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RADIAL DIFFUSION METHOD FOR DETERMINING TANNIN IN PLANT EXTRACTS

ANN E. HAGERMAN

Department of Chemistry
Miami University
Oxford, Ohio 45056

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Abstract—Tannin in plant extracts can be determined by reacting the tannin with a protein and quantitating the precipitated complex. In the new assay described here, a tannin-containing solution is placed in a well in a protein-containing agar slab. As the tannin diffuses into the gel and complexes with protein, a visible ring of precipitation develops. The area of the ring is proportional to the amount of tannin in the extract. The detection limit of the method is 0.025 mg tannic acid or condensed tannin and the precision is 6% (relative standard deviation). Tests with extracts of a variety of plants show that the new method gives results comparable to other precipitation methods and that the new method is superior for samples of unusual composition, such as aspen buds. The method has several advantages over other methods for determining tannin: The new method is very simple and requires neither complex reagents nor instruments. Components of the plant extract such as non-tannin phenolics or water-insoluble compounds do not interfere with the method. The assay is not subject to interference from the organic and aqueous solutions which are commonly used to extract tannin from plants.

Key Words—Tannin, proanthocyanidin, protein precipitation, digestibility-reducing substances, phenolic analysis.

INTRODUCTION

Tannins, like many other secondary compounds, are thought to defend plants from herbivores (Swain, 1979). To establish the role of tannin as a defensive compound, the tannin content of various plants must be correlated with patterns of herbivory. Sensitive, specific tannin assays which can easily be run on large numbers of samples are required for such studies of tannin as a defensive compound.

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The analytical methods currently available for determining tannin have several disadvantages. The functional group methods do not have satisfactory specificity. For example, the redox methods such as the Folin-Denis assay (Folin and Denis, 1915) are not specific for tannin, but detect any phenolic compound. On the other hand, the proanthocyanidin and vanillin assays (Bate-Smith, 1975; Price et al., 1978) are too selective. The hydrolyzable tannins, which are gallic acid derivatives (Haslam, 1979), do not react with acidic butanol or vanillin. Only the flavonoid-based condensed tannins (Haslam, 1979) can be detected with these reagents.

Precipitation assays also have disadvantages. Several methods for determining protein precipitated by the tannin have been described (Bate-Smith, 1973; Hagerman and Butler, 1980a; Martin and Martin, 1983). Although selective, these methods are inconvenient; they may include multiple steps for forming and isolating the precipitate, or they may require special materials such as radiolabeled compounds. Two simple precipitation methods have been described. In one method, a dye-labeled protein is used, and the amount of protein precipitated by the tannin is determined spectrophotometrically (Asquith and Butler, 1985). In another method, the tannin precipitated by excess protein is measured spectrophotometrically after reaction with ferric chloride (Hagerman and Butler, 1978). Although these methods are straightforward, sample preparation for these assays is complicated. Some solvents, such as acetone, interfere with the precipitation and must be removed from the extract before analysis. In addition, water-insoluble compounds frequently found in the tannin extract interfere with precipitation assays (Hagerman and Butler, 1978; Asquith and Butler, 1985).

A new protein precipitation assay that overcomes these problems is described here. In the assay, tannin diffuses through a protein-containing gel, and a visible disk-shaped precipitate develops as the tannin interacts with the protein. The method is simple, sensitive, and specific, and should be especially applicable to studies in which large numbers of samples are to be analyzed.

METHODS AND MATERIALS

Reagents. All reagents were analytical grade or the best grade available. Agarose (type I), bovine serum albumin (BSA) (fatty acid-free fraction V), and catechin were obtained from Sigma Chemical Co. (St. Louis, Missouri). Condensed tannin was prepared from *Sorghum vulgare* IS 4225 by the method of Hagerman and Butler (1980b). Hydrolyzable tannin was purified from commercial tannic acid as described by Hagerman and Klucher (1986). Buffer A consisted of 50 mM acetic acid and 60 μM ascorbic acid adjusted to pH 5.0.

Assay Method. A 1% (w/v) solution of agarose was prepared in buffer A