# CHEMICAL STUDIES ON A TOXIC CONCENTRATE FROM THE BIG BEND LOCOWEED

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A preliminary study on the use of ion exchange resins with locoweeds indicated the possibility of their value in finding a method for the isolation of the toxic principle of the Big Bend locoweed (1). The present paper reports a new ion exchange procedure successfully applied to the locoweed, and also chemical studies performed on the toxic concentrate obtained after treatment of the locoweed extract with the Amberlite resin.

## EXPERIMENTAL

Each liter of an aqueous extract of Big Bend locoweed was diluted to two liters with distilled water, and 50 ml. of 20% sodium hydroxide solution was stirred into the diluted extract. This alkaline extract was then passed through the column containing the ion exchange resin. A typical column consisted of a glass tube 4 cm x 120 cm, packed with Amberlite IRC-50 (Rohm and Haas Co., Philadelphia), with an effective height of 75 cm. Passage was at the approximate rate of 50 ml. per minute. The toxic substance remained on the Amberlite. The extracts from about 800 gm. of weed were required for the apparent exhaustion of the column. The column was washed with 500 ml. of water, then 5% sulfuric acid solution was started through to remove the toxic substance. When about 1.2 liters of acid had been used, a dark line reached the bottom of the column. Collection of the eluate was started at this time. The acid was followed by water, until about 800 ml. of eluate had been collected.

The processes of extracting the weed with water and treating the alkaline extracts with the Amberlite resin were continued until an eluate fraction was obtained which represented 8 kg. of the dry weed. Faulkner and Smith (2), in tests on cats, found the neutralized eluate to be toxic.

The use of phosphotungstic acid precipitation was found to yield inconsistent results in further purification of the toxic eluate.

The toxic eluate was further treated to yield a crystalline substance plus a residue. The procedure for this separation was as follows:

The eluate was evaporated under reduced pressure and at temperatures below 60° C. to a moist solid. This solid was then extracted thoroughly with absolute alcohol. The absolute alcohol extract was treated with saturated alcoholic silver nitrate solution. The gray precipitate obtained was centrifuged and the liquid decanted. This organic precipitate was recrystallized to a high degree of purity, but proved to be non-toxic (2). The supernatant liquid contained the toxic substance.

Further chemical studies on the toxic fraction have been pursued. An aqueous solution of mercuric chloride produced a precipitate with the toxic fraction. A flavianate was prepared by adding an equeous solution of flavianic acid (2,4-dinitro-1-naphthol-7-sulfonic acid) to an absolute alcohol solution of the toxic material. The yellow flavianate decomposed at 185° C. Flavianic acid itself melts at 151° C. A precipitate was also obtained on treatment of an alcohol solution of the toxic substance with sodium-quinizarin-2-sulfonate. Animal tests are in progress to determine in each of these cases whether or not the precipitate or the remaining filtrate contains the toxic fraction.

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## LITERATURE CITED

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2. FAULKNER, KENNETH K. and JAMES W. H. SMITH. 1950. Preliminary Studies of the Toxicity of Locoweed Extracts. Proc. Oklahoma Acad. Sci. *31*: 48-50.