Molecular Classification of Banana Exudates[1]

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Abstract: Banana plants exude liquid from cuts on either their leaves or their fruit stalks. These exudates can stain clothing, tools, and the fruit itself. The liquid turns to a solid or semisolid over time after exposure to the atmosphere. Economic ramifications of this exudation include discolored fruit that must be discarded, added steps in production to remove the exudate before staining through sealing and washing, and cost to the environment of the aqueous waste from exudate removal. In order to elucidate the molecular structure of banana exudates, we have examined seven exudate samples from four different sources by nuclear magnetic resonance spectroscopy, both in the solid state with the carbon-13 nuclide and in solution with the hydrogen-1 nuclide. Remarkably, the samples proved to constitute four distinct molecular types, depending on the plant part and on the processing methods. The solidified exudate from the rachis proved to be a phenolic (polymeric material containing phenol components), which can be highly colored. The other materials were a wax (organic ester of long-chain hydrocarbons), a gum (high molecular weight polycarbohydrates), and resins (terpenoid hydrocarbon polymers).

Key Words: banana, exudate, gum, latex, nuclear magnetic resonance spectroscopy, phenolic, rachis, resorcinol, terpenoid resin, wax

Bananas, which originated in Southeast Asia, are cultivated intensively in many countries in the Western Hemisphere, particularly in the Caribbean Basin including both the islands and the adjacent mainland countries of North, Central, and South America (Peña et al. 2018, Robinson 1999). Bananas used primarily for cooking, often referred to as plantains (AAB group), are starchier and less sweet than the so-called dessert bananas (AAA group) popular for their sweetness in Europe and the Americas. All such plants are monocotyledons (monocots) that belong to the genus *Musa* from the family Musaceae of the order Zingiberales.

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There are about 70 species in the genus *Musa*, but only a few produce commercial bananas. Most of these are cultivars of *Musa acuminata*, particularly those from the Cavendish subgroup, which include the Grand Nain and other selections of Giant Cavendish.

Banana plants resemble trees but in fact are entirely herbaceous, possibly the largest such extant plants on Earth. The pseudostem of the banana comprises rolled bases of leaves and corresponds to the trunk of trees. To achieve an erect posture without a woody structure, the plants maintain a very high turgor pressure, or swelling within the plant walls from its fluid contents. Consequently, a wound on almost any part of the banana plant, caused for example by the harvesting process, results in generous exudation of phloem as a clear or milky liquid sometimes referred to as latex. Although harmless if washed off quickly, the exudate can stain skin, clothing, tools, or the surface of the fruit as it dries. Whereas stained clothing is an inconvenience to the worker, stained fruit tends to be rejected by the consumer. Banana exudates therefore have important economic consequences. If the exudate is collected and allowed to dry, it eventually forms a sticky to semi-solid material.

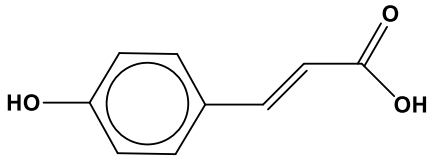
Bananas are harvested commercially in the unripe or green stage, so that the fruit may ripen about the time it reaches the consumer. They are harvested initially as large stalks, typically containing some 250 individual bananas. Each stalk is reduced to a number of bunches (also called hands, and the banana fruits referred to as fingers) for efficiency in packing, so that multiple cuts are made during processing, with resulting exudation (see Stover and Simmons 1987 for banana morphological nomenclature). The initial cut at the top of the stalk (the narrow connection from the pseudostem down to the first fruit, called the neck, peduncle, or pedicel) typically is covered for transportation of the whole stalk to the local packing facility. Cut surfaces continue to exude a liquid, in this context referred to as latex, for several minutes, until the turgor pressure reaches equilibrium. To avoid staining of surfaces in general, companies developed procedures for initially immersing the fruit in tanks of running water to wash away the exudate. These procedures have been detailed in unpublished research reports by scientists with the United Fruit Company. The reports were deposited in the Fundación Hondureña de Investigaciones Agrícolas (FHIA) in La Lima, Honduras, and are in the public domain. United Fruit Company researchers identified the presence of organic compounds containing unsaturated functionalities using paper chromatography (Jones 1966)[6]. The quantity of fresh water required for exudate removal is considerable, estimated at 8-10 liters per kilo of fresh fruit exported. The report estimates that an average farm releases about 350 kg of organic matter per harvest day into local waste water. In most countries, the waste moves directly into local drainage systems without treatment and thence into downstream ecosystems. The effluent can cause short-term

[6] The depository is housed at the former United Fruit Company Tropical Research Center.

oxygen deprivation and medium-term eutrophication in these systems. Thus, the exudation process has significant environmental ramifications.

When exposed to air, the exudate from the cuts begins to oxidize and polymerize, a process that intensifies the dyeing properties. Our own experiments confirm FHIA reports from the United Fruit Company that bananas produce about 3 g of dried exudate solid after curing per kilo of harvested fruit. The fresh latex normally is absorbed by a large volume of water, into which the banana hands and fingers are immersed. When air-collected exudate is allowed to settle, it forms three distinct fractions: a silty white precipitate, a reddish low-viscosity solid, and a highly viscous, white, sticky material. The third fraction is the adhesive material that sticks to any available surface of the processing facility, including skin, clothing, tools, and the fruit itself. All three fractions are primarily organic but are rich in inorganic elements as well.

Some chemical examination of banana exudates was carried out by Von Loeseke (1950). He reported that the latex from the peel of green bananas consists of about 85% water and 15% organics and produces about 1% inorganic ash, which contains chlorides, phosphates, carbonates, and sulfates of potassium, calcium, and magnesium. El-Sayed et al. (2001) carried out a gas chromatographic/mass spectrometric study on juice extracted from the pseudostem of bananas that the authors identified only as being paradica (possibly the hybrid *Musa* X *paradisiaca*) and mughraby (otherwise unidentified). They identified four compounds only from the masses of their presumed parent peaks. These were octyloxybenzene (the octyl ester of phenol) and three aromatic amines (the nitrogen counterpart of phenols). Pothavorn et al. (2010) used high-performance liquid chromatography/electrospray ionization mass spectrometry to identify organic molecules in the latex of several *Musa* species. They employed methods to avoid oxidation, so that the original molecules could be identified. They found a hydroxycinnamic acid (*p*-coumaric acid, **1**), flavones (**2**), flavonols (**3**, in which *glycosides* represent carbohydrate components), and dopamine (**4**). Under natural conditions, the phenolic exudates polymerize to form polyphenols, which are the active dyes but in addition may have positive physiological properties such as antimicrobial, anti-inflammatory, blood coagulative, or wound-healing activity (Pothavorn et al. 2010). Indeed, Ranjan-Kumara et al. (2014) reported antimicrobial properties of sap from the banana pseudostem with various fungal and bacterial strains. Nagarajan et al. (2013) found that the antimicrobial activity was caused by compounds synthesized in the secondary metabolism of the banana plant. In a more botanical study, Kallarackal et al. (1986) used microscopic and cytochemical methods to elucidate primarily the cell structure of latex components. Baker et al. (1990) carried out inorganic analyses of banana latex, finding, like Von Loeseke, primarily the chlorides and nitrates of potassium and magnesium.



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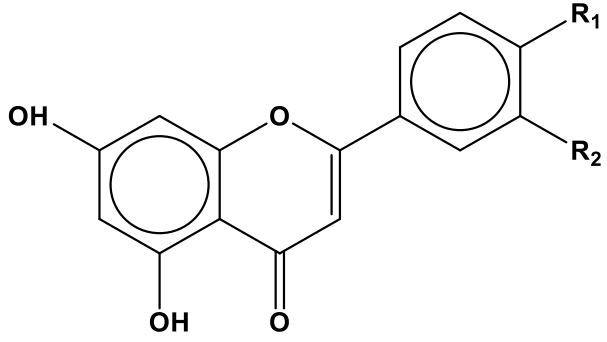

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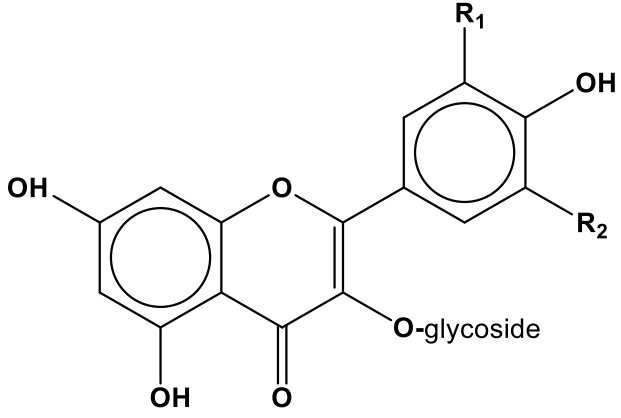

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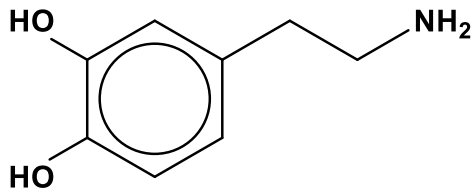

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There has been little recent scientific examination of banana exudates. Much of the earlier research was driven by the economic agenda of the large banana producers. Nonetheless, there have been isolated analyses, both inorganic (Baker et al. 1990) and organic (Pothavorn et al. 2010). The study of Pothavorn et al. was extremely successful in identifying almost a dozen specific molecular components in unoxidized banana latex. Their objectives were to identify components in the original latex, rather than in the oxidized form that is the natural residue. Phenolic components of the latex polymerize through catalysis by polyphenol oxidase (PPO). The effectiveness of the enzyme may be inhibited in solution by the presence of salt (NaCl), ascorbic acid, citric acid, or sodium bisulfite ($Na_2S_2O_5$). The use of these materials by Pothavorn et al. in the isolation process therefore enabled them to identify molecules from the original latex. Their method involved initial dissolution of the sap in 80% ethanol containing the inhibitors, heating to 80°C for 30 min, centrifugation, collection of the supernatant, concentration through rotary evaporation, and redissolution in 80% ethanol to produce the material to be subjected to analysis through HPLC and ESI MS. This procedure involved treatment at a temperature much higher than banana plants normally experience, which might be 45°C in the extreme. Only the supernatant was analyzed, so that higher molecular weight components were left in the centrifuge tube. The relative amounts of residue and supernatant were not specified. Although the percentages were normalized to 100%, the proportions do not reflect proportions in the original exudate or even in the treated exudate when the residue is included. No control was reported for solutions not treated with the inhibitors of PPO. Although the results provide outstanding insight into the identity of molecules in the original sap, the study left a large portion of the exudate unexamined. In commercial processing, no inhibitors are included other than aluminum sulfate to coagulate the suspended organic material, so that the exudates that are removed by treatment in water tanks are rather different from those studied by Pothavorn et al. (2010).

It is the purpose of the present study to examine oxidized banana exudates with nuclear magnetic resonance (NMR) spectroscopy (Lambert et al. 2019). This technique can examine materials in either the solid or the solution state. We report such examination of seven banana exudate samples. When

exudates are examined in the solid state, the analysis refers to the entire sample. There is no purification or selection process (Lambert et al. 2008). When the exudates are examined in solution, the results reflect the dissolved portion of the exudate and are strongly dependent on the nature of the solvent.

Methods

For this study we examined seven samples of banana exudates. One was a museum sample, one was collected more or less adventitiously, and the remaining five were prepared in controlled fashions. Each is identified herein by the accession number in the Trinity University collection of modern and fossilized resins. Sample 1192 was drawn from the collection of the National Herbarium of the Netherlands (Leiden branch). It was dated November 1932 and carried the label *Musa sumatrana*, the blood banana, so-called because of the red patches in its otherwise green leaves. The species name today is *M. acuminata* var. *zebrina* (L. van Houtte ex Planchon) Nasution, a wild banana native to Sumatra, Indonesia. The sample label also carried the words "Pisangwar B'2org ivory." Although these words are essentially uninformative, it should be noted that *pisang* is the Malay word for banana, suggesting a source in Malaysia. The Dutch had historical presences in both Malaysia and Indonesia. Sample 379 differed from our others by being granular in nature. It was collected by S. Shaffer and author JASB in 2005 as found on a banana plant growing in Washington, DC. It was identified simply as *Musa* sp., although it presumably was *M. acuminata*. The sample was solid and easy pulverized.

The remaining samples were obtained and prepared by author RCY. They were identified as the Grand Nain cultivar of the Cavendish subgroup, which today is given the species name *M. acuminata* 'Grand Nain' and previously was *M. cavendishii*. Samples 1752 and 1753 were opaque black with a tinge of red. They were harvested as whole latex by attaching polyethylene bags to the severed ends of cut banana rachides on plants grown at the Tropical Research and Education Center, University of Florida, Homestead, Florida (Figure 1). The rachis (singular, *rachides* plural) is the stem below the neck or peduncle of the stalk, to which the banana hands or bunches are attached. The bag was left open at ambient temperature for about one week to oxidize and dry naturally. No heating was applied. The sticky but solidified material was removed with a spatula and stored in polyethylene bags. These plants were grown in calcareous Krome soil with a relatively high pH. Samples 1752 and 1753 were distinguished only by coming from different plants. Provenance and location of the two plants were identical, and both fruits had reached the unripe, harvestable stage of maturity.

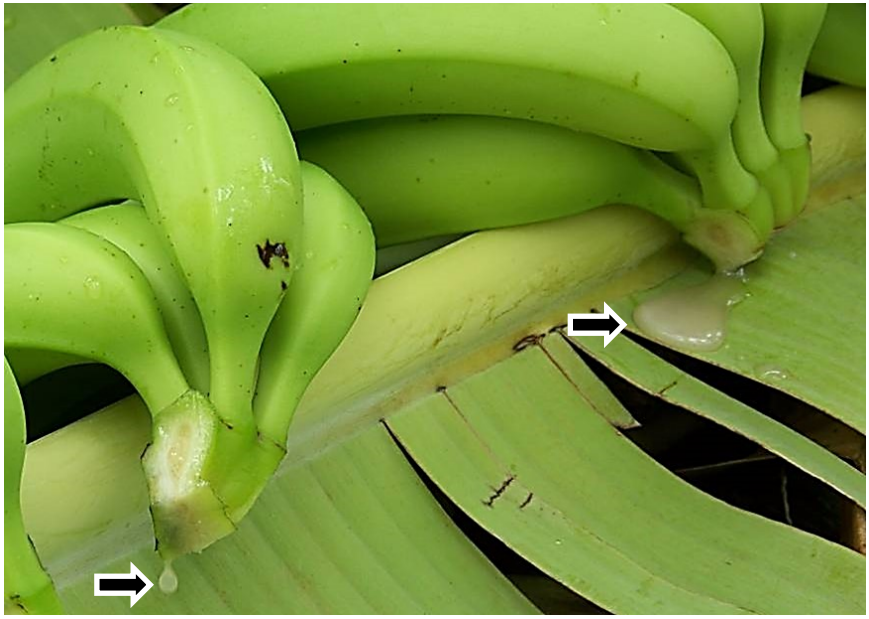

Figure 1. Cut banana bunches, showing cut rachides with exudation (arrows). Photograph by Richard C. Yudin.

Samples 1782-4 were replicates collected by author RCY from the Cortijo farm in the Uraba region of Colombia (latitude 7.82° north, longitude 76.66° west). Each sample comprised a half gram of the dried residue of the lipid-rich *chicle* floating portion of the banana phloem collected from the cut rachides of freshly harvested immature fruit. Each exudate was collected by allowing the cut surfaces to drip directly into steam-sterilized glass baby food jars with a screw top and left to settle so the three fractions separated prior to air transport to Florida.

Spectra were taken in four modes. Carbon (^{13}C) spectra were recorded with full decoupling (removal of interactions with hydrogen atoms, referred to herein as protons) and with interrupted decoupling (also called dipolar dephasing), a technique to select for quaternary carbons (those lacking C—H bonds), although some rapidly moving carbons also appear in the spectrum. Both the normal one-dimensional (1D) proton (^{1}H) was recorded and the two-dimensional COSY (correlation spectroscopy) spectrum.

Solid state ^{13}C data were recorded on a 400 MHz Varian NMR System at Northwestern University with a 5 mm T3 PENCIL probe or on a 400 MHz Bruker Avance III HD NMR Spectrometer with a 4 mm HX probe. The magic angle spinning rate was set to 5000 Hz. The cross polarization

(CP) pulse sequence was used for normal proton decoupling on both spectrometers. For interrupted decoupling (dipolar dephasing), a 50 μs (Varian) or a 48 μs (Bruker) delay was applied in the ^{1}H channel just before the 180° pulse in the ^{13}C channel. We used adamantane (Varian) or glycine (Bruker) to adjust the Hartmann-Hahn matching condition for normal CP experiments and to adjust the observation pulse and the delay time for dipolar dephasing. A typical parameter set was as follows: spectrum frequency 100.544 MHz (Varian) or 100.524 MHz (Bruker), spectral width 296 ppm, pulse width 3.4 μs for the 90° pulse for both ^{1}H and ^{13}C (Varian) or 2.5 μs for ^{1}H and 4.0 μs for ^{13}C (Bruker), pre-delay time 5 s, contact time 5 ms, acquisition time 50 ms, scan number 256, carrier frequency 110 ppm, and a ramped pulse with 83 Watts used in the ^{1}H channel during contact time. Solid state ^{13}C spectra were referenced to an external adamantane peak at δ 38.3 (Varian) or to an external glycine methylene peak at δ 43.4 (Bruker) and were referenced to tetramethylsilane at δ 0.0. Proton spectra were obtained at 500 MHz on a Varian Inova-500 spectrometer at room temperature without spinning at Trinity University. Spectra were referenced in $CDCl_3$ to TMS at δ 0.0. Typical 1D parameters were as follows: spectral width 12,000 Hz, pulse width 60°, delay time 1.0 s, acquisition time 1.0 s, and scan number 4.

Results and Discussion

The granular material of sample 379 proved to be a gum. Such materials constitute the second most common type of exudate in Nature, after terpenoid resins, and are composed of polymerized sugar units (Nussinovitch 2010). In a carbohydrate, every carbon is attached to an oxygen atom, but the so-called anomeric carbon is attached to two. In hexose sugars (those with six total carbon atoms), there is one anomeric carbon and five carbons with single oxygen attachments for each sugar unit. Figure 2 presents the ^{13}C spectrum of sample 379. The two major peaks result from the C—O carbons in the sugars at δ 73 and the anomeric carbon at δ 105, very normal positions for gums. There are some other, smaller peaks in the C—O region and some small peaks at δ ca. 170 in the carbonyl region. The absence of a peak at δ 93 demonstrates that the material is not simply sucrose, which exhibits a peak at that position. The spectrum is definitive for a gum, although there additionally are some resinous peaks in the δ 15-50 region, unsaturated peaks in the δ 115-140 region, and carbonyl peaks in the δ 165-185 region. The material was insoluble in $CDCl_3$, so the ^{1}H spectrum contained only solvent peaks. The gum is not responsible for the dying effects of banana exudates.

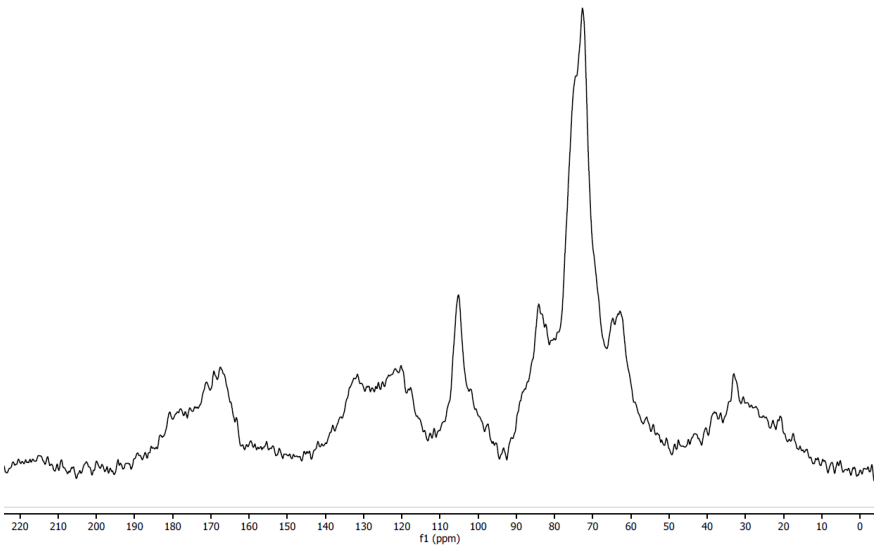

Figure 2. The solid state 100 MHz ^{13}C spectrum of sample 379 with cross polarization and magic angle spinning.

The historical sample 1192 from the Netherlands proved to be a wax. Since we had no information from the museum as to what part of the banana plant was sampled, we cannot relate this result to other factors. The ^{13}C spectrum has been published as Figure 17 in our study of monocots (Lambert et al. 2015). The only peaks are in the saturated region, in which the carbon atoms are attached only to hydrogens and other carbon atoms. The methylene (CH_2) and methine (CH) carbons appear as the large, broad, featureless peak centered at δ 32. The breadth of the peak (from δ 20 to 40), however, encompasses the region of carbons next to carbonyl groups, as in ketones (acetone δ 30), carboxylic acids (acetic acid δ 19), and esters (methyl acetate δ 18). There also is a small, sharp peak at δ 16 from methyl (CH_3) carbons. The proton spectrum in $CDCl_3$ (Figure 3) contains a small number of sharp peaks, not resembling other exudates except resins in a few features. Like the carbon spectrum, it suggests a wax. Indeed, a white, powdery wax is visible on the outer surfaces of younger plants of newly emerged leaf petioles, which attaches the leaf to the plant stem (Figure 4). It appears to have a biological function as a lubricant for the coiled growing leaves as they push their way up through the bundle of older petioles that comprise the rigid vertical pseudostem. It has no dying properties. It is possible to collect this powder by scraping. We can only hypothesize that sample 1192 was collected in this manner.

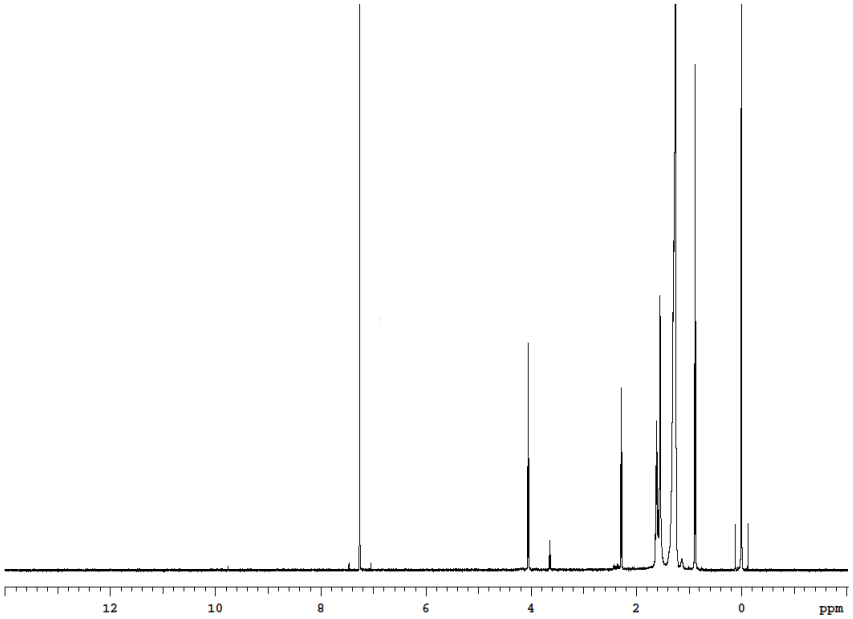

Figure 3. The 500 Hz ^{1}H spectrum in $CDCl_3$ of sample 1192.

Figure 4. Leaves on a growing banana plant, exhibiting the waxy exudate as the surface white substance. Photograph from collection of Richard C. Yudin.

The large peak at δ 7.3 in the ^{1}H spectrum (Figure 3) is from residual $CHCl_3$ in the solvent $CDCl_3$. The lowest frequency grouping (at the far right) is a narrow set of peaks at δ 0.9 composed of two or three sharp peaks. The resonance position strongly suggests methyl hydrogens, consonant with the small carbon resonance at δ 16. The largest grouping in the spectrum by far is at δ 1.3, composed of multiple sharp peaks several times the intensity of all other peaks. Presumably these are methylene (CH_2) groups corresponding to the lower frequency positions of the large carbon resonance at δ 15-44. Next is a small pair of peaks at δ 1.6 and 1.7, the former composed of multiple peaks but the latter a simple singlet. These resonances may come from methinyl (CH) groups or from methylene groups beta to oxygen. The next higher frequency grouping is at δ 2.3, which most likely corresponds to methylene groups next to carbonyl [—CH_2(C=O)—]. Finally, the highest frequency peak grouping is at δ 4.1, composed of multiple sharp peaks. Only ester functionalities resonate at such a high frequency, because of an additional electron-withdrawing effect provide by electronic interactions between the ester oxygen and the ester carbonyl. This overall resonance pattern is explicitly representative of waxes.

Noller (1957) defines this class of organic compounds as follows: "esters of high molecular weight monohydric [one hydroxyl group] alcohols with the common higher fat acids. Hence they have the general formula of RCOOR'. Actually, the natural waxes are mixtures of esters and frequently contain hydrocarbons as well." The approximate formula for beeswax is $C_{15}H_{31}COOC_{30}H_{61}$. Spermaceti from the head of the sperm whale is composed predominately of cetyl palmitate, $C_{15}H_{31}COOC_{16}H_{33}$. Carnauba or Brazil palm wax has a similar average formula with both the alcohol (R' above) and carboxylic acid (R) in the C26-C30 range. The actual structure of the wax molecule(s) should be readily amenable to mass spectrometric methods.

The ^{13}C spectra of samples 1782-1784 (Figure 5 for 1782) were nearly identical and contained significant resonances only in the saturated region typical of a terpenoid resin. As a class, resins constitute the largest type of plant exudates (Langenheim 2003). Other regions are not devoid of peaks, but all are weak. Such materials are hydrocarbon in nature and tend to be soluble in chloroform and insoluble in water. Indeed, the samples gave nearly identical ^{1}H spectra in $CDCl_3$ (Figure 6 for 1782). The resonances fall into four or five narrow groupings, indicative of a modest number of distinct compounds, consistent with the sharp peaks in the solid-state ^{13}C spectra. As the exudates become molecularly more complex, peak overlap increases in both ^{1}H and ^{13}C spectra. The ^{1}H spectra of 1782-1784 in D_2O was nearly empty. The ^{1}H in DMSO-d_6 [$(CD_3)_2SO$, which has polarity intermediate between chloroform and water] was similar to that in $CDCl_3$. Heating the sample to 60°C resulted in a few weak peaks in D_2O in the region δ 0.7-3.9, but these may result from decomposition rather than reflect higher solubility. Normally resins have no dying properties.

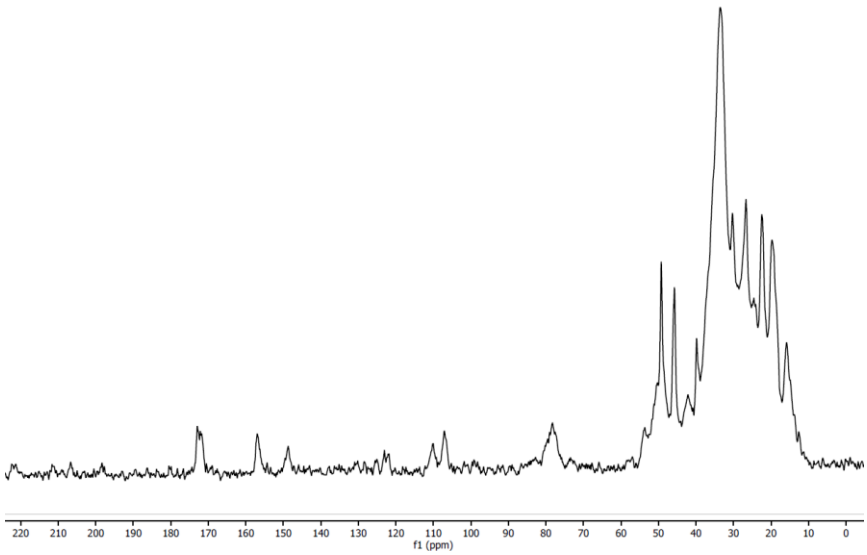

Figure 5. The solid state 100 MHz ^{13}C spectrum of sample 1782 with cross polarization and magic angle spinning.

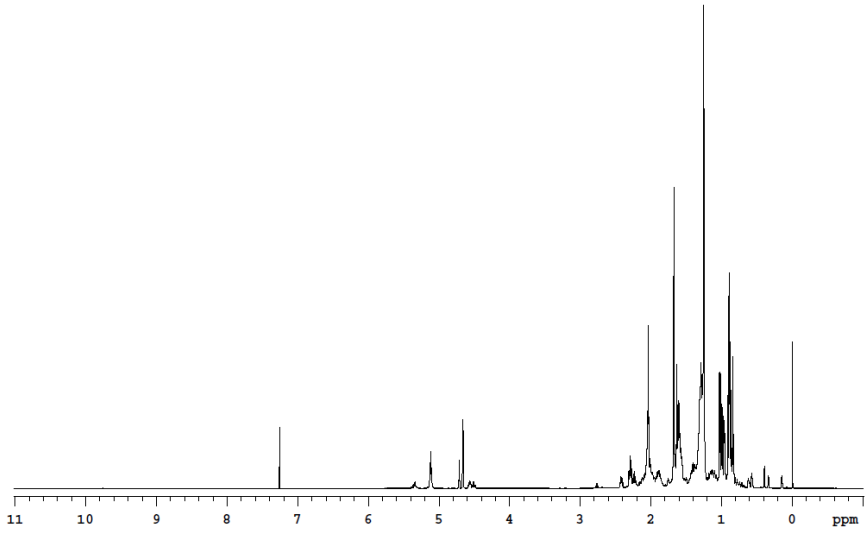

Figure 6. The 500 MHz ^{1}H spectrum in $CDCl_3$ of sample 1782.

Samples 1752 and 1753 comprise whole latex harvested from rachides of *M. acuminata* 'Grand Nain' and allowed to dry under ambient conditions. The ^{13}C spectra of these samples in the solid differ fundamentally from anything related to a resin, gum, gum resin, or wax, as illustrated in Figures 7 and 8 for 1752 and 1753, respectively. Mills and White (1994), in their survey of the materials of museum objects, cite only four types of exudate museum objects: resins (mostly hydrocarbons derived from terpenes), gums (polycarbohydrates), gum resins (a mixture of the two), and waxes (esters of long-chain fatty acids). The ^{13}C spectrum of a gum is illustrated in Figure 2. That of wax 1192 has been published in Lambert et al. (2015). The spectra of resins have been discussed extensively (for example, Lambert et al. 2007a).

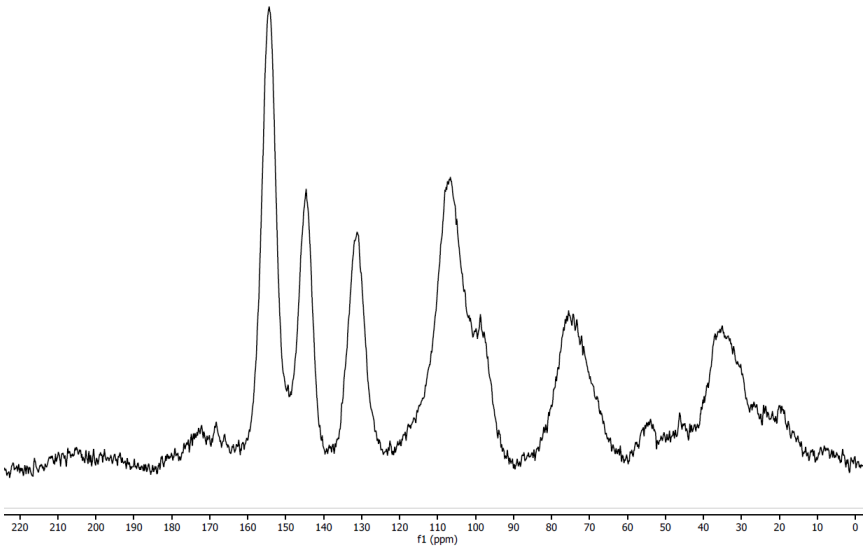

Figure 7. The solid state 100 MHz ^{13}C spectrum of sample 1752 with cross polarization and magic angle spinning.

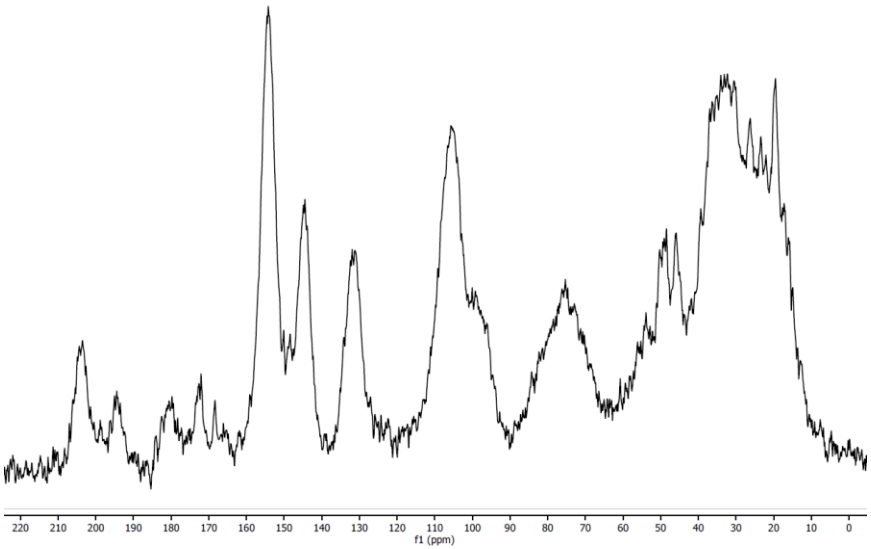

Figure 8. The solid state 100 MHz ^{13}C spectrum of sample 1753 with cross polarization and magic angle spinning.

Langenheim (2003) includes considerable information about a fifth type of exudate. She uses the traditional chemical term *phenolic resin*, which derives from polymer chemistry and refers, however, to copolymers of phenol and formaldehyde, very different structures from those found in terpenoid resins and from the present materials, which lack the formaldehyde co-monomer. In order to avoid confusion between these various types of polymeric materials, we prefer to omit the word *resin* from *phenolic resins* in the context of exudates, so that the word *resin* is reserved for terpenoid polymers and these phenol-containing exudates without formaldehyde are termed simply *phenolics*. Mills and White (1994) made no mention of the phenolic exudate group. These materials are defined by the presence of phenolic functional groups, which requires a hydroxy group (—OH) attached directly to a benzene ring (**5**, the parent compound phenol). Phenolic functionalities are present are **1**-**4**. The properties of phenolic hydroxy groups are very different from alcoholic hydroxy groups, particularly with regard to acidity. The phenolic functionality is found widely in naturally occurring organic materials. The ^{13}C chemical shift of the aromatic carbon to which hydroxy is attached in monohydroxylic phenols is δ 155, whereas the other five carbons in such aromatic rings resonate in the region δ 115-130, more typical for aromatic carbons. The only other functionalities that can shift the resonance position to this high frequency (δ 155) are fluoro, amino, and nitro, which are much less common on aromatic rings in nature. Consequently, a resonance in the range δ 150-160 (a range that allows for effects of other substituents on the aromatic ring) is strongly diagnostic for a phenolic exudate. The first time we

saw such spectra were in the Australian kinos (Lambert et al. 2007b).

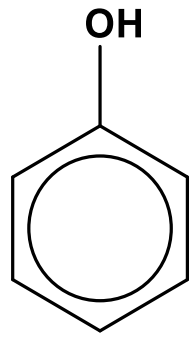

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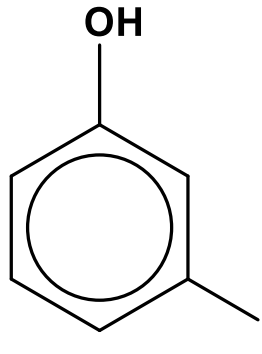
Figure 7 reproduces the ^{13}C spectrum of sample 1752. The characteristic phenolic peak is the largest in the spectrum, centered at δ 154, and resonances from the other aromatic carbons are found at δ 122. The peaks in the region from δ 15 to 60 are indicative of resinous (terpenoid) functionalities. The peaks in the broad range from δ 65 to 90 indicate carbons attached to oxygen, as in alcohols, ethers, and esters. These include the C—O bonds in carbohydrates, as found in gums, but we cannot differentiate here between carbohydrates and simple alcohols (ROH), ethers (ROR), and esters (R'(CO)OR). Normally gums may be identified by a characteristic peak at δ ca. 105 from the anomeric carbon attached to two oxygen atoms, O—C—O). There are multiple strong peaks in the region δ 96-116, which could correspond to the anomeric carbon. The size of the resonance, however, is much too large. Normally the anomeric resonance is a fifth the size of the C—O resonance in the spectra of carbohydrates, reflecting the atom ratio within sugar rings, but here the peak around δ 105 is considerably larger than the C—O resonance at δ 75. Although there may be small anomeric peaks, a more likely explanation is that there are strong contributions from resorcinol functionalities, which are phenols with two hydroxy groups 1,3 (meta) to each other, as in the parent compound (**6**). In resorcinol the two carbons attached to hydroxyl resonate at δ 158 and fall within the diagnostic range for phenolics. The carbon between (ortho to) the two hydroxy groups, however, resonates at δ 104, and the carbons just on the other side of (ortho to) the hydroxyls resonate at δ 109. These three carbons are responsible for the large peak at δ 96-116, which also includes any anomeric carbons. The carbon that is 1,3 (meta) to both hydroxy groups in resorcinol resonates at δ 132 and is found within the same peak derived from other phenolic carbons. It is noted that both flavones (**2**) and flavonols (**3**) contain resorcinol components. The ortho carbon of phenol itself resonates at δ 115, a largely empty region in these spectra, so it is likely that most of the phenols in these exudates are dihydroxylic (resorcinols).



6

The sample also may contain cinnamyl components (**1**), which are common in phenolics and have the general structure Ph—CH=CH—O(CO)R). In a sense, the double bond has been interposed between the aromatic ring (Ph stands for the simplest such ring, C_6H_5—, called phenyl) and the hydroxy functionality. Here the OH group has been esterified to form the ester functionality —O(CO)R, in which R normally is an alkyl group. For example, when R = CH_3, the CH carbon attached to oxygen in methyl cinnamate resonates at δ 142. Thus the large resonance at δ 145 may indicate the cinnamyl component. It should be noted that, in solid state ^{13}C spectra, carbon atoms on double bonds create spinning sidebands that corresponding to the spinning rate of the sample (here 5000 Hz or 50 ppm). Thus, the peaks at δ 206, 196, 180, and 57 likely are spinning sidebands and are false peaks, to be ignored. There are other spinning sidebands that fall underneath large peaks and are hidden.

The picture as a whole for phenolics corresponds to a mixture of phenols, resorcinols, cinnamates, sugars, and alkanes (possibly of a terpenoid source). The spectrum of sample 1753 (Figure 8) is nearly identical to that of 1752 (Figure 7) in the entire unsaturated and carbonyl regions from δ 100 to 210. The major differences between the two are that the saturated region of 1753 is about three times larger than that of 1752, and the cinnamyl peak at δ 145 is slightly smaller in 1753. The two spectra are very similar to that of *Eucalyptus sideroxylon* A.Cunningham ex Woolls (Myrtaceae) (Lambert et al. 2007b and Figure 9, below) in terms of peak locations, but with large differences in relative intensities.

Sample 1752 of *M. acuminata* gave a poor ^1H spectrum in $CDCl_3$, containing just a few peaks in the saturated region, probably representing the terpene portion of the sample that dissolved is this organic solvent. In addition, there is a small peak at δ 4.7, in the alkenic region, possibly from a cinnamyl functionality. The aromatic region is completely empty. Chloroform apparently is able to dissolve only resinous materials and excludes the phenolic components. In the highly polar and hydroxylic solvent D_2O the reverse occurs (Figure 10). Water dissolves very different materials from what chloroform dissolves. A very rich ^1H spectrum of

1752 (Figure 10) was obtained in D_2O, which is very effective in dissolving phenols, carboxylic acids, and similar hydroxylic materials. There are many peaks in the region δ 3-4, and somewhat less intense peaks in the aromatic region, δ 6.6-7.6. Phenolic aromatic peaks resonate in the region δ 6.9-7.2 and can account for all the aromatic resonances except the single at δ 8.3, which may be aldehydic. The ^1H spectrum suggests that the cinnamyl esters are more soluble than the phenolic esters, as expected. The spectra of 1752 and 1753 are nearly identical in both respective solvents, except that the saturated region of 1752 in $CDCl_3$ is weaker than that of 1753, presumably reflecting the lower proportion of resinous material in this sample. The large, off-scale peak at δ 4.7 is from residual HOD in the fully deuterated solvent, and the sharp peaks at δ 1.8 and 2.1 are impurities in the solvent. There are almost no resonances in the saturated region, δ 0.5-2.5, as nonpolar materials like terpenes, which produce resonances in that region, are poorly soluble in water. The richest part of the spectrum is the electron-withdrawing region, δ 2.5-4.5, in which resonances from hydrogens on carbons attached to oxygen (H—C—O) or even carbonyl (H—C—C=O) and alkenic (H—C—C=C) groups are found. There also is a rich but weaker set of resonances in the aromatic region, δ 6.5-8.0, attributable to phenolic functionalities. The overall picture from the proton spectrum is less definitive than that from the carbon spectrum, but is consistent with materials dominated by oxygen and, less so, aromatic functionalities.

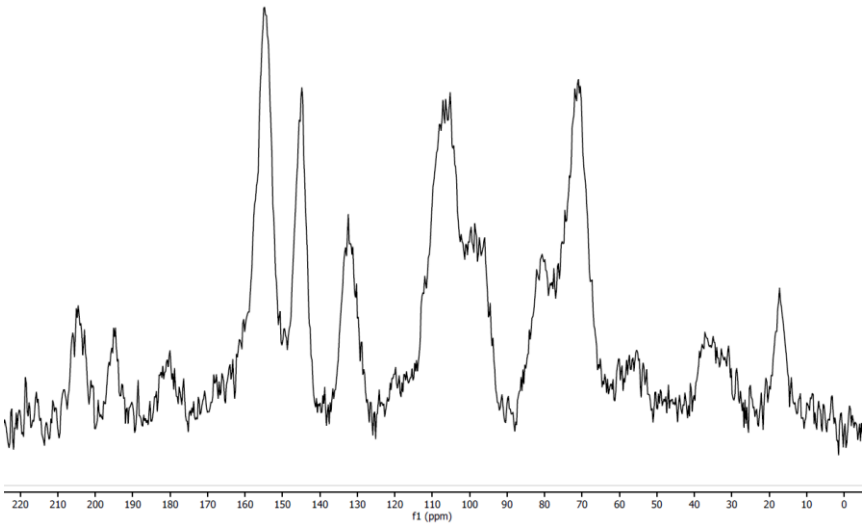


Figure 9. The solid state 100 MHz ^{13}C spectrum of sample 1288, *Eucalyptus sideroxylon* from the Boyce Thompson Arboretum, Superior, Arizona, USA collected by author JASB.

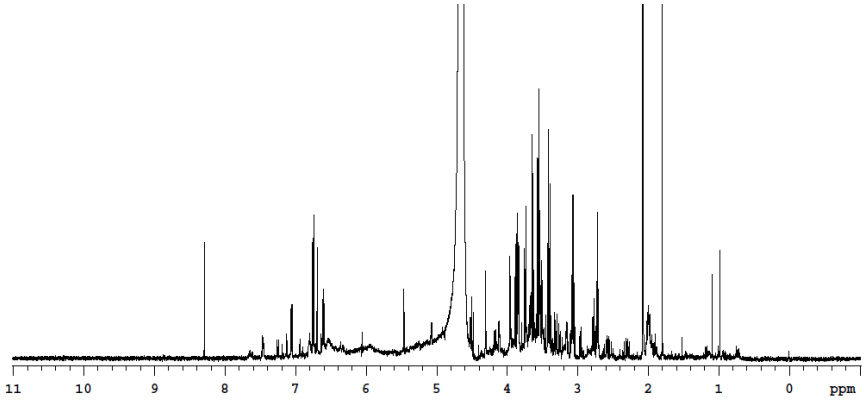


Figure 10. The 500 MHz ^{1}H spectrum in D_2O of sample 1752.

Conclusions

The messy exudates of commercial bananas have significant negative impact from both economic and environmental considerations. The materials produced by severing banana stalks, leaves, or fruit stain most anything with which the plant parts come into contact. Additional production steps that are required to remove these materials at the time of harvesting increase the cost of production and create a burden on the aqueous environment. We have carried out the current study in order to characterize these exudates molecularly through analysis of their NMR spectra in both the solid state and solution. Significantly, we have identified four different molecular types, indicating a variety of molecular biological processes that produce the exudates. Sample 379 is predominately a polycarbohydrate, an exudate classification called a gum. This sample was harvested from the leaf of plant on public property. Sample 1192 is a wax, typically a mixture of organic materials with the overall formula RCOOR', in which R and R' are long-chain hydrocarbons. This sample is historical, as it was obtained from a museum collection probably harvested in 1932. The part of the plant that produced the exudate was not provided. Samples 1782-4 were obtained from the lipid-rich chicle floating portion of banana phloem. These materials proved to be terpenoid resins, composed entirely of saturated hydrocarbons. The exudates from samples 1752 and 1753 were harvested from the severed ends of harvested banana rachides and allowed to dry. Hence, they represent the primary source of banana stains. They proved to be phenolics, a class of exudates containing polyphenols known to include dyes. Thus, we have documented four distinct molecular types of banana exudates: wax, gum, resin, and phenolic. The phenolic portion is the most important, as it is responsible for the stains during commercial processing of bananas. The authors have encountered no other plant species from our collection of over a thousand that produce more than two different molecular groups of exudates.

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