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Taxonomic and Chemical Relationships Revealed by Nuclear Magnetic Resonance Spectra of Plant Exudates

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Exudates collected from 65 species of gymnosperms and angiosperms were examined by solid-state carbon-13 (^{13}C) nuclear magnetic resonance (NMR) spectroscopy. Diagnostic criteria were developed to distinguish resins, gums, and gum resins. The typology generated from the exudate spectra generally follows current taxonomic classifications, suggesting that ^{13}C NMR spectroscopy may have applications in exudate identification, at least at the familial level, and in some cases at the generic or specific levels.

Throughout history, numerous cultures have used and valued plant exudates, including resins, gums, gum resins, and latexes.[1−6] Rubber, turpentine, perfumes, paint solvents, and cosmetics contain exudates and have had considerable economic value. Exudates have been used for religious purposes,[7] for imparting luster to paper and textiles, as an admixture in construction materials, as dental adhesives, as lubricants in surgical instruments, as bow rosin for some stringed musical instruments, as a stabilizer of wine, and simply as objects of beauty in jewelry. They are produced by cells and released onto the surface of plants, usually as the result of injury.[8] For the purpose of this study, exudates are limited to organic compounds that do not require chemical extraction.[9−12] In other words, these materials may be picked or scraped from the surface of plants.[13]

Exudates may have very similar physical appearances and properties and yet very different molecular structures. It was the objective of the present study to provide simple criteria by nuclear magnetic resonance (NMR) spectroscopy for distinguishing types of exudates. Furthermore, we sought to document chemical differences at the family, genus, and species level by the same technique. To date, there have been no simple and direct methods to specify exudate type (gum, resin, gum resin, latex), nor has taxonomy been related in any way to NMR properties.

Resins are water-insoluble mixtures largely of terpenes (organic derivatives composed of isoprene building blocks).[1] Fossilization renders resins extremely resistant to dissolution, and such materials usually are referred to as *fossil resins*, *resinites*, or, inappropriately in the general sense, *amber*.[14] *Gums* are water-soluble or water-dispersible mixtures of high molecular weight polysaccharides.[15] Gums have been used as adhesives, watercolor media, and foodstuffs. The most famous example probably is gum Arabic from *Acacia* species. *Gum resins* such as myrrh and frankincense contain both terpenoid and carbohydrate components. *Latexes* are usually whitish, milky fluids consisting of tiny droplets of organic compounds suspended or dispersed in an aqueous medium. The naturally occurring latex material called gum elastic or caoutchouc, from the plant *Castilla elastica*, was developed in the pre-Hispanic New World for balls, bands, shoe soles, and small figurines. Processed into rubber, this material became widely used in Europe after the development of vulcanization, which, through cross-linking with sulfur atoms, generates a stronger and more elastic material. Other exudates include waxes and natural lacquers.[1]

Our interest in modern resins arose from our examination of fossilized resins or amber.[16] With a series of samples

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from Australia and New Zealand, we were able to follow chemical structural changes, by their solid-state ^{13}C NMR spectra, from modern *Agathis* resin through semifossilized samples (thousands of years old) to fully fossilized materials (millions of years old).[17,18] Subsequently, we reported the ^{13}C spectra of single samples of six other modern genera.[19] We found that the spectral signatures of these materials varied from genus to genus. On the basis of this small sampling, it appeared that NMR may provide a new diagnostic for taxonomic relationships among plants.

Consequently, we have amassed a large inventory of modern resins, along with related exudates such as gums and gum resins. All three categories often are indistinguishable to the eye in the field. The three types together, however, may be distinguished visually from latexes, which are milky liquids and hence were not included in this study. Even so, we can easily identify latexes by their distinctive NMR spectrum of linear polyisoprene.

We have developed herein what may be called a library of chemical signatures, which provides a unique chemical perspective on taxonomic relationships. We report the results for 65 species from 20 plant families. Moreover, these materials constitute the starting point in the process of fossilization and may shed light on the sources of various fossilized resins. The spectral signatures also provide a measure of the authenticity of resinous materials lacking provenance. The spectra of both resins and gums are quite distinct from those of synthetic polymers that can resemble them in appearance and constitute fraudulent commercial samples,[20,21] possibly even with artificially entombed plants or animals.

The NMR experiment is carried out on the carbon portion of the sample. NMR provides a census of the structural types of a particular nucleus in a sample.[23] Carbon provides the molecular skeleton of these organic materials and hence is rich in structural information. Although the experiment may be performed on either solids or liquids, we chose the solid-state method with magic angle spinning and cross polarization. Many modern resins are poorly soluble; all fossilized resins are nearly insoluble. Whereas many resins dissolve in organic solvents, gums either dissolve sparingly in water or are entirely insoluble in all solvents. Thus a variety of conditions would be necessary to examine samples in solution, and many samples would be excluded because of poor solubility. The solid-state experiment examines the bulk material directly, either crushed or powdered, with all samples examined in exactly the same way. Only examination in the solid state provides a single experiment under constant conditions for all samples, whether modern or fossilized, whether resin or gum. Dissolution would be selective, since different samples vary widely in solubility.

The mass spectrometric (MS) experiment requires the sample to be volatilized. Although considerable information is so obtained, the MS experiment is highly selective in the extent to which a given sample provides gaseous material. Typically, small- to medium-sized molecules outgas, but polymer molecules, particularly when cross-linked, fail to leave the sample. While the resulting MS experiment is valuable, it does not reflect the bulk of the sample. MS comparisons between very different samples then may be inappropriate.

Each sample was examined under two distinct sets of NMR conditions. In spectra with *normal decoupling*, the coupling interaction between carbons and their attached protons is spectroscopically removed. The process also increases the sensitivity of the experiment somewhat. The spectroscopic result is a signal from all types of carbon, independent of the number of attached hydrogens (C, CH, CH_2, CH_3). In spectra with *dipolar dephasing* (also called *interrupted decoupling*),[22] signals occur only from carbons entirely lacking attached hydrogens and from some carbons (usually methyl groups) with attached hydrogens that are moving rapidly in the solid state. The result of the two experiments is a pair of spectral diagnostics or fingerprints for the material under examination.

Exudate production is geographically, temporally, and taxonomically widespread in plants. Exudates have been collected as far north as 80° N (Axel Heilberg Island, Canadian Arctic) and as far south as ca. 45° S (New Zealand).[24] Latitudinal or other geographical features of exudate production have not yet been discerned. Possible plant exudates have been attributed to medullosan seed ferns, *Myeloxylon*, from as early as the Carboniferous [354−290 million years ago (Ma)].[25] Vascular plant exudates may have existed as early as the Early Devonian (ca. 395 Ma).[26] For many fossilized resins, the plant source of the exudate is unknown or controversial. Various ecological reconstructions depict amber forests near sea level,[27] and resin production appears to have been associated with aqueous habitats.[28]

Numerous species are reported to produce exudates, and in some cases exudate production is the general rule within a genus.[29,30] Thus "a hundred or more species of *Acacia* are known to yield gum".[31] An as yet unpublished compilation of worldwide exudate production lists over 600 genera of exudate-producing plants in approximately 160 vascular plant families.[32]

Vascular plants are the dominant producers of exudates. The gymnospermous families (those with naked seeds, roughly, the evergreens) having the most genera of resin producers are the Cupressaceae and Pinaceae. The most common angiospermous (flowering plants) exudate producers include the Anacardiaceae, Burseraceae, Dipterocarpaceae, Euphorbiaceae, and Fabaceae/Leguminosae. Whereas gymnosperm genera are primarily resin producers, the angiosperms may produce resins, gums, or latexes. Gums are especially commonly produced by the Fabaceae/Leguminosae and the Sterculiaceae. Latexes are commonly produced by the Apocynaceae, Asclepiadacee, Euphorbiaceae, and Sapotaceae.

Exudates are produced mainly by trees. In the large compilation by Shiva,[10] approximately 95% of the listings are from trees and 5% from herbs; contributions from other vascular plants (ferns, climbers, grasses, and shrubs) are negligible. To our knowledge, neither nonplants, like fungi and lichens, nor nonvascular plants, like mosses, liverworts, and hornworts, have been implicated unequivocally in exudate production. Exudates usually occur on the above-ground portion of the trees. Resin production from roots is uncommon, although it has been reported and is a prime suspect to explain the abundance of soil-dwelling organisms, such as fossorial millipedes, in amber.[33] Plants that produce root exudates include the trees *Pinus strobus* (Pinaceae) and *Liquidambar styracifolia* (Hammamelidaceae).[32]

Exudate production has been associated overwhelmingly with injury or infection of plant tissues,[31] including accidents such as lightning or fire,[34] as well as with extreme environmental conditions. Poor soil and dry climate, for example, are correlated with gum production from *Acacia senegal* in the Sudan.[31]

The anatomical basis of exudate production varies with the type of exudate and the identity of the major plant

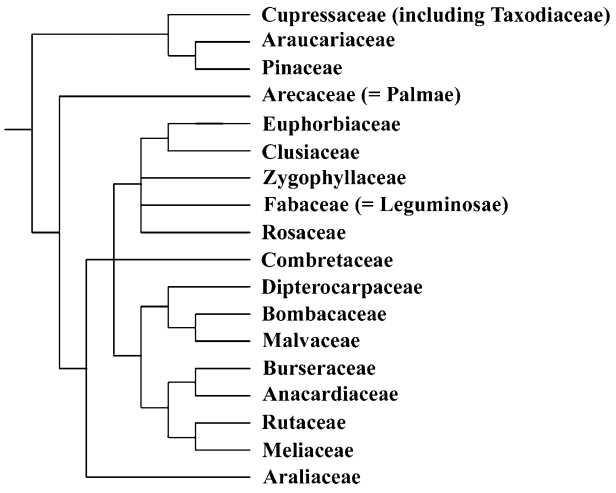


Figure 1. Plant phylogeny for families in this study.

group, i.e., gymnosperm or angiosperm. Resins are produced by epithelial derivative cells adjacent to parenchymatous cells, whose contents are released into elongated resin canals (for gymnosperms) or into more globular resin pockets or blisters (for angiosperms).[35,36] Gums are produced by parenchymatous cells derived from the cambium. Latexes are produced by cells, known as laticifers, from several plant tissues, such as the parenchyma and the secondary phloem.

Results and Discussion

Since our objective is to relate the ^{13}C spectral signatures to plant taxonomy, initially we must specify the taxonomic relationships of the plant families we have examined. Figure 1 provides a dendrogram of such relationships.[37] The first three families [Cupressaceae (including the Taxodiaceae), Araucariaceae, Pinaceae] are gymnosperms. This group contains the familiar conifers or evergreens such as pines, firs, spruces, redwoods, and cypresses. The remaining families listed in Figure 1 are angiosperms. Of the angiosperms studied, only the Arecaceae/Palmae are monocotyledonous; the remaining angiosperm families are dicotyledonous.

We divide our discussion according to the types of exudates: resins, then gums, and finally gum resins. Some families can produce more than one of these materials and accordingly will be discussed in more than one section.

Resins. Resinous exudates are composed of various terpenoid molecules. Saturated (C−C) terpenoid carbons attached only to hydrogen or other carbons produce resonances in the region δ 10−50. Unsaturated carbons (C=C) resonate in the region δ 110−150. Saturated carbons bonded to oxygen (C−O) appear in the region δ 60−80. Occasionally, oxidation of carbon atoms produces carbonyl groups (C=O), found in the region δ 170−210. Specifically absent in resins are resonances at δ 95−105 for carbons attached to two oxygens through single bonds (OCO). This structural entity occurs in all sugars (carbohydrates) and is diagnostic for gums and gum resins.

Cupressaceae Including the Taxodiaceae. Our discussion of resins follows the sequence of families in Figure 1, starting with the conifers. The first several families to be discussed exude diterpenoid resins. Until recently, the Cupressaceae and the Taxodiaceae constituted two distinct families.[38,39] Current classification, however, combines them into a single family, the Cupressaceae. This large gymnospermous family contains the cypresses, cedars, junipers, and sequoias.

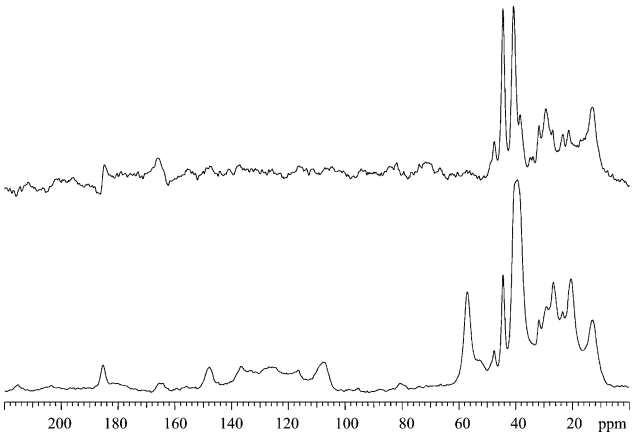


Figure 2. The 75 MHz ^{13}C NMR spectra of the exudate from *Cupressus arizonica* with normal decoupling (lower) and with dipolar dephasing (upper), resin Group CA.

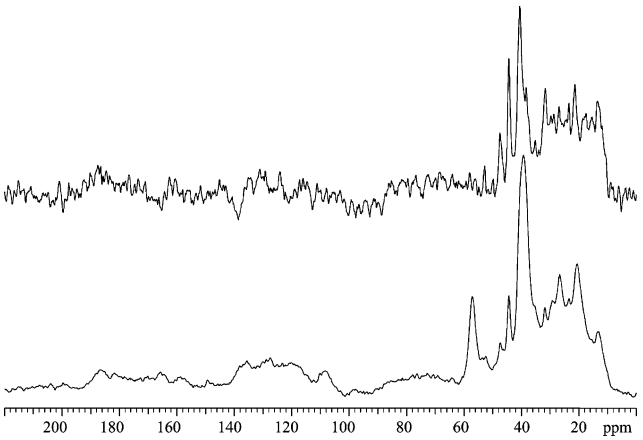


Figure 3. The 75 MHz ^{13}C NMR spectra of the exudate from *Metasequoia glyptostroboides* with normal decoupling (lower) and with dipolar dephasing (upper), resin Group CA.

The spectra for *Cupressus arizonica* (Figure 2, Arizona cypress) are representative of these species. In all such displays, the spectrum with normal decoupling is at the bottom, and that with dipolar dephasing (interrupted decoupling) is at the top. There is only a small carbonyl resonance in Figure 2. The unsaturated region (δ 110−150) contains peaks of small intensity, including clear exomethylene resonances at δ 108 and 148 (respectively, the CH_2 and C in C=CH_2). There are no C−O or OCO peaks. Thus most of the spectrum is concentrated in the saturated region, which is dominated by the strong peak at δ 39. There are several other sharp peaks, particularly at δ 13, 20, 26, 44, and 57. With interrupted decoupling, only peaks from saturated carbons survived, none dominant, particularly at δ 13, 30, 40, and 44.

Remarkably, almost every species from the Cupressaceae gave very similar spectra. This close resemblance indicates that the terpene contents are nearly identical in terms of molecular structure. Even the spectra of the species *Metasequoia glyptostroboides* (Figure 3, dawn redwood), formerly placed in the Taxodiaceae, are nearly identical to those of *Cupressus arizonica* (Figure 2), supporting the recent incorporation of this species into the Cupressaceae.[38]

There are small differences between the spectra of Figures 2 and 3. The carbonyl peak at δ 185 and the exomethylene peak at δ 147 (the C in *C*=CH_2) are more pronounced for *Cupressus arizonica* than for *Metasequoia glyptostroboides*. The Cupressaceae species *Calocedrus decurrens* (incense cedar), *Chamaecyparis lawsoniana* [Port

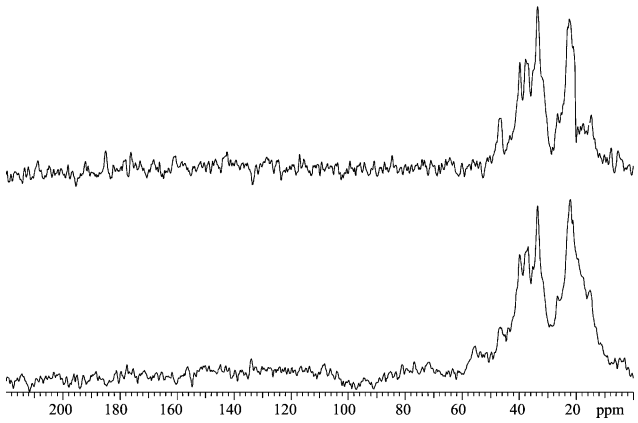


Figure 4. The 75 MHz ^{13}C NMR spectra of the exudate from *Sequoia sempervirens* with normal decoupling (lower) and with dipolar dephasing (upper), resin Group S.

Orford cedar, Oregon cedar, or white cedar or Lawson (false) cypress], *Cupressus sempervirens* (Italian cypress), *Juniperus deppeana* (alligator juniper), and *Thuja plicata* (giant arborvitae or western red cedar) have spectra most similar to those of *Metasequoia glyptostroboides*, whereas *Chamaecyparis formosensis* (Formosan cypress), *Chamaecyparis obtusa* (Hinoki (false) cypress), and *Cupressus montana* (often considered to be a variety of the Arizona cypress) have spectra most similar to those of *Cupressus arizonica*. The spectrum with normal decoupling of the Hinoki cypress, however, has very weak peaks outside the saturated region and a new sharp peak at δ 33, which survives with dipolar dephasing.

The spectra of the species *Sequoia sempervirens* (Figure 4, coast redwood), previously belonging to the Taxodiaceae, are clearly different from those of *Metasequoia glyptostroboides* (Figure 3) and the other Cupressaceae. There are peaks only in the saturated region, now with two dominant contributors at δ 20 and 32 and smaller peaks at δ 15, 27, 38, 40, 47, and 56. Most of the peaks, except that at δ 56, survive with dipolar dephasing. This pattern represents, to date, a unique fingerprint, which we call Group S. Thus the Cupressaceae give at least two quite distinct spectral fingerprints, one major and one minor, which could be indicative of unresolved taxonomic problems.

Araucariaceae. In previous work,[18,19] we reported the spectra of five *Agathis* species [*australis* (kauri pine), *atropurpurea* (blue (kauri) pine), *lanceolata* (Koghis kauri), *macrophylla* (kauri variant), and *moorei* (Moore kauri)] and of *Wollemia nobilis* (Wollemi pine). We found that these six samples, along with two types of Manila copal (Pangalinan and Loba), gave a common spectral signature. Figure S1 in the Supporting Information presents the previously unpublished spectra of Loba and is representative of these materials. Rice[40] states that Manila copal does indeed derive from *Agathis* sources in Indonesia or the Philippines and that the term *Loba* is used for Philippine resin whose harvesting is delayed for several weeks after tapping to allow it to harden.

The spectra of Loba in Figure S1 are similar if not identical to those described above for *Cupressus arizonica* (Figure 2), *Metasequoia glyptostroboides* (Figure 3), and indeed for the entire Cupressaceae except for *Sequoia sempervirens* (Figure 4). The defining pattern in the spectra with normal decoupling of many members of the Cupressaceae and the Araucariaceae is the dominant peak at δ 38−9 with two significant peaks to the left and three significant peaks to the right in the saturated region, plus pronounced unsaturated peaks between δ 108 and 148 and usually a single carbonyl peak at δ 185. Most diagnostic of this group in the spectra with dipolar dephasing are the two sharp, closely spaced peaks at δ 40 and 44 and often but not always two shorter and broader peaks at δ 13 and 29. These characteristics are well represented in Figures 2 and S1.

In an important variant of this pattern, the unsaturated peak at ca. δ 150 with normal decoupling is weak or entirely missing, and there are one or two additional peaks with dipolar dephasing, as illustrated in the Cupressaceae by *Metasequoia glyptostroboides* (Figure 3), *Calocedrus decurrens*, *Chamaecyparis lawsoniana*, and *Thuja plicata*, among others. We also find that *Araucaria araucana* (monkey-puzzle tree) in the Araucariaceae shares these features (Figure S2 in the Supporting Information). We return to these spectra when we discuss other members of the genus *Araucaria*.

The common features of all these spectra justify identifying a large group of plants that produce essentially identical resins (according to the carbon skeleton), which we shall call Group CA (for Cupressaceae-Araucariaceae). The single species *Sequoia sempervirens* of the Cupressaceae (formerly Taxodiaceae) then constitutes the small, separate Group S.

Pinaceae. Our previous study[19] examined only the single species *Pinus monticola* (western white pine) from this family. We did not know whether the observed spectral patterns would be generally characteristic of *Pinus* samples from other species, much less those of other Pinaceae genera. We did, however, note that several examples of commercial rosin (U.S. Rosin, Chinese gum rosin, commercial violin rosin), sea amber (so-called because it had spent considerable time in some body of water before being harvested), a resin collected in Chichicastenango, Guatemala, without reported species, and a sample called Bical but said to be Manila copal all exhibited spectra similar to those of *Pinus monticola*. Since Manila copal normally comes from *Agathis* sources,[40] the result with Bical was suspicious. The term "Bical" may refer to the Biomass Industrial Crops Ltd., and the material may not have been true Manila copal.

In addition to *Pinus monticola* and the various resins listed above, we now have recorded the spectra of 13 pinacean species from five genera: *Abies koreana* (Korean fir), *Abies sachalinensis* (Sakhalin fir), *Cedrus atlantica glauca* (blue atlas cedar), *Cedrus libani* (cedar of Lebanon), *Picea abies* (Norway spruce), *Picea koyamai* (Koyama spruce), *Picea pungens* (Colorado blue spruce), *Pinus elliotii* (slash pine), *Pinus nigra nigra* (European black pine called Austrian pine), *Pinus rudis* (Mexican red pine), *Pinus strobus* ((eastern) white pine), *Pinus thunbergiana* (Japanese black pine), and *Pseudotsuga menziesii* (Douglas fir). Also, we have found that a sample called Moroccan copal falls into this group.

All these materials generated remarkably similar spectra, of which Figure 5, of *Pinus rudis*, is representative. The saturated region with normal decoupling is characterized by large peaks at δ 17, 24, 38, and 48. There is a low-intensity, broad peak in the C—O region δ ca. 70, nothing in the OCO (sugar) region, unsaturated peaks mostly between δ 114 and 138 with only very small exomethylene carbon peaks (C=CH_2) at δ ca. 110 and 146, and a small, sharp carbonyl peak at δ 187. The spectrum with dipolar dephasing, as we have already noted,[19] is highly diagnostic. The saturated region contains four sharp peaks at δ 17, 24, 38, and 48 (the peak at δ 38 often is split in two, giving

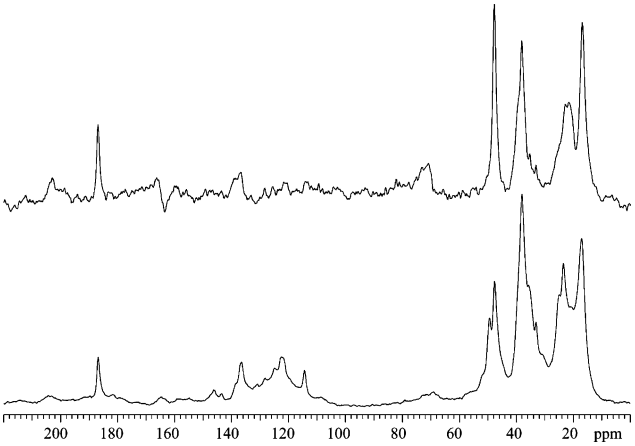


Figure 5. The 75 MHz ^{13}C NMR spectra of the exudate from *Pinus rudis* with normal decoupling (lower) and with dipolar dephasing (upper), resin Group P.

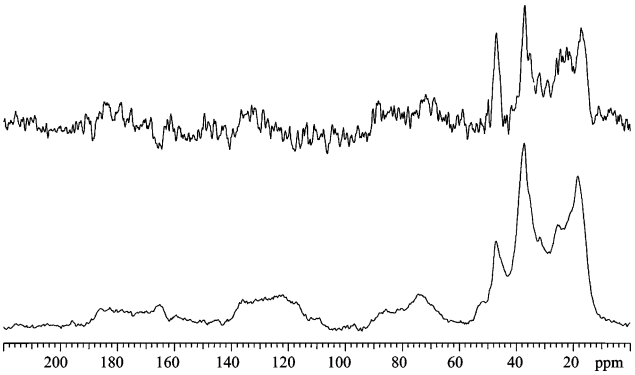


Figure 6. The 75 MHz ^{13}C NMR spectra of the exudate from *Pseudotsuga menziesii* with normal decoupling (lower) and with dipolar dephasing (upper), resin Group P.

a total of five peaks). A small C—O peak survives at δ ca. 70, and the carbonyl peak at δ 187 is enhanced. The spectra of *Picea pungens* in Figure S3 (in the Supporting Information) is almost identical in all aspects to those in Figure 5, as are the spectra of Moroccan copal in Figure S4 (in the Supporting Information) and the unillustrated spectra of *Pinus nigra nigra* and *Pinus monticola* (with a larger peak at δ 146 with normal decoupling). Since the botanical source of African copal normally is the genus *Copaifera* or *Hymenaea* (Fabaceae/Leguminosae), Moroccan copal (Figure S4, clearly Pinaceae) is deviant. Rice[40] states that African copal does not come from Morocco and that samples attributed to that source proved to be modern synthetic plastics. The material we examined is not a modern synthetic, but its identity as Pinaceae suggests possibly an introduced or mislabeled source. We cannot comment further without additional, independent samples.

A second group of pinacean resins exhibits similar, but slightly different spectral patterns. *Pseudotsuga menziesii* in Figure 6 provides a somewhat broadened version of the spectra just discussed. The dominant peaks in the saturated region with normal decoupling still are present, although that at δ 24 is reduced in intensity. The C—O region is larger. The peaks in the unsaturated region have merged into a single broad band. The highly diagnostic saturated region with dipolar dephasing, however, retains its overall four-peak pattern (with broadening of the peak at δ 24). Other samples with the overall appearance exemplified by *Pseudotsuga menziesii* include *Abies koreana*, *Abies skalinensis* (with some additional peaks in the saturated region), *Cedrus atlantica* (very broad, with a new peak at δ 55), *Cedrus libani*, *Picea abies*, *Picea koyamai*, *Pinus elliottii*, *Pinus thunbergiana*, U.S. Rosin, and Chinese gum rosin. The five-peak variant with interrupted decoupling is clearly seen with *Pinus strobus* (with additional small peaks at δ 26 and 52 with normal decoupling), Manila copal Bical (three separate samples were examined; see Figure S5 in the Supporting Information), Moroccan copal, and violin rosin.

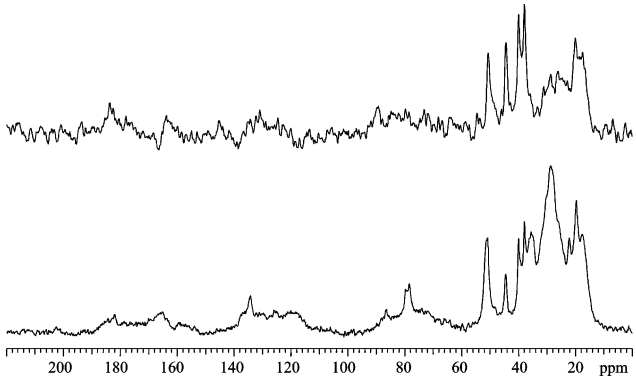


Figure 7. The 75 MHz ^{13}C NMR spectra of the exudate from *Euphorbia tirucalli* with normal decoupling (lower) and with dipolar dephasing (upper), resin Group E.

The unique pattern provided by the Pinaceae serves to define Group P. This family completes our examination herein of the gymnosperms. The angiosperms or flowering plants that produce resins are represented in this study by numerous families, all dicotyledonous.

Euphorbiaceae. This family is represented herein by the single species *Euphorbia tirucalli* (milkbush, Indiantree spurge, pencil tree, aveloz). The Euphorbiaceae have been associated primarily with latexes rather than resins.[41] Latex comprises the linear polymer of isoprene, plus, in the unprocessed form, some proteins. The ^{13}C spectrum of latex is simple, consisting of two sharp peaks between δ 130 and 140 and three sharp peaks between δ 25 and 40,[42] corresponding to the five carbon atoms in polyisoprene. These peaks do not contribute significantly to the spectrum of the material harvested from *Euphorbia tirucalli* (Figure 7). The complex saturated region with normal decoupling is typical of resins in general and is dominated by a strong peak at δ 30. In addition, there are important but broad peaks in the C—O and unsaturated regions. The spectrum with dipolar dephasing exhibits four sharp peaks between δ 38 and 51, sufficiently diagnostic to distinguish the spectrum from all other patterns discussed so far. Thus *Euphorbia tirucalli* provides a new pattern we call Group E.

Clusiaceae. Similarly, we have examined only one representative of this family, *Clusia rosea* (pitch apple), to date. Its spectra (Figure 8) represent a unique pattern, characterized (with normal decoupling) by a pair of strong peaks in the saturated region at δ 18 and 27, small peaks in the C—O region, and moderately strong peaks in the unsaturated region. The two strong peaks in the saturated region survive with dipolar dephasing and provide a nice contrasting diagnostic from all other spectra discussed so far. We refer to this pattern as Group C.

Fabaceae/Leguminosae (subfamily Caesalpinioideae). In our previous study,[43] we reported the spectra of *Hymenaea courbaril* ("jatoba," copal, "guapinol," Brazilian cherry/locust, stinking toe) from the Dominican Republic in the Caribbean and *Copaifera* sp. from an African source. We found that the two materials gave essentially identical spectra, an interesting commentary on continental drift.[44] In addition, copals from Colombia, Kenya, Tanzania,

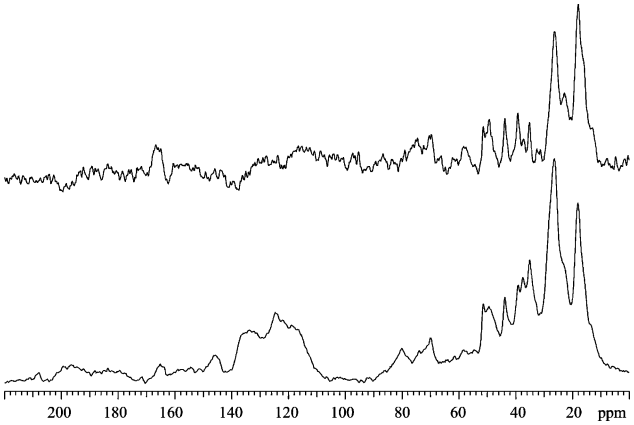


Figure 8. The 75 MHz ^{13}C NMR spectra of the exudate from *Clusia rosea* with normal decoupling (lower) and with dipolar dephasing (upper), resin Group G.

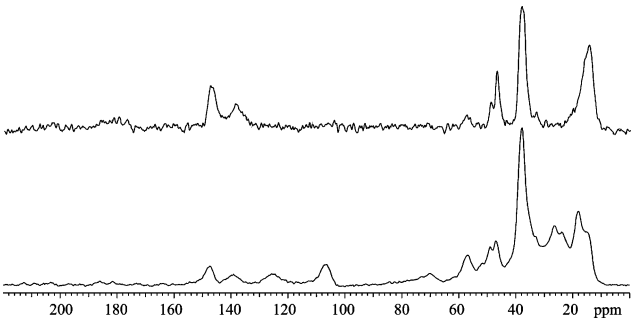


Figure 9. The 75 MHz ^{13}C NMR spectra of the exudate from *Copaifera* sp. with normal decoupling (lower) and with dipolar dephasing (upper), resin Group FL.

Congo, and Madagascar had the same spectra (the only variant being the intensity of a peak at δ 33 with interrupted decoupling). Figure 9 gives the spectra of *Copaifera* sp. The peak at δ 38 dominates the spectrum with normal decoupling. In addition, there are five other groupings of peaks in the saturated region (δ 14−18, 24−27, 33 (the variable sharp peak, very small in this particular spectrum), 46−50, and 57). The unsaturated region has four peaks at about δ 107, 125, 139, and 148. There are strong similarities with the *Agathis* spectrum, but these similarities do not carry over to the spectra with dipolar dephasing. The latter spectrum of *Copaifera* (Figure 9, upper) contains three prominent peaks at δ 13, 38, and 46 (in addition, the peak at δ 33 can be equally prominent in other Fabaceae/Leguminosae resins). The alkenic peaks at δ 139 and 148 also survive with dipolar dephasing.

In addition to these samples, we now have examined copals of unidentified botanical origin from Guyana in South America and Mauritania in West Africa. These materials give patterns nearly identical to the previous *Hymenaea* and *Copaifera* samples, although the Guyana sample gives the most prominent peak yet observed at δ 33. New World samples have been associated primarily with *Hymenaea*, whereas African samples have been associated variously with *Hymenaea*, *Copaifera*, or *Trachylobium*. Although we cannot assign the copals to distinct species, the spectral patterns are clearly the same as those of the other Fabaceae/Leguminosae. These materials, which now define Group FL, exhibit a common and distinctive spectral signature.

Dipterocarpaceae. The Dipterocarpaceae are widespread producers of triterpenoid resins, including the dammars. We have examined samples of *Dipterocarpus alatus* (yang tree), *Shorea* sp., and commercial dammar.

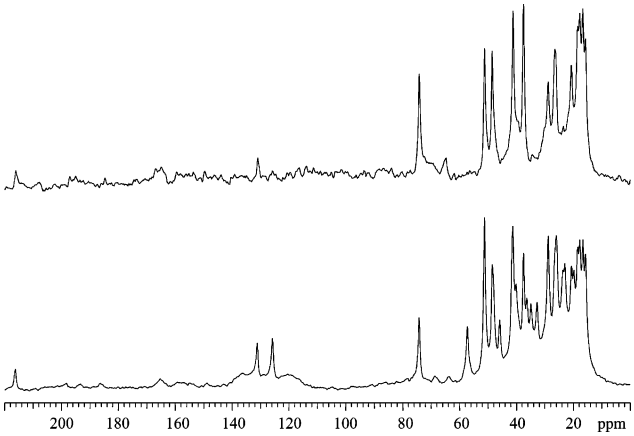


Figure 10. The 75 MHz ^{13}C NMR spectra of the exudate from *Dipterocarpus alatus* with normal decoupling (lower) and with dipolar dephasing (upper), resin Group D.

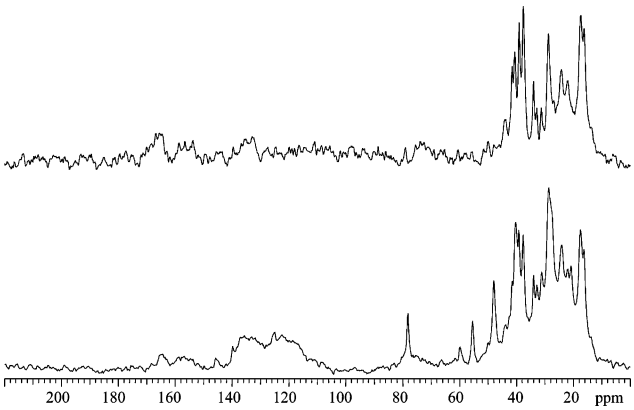


Figure 11. The 75 MHz ^{13}C NMR spectra of the exudate from *Dacryodes excelsa* with normal decoupling (lower) and with dipolar dephasing (upper), resin Group B.

Figure 10 illustrates the spectra of *Dipterocarpus alatus* from Cambodia. The most striking aspect of the spectrum with normal coupling is the sharpness and number of peaks, contrasting with all the previous resins. There are no carbonyl (C═O) or carbohydrate (OCO) peaks. There is a single sharp C—O peak at δ 74 and a pronounced C═C region that includes two sharp peaks at δ 126 and 131. The saturated region contains numerous sharp peaks between δ 15 and 58. The spectrum with dipolar dephasing retains the peak at δ 74, three doublets in the region δ 25−55, and several overlapping peaks in the region δ 15−22. The spectrum of commercial dammar has broader peaks than those in Figure 10 but nonetheless resembles it closely. The same may be said for a sample of Philippine *Shorea*. We refer to these materials as Group D, which are easily distinguished by NMR from all previous groups.

Burseraceae. This family is pantropical, with many species in tropical America, northeast Africa, and Malaysia. The genus *Bursera* has been used as incense in Mesoamerica from ancient to modern times. We have examined several samples from this triterpenoid-producing family, including *Dacryodes excelsa* (Figure 11). Members of this family, including *Dacroydes*, often have been called elemis. The sample of *Dacryodes excelsa* was collected in Puerto Rico, where it is known as "tabonuco". The spectra have some resemblances to those of the Dipterocarpaceae (Figure 10), including a pronounced C═C (without the two sharp peaks), the sharp C—O peak at δ 78, and a series of sharp peaks in the saturated region. There is less resemblance between the spectra of the Dipterocarpaceae and the Burseraceae with dipolar dephasing, in particular the

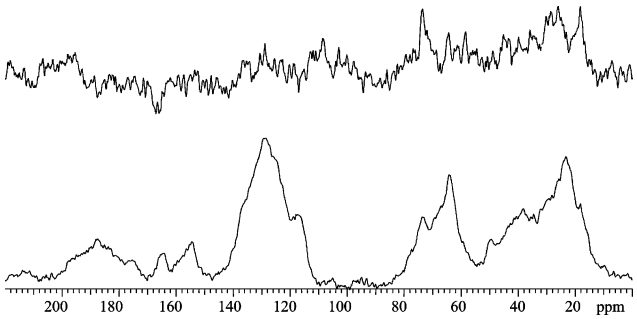
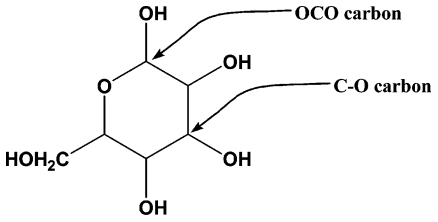


Figure 12. The 75 MHz ^{13}C NMR spectra of the exudate from *Amyris elemifera* with normal decoupling (lower) and with dipolar dephasing (upper), resin Group R.

absence of the pair of peaks at δ 49 and 52. A second *Dacryodes* sample of unidentified species gave very much the same spectra. A commercial sample from the O. G. Innis Corp. also gave the same spectra, with some minor differences in the unsaturated region. Samples from Mexico and from Guatemala (Chichicastenango), presumed to be *Bursera*, gave spectra very similar to those in Figure 11, but not identical. We call this set of materials Group B.

Rutaceae. This family is represented herein by the single species *Amyris elemifera*, which is a source of elemi. Its spectra (Figure 12) are quite different from those of the other triterpenoid resins. The peaks are very broad, and the resonances in the unsaturated and C—O regions are much larger than in other samples. There is no peak, however, in the OCO region (δ ca. 100). This sample thus contains no sugars and cannot be classified as a gum resin. The spectra provide a unique pattern, which we call Group R.

Gums. The principal component of gums is sugars (carbohydrates), present as oligomers or polymers (that is, oligosaccharides or polysaccharides). There normally is no hydrocarbon (terpene) component in the molecules. Consequently, the saturated region of the ^{13}C NMR spectrum is empty. Occasionally water is lost from a sugar to produce unsaturated molecules such as ascorbic acid, and oxidation can yield glycaric acids that contain the carbonyl group. The main functionalities in sugars are illustrated by the following general hexose structure **1**:



1

Sugars contain several C—O carbons (one is pointed out in the structure), which resonate in the region δ 60−80, and a single OCO carbon (also pointed out), which resonates in the region δ 95−105. As simple alcohols, esters, and ethers also resonate in the C—O region, it is the unique OCO region (found in very few other organic compounds) that is highly diagnostic for sugars.

We have found spectra characteristic of gums for 15 species belonging to 13 genera in 10 families, all of which are angiosperms (flowering plants). The spectra are not so distinctive as those of resins. Figure 13 gives the spectra for *Prunus armeniaca* (apricot) of the Rosaceae. There are no resonances below δ 60, indicating the absence of resins. The dominant features are in the C—O region from δ 60 to

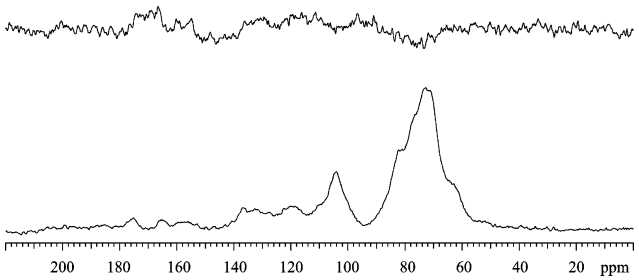


Figure 13. The 75 MHz ^{13}C NMR spectra of the exudate from *Prunus armeniaca* with normal decoupling (lower) and with dipolar dephasing (upper), a gum.

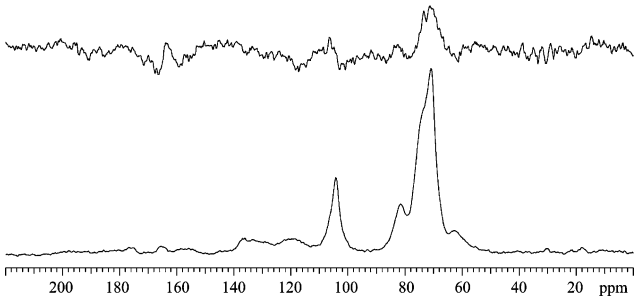


Figure 14. The 75 MHz ^{13}C NMR spectra of the exudate from *Pleiogynium timoriense* with normal decoupling (lower) and with dipolar dephasing (upper), a gum.

85, which is broad with a double maximum at δ 71 and 74 and two shoulders at δ 62 and 82. Most critical, however, is the presence of the medium-sized peak at δ 104, indicative of the OCO carbon. It is possible to have C—O resonances in the absence of sugars, as we have seen with several resins. These C—O peaks generally are smaller and sharper than in gums. Moreover, the absence of the OCO peak confirms the nonsugar source of such peaks. There also are small unsaturated and carbonyl peaks in Figure 13. When the spectrum is taken with dipolar dephasing, almost all the peaks disappear. There are no quaternary carbons (lacking attached hydrogens) in most sugars, as seen in sugar structure **1**. The spectra of *Prunus avium* (wild or sweet cherry) and of *Prunus sargentii* (Sargent cherry) are essentially identical to those of *Prunus armeniaca*.

The spectra of *Pseudobombax ellipticum* (shaving brush tree) from the Malvaceae, of *Munroidendron racemosum* (a short Hawaiian tree sometimes called the false 'ohe) from the Araliaceae, of *Acacia tortuosa* (twisted acacia, "poponax", or "huisachillo") from the Fabaceae/Leguminosae, and of *Enterolobium* sp. (from Mexico, probably *Enterolobium cyclocarpum*, previously published[19]) also from the Fabaceae/Leguminosae are very similar to those of Figure 13. The spectrum of *Munroidendron* contains the most fully defined trio of C—O peaks, which now may be placed roughly at δ 62, 71, and 83 (the other maximum at δ 74 is gone, but the overall appearance is very similar). In the spectra of most of these species, there is a small peak in the saturated region at δ 18.

A somewhat different spectrum is represented in Figure 14 by *Pleiogynium timoriense* (Burdekin or sweet plum) of the Anacardiaceae. The central C—O peak at δ 71 is very prominent (its partner at δ 74 appears as a shoulder), and the other two C—O peaks, at δ 63 and 82, are much reduced in intensity. The OCO peak also is larger. The spectrum of *Swietenia mahogany* (mahogany) of the Meliaceae is quite similar to that of *Pleiogynium timoriense*, as is that of *Chorisia speciosa* (silk floss tree) of the Bombacaceae, although with somewhat broader peaks.

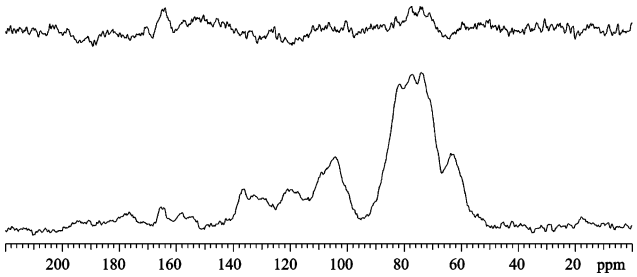


Figure 15. The 75 MHz ^{13}C NMR spectra of the exudate from *Terminalia bentzoe* with normal decoupling (lower) and with dipolar dephasing (upper), a gum.

Yet another variation is found with species of the Combretaceae: *Terminalia bentzoe* (Figure 15, no common name), *Bucida buceras* (black olive tree) (previously published[19]), and *Bucida* × Pitt #3 (a hybrid cultivar). The three spectra are almost identical, characterized by four distinct peaks in the C—O area, at roughly δ 63, 74, 78, and 81 (the previous maximum at δ 71 appears as a shoulder). The new peak is the one at δ 78, and it is of equal intensity to those at δ 74 and 81 (that at δ 63 is smaller). There are significant unsaturated carbons in the region δ 110−140, and the small saturated peak appears at δ 18. A sample from Puerto Rico of an unidentified genus and species has a very similar spectrum, although the unsaturated carbons are absent.

The spectra of the monocotyledonous *Syagrus botryophora* (a Brazilian palm with no common name) of the Arecaceae/Palmae are similar to those of several of these species but with a four-peak pattern in the C—O region. In this case the peak at δ 77 rather than δ 71 or 74 is the largest. In addition, the lowest frequency peak is at δ 66 rather than δ 62.

We have seen that the Fabaceae/Leguminosae contains several resin-producing trees and some gum-producing plants, all in the subfamily Caesalpinoideae. In addition, this family contains the gum-producing *Prosopis glandulosa* (honey or Texas mesquite) from the subfamily Mimosoideae with a slightly different spectrum, as seen in Figure S6 (in the Supporting Information). It offers the richest gum spectrum to date, as peaks occur at δ 62, 66, 75, 79, and 83. In addition there are two OCO resonances, at δ 98 and 104, as well as small unsaturated peaks.

Detailed examination of these superficially similar spectra of gums shows that there are identifiable peaks at δ ca. 62, 66, 71, 74, 78, and 82. The variation of the intensities of these peaks provides some distinguishing features from species to species.

Gum Resins. The NMR identification of resins and gums is straightforward and unambiguous. Providing an operational diagnostic by NMR spectroscopy for gum resins is no more difficult. The spectra must exhibit characteristics of both groups. To be a gum, the material must give a spectrum that (1) contains a dominant C—O resonance and a clear OCO resonance and (2) lacks all saturated (terpenoid) resonances. To be a resin, the material must give a spectrum that (1) contains a dominant band of resonances in the saturated region and (2) lacks the OCO resonance, although a small C—O resonance may be present. In addition, both resins and gums can give unsaturated and carbonyl resonances. Thus, the gum resin must contain significant saturated resonances, a large C—O resonance, and a significant OCO resonance. One problem is that there can be an exomethylene (C=CH_2) resonance at δ ca. 108, as well as an OCO resonance as high as δ 105. These positions are close enough together to cause ambiguity.

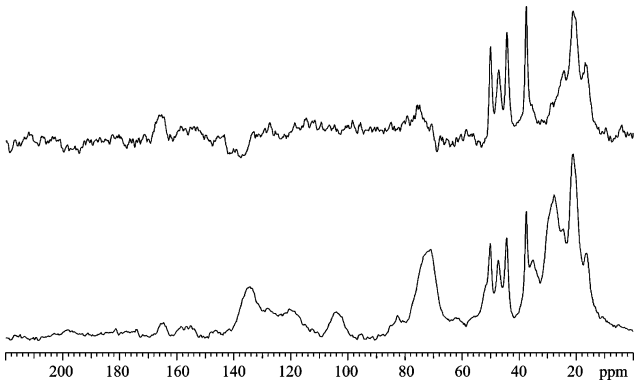


Figure 16. The 75 MHz ^{13}C NMR spectra of the exudate from *Schinus molle* with normal decoupling (lower) and with dipolar dephasing (upper), a gum resin.

Since the corresponding C=CH_2 resonance at δ ca. 150 must be present to complete the exomethylene group, it serves as confirmation of the identity of a peak in the range δ 100−110.

Figure 16 provides the spectra of *Schinus molle* (California pepper tree) from the Anacardiaceae, with classic features of both gums and resins. With normal decoupling there are a series of terpenoid peaks in the region δ 15−50, a large C—O peak centered at δ 72, and a pronounced OCO peak at δ 104, confirmed not only by its frequency but also by the absence of the exomethylene peak at δ ca. 150. The terpenoid peaks survive with dipolar dephasing.

It should be noted that there never has been a simple, objective physical criterion for classifying an exudate as a gum resin (or for that matter as a gum or a resin). The above NMR criteria provide just that. Extraction by water might indicate gum properties, and extraction by an organic solvent might indicate resin properties, but such approaches are both qualitative and unreliable. GC/MS also can provide reliable criteria, if the wealth of resulting GC peaks can be easily identified and ascertained to characterize the exudate bulk (i.e., a very small resinous component can appear in the GC trace but would not render the bulk a gum resin). MS cannot, however, provide a quantitative assessment of the relative amounts of the resin and gum components. Figure 16 constitutes a direct, unequivocal assignment of the identity of this exudate as a gum resin *and provides a measure of the resin/gum ratio*.

The spectra of *Pistacia lentiscus* of the Anacardiaceae (Figure S7 in the Supporting Information; both the tree and the exudates are called mastic) have some similarities to those in Figure 16. Indeed, *Schinus molle* has been called American mastic. Mills and White[1] classify *Pistacia lentiscus* as a resin and *Schinus molle* as a gum resin. NMR spectroscopy now indicates that the exudates have nearly identical chemical constituents, in terms of the proportion of terpenoid to sugar resonances. The terpenoid (saturated) region of *Pistacia lentiscus*, however, is quite distinct from that of *Schinus molle*, so the two materials are distinguishable by NMR.

Given these objective criteria, it follows that several samples from the genus *Araucaria* are gum resins. The spectra of *Araucaria cunninghami* (hoop pine) are illustrative (Figure 17). In addition to the resin peaks in the region δ 10−60, there is a large C—O resonance at δ 65−86. The clear peak at δ 102−108 may be assigned to OCO, as confirmed by the presence of only a small peak at δ 148. Examination of our previously published spectrum of *Araucaria columnaris* (Cook or New Caledonia pine) (Lam-

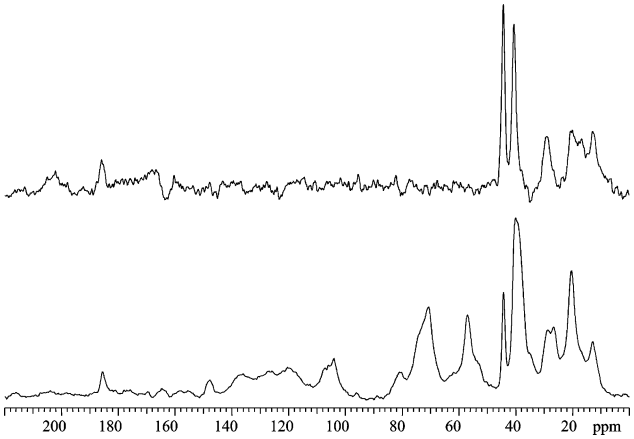


Figure 17. The 75 MHz ^{13}C NMR spectra of the exudate from *Araucaria cunninghami* with normal decoupling (lower) and with dipolar dephasing (upper), a gum resin.

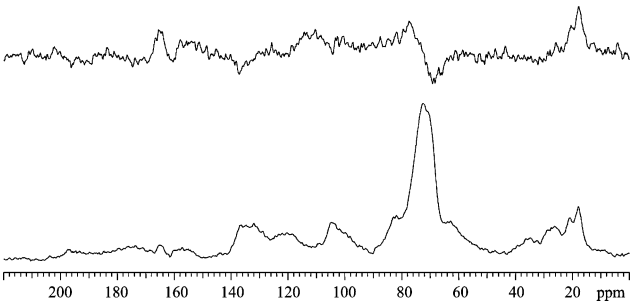


Figure 18. The 75 MHz ^{13}C NMR spectra of the exudate from *Commiphora* sp. (myrrh) with normal decoupling (lower) and with dipolar dephasing (upper), a gum resin.

Figure 19. The 75 MHz ^{13}C NMR spectra of the exudate from *Boswellia serrata* (frankincense) with normal decoupling (lower) and with dipolar dephasing (upper), a gum resin.

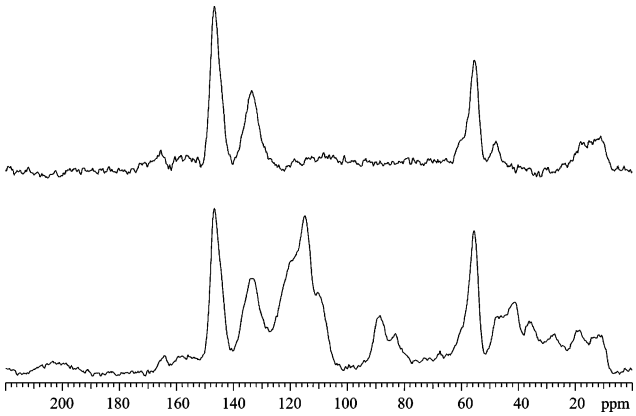


Figure 20. The 75 MHz ^{13}C NMR spectra of the exudate from *Guiacum officinale* with normal decoupling (lower) and with dipolar dephasing (upper), a gum resin.

bert et al. 1999, 2002) leads to the same conclusion, confirmed by a new sample from a different source. The spectra of *Araucaria laubenselsis* (no common name) are very similar to those of *A. cunninghami* and *A. columnaris*, implying that this exudate also is a gum resin. The spectra of these three *Araucaria* species contrast with those of *Araucaria araucana* (Figure S2). Although the resin resonances in Figures S2 and S7 are similar, *A. araucana* has very few C—O resonances and nothing for OCO at δ ca. 105. We therefore retain *A. araucana* as a resin producer, but the other *Araucaria* species are gum resin producers. All the Cupressaceae and Araucariaceae (resin Group CA) and the gum resins just enumerated give characteristic spectra with normal and interrupted decoupling. The resin peaks in Figure 17 are very similar to those of the Group CA samples in Figures 2, 3, S1, and (particularly) S2, as well as those previously published.[16,18,19] Figure 17 is basically the superposition of the resin spectra of Group CA and the gum peaks at δ 60−85 and 105. The NMR spectra suggest that all the Cupressaceae and Araucariaceae species produce the same terpenoid resinous material, whether gum or gum resin. Whereas most *Araucaria* species also exude a saccharide component, *A. araucana* does not.

Myrrh has been classified as a gum resin by Mills and White.[1] Its spectrum (Figure 18, from a commercial sample, presumably *Commiphora* sp. of the Burseraceae) with normal decoupling contains the same gum resonances as seen in the spectra of *Pleiogynium timoriense* (Figure 14), *Swietenia mahogani*, and *Chorisia speciosa*. In addition, however, there are clear terpenoid resonances in the saturated region, smaller than those seen for the mastics and for *Araucaria* species but surviving with dipolar dephasing. Thus the gum proportion of myrrh is higher than that of mastic.

In contrast, frankincense tends toward a higher resinous component. The spectra of *Boswellia serrata* of the Burseraceae are dominated by the terpenoid peaks, but sugar peaks are clearly present (Figure 19). The gum proportion in frankincense is lower than that of myrrh or mastic. Samples of *Bursera simaruba* (gumbo-limbo) of the Burseraceae follow this trend further. The terpenoid resonances are even larger, but relatively small sugar resonances are clearly discerned (Figure S8 in the Supporting Information).

A second species of mesquite (*Prosopis juliflora*) from the Fabaceae/Leguminosae, native to Latin America but not to the United States, produces a gum resin rather than a gum (as from the Texas mesquite, *Prosopis glandulosa*, Figure S6). The sugar resonances, however, are clearly larger than the terpenoid resonances.

Thus it is possible on the basis of the ^{13}C NMR spectra to delineate a series of gum resins according to the resin/gum ratio, as follows from highest to lowest resin proportion: gumbo-limbo (*Bursera simaruba*), frankincense (*Boswellia serrata*), mastic (*Pistacia lentiscus*), *Araucaria* (all species examined except *A. araucana*), mesquite (*Prosopis juliflora*), and myrrh (*Commiphora* sp.). Such an ordering has not been possible by previous techniques.

Not all spectra are easily classified. The exudates from *Guaiacum officinale* (Figure 20) and *Guaiacum sanctum* (both called the tree or wood of life, from the Caribbean) from the Zygophyllaceae produce identical spectra with general characteristics of gum resins. There are numerous

Table 1. Exudate Samples Studied

exudate type	family (subfamily)	scientific name[a] genus and species, authorship, subspecies, variety, cultivar if available	common name	sample number	source
RESINS	Araucariaceae	*Agathis australis* (D. Don) Loudon	kauri pine	128	Waiporra Kauri Forest, New Zealand; G. O. Poinar, Jr.
		Agathis atropurpurea B. P. M. Hyland	blue kauri or blue pine	132	Royal Botanic Gardens, Sydney, Australia; G. O. Poinar, Jr.
		Agathis lanceolata (Lindley ex Warburg)	Koghis kauri	142	Royal Botanic Gardens, Sydney, Australia; G. O. Poinar, Jr.
		Agathis macrophylla (Lindl.) Mast.	kauri variant	150	Royal Botanic Gardens, Sydney, Australia; G. O. Poinar, Jr.
		Agathis moorei (Lindl.) Mast.	Moore kauri	133	Royal Botanic Gardens, Sydney, Australia; G. O. Poinar, Jr.
		Agathis sp.[b]	Manila copal Loba	58	C. W. Beck
		Agathis sp.[b]	Manila copal Pangalinan	59	C. W. Beck, P. Perez
		Araucaria araucana (Molina) C. Koch	monkey-puzzle tree	303	University of California Botanical Garden, Berkeley, CA; J. A. Santiago-Blay
		Wollemia nobilis W. G. Jones, K. D. Hill, & J. M. Allen	Wollemi pine	201	Royal Botanic Gardens, Sydney, Australia; K. Hill
	Burseraceae	*Dacryodes excelsa* Vahl	"tabonuco"	241	Caribbean National Forest, Río Grande, Puerto Rico; J. A. Santiago-Blay
		Dacryodes sp.		234, 237	G. O. Poinar, Jr.
		unknown[b]	Innes gum	206	O. G. Innes Corp.
		unknown[b]	incense	207	Mexico; G. O. Poinar, Jr.
		unknown[b]	incense	225, 226	Chichicastenango, Guatemala; C. J. Welch
	Clusiaceae	*Clusia rosea* Jacq.	pitch apple	283	Fairchild Tropical Botanical Garden, Coral Gables, FL; J. A. Santiago-Blay
	Cupressaceae	*Calocedrus decurrens* (Torr.) Florin	incense cedar	301	Ashland, OR; J. A. Santiago-Blay
		Chamaecyparis formosensis Matsum	Formosan cypress	285	University of California Botanical Garden, Berkeley, CA; J. A. Santiago-Blay
		Chamaecyparis lawsoniana (A. Murray bis) Parl.	Port Orford (OR) cedar, white cedar, or Lawson (false) cypress	312	Holden Arboretum, Kirtland, OH; J. A. Santiago-Blay
		Chamaecyparis obtusa (Siebold & Zucc.) Endl.	Hinoki (false) cypress	316	United States National Arboretum, Washington, DC; J. A. Santiago-Blay
		Cupressus arizonica Greene	Arizona cypress	259	New Mexico; J. A. Santiago-Blay
		Cupressus montana Wiggins	var. of Arizona cypress	260	New Mexico; J. A. Santiago-Blay
		Cupressus sempervirens L.	Italian cypress	317	Universidad Autónoma "Antonio Narro", Agraria Coahuila, Mexico; J. A. Santiago-Blay
		Juniperus deppeana. Steud	alligator juniper	315	United States National Arboretum, Washington, DC; J. A. Santiago-Blay
		Metasequoia *glyptostroboides* Hu and W. C. Chang, formerly in Taxodiaceae	dawn redwood	318	National Museum of Natural History, Washington, DC; J. A. Santiago-Blay
		Sequoia sempervirens (D. Don) Endl., formerly in Taxodiaceae	coast redwood	224	United States National Arboretum, Washington, DC; J. A. Santiago-Blay
		Thuja plicata Donn ex D. Don	giant arborvitae or western red cedar	310	Holden Arboretum, Kirtland, OH; J. A. Santiago-Blay
	Dipterocarpaceae	*Dipterocarpus alatus* A. DC.	yang tree	256, 257	Cambodia; C. Lampert
		Shorea sp.	dammar	270	G. O. Poinar, Jr.
		Shorea sp.[b]		231	G. O. Poinar, Jr.
	Euphorbiaceae	*Euphorbia tirucalli* L.	milkbush, Indiantree spurge, pencil tree, Aveloz	299	Fairchild Tropical Botanical Garden, Coral Gables, FL; J. A. Santiago-Blay

Table 1 (Continued)

exudate type	family (subfamily)	scientific name[a] genus and species, authorship, subspecies, variety, cultivar if available	common name	sample number	source
	Fabaceae/ Leguminosae (Caesalpinioideae)				
		Copaifera sp.		235	G. O. Poinar, Jr.
		Hymenaea courbaril L.	"jatoba," copal, "guapinol," Brazilian cherry/locust, stinking toe	63, 164	Dominican Republic; G. O. Poinar, Jr.
		Hymenaea sp.[b]	Colombian copal	173, 174	M. Santander and A. Nisbet
		Hymenaea sp.[b]	Congo copal	57, 240	C. W. Beck
		Hymenaea sp.[b]	Kenyan copal	166	Mombasa, Kenya; A. Graffin
		Hymenaea sp.[b]	Madagascar copal	242, 243, 244	G. O. Poinar, Jr.
		Hymenaea sp.[b]	Tanzanian copal	168	G. O. Poinar, Jr.
	Pinaceae	*Abies koreana* E. H. Wilson	Korean fir	309	Holden Arboretum, Kirtland, OH; J. A. Santiago-Blay
		Abies sachalinensis (F. Schmidt) Mast.	Sakhalin fir	272	Japan
		Cedrus atlantica (Endl.) Manetti ex Carrière subsp. *glauca*	blue atlas cedar	300	Winterthur Garden, Winterthur, DE; J. A. Santiago-Blay
		Cedrus libani A. Rich	cedar of Lebanon	292	Holden Arboretum, Kirtland, OH; J. A. Santiago-Blay
		Picea abies (L.) H. Karst	Norway spruce	305	Salem, VA; J. A. Santiago-Blay
		Picea koyamai Shirasawa	Koyama spruce	291	United States National Arboretum, Washington, DC; J. A. Santiago-Blay
		Picea pungens Engelm.	blue spruce	264	Salem, VA; J. A. Santiago-Blay
		Pinus elliottii densa (Little & K. W. Dorman) E. Murray	slash pine	297	Fairchild Tropical Botanical Garden, Miami, FL; J. A. Santiago-Blay
		Pinus monticola Douglas ex. D. Don	western white pine	208	California, G. O. Poinar, Jr.
		Pinus nigra nigra Arnold	European black pine called Austrian pine	274	Central Europe
		Pinus rudis Endl.	Mexican red pine	266	Coahuila, Mexico; J. A. Santiago-Blay
		Pinus strobus L.	(eastern) white pine	258	Salem, VA; J. A. Santiago-Blay
		Pinus thunbergiana (Parl.) Franco	Japanese black pine	304	New York Botanical Garden, Bronx, NY; J. A. Santiago-Blay
		Pinus sp.[b]	Manila copal Bical	60	C. W. Beck, P. Pérez
		Pinus sp.[b]	Bical	222, 223	C. W. Beck
		Pinus sp.[b]	Moroccan copal	245	
		Pinus sp.[b]	Chinese gum rosin	71	C. W. Beck
		Pinus sp.[b]	U. S. rosin	56	C. W. Beck
		Pinus sp.[b]	Violin rosin	209	G. O. Poinar
		Pseudotsuga menziesii (Mirb.) Franco	Douglas fir	290	Coahuila, Mexico; J. A. Santiago-Blay
		unknown[b]		227, 228	Chichicastenango, Guatemala; C. J. Walsh
	Rutaceae	*Amyris elemifera* L	elemi	307	Bosque Guánica, Puerto Rico; Miguel Canáls
GUMS	Anacardiaceae	*Pleiogynium timoriense* (A. DC.) Leenh.	Burdekin or sweet plum	279	Fairchild Tropical Botanical Garden, Miami, FL; J. A. Santiago-Blay
	Araliaceae	*Munroidendron racemosum* (C. N. Forbes) Sherff	false 'ohe	277	National Tropical Botanical Garden, Kauai, HI
	Arecaceae/Palmae	*Syagrus botryophora* (Mart.) Mart.	Brazilian palm	319	Montgomery Botanical Center, Coral Gables, FL; J. A. Santiago-Blay
	Bombacaceae	*Chorisia speciosa* A. St.-Hil	silk floss tree	295	Fairchild Tropical Botanical Garden, Miami, FL; J. A. Santiago-Blay
	Combretaceae	*Bucida buceras* L.	black olive tree	247	Guyanabo, Puerto Rico; A. Blay and R. González

Table 1 (Continued)

exudate type	family (subfamily)	scientific name[a] genus and species, authorship, subspecies, variety, cultivar if available	common name	sample number	source
		Bucida sp. (× Pitt #3)	hybrid	296	Fairchild Tropical Botanical Garden, Coral Gables, FL
		Terminalia bentzoe (L.) Pers.		284	National Tropical Botanical Garden, Kauai, HI, M. H. Chapin
		unknown[b]		211	Puerto Rico
	Fabaceae/ Leguminosae (Mimosoideae)				
		Acacia tortuosa (L.) Willd.	twisted acacia, "poponax", or "huisachillo"	298	Fairchild Tropical Botanical Garden, Coral Gables, FL; J. A. Santiago-Blay
		Enterolobium sp.		165	Totolapa, Mexico; T. A. Lee, Jr.
		Prosopis glandulosa Torr.	honey or Texas mesquite	267	Coahuila, Mexico; José Villanueva Díaz
	Malvaceae	*Pseudobombax ellipticum* (Kunth) Dugand	shaving brush tree	294	Fairchild Tropical Botanical Garden, Coral Gables, FL; J. A. Santiago-Blay
	Meliaceae	*Swietenia mahogani* C. DC.	mahogany	287	National Tropical Botanical Garden, Kauai, HI; M. H. Chapin
	Rosaceae	*Prunus armeniaca* L.	apricot	269	Universidad Autónoma Agraria "Antonio Narro", Saltillo, Coahuila, Mexico; J. A. Santiago-Blay
		Prunus avium (L.) L.	wild or sweet cherry	311	Holden Arboretum, Kirtland, OH; J. A. Santiago-Blay
		Prunus sargentii Rehder	Sargent cherry	286	United States National Arboretum, Washington, DC
GUM RESINS					
	Anacardiaceae	*Schinus molle* L.	California pepper tree	280	Universidad Autónoma Agraria "Antonio Narro", Saltillo, Coahuila, Mexico; J. A. Santiago-Blay
		Pistacia lentiscus L.	mastic	268	Langley Park, MD
	Araucariaceae	*Araucaria columnaris* Hook.	Cook or New Caledonia pine	134, 288	Royal Botanic Gardens, Sydney, Australia; G. O. Poinar; Montgomery Botanical Center, Coral Gables, FL; J. A. Santiago-Blay
		Araucaria cunninghamii Aiton ex D. Don	hoop pine	263	
		Araucaria laubenselsis		281	Montgomery Botanical Center, Coral Gables, FL; J. A. Santiago-Blay
	Burseraceae	*Boswellia serrata* Roxb.	frankincense	306	Dhufar province, Muscat and Oman (Ray Cleveland Collection)
		Boswellia sp.	frankincense	232	G. O Poinar
		Bursera simaruba (L.) Sarg.	gumbo-limbo	282	Fairchild Tropical Botanical Garden, Coral Gables, FL; J. A. Santiago-Blay
		Commiphora sp.	myrrh	271	commercial
	Fabaceae/ Leguminosae (Mimosoideae)				
		Prosopis juliflora (Sw.) DC.	Latin American mesquite	289	Fairchild Tropical Botanical Garden, Coral Gables, FL; J. A. Santiago-Blay
	Zygophyllaceae	*Guaiacum officinale* L.	tree of life	254	Guánica, Puerto Rico; M. Álvarez
		Guaiacum sanctum L.	tree of life	273	Fairchild Tropical Botanical Garden, Coral Gables, FL; J. A. Santiago-Blay

[a] Global databases, such as the International Plant Index (IPNI, http://www/uk.ipni.org/index.html) and Tropicos (http://mobot.mobot.org/W3T/search/vast.html), were used to determine authorship data. [b] Species assigned by the NMR spectra.

saturated peaks, as well as a small C—O peak. The surprise is the very large peaks in the unsaturated region, which tail into the OCO region. The size of the OCO resonance as judged by the size of the C—O resonance is too small to see at δ ca. 104, so that the identity of the material is not easily assigned.

Summary and Conclusions

Nuclear magnetic resonance spectroscopy, applied to the bulk material of solid exudates, readily identifies the materials as resins, gums, or gum resins. Resins from the families Cupressaceae and Araucariaceae give the same spectra, which we label Group CA. It includes the cypresses, junipers, kauris, dawn redwoods, and some cedars. The single species *Sequoia sempervirens* (coast redwood) offers a unique spectrum (Group S). The Pinaceae, which include the firs, spruces, pines, and some cedars, give the same spectral pattern, labeled Group P. These three groups constitute the gymnosperms that we have examined to date. The remaining resin-producing species studied are angiosperms or flowering plants. The single species *Euphorbia tirucalli* from the Euphorbiaceae constitutes Group E, and the single species *Clusia rosea* (the evergreen pitch apple) from the Clusiaceae, Group C. The Dipterocarpaceae, including the dammars, constitute Group D, and the Burseraceae, including many *Bursera* incense species, Group B. The Fabaceae/Leguminosae, with many examples from both Africa and the Americas, constitute Group FL. The single species *Amyris elemifera* from the family Rutaceae, which includes the elemis, provides unique spectra characteristic of Group R.

To date we have defined nine groupings of resins, each with a unique spectral signature. It is likely that many species will be added to these groupings, that there will be many additional groupings, and that other adjustments will be made. We have demonstrated that the complex mixtures of terpenes found in all resins vary across many families but give specific fingerprints or signatures that reflect the specific terpene constituents. Taxonomically related genera tend to have similar or identical spectra. Usually, a specific spectral type is characteristic of a single family, as is the case with the Pinaceae and most other families. In the case of the Cupressaceae and the Araucariaceae, representatives from both families give the same spectrum.

Gums, all produced by angiosperms, provide an entirely different type of NMR spectrum, lacking resonances from saturated hydrocarbons but containing resonances from OCO carbons characteristic of sugars. Representatives from the Rosaceae, Malvaceae, Araliaceae, and Fabaceae/Leguminosae families provided one particular spectral signature. Samples from the Anacardiaceae, Meliaceae, and Bombacacae families exhibited slightly different spectra. A sample from the Combretaceae gave slight variants, as did one from the Arecaceae/Palmae. The Texas mesquite (*Prosopis glandulosa*) gave yet another variant. The spectra of gums are very similar to each other, in contrast with the spectra of resins, but nonetheless have defining features.

Gum resins exhibit characteristics of both classes, with spectra containing saturated hydrocarbon and sugar resonances. The materials that we examined with these characteristics included mastic (Anacardiaceae), myrrh (Araucariaceae), frankincense (Burseraceae), Mexican mesquite (Fabaceae/Leguminosae), and the tree of life (Zygophyllaceae).

This study is the broadest to date that attempts to relate chemical structure of exudates to taxonomy. The spectroscopic topology generally matches our current understanding of plant classification. Thus ^{13}C NMR spectroscopy offers valuable applications in exudate identification, particularly at the family level when provenance is unknown or unavailable. In addition, it provides an unambiguous means to characterize an exudate as a resin, gum, gum resin, or latex.

Experimental Section

Plant Material. Most samples were collected from or provided by major botanical gardens or arboreta with permission of the institutions. These samples had been authenticated by the curators. A few samples were collected from private or public land, with permission, and authenticated by vegetative materials. The exudates were removed from the plant surface by hand or with the help of a knife or other sharp object. This protocol does not produce incisions in plants, as occurs in some commercial processes.[45] Small samples (1−5 g) were collected, of which the NMR experiment requires less than 100 mg. Although the material is powdered for spectroscopic examination, the experiment is entirely nondestructive. The materials will remain in the laboratory at Northwestern University for continued experiments but can be made available on request.

NMR Data Acquisition. Data were obtained on a Varian VXR-300 NMR spectrometer using a Doty wide-bore solid-state probe and operating at 75.413 MHz for carbon. Samples were ground to a fine powder and loaded into a 5 mm Zirconia rotor sealed with Aurum caps. Each sample load required less than 100 mg of material. The magic angle spinning rate (to narrow the signals) was set to about 4 kHz (determined by spectrometer limitations). The cross-polarization pulse sequence was applied to increase sensitivity, and the experiment was carried out with either of the two decoupling modes: normal and interrupted decoupling.[22] Sideband suppression was not necessary. Typical parameters were spectral width 30 kHz, 90° pulse width 4.9 μs, delay time 5 s, contact time 2.2 ms, acquisition time 150 ms, and scan number 512. The delay time in the dipolar dephasing experiment (interrupted decoupling) was 50 μs. For dipolar dephasing, the standard Varian sequence was used, which is a variant of the original Opella−Frey sequence.[22] The decoupler power level was set at 91 during cross-polarization (Level 1) and 100 during acquisition time (Level 2). Approximately 45 min of spectrometer time was required to acquire both spectral modes. Spectra were referenced to external adamantane at δ 38.3 and converted to the normal scale based on TMS at δ 0.

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Supporting Information Available: Figures S1−S8. This material is available free of charge via the Internet at http://pubs.acs.org.

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