroform eluate were combined and evaporated to dryness *in vacuo*. Crystallization of the amorphous residue from ether yielded colorless needles, 300 mg., m.p. 230–232° dec. Recrystallization from *n*-butyl chloride yielded germitetrine B in the form of rectangular plates, m.p. 233–234° dec., $[\alpha]^{22}D$ −69° (*c* 2.00, pyr.).

Anal. Calcd. for $C_{41}H_{63}O_{14}N$: C, 62.02; H, 8.00. Found (after drying *in vacuo* at 120°): C, 62.08; H, 7.99.

The mixed melting point with an authentic specimen of germitetrine B[13] was not depressed. The infrared spectra and X-ray diffraction patterns[14] of the respective samples were identical.

Isolation of Protoveratrine A and Protoveratrine B by Chromatography of Protoveratrine.—Crude protoveratrine (the ether-insoluble fraction mentioned above) was purified by repeated crystallization from chloroform–petroleum ether and by reprecipitation with aqueous ammonia from dilute alcoholic acetic acid solution. By this procedure, protoveratrine melting at 265–267° dec. was obtained.

A solution of protoveratrine (5 g.) in chloroform (150 ml.) was chromatographed on alumina (125 g. in a column of 20 mm. diameter). The forerun of 200 ml. of chloroform contained no solid material; the next four 100-ml. fractions eluted with chloroform were combined and evaporated to dryness *in vacuo*. Upon addition of ether to the semicrystalline residue, 1.8 g. of crystalline solid separated. Recrystallization from chloroform–petroleum ether yielded protoveratrine A as colorless plates, m.p. 270–271° dec., $[\alpha]^{25}D$ −40° (*c* 2.00, pyr.). This material did not depress the melting point of an authentic specimen of protoveratrine A,[13] and the infrared spectra of the two samples were identical.

The material recovered from the following four 100-ml. fractions proved to be mixtures when analyzed by means of 14-plate countercurrent distribution using chloroform–2% acetic acid solution. Rechromatography afforded additional protoveratrine A.

Chloroform–1% methanol (that is, 99% Merck reagent chloroform +1% methanol) was then added to the column, and four 100-ml. fractions were collected, combined and evaporated to dryness *in vacuo*. After addition of ether to the semi-crystalline residue, 1.3 g. of crystalline solid was obtained. After two recrystallizations from acetone, 1.0 g. of protoveratrine B was obtained as prisms, m.p. 267–269° dec., $[\alpha]^{24}D$ −37° (*c* 2.00, pyr.). The mixed melting point with an authentic specimen of protoveratrine B[13] was unchanged, and the infrared spectra of the two samples were identical.

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A Study of the Mannich Base–Indole Condensation

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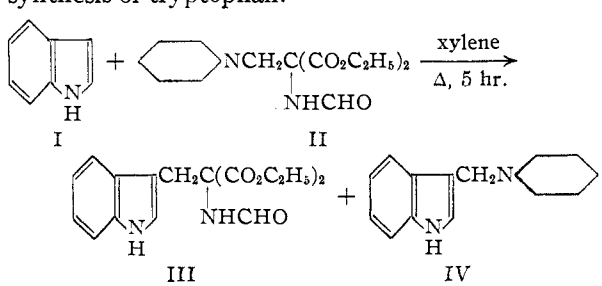
The condensation of indole with diethyl (1-piperidylmethyl)-formamidomalonate to produce diethyl skatylformamidomalonate apparently proceeds with the intermediate formation of α-(1-piperidyl)-skatole. The scope of the condensation is found to be limited.

Many syntheses of the essential amino acid tryptophan involve the condensation of a Mannich base of indole with a derivative of an active methylene compound.[1–4] Thus, for example, 3-dimethylaminomethylindole can be condensed with formamidomalonic ester[4] to give diethyl skatylformamidomalonate (III), in 96% yield, which can be hydrolyzed and decarboxylated to tryptophan. More recently it has been found that indole (I) can be condensed with the Mannich base diethyl (1-piperidylmethyl)-formamidomalonate (II) to give III,[5] thus providing a new approach to the synthesis of tryptophan.



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