Nuclear Magnetic Resonance Spectroscopic Characterization of Legume Exudates

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Exudates from the plant family Fabaceae have been characterized by proton and carbon-13 nuclear magnetic resonance spectroscopy. The 79 identified species from 38 genera represent all three subfamilies of this widespread and economically important angiospermous (flowering) family. The observed exudates include resins, gums, kinos, gum resins, and a few materials as yet unclassified molecularly. Exudates from the subfamily Caesalpinoideae are primarily resins, whereas those from the Mimoisoideae and Faboideae are primarily gums. Three species of the Mimoisoideae produce both gums and kinos.

The Fabaceae (or Leguminosae)[1] constitute the third largest living flowering plant family. According to Lewis et al.,[2] this morphologically diverse family contains approximately 725 genera and 19 300 species found in both temperate and tropical locations. With few exceptions, members of this family are characterized morphologically by the presence of a one-chambered, pod-like fruit, the legume. Molecular studies cited by Lewis[2] support the monophylesis (descent from a common ancestor) of this enormous group of plants. The legumes are second only to the grasses (the Poaceae or Gramineae) in economic importance.[3] In symbiosis with soil bacteria, numerous legume species are involved in nitrogen fixation, without which life on Earth would be greatly altered.[4] Prominent legumes include the peanut (*Archis hypogaea*), the soybean (*Glycine max*), garden beans (*Phaseolus* spp. and *Vigna* spp.), alfalfa (*Medicago sativa*), forage clover (various species from the genus *Trifolium*), Mendel's pea (*Pisum sativum*), the dye indigo (*Indigofera*), the pesticide rotenone (*Derris*), gum Arabic (*Acacia*), copal and incense [*Hymenaea*, *Commiphora* (myrrh), and *Boswellia* (frankincense)], mesquite trees (*Prosopis* species), and kudzu (*Pueraria montana* var. *lobata*, infamous in the southern United States as an invasive vine).

Many legumes produce exudates, which are sticky materials released by a plant usually as the result of damage or disease.[5,6] Solidified exudates often have very similar physical appearance but take many molecular forms, including resins (primarily hydrocarbon terpene derivatives), gums (polysaccharides), gum resins (with both resin and gum components), and kinos (with phenolic and other aromatic constituents). Chemical aspects of exudates may be studied by either nuclear magnetic resonance (NMR) spectroscopy or mass spectrometry. A comparison of the two methods has appeared recently.[7] We have reported a comprehensive molecular survey by NMR spectroscopy of exudates from many important coniferous gymnosperms, including the Pinaceae,[8,9] Cupressaceae, and Auracariaceae.[9,10] We also have reported a survey of exudates from some eucalyptus species from the Myrtaceae, which comprise mostly kinos.[11] Our *modus operandi* is to record carbon-13 (^{13}C) spectra of solid exudates to characterize the bulk structure and to record the proton (^{1}H) spectra of solutions to study the soluble portions. To date, few solid state ^{13}C spectra and no ^{1}H spectra have been reported of exudates of flowering plants (angiosperms) other than the eucalypts.[9] As the Fabaceae have been cited as a family rich in exudates,[5,6] we have focused on legumes as our initial angiosperm subject for NMR spectroscopic characterization. We report herein the results of both ^{13}C and ^{1}H spectroscopic studies.

Legumes have traditionally been classified into three large subfamilies: the Caesalpinoideae, the Mimosoideae, and the Faboideae (or Papilionoideae).[1] Figure 1 summarizes the genera included in this study, arranged according to taxonomic relationships suggested primarily by biomolecular characteristics.[2] For the large genus *Acacia*, we have followed the new division into five separate genera.[12]

Results and Discussion

The 79 species in this study represent 38 genera. Nine further samples were identified according to genus but not species, and two additional samples were distinct variants, bringing the total materials to 90. In addition, there are duplicate, triplicate, and quadruplicate samples for many species, representing either distinct geographical sources or replication of the same sample, for a total of 113 total samples. There are 16 genera from the subfamily Caesalpinoideae, 11 from the Mimosoideae, and 11 from the Fababoideae. Table S1 lists all the samples according to subfamily and genus and provides information about authorship and sources. Each species was characterized by solid state ^{13}C NMR spectroscopy, by 1D ^{1}H NMR spectroscopy in chloroform and dimethyl sulfoxide (DMSO), and by ^{1}H−^{1}H 2D COSY in both solvents (solubility permitting for all solution spectra). Figure 1 provides taxonomic relationships of the genera, lists the subfamilies and tribes, and summarizes the types of exudates obtained from each genus.

Whereas conifer exudates are almost entirely resins[8−10] and eucalyptus exudates are kinos,[11] Fabaceae exudates exhibit an impressive variety. Our discussion follows plant subfamily classification. For all subfamilies, it should be kept in mind that not all species produce exudates.

Caesalpinoideae. This subfamily includes at least 170 genera and 2000 species. The 45 samples (including replicates) from this subfamily represent 16 genera, 31 identified species, two species variants, and three identified only according to genus (Table S1). Of these, 32 proved to be resins, eight gums, three kinos, and two unique materials. The characteristic NMR patterns of the three main types of exudates have been described previously.[7−11] Figure 2 illustrates typical ^{13}C resin patterns for the solid exudate from *Guibourtia copallifera*. The spectrum with normal ^{1}H decoupling (lower) is dominated by saturated resonances in the region δ 17−80. There are distinct but weak unsaturated peaks in the region δ 100−150, including clear but weak exomethylene (C=CH$_2$) resonances at δ 107 and 148. In addition, there are important carbonyl resonances at δ 186 and 198. Dipolar dephasing (upper)

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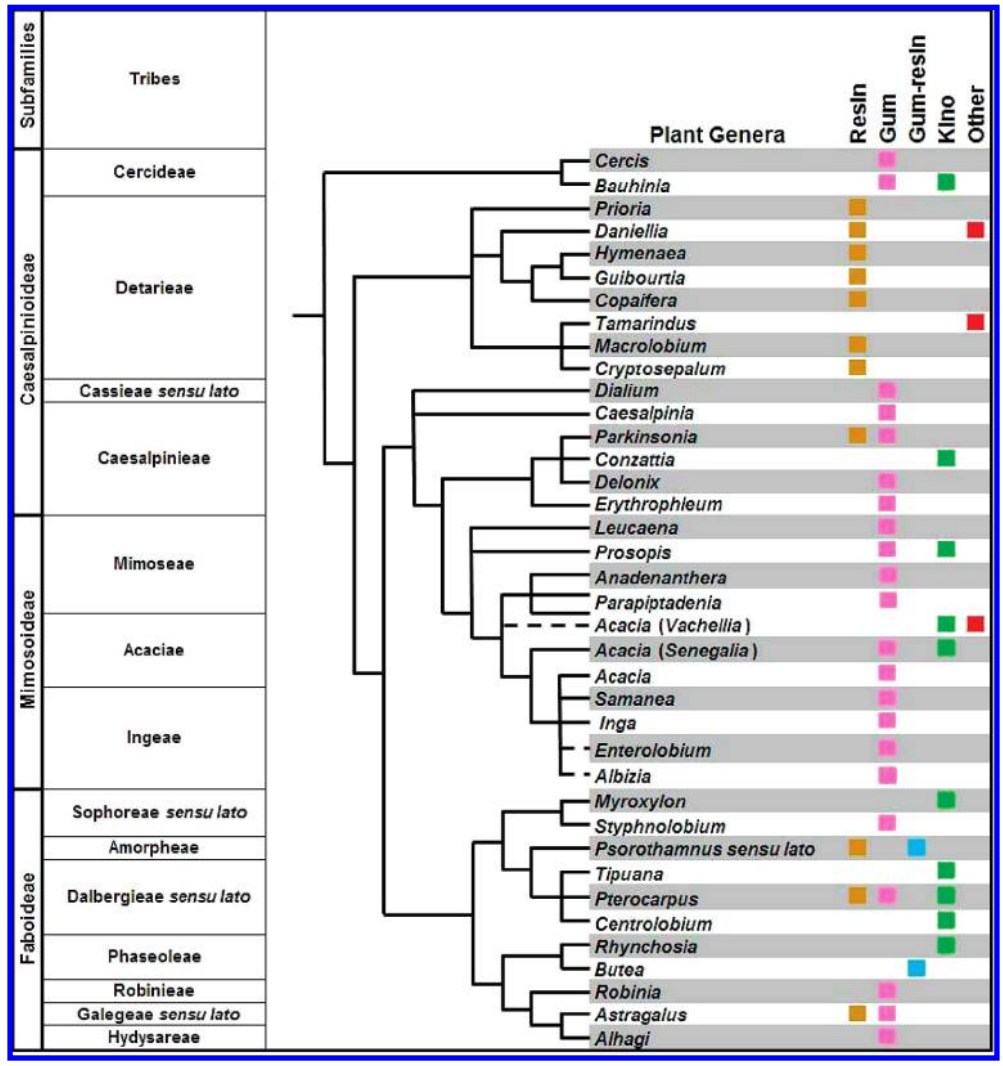

Figure 1. Taxonomic relationships between exudate-producing species in this study.

selects quaternary carbons and some rapidly moving carbons. The resulting spectrum provides an alternative fingerprint and for *G. copallifera* contains three dominating saturated peaks at δ 16, 38, and 48. In our earlier, general publication on exudates,[9] the spectrum of a sample identified as *Copaifera* sp. exhibited very similar spectra (Figure 9 in ref 9). This pattern is seen additionally in the current study for the spectra of all the exudates from the genera *Copaifera*, *Daniellia*, *Guibourtia*, and *Hymenaea*, all members of the tribe Detarieae. These species come from tropical sources all over the world. The African and South American samples often are referred to as varieties of copal.

Although all the samples from these species exhibit the three diagnostic peaks seen in the upper part of Figure 2 (with dipolar dephasing), some of the samples have additional peaks. A peak at δ 22 is seen in the exudates from *Daniellia alsteeniana*, *G. copallifera*, *Hymenaea oblongigolia*, *H. rubriflora*, and *Hymenaea* sp. from Borneo, representing samples from South America, Africa, and Asia. Samples from *D. alsteeniana*, *H. retuculata*, and *H. rubriflora* have an additional peak at δ 32−34. The *Hymeanea* sample from Borneo also has a peak at δ 45. A small peak at δ 75 in the sample from *Copaifera mildbraedii* likely indicates a small carbohydrate component. Nonetheless, the repetitive appearance of the pattern of peaks at δ 16, 38, and 48 characterizes a widespread type of exudate, particularly with African and South American sources.

Not all Caesalpinoideae resins exhibit the African/American copal pattern of Figure 2 (called Group FL in ref 9). Five quite different patterns are seen respectively in the spectra of *Parkinsonia praecox* (sample 2 in Table S1, Figure S1), *Cryptosepalum pseudotaxus* (Figure S2), *Daniellia oliveri* (Figure S3), *Macrolobium acaciaefolium* (Figure 3), and *Prioria copaifera* (Figure S4). The spectra of *P. praecox*, *C. pseudotaxus*, *M. acaciaefolium*, and *P. copaifera* exhibit standard resin patterns, each different from the other, with strong saturated resonances (δ 0−65) and only small resonances elsewhere. The spectra of *D. oliveri* contain these resonances but additionally have numerous strong resonances in the unsaturated and carbonyl regions, from δ 95 to 200. This pattern is currently inexplicable in terms of our existing structural categories, so we have set it aside in Table S1 as "other."

As resins are highly soluble in organic solvents, we recorded the ^{1}H spectra in chloroform and DMSO in one and two (COSY) dimensions for all 25 samples. Figures 4 and 5 illustrate the 1D ^{1}H and 2D COSY spectra for *G. copallifera*. In addition to a rich saturated region, the African/American (Detarieae) copals all have resonances at δ 4.5 and 4.8 and an AX quartet of varying intensity at δ 3.1 and 3.4. The aromatic region usually is empty, in contrast to most conifers.[8,10] The COSY spectra regularly show cross-peaks at 3.1/3.4 and 5.1/6.3. The illustrated spectra are in chloroform, but those in DMSO are very similar, as expected for essentially hydrocarbon materials. The three unique resins, *Parkinsonia praecox* (sample 2), *Macrolobium acaciaefolium*, and *Prioria copaifera*, exhibit different patterns from the African/American copals, as was the case for the ^{13}C spectra.

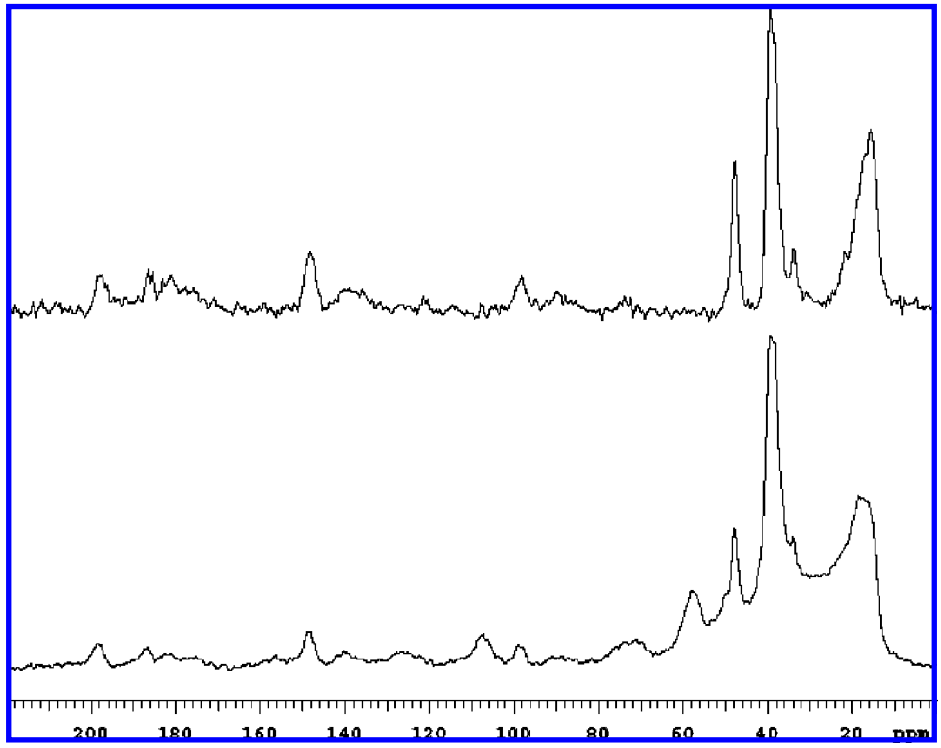

Figure 2. 100 MHz solid state ^{13}C spectra of G*uibourtia copallifera*, (bottom) with normal decoupling and (top) with dipolar dephasing.

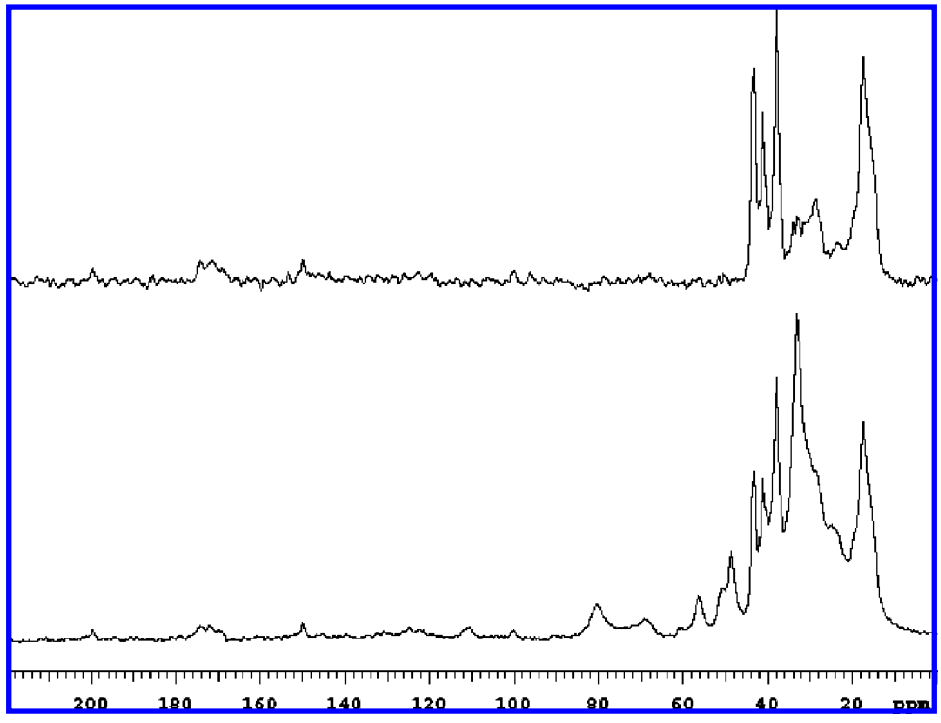

Figure 3. 100 MHz solid state ^{13}C spectra of *Macrolobium acaciaefolium*, (bottom) with normal decoupling and (top) with dipolar dephasing.

The ^{13}C spectra of the eight gums (*Bauhinia carronii*, *Caesalpinia ferrea*, *C. kavaiensis*, *Cercis racemosa*, *Delonix regia*, *Dialium* sp., *Erythrophleum suaveolens*, and *Parkinsonia praecox*) exhibit a common pattern, as illustrated in Figure 6 for *C. racemosa*. There are two large groupings in these spectra, as with all gums: one with a maximum near δ 70 for the O−C peaks in the polysaccharides and a smaller one with a maximum near δ 105 for the anomeric O−C−O peaks. Shoulders or weakly defined peaks also are found at δ 110, 82, and 63. The spectra of *Caesalpinia kavaiensis* and *Delonix regia* represent extremes. Whereas the spectrum of *C. kavaiensis* has only the two major peaks at δ 72 and 104, *D. regia* exhibits nearly equal small, overlapping peaks at δ 104 and 110, nearly equal large, overlapping peaks at δ 71 and 82, and a small maximum or shoulder at δ 63. All gum spectra also exhibit a small carbonyl peak at δ 176. All peaks except the small carbonyl peak disappear with dipolar dephasing (Figure 6, upper). Although all gums have poor solubility, some produce weak ^{1}H spectra in DMSO.

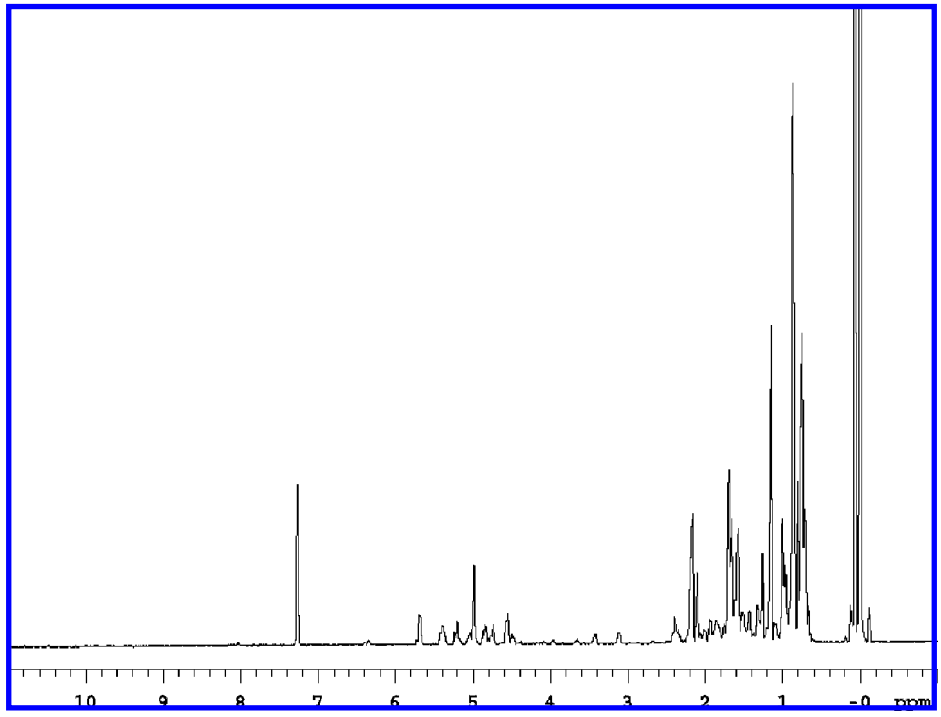

Figure 4. 500 MHz ^{1}H spectrum of *Guibourtia copallifera* in $CDCl_3$.

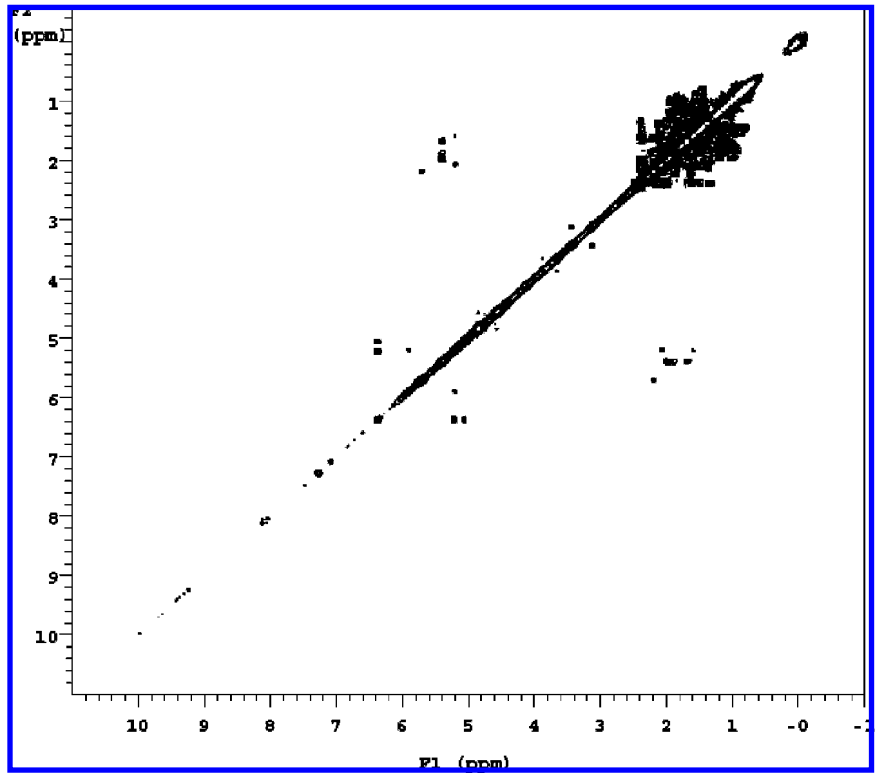

Figure 5. 500 MHz ^{1}H−^{1}H COSY spectrum of *Guibourtia copallifera* in $CDCl_3$.

Two of the species (*Bauhinia racemosa* and *Conzattia multiflora*) exhibit classic kino patterns. Kino exudates are characterized by deep red, violet, or black colors. They often are strong dyes and have been used medicinally.[13] The NMR pattern ("strong unsaturated regions including aromatic as well as alkenic components, important carbonyl resonances, and a usually weak saturated region") was first discovered in a study of *Eucalyptus* (Myrtaceae) and related samples.[11] The ^{13}C spectrum of *B. racemosa* is illustrative (Figure 7), with carbonyl resonances in the region δ 160−200, dominant unsaturated resonances in the region δ 100−160, two characteristic, strong peaks at δ 145 and 156, weak saturated resonances in the region δ 0−60, and a large peak from an electron-withdrawing functionality in the region δ 70−90. The pattern of Figure 7 is very similar to those of the eucalypts in Figures 1−3 of ref 11. The typical 1D ^{1}H kino pattern is illustrated in Figure 8 for *C. multiflora*. The most distinctive peak is from the

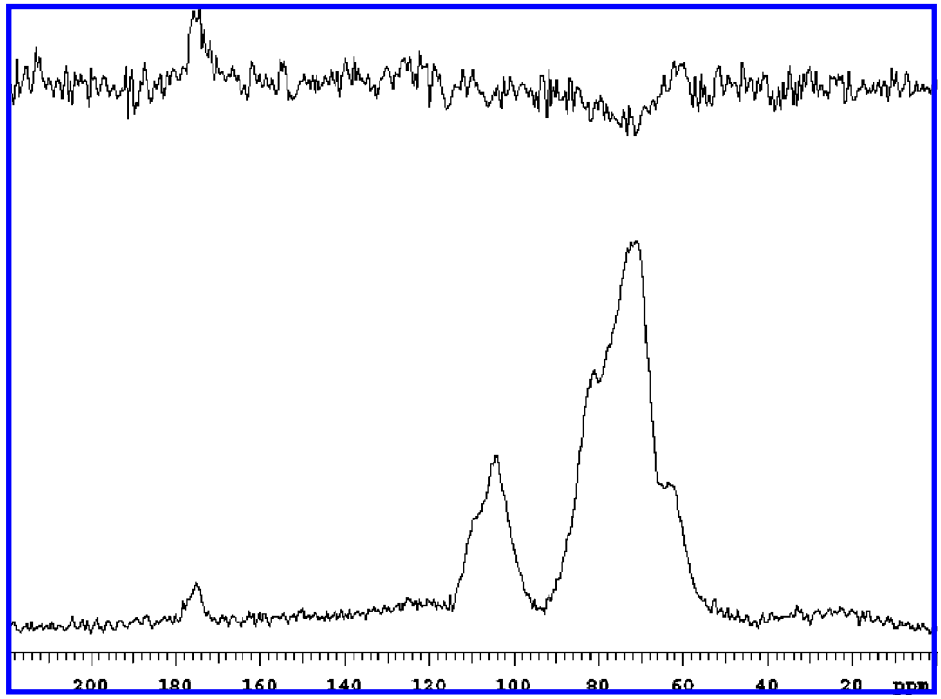

Figure 6. 100 MHz solid state ^{13}C spectra of *Cercis racemosa*, (bottom) with normal decoupling and (top) with dipolar dephasing.

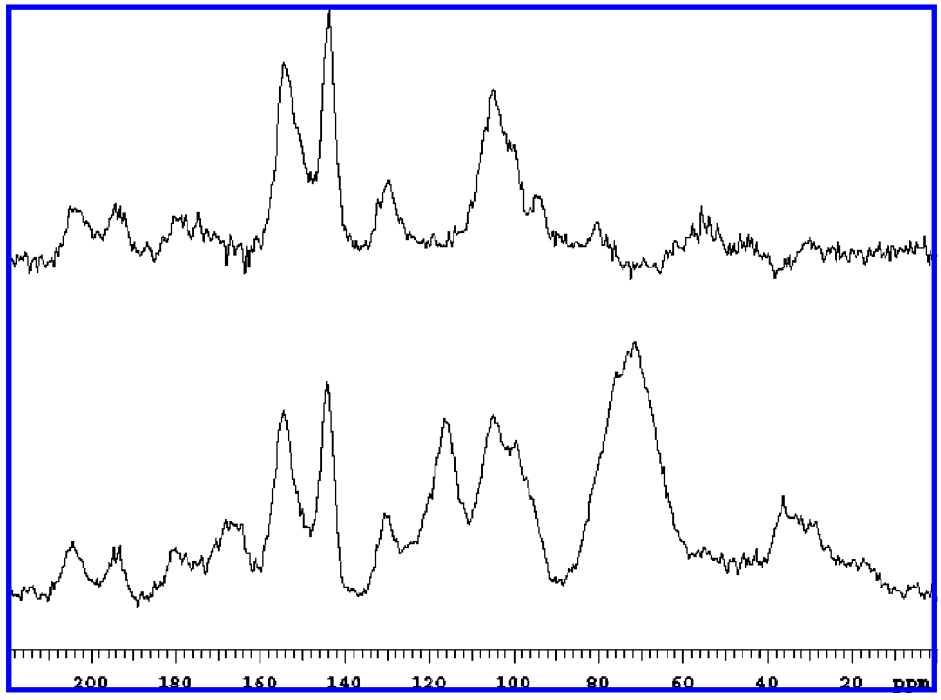

Figure 7. 100 MHz solid state ^{13}C spectra of *Bauhinia racemosa*, (bottom) with normal decoupling and (top) with dipolar dephasing.

phenolic OH found only in DMSO at ca. δ 8.5−9.0. The overall pattern most resembles that of Class B eucalypts such as *Eucalyptus muelleriana* (Figure 6 in ref 11), which generally lack saturated resonances.

The spectra of *Tamarindus indica* fall into none of the existing categories (Figure S5). Its small ^{13}C resonances at δ 80 and 105 resemble a gum, but there also are significant saturated resonances at δ 30 and unsaturated resonances at δ130. The greatest surprise, however, is that the largest group of peaks in the spectrum is from carbonyls, at δ 176−186. The material is insoluble and so gave no useful ^{1}H spectra. Until further information is available, this pattern is not classifiable ("other" in Table S1).

Mimosoideae. This subfamily contains over 80 genera and 3200 species. The 47 samples represent 11 genera, 34 identified species, and three identified by genus only (Table S1). Of these, 38 gave gum patterns, seven gave kino patterns, two gave new patterns, and none gave the resin pattern. Thus the Mimosoideae overwhelmingly produce gums. The gums include all the identified species of the genera *Acacia* and *Albizia*, four species of the genus *Senegalia*, five species of the genus *Vichellia*, Talha gum, *Anadenanthera colubrina*, *Inga preusii*, *Leucaena leucocephala*, and several of the *Prosopis* samples. Until recently, all acacia (wattle) species were combined in the single, large genus *Acacia*, which now, however, has been broken up into five genera,[13] of which three (*Acacia,*

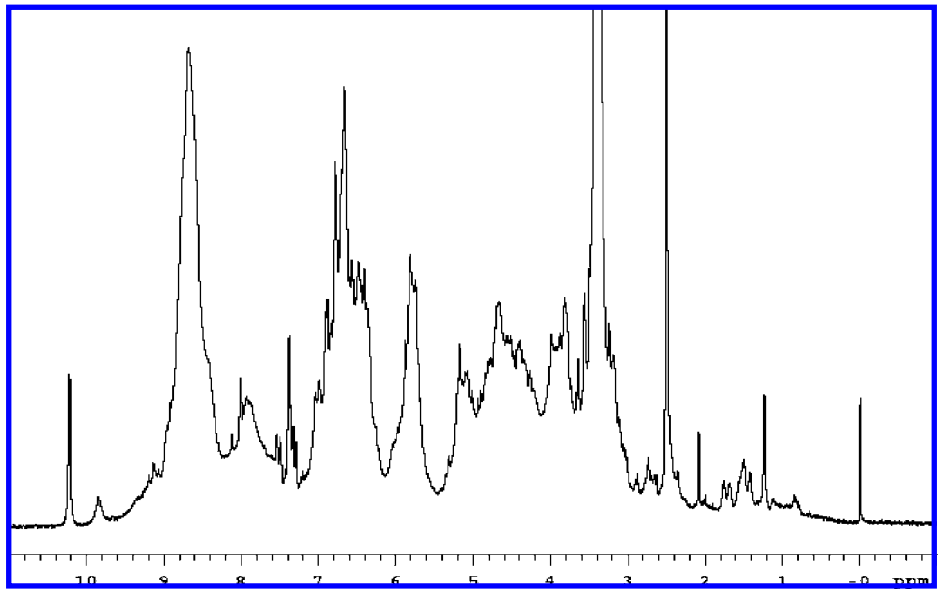

Figure 8. 500 MHz ^{1}H spectrum of *Conzattia multiflora* in DMSO-d_6. Solvent and water peaks are at δ 2.5 and 3.4, respectively.

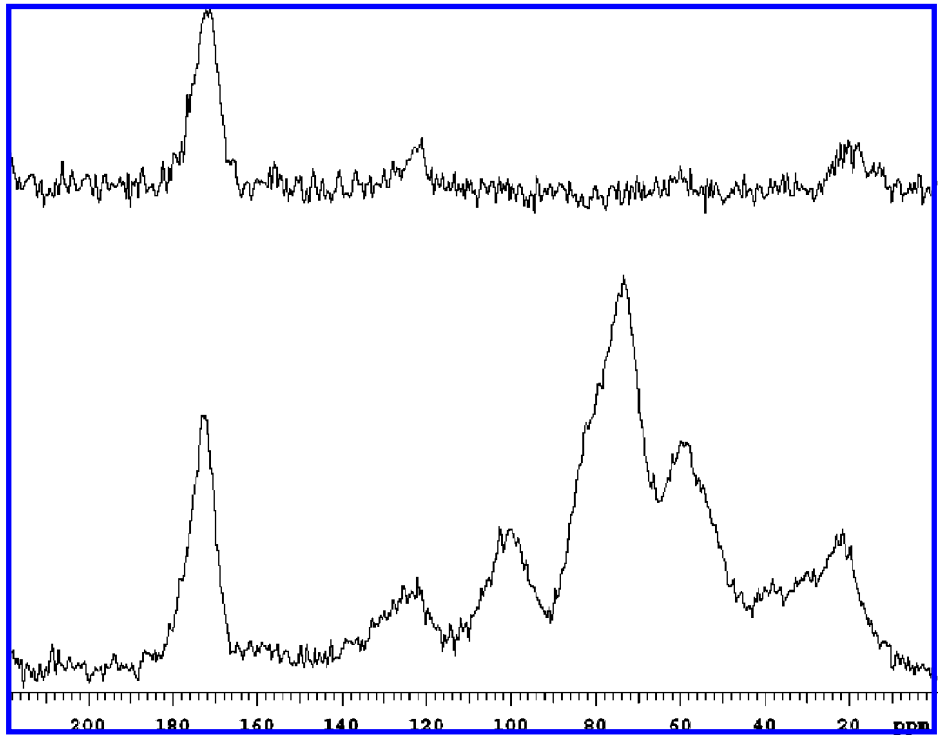

Figure 9. 100 MHz spectra of *Vachellia leucophloea*, (bottom) with normal decoupling and (top) with dipolar dephasing.

Senegalia, and *Vachellia*) are represented here. All these materials exhibited variations of the basic gum pattern, as in Figure 6. That of *Acacia biserrata* (Figure S6) is particularly sharp for any gum we have studied.

The COSY spectra of gums are generally unavailable in chloroform because of poor solubility, but some cross-peaks are visible in DMSO. The only representative cross-peaks are found regularly at 4.2−4.3/6.3−6.5 and 4.8−5.0/6.0−6.1. Both these peaks represent coupling between the anomeric proton (O−CH−O) and the adjacent ring proton (CH−O). They were observed for *Acacia dealbata, A. decurrens*, *A. microbotrya, A. vestita, Senegalia modesta*, and *Vachellia stenocarpa.*

We previously observed that the genus *Prosopis* generates kino exudates, similar to those of the eucalypts.[11] Four species in the current study (*Prosopis alba*, *P. juliflorma*, *P. velutina*, and *Senegalia catechu*) gave the kino pattern exclusively, all closely resembling the spectra of eucalypts. Interestingly, different samples of three other species produced both gum and kino patterns (*Vachellia farnesiana*, *Prosopis chilensis*, and *P. glandulosa*). We routinely take and analyze multiple samples, to test the uniformity of exudate production. Generally, duplicate samples give nearly identical ^{13}C spectra and very similar ^{1}H spectra. We previously observed the duality of exudate identity for *P. glandulosa*, that is, the ability to produce two molecularly distinct exudates.[11] These two additional species now confirm the phenomenon of kino/gum duality within a species and suggest that it is not uncommon.

The spectrum of *Vachellia leucophloea* (Figure 9) is quite distinct from all others we have observed. There appears to be a large gum component, indicated by the dominant, broad peak at δ 70−80 and the anomeric peak at δ ca. 100. Typically, gums have no other

peaks (Figure 6), but this species additionally has an unusually large carbonyl peak at δ 176, some saturated (resin-like) peaks at δ 18−40, some alkenic or aromatic resonances at δ 120−130, and a significant peak in the region for saturated functionalities attached to electron-withdrawing groups (δ 50−65). This is not a kino pattern, which is dominated by unsaturated peaks in the δ 110−160 region (Figure 7). One might be tempted to call it a gum resin, but no gum resin exhibits the large carbonyl resonance.[9] It has some resemblance to the spectra of *Tamarindus indica* (Figure S5), but there are several important differences (the spectra of *T. indica* are sharper, the carbonyl and unsaturated peaks are at different frequencies, and *T. indica* lacks the resonance at δ 50−65). There are modest similarities also with the spectra of *Amyris elemifera* and of *Liquidambar styraciflua* (Figures 12 and S18 in the eucalypt study[11]), but the differences are overwhelming (*A. elemifera* has a large peak at δ 130 and very large saturated resonances). The spectra of *V. leucophloea* were *sui generis* until we examined those of a sample of *Acacia* unidentified by species obtained from Koko Crater, Oahu, HI. The spectra of the two samples with both decoupling modes (Figures 9 and S7) are identical, peak for peak, with small differences in intensities. The sample of *V. leucophloea* had come from the Field Museum (Chicago, IL), which in turn had specified its source as The India Museum. Thus the nearly fresh sample from Hawaii and the old sample from India have corresponding spectra of this new, unique type.

Faboideae. This is the largest legume subfamily, with at least 170 genera and 14 000 species. The 21 samples represent 11 genera, 14 identified species, and three identified by genus only (Table S1). Of these, 11 gave gum patterns, three likely gave gum resin patterns, six gave kino patterns, and one gave a resin pattern.

All four identified samples from the genus *Astragalus* (*A. gummifer, A. leioclados, A. verus,* and *A. virens*) are gums with unremarkable spectral properties. Other gums come from *Enterolobium cyclocarpum, Pterocarpus* sp., *Robinia pseudoacacia,* and *Styphnolobium japonicum.* A probably processed sample from a commercial source (referred to in Table S1 as *Astragalus* sp. and by the supplier as tragacanth) is a white powder, whereas natural gums typically are yellowish, translucent, conchoidal solids. The ^{13}C spectra of *Astragalus* sp. have, in addition to the standard gum resonances, a very small resonance in the saturated region, not sufficient to classify it as a gum resin. Nonetheless, in solution, resin resonances dominate, because of the general insolubility of the larger gum component. As a result, ^{13}C and ^{1}H gave apparently contradictory assignments (Table S1). Processing clearly had altered the natural structure and should always be viewed with caution. Whereas the solid state ^{13}C spectrum always reflects the bulk, the solution ^{1}H spectrum reflects only the soluble portion. Resins in general are highly soluble, but gums have poor to zero solubility, varying from sample to sample, depending on the molecular weight of the polysaccharide components.

Pterocarpus marsupium is the only member of the Faboideae in this study to produce a resin exudate. Its spectra (Figure S8) are distinct from those of the African/American copals of the Caesalpinoideae. The ^{13}C spectrum is dominated by a huge saturated peak from δ 10 to 60, plus a significant peak centered at δ 80. This latter peak is not part of a carbohydrate component, as the anomeric region δ 100−110 is empty. There are weak unsaturated resonances from δ 120 to 140 but no exomethylene resonances. The ^{1}H spectrum in both chloroform and DMSO exhibits an aromatic AX quartet at δ 7.5 and 7.8, corroborated by a large 2D cross-peak at 7.5/7.8. The spectrum in both solvents contains very strong, sharp peaks between 0.7 and 1.2, tailing to δ 2.3. There are minor unsaturated resonances at δ 5.1, 5.2, and 5.5. The largest COSY cross-peak is at 1.9/5.2. This pattern is unique within the resin group. This species is widely referred to as a kino tree. By our definition of kinos, based on the Australian eucalypt model,[11] this exudate is not a kino. The traditional use of the term *kino* was based on medicinal properties, but we advocate the use of a molecularly based definition.

Five species gave clear signatures of kinos. The spectra of *Centrolobium tomentosum* have been published in our earlier study of eucalypts (Figures 10 and 14 in ref 11). The spectra of *Pterocarpus erinaceus* resemble the eucalypt Class C. The ^{13}C spectra of *Rhynchosia* sp. and of *Myroxylon balsamum* resemble the eucalypt Class A. There was insufficient material for a ^{13}C spectrum of the material from *Tipuana tipu*, but the ^{1}H spectrum exhibits the classic kino pattern.

The spectra of *Butea monosperma* and *Psorothamnus spinosus* did not fall into the classic resin, gum, or kino patterns. The ^{13}C spectrum of *B. monosperma* with normal decoupling (Figure S9) has many of the traditional resin resonances, with weak carbonyl and unsaturated peaks, a strong, broad saturated peak in the region δ 20−40, and medium, sharp peaks in the electron-withdrawing region δ 40−60. Assignment as a gum resin rests on the additional presence of the traditional gum and resin resonances respectively at δ 77 and at δ 104. There are two contrasts with the spectra of *Pterocarpus marsupium*, which we assigned as a pure resin. (1) The resonance at δ 78 for *P. marsupium* is not accompanied by an anomeric peak at δ 100−110. (2) The resonance at δ 77 for *B. monosperma* disappears entirely with dipolar dephasing, as occurs invariably with gums, whereas the resonance at δ 78 for *P. marsupium* survives weakly with dipolar dephasing. These observations suggest that the exudate from *B. monosperma* is a gum resin with a large resin/gum ratio, similar to the case of *Boswellia serrata* (frankincense, Figure 19 in ref 9) and *Schinus molle* (mastic, Figure 16 in ref 9). The ^{1}H spectra have the appearance of a typical resin, as the gum portion is not soluble.

The ^{13}C spectra of *Psorothamnus spinosus* (Figure S10) do not allow an obvious interpretation. There are strong carbonyl peaks, reminiscent of kinos, but the spectrum with normal decoupling entirely lacks the unsaturated peaks found invariably and strongly at δ 145 and 156 in the ^{13}C spectra of all kinos (Figure 7). Moreover, the ^{1}H spectrum lacks the hallmark phenolic OH resonance at δ 8−9.5 in DMSO. The ^{13}C and ^{1}H spectra both have strong, saturated resinous peaks (the ^{1}H spectrum of course more so) indicative of resins. The ^{13}C spectrum has a strong carbohydrate peak at δ 77, confirmed by an anomeric peak at δ 104. These latter peaks, however, did not disappear entirely with dipolar dephasing, indicating either that gums in fact are not present or that other resonances underlie these two gum resonances. Our most likely conclusion is that the material is a gum resin, with about equal amounts of gum and resin. The alternative conclusion is that the exudate is of an as yet unclassified nature.

Conclusions

Our earlier studies of exudates from over 130 conifer species in the families Pinaceae,[8] Araucariaceae, Cupressaceae, and Podocarpaceae[10] found that these gymnospermous plants produce resins almost exclusively. Only a few species of the genus *Araucaria* produce gum resins.[9] The present study constitutes the first intensive NMR examination of an angiosperm (flowering plant) family, including the first use of ^{1}H NMR spectroscopy, aside from our report on eucalypt exudates.[11] We chose the family Fabaceae as the subject for the first NMR examination of angiospermous exudates because of the large size of the family and its reported extensive resin production.[5,6] In addition, legume resins have in common with conifer resins the generally diterpenoid structure of the molecular constituents, whereas other angiosperms, such as the Dipterocarpaceae (dammars), Burseraceae (elimis), and Anacardiaceae (mastics), produce triterpenoid resins.[5,6]

Mills and White[5] state that only the subfamily Caesalpinoideae produce resins. We have found this pattern generally to be true, with the single exception of *Pterocarpus marsupium* from the Faboideae. Eight of the 16 genera produce resins, which represent

32 of our 45 samples from this subfamily. The remaining exudates are primarily gums. The Fabaceae resins, however, are not all molecularly homogeneous. The African/American copals from the genera *Copaifera*, *Daniellia*, *Guibourtia*, and *Hymenaea* (from the tribe Detarieae) constitute a relatively homogeneous group of resins (^{13}C Group FL[9]), but exudates from *Parkinsonia praecox, Cryptosepalum pseudotaxus*, *Macrolobium acaciaefolium*, and *Prioria copaifera* of the Caesalpinoideae, as well as *Pterocarpus marsupium* from the Faboideae, exhibit distinct resin patterns. Thus to date we have found six distinct resin types, including the large African/American group. Mass spectral studies of these resins would be worthwhile to identify specific molecular classes.

Gums constitute the dominant exudate for the Mimosoideae and, to a lesser extent, for the Faboideae. They are produced by nine out of 10 Mimosoideae genera and by five out of 11 Faboideae genera, but also by seven out of 16 Caesalpinoideae genera. Thus if one can speak of a dominant exudate type from the Fabaceae as a whole, it is the gums. In general, the ^{13}C NMR gum patterns are relatively similar. Analysis of ^{1}H spectra is somewhat less reliable, because of limited solubility of gums. There is, however, some spectral variability that might prove useful in distinguishing various gums.

The kino spectral signature is remarkably constant. Found originally in and spectrally defined by the genera *Eucalyptus* and *Corymbia* of the Myrtaceae, the kino pattern also was found in the genera *Prosopis* and *Centrolobium* of the Fabaceae and in the genus *Guaiacum* of the Zygophyllaceae.[11] Herein, we have found kinos in all three subfamilies: three in the Caesalpinoideae, seven in the Mimosoideae, and six in the Faboideae. This type of exudate appears to represent a widespread, but minor proportion of Fabaceae exudates.

We classified two Faboideae species as gum resins, without complete conviction: *Butea monosperma* and *Psorothamnus spinosus*. It is clear, however, that these exudates combine elements of other classes or demand new categories.

The tripartite division of exudates into resins, gums, and kinos is incomplete, but as yet we have not perceived patterns that suggest any broad new classes, discounting the gum resins as a combination mode. Previously, we found exceptional spectra for *Liquidambar styraciflua* of the Hamamelidaceae and *Amyris elimifera* of the Rutaceae[11] and herein from the Fabaceae for *Daniellia oliveri*, *Tamarindus indica*, *Vachellia leucophloea*, and *Acacia* sp. These spectra lack phenolic resonances of kinos but have strong carbonyl resonances not found with gums or resins. Both unsaturated and saturated resonances are present but variable. Discernment of any broad new classifications must await further surveys of the flowering plants, currently ongoing in our laboratories.

Experimental Section

Plant Materials. Samples were collected from public and private botanical gardens or arboreta with permission of the institutions. Nomenclature and other characteristics of the exudates are collected in Table S1. Samples were removed from the plant surface by hand or with the help of a knife without producing any harm to the plant such as causing an incision. Samples collected typically were 1−5 g. Although the material is powdered or dissolved for NMR analysis, it is fully recoverable. The samples will remain in the laboratory at Northwestern University for continued experiments but can be made available on request.

Sample Preparation. Samples were ground into a fine powder for solid state ^{13}C experiments and were loaded into a Varian 5 mm general purpose Zirconia rotor sealed with Vespel caps. Each sample load required about 160 mg of material, although smaller sample sizes were possible (down to 30 mg). For ^{1}H spectra, approximately 55 mg of exudate was transferred from its original container to a small, glass vial, and about 1 mL of deuterated chloroform-d_6 or dimethyl sulfoxide (DMSO-d_6) was added to each vial before transfer to the NMR tube.

NMR Data Acquisition. Solid state ^{13}C NMR data were recorded on a 400 MHz Varian NMR system. The DirectDrive console had a clean rf architecture with a powerful digital receiver and utilized advanced phase-amplitude modulation. The system had a 5 mm T3 PENCIL probe. The magic angle spinning rate was set to 5000 Hz. The cross-polarization pulse sequence called tancpx was used for normal proton decoupling. For interrupted decoupling, the pulse sequence tancpxidref was used, in which a 50 μs delay was applied in the ^{1}H channel directly before the 180° pulse in the ^{13}C channel. A typical parameter set was as follows: spectrum frequency 100.544 MHz, spectral width 50 kHz, pulse width 3.4 μs for the 90° pulse for both ^{1}H and ^{13}C, delay time 5 s, contact time 2 ms, acquisition time 20.5 ms, and scan number 256. Solid state ^{13}C spectra were referenced to an external adamantane peak at δ 38.3 and were converted to tetramethylsilane at δ 0.0. Data were collected and processed with the software VnmrJ 2.1B. Proton spectra were obtained on a Varian Inova-500 NMR spectrometer at room temperature without spinning. Typical one-dimensional 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. Spectra were referenced in $CDCl_3$ to TMS and in DMSO to the DMSO peak at δ 2.5. Typical two-dimensional parameters without pulsed field gradients were as follows: spectral width 12 000 Hz, pulse width 90°, delay time 1.0 s, scan number 4, and increment number 256.

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Supporting Information Available: Table S1, Figures S1−S10, the ^{13}C and ^{1}H spectra of various legumes. This material is available free of charge via the Internet at http://pubs.acs.org.

References and Notes

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