A GENERAL USE OF AMBERLITE XAD-2 RESIN FOR THE PURIFICATION OF FLAVONOIDS FROM AQUEOUS FRACTIONS

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A very important step in any isolation sequence for plant phenolics from aqueous extracts is the elimination of the bulk of water soluble contaminants. For flavonoids, various procedures have been reported, including preparative paper chromatography, adsorption on charcoal, and polyamide chromatography (1,2). Very often, these procedures may lead to losses due to irreversible adsorption (charcoal), to incomplete removal of contaminants, or to contamination with oligomeric extractives from the adsorbents (cellulose, polyamide).

We now wish to report an alternative method based on adsorption on the nonionic resin Amberlite XAD-2.[1] We found that this method is useful not only for obtaining the flavonoid fraction from plant extracts but also for purifying individual flavonoid fractions after chromatography on other adsorbents.

The Amberlite XAD-2 resin has been routinely used in the past 10-15 years for the reversible adsorption of a variety of drugs from body fluids and is also known to adsorb polyphenolic compounds such as tannic acid (3,4). The XAD-2 purification process could be of value in any screening procedure of plant extracts, and in many sequences, it may be the key step.

Previously, the resin had also been used for the separation of flavonoids by gradient elution chromatography with H_2O-EtOH mixtures. Under these conditions, mixtures of two to four pure substances were separated in jacketed columns at a constant temperature of 45° (5). This use of the resin does not work satisfactorily if crude extracts are processed.

In our use of XAD-2, crude aqueous extracts are passed over the column, and the adsorbed material is washed with H_2O only. Instead of using a gradient of H_2O-alcohol mixtures, we start with 50% MeOH to elute all of the adsorbed compounds in bulk and then change to 100% MeOH to complete the elution process. After the flavonoid fraction has thus been purified, other adsorbents such as polyamides or polyvinylpyrrolidone are the preferred agents for the separation of individual compounds. Sometimes, depending on the purity of these other adsorbents, the eluted compounds can become contaminated with soluble impurities. They can then be purified again by adsorption and desorption using Amberlite XAD-2. Since the Amberlite XAD-2 resin can be cleaned on the column with 5% sodium hypochlorite after each run, its use is also very economical. The following experimental data describe the general procedure we employ in our ongoing research in plant phenolics.

EXPERIMENTAL

A 6×100 cm Amberlite XAD-2 column was adjusted to pH 2 with HCl. The aqueous plant extract (1 liter) was also acidified and passed over the column. The column was washed with 4 liters of H_2O at pH 2, followed by 4 liters of neutral H_2O. Four liters each of 50% MeOH and absolute MeOH were used successively to elute the column. These eluates were taken to dryness under reduced pressure. The initial acidification of the extract and the wash-H_2O is usually not necessary but indirectly improves the capacity of the resin. A judgment whether neutral or acidic conditions are indicated is made separately for each extract and is based on preliminary small-scale runs and the specific objectives of the investigator.

LITERATURE CITED

1. T.J. Mabry, K.R. Markham, and M.B. Thomas, "The Systematic Identification of Flavonoids," Springer-Verlag, NY, 1970, p. 13.
2. H. Rosler, T.J. Mabry, and J. Kagan, *Chem. Ber.*, **98**, 2193 (1965).
3. M.P. Kullberg, W.L. Miller, F.J. McGowan, and B.P. Doctor, *Biochem. Med.*, **7**, 323 (1973).
4. R.L. Gustafson, R.L. Albright, J. Heisler, J.A. Sirio, and O.T. Reid, Jr., *Ind. Eng. Chem. Prod. Res. Develop.*, **7**, 107 (1968).
5. M. Hori, *Bull. Chem. Soc. Jap.*, **42**, 2333 (1969).

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[1]The Amberlite XAD-2 was obtained from Rohm and Haas Co., Philadelphia, PA.