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Classification of Modern Resins by Solid State Nuclear Magnetic Resonance Spectroscopy

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The carbon-13 nuclear magnetic resonance (NMR) spectra of modern resins have been used to characterize their botanical sources. Resins from four of the principal diterpenoid resin-producing genera, *Agathis, Hymenaea, Pinus,* and *Araucaria*, give distinct NMR spectra under conditions of normal and interrupted decoupling. The genus *Wollemia* gives the same spectra as *Agathis*. Samples from the triterpenoid resin-producing family Burseraceae and of gum resins were recorded for comparison. Resin samples from unknown or uncertain sources then were examined and assigned to their botanical sources.

Resinous material exuded by woody plants as a metabolic product but useful for protective purposes often congeals relatively rapidly to form a solid (*1*,*2*). Even surface stickiness can be lost, yielding a translucent, glassy substance. Although hard to the touch, the material is easily worked by tools. It dissolves in organic solvents but not water, and it burns. As the material ages, it becomes harder, less soluble, and less combustible. Over millions of years, through loss of small molecules, polymerization, ring formation, oxidation, decarboxylation, and other processes, fossil resins often called amber can be formed.

Modern resins are of particular interest when they resemble amber most closely, possessing a light yellow to brown color and a rocklike hardness. Such materials are found the world over and can come from a variety of botanical sources. When harvested directly from or under the source tree, there is no doubt about the botanical identity. Even many modern resins, however, cannot always be associated with the botanical source. Resinous materials found washed up on shores may be either recent or fossilized and have been referred to as sea amber. Recent materials also may appear as commercial products, devoid of provenance (and less valuable than products made from the more robust fossil resin). Even when harvested and sold in a local market, the tree source may not be clear. When the resin is to be burned as incense, the raw

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material may be mixed with other materials such as fragments of kindling wood, further confusing the botanical question. After hundreds or thousands of years, the resins still are considered to be recent (not fossilized), but association with the source most likely has been lost.

Knowledge of the identity of botanical sources aids our understanding of harvesting methods, trade patterns, and relationships between plant and end function. Both in the ancient world and in the present, resins have been valued as adhesives, linings, coatings, incense, additives, and preservatives (as in wine). In their hardened forms, they have been carved to form decorative objects such as beads or small sculptures. Development of a method for characterizing botanical sources of modern resins may assist in the identification of the botanical sources of these fossil resins, which have been altered through natural degradation. For these reasons, we have sought to develop a method for distinguishing botanical sources of modern resins through the measurement of their carbon-13 (^{13}C) nuclear magnetic resonance (NMR) spectra in the solid state. Incomplete and variable solubility renders spectra in solution less diagnostic. In the past, gas chromatography coupled with mass spectrometry (GC/MS) has been the primary tool for identifying resin sources (*3*). This technique is destructive and examines only the portion that is sufficiently volatile to enter the mass spectrometer. Infrared spectroscopy also has been used but has proved to be insufficiently diagnostic (*4*). NMR offers a formally nondestructive alternative, which retains all molecular components by examining the material directly as the solid (the material normally, however, must be powdered).

Resins are composed of the molecular class of organic compounds known as terpenes. This class is built up from the five-carbon compound isoprene ($CH_2{=}CH{-}C(CH_3){=}CH_2$). Molecules made of two such units contain 10 carbon atoms and are called monoterpenes, with a variety of molecular skeletons. Monoterpenes give rise to the fluidity of the initially formed resin and are lost over time through volatilization. Distillation of natural pine resin produces a liquid mixture of monoterpenes known as oil of turpentine. The solid residue is composed of larger molecular weight constituents and is called rosin or colophony.

Because liquid monoterpenes are lost during the process of solidification, the molecules of interest in this study are of higher molecular weight. Molecules based on four isoprene units, with 20 carbon atoms, are called diterpenes and usually are solid. Those with six isoprene units and 30 carbon atoms are called triterpenes, and so on. This study has focused on diterpenoid resins. Triterpenoid resins such as the dammars and mastics, and gum resins such as myrrh and frankincense will await another study.

Diterpenoid resins derive primarily from the large families Coniferae (conifers) and Leguminosae (angiosperms) (*1,2*). Three Coniferae subfamilies, Pinaceae, Cupressaceae, and Araucariaceae, all produce resins, and of these we have obtained samples from the Pinaceae genus *Pinus* and the Araucariaceae genera *Agathis*, *Araucaria*, and *Wollemia*. We also have obtained samples from the genus *Hymenaea* of the Leguminosae family. These five genera are among the best known and most prolific resin producers. For comparison, we have obtained resins thought to be triterpenoid from the Burseraceae family (sometimes called elemis) and gum resins from the Combretaceae family. These samples were drawn from all continents except Europe and Antarctica.

We report the solid state NMR spectra in order to provide a new analytical method for the identification of modern resins. After characterizing the authentic materials, we are able to identify the botanical sources of various unprovenanced resins.

MATERIALS AND METHODS

Resin from several species of the genus *Agathis* was harvested by G. O. Poinar, Jr., directly from or under trees in New Zealand (*australis*, the kauri tree) and Australia (*atropurpurea* Hyland, *moorei* (Lindley) Masters, *lanceolata* Lindley ex Warburg, and *macrophylla* (Lindley) Masters). A sample of *Araucaria columnaris* (Foster) Hooker was obtained from a tree growing in the Royal Botanic Gardens (Sydney) by G. O. Poinar, Jr. A sample of Wollemi pine (*Wollemia nobilis*) was provided by K. Hill of the Royal Botanic Gardens (Sydney). A sample of *Hymenaea courbaril* was collected in the Dominican Republic by G. O. Poinar, Jr. A sample of *Pinus monticola* was collected in California by G. O. Poinar, Jr. A sample from the family Burseraceae originated from the O. G. Innes Corp. without further attribution. A second sample said to be from this family originated from Mexico.

In addition to these authentic materials, we have examined a number of modern and recent resins of largely unknown plant origin. In this study we endeavor to assign the origins of all of these materials by comparison of their ^{13}C NMR spectra with those of materials from known sources. Several samples of rosin (which is normally *Pinus*) were obtained from commercial sources. In this study we refer to them as U.S. Rosin (provided by C. W. Beck from the Vassar stockroom without indication of the manufacturer), Chinese gum rosin, and two samples of commercial violin rosin (Poinar and Hill). Additional samples came from the following wide variety of sources: Congo copal and Manila copal Loba (O. G. Innes Corp., provided by C. W. Beck), Manila copals Pangasinan and Bical (Philippine Forestry Service, provided by C. W. Beck and P. Perez), abietic acid dimer (Hercules Corp., tradename Dymerex), Chiapas (Mexico) and Chichicastenango (Guatemala) sticky resin (provided by C. J. Welch, so-called because it had not fully hardened and retained considerable stickiness), Chichicastenango incense resin (provided by C. J. Welch, mixed with kindling wood pieces), Colombia (collected by M. and A. Nisbet from the state of Santander), Kenya (provided by A. Graffin of Geological EnterPrises, Ardmore, OK, and attributed to Mombasa), Madagascar (provided by G. O. Poinar, Jr.), and two samples of deep sea amber (resin recovered from the sea off the Eastern U. S. coast and provided by B. Kobus). For contrast, we have recorded the spectra of two gum resins, one from Totolapa, Mexico (*Enterolobium*, family Leguminosae, provided by T. A. Lee, Jr.) and one from Puerto Rico (*Bucida buceras*, family Combretaceae, collected by R. González and A. Blay in Guaynabo, Puerto Rico), to illustrate the effect of the presence of carbohydrates on the spectrum.

Samples were powdered by grinding in an agate mortar, a process that was straightforward for all but the Chiapas sticky resin. About 70 mg was loaded into a silicon nitride rotor, which was closed with Kel-F caps. Samples were spun at approximately 7 kHz in a Varian 300 MHz spectrometer (^{13}C at 75 MHz). Spectra were obtained with a 4.9-μs pulse, a 450-μs contact time, a 5-s repetition delay, a 0.15-ms acquisition, a 100 kHz spectral window, usually 512 transients, and, for interrupted decoupling, a 50-μs delay. Adamantane was used as an external reference, converted to tetramethylsilane (TMS, $(CH_3)_4Si$) at δ 0.0.

RESULTS AND DISCUSSION

Agathis and Wollemia. Agathis is very widespread in the southern hemisphere, particularly in Australasia. We previously published spectra of modern *Agathis* from New Zealand and Australia (*5*), and Fig. 1 contains typical spectra. At the bottom is the spectrum with normal decoupling, in which all carbons are represented. At the top is the spectrum with interrupted decoupling, in which only carbons appear that lack attached protons or are very rapidly moving. Such an edited spectrum provides an alternative fingerprint. All resins discussed herein have been characterized by both types of spectra.

The representative *Agathis* spectrum with all carbons exhibits resonances in three general areas. The alkene region (unsaturated carbons) occurs from δ 100 to 150. The peaks centered at about δ 110 and 150 come from a particular type of unsaturation corresponding to the $>C{=}CH_2$ group, called exomethylene when the $>C$ is part of a ring. The grouping between δ 120 and 140 comes from trisubstituted ($>C{=}CH$-) and disubstituted (-$CH{=}CH$-) double bonds. It is characteristic of most modern or recent resins to have strong exomethylene resonances. As a result, the peaks around 110, 130, and 150 have similar intensities. The second general region occurs from δ 10 to 80 and contains resonances of saturated carbons (alkanes), those not part of multiply bonded functionalities. It is dominated by a strong peak at δ 38. In addition there are distinct but weaker peaks at δ 16, 22, 28, 47, and 57, and a very broad peak around δ 75. The peaks at δ 57 and 75 probably imply a small number of carbons attached to an electronegative element such as oxygen (C-O). The third region, the carbonyl ($C{=}O$), occurs between δ 170 and 210 and is not strongly populated in the *Agathis* spectrum.

Application of interrupted decoupling deletes or simplifies resonances from certain regions of the spectrum. Only fully substituted carbons (no attached hydrogens) remain in the alkene region (peaks at δ 136 and 150, the latter coming from the $>C$ part of the exomethylene group). The resonance in the carbonyl region at around δ 185 becomes enhanced. The alkane region contains both quaternary carbons (no attached hydrogen) and rapidly moving methylene (CH_2) and methyl (CH_3) carbons, including two strong, sharp peaks at δ 38 and 44, a medium peak at δ 26, and a medium, broad peak at δ 14–20.

All of the authentic, modern *Agathis* samples in our earlier study exhibited this pattern for both normal and interrupted decoupling, as did most of the semifossilized resins from New Zealand, with dates ranging up to 37,000 BP (*5*). In our current survey of related materials, we found that Loba and Pangasinan (Manila) copal from the Philippines and Wollemi pine matched this pattern. Figure 2 contains the spectra of Pangasinan copal and Wollemi pine. (Loba is essentially the same.) Manila copal usually comes from *Agathis dammara* or *alba* (*1*). Wollemi pine comes from a different genus of the family Araucariaceae, *Wollemia nobilis* Jones, Jill, and Allen. These spectra are closely similar to that of the *Agathis* standard. Thus resin from both Araucariaceae genera are sufficiently similar to give essentially identical NMR spectra.

The distinguishing characteristics of *Agathis* and *Wollemia* spectra with normal

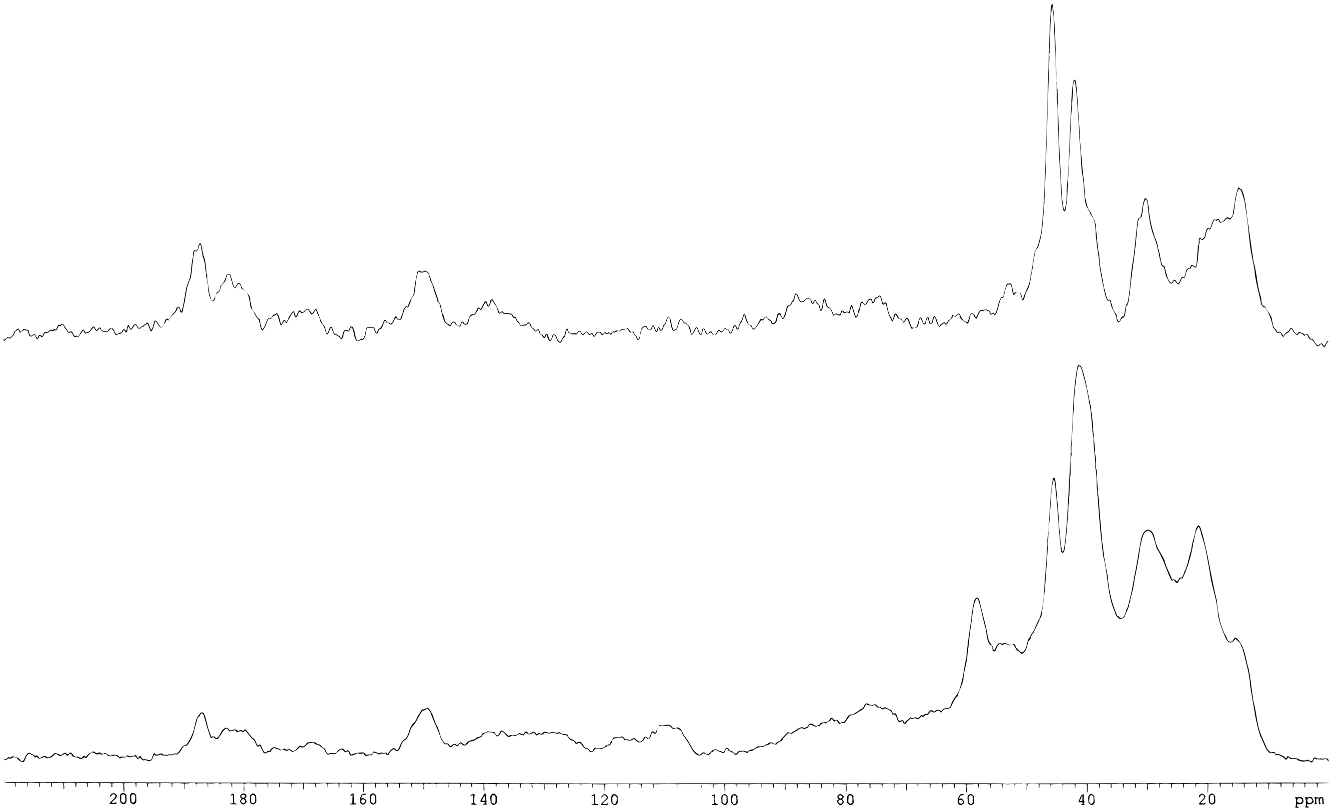

FIG. 1. The ^{13}C NMR spectrum with normal (lower) and interrupted decoupling of resin from *Agathis lanceolata* lindley ex Warburg.

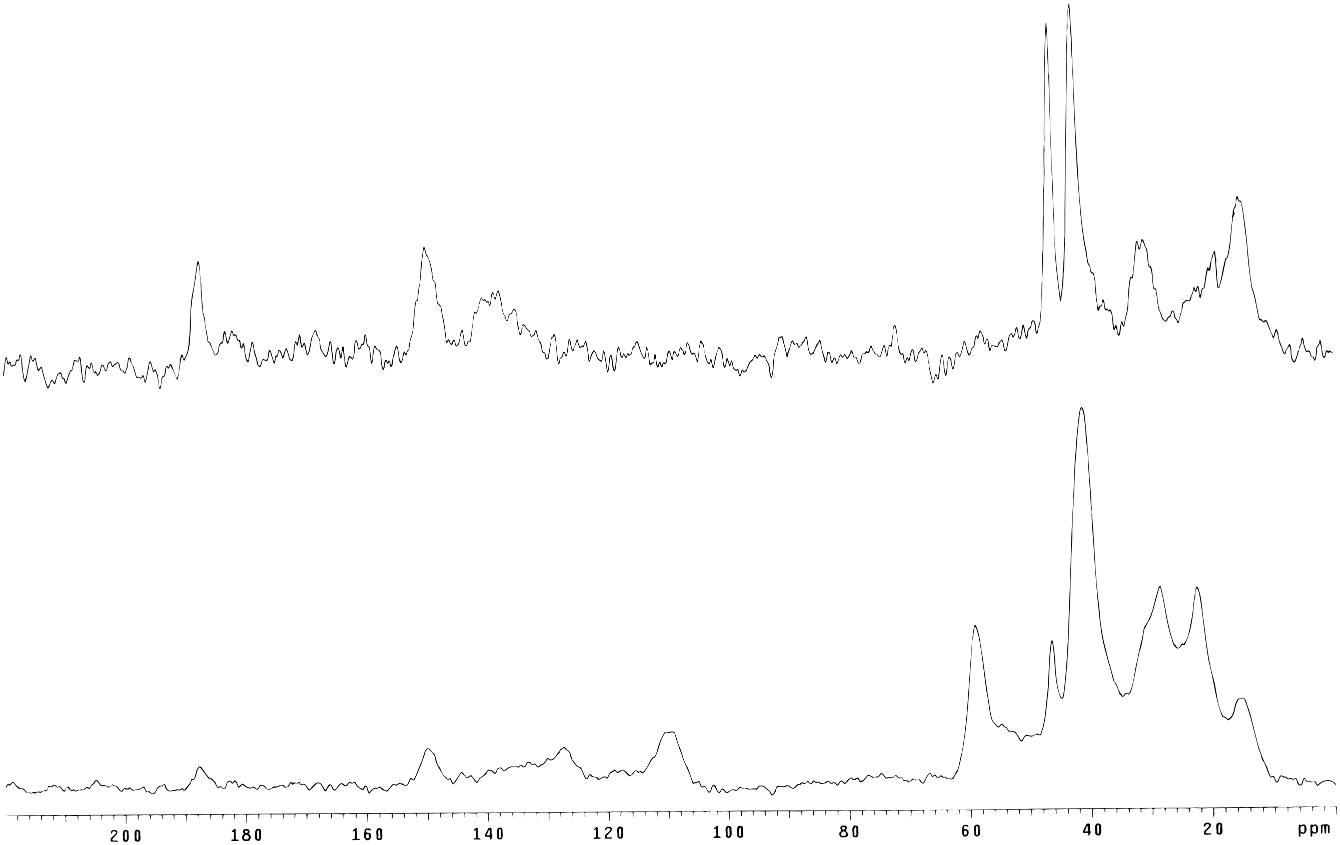

FIG. 2. The ^{13}C NMR spectra with normal and interrupted decoupling of Pangasinan copal from the Philippines (top two) and Wollemi pine resin (bottom two). The normal spectrum is always the lower of the pair.

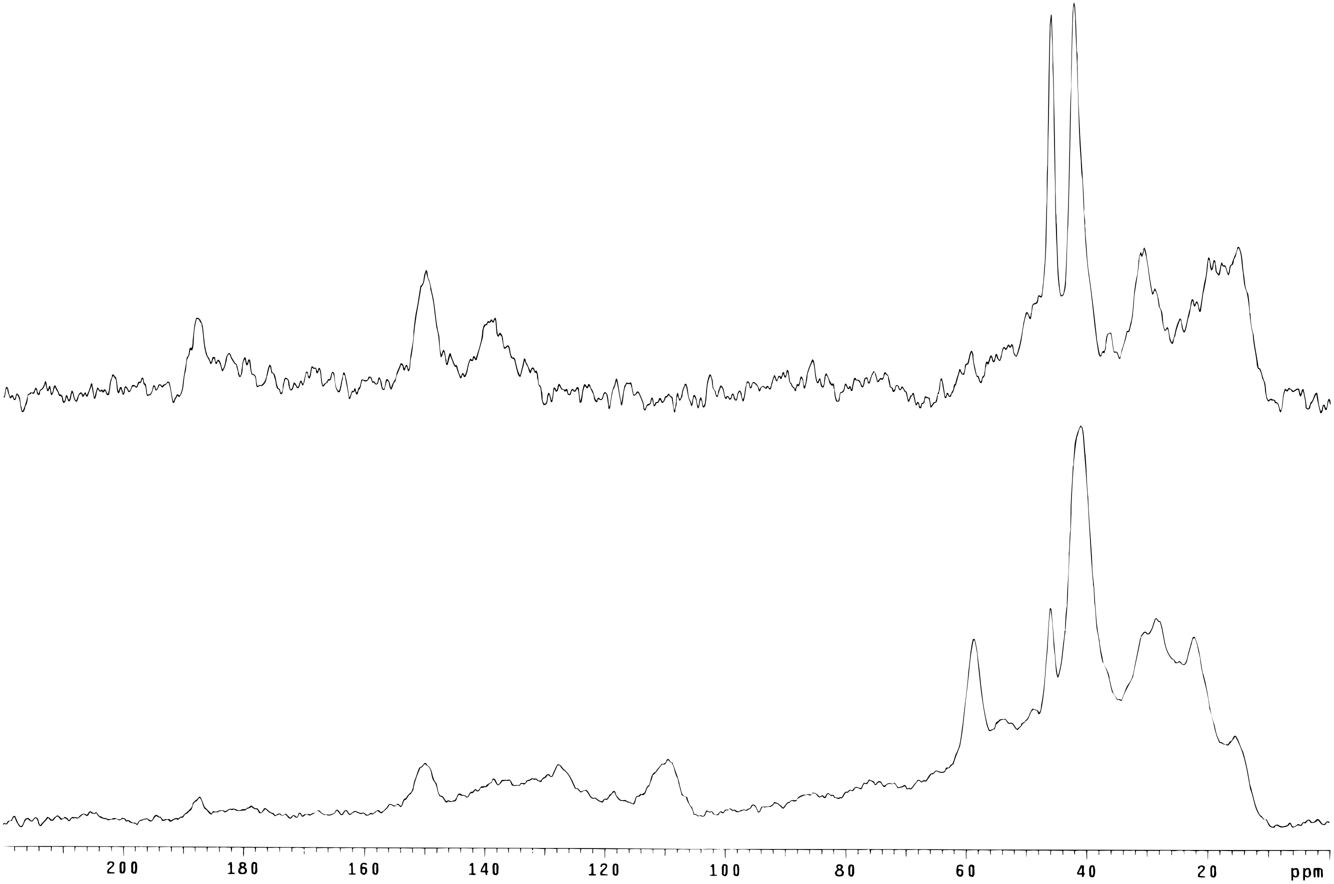

FIG. 2.—*Continued*

decoupling are strong exomethylene resonances at δ 110 and 150, a strong, sharp resonance at δ 38, and five other resonances in the saturated region from δ 15 to 60. In the interrupted decoupling spectrum, the distinguishing features are two sharp peaks at δ 40 and 46 of similar intensity, two smaller peaks at δ 15 and 30, and two medium peaks in the alkene region at δ 138 and 150. These peak positions can vary over a range of 2–4 ppm because of difficulties in calibration, but the pattern remains the same.

Hymenaea. This angiosperm genus produces most of the resins of tropical America but also is found in East Africa. The Aztecs called the resin *copalli*, which has given rise to the term copal, often used for incense in Mesoamerica. Figure 3 presents the spectra for a sample of *Hymenaea courbaril* from the Dominican Republic, a material whose spectra we have discussed previously (*6*). The alkene region is characterized by relatively strongly exomethylene resonances (δ 108 and 148). There also are resonances from di- or trisubstituted double bonds in the intermediate region, at δ 126 and 140. The alkane region is dominated as usual by the peak around δ 38. In addition, there is more detail in the less intense peaks than was the case for the *Agathis* spectra. In particular, there are five groupings of peaks, at δ 16–20 (usually two peaks), 24–27 (usually two peaks), 33 (a small, sharp peak), 46–50, and 57. With interrupted decoupling, the alkene region collapses to two peaks at δ 140 and 148, very similar to the *Agathis* spectrum. The alkane region, however, is quite different. It consists reproducibly and diagnostically of four peaks, two large ones at δ 17 and 38 and two sharp, medium ones at δ 34 and 48. The region δ 60–100 is empty. A weak carbonyl peak around δ 180 also is evident.

We already have commented that resins from the state of Santander, Colombia, provide essentially identical spectra to *Hymenaea* and hence appear to be recent resins (*6*). In this same study, we found that resins from Kenya, Tanzania, and the Congo give the same pattern. Although East Africa is a source of *Hymenaea verrucosa*, the region also produces resin from an alternative member of the Leguminosae family, the genus *Copaifera*. We do not know if the African materials we have examined are *Hymenaea* or *Copaifera*. Clearly it would be useful to examine a sample of authentic *Copaifera* material.

Figure 4 contains the spectra from the Congo resin. All the characteristics of the *Hymenaea* spectra are present, except that the peak at δ 34 with interrupted decoupling is weaker, and a shoulder has appeared at δ 20 with interrupted decoupling. The Madagascar sample exhibits major differences in intensities but not peak positions. Madagascar resin also has been identified with *Trachylobium* rather than *Hymenaea*. In the spectrum with interrupted decoupling, the peak at δ 48 is much diminished, that at δ 34 is now the same size as that at δ 38, that at δ 17 also is diminished, and the shoulder at δ 20 has become a significant peak. Similar differences are present in the spectrum with normal decoupling. Whether *Hymenaea*, *Trachylobium*, or another genus, Madagascar resin has a clear resemblance to the *Hymenaea* standard.

The signature for *Hymenaea* resins is strong exomethylene resonances, a dominating δ 38 peak, and five other alkane resonances in the spectrum with normal decoupling (not the same five peaks as for *Agathis*), and a particularly diagnostic four-peak alkane region in the spectrum with interrupted decoupling. The region from δ 60 to 100 is empty.

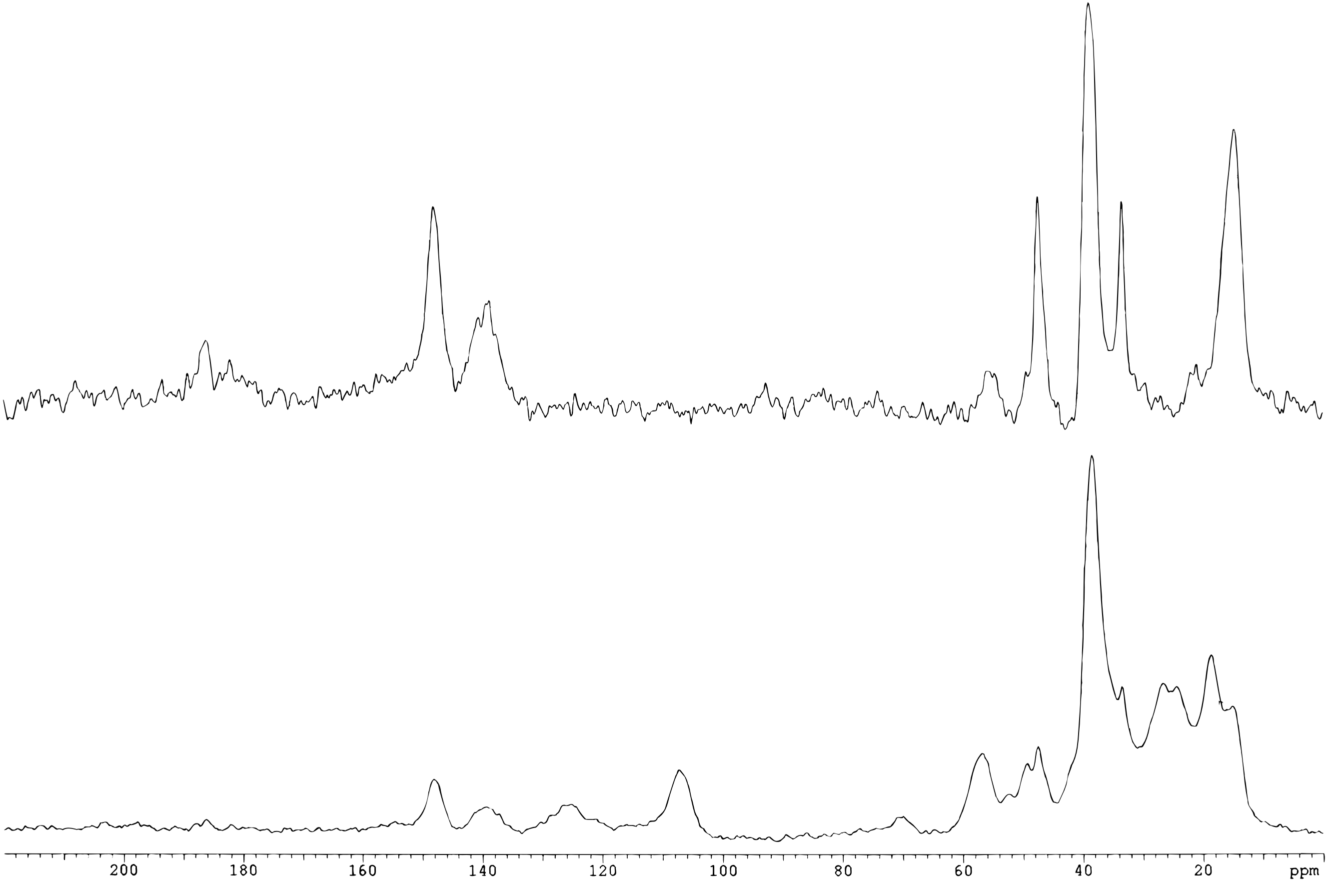

FIG. 3. The ^{13}C NMR spectrum with normal (lower) and interrupted decoupling of resin from *Hymenaea courbaril*.

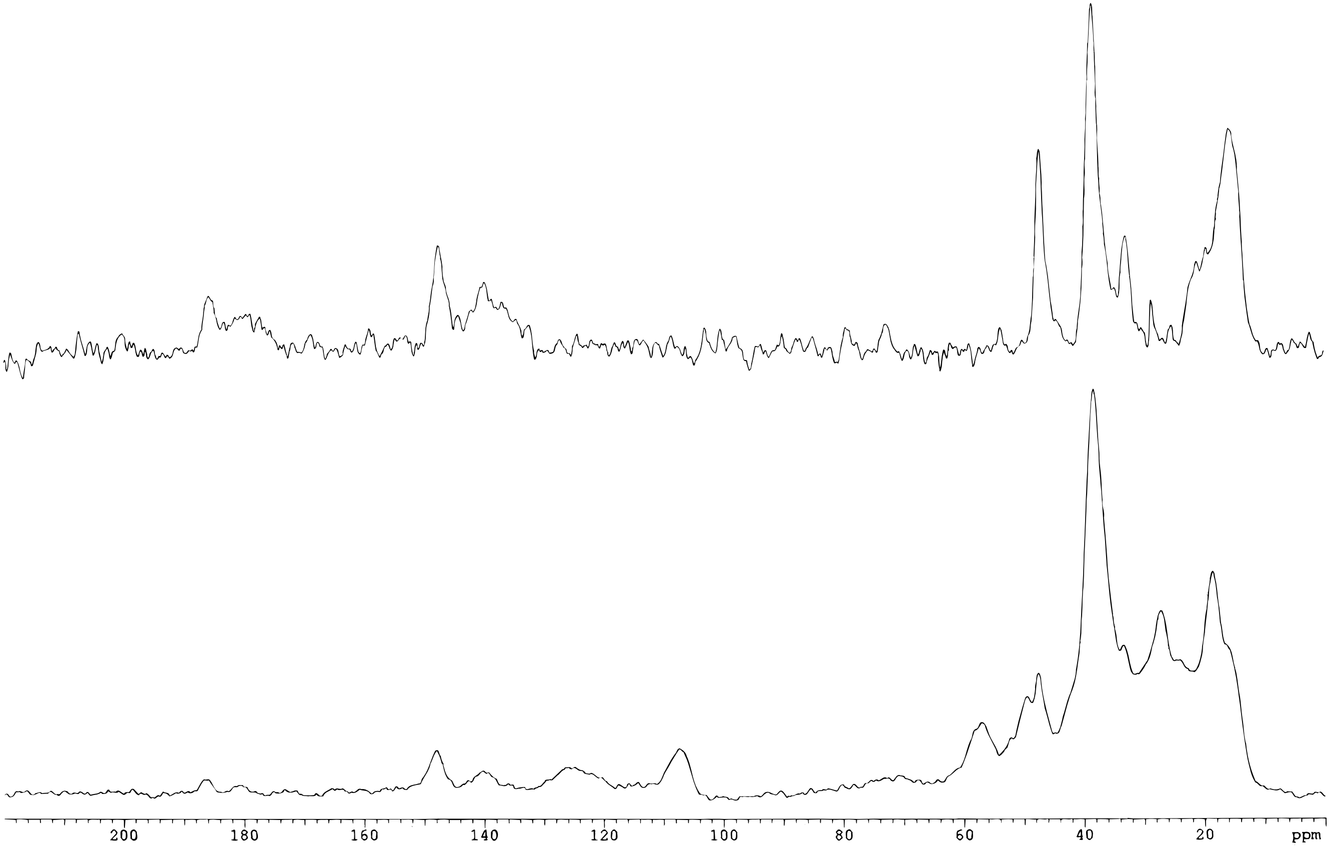

FIG. 4. The ^{13}C NMR spectra with normal and interrupted decoupling of resin from the Congo (top two) and from Madagascar (bottom two). The normal spectrum is always the lower of the pair.

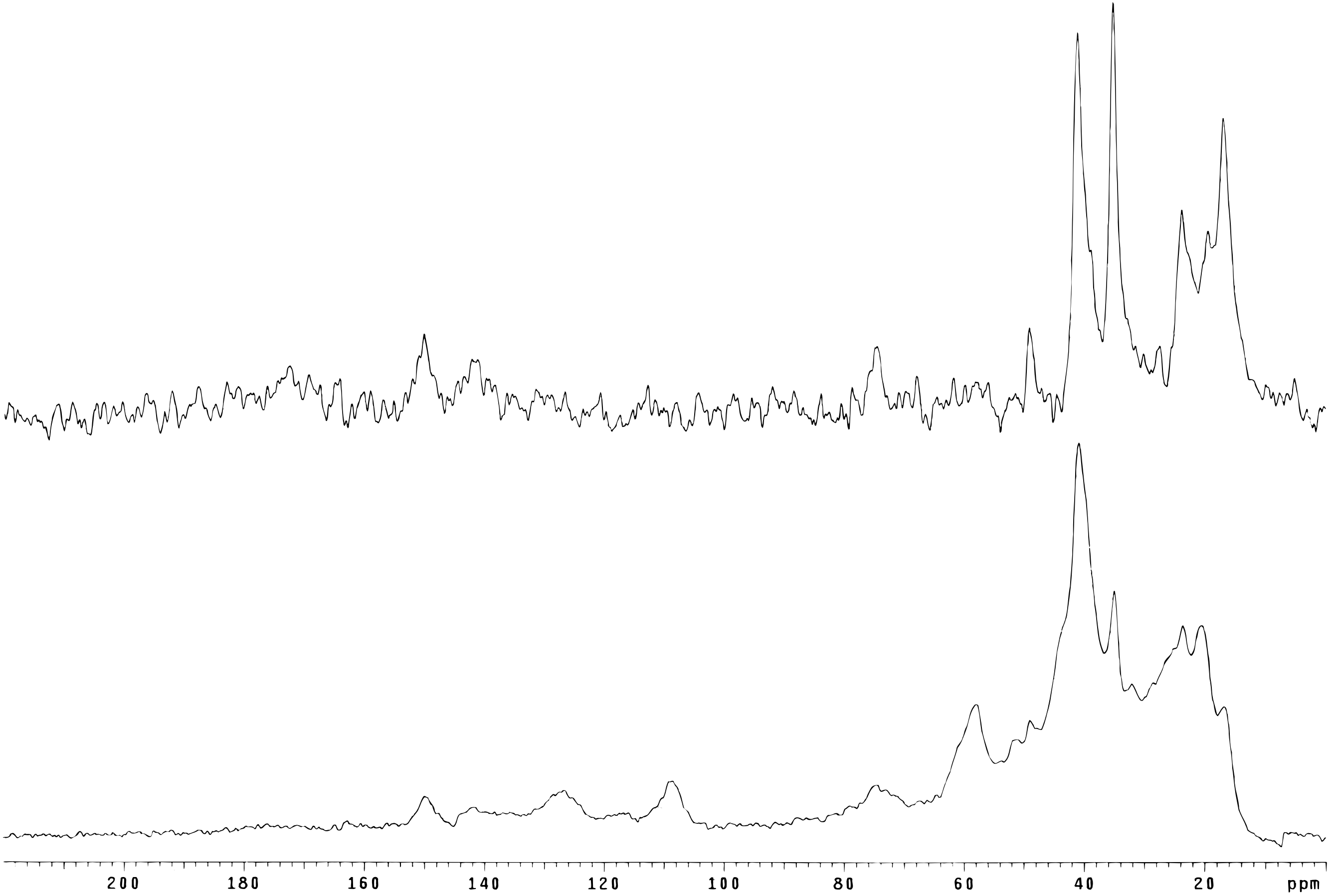

FIG. 4.—*Continued*

Pinus. Numerous Pinaceae genera produce resins, but of these *Pinus* probably is the most prolific. *Pinus* is found in numerous northern temperate zones and mountainous northern tropical zones. Figure 5 contains the spectra of pine resin obtained directly from a tree (*Pinus monticola*) in California. The spectrum without interrupted decoupling has been reported (*7*), although without the genus specified. The results are similar to those of Fig. 5 (bottom). The exomethylene resonances at δ 108 and 148 are very weak, but other alkene bands are present between δ 120 and 140. A carbonyl resonance at δ 186 is clearly present, although weak. The alkane region is composed of three groupings of peaks: a large pair of peaks at δ 16–26, a strong peak at δ 38 with a shoulder at δ 36, and a complex of peaks at δ 44–54. With interrupted decoupling, the carbonyl peak and one alkene peak become more pronounced. The alkane region contains four prominent peaks (δ 18, 22, 38, and 48) and one smaller peak at δ 33, all sharp.

All of our rosin sources produced the same spectra: U.S. Rosin, Chinese gum rosin, and commercial violin rosin, with some small variations. Figure 6 contains the spectra for violin rosin. Two samples of sea amber have spectra (also illustrated in Fig. 6) very similar to the *Pinus* standard and to rosin. The only differences are in peak intensity, e.g., δ 33. Surprisingly, several samples of alleged Manila copal Bical also had identical spectra. Since the Philippines are more likely to be the source of *Agathis* than *Pinus*, either the samples were misidentified or *Pinus* resins are present in Southeast Asia. Resins collected in Chichicastenango, Guatemala, also had spectra similar to the *Pinus* standard, although the exomethylene peak at δ 148 (but not 108) and the oxygen-substituted region around δ 75 were much stronger. The latter resonance is characteristic of gums, which contain oxygen-rich carbohydrates and are discussed below. In fact the *Agathis* (Fig. 1), *Hymenaea* (Fig. 3), and *Pinus* (Fig. 5) standards each have small peaks around δ 75. The sample of the Hercules product Dymerex, said to be composed largely of the abietic acid dimer, gives spectra (Fig. 7) very similar to the *Pinus* standard, suggesting a possible chemical component of these resins. Indeed, GC/MS analysis of *Pinus* has found >80% of abietanes and low levels of labdanes (*2*).

The genus *Pinus* thus represents a widespread source of resin. The five peaks in the alkane portion of the spectrum with interrupted decoupling provide the most diagnostic indicator.

Araucaria. This genus of the family Araucariaceae, found in Australasia and South America, is represented by a single sample of *Araucaria columnaris* (Foster) Hooker (Fig. 8). The spectra are clearly distinct from those of *Hymenaea* and *Pinus*. Although more closely similar to *Agathis*, there nonetheless are distinct differences. There are essentially no alkene carbons other than exomethylene. Thus the region between δ 120 and 140 is almost a void. There is a strong peak around δ 72 with a shoulder at δ 74 and another smaller peak at δ 82. This last peak remains with interrupted decoupling. This region contains resonances of carbons attached to oxygen, as is found in carbohydrates. These features suggest that *Araucaria* may contain more carbohydrates mixed with the terpenes in the resin. The alkane regions of *Araucaria* and *Agathis* are somewhat similar (compare Figs. 1 and 8). In the interrupted decoupling spectrum there are two sharp peaks (as with *Agathis*) but three medium-sized peaks, including one at δ 23 that is absent in the *Agathis* spectrum.