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Molecular classification of the natural exudates of the rosids

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ABSTRACT

Exudates of the rosid clade of the eudicots have been surveyed and characterized by carbon-13 and proton nuclear magnetic resonance spectroscopy. Of 554 samples divided roughly equally between the subclades fabids and malvids, about two-fifths are resins, a third gums, one-ninth gum resins, one-twelfth kinos, and the remaining not affiliated with these four main molecular classes. Two small new molecular classes, respectively from the Clusiaceae (xanthics) and the Zygophyllaceae (guaiacs), are identified and described.

1. Introduction

Plant exudates are complex mixtures of organic compounds that are secreted into the extracellular matrix and normally appear on the surface of the organism. Released by plants in response to damage, disease, or functions associated with reproduction and early development (Cunningham, 2001; Durkee et al., 1984; Raghavan, 1997; Schmidt-Adam, 1999), exudates usually form as sticky, viscous, substances that solidify over time, although they also are produced as powders. They are widespread in the botanical world (Lambert et al., 2008; Langenheim, 2003; Nussinovich, 2010) and are produced by numerous plant organs, from the shoot (Machado et al., 2013; Pennisi et al., 1999; Wagner et al., 2004) to the root (Neumann and Römheld, 2010; Popovici et al., 2010; Thorpe et al., 2009). These complex phytochemical materials have proved to have utilitarian, medicinal, and cultural uses for a wide variety of peoples (de Faria Lima Santos et al., 2012). They tend to have fairly constant and reproducible chemical constitutions from sample to sample within a given species, but these constitutions can vary enormously between species, genera, and families (Lambert et al., 2007b).

Both nuclear magnetic resonance (NMR) spectroscopy and mass spectrometry (MS) have been used to characterize exudates, and both methods have considerable strengths (Lambert et al., 2008, 2011). NMR spectroscopy was used herein because of the ease and rapidity of the analysis and characterization and because of the richness of analytical information. Sample preparation takes only a few minutes, and the analysis itself requires only about 20 min for examination of carbon-13 (^{13}C) nuclei in the solid state and less time for examination of hydrogen (or proton, ^{1}H) nuclei in solution. The spectra were used for the identification of the molecular class (discussed below), a process that often may be done immediately by inspection, and to detect more subtle distinctions within molecular classes. NMR spectroscopy provides characteristic patterns for these classes rather than identification of individual molecules. A considerable advantage of the ^{13}C NMR spectroscopic method is that it examines the unaltered bulk of the sample, whereas solution and gas-phase methods always involve some sample loss and alteration, depending on degree of solubility, totality of vaporization, and harshness of conditions. Many exudates are entirely insoluble, particularly in chloroform. Consequently, solid state ^{13}C NMR spectroscopy proved to be the only practical analytical technique for a wide-ranging survey. The ^{1}H spectra of exudate solutions are, however, also examined when possible for the

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important information it can provide, such as distinctions between aromatic and alkenic functionalities. Moreover, ^{1}H NMR spectroscopy offers not only the more common one-dimensional version, but also two-dimensional spectra that highlight relationships between hydrogen atoms. Both 1D and 2D methods are used. Since the solid state ^{13}C method is the most useful, the studies herein were restricted to materials that fully solidify, either before or after harvesting, excluding all sticky, semi-solid, or rubbery samples.

Previous studies focused on the conifers (Lambert et al., 2007a,b), the eucalypts (Lambert et al., 2007c), and the legumes (Lambert et al., 2009). The eucalypts (part of the Myrtales) and the legumes (Fabaceae of the Fabales) are members of the flowering plant clade known as the rosids, representing part of only two of its 17 orders. After the gymnospermous conifers, the rosids produce the largest relative abundance of exudates. Because of the plethora of exudate-producing species throughout the rosid clade, the objectives of this study were to find, to sample, and to characterize exudates representing as fully as possible this rich group. In the Angiosperm Phylogeny Group III (APG III) system (Angiosperm Phylogeny Group, 2009), rosids are classified among the eudicots, or true dicotyledons, which are distinguished from the basal angiosperms, the magnoliids, and the monocots. The eudicots in turn are divided into two large clades, the rosids and the asterids. The rosids contain some 70,000 species, more than a quarter of the flowering plants. Of the 59 orders recognized in the APG III system, 17 fall within the rosid clade. These comprise eight orders each under the fabid (or eurosids I) subclade and the malvid (or eurosids II) subclade, plus the order Vitales that is not affiliated with either the eurosids I or II. The fabids contain some 73 families, the malvids some 59 families, and the order Vitales contains the single family Vitaceae. Thus, 554 samples were characterized from 11 of these orders, representing the largest survey of rosid exudates to date (see Table S1 in the Supporting information for a complete list). Although certainly not inclusive of all exudate-producing rosid families, this study, by its size, should be reasonably representative of the relative incidence of exudate production within the rosid clade. It is recognize, however, that no study is statistically comprehensive without examining most of the 70,000 species for exudates an impossible goal.

The spectroscopic examination of rosid exudates herein includes one sample from the Vitaceae of the order Vitales, 263 samples from 19 families and 6 orders of the eurosids I, and 290 samples from 14 families and 4 orders of the eurosids II. The 554 examined exudates include all the previously known molecular classes, two new molecular classes, and a few unclassified materials. The exudates of most of these species have never been examined or characterized molecularly before, and for many cases never previously have been reported.

Although the chemical makeup, timing and locus of production, functional significance for the plant, and amount of exudate produced are extremely diverse (Wollenweber and Dörr, 2009, for the Juglandaceae), analysis of over 1000 exudates from all plant classes by ^{13}C NMR spectroscopy has generated a relatively small number of molecular categories of exudates. The earliest molecular classes characterized were resins, gums, and a natural mixture of the two (gum resins) (Mills and White, 1994). Resins are made

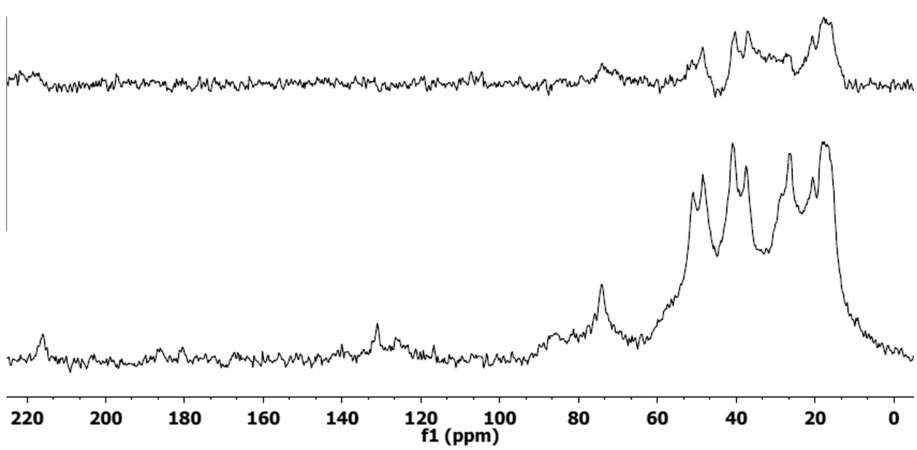

Fig. 1. The solid state ^{13}C NMR spectrum of the exudate from *Dipterocarpus costulatus* of the Dipterocarpaceae with normal decoupling (lower) and with dipolar dephasing (upper). This material is a typical resin.

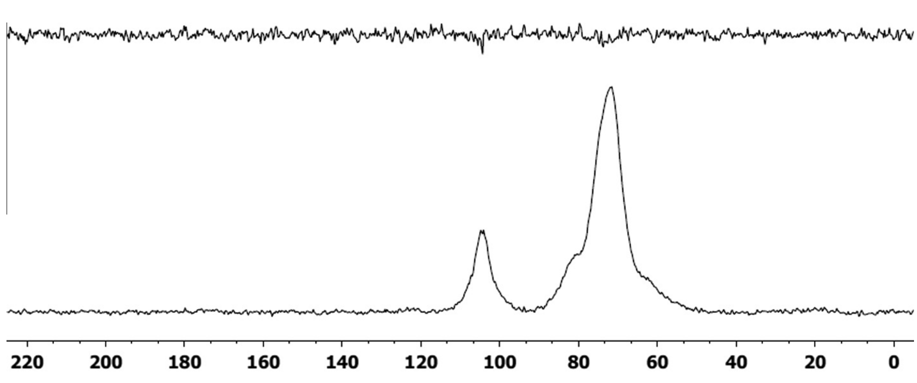

Fig. 2. The solid state ^{13}C NMR spectrum of the exudate from *Anacardium occidentale* of the Anacardiaceae with normal decoupling (lower) and with dipolar dephasing (upper). This material is a typical gum.

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