[CONTRIBUTION FROM THE INSECTICIDE DIVISION, BUREAU OF ENTOMOLOGY AND PLANT QUARANTINE, U. S. DEPARTMENT OF AGRICULTURE]

# The Preparation of Picrotoxin

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Picrotoxin may be prepared conveniently by the method outlined below. The simplicity of the procedure and the uniform results obtained with it make it superior to previously published methods.

In the course of the work with picrotoxin which led to the development of the procedure under discussion, the optical crystallographic characteristics, as determined by the immersion method, were obtained for picrotoxin and its two principal derivatives, picrotin and picrotoxinin.[1] These data are recorded because of their value in determinative work.

One kilogram of ground fish berries, *Anamirta cocculus* (L) Wight and Arn., covered with 2 liters of 95% ethanol is heated for forty-five minutes at the boiling point of the alcohol. The hot extract is separated from the drug by filtration, and the residue on the filter is washed with three 750-cc. portions of hot alcohol. The combined extract and washings are concentrated to a volume of 1 liter. Two volumes of water at approximately 75° are added with stirring to the hot concentrate, after which ice is added to make a volume of 5 liters. As soon as the ice is melted, the liquid is separated from the insoluble fatty material by filtration through folded filter paper. The residue is washed with a liter of water, and the combined filtrates are passed through a thin layer of norit in a small Büchner funnel. The object of the second filtration is to remove impurities, which, if allowed to remain, cause foaming in the subsequent concentration. The clear filtrate is then concentrated under reduced pressure to approximately 600 cc. During this process picrotoxin gradually crystallizes and must be removed from the flask from time to time. After standing overnight, the crystals are separated from the mother liquor, washed with a little cold water, and dried. Upon further concentrating the mother liquor a second crop of crystals is obtained. The yield is usually 1.4% of the drug. Except for a little coloring matter, the crude picrotoxin is quite pure.

Upon recrystallization in the following manner an analytically and optically pure product is obtained. A solution of 10 g. of picrotoxin in 30 cc. of hot acetone is filtered through a thin layer of norit upon a small Hirsch funnel, and the adhering substance is washed from the apparatus with 15 cc. of hot acetone. The combined filtrate and washings are again heated to boiling, and 3 volumes of hot water are added. Upon cooling, the picrotoxin separates as well-formed prismatic crystals having a melting point of 203–204°. The yield is 85%, but upon concentrating the mother liquors an additional 14% is obtained. The crystals exhibit straight extinction and positive elongation. With crossed nicols (convergent polarized light) partial interference figures are occasionally shown, indicating that the substance is biaxial: $\eta_\alpha$, 1.520 (crosswise); $\eta_\gamma$, 1.565 (lengthwise).

Picrotin prepared by the method of Horrmann [*Arch. Pharm.*, **258,** 209 (1920)] and recrystallized from water, m. p. 252°, has the following optical properties: colorless rods with straight extinction and positive elongation, $\eta_\alpha$, 1.535 (crosswise); $\eta_\beta$, indeterminate; $\eta_\gamma$, 1.565 (lengthwise).

Picrotoxinin, also prepared by Horrmann's procedure and recrystallized from water, m. p. 209.5°, consists of colorless rods with straight extinction and negative elongation. In parallel polarized light (crossed nicols) many rods do not extinguish sharply. In convergent polarized light (crossed nicols) many rods show partial biaxial interference figures with the optic axis inclined: $\eta_\alpha$, 1.550, not common, but found on crystals lengthwise; $\eta_\beta$, approximately 1.555; $\eta_\gamma$, 1.570, uncommon, crosswise.

(1) These values were determined by George L. Keenan, of the Food and Drug Administration of the U. S. Department of Agriculture, and are accurate to ±0.003.

WASHINGTON, D. C. RECEIVED APRIL 17, 1935