Characterization of Plant Exudates by Principal-Component and Cluster Analyses with Nuclear Magnetic Resonance Variables

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Principal-component and cluster analyses have been applied to nuclear magnetic resonance data for exudates derived from both conifers and angiosperms in order to classify these materials on the basis of molecular structure. The method succeeds in distinguishing resins produced by the conifer families Araucariaceae, Cupressaceae, and Pinaceae from each other and from resins produced by the angiosperm family Fabaceae. Other exudate types, including gums, gum resins, and kinos, also are distinguished from each other and from the resins.

Plants exude materials with a variety of molecular structures, usually as the result of damage or disease. In our own unpublished tally, we have found approximately 160 vascular plant families that produce exudates, demonstrating that exudation is a widespread phenomenon. Many of these materials are sticky but solidify with time. We have chosen to focus our study on solid plant exudates, which are more easily harvested by hand, have excellent molecular stability over decades, and have found numerous practical and esthetic applications. In contrast, many liquid plant products are the result of human processing, whereby molecular structure is altered. Our intention has been to examine only unprocessed materials, in their natural molecular state, in order to characterize and categorize plant exudates according to family, genus, and species.

There are a number of distinct molecular classes of such solids, indeterminate by visual examination.[1] There is no universal agreement on the names of the molecular classes, but, with one exception, we adopt those used by Langenheim[2] and by Mills and White.[3] First, *resins* consist of terpenoid hydrocarbons with minor heteroatom functionalities. They are readily soluble in organic solvents and are insoluble in water. Solid resins may be predominantly diterpenoid (C_{20}), including those from conifers and legumes, or triterpenoid (C_{30}), from angiosperms, although actual resin structure is far more complex, including not only sesquiterpenes but other natural products. Monoterpenoid (C_{10}) plant products are liquids, often referred to as *oils*. When processed, they become common commercial products such as turpentine. In our study, we exclude both processed and unprocessed oils. We additionally exclude *latexes*, which are water-soluble, milky liquids (emulsions) exuded by some plants.

Polysaccharides provide a second major class of solid exudates, called *gums*.[4] These materials are insoluble in organic solvents but can dissolve in highly polar solvents such as water, depending on the molecular weight. Carbohydrates sometimes combine with terpenoid components to form the mixed exudate class called *gum resins*, which we consider a third major class. The term *gum* in popular usage can refer to a variety of plant and synthetic products, but we restrict it to polysaccharides. Chewing gums include polyterpenoid mastics and natural or synthetic rubber. Eucalyptus trees in Australia are referred to as gum trees. Their exudates, however, are neither polyterpenoid nor polysaccharide but rather polyphenolic, which constitutes a fourth major class of solid exudates. Phenols are aromatic compounds in which the benzene ring possesses at least one attached hydroxy (OH) group. This distinct group of exudates, which extends well beyond the eucalypts, is not considered by Mills and White,[3] and Langenheim[2] calls them *phenolic resins*. In chemical industry today, however, this term refers to the polymeric products of phenols with aldehydes, such as Bakelite, so it is inappropriate in the present context. For phenolic exudates, we therefore prefer the term *kino*, which has been used historically to describe eucalypt and related exudates. Processed phenolic materials provide commercial, liquid materials such as clove oil (yet another use of the term *oil*). Although resins, gums, gum resins, and kinos constitute the four major molecular classes of exudates, we have found isolated examples of materials that have entirely different molecular constitutions.

Because molecularly distinct solid exudates can have remarkably similar physical appearances, spectroscopy (including mass spectrometry) is the preferred method for classification. The volatile molecular constituents of exudates have been separated chromatographically and analyzed by mass spectrometry.[1] NMR spectroscopy offers a general and reliable approach for the analysis of materials in bulk.[1] We employed solid-state ^{13}C NMR methods in our initial investigations into the molecular structure of exudates.[5,6] By examination of the material as the bulk powder, we could characterize it without selection associated with volatilization or solubilization for gas phase and liquid phase analysis, respectively. In our initial survey of three conifer (gymnosperm) families and 15 flowering plant (angiosperm) families, we found distinct ^{13}C NMR patterns for almost every family.[5] Several families, however, were represented by only a single sample, so that the survey was by no means comprehensive.

Although liquid phase methods examine the exudate bulk nonselectively only when the material is completely soluble, the use of ^{1}H NMR methods on solutions has many advantages. Higher sensitivity allows spectra to be recorded on a few milligrams, whereas solid-state ^{13}C NMR analysis requires at least 50 mg. Two-dimensional methods expand the modes of molecular characterization. Materials that fail to form powders can be examined. Peaks are sharper and permit higher resolution spectra. The only drawback to solution ^{1}H NMR analysis is its selection against insoluble components.

Our first such experiments were with the Pinaceae, a family of conifers,[7] followed by an expanded study of all conifer families.[8] We found ^{1}H NMR spectroscopic patterns that distinguished the Pinaceae, Araucariaceae, and Cupressaceae families. Most conifer exudates are resins, but gum resins are found in the Araucariaceae. Our initial examination of flowering plants focused on the Myrtaceae (eucalypts)[9] and the Fabaceae (legumes).[10] Exudates from eucalyptus and related trees are primarily kinos. The Fabaceae, one

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of the largest families of flowering plants, produce resins, gums, gum resins, kinos, and materials that do not fit into any of these molecular classes.

Thus, exudates have been characterized according to molecular classes by the specifics of their ^{13}C and ^{1}H NMR spectra.[5−10] For ^{1}H NMR, both 1D and 2D (COSY, for COrrelation SpectroscopY) spectra were recorded. Thus resins exhibit peaks in the saturated, alkenic, and, occasionally, aromatic spectral regions, whereas gums have peaks only for CH groups attached to oxygen. Gum resins have elements of both types. Kinos have large aromatic resonances. All materials could be examined by ^{13}C NMR methods, but solubility prevented examination of most gums by ^{1}H NMR methods. Kinos could be examined in solution only in highly polar solvents such as DMSO. Gum resins produced solution spectra typical of resins, since the gum component often failed to dissolve.

It is not possible to mention the legion of studies that use NMR or GC/MS to identify specific natural product components, since our focus is characterization of the bulk. NMR has been used to characterized bulk resins in the archaeological context.[11,12]

All our conclusions were based on specialized knowledge and interpretation of NMR peak positions. For example, resins exhibit several spectral types that can be distinguished only by subtle spectroscopic properties, such as the aromatic patterns of pinaceans. We recognize that many botanists, horticulturalists, and others in the biological sciences are not acquainted with the details of NMR spectroscopy or may not have access to the necessary equipment. Consequently, there may be some difficulty in applying this method to exudate classification according to molecular structure. We describe herein a statistical approach using principal-component analyses (PCA), which helps to visualize and expand the spectroscopic classification of plant exudate groups. We have sought thereby to recognize distinctions among different molecular classes and to define these classes solely on the basis of NMR observables, without reference to specialized structural associations ("aromatic region" and so on).

The methods of PCA and the analysis of variance (ANOVA) have been used widely in the sciences to distinguish classes of materials on the basis of simple observables. The elemental content of archaeological materials (pottery, copper, marble), for example, can distinguish geographical sources of the raw materials by PCA.[13] PCA has seen some use in chemistry, for example, for the correlation of solvent properties.[14−16] The PCA method reduces a large number of dependent variables (elemental content, solvent parameters) to a smaller number of uncorrelated (orthogonal) variables, which are called *principal components*. Many of the original variables (or loading factors) covary, either positively or negatively, and hence have built-in redundancies. The principal components are weighted averages of the loading factors. They reduce the redundancies and focus the analysis of variance on fewer variables. The first principal component (PC1) includes most of the variability of the multivariate data set, the second principal component (PC2) the next most, and so on. The power of PCA is to characterize study groups according to their response to the smaller number of focused variables, or principal components. Thus, in archaeology the raw materials from which artifacts are composed can be associated with specific geographical sources according to their response to the principal components. To be sure, classifications could rely entirely on the raw proportions of specific elements, but the use of orthogonal combinations of elements often reveals relationships not discernible by specific elements alone. In the same fashion, reaction mechanisms in chemistry can be sorted according to kinetic responses to solvent parameters.

NMR variables rarely have been utilized for principal-component analysis, and almost entirely in medical applications.[17] For example, disease states could be identified by the PCA of NMR parameters of urine samples of rats.[18,19] From these thin precedents, we decided to utilize NMR observations from three distinct experiments to characterize and distinguish molecular classes of exudates, possibly with insight into taxonomic relationships. 2D ^{1}H NMR data have not been used previously in PCA for any purpose. There have been a few reports of the use of MS data for the study of exudates by PCA,[20−22] although they have been restricted to resinous materials from archaeological sources. We have found no previous studies that encompass the full range of exudate materials from unprocessed botanical sources, with the specific aims of distinguishing types of exudates and plant genera.

Table 1. Exudates Included in the Study Group

exudate class and plant family	genus and species	sample number[a]
resins from the Pinaceae	*Abies homolepsis*	390
	Larix kaempferi	386
	Picea abies	305
	Picea pungens	264
	Pinus monticola	208
	Pinus strobus	258
resins and gum resins from the Araucariaceae	*Agathis atropurpurea*	132
	Agathis australis[b]	128
	Agathis robusta	348
	Araucaria angustifolia[b]	418
	Araucaria columnaris[b]	288
	Araucaria heterophylla	326
	Araucaria laubenfelsii	281
	Wollemia nobilis	517
resins from the Cupressaceae	*Callitris columellaria*	355
	Chamaecyparis lawsoniana	312
	Cupressus bakeri	395
	Juniperus chinensis	394
	Juniperus deppeana	315
	Metasequoia glyptostroboides	318
	Thuja occidentalis	344
resins from the Fabaceae	*Copaifera demeusii*	543
	Hymenaea courbaril	473
	Parkinsonia praecox (*Cercidium viridis*)	532
	Trachylobium hornimannianum	569
	Trachylobium sp. (Borneo)	570
	Trachylobium sp. (Tanzania)	541
gum resins	*Ailanthus* sp. (Simaroubaceae)	614
	Boswellia serrata (Burseraceae)	611
	Bursera microphylla (Burseraceae)	616
	Commiphora opopanax (Burseraceae)	613
gums from the Fabaceae	*Acacia biserrata*	513
	Acacia dealbata	564
	Acacia decurrens	538
	Acacia penninervis	533
	Astragalus gummifer	509
	Prosopis glandulosa	542
	Senegalia (*Acacia*) *modesta*	566
	Senegalia (*Acacia*) *senegal*	567
	Vachellia (*Acacia*) *stenocarpa*	535
kinos from the Myrtaceae	*Corymbia maculata*	383
	Eucalyptus muelleriana	431
	Eucalyptus resinifera	432
	Eucalyptus rubida	433
	Eucalyptus sideroxylon	334

[a] See Table S1 for full characterization. [b] Gum resin.

Results and Discussion

We selected a study group of 45 exudates with representative compositions from the several classes we have described (Table 1). The study group includes resins from the three major conifer families (the Pinaceae, Cupressaceae, and Araucariaceae), resins from one flowering family (the Fabaceae), gums from the Fabaceae, gum resins from three families (the Burseraceae, Simaroubaceae, and Araucariaceae), and kinos from the Myrtaceae. We selected 73 NMR parameters as loading factors (variables), including 19 ^{13}C NMR chemical shifts, 28 ^{1}H NMR chemical shifts, and 26 COSY cross-peaks. These parameters were selected for their abilities to distinguish the various classes and families according to our previous studies.[7−10] For example, resins from the Pinaceae

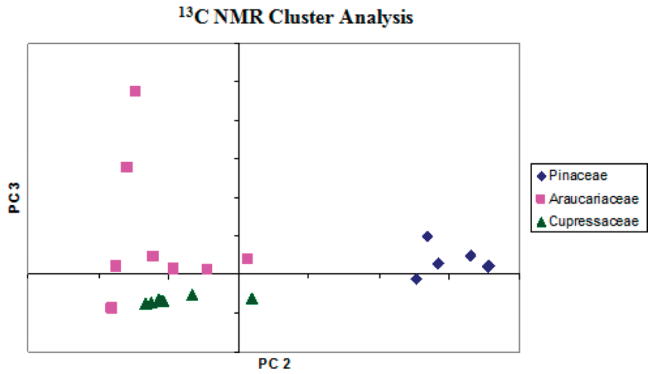

Figure 1. Plot of principal components 2 and 3 for the ^{13}C NMR data for resins from the three conifer families, Pinaceae, Araucariaceae, and Cupressaceae.

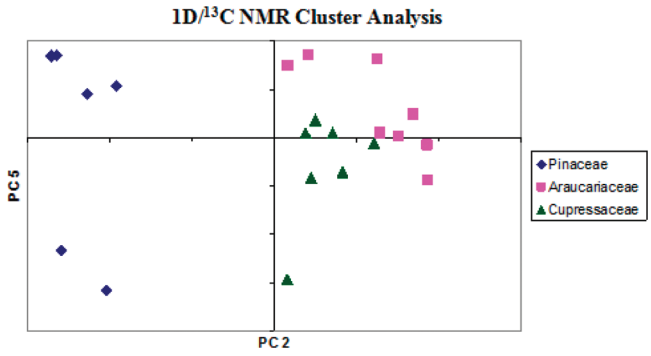

Figure 2. Plot of principal components 2 and 5 for the combined 1D ^{1}H and ^{13}C NMR data for resins from the three conifer families.

almost always exhibited ^{1}H NMR resonances at δ 0.8−0.9, 1.2−1.3, 1.6−1.7, 2.9, 4.9, 5.1−5.2, 5.3−5.4, 5.8, 6.9, 7.0, 7.2, and 7.9. The Cupressaceae exhibited resonances at δ 0.6, 0.8, 1.2, 1.6, 3.7, 4.5, 4.8, 5.1, and 5.8 but lacked peaks at δ 2.9, 7.0, and 7.2 (PCA provides negative as well as positive correlations). The Araucariaceae were characterized by COSY cross-peaks at δ/δ 5.1/5.9 and 5.2/5.9. The Fabaceae had distinguishing cross-peaks at δ/δ 3.1/3.4, 4.2−4.3/6.3−6.5, 5.1/6.3, and 4.8−5.0/6.0−6.1. Gums invariably were dominated by ^{13}C resonances at δ ca. 73 and 104, and kinos exhibited numerous characteristic ^{1}H and ^{13}C NMR peaks in the unsaturated region. The complete list of NMR factors is given in the Experimental Methods.

Figure 1 illustrates the plot of two principal components for just the three conifer families using only ^{13}C NMR data. The axes represent ratios and hence lack units. The Pinaceae form a tight group that is well separated from the other two families, which are separated but close together. Inclusion of the ^{1}H NMR chemical shifts in addition to the ^{13}C NMR chemical shifts also provides good separation, again with the Pinaceae well apart from the other two families (Figure 2). Inclusion of the 2D cross-peaks in addition to the 1D ^{1}H and ^{13}C NMR chemical shifts shows even better separation (Figure 3). Thus, PCA is effective in separating the three conifer groups by means of any of the spectroscopic methods, although the Cupressaceae and the Araucariaceae are always close together. We were not able to discern any meaningful difference between the ^{13}C NMR spectra of Cupressaceae and Araucariaceae samples in our original report.[5] Thus PCA is able to discern distinctions not able to be gleaned from the spectra themselves.

The Fabaceae, like the conifer families, produce resins abundantly.[10] Analysis of the data for all four resin-producing families yields good separation of the Pinaceae and the Fabaceae (Figure 4 for the set composed of ^{13}C chemical shifts and COSY

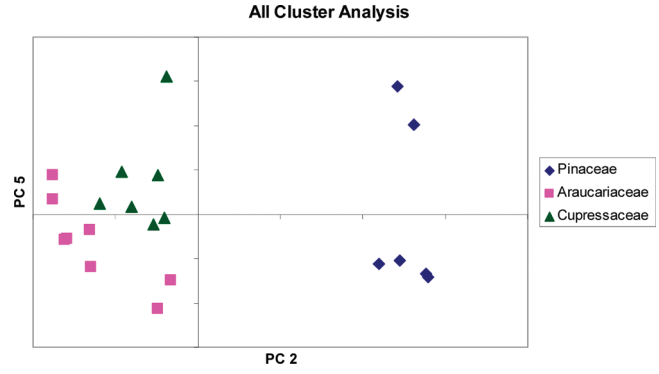

Figure 3. Plot of principal components 2 and 5 for the combined 1D ^{1}H NMR, ^{13}C NMR, and 2D COSY data for resins from the three conifer families.

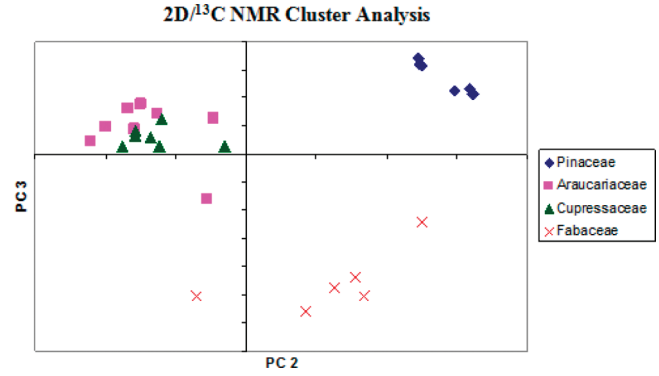

Figure 4. Plot of principal components 2 and 3 for the combined ^{13}C NMR and COSY data for resins from the three conifer families and the Fabaceae family of flowering plants.

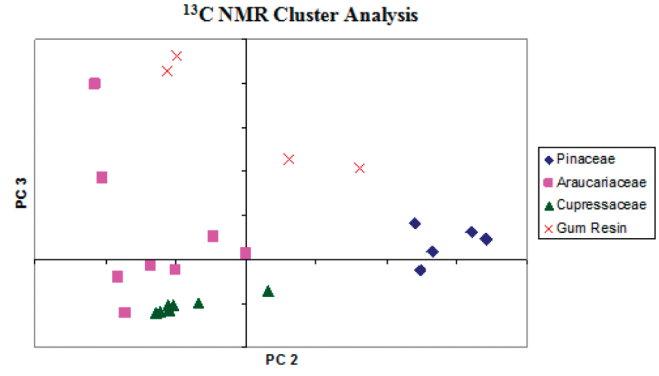

Figure 5. Plot of principal components 2 and 3 for the ^{13}C NMR data of resins from the three conifer families and gum resins from several families.

cross-peaks). The Araucariaceae and Cupressaceae exhibit some overlap in Figure 4.

Combining the three conifer families with the gum resins provides similar separation (Figure 5, for the data set from ^{13}C NMR chemical shifts alone). This plot reveals more about the Araucariaceae. Some of the members of the genus *Araucaria* in fact are not pure resins. *Araucaria angustifolia* is a gum resin in which the major component is gum; *A. columnaris* also is a gum resin but with somewhat more resin than gum; *A. laubenfelsii* is a resin with possibly a small amount of gum; *A. heterophylla* is a pure resin. In Figure 5, the data for *A. angustifolia* provide the point closest to the upper left corner, adjacent to two gum resins on the left whose major component is gum (*Commiphora opopanax* and *Bursera microphylla*). The data for *A. columnaris* provide the other point above the remaining six Araucariaceae, closer to the gum resins.

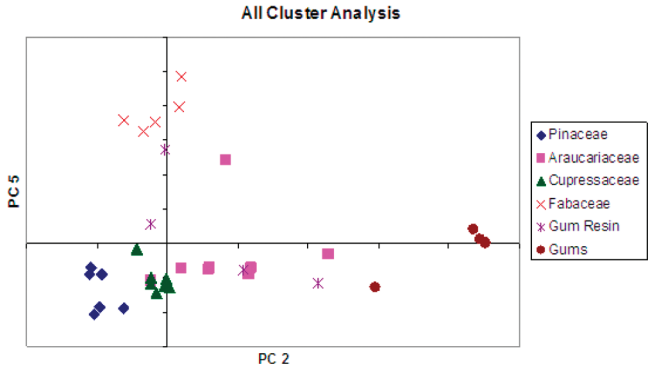

Figure 6. Plot of principal components 2 and 5 for the combined 1D ^{1}H NMR, ^{13}C NMR, and 2D COSY data for resins from the three conifer families and the Fabaceae, gums from the Fabaceae, and gum resins from two families.

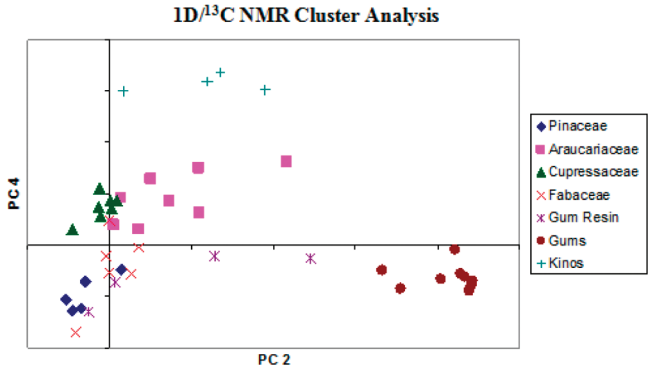

Figure 7. Plot of principal components 2 and 4 for the combined 1D ^{1}H and ^{13}C NMR data for resins from the three conifer families, resins from the Fabaceae, gums from the Fabaceae, gum resins from two families, and kinos from the Myrtaceae.

These two species show separation from the other Araucariaceae in other plots, such as Figure 1. The other two gum resins, on the right (*Ailantus* sp. and *Boswellia serrata*), are primarily resin and are located in Figure 5 to the left of the Pinaceae and to the right of the Araucariaceae and Cupressaceae, i.e., with a strong affinity to resins. Figure 5 is based on the ^{13}C NMR data from bulk material, so that resonances from both gum and resin materials are present. On the other hand, the poor solubility of gum components means that any ^{1}H NMR data (not represented in Figure 5) would derive primarily from the resin component.

Inclusion of gums generally gives good separation (Figure 6 for the combined three data sets). The gums are found on the right, the Fabaceae at the top, the Pinaceae on the far left, the Araucariaceae and Cupressaceae at the center with some overlap, and the gum resins interspersed with the latter resins. One point each from the Araucariaceae (*Araucaria angustifolia*) and from the gum resins lie close to the gums and in fact come from species that are predominantly gum. The kinos also generally give good separation, as in Figure 7 for ^{1}H and ^{13}C NMR chemical shift data. Here the kinos are at the top, the gums are on the right, the resins are scattered around the center with generally good internal separation, and the gum resins are intermediate between the gums and the resins. Not all data were available for all classes. Thus 2D data were not found to be useful for the kino materials[9] and were not available for all gums.

Dendrograms provide another way to visualize data variance and, moreover, to utilize all the data (principal components are selective, as dictated by their loading factors). Whereas the PC plots may provide better separation through focused relationships, the dendrograms represent the complete data set. Figure 8 provides a dendrogram for the three conifer families, based on the ^{1}H NMR

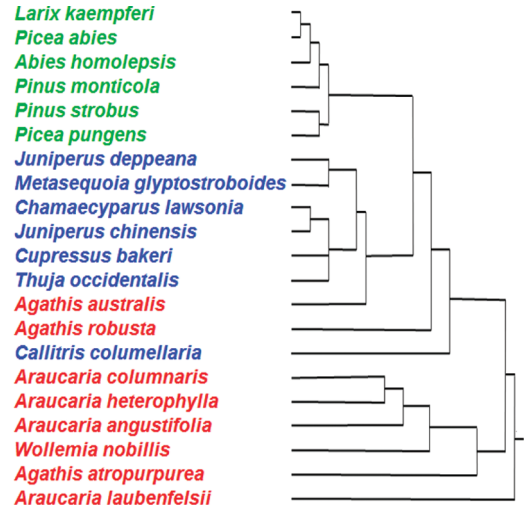

Figure 8. Dendrogram from hierarchical cluster analysis, illustrating separation of the three conifer families according to their 1D and 2D ^{1}H NMR variables: Pinaceae (green), Cupressaceae (blue), and Araucariaceae (red).

data (both 1D and 2D). As with the PC plots, the Pinaceae separate out nicely, and there is nearly complete separation between the Cupressaceae and the Araucariaceae. These data come from dissolved materials, so that the resin portion of the *Araucaria* gum resins predominates in solution. As a result, all the Araucariaceae clump together, with one outlier from the Cupressaceae, indicating that the resin portions of the *Araucaria* gum resins closely resemble the resins of the *Agathis* and *Wollemia* samples.

The dendrogram based on the ^{13}C NMR chemical shifts provides good separation for most classes (Figure 9). The gums and kinos clump together at the top of the dendrogram. Mixed with the gums is *Araucaria angustifolia*, which in fact is a gum resin with largely gum character. The two gum resins (*Commiphora opopanax*, *Bursera microphylla*) with predominant gum character follow directly after the gums. The resins are well separated into distinct groups for the Pinaceae, Fabaceae, Cupressaceae, and Araucariaceae, with two exceptions. In our study of conifers,[8] we noted that the ^{1}H NMR spectrum of *Thuja occidentalis* contained some peaks that distinguished it from other Cupressaceae: "weak alkene peaks and an AX quartet ... at δ 6.5 and 7.0". Its ^{13}C NMR spectrum also is somewhat different from other Cupressaceae. As a result, it falls within the Araucariaceae in Figure 9. The sample from *Parkinsonia praecox* (*Cercidium viridis*) of the Fabaceae is an outlier at the bottom of the dendrogram. Indeed, it was noted in the study of the Fabaceae that the ^{13}C NMR spectrum of this material is unique.[10] The spectrum is clearly of a resin, but is distinct from those of the common spectral type of most remaining Fabaceae, referred to as African-American resins.[10] All the remaining Fabaceae in Table 1 were of this common type. Finally, the two gum resins with predominantly resin content (*Ailanthus* sp. and *Boswellia serrata*) form an outlier group within the other resins. The dendrograms are useful to demonstrate that these two different visualizations, one based on comparisons of specific principal components (Figures 1−7) and another based on analysis of all data (Figures 8 and 9), both provide separation of the different exudate groups.

Summary

Principal-component analysis of NMR data effectively separates the groupings of plant exudates studied to date. The conclusions generally are independent of whether ^{13}C NMR chemical shifts, ^{1}H NMR chemical shifts, or COSY cross-peaks are used. When just conifer resins are examined, the Pinaceae form a tight group well separated from the other two conifer families (Figures 1−3).

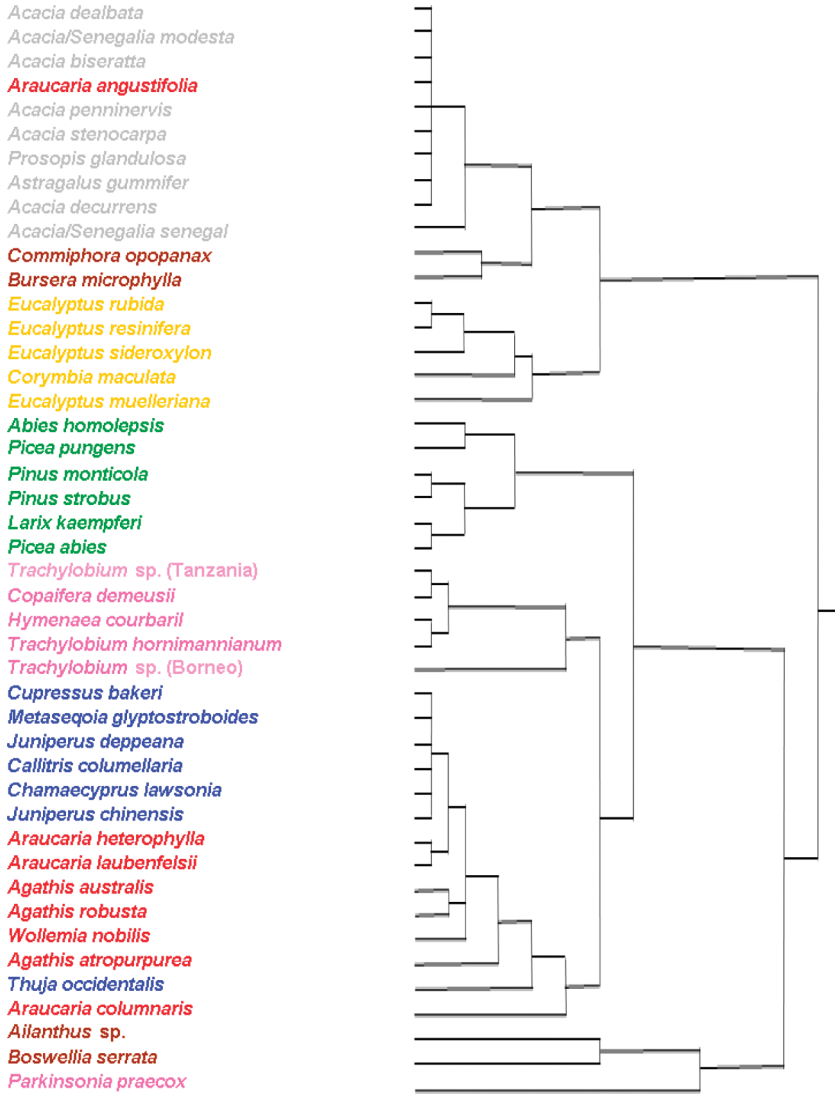

Figure 9. Dendrogram from hierarchical cluster analysis, based on ^{13}C NMR data alone, illustrating separation of gums (gray), gum resins (brown), kinos (yellow), resins from the Pinaceae (green), resins from the Fabaceae (pink), resins from the Cupressaceae (blue), and resins from the Araucariaceae (red).

The Araucariaceae and Cupressaceae resins generally are distinguished in the PCA plots, whereas ^{13}C NMR spectra alone provided no ability to distinguish them.[8] Nevertheless, they are close together in the PC plots and often overlap. Inclusion of resins from the Fabaceae yields plots in which the Pinaceae and the Fabaceae are well separated from each other and from the overlapping Cupressaceae and Araucariaceae (Figure 4). Inclusion of gum resins with the conifers yields similar results (Figure 5). Combination of all the resins with the gums and the gum resins yields plots in which the Pinaceae, the Fabaceae, and the gums are well separated, but the Cupressaceae, the Araucariaceae, and the gum resins overlap (Figure 6). In a plot of all classes, the kinos and gums are well separated from the somewhat overlapping resins, with the gum resins spread over all the resins (Figure 7).

The dendrogram of the resins alone, based on ^{1}H NMR data (Figure 8), generally gives good separation, even of the Cupressaceae and the Araucariaceae. The dendrogram from the ^{13}C NMR spectra for all samples (Figure 9) not only separates the gums, kinos, and resins completely, but even distinguishes gum resins on the basis of their dominant constituent. Those with predominantly gum character are adjacent to the gums; those with predominantly resin character fall within the resins. The gum resin character of *Araucaria angustifolia* of the Araucariaceae is revealed by its placement within the gums. The Cupressaceae and the Araucariaceae are almost completely separated in Figure 9. The somewhat anomalous natures of the spectra of *Thuja occidentalis* of the Cupressaceae and *Parkinsonia praecox* of the Fabaceae are indicated by their placement within the resins.

Experimental Methods

NMR Data. Spectroscopic data from previous studies[5,7−10] were supplemented by unpublished data as indicated in Table S1. Although data were attempted in two solvents ($CDCl_3$ and DMSO-d_6) for all samples, solubility prohibited collection of data for some samples. For this study, only data from $CDCl_3$ were included for the resins and gum resins, and only data from DMSO-d_6 were included for gums and kinos, as these were the most complete data sets. As reported,[9] COSY experiments gave no useful results for the kinos, so no data for them were included in this study.

Principal-Component Analysis. From previously recorded ^{13}C NMR spectra,[5,9,10] 19 chemical shifts or chemical shift ranges were selected on the basis of the frequency of their occurrence and their abilities to distinguish different classes or genera: δ 13, 18−20, 25, 28, 30−31, 34, 38−40, 43−44, 46, 48−50, 52, 56−58, 72−74, 104−105, 106−108, 145, 154, 179, and 204. Ranges are reported for peaks that clearly are related structurally but do not always resonate at a precise position. From one-dimensional ^{1}H NMR spectra,[7−10] 28 chemical shifts or chemical shift ranges similarly were selected: δ 0.6, 0.8−0.9, 1.0, 1.1, 1.2−1.3, 1.6−1.7, 2.9, 3.5, 3.6, 3.7, 3.8, 4.0, 4.4, 4.5, 4.6, 4.8−4.9, 5.0−5.2, 5.3−5.4, 5.5, 5.7−5.9, 6.4−6.5, 6.7−6.8,

6.9, 7.0, 7.1, 7.2, 7.4−7.6, and 7.9. From the COSY spectra,[7−10] 26 cross-peaks were selected: δ/δ 1.2/2.8, 1.2/3.1, 1.5−1.6/2.9, 1.5−1.7/4.5, 1.7/5.2−5.3, 1.8/2.7−2.9, 2.1/4.8, 2.1/5.4, 2.4/5.4, 2.5/2.8, 3.1/3.4, 3.4/3.8, 4.2−4.3/6.3−6.5, 4.2/5.4, 4.5/4.9, 4.8−5.0/6.0−6.1, 4.9−5.8, 4.9/6.3, 5.0/6.3, 5.1/5.9, 5.1/6.3, 5.2/5.9, 6.6/6.7, 6.9/7.2, 7.0/7.2, and 7.4/7.7.

For the ^{13}C and 1D ^{1}H NMR chemical shifts, intensities were measured on a scale of 1 to 10 (Table S2). When multiple peaks occurred within the range, as was often the case with 1D ^{1}H NMR spectra, the most intense peak was chosen for the intensity measurement. Each COSY cross-peak was assigned an intensity on a four-point scale: absent (0), weak (1), medium (2), and strong (3) (Table S2). These 73 variables provided the input data for PCA analysis.

The three sets of decimalized NMR parameters were uploaded into SPSS 16.0 for Windows. Principal-component calculations for the plots in Figures 1−7 were based on the component matrices. The axes were scaled according to the relative values of the component matrices. The first few principal components generally included the majority of data variance, but up to seven principal components were calculated. Table S3 gives the factor score coefficient matrix (weighting or loading factors for the resulting principal components). Table S4 provides the amounts of variance retained by each principal component. For the most part, the first principal component, PC1, was dominated by data from the saturated functionalities (δ 0−2.5 for protons and δ 0−40 for carbons). As these were present in all of the resins, gum resins, and kinos, this principal component was not useful in distinguishing these groupings and is not represented in the plots.

Seven data sets were used: ^{13}C chemical shifts alone, ^{1}H NMR chemical shifts alone, COSY cross-peaks alone, ^{13}C and ^{1}H NMR chemical shifts together, ^{1}H NMR chemical shifts with COSY cross-peaks, ^{13}C NMR chemical shifts with COSY cross-peaks, and all three methods combined. Separate analyses were carried out for four different plant groups: conifer resins alone (Figures 1−3), conifers with Fabaceae resins (Figure 4), conifers with gum resins (Figure 5), and all exudates (resins, gums, gum resins, kinos, Figure 7). Calculations with gums were limited by solubility considerations. Each of these 28 calculations generated a different set of loading factors and principal components (Tables S3 and S4). Plots were constructed between pairs of principal components in two dimensions (Figures 1−7). Hierarchical cluster analysis using the Ward method or average linkage produced dendrograms based on all variance, demonstrating the relationships between all materials (Figures 8 and 9).

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Supporting Information Available: Table S1, listing the sources of the 45 exudates and citations for any previous publication of the NMR data. Excel Table S2, listing the NMR intensity values for 1D proton, 2D COSY, and 1D carbon spectra. Excel Table S3, listing the factor score coefficient matrices for all principal-component plots. Excel Table S4, listing the amounts of variance retained by each principal component. This material is available free of charge via the Internet at http://pubs.acs.org.

References and Notes

(1) Lambert, J. B.; Santiago-Blay, J. A.; Anderson, K. B. *Angew. Chem., Int. Ed.* **2008**, *47*, 9608–9616.
(2) Langenheim, J. H. *Plant Resins*; Timber Press: Portland, OR, 2003.
(3) Mills, J. S.; White, R. *The Organic Chemistry of Museum Objects*, 2nd ed.; Butterworth-Heineman: Oxford, UK, 1994.
(4) Nussinovitch, A. *Plant Gum Exudates of the World: Sources, Distribution, Properties, and Applications*; CRC Press: Boca Raton, FL, 2010.
(5) Lambert, J. B.; Wu, Y.; Santiago-Blay, J. A. *J. Nat. Prod.* **2005**, *68*, 635–648.
(6) Santiago-Blay, J. A.; Lambert, J. B. *Am. Sci.* **2007**, *95*, 150–157.
(7) Lambert, J. B.; Kozminski, M. A.; Fahlstrom, C. A.; Santiago-Blay, J. A. *J. Nat. Prod.* **2007**, *70*, 188–195.
(8) Lambert, J. B.; Kozminski, M. A.; Santiago-Blay, J. A. *J. Nat. Prod.* **2007**, *70*, 1283–1294.
(9) Lambert, J. B.; Wu, Y.; Kozminski, M. A.; Santiago-Blay, J. A. *Aust. J. Chem.* **2007**, *60*, 862–870.
(10) Lambert, J. B.; Heckenbach, E. A.; Hurtley, A. E.; Wu, Y.; Santiago-Blay, J. A. *J. Nat. Prod.* **2009**, *72*, 1028–1035.
(11) Ghisalberti, E. L.; Godfrey, I. M. *Stud. Conserv.* **1998**, *43*, 215–230.
(12) Lambert, J. B.; Shawl, C. E.; Stearns, J. A. *Chem. Soc. Rev.* **2000**, *29*, 178–182.
(13) Lambert, J. B. *Traces of the Past*; Addison-Wesley: Reading, MA, 1997.
(14) Cramer, R. D. *J. Am. Chem. Soc.* **1980**, *102*, 1837–1849.
(15) Maria, P.-C.; Gal, J.-F.; de Franceschi, J.; Fargin, E. *J. Am. Chem. Soc.* **1987**, *109*, 483–492.
(16) Katritzky, A. R.; Fara, D. C.; Kuanar, M.; Hur, E.; Karelson, M. *J. Phys. Chem.* **2005**, *109*, 10323–10341.
(17) Robertson, D. G.; Reily, M. D.; Sigler, R. E.; Wells, D. F.; Paterson, D. A.; Braden, T. K. *Toxicol. Sci.* **2000**, *57*, 326–337.
(18) Pan, Z.; Gu, H.; Talaty, N.; Chen, H.; Shanaiah, N.; Hainline, B. E.; Cooks, R. G.; Raftery, D. *Anal. Bioanal. Chem.* **2007**, *387*, 539.
(19) Schoonen, W. G. E. J.; Kloks, C. P. A. M.; Ploemen, J.-P. H. T. M.; Smit, M. J.; Zandberg, P.; Horbach, G. J.; Mellema, J.-R.; Thijssen-vanZuylen, C.; Tas, A. C.; van Nesselrooij, J. H. J.; Vogels, J. T. W. E. *Toxicol. Sci.* **2007**, *98*, 286–297.
(20) Hayek, E. W. H.; Krenmayr, P.; Lohninger, H.; Jordis, U.; Moche, W.; Saute, F. *Anal. Chem.* **1990**, 2038–2043.
(21) Colombini, M. P.; Modugno, F.; Ribechini, E. *J. Mass Spectrom.* **2005**, *40*, 675–687.
(22) Modugno, F.; Ribechini, E.; Colombini, M. P. *Rapid Commun. Mass Spectrom.* **2006**, *20*, 1787–1800.

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