Amber

DOI: 10.1002/anie.200705973

Chemical Signatures of Fossilized Resins and Recent Plant Exudates

Joseph B. Lambert, Jorge A. Santiago-Blay,* and Ken B. Anderson**

amber · gas chromatography · mass spectrometry ·
NMR spectroscopy · resins

Amber is one of the few gemstones based on an organic structure. Found over most of the world, it is the fossil form of sticky plant exudates called resins. Investigation of amber by modern analytical techniques provides structural information and insight into the identity of the ancient plants that produced the source resin. Mass spectrometric analysis of materials separated by gas chromatography has identified specific compounds that are the basis of a reliable classification of the different types of amber. NMR spectroscopy of bulk, solid amber provides a complementary classification. NMR spectroscopy also can be used to characterize modern resins as well as other types of plant exudates such as gums, gum resins, and kinos, which strongly resemble resins in appearance but have very different molecular constitutions.

1. Introduction

The familiar odor of evergreens, whether in the wild or on a Christmas tree lot, comes in part from a sticky material termed a resin, often secreted in response to damage or disease. Such plant materials are part of a more general group of materials called exudates, which also includes gum arabic, myrrh, frankincense, and kino dyes (Figure 1).[1,2] Exudates are viscous liquids when released by plants. Some harden in a matter of days or weeks, while others remain sticky. Under favorable circumstances in soils and sediments, hardened resins can be preserved for up to hundreds of millions of years. Fossilized resins recovered from sediments are popularly known as amber (Figure 2).[2–4]

Our groups independently have been collecting and examining both fossilized and recent samples to answer many fundamental questions about them. Of what chemical classes or even molecular constituents are ambers or recent exudates com-

Figure 1. Exudate (ca. 4 cm long) from a cherry tree (*Prunus* sp.) on the Tidal Basin of Washington, DC. This tree is part of the plantings responsible in the spring for the Cherry Blossom Festival. This material is a gum. Photograph by Chip Clark, National Museum of Natural History (Washington, DC).

---

[*] Prof. J. B. Lambert
Department of Chemistry
Northwestern University
Evanston, IL 60208 (USA)
Fax: (+1) 847-491-7713
E-mail: jlambert@northwestern.edu

Dr. J. A. Santiago-Blay
Department of Paleobiology
National Museum of Natural History
Smithsonian Institution
P.O. Box 37012, Washington, DC 20013-7012 (USA)
Fax: (+1) 202-786-2832
E-mail: blayj@si.edu

Prof. K. B. Anderson
Department of Geology
Southern Illinois University Carbondale
Carbondale, IL 62901 (USA)
Fax: (+1) 618-453-7393
E-mail: kanderson@geo.siu.edu

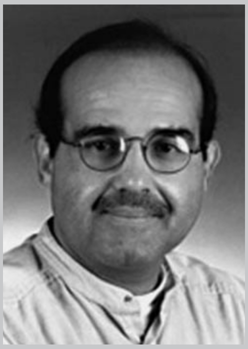
Figure 2. Left: Necklace of Baltic amber. Top: Round-beaded necklace made of Dominican amber. Bottom center: Necklace of Dominican amber. Right: Bracelet of Baltic amber. Photograph by Chip Clark, National Museum of Natural History (Washington, DC).

posed? Can chemical and spectroscopic analyses relate an amber piece to its plant source? Are ambers from different sources sufficiently distinct that chemical or spectroscopic signatures can establish geologic (temporal) or geographic (spatial) provenance? How do chemical or spectroscopic signatures of recent exudates vary taxonomically, that is, from species to species, genus to genus, and family to family? The subject thus represents the multifaceted interfaces of chemistry with botany, geology, and even archaeology.

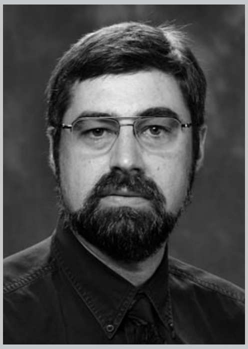
Plant exudates and their fossilized analogues represent challenging subjects for chemical analysis. As harvested from a plant, they constitute a mixture that is generally consistent within a given species wherever found, because they are produced by strictly defined botanical processes.[1] How they might be contaminated with foreign materials, how they might vary within a species, how they might compare with exudates from other species, genera, and families, and how they might change with time are considerations to be addressed by chemical and spectroscopic tools. Molecular constituents of amber or of recent resin often can be separated by gas chromatography (GC) and identified by mass spectrometry (MS). Although it is a difficult or impossible task to identify every molecular component in a recent or fossilized resin, in some cases nearly all major components may be identified. Furthermore, the occurrence of specific compounds (biomarkers) or of a specific pattern of compounds sometimes can serve as a botanical signature. IR and NMR techniques, on the other hand, can be applied to bulk materials. They provide a spectroscopic signature that is not dependent on factors associated with separation of components and thus reflects an average of all molecular constituents. Raman spectroscopy and X-ray diffraction are used less commonly on bulk materials with similar objectives.[5–8] Investigations of separate molecular constituents and of bulk structure are complementary, and both can identify the chemical classes that make up the exudate.

Curt Beck of Vassar College was an early pioneer in this field. His comprehensive IR studies of ambers revealed a unique spectroscopic signature for Baltic amber, the most commonly traded amber throughout much of Europe from Neolithic times to Classical Greece and Rome and through to the present.[9] At the same time, Jean H. Langenheim of the University of California Santa Cruz investigated the plant sources of many resinous materials; her work culminated in her monumental summary in 2003.[1] John S. Mills and Raymond White of The National Gallery, London, were among the first to apply GC–MS to the analysis of resins and ambers as part of their broader investigations of a wide variety of organic materials that have found their way into museums, both as curated objects and as scientific tools such as preservatives.[2,10] In this minireview, we discuss GC–MS studies of ambers and resins, and NMR studies of these materials and several additional classes of exudates.

Joseph B. Lambert was educated at Yale University (BS, 1962) and the California Institute of Technology (PhD, 1965). He has worked continuously at Northwestern University in Evanston, Illinois, since 1965, except for a sabbatical in 1973 at the British Museum to learn techniques of archaeological chemistry. He is Clare Hamilton Hall Professor of Chemistry and serves as Editor-in-Chief of the Journal of Physical Organic Chemistry. His major areas of research are organosilicon chemistry, reaction mechanisms, and NMR spectroscopy.



Jorge A. Santiago-Blay finished his MS in Biology, with emphasis in zoology, at the University of Puerto Rico. In 1990, he completed his MA in Botany and PhD in Entomology at the University of California at Berkeley. Currently he is Associate Professor at Gallaudet University. He pursues his research interests in arthropods and plants as a Research Collaborator in the Department of Paleobiology at the National Museum of Natural History (Washington, DC). In addition, he serves as Editor-in-Chief of Terrestrial Arthropod Reviews and Editor of Entomological News.



Ken B. Anderson received both his BSc-(Hons) and PhD degrees (organic chemistry) from the University of Melbourne, Australia. He joined the faculty at Southern Illinois University in 2003 after working in the petroleum industry and at Argonne National Laboratory. He is co-Editor-in-Chief of Geochemical Transactions and a member of the council of the American Chemical Society. His research interests focus on organic geochemistry, especially including analytical methods and the chemistry of ambers and coals.

2. GC–MS and Pyrolysis GC–MS of Ambers

2.1. Introduction

Throughout human history, fresh resins have been used for a variety of purposes, for example as adhesives, lubricants, perfumes, fuel, incense, medicines, and wine additives.

Ambers have been prized and traded for millennia as jewelry, as an artistic medium, and occasionally for their purported medicinal or even magical properties.

Most natural resins, and hence most ambers, consist of moderately complex mixtures of terpenes. These mixtures often include components that readily polymerize when exposed to light or oxygen,[1,11] giving rise to the hardened masses commonly observed on many types of plants. The hardened resins often consist of a mixture of macromolecular material containing occluded, nonpolymerizable, lower molecular weight components.

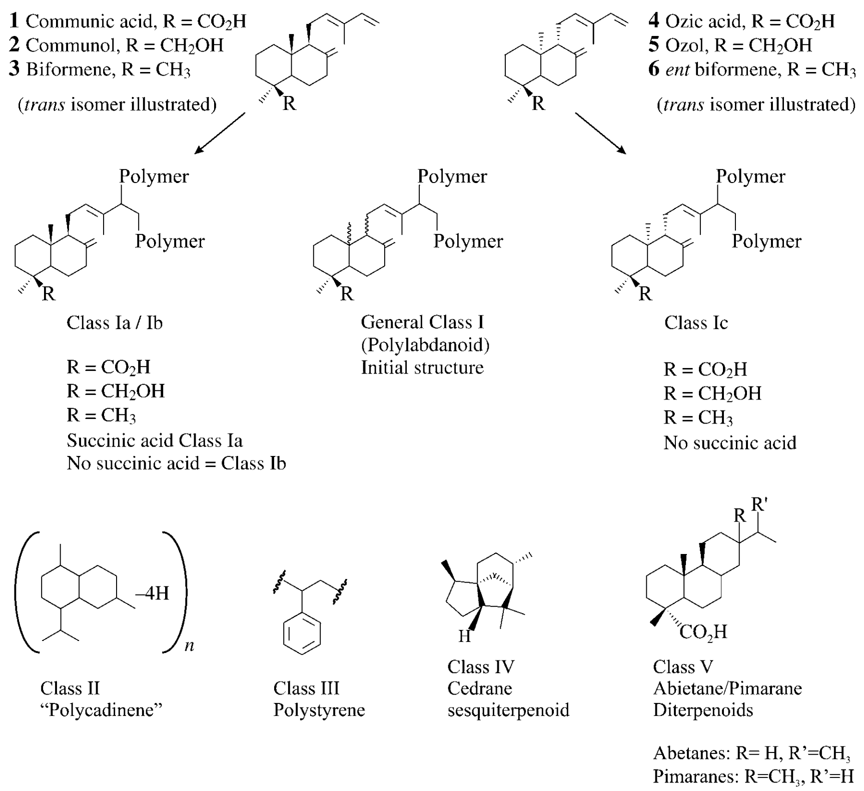
Conventional GC–MS analysis of volatile terpenes found in resins and ambers consists of three steps: 1) extraction with an organic solvent, 2) derivatization to convert polar analytes to more GC-amenable derivatives, and 3) chromatographic separation of individual components followed by identification of the components by MS. Both qualitative and quantitative information can be obtained in a single analysis, allowing the abundance and the structure of individual components to be determined. There are, however, significant limitations to the use of this methodology for analysis of ambers. Although use of microextraction and derivatization techniques has reduced sample requirements significantly, minimum sample sizes of >10 mg are typical. Once exposed to solvent, the sample cannot be recovered in its unaltered state for further analyses. This sample size, although small, still often exceeds the total amount of sample available, especially for rare or precious samples. Most critically, this methodology is limited to analysis of extractable low-molecular-weight components present in the resin or amber. Insoluble macromolecular materials and even soluble oligomeric materials, which can constitute a large fraction of some samples, are not observed by this technique.

To circumvent these limitations, conventional GC–MS analyses of ambers have been supplemented by pyrolytic injection techniques: pyrolysis GC–MS (Py GC–MS).[12] In this approach a small amount of sample is heated rapidly, and the resulting volatile material is swept directly into the injector of a GC. Sample requirements for Py GC–MS are very small. Exact requirements depend on the level of volatile and volatilizable materials present, but sample loadings for ambers of 200–500 μg are typical. This sample size is sufficiently small that samples not amenable to analysis by most spectroscopic methods or by conventional extraction can be readily analyzed. Furthermore, unlike conventional GC–MS methods, Py GC–MS analyses also can provide specific information on the composition of the macromolecular components of ambers. The temperature of pyrolysis can be varied such that only occluded materials are distilled away from the macromolecular components, or raised such that decomposition of the macromolecular phase occurs as well. Used in combination with in situ derivatization methods[12] to enhance the chromatographic behavior of polar analytes, these procedures provide highly detailed information regarding both occluded low-molecular-weight materials and the composition of the macromolecular phase.

2.2. Classification of Fossil Resins

Inorganic minerals are defined on the basis of their crystal structure and composition. Ambers lack a definable crystal structure and hence cannot be classified as true minerals. That is not to say, however, that ambers lack definable structural characteristics. Py GC–MS and other studies of ambers from all over the world indicate that most ambers fall into a small number of structural families, within which samples share common structural characteristics. Based on these observations, a structurally defined classification system for ambers has been proposed (Scheme 1).[13–16]

Ambers whose macromolecular structure is based on polymers or copolymers of labdanoid diterpenes are classified as Class I. These are by far the most common fossil resins found in the geosphere, both in terms of geographic distribution and distribution across geologic time. This type of resin structure appears to have evolved early and been conserved by subsequent evolution. Hence, a broad range of genera and families produce and have produced resins with



Scheme 1. Classification system for fossil resins, including structures of major precursors for Class I ambers.

this general macromolecular structure. This class is subdivided on the basis of the stereochemical characteristics of the labdanoids that make up the macromolecular structure and on the presence or absence of succinic acid within the structure.

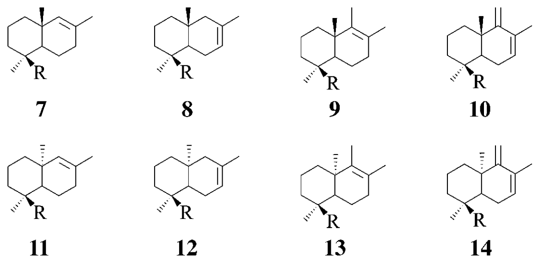
Class Ia ambers are based on labdanoid polymers with a so-called regular configuration (11β,19β,20β; hereafter referred to as βββ), for example polymers and copolymers of communic acid (**1**), communol (**2**), and biformene (**3**); in addition they incorporate significant amounts of succinic acid within their macromolecular structure. The well-known and abundant ambers found in the Baltic region and northern Europe are generally of this type. The structural role of succinic acid in these materials is not clear, but it may function primarily as an ester cross-linking agent between communol units within the macromolecular structure. Regular polylabdanoid ambers that lack succinic acid are classified as Class Ib and are arguably the most common form of amber in the geosphere. Class Ic ambers are based on polymers or copolymers of labdanoid diterpenes that have a 19β,11α,20α stereochemistry (hereafter referred to as βαα), for example ozic acid (**4**), ozol (**5**), and *ent*-biformene (**6**); they do not incorporate succinic acid within their structure. Polylabdanoid amber based on βαα monomers that does incorporate succinic acid also is known. At present such materials have been obtained only from a single site[17] and cannot be considered to be a general class. If confirmed, such materials would be designated Class Id.

The second most common type of amber on a global basis (Class II) is based on polymers of sesquiterpenoid hydrocarbons related to cadinene (Scheme 1). The most often cited structure for these materials[18,19] has been shown by 2D NMR analyses to be inaccurate.[20] Ambers of this type are common in Southeast Asia and the western and southern regions of the United States.

The remaining classes of ambers are far less common. Class III ambers are fossil polystyrenes.[13,21] These are confirmed from only two areas: Germany, where they are known as siegburgite, and the Atlantic coastal plain in the U.S., where they co-occur with other ambers. Class IV and V ambers are composed of terpenoids that lack the structural characteristics necessary for spontaneous polymerization and hence are not based on macromolecular structures. As a consequence, these materials tend to be softer and more friable than ambers of Classes I–III, and hence they are less able to survive, in recognizable form, the geologic processes that accompany burial. For this reason these materials are generally rare. Class IV ambers are based on cedranes and related materials. Class V ambers consist of mixtures of abietane, pimarane/isopimarane, and related diterpenoids commonly associated in modern taxa with pinaceous species.

Ambers are most conveniently classified by PyGC–MS, although the classifications are not defined on the basis of this type of analysis and PyGC–MS is by no means the only method capable of differentiating ambers of these various classes. The minimal sample requirements and detailed molecular level information accessible by this technique, however, make PyGC–MS the methodology of choice for characterization and classification of ambers.

Class I ambers are readily recognized in PyGC–MS analysis by the presence in their pyrolysates of a characteristic series of bicyclic compounds (Scheme 2) derived from the A/B rings of the labdanoids from which the macromolecular structure of these ambers was originally derived. Compounds **7**–**10** are characteristic of Class Ia and Ib ambers, and the epimeric compounds **11**–**14** are characteristic of Class Ic ambers. In mature samples, A-ring functionalization (R) also can be lost, and the remaining methyl group can be retained in either the α or the β configuration.[16] Classes Ia and Ib ambers are readily distinguished on the basis of the presence or absence of succinic acid.



R = CH_3, CH_2OH, CH_2OCH_3, CO_2H, CO_2CH_3

Scheme 2. Structures of characteristic bicyclic products observed in the pyrolysates of Class I ambers. Compounds **7**–**10** are characteristic of Class Ia and Ib ambers, compounds **11**–**14** are characteristic of Class Ic ambers.

Occluded compounds are readily differentiated from products derived from the macromolecular phase of the amber by variation of pyrolysis temperatures. Pyrolysis at 300–360 °C results in distillation of volatile components present in the amber but is insufficiently severe to result in dissociation of the macromolecular phase. Pyrolysis at higher temperatures (480 °C is typical in our laboratory) results in disruption of the macromolecular phase and release of compounds **7**–**10** or **11**–**14**. Pyrolysis at temperatures exceeding 500 °C tends to increase the formation of secondary pyrolysis products.[12]

Pyrolysis of Class II ambers generates characteristic but complex mixtures of hydrocarbons related to cadinene, and Class III ambers produce only styrene and related dimeric and trimeric compounds when subjected to pyrolysis.[13] Hence, these classes of ambers also are readily assigned on the basis of PyGC–MS analyses. Class IV ambers have not been studied by PyGC–MS to date. Class V ambers readily distill on low-temperature pyrolysis and are easily analyzed.

2.3. Maturation

All forms of organic matter undergo changes in sedimentary systems over geologic time. This general phenomenon is referred to as maturation. For example, vegetable matter deposited in sediments matures from biomass to peat to lignite to sub-bituminous coal to bituminous coal to anthracite, with each stage being accompanied by both physical and chemical changes in the structure of the material. Ambers undergo similar processes of maturation. The initial structural characteristics of polylabdanoids appear to be fairly well understood, based on studies of components of recent resins.[22,23] Communic acid and related compounds (**1**–**3**), for example, polymerize across a terminal double bond, resulting in a general polymer structure of the type illustrated in Scheme 1 for Classes I a/I b. Likewise, ozic acid and its analogues (**4**–**6**) polymerize to give a comparable macromolecular structure, which, however, incorporates the alternative stereochemical characteristics of these compounds (Class I c in Scheme 1).

Rapid polymerization results in the formation of high-molecular-weight polymers in hours to days, as indicated by the rapid hardening of fresh resins on the outer surfaces of trees.[22,23] Over geologic time, however, these initial polymers undergo further, subtle structural changes, possibly including additional cross-linking, in response to prolonged exposure to moderately elevated temperatures. The precise details of the structural changes that occur in Class I fossil resins in response to increasing maturation are not yet completely clear, but analytical evidence for these changes is well established.

A number of groups[5,13,24–26] have reported spectroscopic analyses of series of related Class I ambers of different levels of maturity and have demonstrated progressive loss of exomethylene ($C{=}CH_2$) structures with increasing maturity. In Py GC–MS data, maturation results in a change in the relative abundances of the characteristic C_{14} and C_{15} bicyclic products, e.g., (**7**+**8**)/(**9**+**10**) in Scheme 2. With increasing maturity, the relative abundance of C_{15} bicyclic products increases significantly to the point at which in mature samples C_{14} products are minimal or completely absent.[13] We also have observed a general trend of decreasing levels of occluded materials with increasing maturity, probably because these products migrate slowly into surrounding sediments.

3. NMR Spectroscopy of Ambers

As mentioned earlier, spectroscopic methods such as NMR and IR characterize the bulk composition of an amber sample, thus combining occlusions and polymeric materials. Although there is some advantage in examining the bulk of a material, the result is not a molecular analysis but rather a spectroscopic fingerprint. Thus, whereas GC–MS provides a (partial) analysis at the molecular level, IR and NMR spectroscopy provide an empirical or phenomenological assessment of the entire material with minimal consideration of molecular structure.

3.1. Solid-State ^{13}C NMR Spectroscopic Analysis of Ambers

In our early work, inspired by and carried out originally on samples provided by Beck, we set out to examine Baltic amber (succinite) and other ambers by the then newly developed technique of high-resolution solid-state ^{13}C NMR spectroscopy.[27] Many ambers are very poorly soluble in all solvents, so that solution NMR experiments would be selective if not impossible. The solid-state spectra of several samples of Baltic amber proved to be very consistent (Figure 3). The spectra of these samples are dominated by a reproducible sequence of peaks in the saturated region ($\delta = 0$–80 ppm). They also contain weak carbonyl resonances ($\delta = 170$–210 ppm) and very characteristic but weak alkenic resonances ($\delta = 110$–150 ppm). In addition to alkenic peaks in the region $\delta = 115$–140 ppm from di- and trisubstituted double bonds, the spectra contained peaks at $\delta = 110$ and 150 ppm from the functionality $C{=}CH_2$. This exomethylene functionality is found in the labdanoid diterpenes that define Class I (Scheme 1, compounds **1**–**6**) and in some other diterpenes such as agathic acid, but it does not occur in tricyclic diterpenes such as abietic acid and pimaric acid. Figure 3 contains not only the spectrum of Baltic amber with complete proton decoupling but also the spectrum with partial decoupling due to dipolar dephasing. This latter technique allows selection primarily of quaternary carbons, those not bonded to hydrogen, although some non-quaternary carbons in rapidly moving groups leak through. The two decoupling methods provide distinct patterns and help define phenomenological differences.

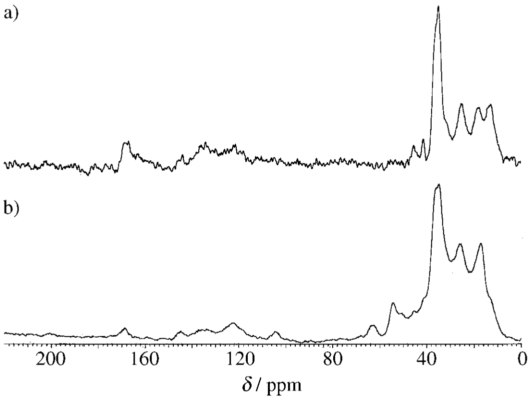


Figure 3. The ^{13}C NMR spectrum of Baltic amber (Class Ia, equivalent to NMR Group C) with a) full decoupling and b) dipolar dephasing.

A few ambers, such as beckerite, proved to have spectra nearly identical to that of succinite and may be considered to be the same material chemically.[28] Just as Beck observed with the IR spectra of succinite,[9] we found its NMR spectra to be rather different from those of European ambers other than succinite (Figure 4).[29] Differences are found in the alkenic region, which for non-succinite European ambers is devoid of exomethylene resonances at $\delta = 110$ and 150 ppm. In our 2002 summary,[30] we considered these differences sufficient to define two types of amber. We labeled the larger set, often lacking exomethylene resonances, Group A, and succinite and related materials, always with exomethylene resonances, Group C.

These phenomenological groups based on NMR spectra have correspondences with the molecular classifications based on GC–MS and other techniques. Thus Group A corresponds to Class I b (the largest in both classifications)

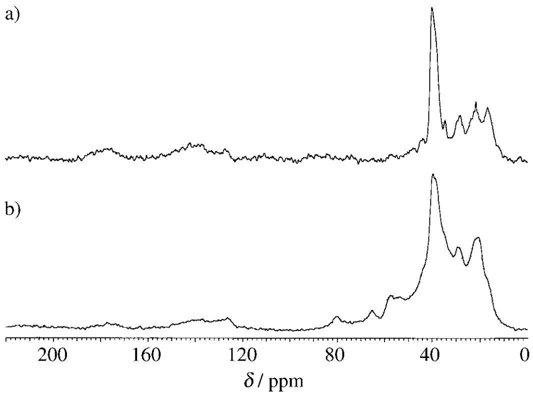


Figure 4. The ^{13}C NMR spectrum of walchowite (Class I b, equivalent to NMR Group A) with a) full decoupling and b) dipolar dephasing.

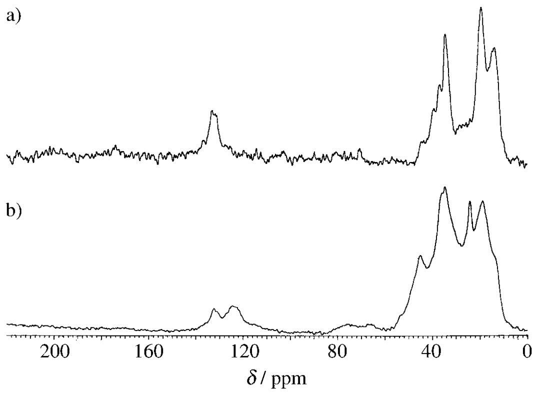


Figure 5. The ^{13}C NMR spectrum of amber from the Claiborne Formation in Arkansas, USA (Class II, equivalent to NMR Group B) with a) full decoupling and b) dipolar dephasing.

and Group C to Class I a (largely Baltic amber and related materials). Indeed, these materials differ only in the presence of succinic acid for Class I a and its absence for Class I b. The ^{13}C NMR resonance of the carboxyl carbon of succinic acid occurs at about $\delta = 170$ ppm, a relative low frequency value for carboxyl groups. Such a resonance appears in the spectra of Baltic amber (Figure 3) but not in the spectra of other European ambers (Figure 4) or of the more recent, related New Zealand ambers. Thus succinic acid appears to provide a distinguishing characteristic in both GC–MS and NMR classifications.

Subsequently, we undertook a worldwide survey of ambers by solid-state ^{13}C NMR spectroscopy.[30,31] We found many examples of the Group A NMR pattern for Class 1 b ambers from all over the world, including Alaska, Canada, the continental United States, Greenland, western Europe, Southwest Asia (Jordan, Lebanon, Iran), China (Liaoning province), Siberia, Southeast Asia (Burma),[34] Australia, and New Zealand. In general, the carbonyl resonance at $\delta = 170$ ppm was absent, and in older (especially Cretaceous) samples the exomethylene resonances at $\delta = 110$ and 150 ppm were minimal or absent (compare Figures 3 and 4).

A second worldwide NMR spectral pattern emerged from this study for samples, then termed Group B, corresponding to the molecular Class II.[30–33] This distinct NMR pattern (Figure 5) exhibits stronger resonances at the low-frequency end of the saturated region than the NMR patterns referred to as Groups A and C for Class I ambers. These materials also exhibit weak alkenic resonances but no exomethylene peaks. Samples of this type were found in the United States (Arkansas), India (Gujarat), Borneo, Sumatra, Papua New Guinea, and Australia.

Caribbean amber, usually from mines in the Dominican Republic, proved to have a ^{13}C NMR signature different from the other three groups (Figure 6).[25] The exomethylene resonances varied from strong in Dominican mines containing more recent amber to absent in nearby mines in Chiapas, Mexico.[35] The saturated region, however, remained relatively constant in all these samples, particularly when the spectra were recorded with the selective decoupling provided by dipolar dephasing. This pattern was designated as spectral Group D, which corresponds to the molecular Class I c. Many ambers from South America and Africa proved to have the same pattern, including materials from Kenya, Tanzania, and Madagascar.[36]

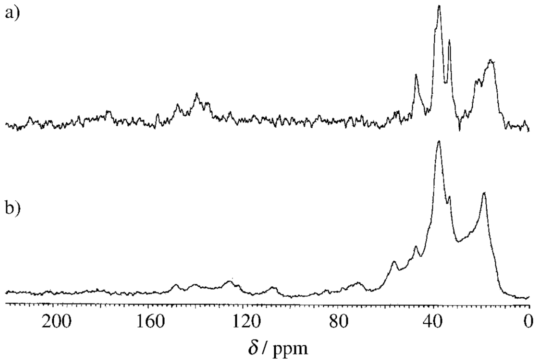


Figure 6. The ^{13}C NMR spectrum of amber from the La Aguita mine, Dominican Republic (Class Ic, equivalent to NMR Group D) with a) full decoupling and b) dipolar dephasing.

NMR spectra have been reported of fossil polystyrene from New Jersey (Class III).[31] No phenomenological grouping was assigned to this material.

3.2. NMR Spectroscopic Analysis of Modern Exudates

Since amber derives from resins, it is important to characterize as many recent exudates as possible to make comparisons. Numerous GC–MS studies of resins have been reported.[1–2] Even as recently as 2005, however, the solid-state ^{13}C NMR spectra had been reported for very few resins.[38] The ^{13}C NMR spectra of nearly 50 resin samples showed that the conifer families Cupressaceae and the Araucariaceae in general give nearly identical spectra, distinct from the spectra of the coniferous Pinaceae in particular.[38] The angiospermous Fabaceae and Burseraceae had quite different spectra from those of the three gymnospermous families and from each other.

Resins are just one class of exudates, and our survey of ^{13}C NMR spectra provided a means to distinguish quickly between materials that have little visual or tactile difference.[38] Mills and White[2] discuss three types of plant exudates: resins, gums, and gum resins. Even after having harvested hundreds of exudates in the wild, Santiago-Blay says that resins and gums are difficult or impossible to distinguish. Whereas resins derive from terpenes and have basically hydrocarbon structures, hardened plant gums are generally insoluble, high-molecular-weight polysaccharides. Gums have been used as watercolor media, as adhesives on postage stamps, and as components of food. Gums are produced commonly by plants of the genus *Acacia* of the Fabaceae and by trees of the genus *Prunus* of the Rosaceae, known for their fruit production (Figure 1). No conifers produce gums, although we have identified an araucarian gum resin. Figure 7 gives the spectrum of the well-known gum arabic as an example of a gum.[38] There is a large peak from carbons attached to a single oxygen centered at $\delta = 70$ ppm and a small peak from the anomeric carbon, which is attached to two oxygens, at $\delta = 105$ ppm. There are almost no hydrocarbon peaks, either saturated or unsaturated.

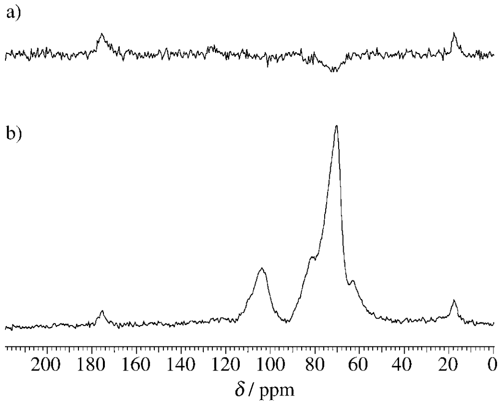


Figure 7. The 100 MHz ^{13}C NMR spectrum of an example of a gum, *Acacia senegal* (gum Arabic), with a) normal decoupling and b) dipolar dephasing.

In our survey we also found several examples of gum resins.[38] Chemically, these materials contain both terpenoid hydrocarbons, as do resins, and polysaccharides, as do gums. Frankincense and myrrh are famous examples of this category. Their NMR spectra also provide an immediate measure of the relative amounts of gum and resin, information not easily available from other techniques.

A fourth general exudate category emerged from the initial study[38] and was confirmed later.[39] Their solution ^{1}H and solid-state ^{13}C NMR spectra are dominated by resonances of unsaturated functionalities. Aromatic resonances attributable to phenols were evident in several samples from the Myrtaceae (genera *Eucalyptus* and *Corymbia*), generally referred to as eucalyptus trees, but also from the Fabaceae (*Prosopis*, *Centrolobium*) and the Zygophyllaceae (*Guaiacum*). We termed all of these materials kinos, although in the past the term has been used only for a few specific exudates.[39] The eucalypt materials are known as gum in Australia, but it seemed to us that the term should be reserved for polysaccharide exudates.

Because many modern resins generally are soluble in organic solvents, we also began systematically examining the solution-phase ^{1}H NMR spectra of conifer resins.[40,41] Such experiments, like non-pyrolytic GC–MS methods, have limitations with regard to sampling. They provide a phenomenological approach to characterizing many types of modern resins quickly. In the 1D spectra, we found 13 peaks present in most spectra of species of the Pinaceae, nine for the Cupressaceae, and five for the Araucariaceae (these are the three largest conifer families), with little overlap of the defining peaks between families. In the 2D COSY spectra, we found ten cross peaks characteristic of the Pinaceae, ten of the Cupressaceae, and six of the Araucariaceae, again with little overlap. Although some of these peaks and cross peaks occur in more than one family, it is clear that ^{1}H NMR spectroscopy generally can distinguish samples from the three families. Of the 21 other cross peaks considered diagnostic for one or more of these families, only three were diagnostic for two families. Thus 18 cross peaks could be used to identify particular families.

4. Botanical Sources of Ambers

Another dimension of the study of fossilized resins is the identification of the plant species responsible for the original exudate. Such identification is based primarily on comparison of ancient material with modern botanical counterparts. Unfortunately, there is no guarantee that the ancient plant is extant or even that closely related species still exist. In such cases, any identification is problematic. Secondly, maturation of the fossilized resins during burial over geologic times can produce changes that render comparisons with modern counterparts difficult. Nonetheless, it is important to endeavor to identify species responsible for exudates, because of possible ramifications in our understanding of paleoenvironments and possible clarification of phylogenetic or evolutionary relationships between extant and extinct taxa. Two general methods have been applied to this problem: identification of fossil plant parts associated with amber and comparison of chemical or spectroscopic characteristics of ancient and modern materials.

4.1. Identification of Fossil Plant Parts

Attempts to associate plant fossils with co-deposited amber suffer from a degree of uncertainty unless the amber is in unequivocal association with the plant fossils. It is possible, for example, that the dominant plant species present in an ecosystem may not have produced significant quantities of resin and that a relatively minor species was a copious resin producer. Abundant amber from the Fossil Forest deposits in the Canadian Arctic provide an excellent example of this problem.[17] Studies of well-preserved macrofossils have established that the original flora was dominated by *Metasequoia*, but analysis of fossil resins found in association with cone scales established that the ambers in these deposits were derived primarily from *Pseudolarix*, a relatively minor species in these ecosystems.

In most cases, paleobotanical association between ambers and co-deposited tissues can provide only circumstantial evidence for the botanical origins of the ambers. Analysis of Burmese amber (burmite) illustrates the methodology.[34] Fossil wood associated with the amber exhibited tracheal pits known to occur only in wood of the family Araucariaceae, and particularly in the wood of the genus *Agathis* (although the genus *Araucaria* is similar), suggesting this family as a potential paleobotanical source for this amber. In these cases, chemical or spectroscopic experiments on the amber itself are useful in providing supporting information (see Section 4.2).

Numerous workers have attempted to draw tentative conclusions regarding the paleobotanical sources of ambers. Langenheim[1] has reviewed the many controversies concerning paleobotanical assignments of ambers, so we will mention only a few of the more certain cases. Ambers of Class I c have been associated with the genus *Hymenaea* of the Fabaceae, a family of flowering plants (angiosperms), in contrast to the coniferous or gymnospermous sources of Class I b ambers. Langenheim found plant parts characteristic of *Hymenaea* in amber from Chiapas,[1] Grimaldi et al.[42] identified *Hymenaea* plant parts associated with Dominican amber, and Poinar[43] made similar observations with African materials. These conclusions are supported by spectroscopic investigations (see Section 4.2).

Probably the longest running and most controversial question on the subject of paleobotanical origins of amber relates to identification of the source of the rich Class I a amber deposits around the Baltic Sea. No modern resin analogous to these ambers is known. Langenheim's detailed review[1] came to no firm conclusion, but described possibilities within the gymnospermous families Pinaceae and Araucariaceae. Ambers derived from *Pseudolarix* found in the Eocene forests on Axel Heiberg Island in the Canadian Arctic contain succinic acid but are based on a macromolecular structure derived from labdanoids with a $\beta\alpha\alpha$ rather than the $\beta\beta\beta$ configuration found in typical Class I a ambers. Hence, *Pseudolarix* can be excluded as a potential source for Baltic amber.[17]

4.2. Chemical and Spectroscopic Comparisons of Ancient and Modern Resins

In some cases, chemical and spectroscopic analyses can provide strong evidence supporting the paleobotanical assignment of specific ambers. Class I c amber illustrates one relatively straightforward case for identification of the source genus. Fossil plant parts are consistent with a source from the genus *Hymenaea* of the angiospermous Fabaceae.[1] The IR spectra[44] of modern *Hymenaea courbaril* samples, their solid-state ^{13}C NMR spectra,[25,36] and their molecular components according to Py GC–MS[13] showed strong similarities to the data from Dominican and African ambers. Py GC–MS studies comparing Dominican and Mexican (Chiapas) ambers showed indistinguishable polylabdanoid skeletons characteristic of Class I c.[16] Dominican, Mexican, and South American amber additionally gave ^{13}C NMR spectra with strong similarities to each other.[25,36]

The paleobotanical origins of Class I b ambers are complex. An array of modern genera produce labdanoid-containing resins[45] that in principle could be precursors of Class I b ambers. It is also possible that extinct genera with no modern analogues could have produced polylabdanoid resins that now are preserved in the fossil record as Class I b ambers. There are a few cases (see below) with strong evidence associating particular ambers with defined botanical sources, but, in general, identification of paleobotanical sources of Class 1 b ambers has not been achieved with any level of confidence.

Py GC–MS analysis of upper Cretaceous amber from the Raritan Formation in New Jersey, USA, suggests a cupressean origin.[46] This conclusion is based on the presence of specific biomarkers that in modern species occur primarily in this family. This same study also was able to differentiate two related but distinct forms of amber in these deposits, based on variation in the distribution of diterpenes occluded within the macromolecular structure. On this basis it was suggested that these ambers are derived from at least two discrete, but likely closely related, cupressean species.[46]

Studies of ambers from New Zealand strongly suggest an association with the araucarian genus *Agathis*. Both NMR[26] and Py GC–MS[13] analysis indicate that these samples represent a nearly continuous series of Class I b ambers deposited from the Eocene on. These studies indicate strong correlation between mature samples and recent or even modern *Agathis* resins. The similarity of the NMR spectra of several burmite samples with those of some of the New Zealand samples supports the conclusion, based on tracheal pits observed in associated fossil wood, that burmite also was formed from araucarian species.

Although the situation is no clearer for Class II ambers,[1] the preponderance of evidence, primarily from IR and fossil plant studies, is that the source or sources of these ambers probably were angiospermous rather than gymnospermous.

5. Summary and Outlook

Modern chemical and spectroscopic techniques have provided reliable means to distinguish the several types of modern plant exudates. These include terpenoid resins, polysaccharide gums, hybrid gum resins with both terpenes and polysaccharides, and phenolic kinos. Only resins appear to persist over geological time to form amber. Py GC–MS has provided a general and reliable classification of amber types based on specific molecular constituents. Solid-state NMR spectroscopy is able to distinguish the various types of modern exudates and generally reflects the molecularly based GC–MS classification of ambers. NMR data also can provide many distinctions at the family, genus, and sometimes species level for modern resins.

The strongest challenge in this field at present is the reliable identification of ancient species responsible for the different types of amber. Only the modern plants responsible for amber in Class I c have been identified with reasonable reliability (the genus *Hymenaea* of the angiospermous family Fabaceae). Controversy continues over the most famous type of amber, found around the Baltic Sea (Class Ia), although all experts agree on a coniferous (gymnospermous) source. Class Ib ambers likely have many suggested coniferous sources. Paleobotanical affiliations need to be assessed on a case-by-case basis. Class II ambers appear to have an angiospermous source, but reliable and general attribution to specific plant families remains to be determined. Such research requires collaboration among geologists, archaeologists, botanists, and chemists in order to find the ancient materials with reliable provenance, identify associated ancient plant parts, obtain samples of modern plant exudates from thousands of candidates, characterize the modern exudates by chemical or spectroscopic techniques, and draw conclusions based on comparisons of the ancient and modern materials.

We gratefully acknowledge the financial assistance of the National Science Foundation (CHE-0349412), the Alumnae of Northwestern University, Gallaudet University, and Southern Illinois University Carbondale. We also are enormously indebted to the many people who have provided samples of ambers and modern exudates for analysis and to the many botanical gardens that have allowed us to collect on their premises. We thank P. S. Bray for helpful comments during the preparation of this manuscript and Dr. Yuyang Wu for recording many of the illustrated NMR spectra.

Received: December 28, 2007

---

[1] J. H. Langenheim, *Plant Resins: Chemistry, Evolution, Ecology, and Ethnobotany*, Timber Press, Portland, OR, **2003**.

[2] J. S. Mills, R. White, *The Organic Chemistry of Museum Objects*, 2nd ed., Butterworth Heinemann, Oxford, **1994**.

[3] P. C. Rice, *Amber the Golden Gem of the Ages*, Van Nostrand Reinhold, New York, **1980**.

[4] H. Fraquet, *Amber*, Butterworths, London, **1987**.

[5] J. W. Frondel, *Nature* **1967**, *215*, 1360 – 1361.

[6] J. W. Frondel, *Science* **1967**, *155*, 1411 – 1413.

[7] R. H. Brody, H. G. M. Edwards, A. M. Pollard, *Spectrochim. Acta Part A* **2001**, *57A*, 1325 – 1338.

[8] Y. M. Moreno, D. H. Christensen, O. F. Nielsen, *Asian J. Spectrosc.* **2000**, *4*, 49 – 56.

[9] C. W. Beck, *Appl. Spectrosc.* **1986**, *22*, 57 – 110.

[10] J. S. Mills, R. White, L. J. Gough, *Chem. Geol.* **1984**, *47*, 15 – 39.

[11] C. Lagercrantz, M. Yhland, *Acta Chem. Scand.* **1962**, *16*, 505 – 506.

[12] K. B. Anderson, R. E. Winans, *Anal. Chem.* **1991**, *63*, 2901 – 2908.

[13] K. B. Anderson, R. E. Winnans, R. E. Botto, *Org. Geochem.* **1992**, *18*, 829 – 841.

[14] K. B. Anderson, *Org. Geochem.* **1994**, *21*, 209 – 212.

[15] K. B. Anderson, J. C. Crelling in *Amber, Resinite, and Fossil Resins*, *ACS Symposium Series No. 617* (Eds.: K. B. Anderson, J. C. Crelling), American Chemical Society, Washington, DC, **1995**, pp. ix–xvii.

[16] K. B. Anderson, in *Amber, Resinite, and Fossil Resins*, *ACS Symposium Series No. 617* (Eds.: K. B. Anderson, J. C. Crelling), American Chemical Society, Washington, DC, **1995**, pp. 105 – 129.

[17] K. B. Anderson, B. A. LePage, in *Amber, Resinite, and Fossil Resins*, *ACS Symposium Series No. 617* (Eds.: K. B. Anderson, J. C. Crelling), American Chemical Society, Washington, DC, **1995**, pp. 170 – 192.

[18] B. K. G. van Aarssen, H. C. Cox, P. Hoogendoorn, J. W. de Leeuw, *Geochim. Cosmochim. Acta* **1990**, *54*, 3021 – 3031.

[19] B. G. K. van Aarssen, J. W. de Leeuw, M. Collinson, J. J. Boon, K. Goth, *Geochim. Cosmochim. Acta* **1994**, *58*, 223 – 229.

[20] K. B. Anderson, J. V. Muntean, *Geochem. Trans.*, **2000**, *1*, 1.

[21] M. A. Wilson, J. V. Hanna, K. B. Anderson, R. B. Botto, *Org. Geochem.* **1993**, *20*, 985 – 999.

[22] R. M. Carman, D. E. Cowley, R. R. Marty, *Aust. J. Chem.* **1970**, *23*, 1655 – 1665.

[23] R. M. Carman, N. Dennis, *Aust. J. Chem.* **1967**, *20*, 157 – 162.

[24] A. Cunningham, I. D. Gay, A. C. Oehlschlanger, J. H. Langenheim, *Phytochemistry* **1983**, *22*, 965 – 968.

[25] J. B. Lambert, J. S. Frye, G. O. Poinar, Jr., *Archaeometry* **1985**, *27*, 43 – 51.

[26] J. B. Lambert, S. C. Johnson, G. O. Poinar, Jr., J. S. Frye, *Geoarchaeology* **1993**, *8*, 141 – 155.

[27] J. B. Lambert, J. S. Frye, *Science* **1982**, *217*, 55 – 57.

[28] C. W. Beck, J. B. Lambert, J. S. Frye, *Phys. Chem. Miner.* **1986**, *13*, 411 – 414.

[29] J. B. Lambert, C. W. Beck, J. S. Frye, *Archaeometry* **1988**, *30*, 248 – 263.

[30] J. B. Lambert, G. O. Poinar, Jr., *Acc. Chem. Res.* **2002**, *35*, 628 – 636.

[31] J. B. Lambert, S. C. Johnson, G. O. Poinar, Jr., *Archaeometry* **1996**, *38*, 325 – 335.

[32] J. B. Lambert, C. E. Shawl, S. C. Johnson, G. O. Poinar, Jr., *Ancient Biomol.* **1999**, *3*, 29 – 35.

[33] J. B. Lambert, J. S. Frye, G. O. Poinar, *Geoarchaeology* **1990**, *5*, 43 – 52.

[34] G. Poinar, Jr., J. B. Lambert, Y. Wu, *J. Bot. Res. Inst. Texas* **2007**, *1*, 449 – 455.

[35] J. B. Lambert, J. S. Frye, T. A. Lee, Jr., C. J. Welch, G. O. Poinar, Jr., in *Archaeological Chemistry IV*, *Adv. Chem. No. 220* (Ed.: R. O. Allen), American Chemical Society, Washington, DC, **1989**, pp. 381 – 388.

[36] J. B. Lambert, S. C. Johnson, G. O. Poinar, Jr., in *Amber, Resinite, and Fossil Resins*, *ACS Symposium Series 617* (Eds.: K. B. Anderson, J. C. Crelling), American Chemical Society, Washington, DC, **1995**, pp. 193 – 202.

[37] J. B. Lambert, C. E. Shawl, G. O. Poinar, Jr., J. A. Santiago-Blay, *Bioorg. Chem.* **1999**, *27*, 409 – 433.

[38] J. B. Lambert, Y. Wu, J. A. Santiago-Blay, *J. Nat. Prod.* **2005**, *68*, 635 – 648.

[39] J. B. Lambert, Y. Wu, M. A. Kozminski, J. A. Santiago-Blay, *Aust. J. Chem.* **2007**, *60*, 862 – 870.

[40] J. B. Lambert, M. A. Kozminski, C. A. Fahlstrom, J. A. Santiago-Blay, *J. Nat. Prod.* **2007**, *70*, 188 – 195.

[41] J. B. Lambert, M. A. Kozminski, J. A. Santiago-Blay, *J. Nat. Prod.* **2007**, *70*, 1283 – 1294.

[42] D. A. Grimaldi, E. Bonwich, M. Dellanay, S. Doberstern, *Am. Mus. Novit.* **1994**, *3097*, 1 – 31.

[43] G. O. Poinar, Jr., *Experentia* **1991**, *47*, 1075 – 1082.

[44] J. H. Langenheim, *Science* **1969**, *163*, 1157 – 1169.

[45] A. Otto, V. Wilde, *Botanical Rev.* **2001**, *67*, 141 – 238.

[46] K. B. Anderson, *Geochem. Trans.* **2006**, 7, 1 – 9.