

## PRELIMINARY STUDIES ON THE USE OF AMBERLITE RESINS IN LOCOWEED INVESTIGATIONS

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**INTRODUCTION.** Several species of the plant genera *Astragalus*, commonly called "locoweeds", have caused considerable economic losses among stockmen in the Great Plains states (Marsh 1919).

Chemical investigations of the plant have been conducted by Couch (1), Fraps and Carlyle (3), Stempel and Elderfield (5), and Fraps and Wender (2).

Several instances of applications of synthetic ion exchange resins to the separation of physiologically active materials are summarized in an article by Winters and Kunin (6). The present work is concerned with preliminary efforts to find if the toxic material in locoweed can be isolated or purified by such means.

**EXPERIMENTAL.** Two different Amberlite resins have thus far been utilized in the present investigation: IRC-50 and IRA-400, synthesized by the Resinous Products Division, Rohm and Haas Co.

**PREPARATION OF COLUMNS.** Ordinary 19 x 240 mm glass chromatography columns were used for preliminary studies. A 150 ml separatory funnel, fitted to the top of the column by means of a rubber stopper, served as a reservoir for solutions being passed through the column. Approximately 50 ml of IRC-50 were washed with several portions of water, then with 1%  $H_2SO_4$ , next with more water. The resin, in a water slurry, was poured into the column to a depth of 20 cm. Fifty ml of 1%  $H_2SO_4$  were then passed through the column, followed by several hundred milliliters of water, until the washings were neutral and gave no characteristic absorption curve with the spectrophotometer. During use, the resin was not allowed to drain dry.

A column of IRA-400 was prepared in a similar manner, except that 4% NaOH was used instead of the dilute acid.

**PREPARATION OF LOCOWEED EXTRACT.** Extracts were prepared by heating, with stirring, 1 kg of the dry, ground Big Bend locoweed with 10 liters of water for 1½ hours. The mixture was strained through parachute cloth, passed twice through the Webcell continuous dialysis apparatus, and the dialysate concentrated to a viscous syrup. Absolute alcohol and benzene were stirred into the liquid, and the mixture again distilled to a thick syrup. This process was repeated until the material was in an absolute alcohol solution with a volume of 200 ml. This solution was allowed to stand in the freezing compartment of a refrigerator overnight, whereupon a semi-solid mass of material settled out. The clear, darkly colored alcohol solution was decanted off, and the residue was warmed and shaken with 50 ml of absolute alcohol. This was again allowed to separate in the refrigerator, and the alcohol decanted and added to the previous solution. This process was repeated until the alcohol layer separated out nearly colorless. The combined alcohol solutions were concentrated to a syrup, and made up to 300 ml with distilled water. This solution was the concentrated extract used for study with the resins.

**Absorption Spectra.** Fraps and Wender (3) had found that all of their solutions which were toxic when fed to cats exhibited ultraviolet absorption spectra with maxima in the region of 264-275  $m\mu$  and minima in the region of 240-250  $m\mu$ . In the present work, therefore, progress was followed by means of determination of spectral absorption curves of the fractions, using a Beckman Model DU Spectrophotometer, with ultraviolet attachments.

The extract as prepared above gave a spectral absorption curve with a rather flat maximum at 263-266  $m\mu$ , and a minimum at 245-246  $m\mu$ .

**ION EXCHANGE.** IRC-50. 10 ml of the locoweed extract as prepared above were diluted to 100 ml to give a slightly yellow-brown colored solution with a pH of about 5.2. This solution was passed, at the approximate rate of one drop each two seconds, through the IRC-50 column, which was now in the hydrogen form. The first 20 ml of effluent liquid were discarded, and the last of the effluent solution was washed through the column with 50 ml of distilled water.

This latter effluent solution was still colored. It now had a spectral curve similar to that of the extract before passage through the column, with a slightly sharpened maximum at 263-264  $m\mu$  and minimum at 249  $m\mu$  (Fig. 1).

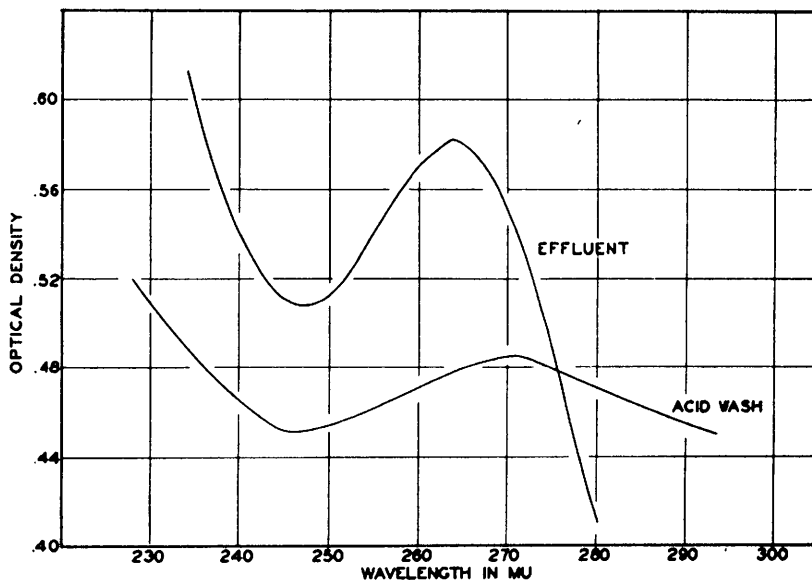


FIGURE 1.

The column was next washed with water until washings no longer gave the characteristic curve with the spectrophotometer, then was regenerated with 30 ml of 1%  $H_2SO_4$  (passed through at the same rate as the previous solution), followed by 50 ml water. The acid wash yielded a spectral absorption curve with a broad maximum in the region of 270-272  $m\mu$  and minimum at 246  $m\mu$  (Figure 1).

The above experiments were supplemented by the preparation of blanks. With a duplicate column of IRC-50, using water instead of the extract, the effluent solution and also the solution obtained by passing 1%  $H_2SO_4$  through the column were used as blanks in the spectrophotometer for corresponding solutions of extract. This procedure indicated that the blanks were apparently not essential.

A limited amount of work has also been carried out using the resin in the sodium form. The sodium form was prepared by leaching the IRC-50 with 4% NaOH and washing it thoroughly. When a solution of the extract at pH 7.2 was passed through this column, the effluent solution exhibited an absorption maximum of 263-265  $m\mu$ , and a minimum of 250  $m\mu$ . The solution which

resulted when the column was regenerated with 4% NaOH gave a maximum of 273-274  $m\mu$ , and minimum of 258-260  $m\mu$ .

**pH EFFECT WITH IRC-50.** The experiment with the IRC-50 column in the hydrogen form was repeated using similar starting solutions at pH's of 6.0, 6.8, 7.0, 7.9, 8.9, and 9.8. In all cases, similar results were obtained: the effluent solutions exhibited maxima at 264-265  $m\mu$ , and minima at 245-249  $m\mu$ , while the acid washed showed slightly varying maxima from 270 to 280  $m\mu$  and minima at about 246  $m\mu$ . No correlation could be seen between pH of the solutions before being put through the column and the slight differences in the absorption maxima of effluent solutions.

**ION EXCHANGE, IRA-400.** The solution which had passed the IRC-50 column, and which was at pH 10, was next passed through a column of IRA-400 at the same previously used rate. The effluent was colorless, and exhibited a sharp absorption maximum at 271  $m\mu$  (Fig. 2). When this alkaline solution was acidified, the maximum was shifted to 264  $m\mu$  (Fig. 2); when it was again made alkaline, the maximum shifted back to 271  $m\mu$ .

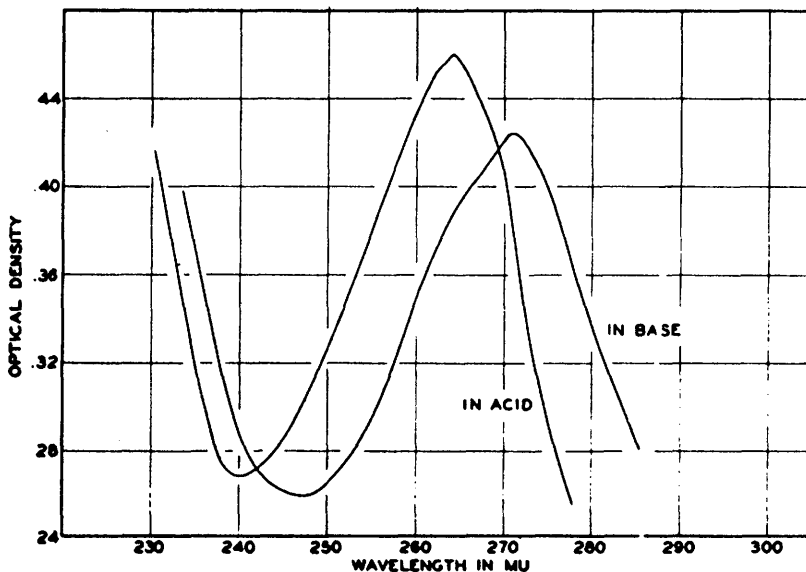


FIGURE 2

The effluent solution from IRA-400 was again put through the two resins (IRC-50 and IRA-400) as before. The resulting solution, which had alternately passed through both resins twice, now exhibited a maximum at 271  $m\mu$ ; upon acidification the maximum did not shift, but a break in the curve appeared at 264  $m\mu$ .

As this procedure of consecutive use of resins was repeated upon other portions of extracts, solutions were sometimes obtained which yielded an absorption curve with two maxima, one at 271  $m\mu$  and another 264  $m\mu$ , or curves with a maximum at 271  $m\mu$  and a break at 264  $m\mu$ , or vice versa. Supplementary attempts at purification, such as extraction or evaporation to dryness and

taking up in alcohol, generally gave solutions with sharp maxima at either 264  $m\mu$  or 271  $m\mu$ , with no breaks, and with no shifts accompanying changes in acidity.

Regeneration of the IRA-400 column with 4% NaOH yielded indefinite results. A solution with a maximum at 255-258  $m\mu$  was obtained in some cases; however, solutions with similar curves were obtained merely by washing an unused portion of the resin with the dilute base.

**CONCLUSION.** The preliminary studies with Amberlite resins indicate that the extracts possibly contain two or more substances exhibiting different absorption curves in the ultraviolet. At least one of these undergoes a reversible shift of maximum or complete loss of absorption when changed from acidic to basic solution.

With further study, the Amberlite resins may prove to be of value in the separation of the toxic materials from locoweed.

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