PROTEIN-PRECIPITATING CAPACITY OF TANNINS IN *Shorea* (DIPTEROCARPACEAE) SEEDLING LEAVES

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Abstract—The protein-precipitating capacities of tanniferous extracts from immature and mature leaves of three *Shorea* spp. (Dipterocarpaceae) seedlings were measured by an adaptation of Goldstein and Swain's β-glucosidase precipitation assay. Protein precipitation by the extracts was not correlated with total phenolics (Folin-Denis assay) or proanthocyanidin content (BuOH—HCl assay) as measured in an earlier study. Extracts of *S. maxwelliana* mature leaves had much lower protein-precipitating capacity than those of *S. acuminata* and *S. leprosula*, but fewer insect species feed on and cause less damage to the foliage of *S. maxwelliana* compared with the other species' foliage. Immature leaf extracts of *S. leprosula* and *S. acuminata* had substantial protein-precipitating capacities which in the latter species exceeded that of its mature leaf extracts. Leaf extracts precipitated less protein when initial protein concentration was reduced, although not limiting, but no effect or the reverse effect occurred with quebracho tannin and tannic acid. Problems in the characterization of foliage astringency and the interpretation of its role as a potential antiherbivore defense are discussed.

Key Words—Tannins, *Shorea*, Dipterocarpaceae, insect herbivores, protein precipitation, β-glucosidase, phytochemical defenses.

INTRODUCTION

Tannins adversely affect the feeding, growth, or survival of certain insects (Bennett, 1965; Bernays, 1981; Bernays and Chamberlain, 1980; Bernays et al., 1980; Chan et al., 1978; Feeny, 1970; Fox and Macauley, 1977; Maxwell et al., 1967) and so may defend plants to some extent against herbivores (Feeny, 1976; Rhoades and Cates, 1976). An earlier study (Becker, 1981) found no correlation between insect attack and the concentrations of total phenolics and.
(Folin-Denis assay) or proanthocyanidins (BuOH-HCl assay) in seedling leaves of Shorea maxwelliana, S. acuminata, and S. leprosula from a Malaysian rainforest. The assays employed in that study have serious shortcomings as indices of tannin content (Swain, 1979) and, moreover, equal amounts of different tannins may have quite different protein-precipitating capacities (Haslam, 1974). Since the potential defensive function of plant tannins against herbivores may depend on their capacity to form complexes with proteins (Feeny, 1970), we compared this property in the three Shorea species by adapting Goldstein and Swain's (1965) β-glucosidase precipitation assay to the analysis of leaf extracts. In this assay tannins in a leaf extract cause the precipitation of β-glucosidase from solution by forming a complex with the enzyme. After centrifugation, the amount of protein precipitated, as enzyme, is measured by the difference between the enzyme activities of the supernatant and an appropriate control.

Although leaves of the three Shorea spp. studied have similar concentrations of both total phenolics and proanthocyanidins (Becker, 1981), the potencies of their tannins, as measured by the β-glucosidase precipitation assay, differed markedly. Protein-precipitating capacity of the leaf extracts depended on the initial protein concentration, so we further investigated this effect using commercial tannins.

METHODS AND MATERIALS

Preparation of Extracts. Mature and immature leaves were collected from 0.1–0.4-m-tall seedlings of the 1976 mast crop growing in the shaded, primary forest understory of Pasoh Forest Reserve (Negeri Sembilan, W. Malaysia) during October and November 1979. (See Becker, 1981, for details of the collection protocol; insufficient S. maxwelliana immature leaves remained after previous phytochemical assays for use in this study.) Dried leaves were ground in a Culatti® microbeater and mortar to pass a 0.25-mm (No. 60) sieve and stored for 17 months at room temperature. Samples were oven-dried to constant weight at 70–72°C prior to extraction. All solutions with tannins were processed in glassware. All solutions less than 5 ml in volume were dispensed by adjustable Pipetmans®. Leaf powder (80.0 mg dry wt) was extracted twice for 8 min with 3.4 ml of boiling 50% (v/v) aqueous methanol in a marble-capped centrifuge tube at 90–95°C. The combined extracts were centrifuged (12,000 g, 15 min, 5°C), and the resulting pellet was resuspended in 2 ml of 50% methanol and centrifuged as before. The volume of the combined supernatants was brought to 10.0 ml with 50% methanol. Because methanol inhibits β-glucosidase, solvent was removed from 5.0 or 10.0 ml of this extract at 27–38°C under reduced pressure in a rotary concentrator. The slightly moist residue was redissolved in 5.0 ml of acetate