Further Fractionation of a Toxic Concentrate From Big Bend Locoweed

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Chervenka and Wender (1), using ion exchange resins, isolated a toxic concentrate from the Big Bend locoweed, and found that this toxic fraction produced a precipitate with flavianic acid (2,4-dinitro-1-naphthol-7-sulfonic acid). The present paper reports further fractionations of the flavianate thus obtained.

Experimental

Two hundred ml. of an alcoholic extract from a total of 8 kg. of weed was prepared by the method of Chervenka and Wender (1), continuing on through the removal of the non-toxic precipitate from the addition of silver nitrate and subsequent removal of the excess silver ion with concentrated hydrochloric acid. This alcoholic supernatant liquid contained the toxic principle.

The flavianate was prepared by heating the alcoholic solution to boiling for 3 minutes with flavianic acid in the proportion of 1 gm. flavianic acid to 50 ml. of alcoholic solution. The mixture was then placed in an ice bath. Yellow crystals deposited. These turned black at 210°C and did not decompose before 275°C. The original flavianic acid melted at 149°C.

These flavianates can be decomposed with concentrated hydrochloric acid, by adding 5 ml. of the acid to 1 gm. of the flavianate and refluxing for 1 hr. Upon cooling, most of the flavianic acid precipitates.

The filtrate from the flavianate corresponding to 15 ml. of the alcoholic extract was partially neutralized to *p*H 4 by the addition of solid potassium carbonate. An orange precipitate formed; this was filtered off. The filtrate dissolved no more potassium carbonate; thereupon alcoholic potassium hydroxide was added. A yellow precipitate now formed immediately. At *p*H 7, the addition of the alcoholic potassium hydroxide was stopped, and the yellow precipitate was separated. (Crystals of this precipitate turned black at 210°C.). The filtrate was then passed through a 2 cm x 18 cm column of alumina and the column developed with 95% ethanol. The column had three bands upon it of various shades of orange. The lower two bands were lightly adsorbed and were taken off in one fraction which was an amber colored liquid. This solution fluoresced under an ultraviolet light. The next fraction was a yellow liquid containing a few white needle crystals. In order to move the rest of the bands, benzene was added; then ethyl acetate. Neither had any noticeable tendency to move the bands. A solution of hydrochloric acid in alcohol, however, cleared the column rapidly. The latter fractions were then combined.

The fraction containing the first eluted bands was then placed on a fresh column of alumina, 2 cm x 18 cm with 95% ethanol as the solvent. An amber colored liquid moved rapidly through the column. Then a pale yellow liquid moved off the column, leaving an orange band adsorbed on the alumina. The yellow eluate was concentrated to 25 ml. by distillation and set aside. Upon standing, it deposited white crystals. The amber filtrate passed through another alumina column 2 cm x 18 cm, again using 95% ethanol as the solvent. A dark yellow liquid came through, and a faint yellow band was adsorbed.

All fractions from various trials were then combined with the exception of the last two eluates. These fractions amount to the extract of 5 kg. of the weed. The resulting amber colored acidic solution (*p*H 3) was filtered. The precipitate was thrown away (mostly inorganic salts). To the filtrate was added 1 gm. of flavianic acid. A yellow precipitate formed immediately. The solution was filtered, concentrated, and 0.5 gm. of flavianic acid was added to the solution. This was heated to boiling, and cooled. Another yellow precipitate separated again. The solution was filtered and alcoholic potassium hydroxide added. A yellow precipitate formed immediately, and at *p*H 4, the solid was filtered. Alcoholic potassium hydroxide was added until *p*H 7. A large quantity of a tan solid formed. This was filtered, and the filtrate placed on a 5 cm x 45 cm column of alumina. Using 95% ethanol as a solvent, two bands developed, a brown one at the top, and an orange band below it. The two bands were rather strongly adsorbed and no noticeable movement was observed for a few hours. A third band, however, developed quickly which moved down the column at a rapid rate. This band gave an amber liquid. It was concentrated from 2 liters to 25 ml. Two liters more were taken and concentrated similarly (by distillation).

Still another yellow band separated from the orange band and moved off the column. Between the third and fourth fraction there was a period where 150 ml. of clear alcohol was obtained. The fourth fraction was concentrated and saved. A brown residue was left on the column and the alumina saved. Studies on these fractions are now in progress.

This investigation was supported in part by a Frederick G. Cottrell grant from the Research Corporation.

LITERATURE CITED

1. Chervenka, Charles H. and Simon H. Wender. 1950. Chemical studies on a toxic concentrate from the Big Bend Locoweed. Proc. Oklahoma Acad. Sci. 31:99.