Ion Exchange Studies on the Flavonoid Fraction from Licorice Root

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In preliminary attempts to isolate the flavonoid fraction from licorice root, *Glycyrrhiza glabra*, using cation exchange resins (2), the flavonoid remained contaminated with a brown material which was difficult to remove. The flavonoid material was not adsorbed on Amberlite IRA-400(Cl), when that method of purification was used. When the extract was passed through a column of Amberlite IRA-400(OH) and then onto a column of Amberlite IRC-50(H), the eluted flavonoid fraction was precipitated as a brown, tarry mass, thus complicating purification.

This paper reports the finding of a successful ion exchange procedure for removing the persisting contaminant from the flavonoid fraction of licorice root, by making use of the anion exchange resin Dowex I-XI.

Experimental

One kilogram of powdered licorice root (S. B. Penick and Co., New York) was extracted with 38 liters of boiling water. The extract was filtered through a flannel bag, and then passed through a 7.6 cm. x 102 cm. column of Amberlite IRC-50(H) (Rohm and Haas, Philadelphia, Pa.). Although many impurities passed through the column, the flavonoid fraction did not. The column was thoroughly washed by passing distilled water through it, and the flavonoid fraction was next eluted with 1.75 liters of ethyl alcohol. This alcohol solution, containing the flavonoid fraction, was divided into four portions for further study separately with Dowex I-XI (Cl); Dowex I(Cl), (Dow Chemical Company, Midland, Michigan); Amberlite IRB-4(OH), and Amberlite IRA-410(OH). Each of these resins was filled to a depth of 25 cm. in individual glass columns of 2.5 cm. diameter. A portion of the eluted alcohol solution containing the flavonoid fraction was passed onto each resin. The effluent liquid from three of the columns gave a positive reduction test, indicating that the flavonoid had already passed through the column. The effluent from the Dowex I-XI, however, contained no detectable flavonoid, and the flavonoid fraction could be detected at the top of this Dowex I-XI column. About 250 ml. however, of the solution would saturate this column.

Further washing of the Dowex I-XI column containing the flavonoid was performed with a 5 per cent potassium hydroxide solution. A dark brown eluate resulted, but no flavonoid was detected therein. Distilled water was next passed through the column until the washings were neutral. To remove the flavonoid from the Dowex I-XI column, 500 ml. of ethyl alcohol containing 75 ml. of concentrated hydrochloric acid, was passed through the column. After the resin had been transformed into the chloride form, the flavonoid appeared in the effluent liquid, as evidenced by a positive reduction test. Two hundred and fifty milliliters of eluted solution containing the flavonoid was collected. After neutralization with sodium bicarbonate, the solution was filtered and the filtrate concentrated to a volume of 40 ml. The concentrate was diluted with an equal volume of water, and placed in the refrigerator overnight. A yellow flavonoid solid separated. After drying, this solid was dissolved in anhydrous acetone, and chromatographed on a column containing Magnesol. Elution was performed according to the procedure of Ice(1), using ethyl acetate saturated with water. The second visible band, yellow in color, proved to be one containing the desired flavonoid compound. Paper chromatography, using 15 per cent acetic acid solution, indicated that the compound was liquiritin (the 4'-glucoside of 4',7-dihydroxy flavanone), Rf, 0.43, and which had

already been proved by Shinoda and Ueeda (3), to be present in licorice root. After hydrolysis with 5 per cent sulfuric acid, the aglucone liquiritigenin, Rf 0.20, was identified by paper chromatography, using an authentic sample for comparison.

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LITERATURE CITED

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