CHAPTER 16

ACID BUTANOL ASSAY FOR PROANTHOCYANIDINS (CONDENSED TANNINS)

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1. INTRODUCTION

Tannins are a major class of secondary metabolites that are widespread in plants (Waterman & Mole 1994, Kraus et al. 2003a). They are water-soluble polyphenolics with molecular weights typically ranging from 1000 to 3000 (Swain 1979). By definition, tannins are capable of complexing and subsequently precipitating proteins (cf. Chapter 15), and they can also bind to other macromolecules (Zucker 1983). Two main, chemically distinct groups are commonly distinguished in vascular plants: hydrolysable tannins, which are further divided into the gallotannins and ellagitannins, and condensed tannins, or proanthocyanidins, which cannot be hydrolyzed (Waterman & Mole 1994, Hättenschwiler & Vitousek 2000). Proanthocyanidins are the most widely distributed tannins in woody plants. They are usually also the most abundant group. Their diversity both within and among species is remarkable; however, the polymeric structures of proanthocyanidins can be derived from relatively few building blocks of low-molecular weight compounds. The most important monomers are flavan-3-ols such as catechin, epicatechin, gallocatechin and epigallocatechin; they react with one another in various ways, leading to either linear or branched polymers (Fig. 16.1).

Discussions on the ecological functions of tannins have mainly revolved around their capacity to bind to proteins and precipitate them (Zucker 1983). Both vertebrate and invertebrate herbivores can be affected. Herbivores also tend to prefer diets with low tannin concentrations, suggesting that tannins act as feeding deterrents to these consumers, although evidence supporting this tenet is inconclusive (Ayres et al. 1997). A range of additional general ecological functions at both the organismic and ecosystem level have been proposed (Hättenschwiler & Vitousek 2000, Kraus et al. 2003a). These include the role of tannins as antioxidants, mediators of nutrient availability in soils, and regulating factors of
litter decomposition. In addition, as Zucker (1983) pointed out more than two decades ago, the chemical structure of tannins suggests that there is tremendous scope for specific chemical interactions of tannins both within organisms and in ecosystems. This view of multiple ecological roles for tannins is now widely accepted but data that would allow assembling a clear overall picture of tannin function are still limited (Hättenschwiler & Vitousek 2000, Kraus et al. 2003a).

Figure 16.1. Flavan-3-ols (+)-catechin and (-)-epigallocatechin, examples of monomeric precursors that polymerize to form macromolecular products such as linear proanthocyanidins composed of monomeric flavanoid units connected by C4-C8 linkages.

If tannins remain in leaves following abscission (Table 16.1), similar mechanisms as in plant-herbivore interactions would be expected for trophic interactions between leaf litter and detritivores (e.g. Stout 1989, Ostrofsky 1997, Kraus et al. 2003a), with consequent effects on detritivore performance (Zimmer et al. 2002). There is evidence, moreover, that tannins interact with microbial decomposers (Kraus et al. 2003a), indicating that there is significant potential for tannins to affect litter decomposition in both terrestrial (Horner et al. 1988) and aquatic environments (Stout 1989, Ostrofsky 1993, Campbell & Fuchshuber 1995). Tannin concentration thus could be an important indicator of chemical litter quality when addressing a variety of ecological questions relating to litter use and turnover.

The structural diversity of proanthocyanidins provides challenges for accurate quantitative analyses. Chromatographic characterization of cleavage products is therefore increasingly being used (Waterman & Mole 1994, Hernes & Hedges 2000), especially when specific functions of tannins are to be elucidated. Nevertheless, two simple methods for determining total proanthocyanidins, are