Ferns, Cycads, Ginkgo, and Gnetophytes: Nuclear Magnetic Resonance Characterization of Exudates from Exotic Plant Sources[1]

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Abstract: Rarely encountered exudates from the spore-bearing ferns and from the seed-bearing living-fossil cycads, ginkgo, and gnetophytes have been examined in the bulk solid by carbon-13 nuclear magnetic resonance (NMR) spectroscopy and in some cases in solution with hydrogen NMR spectra. All 18 cycad samples proved to be gums, i.e., polycarbohydrates, as was one of the ferns. The two ginkgo samples and the other two ferns produced phenolic-based exudates. The single gnetophyte exudate was of an unknown and unique composition containing carbohydrate, saturated, and unsaturated components. None of the exudates proved to be resins (terpene-based materials), which are the most common molecular composition of exudates produced by conifers and flowering plants.

Key Words: cycad, exudate, fern, ginkgo, gnetophyte, gum, nuclear magnetic resonance spectroscopy, phenolic

Introduction

Plant exudates are materials that emerge on the surface of a plant, usually as the result of injury or disease. Most such materials are produced by seed-bearing plants (spermatophytes), which are represented most prominently by the cone-bearing (conifers) and flowering (angiosperms) plants (Kenrick and Crane 1997, Taylor et al. 2009, Friis et al. 2011, Judd et al. 2016). The exudates of these groups have been comprehensively reviewed by Langenheim (2003) and Nussinovitch (2010). Spermatophytes are genetic siblings to the ferns, and the two groups (clades or branches) together are classified as euphyllophytes. In addition to the two large clades of the conifers and angiosperms, there are three other extant seed-bearing clades: the cycads, ginkgo, and the gnetophytes. These relationships are summarized in Figure 1 according to one phylogenetic classification (Kenrick and Crane 1997, Lee et al. 2011).

[1] Submitted on November 2, 2016. Accepted on November 13, 2016. Last revisions received on November 22, 2016

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DOI: 10.9784/LEB4(3)Lambert.01
Electronically available on November 28, 2016. Mailed on November 28, 2016.

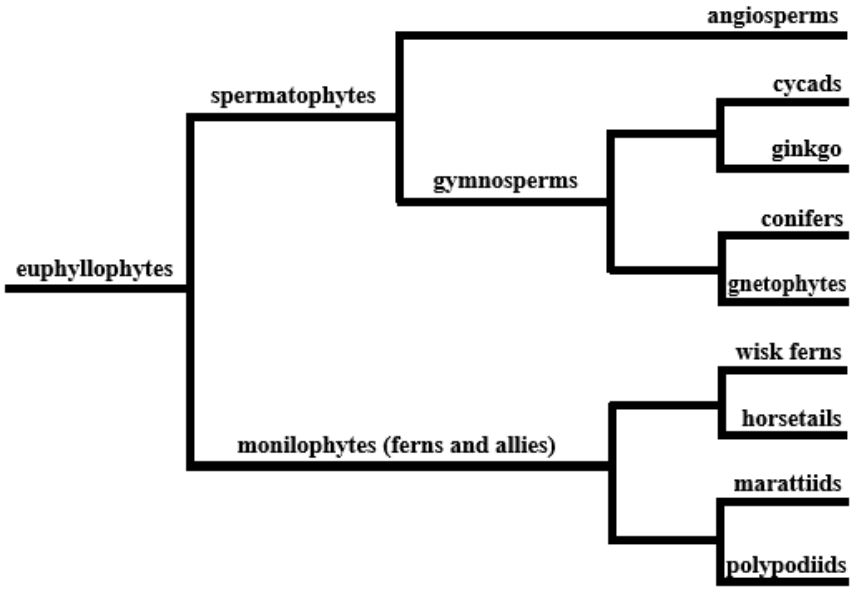

Figure 1. A simplified phylogenetic relationships of the extant seed-bearing plants (spermatophytes) and the ferns (Kenrick and Crane 1997, Lee et al. 2011).

The widely found exudates of the conifers and the angiosperms have been extensively examined in terms of their molecular makeup by nuclear magnetic resonance (NMR) spectroscopy, mass spectrometry (MS) (Lambert et al. 2008), and infrared spectroscopy (Tappert et al. 2011). Comprehensive studies have been carried out on both the conifers (Lambert et al. 2007a, 2007b) and the angiosperms (Lambert et al. 2007c, 2009, 2013a, 2013b, 2015) by NMR methods. To date, there has been no such study on the remaining groups of spermatophytes (cycads, ginkgo, and gnetophytes) or on the ferns. We report herein the first such examination of exudates from these groups, using NMR methods for identifying the molecular classes of exudates. Past work with conifers and angiosperms has found several large molecular groups of exudates. Resins, composed of terpene building blocks, are basically hydrocarbons and are highly soluble in organic solvents (Langenheim 2003). Gums are high polymers of carbohydrates and are partially soluble in water but insoluble in organic solvents (Nussinovitch 2010). Gum resins are mixtures of the two classes. Phenolics contain significant amounts of aromatic constituents, along with other constituents, and usually are soluble in organic solvents. There are numerous subgroupings of phenolics, which are quite distinct in chemical composition. NMR methods easily distinguish each of these major groups, as well as the subgroups. Carbon-13 (^{13}C) magnetic resonance spectra can be taken directly on the solid exudate, so that the sample bulk is examined directly (Lambert et al. 2005). Proton/hydrogen (1H) magnetic

resonance spectra are taken on solutions of the exudate, so some degree of solubility is required and there is the possibility that important information is lost with the insoluble portion (Lambert et al. 2007a).

Methods

Samples were collected from a wide variety of sources. Table 1 presents the genus and species of each sample, along with its source and other information. Authorships are included in the table and are not repeated elsewhere. Detailed description of the methods have been published previously (Lambert et al. 2013b). Each sample was subjected to four different NMR experiments. (1) Observation of ^{13}C nuclei of powdered, solid samples with full decoupling of carbon from hydrogen, that is, removal of the scalar coupling interactions between these nuclei. By examination of the bulk, this analysis is assured to characterize the entire sample. (2) Observation of ^{13}C nuclei of solid state samples with partial decoupling of carbon from hydrogen, using the technique known as dipolar dephasing or interrupted decoupling (Opella and Frey 1979). This experiment selects largely for carbon nuclei that are not attached to a hydrogen and provides an alternative method to distinguish spectral classes. (3) Standard one-dimensional (1D) observation of ^{1}H nuclei in solution state, usually with deuterated chloroform ($CDCl_3$) as the solvent. Examination of the solution phase may involve some loss of material due to partial insolubility. (4) The two-dimensional (2D) ^{1}H method known as COSY (COrrelation SpectroscopY), in which both Cartesian coordinates represent the frequency of ^{1}H resonances. Proton NMR spectra can provide distinctions sometimes not apparent from ^{13}C spectra (Lambert et al. 2007a, b).

For ^{13}C NMR measurements, samples were ground into a fine powder and loaded into a Varian 5 mm general purpose Zirconia rotor sealed with Vespel caps. The optimal sample load is about 150 mg of material, but smaller sample sizes (as little as 50 mg) required larger scan numbers. For ^{1}H spectra, approximately 55 mg of powdered exudate (recovered from ^{13}C analysis) was transferred to a small, glass vial. About 1 mL of deuterated chloroform-*d* was added to each vial. The material was stirred at room temperature and allowed to sit overnight. The supernatant was pipetted out and transferred to the NMR tube. The solutions were evaporated to retrieve the sample, and all powders have been retained, along with unused materials, in the archive at Trinity University (San Antonio, Texas, USA).

Table 1. Exudates Examined by Nuclear Magnetic Resonance Spectroscopy.

Number	**Clade**	**Order**	**Family (Subfamily: Tribe)**	***Genus species* Authorship**	**Exudate Type**	**Source***
660	fern	Marattiales	Marattiaceae	*Marattia* sp.	gum	M. Tomescu, Humboldt State University, Arcata, California
962	fern	Cyatheales	Cibotiaceae	*Cibotium glaucum* (Sm.) Hook. and Arn.	phenolic	JASB, Kauai, Hawaii
1593	fern	Cyatheales	Dicksoniaceae	*Dicksonia squarrosa* (G. Forst.) Sw.	phenolic	JASB, Denver Botanical Garden, Denver, Colorado
471	cycad	Cycadales	Zamiaceae (Eucephalartoideae: Diooeae)	*Dioon mejiae* Standl. and L. O. Williams	gum	JASB, National Tropical Botanical Garden, Allerton Garden, Kauai, Hawaii, 910497 001
460	cycad	Cycadales	Zamiaceae (Eucephalartoideae: Encephalarteae)	*Encepharlartos ferox* (G. Bertol) Lehm.	gum	JASB, National Tropical Botanical Garden, Allerton Garden, Kauai, Hawaii, 920188 009
463	cycad	Cycadales	Zamiaceae (Eucephalartoideae: Encephalarteae)	*Encepharlartos lebomboensis* Verd.	gum	JASB, National Tropical Botanical Garden, Allerton Garden, Kauai, Hawaii, 920088 007
466	cycad	Cycadales	Zamiaceae (Eucephalartoideae: Encephalarteae)	*Encepharlartos manikensis* Gilliland	gum	JASB, National Tropical Botanical Garden, Allerton Garden, Kauai, Hawaii, 990017 003
472	cycad	Cycadales	Zamiaceae (Eucephalartoideae: Encephalarteae)	*Encepharlartos transvenosus* Stapf and Burtt-Davy	gum	JASB, National Tropical Botanical Garden, Allerton Garden, Kauai, Hawaii, 920299 002

966	cycad	Cycadales	Zamiaceae (Eucephalartoideae: Encephalarteae)	*Lepidozamia hopei* Regel	gum	JASB, Waimea Arboretum and Botanical Garden, Oahu, Hawaii
562	cycad	Cycadales	Zamiaceae (Encephalartoidea: Macrozamiinae)	*Macrozamia spiralis* (Salisb.) Miq.	gum	R. T. Baker, Field Museum, Chicago, Illinois, 271160
340	cycad	Cycadales	Zamiaceae (Zamioideae: Zaminae)	*Microcycas calocoma* (Miq.) A.DC.	gum	S. Schaffer and JASB, Montgomery Botanical Center, Coral Gables, Florida, 77404E
1040	cycad	Cycadales	Zamiaceae (Zamioideae: Zaminae)	*Zamia elegantissima* Schutzman, Vovides, and R. S. Adams	gum	University of Panama, Panama City, Panama
336	cycad	Cycadales	Zamiaceae (Zamioideae: Zaminae)	*Zamia furfuracea* L.f.	gum	S. Schaffer and JASB, Montgomery Botanical Center, Coral Gables, Florida, 98997-1
1042	cycad	Cycadales	Zamiaceae (Zamioideae: Zaminae)	*Zamia hamannii* A. S. Taylor, J. L. Haynes, and Holzman	gum	University of Panama, Panama City, Panama
1039	cycad	Cycadales	Zamiaceae (Zamioideae: Zaminae)	*Zamia imperialis* A. S. Taylor, J. L. Haynes, and Holzman	gum	University of Panama, Panama City, Panama
1041	cycad	Cycadales	Zamiaceae (Zamioideae: Zaminae)	*Zamia nesophila* A. S. Taylor, J. L. Haynes, and Holzman	gum	University of Panama, Panama City, Panama

1211	cycad	Cycadales	Zamiaceae (Zamioideae: Zaminae)	*Zamia skinneri* Warsz. ex A. Dietr.	gum	C. Ritchie and JASB, San Diego Zoo, San Diego, California, 2001-0215-P
1038	cycad	Cycadales	Zamiaceae (Zamioideae: Zaminae)	*Zamia* sp. (common name, El Blanco)	gum	University of Panama, Panama City, Panama
608	cycad	Cycadales	Cycadaceae	*Cycas circinalis* Linnaeus	gum	JASB, Queen Sago, Foster Garden, Oahu, Hawaii, 71.0844
1603	cycad	Cycadales	Cycadaceae	*Cycas circinalis* Linnaeus	gum	JASB, Denver Botanical Garden, Denver, Colorado
965	cycad	Cycadales	Cycadaceae	*Cycas rumphii* Miq.	gum	JASB, National Tropical Botanical Garden, Allerton Garden, Kauai, Hawaii
1469	ginkgo	Ginkoales	Ginkgoaceae	*Ginkgo biloba* Linnaeus	phenolic	E. Miller and JASB, Blandy Experimental Farm, Boyce, Virginia
1678	ginkgo	Ginkoales	Ginkgoaceae	*Ginkgo biloba* Linnaeus	phenolic	JASB, Salisbury, Hawaii
601	gnetae	Gnetales	Gnetaceae	*Gnetum gnemon* Linnaeus	other	JASB, Foster Botanical Garden, Oahu, Hawaii 64.189

*The sources indicated by "JASB" refer to the author, Jorge A. Santiago-Blay.

Ferns

Ferns are vascular plants that reproduce via dispersal of spores rather than seeds. The broader term pteridophyte refers to a polyphyletic assemblage that includes the ferns, horsetails, clubmosses, and other plants. The two most prominent groups of ferns are the species-poor subclass of the Marattiidae (equivalent to the earlier class Marattiopsida) and the species-rich subclass of the Polypodiidae (equivalent to the earlier class Pteridopsida or Polypodiopsida) (Smith 2006, Christenhusz and Chase 2014). We have rarely encountered exudates among the ferns, but we have been fortunate enough to obtain one sample from the Marattiidae and two from the Polypodiidae.

The Marattiidae contain one order, the Marattiales, with a single family, the Marattiaeae. Traditionally, the family contained four genera, now expanded to six, with about 135 species. These are the larger ferns, with the largest known fronds and fleshy roots. Our single sample (no. 660 in the Trinity collection) was identified as Marattia sp. Droplets formed at the base of the frond when it was cut from the stem or rachis with the permission of the source. The droplet solidified very quickly and later could be powdered for examination by ^{13}C NMR spectroscopy of the solid (Figure 2).

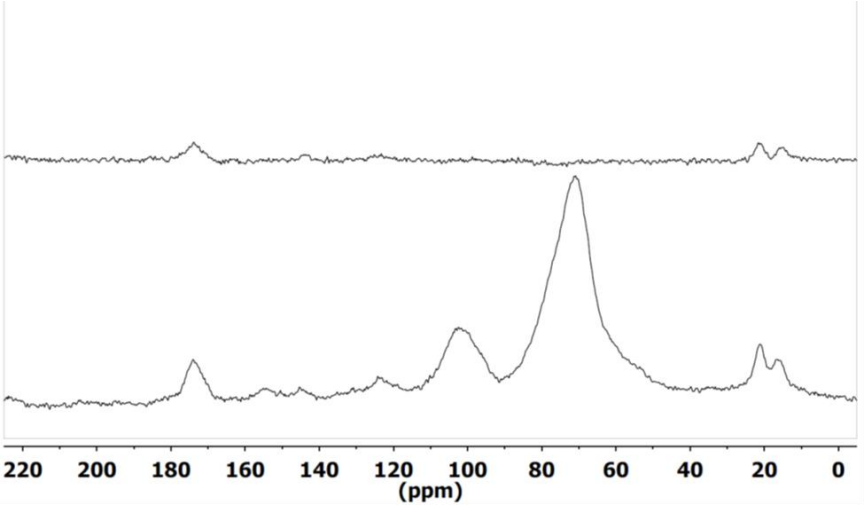

Figure 2. The ^{13}C spectra of *Marattia* sp. (sample 660) in the solid state, with normal decoupling (lower) and with dipolar dephasing (upper).

This spectrum is typical for a gum, which as a class consists of high molecular weight carbohydrates and is found commonly among the angiosperms (Lambert et al. 2013a, 2013b). The main peak centered at δ 72 comes from all the carbon atoms connected to a single oxygen (C—O), and the smaller peak at δ 102 comes from the single carbon in a given carbohydrate ring, known as the anomeric carbon, that is attached to two oxygens (O—C—O). In common sugars, all

carbons are bonded to at least one oxygen. The spectrum also contains weak peaks in the carbonyl region at δ 174 and in the region of saturated carbons not attached to electron-withdrawing groups at δ 16-22. With dipolar dephasing, the carbohydrate peaks disappear but the carbonyl and saturated resonances persist. This result is normal for carbonyl resonances, which, except for aldehydes, lack attached protons, but is not expected for most saturated carbons, unless they are quaternary or have particularly rapid motion. The sample was insoluble, so that no hydrogen spectrum was obtained.

The Polypodiidae comprise over 8000 species from seven orders, of which our two samples are both from the Cythales. Sample 962 is from *Cibotium glaucum*, the Hawaiian tree fern, of the Cibotiaceae. This family contains the single genus with 11 species, all tropical tree ferns. Sample 1593 is from *Dicksonia squarrosa*, the rough tree fern of New Zealand, of the Dicksoniaceae. This genus has about two dozen species found in Southeast Asia and the Pacific. Despite the difference in families and the distance between the native locations, the two exudates produced essentially identical ^{13}C spectra, with only minor differences in individual intensities (Figures 3 and 4). Such spectra belong to the molecular class of exudates called phenolics, which as a group exhibit considerable variation, but always with a dominant peak near δ 150 for the carbon by which the OH group (the defining group for phenols) is attached to a benzene (aromatic) ring. The peak always survives with dipolar dephasing as the carbon lacks an attached hydrogen. We first observed phenolic exudates in our study of the eucalypts (Lambert et al. 2007c). The details of eucalypt spectra are found in numerous other species as well, so that we called this exudate group *kinos*, a term used widely in Africa and Asia for such materials. For example, *Myristica globosa* (sample 556 related to nutmeg, illustrated in Lambert et al. 2015) exhibits spectra identical to those of eucalypts. The kino spectral pattern, however, is distinct from that of Figures 3 and 4, although the region δ 20-90 (the saturated region) is very similar. These saturated peaks entirely disappear with dipolar dephasing. The hydrocarbon portions of the exudates from the eucalypts and these ferns may be very similar, but the remainder is quite different. Thus ferns and kinos represent different subgroups of the more general classification of phenolics. Figures 3 and 4 additionally include a very large peak at δ 105 that survives with dipolar dephasing (hence is not from carbohydrates). There are two strong carbonyl peaks at δ 178 and 205, possibly from esters and ketones, respectively.

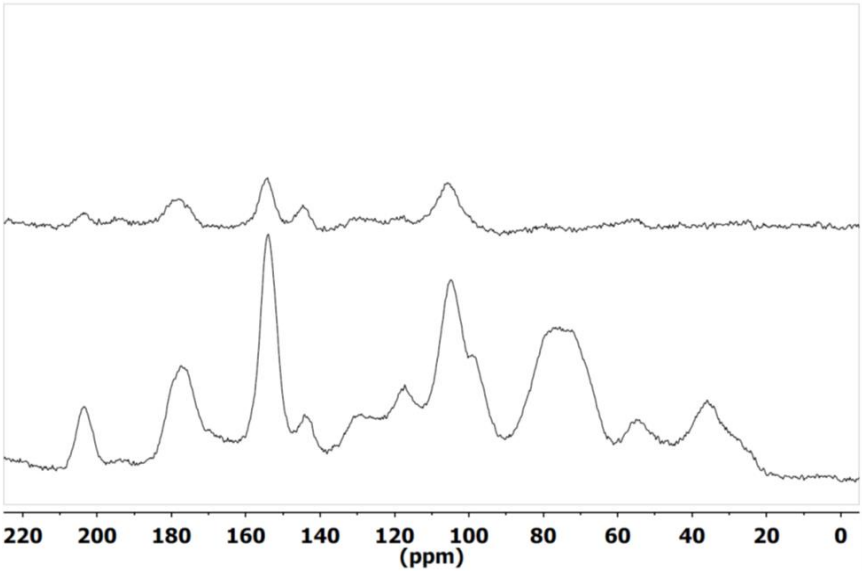

Figure 3. The ^{13}C spectra of *Cibotium glaucum* (sample 962) in the solid state, with normal decoupling (lower) and with dipolar dephasing (upper).

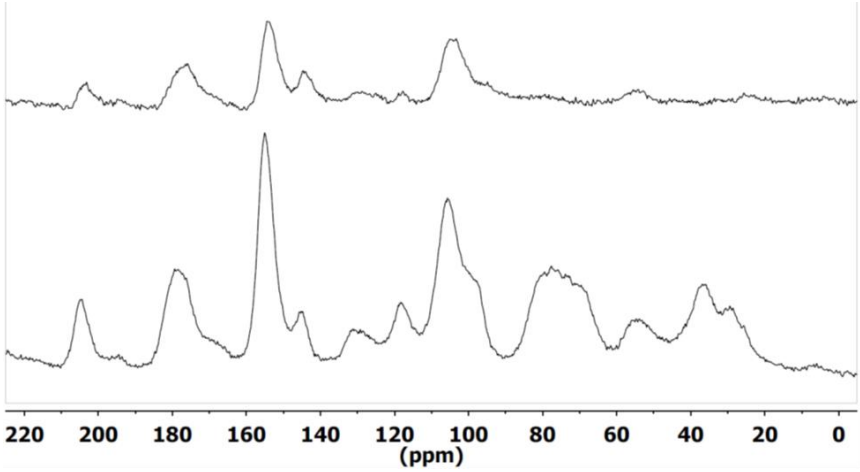

Figure 4. The ^{13}C spectra of *Dicksonia squarrosa* (sample 1593) in the solid state, with normal decoupling (lower) and with dipolar dephasing (upper).

In addition to kinos, there are many other exudates that fall into the phenolic classification. Many have been observed in only a single species, but some, like kinos, occur in multiple species. For example, within the monocotyledons

(monocots), we have examined exudates from four different species of the genus *Xanthorrhoea* from the Xanthorrhoeaceae. A total of nine samples produced nearly identical ^{13}C spectra (Lambert et al. 2015). Several other monocots also give phenolic spectra, each with a different pattern. From other types of flowering plants, seven samples from three genera of the Zygophyllaceae from the order Zygophyllales produced the same phenolic exudates, which we called guaiacs because of the present of guaiacol (2-methoxyphenol) (Lambert et al. 2013b). Their ^{13}C patterns were distinct from the other cited phenolics. Thus phenolics form a rich and diverse group of exudates from across the seed- and spore-bearing plants.

Cycadophyta (Cycads)

This large group of ancient plants, resembling palms, is a rich source of exudates. They possess a crown of large compound leaves attached to a broad trunk. Although minor today in tropical and subtropical regions, they were a dominant plant in the Mesozoic Era and during the Jurassic Period in particular (Jones 2002). Both geological periods sometimes are referred to as the Age of Cycads. Cycads are gymnosperms as their seeds are naked, that is, not enclosed in a fruit, like those of conifers. Cycads possibly evolved from (extinct) seed ferns, and they are not closely related to the conifers. The fossil record indicates an origin at least as early as the Lower Permian (ca. 280 mya), or possibly the Carboniferous. Although the lineage is ancient, extant species most likely evolved more recently.

The cycads today comprise only the single order Cycadales, although there were several extinct orders. The Cycadales contain three extant families today. The Zamiaceae are the oldest, having developed as early as the middle Triassic (ca. 200 mya), followed by the Stangeriaceae as early as the Lower Cretaceous (ca. 135 mya), and finally the Cycadaceae as early as the early Eocene (ca. 54 mya). We have collected 18 cycad exudate samples (Table 1) from two of the three families, including 15 from the Zamiaceae and 3 from the Cycadaceae.

We first consider the Zamiaceae, which contain two subfamilies with eight extant genera and about 150 species. Our 15 samples represent both subfamilies, five genera and 15 different species. The subfamily Encephalartoideae contain two tribes. The tribe Diooeae have only the single genus *Dioon*, from which sample 471 comes. Its ^{13}C spectra demonstrate that the exudate is a gum. The dominant peaks are from polysaccharides, characteristically at δ 74 and 103, but additionally it has small peaks in the saturated region at δ 16-22 and the carbonyl region at δ 175. All these features are shared with the gum spectra of the fern *Marattia* sp., illustrated in Figure 2. The second tribe of the subfamily Encephalartoideae is the Encephalarteae, which contain three genera, of which we have obtained six exudate samples from two genera. All six samples proved to be gums. Five of the samples are from the genus *Encephalartos*, an African plant known as the bread tree or the bread palm because of the material from the

stem processed into a bread-like food (*artos* is the Greek word for bread). The second subfamily of the Zamiaceae are the Zamioideae, which contain two tribes. We have eight samples from the tribe Zamieae. These include one from the genus *Microcycas* of the subtribe Microcycadinae and eight from the genus *Zamia* of the subtribe Zamiinae. All of these exudates proved to be gums.

We have three representatives from the second family, the Cycadaceae. This family has just one genus, *Cycas*, and about 110 species. The three exudates from this family all proved to be gums.

Thus each and every one of the exudates from the 18 cycad samples, representing 17 species, 6 genera, and 2 of the 3 extant families, proved to be gums, the same as the Maratiidae fern 660. Figure 5 illustrates one of these cycads, for sample 608 (*Cycas circinalis*). The similarities to Figure 2 are evident. Despite widespread production of gum exudates from cycads, the comprehensive monograph by Nussinovich (2010) made no mention of cycads among the many hundreds of gum-producing species mentioned. The infrared study of Tappert et al. (2011) examined two samples from the genus *Dioon* and found both of them to be gums.

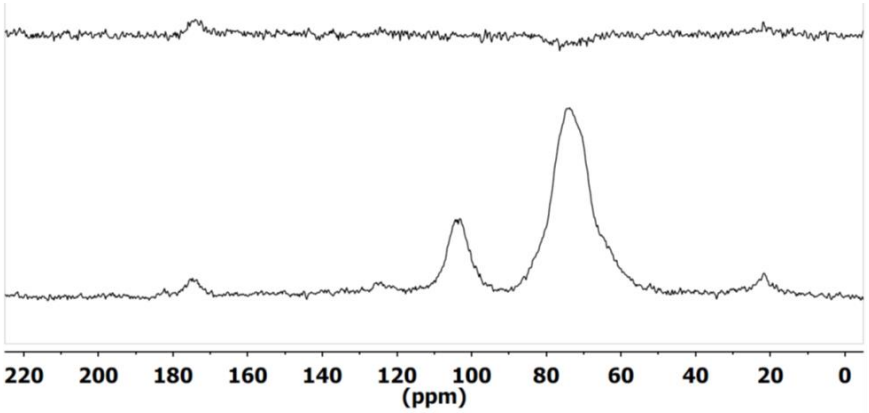

Figure 5. The ^{13}C spectra of *Cycas circinalis* (sample 608) in the solid state, with normal decoupling (lower) and with dipolar dephasing (upper).

Ginkgophytes

Ginkgo biloba is the sole extant species of the genus *Ginkgo* and even of the division Ginkgophyta (Royer et al. 2003). The order first appeared in the Permian, about 280 mya. Some fossil ginkgos bear clear resemblances to the modern species, which therefore is justifiably termed a living fossil, although the species *G. biloba* did not appear until the Early Jurassic, ca. 190 mya. Seed ferns are a plausible ancestor of the ginkgophytes, as is the case with the cycads (Taylor et al. 2009).

We have obtained two samples of exudates harvested from *G. biloba*. Sample 1469 was from the Blandy Experimental Farm, Boyce, Virginia, extracted from the surface of a fructification. The sample appeared to be contaminated with some woody material, which we endeavored to remove by hand. The material is sticky and slightly rubbery, but it powdered to a sufficient extent for direct examination in the solid state by ^{13}C NMR spectroscopy (Figure 6). The large peak at δ 75 most likely is from the C—O carbons of carbohydrates. This assignment is confirmed by the O—C—O (anomeric) peak at δ 106 and by the disappearance of both peaks with dipolar dephasing. Unlike a gum (Figures 2 and 5), however, there are numerous additional peaks. There are five sharp peaks in the saturated region at δ 14-34, probably from methyl or methylene groups. The remaining broad peaks are at δ 99 in the region of resonances in which carbon is attached to electron-withdrawing groups (EWG), at δ 130, 145, and 154 in the region of unsaturated carbons, and at δ 175, 180, 194, and 205 in the region of carbonyl carbons. The unsaturated carbons very likely are phenolic in origin, as in Figures 3 and 4. The richness of the carbonyl region is distinctive. These peaks survive with dipolar dephasing.

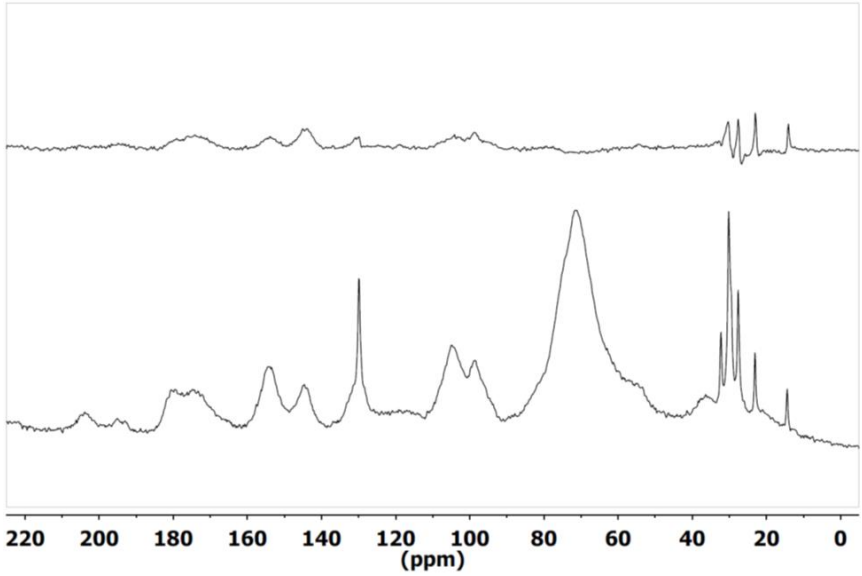

Figure 6. The ^{13}C spectra of *Ginkgo biloba* (sample 1469) in the solid state, with normal decoupling (lower) and with dipolar dephasing (upper).

This material was partially soluble in $CDCl_3$ and produced the ^{1}H spectrum illustrated in Figure 7. It is likely that the carbohydrate portion was insoluble and is not reflected in the spectrum (it would appear in the region δ 3-5, which is

empty, a common result with gums). Aromatic resonances occur in the region δ 6.8-7.3, and sample 1469 has significant resonances in this range (the peak at δ 7.3, however, is from residual undeuterated $CHCl_3$). The peak at δ 5.4 could be from a hydrogen on a double bond (alkenic hydrogen), but it also could be from a phenolic OH group. In phenol itself the OH group resonates at δ 5.35 in $CDCl_3$. There are several peaks in the saturated regions, between δ 0.9 and 2.0, as well as two peaks in the EWG region at δ 2.6 and 3.0. The spectrum also was recorded in $CD_3(SO)CD_3$ (DMSO-d_6). The result was very similar to that in Figure 7, although with slight movement of all the peaks.

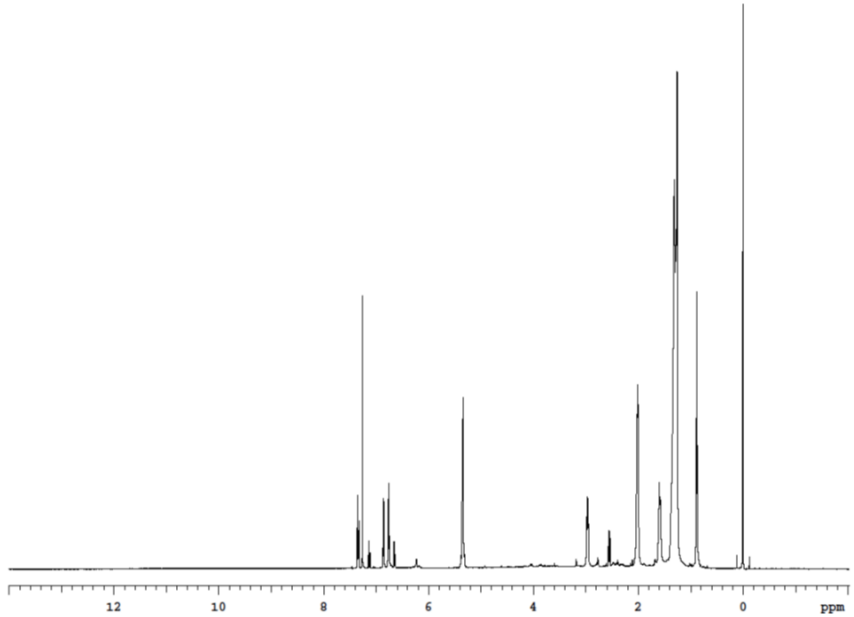

Figure 7. The ^1H spectrum of *Ginkgo biloba* (sample 1469) in $CDCl_3$.

Figure 8 displays the 2D COSY spectrum, in which both axes are hydrogen frequencies. The 1D spectrum appears along the diagonal, and the cross peaks in mirror image relationship from reflection along the diagonal indicate scalar coupling between the hydrogens at the respective frequencies. Thus the cross peaks around δ 7 indicate coupling between hydrogens on aromatic rings, and the cross peaks around δ 1.4-2.0 indicate coupling between saturated hydrogens. The resonance at δ 1.6 has cross peaks with resonances at δ 2.6 and 3.0, which could represent either functionalities of the type $(CO)CH_xCH_y$ or $(aryl)CH_xCH_y$. The spectra indicate that this exudate contains phenolic functionalities as well saturated and carbonyl groups.

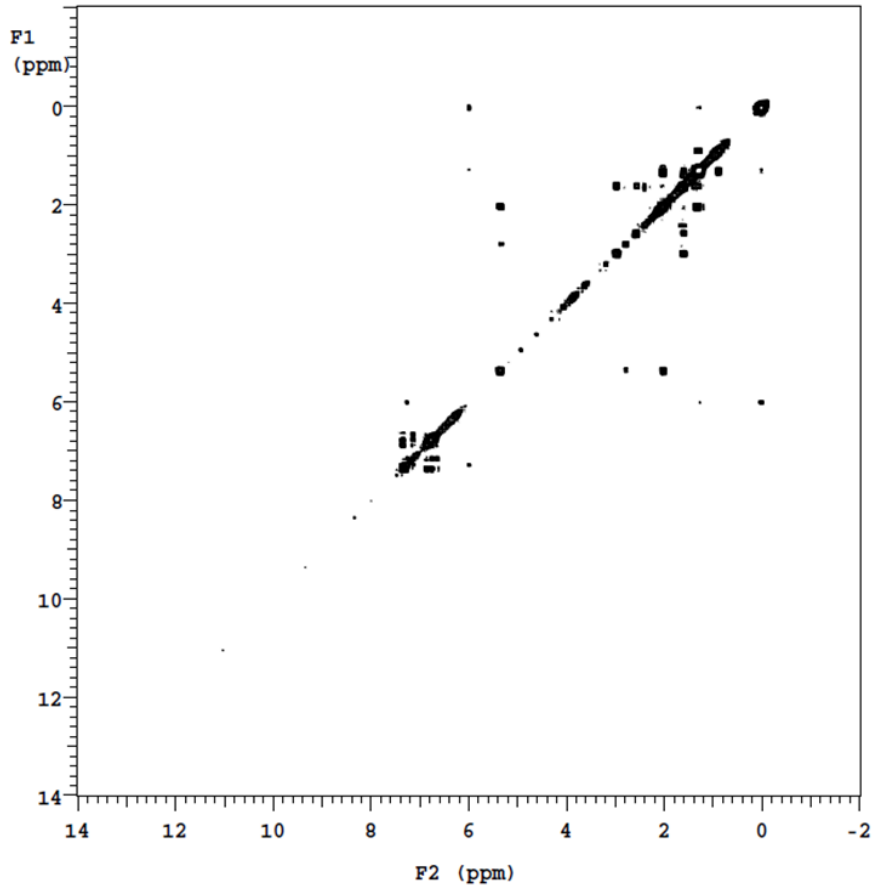

Figure 8. The COSY spectrum of *Ginkgo biloba* (sample 1469) in $CDCl_3$.

Sample 1678 from Salisbury, Maryland, is very fibrous and clingy. Although it did not fully powder, it could be reduced to small particles that could easily be examined by solid state NMR methods. It gives a somewhat different ^{13}C spectrum (Figure 9) from sample 1469 (Figure 7). The common features between the two spectra are the presence of resonances from saturated carbons in the δ 20-40 region (larger for 1678), carbohydrate carbons at δ 74 and 105, unsaturated carbons at δ 128, phenolic carbons at δ 154, and carbonyl carbons at δ 171 and 197 (larger for 1678). The major differences are the presence of a large peak for sample 1678 at δ 58 in the EWG region, an unsaturated carbon resonating around δ 115-125, and in particular a large peak at δ 148 in the unsaturated region, probably from aromatic carbons. Both ginkgo exudates may be classified as

phenolics, but they are not the same, possibly because of the mode of harvesting and the location of the exudate on the plant.

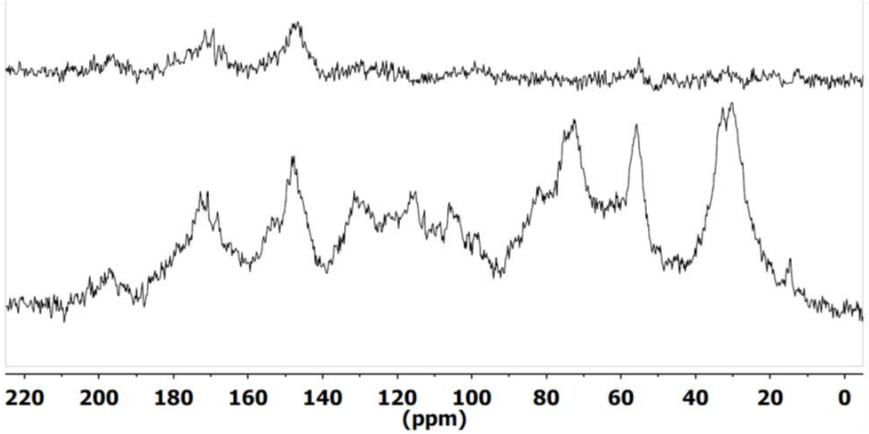

Figure 9. The ^{13}C spectra of *Ginkgo biloba* (sample 1678) in the solid state, with normal decoupling (lower) and with dipolar dephasing (upper). The higher noise level arises from the small amount of sample.

Sample 1678 was nearly insoluble in $CDCl_3$, but aromatic ^1H resonances were discernible in the region δ 6.8-7.0, very similar to the pattern in the spectrum for 1469. The strong saturated peak at δ 0.9 also was present, but the dominant peak for 1469 at δ 1.3 fell under a solvent peak, along with the peak at δ 1.6. The peak at δ 2.0 was visible, but the spectrum of 1678 additionally had peaks at δ 3.9 in the EWG region. As with the ^{13}C spectra, the ^1H spectra of the two ginkgo samples had similarities and differences.

Tappert et al. (2011) characterized a single sample of *G. biloba* by infrared spectroscopy as being a gum. In light of the current results, it is possible that they were observing the carbohydrate portion of the exudate, which we also observed.

Gnetophytes

There are three extant genera and about 60 species of gnetophytes. This is another ancient group of spermatophytes that dates back to the Permian and Triassic (Wang 2004, Ickert-Bond et al. 2009). We have obtained exudate material from a single sample (602) of the species *Gnetum gnemon*. The material has the appearance of dark green scales, which could be converted to a powder. The ^{13}C spectrum (Figure 10) has an unusual nature. The peaks at δ 74 and 104 are characteristic of a carbohydrate component, as found in gums (Figure 2) but also in ginkgos (Figures 6 and 9). The resonances of saturated carbons in the region δ 15-45 resemble those found in resins. Together, these resonances suggest a gum resin, but other factors militate against this interpretation. The large

carbonyl resonance at δ 174 and the broad resonance from saturated carbons attached to an EWG at δ 50-65 are not found in the spectra of gum resins. The carbonyl resonance corresponds to the region of carboxylic acids rather than ketones. Aromatic ethers (Ar—O—**C**H_2—) and aliphatic ethers resonate in this region, as do carbons between an aromatic ring and a carbonyl group, as in phenylacetic acid. There is no phenolic carbon at δ ca. 150, but there is a large, broad resonance in the unsaturated region at δ 115-140, which could be aromatic or alkenic. Gum resins do not have resonances in the unsaturated region. Natural products are more likely to be rich in aromatic groups than alkenic groups, which tend to condense. The cause of the broad, unsaturated resonances is likely to be aromatic and related to benzoic acids. The material was completely insoluble in chloroform and failed to give even weak ^{1}H resonances. Although the hydrogen spectra of gum resins fail to exhibit resonances from the gum portion, they do exhibit resonances from the resin portion. Thus the saturated resonances in Figure 10 do not respond like the terpenoid functionalities of resins or gum resins. It is more likely that the saturated atoms are tied up in a larger molecular piece that resists dissolution. To sum up, the spectra indicate that the gnetophyte exudate contains carbohydrate, aromatic, carboxylic acid, and saturated carbons in a molecular assembly that does not correspond to simple classifications.

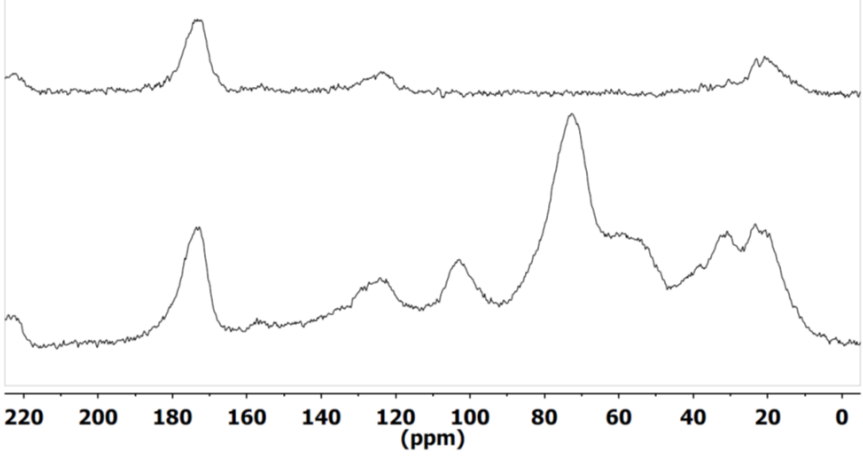

Figure 10. The ^{13}C spectra of *Gnetum gnemon* (sample 601) in the solid state, with normal decoupling (lower) and with dipolar dephasing (upper).

The infrared study of Tappert et al. (2011) examined a single gnetophyte from the genus *Welwitschia*, which is a member of a different family (Welwitschiaceae) from the sample we have examined. They found the material to be a gum.

Summary

Exudates are found in a few ferns and in the so-called living-fossil spermatophytes. We have harvested and analyzed by NMR spectroscopy exudates from three fern samples, 18 cycad samples, two ginkgo samples, and one gnetophyte sample (Table 1). By far the most common molecular type is the gum, found in all 18 cycads and in one fern. Phenolic exudates constitute the second most common type, found in two fern and two ginkgo samples. Exceptional is the single exudate from a gnetophyte, which proved to contain carbohydrate, aromatic, carboxylic acid, and nonresinous saturated groups. None of these species produced resins, which constitute the most common type of exudate in angiosperms and conifers.

Acknowledgments

The authors are grateful to the Welch Foundation (Departmental Grant No. W-0031), the Camille and Henry Dreyfus Senior Scientist Mentor Program, and The Pennsylvania State University, York Campus, for financial support of this research.

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