Studies on Flavonoids of Locoweed

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While investigating the toxic principle of Big Bend Locoweed, *Astragalus earlei*, Chervenka and Wender (1) noted that a bright yellow substance present in extracts of the weed was adsorbed on Amberlite IRC-50 resin. Since there had been no previous reports in the literature concerning the flavonoid constituents of locoweeds, the present study was undertaken.

Two approaches were made toward the identification of the flavonoid constituents of Big Bend Locoweed. In the first, attempts were made to isolate the pigments as pure compounds for characterization. In the second, identification of the flavonoid pigments by chromatographic studies of concentrates was attempted. Studies are now under way to complete each of these investigations in order to identify individual flavonoids.

Experimental

Isolation of Flavonoid Pigments. Seventeen kilograms of the ground weed were extracted with 14 l. of boiling water. The extract was separated from the residue by successive filtration through cheese cloth and muslin, was then clarified in a "Sharples Super-Centrifuge". The extract was concentrated to a thick syrup and extracted with 100 ml. of boiling water in

several portions. A small amount of yellow material crystallized from the water solution. This precipitate, removed by filtration and recrystallized once from dilute ethanol, weighed 15 mg.; m.p. 200-5°.

Determination of Properties of Isolated Pigment. Several color tests were applied to the alcoholic solution of the isolated pigment. Reduction with magnesium and hydrochloric acid gave a reddish-orange coloration. With alcoholic ferric chloride, an olive-green color resulted. Both lead acetate and basic lead acetate gave yellow precipitates. Treatment with either ammonium hydroxide or concentrated sulfuric acid resulted in deep yellow solutions.

The ultraviolet absorption spectrum of an ethanol solution was determined with the Beckman Model DU spectrophotometer. A large absorption maximum at 260-260mμ and a small one at 350-360mμ were observed.

The R_f values of the pigment in three solvent systems as determined by one-dimensional chromatography were: 0.79 in 40% butanol-50% water-10% acetic acid; 0.65 in ethyl acetate, saturated with water; 0.50 in phenol, saturated with water. The pigment exhibited a yellow-brown color when viewed under ultraviolet light after chromatography. When the pigment was treated with the chromogenic spray reagents of Gage, Douglass and Wender (2) the colors tabulated in Table I were obtained.

TABLE I

Colors Produced by Isolated Pigment and Chromogenic Reagents

Reagent	Visible light	Ultraviolet light
Basic lead acetate solution	Yellow	Orange
Lead acetate solution	Yellow	Orange-Yellow
Sodium carbonate solution	Yellow	Yellowish-Brown
Alcoholic aluminum chloride	Yellow	Yellow

Five milligrams of the isolated material were added to 0.5 ml. of 0.6% sulfuric acid and boiled under reflux for 90 minutes. A precipitate separated from the cooled solution. To the supernatant liquid was added an excess of a mixture containing two parts by weight of sodium acetate and three parts of phenylhydrazine hydrochloride. This was allowed to stand in a boiling water bath for 30 minutes. A drop of the liquid was examined under a microscope with the low-power objective. The characteristic crystals of glucosazone were observed.

The amount of precipitate resulting from hydrolysis was too small to make chemical identification possible. A solution of a small amount of the material in 95% ethanol was subjected to paper chromatography and gave R_f values as follows: 0.79 in ethyl acetate, saturated with water, 0.50 in phenol, saturated with water, and 0.80 in 40% butanol-50% water-10% acetic acid.

Chromatography of Flavonoid Concentrate. Two kilograms of the ground weed were extracted with 4 l. of acetone. The acetone was allowed to evaporate at room temperature, and the residue was taken up in 1 l. of boiling water and filtered while hot. When the solution was concentrated to 70 ml. on a boiling water-bath, a red oil separated, and was removed by filtration. The solution was extracted repeatedly with 20 ml. portions of benzene. The benzene gave negative reactions for flavones. The aqueous layer was then exhaustively extracted with 20 ml. portions of ethyl acetate. The ethyl acetate was allowed to evaporate and the resulting syrup was taken up in 3 ml. of 95% ethanol. Ten milliliters of water were added.

Four bands which gave positive flavonoid reactions were observed on paper chromatography in 60% acetic acid. The R_f values of these bands were as follows: 0.41, 0.55, 0.75, 0.86. The colors of these bands in ultraviolet light in the order of increasing R_f values, were as follows: yellow, yellow, brownish-yellow, light yellow. On chromatography in 40% butanol-10% acetic acid-50% water, five such bands were observed. The R_f values of the observed bands were 0.38, 0.50-0.54, 0.79, 0.85, 0.98. Their colors in ultraviolet light, were respectively, yellow, brownish-yellow, brownish-yellow, yellow, light yellow.

The solution, concentrated to a volume of approximately 2 ml., was chromatographed on 32 different paper strips with the butanol-acetic acid-water system. The appropriate bands were cut from each of the strips and the combined zones were extracted with 95% ethanol in a small Soxhlet-type extractor. The pigments have been separated for further identification studies.

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LITERATURE CITED

1. Chervenka, Charles and Simon H. Wender. 1949. Personal communication.

2. Gage, Thomas B., Carl D. Douglass, and Simon H. Wender. 1951. Identification of flavonoid compounds by filter paper chromatography. Anal. Chem. 23:1582.