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Review

Phenolic glycosides of the Salicaceae and their role as anti-herbivore defenses

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ABSTRACT

Since the 19th century the phytochemistry of the Salicaceae has been systematically investigated, initially for pharmaceutical and later for ecological reasons. The result of these efforts is a rich knowledge about the phenolic components, especially a series of glycosylated and esterified derivatives of salicyl alcohol known as "phenolic glycosides". These substances have received extensive attention with regard to their part in plant–herbivore interactions. The negative impact of phenolic glycosides on the performance of many generalist herbivores has been reported in numerous studies. Other more specialized feeders are less susceptible and have even been reported to sequester phenolic glycosides for their own defense. In this review, we attempt to summarize our current knowledge about the role of phenolic glycosides in mediating plant–herbivore interactions. As background, we first review what is known about their basic chemistry and occurrence in plants.

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1. Introduction

Though consisting of barely more than 20 compounds, the phenolic glycosides of the Salicaceae have received disproportionate attention from both phytochemists and chemical ecologists. This can be attributed, at least in part, to their pharmaceutical importance as anti-inflammatory agents which has been known for over 100 years. Other reasons for the great interest are that phenolic glycosides are some of the most abundant secondary metabolites known in plant tissues, and have been identified as important factors in many plant–herbivore studies conducted on poplar, aspen and willows where they have been implicated as toxins and deterrents to a number of insect and mammalian herbivore species.

Here, we summarize the role of phenolic glycosides as anti–herbivore defenses in the Salicaceae. These substances have also been covered in review articles on the secondary metabolites of the Salicaceae (e.g., Chen et al., 2009; Pierpoint, 1994) but have not yet been treated in systematic fashion. Thus, we also describe other aspects of their chemistry and occurrence in plants, including structural properties, methods of chemical analysis, biosynthetic pathways and patterns of genetic, developmental and seasonal variation. However, we do not cover the extensive literature on how phenolic glycosides are influenced by abiotic factors including light, nutrients, water regime and global change variables and their correlation with plant growth (e.g., Harding et al., 2009).

2. Chemical properties of phenolic glycosides

2.1. Structure and terminology

In a broad sense, the word "phenolic glycoside" refers to any molecule containing a sugar unit bound to a phenol aglycone. This description encompasses a vast number of secondary metabolites with only distant chemical or biosynthetic relationships. However, historically the term phenolic glycosides (PGs) has come to be applied just to compounds made up of a core structure consisting of salicyl alcohol and β-D-glucopyranose moieties, with an ether linkage between the phenolic hydroxyl group and the anomeric C-atom of the glucose. This definition will be followed here.

The simplest PG is salicin or D-(−)-salicin (Fig. 1) and hence PGs might best be referred to as "salicinoids". Salicin can be found in many Salicaceae species and is a basic element of the approximately 20 other more complex PGs formed by the esterification of one or more hydroxyl groups (that of the salicyl alcohol function or those of the glucose moiety) with various organic acids. A CAS Scifinder (http://www.cas.org/products/sfacad/index.html) structure search gave 22 compounds (Fig. 1) that matched the structural requirement of a salicin core structure. A few known compounds, namely nigracin, populoside A and salireposide, contain gentisyl alcohol (with an additional free hydroxyl group *para* to the phenolic hydroxyl group of salicin) instead of salicyl alcohol as their basic aglycone (Fig. 1), and these are also regarded as PGs. In contrast, glycosylated derivatives of salicylic acid, rather than salicylic alcohol (e.g., trichocarpin), and other glycosylated phenylpropanoids (e.g., vimalin), phenylethanoids (e.g., salidroside) and benzenoids are not included here even though they may also be specific to the Salicaceae. The esterification of the salicin sub-structure in complex PGs usually occurs at the primary alcohol function of the salicyl alcohol moiety and at the positions 2′ and 6′ of the glucose moiety (Fig. 1) with variable organic acids, commonly benzoic acid and/or 1-hydroxy-6-oxocyclohex-2-en-1-carboxylic acid (HCC), as in tremulacin. Conjugation with these aromatic or aliphatic acids attenuates the hydrophilic character of the salicin core decreasing its water solubility.

The ester bonds of complex PGs are susceptible to chemical and enzymatic hydrolysis, and so these molecules will break down to salicin if not stored appropriately (Lindroth and Pajutee, 1987). Salicin is non-reactive at room temperature, but it has been reported to be light sensitive (Hilden and Morris, 2003) and can be hydrolyzed to yield glucose and salicyl alcohol enzymatically or with dilute acid (Pinto and Diogo, 2008). In contrast to other phenolic compounds in the Salicaceae, such as flavonoids or condensed tannins, most PGs cannot undergo typical anti-oxidative reactions due to the lack of free phenol groups (Zhang et al., 2006) or electron rich double bonds. However, metabolic breakdown of HCC-containing PGs leads to the formation of highly oxidative species (see Section 6).

2.2. Chemical analysis of phenolic glycosides

The preferred way of sampling plant tissue for PG analysis is immediate flash-freezing followed by freeze-drying. Care should be taken to prevent thawing of samples before dryness since hydrolytic reactions in thawed tissue can cause a significant loss of complex PGs (Lindroth and Koss, 1996; Orians, 1995). Alternatively, fresh samples can be vacuum dried without PG loss if they can be processed rapidly (Lindroth and Koss, 1996; Orians, 1995). Dried samples are usually ground and extracted with MeOH or aqueous MeOH. In the latter case, samples should be analyzed promptly to avoid ester hydrolysis. Many authors enhance the extraction process by ultra-sonication (e.g., Förster et al., 2010) or repeatedly extract the tissue to maximize PG recovery.

PG-containing extracts have been analyzed by GC, TLC and HPLC systems. However, GC analysis requires silylation to form volatile derivatives and is therefore rarely used any more. The most frequently reported method is HPLC with gradient elution and UV detection. H_2O spiked with acid and MeOH or acetonitrile are common mobile phases employed with reversed phase C_{18} columns. Standard diode-array detectors are capable of sensitive detection of PGs. The UV chromophore is the aromatic π-electron system of the salicyl alcohol or other aromatic substituents and exhibits the typical absorption spectrum of the benzene ring with three bands caused by π–π^* electron transitions. Many authors use the α-band at about 280 nm which is least sensitive, but most specific for this chromophore. In recent years, the use of LC/MS-systems, typically in the negative ionization mode has become more common for PG analysis.

The unequivocal identification of PGs can only be realized by comparing their retention times to those of authentic standards. Pure standards are also needed to generate curves for absolute quantification. As most PGs are not commercially available, laborious purification from bark or leaves is often necessary. Protocols for purification have been published (Si et al., 2009b; Zhang et al., 2006).

3. Occurrence, patterns of variation and dynamics

Compared to other secondary metabolites, PGs can be very abundant in species of the Salicaceae, and concentrations of up to 30% of plant dry weight have been reported (Donaldson et al., 2006b). However, some species within the Salicaceae do not contain PGs at all (Palo, 1984). The distribution of these compounds within species shows extensive variation with genotype, season and stage of development, and variation among organs is also likely, but less well studied. PGs are commonly found in shoot tissues such as leaves, petioles, internodes, flowers and bark, but neither wood nor root tissue has been investigated thoroughly.

3.1. Genotypic variation of PGs

To date, the more than 20 different PGs described have been found in variable concentrations in members of the Salicaceae. Although some compounds, such as salicortin and salicin, are very widespread, others occur in only a few species (Table 1). Thus,