Distinctions among Conifer Exudates by Proton Magnetic Resonance Spectroscopy

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Proton nuclear magnetic resonance spectra have been recorded of exudates harvested from 12 species from the family Araucariaceae, 40 from the Cupressaceae, and one from the Podocarpaceae. These spectra were compared with the spectra previously recorded of 82 species from the Pinaceae. These four families together represent all major groups of extant, resin-bearing conifers. A common set of 10 COSY two-dimensional cross-peaks generally define samples from the Pinaceae, a different set of six peaks define the Araucariaceae, and yet a third set of 10 peaks define the Cupressaceae, with a few exceptions. It is important that proton spectra can distinguish the Araucariaceae and the Cupressaceae, since carbon-13 spectra do not. The one-dimensional peaks not only confirm these familial distinctions but also often characterize genus and species uniquely.

Conifers (cone-bearing plants) constitute a plant division called either Coniferae or Pinophyta. They are the largest and most important of the four gymnosperm divisions.[1] Although fossil conifers were taxonomically quite varied, living Coniferae constitute a single class, Pinopsida, with one order of taxa, the Pinales. This order contains six to eight families with about 70 genera and 600 species. Although there is no genetic basis for separating these families into more than one order, there are morphological distinctions, particularly in leaf shape, that suggest three broad groups. The family of the Pinaceae (pines, firs, spruces, cedars, and others) constitutes one distinct group (sometimes considered the order Pinales). The Araucariaceae and Podocarpaceae together constitute a second group (sometimes considered the order Araucariales, possibly with a third family, the Phyllocladeceae). The Cupressaceae (cypresses, redwoods, and others) along with the Taxaceae (yews), the Sciadopityaceae, and possibly the Cephalotaxaceae constitute a third group (the Cupressales). We shall refer to these three informal groups respectively as the pinaceans, araucarians, and cupresseans.

Most conifer genera produce a sticky exudate in response to trauma such as damage or disease.[2,3] This exudate often hardens to a solid or semisolid material. Almost all conifer exudates are resins, that is, water-insoluble mixtures of terpenoid compounds. They are diterpenoid and hence can mature to a solid. Numerous uses of resins include wine stabilization, bow rosin for stringed musical instruments, fuels, adhesives, and lubricants. Much of the familiar odor associated with conifers arises from the exudates present at damaged surfaces.

There are several other materials, chemically distinct from resins, which also are produced as exudates. Gums (polysaccharides) and kinos (phenolic resins) do not seem to be produced by conifers, but we have found examples of gum resins (exudates composed of both terpenoid and carbohydrate components) in a few species of the genus *Araucaria*.[4]

Each of these types of exudates may be identified quickly and unambiguously by carbon-13 (^{13}C) nuclear magnetic resonance (NMR) spectroscopy, recorded on the solid exudates.[4] The spectra of resins have numerous resonances in the saturated region (δ 0−50; "saturated" in this context implies saturated carbons attached only to other saturated carbons), small resonances in the region of carbons attached to electron-withdrawing groups (EWGs) such as oxygen (δ 50−110), small or sometimes no resonances in the unsaturated region (δ 110−160), and few or no carbonyl resonances (δ 170−220).

In our initial ^{13}C study of resins from 59 species in nine vascular plant families,[4] we found that most families studied (Burseraceae, Clusiaceae, Dipterocarpaceae, Euphorbiaceae, Fabaceae/Leguminosae, Pinaceae, and Rutaceae) produce spectra with a single ^{13}C pattern, distinct from each of the other genera. The Araucariaceae and the Cupressaceae, however, exhibit a unique pattern common to the two families, with one exception. The coast redwood (*Sequoia sempervirens*) produces a spectrum distinct from the other members of both these families and from other families as well. Thus ^{13}C NMR spectroscopy provides a general tool to identify the resins of many different plant families, including both gymnosperms and angiosperms.

The other types of exudates produce quite different ^{13}C spectra from resins.[4] The spectra of gums contain no saturated, unsaturated, or carbonyl resonances. They are composed entirely of polycarbohydrates, which generate a large, broad peak in the EWG region, centered at about δ 70, due to carbons attached to a single oxygen (C−O), and a much smaller peak at about δ 105 from the anomeric carbon attached to two oxygens (O−C−O). Gum resins produce spectra containing elements of both resins and gums, in varying proportions, depending on the species. Kinos such as those produced by eucalyptus trees have spectra with small or no saturated resonances, strong resonances in the EWG region, strong unsaturated resonances, and small but reliable carbonyl resonances.[5]

Recently, we have found that solution-phase proton (^{1}H) spectra may be recorded for most resins, gum resins, and kinos, but not for most gums, which are generally insoluble. We have reported an extensive study of 82 species from seven genera in the single family Pinaceae.[6] We found 11 peaks in the one-dimensional (1D) spectra and 10 cross-peaks in the two-dimensional (2D) COSY spectra that were present in almost every spectrum. Because of the large number of sharp 1D peaks and 2D cross-peaks, we found numerous intergeneric and interspecific differences.

In the present study, we have examined samples from three more coniferous families, the Araucariaceae, Cupressaceae, and Podocarpaceae. Together with the Pinaceae, these families represent the three broad, extant groups within the conifer order Pinales. Solid samples of resins from the Araucariaceae and the Cupressaceae produce a single ^{13}C spectrum, which was distinct from the spectrum of the Pinaceae.[4] With the higher resolution of ^{1}H spectroscopy, our goal was to determine whether this technique provides distinctions among all three major conifer groups.

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Results and Discussion

Table S1 (Supporting Information) displays the species included in this study, along with their authorships, common names, and sources.

Langenheim states that only four conifer families (the Araucariaceae, Cupressaceae, Pinaceae, and Podocarpaceae) unequivocally produce resin via secretory structures.[3] For plants from other conifer families, however, an injury can produce resin on a small area without such structures. These latter observations are rare, and we have located resin samples from only these four families. Consequently, this study completes a relatively comprehensive examination of conifer resins on a worldwide basis.

The family Araucariaceae contains three genera and about 41 species. Originally a worldwide family, it was limited after the Cretaceous period to the Southern Hemisphere with a few exceptions. All three genera are represented in this study. Of some 21 species of the genus *Agathis*, we have sampled four as well as two without species designation. Of some 19 species of the genus *Araucaria*, we have sampled seven as well as three without species designation. The genus *Wollemia* contains only a single species, which is included in this study. Thus we have sampled all three genera and 12 species in the family. Not all members of the Araucariaceae necessarily produce exudates.

The family Podocarpaceae is closely related to the Araucariaceae. It contains 18 or 19 genera and up to 200 species. Although primarily a Southern Hemisphere family, it is found in a number of Northern Hemisphere locations. Several species produce resin, but Langenheim notes that production is primarily in leaves, with quantities insufficient to justify commercial interest.[3] Mills and White do not even include this family in their discussion of diterpenoid resins.[2] We have found a single example, from the species *Prumnopitys andinus*.

The large family Cupressaceae occurs globally, with about 30 genera and 140 species. We have analyzed 39 species (plus two without species designation) from 15 genera (plus an intergeneric hybrid). Again, not all members of this family produce exudates. The Cupressaceae are divided into seven subfamilies, of which five are represented in this study: Cunninghamioideae (one genus studied out of one known), Sequoioideae (two genera out of three), Taxodioideae (one genus out of three), Callitroideae (two genera out of about 10), and Cupressoideae (nine genera and one hybrid out of about 11). Only the subfamilies Athrotaxidoideae and Taiwanioideae, each containing only a single species, are not represented in this study.

One-Dimensional Spectra. **Family Pinaceae.** Before considering the spectra of these three new families, it is useful to review the properties of the spectra of exudates from the one coniferous family already studied, the Pinaceae.[6] Although each species has its individual characteristics, several features generally are common to the entire family. Figure 1 provides a typical pinacean spectrum, for the species *Pinus griffithi*. The main spectral characteristics are as follows. The saturated region, defined as δ 0−2.2, contains a broad envelope on which are superimposed three large sets of peaks at δ 0.8−0.9, 1.2−1.3, and 1.6−1.7. The middle set usually is the tallest. The region of protons on carbons attached to electron-withdrawing groups (EWGs, roughly δ 2.2−4.0) tails down to the baseline, always with a small peak at δ 2.9. The alkene region is quite variable, but usually has peaks at δ 4.9, 5.1−5.2, 5.3−5.4, and 5.8. The aromatic region is somewhat stronger than the alkene region and almost invariably exhibits peaks at δ 6.9, 7.0, and 7.2, and most have weak peaks at δ 7.9 and 8.1.

Family Araucariaceae, Genus *Agathis*. We have recorded the spectra of seven exudates harvested from plants of the family *Agathis*. Two of these are illustrated in Figures 2 and 3, and all of the chemical shifts are available in the Supporting Information. This set of seven comprises four species, a duplicate of one of these, and two without species identification. The spectra clearly fall into two groups. *Agathis atropurpurea* (Figure 2) and *A. moorei* have very similar spectra, and both are similar to the spectra of the two *Agathis* sp. In fact, the spectrum of *A. atropurpurea* and that of *Agathis* sp. (sample 58) are nearly identical. Since we have not sampled every *Agathis* species, we cannot identify this unknown with certainty, but it very likely is *A. atropurpurea*. The saturated region of all four spectra contains numerous sharp peaks, the largest of which appear at δ 0.6, 1.2, and 2.2. The EWG region contains a single, strong, sharp peak at δ 3.7. The alkene region has three sharp peaks at δ 4.5, 4.9, and 5.7 and almost no other resonances. The aromatic region is nearly empty, other than the solvent chloroform at δ 7.3. Small aldehyde peaks are present at δ 9.8 or 10.0. The spectrum of *A. moorei* has important differences from the other three in the saturated region only, with an additional sharp peak at δ 1.1 (in fact, the most intense peak in the spectrum) and numerous differences in the smaller peaks.

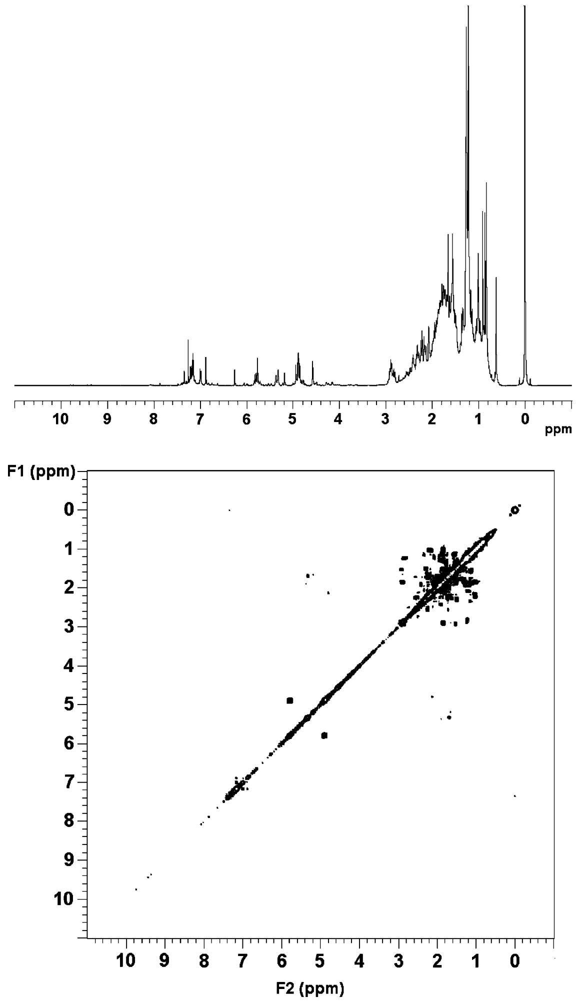

Figure 1. 500 MHz ^{1}H spectrum (top) and COSY spectrum (bottom) of *Pinus griffithi*.

The second group includes *A. australis* and two samples of *A. robusta* (Figure 3). The seven peaks common to all members of the first group (δ 0.6, 1.2, 2.2, 3.7, 4.5, 4.9, and 5.7) are usually present, although often with reduced intensity, particularly for the peak at δ 3.7. The saturated region contains many more peaks than did that of the first group. Although distinct in detail for each species, there are common, sharp peaks at δ 0.8 and 1.0 that are absent in the first group. The spectra of the two samples of *A. robusta* have essentially the same peaks but with somewhat differing relative intensities, and one sample (PR) additionally shows some weak pinacean peaks, including at δ 2.9, 7.2, 7.9, and 8.1 (but not δ 6.9 and 7.0). This sample also has the large pinacean saturated peaks at δ 1.6−1.7. The major difference between the two *Agathis*

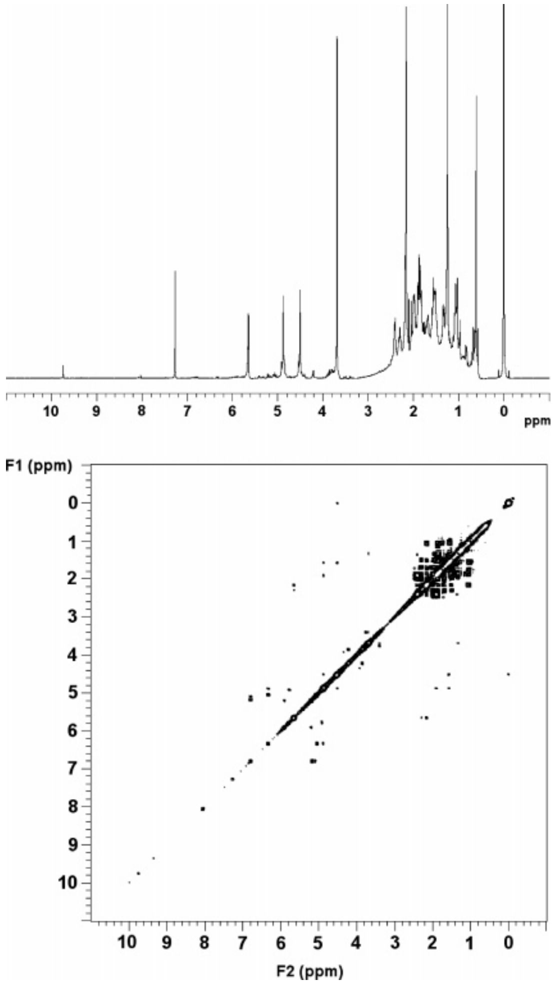

Figure 2. 500 MHz [1]H spectrum (top) and COSY spectrum (bottom) of *Agathis atropurpurea*.

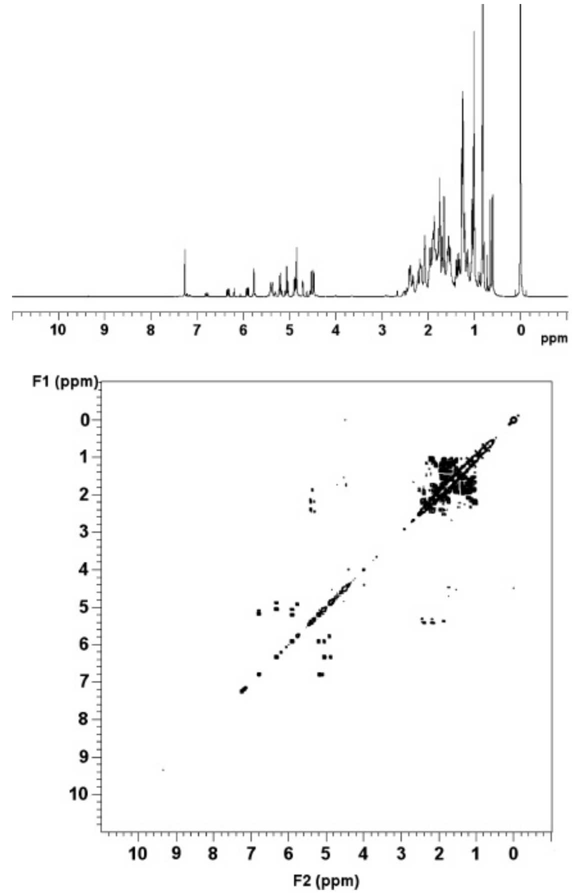

Figure 3. 500 MHz [1]H spectrum (top) and COSY spectrum (bottom) of *Agathis robusta* (sample PR).

groups is that these three samples exhibit a large number of peaks in the alkenic region, in addition to the three peaks seen in the first group. All three samples show clear peaks at δ 5.2 (a doublet) and 5.4, for example. The alkenic regions of the two *A. robusta* samples nonetheless are different from that of the *A. australis* sample, the latter, for example, exhibiting several peaks at δ ca. 4.0 that are absent in the *A. robusta* spectra. All three samples have very weak aromatic regions.

Six of these seven spectra differ dramatically from those of samples from the family Pinaceae. None has so large a saturated envelope. None has the three large sets of peaks characteristic of the pinacean saturated region. None has the invariable pinacean peak at δ 2.9, although a very small peak sometimes occurs at this position. The first *Agathis* group lacks the two pinacean peaks at δ 5.2−5.4. Finally, the three pinacean aromatic peaks at δ 6.9−7.0 are missing entirely here. Thus, of the 11 diagnostic pinacean peaks, only the one at δ ca. 5.8 is present in the *Agathis* spectra. An exception is one of the *A. robusta* samples (CA), which weakly exhibits some, but not all, of the pinacean peaks.

Family Araucariaceae, Genus *Araucaria*. We have recorded the spectra of 14 resins harvested from plants of the genus *Araucaria*. Illustrative spectra are given in Figures 4 and 5, and all chemical shifts are recorded in the Supporting Information. This set of 14 comprises seven species, a triplicate of one of these, duplicates of two of these, and three without species identification. None of the spectra resembles the first group of *Agathis* spectra, in which the alkene region contains only three sharp, characteristic peaks (Figure 2). The saturated region of every sample, however, contains the sharp peaks at δ 0.6 and 1.2, also found for every *Agathis* sample. The third peak found at δ 2.2 in the first *Agathis* group is present in significant intensity only for *Araucaria angustifolia* (both samples), *Araucaria laubenfelsii*, and *Araucaria* sp. (sample MC). Thus the absence of this peak (or its presence only with low intensity) is characteristic of *Araucaria* species in contrast to *Agathis* species. The resonances found at δ 0.8 and 1.0 only for the second group of *Agathis* species are found in many of the *Araucaria* species, but they usually take the form of a set of sharp peaks rather than a single peak. The peak at δ 3.7, which is very large in the first group of *Agathis* spectra, also is important in most *Araucaria* spectra, the exceptions being *A. cunninghamii*, *A. heterophylla*, *A. humboldtensis* (present, but very small), *Araucaria* sp. (sample AL), and *Araucaria* sp. (sample CA). For *A. laubenfelsii* and *Araucaria* sp. (sample MC) this sharp peak is accompanied by a second one at δ 3.6.

In the alkene region, the universal *Agathis* peaks at δ 4.5 and 4.9 (in intensities similar to those of the second *Agathis* group, that is, reduced in comparison with the first group) also are universal in the *Araucaria* spectra. The third universal *Agathis* alkene peak, at δ 5.7, is present in only a few *Araucaria* spectra: *A. angustifolia*, *A. heterophylla* (small), *A. laubensfelsii* (small), and *Araucaria* sp. (sample MC). The doublet observed at δ 5.2 in the second *Agathis* group is present in almost all *Araucaria* spectra, except those of *A. araucana* and *A. cunninghamii*. The second alkene peak found in the second *Agathis* group at δ 5.4 also is present in most *Araucaria* spectra, except those of *A. araucana*, *A. cunninghamii*, *A. heterophylla*, and *Araucaria* sp. (sample AL). A tight cluster of small, sharp peaks is found at δ 5.9 in most *Araucaria* samples, except *A. araucana*, *A. cunninghamii*, and *A. humboldtensis*. A similar cluster is observed at δ 5.1 in a few *Araucaria* samples: *A. columnaris* (all three samples), *A. heterophylla*, and *A. hum-*

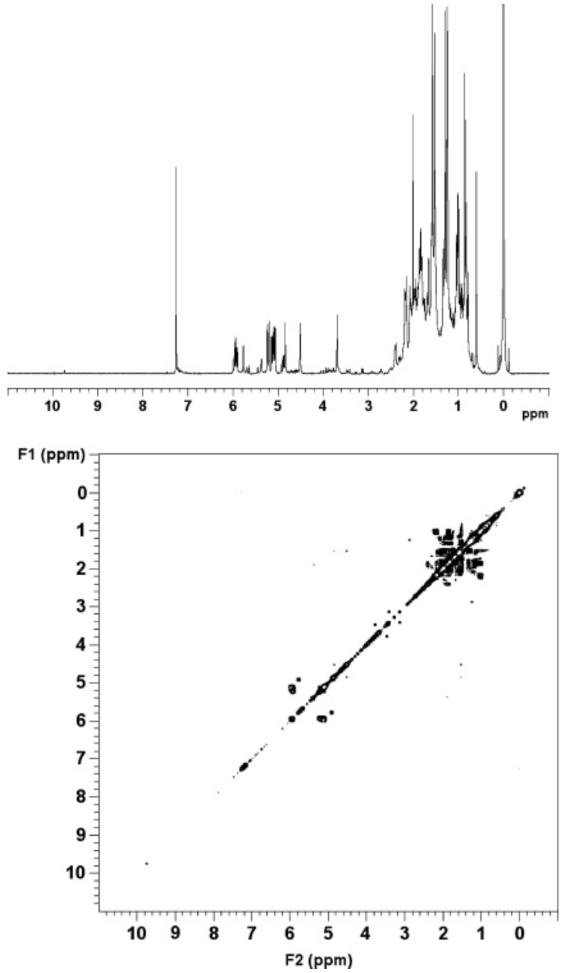

Figure 4. 500 MHz ^{1}H spectrum (top) and COSY spectrum (bottom) of *Araucaria columnaris* (sample OA).

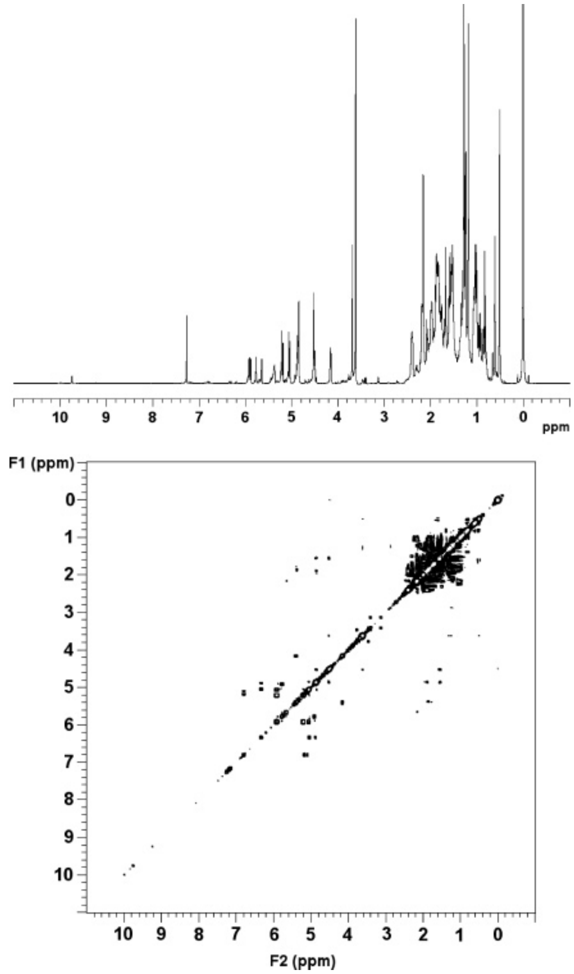

Figure 5. 500 MHz ^{1}H spectrum (top) and COSY spectrum (bottom) of *Araucaria laubenfelsii*.

boldtensis. These and other individual differences thus exist in the alkene region.

There are essentially no aromatic resonances in any of the *Araucaria* samples. An aldehyde peak is present at δ 9.8 or 10.0 in all samples, although they are quite weak in some.

Family Araucariaceae, Genus *Wollemia*. Until 1994, the Araucariaceae contained only the above two genera. In that year, David Noble discovered trees in the Blue Mountains of Australia that resemble fossil Araucariaceae plants.[7] In Noble's honor they were assigned the name *Wollemia nobilis*. The 1D spectrum of its exudate (Figure 6) resembles those of the first group of *Agathis* exudates (Figure 2), with three large saturated resonances at δ 0.6, 1.2, and 2.2, a sharp EWG resonance at δ 3.7, three sharp alkene resonances at δ 4.5, 4.9, and 5.7, and almost empty aromatic and aldehydic regions. In addition, the spectrum contains the peaks at δ 0.8, 1.0, and 5.2 found in the second *Agathis* group but lacks the peak at δ 5.4 for this group. Finally, there is a peak at δ 0.5 found in none of the previous spectra and one at δ 3.6 found only in *Araucaria laubenfelsii* and *Araucaria* sp. (sample MC). The spectrum of the *Wollemia* exudate thus falls clearly within the range of the other Araucariaceae spectra.

Family Araucariaceae 1D Summary. Peaks at δ 0.6, 1.2, 3.7, 4.5, and 4.9 are present in all *Agathis*, *Araucaria*, and *Wollemia* spectra. Peaks at δ 2.2 and 5.7 are observed for all *Agathis* species, for three *Araucaria* samples [*A. angustifolia*, *A. laubenfelsii*, and *Araucaria* sp. (sample MC)], and for *W. nobilis*. The spectral properties of the four *Araucaria* and *Wollemia* samples thus are similar to those of the *Agathis* species and distinct from the other *Araucaria* samples. A second group of three *Agathis* spectra and all the *Araucaria* samples exhibit additional saturated peaks at δ 0.8 and 1.0 and alkene resonances at δ 5.2 (doublet) and 5.4. Most, but not all, *Araucaria* spectra contain peak clusters at δ 5.1 and 5.9. Aromatic resonances are absent in general for all species of the Araucariaceae, and small aldehyde resonances are present for *Araucaria* samples and for *Agathis atropurpurea* only. A peak at δ 3.6 is found in *W. nobilis* and two *Araucaria* species.

Family Podocarpaceae, Genus *Prumnopitys*. The spectrum of our single podocarpacean sample, from *Prumnopitys andinus*, is given in Figure 7. It is completely different from the pinacean and aracucarian spectra. Of the universal and common peaks from the Araucariaceae samples, only δ 1.2 and 4.9 are present. The saturated region has clusters of sharp singlets at δ ca. 0.9 and 1.2 and a singlet of medium intensity at δ 2.1. The EWG region, empty for the Araucariaceae, has clear multiplets at δ 2.8, 2.9, 3.1, and 3.4. The weak alkene region has peaks only at δ 4.9, 5.2, and 5.8. In contrast to the Araucariaceae, there are strong aromatic peaks at δ 6.6 and 6.8 (incidentally, not common for the Pinaceae either) and a small peak at δ 7.9. There are no significant aldehydic peaks. Thus this single example of the family Podocarpaceae is quite different from both the Pinaceae and the Araucariaceae.

Family Cupressaceae, Subfamily Callitroideae. Because of the large size and complexity of this family, we shall examine species according to the subfamily to which they belong. The subfamily Callitroideae contains 10 genera, of which we have sampled two. The spectrum of *Callitris columellaria* (Figure 8) has important differences from those of the Araucariaceae. Of the peaks common to the Araucariaceae, the spectrum of this species lacks those at δ 3.7 and 4.5. In addition, it has major peaks not found in the Araucariaceae, at δ 1.1, 1.3, 1.6 (m), 2.8−2.9 (m), 5.8 (m), 6.9, 7.0 (d), 7.2 (d), and 9.2. Most significant are the three aromatic

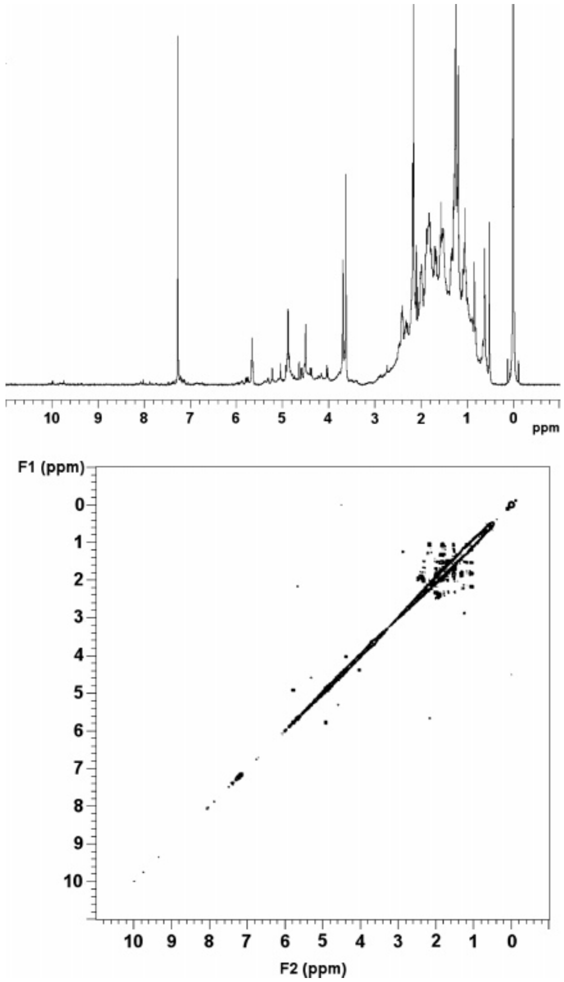

Figure 6. 500 MHz ^{1}H spectrum (top) and COSY spectrum (bottom) of *Wollemia nobilis*.

peaks, as this region was empty in the spectra of the Araucariaceae. This pattern replicates that common to the Pinaceae, and in addition the spectrum contains the two weak pinacean peaks at δ 7.9 and 8.1. With minor variations, the spectrum of *C. preisii* is very similar. The spectrum of *Callitris* sp. is nearly identical in the alkene region to that of *C. columellaria*, but lacks the peak at δ 2.8−2.9 and the three aromatic peaks, that is, the pinacean characteristics. Further differences are seen in the spectrum of *Widdringtonia nodiflora*, which lacks the four pinacean peaks, has a weaker alkenic region, and exhibits a unique saturated region dominated by two sharp singlets close to δ 1.2. The spectra of this subfamily thus exhibit considerable variability but do not resemble those of the Araucariaceae. Although some spectra resemble those of the Pinaceae in the aromatic and EWG regions, the saturated region is not so similar. The Pinaceae exhibit three large sets of peaks at δ 0.8−0.9, 1.2−1.3, and 1.6−1.7 atop a broad envelope, whereas these spectra lack the set at δ 1.6−1.7 and tend to contain more distinct singlets at these and other positions.

Family Cupressaceae, Subfamily Taxodioideae. This subfamily of three genera (previously placed in the family of the Taxodiaceae) is represented here by two species of the genus *Taxodium*. Figure 9 shows the spectrum of *T. distichum*. Two samples of *T. ascendens* from different locations gave nearly identical spectra. All three spectra are quite different from those of the family Araucariaceae and the Cupressaceae subfamily Callitroideae. The saturated region contains several sharp singlets in the region δ 0.8−1.1, a large group of singlets close to δ 1.2, and a singlet at δ 1.7. The multiplet at δ 3.2 is found in all three spectra but is absent in the spectra discussed previously. The alkene region is extremely weak up to δ 6.5. The aromatic region is rich with peaks between δ 6.6 and 8.0, but it

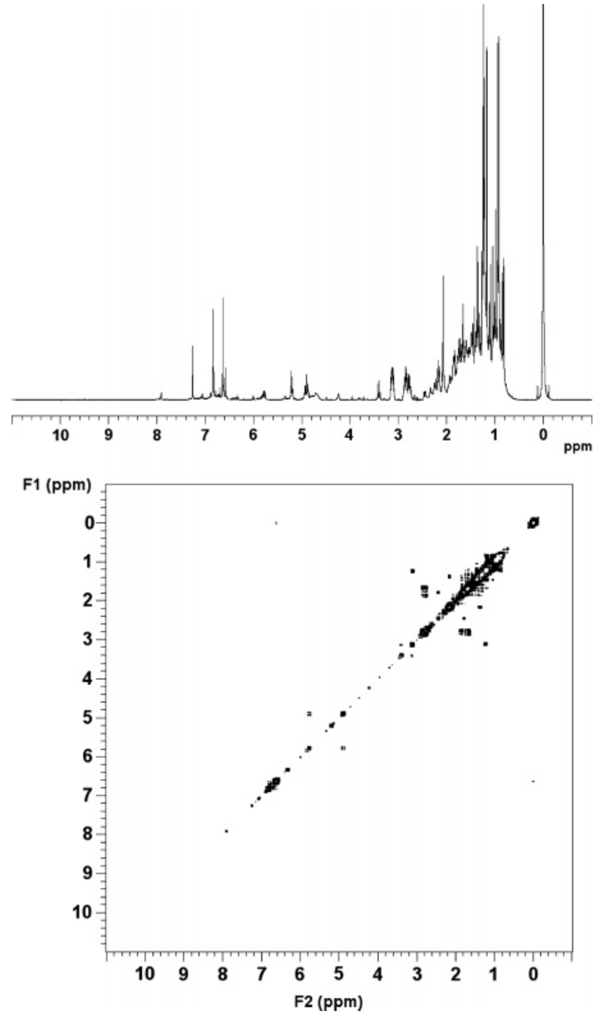

Figure 7. 500 MHz ^{1}H spectrum (top) and COSY spectrum (bottom) of *Prumnopitys andinus*.

lacks the pinacean peaks at δ 6.9, 7.0, and 7.2. The aldehyde region has a peak at δ 9.8 and one at the unusually high-frequency position of δ 10.5. This subfamily thus exhibits a very diagnostic pattern.

Family Cupressaceae, Subfamily Sequoioideae. Two of the three known genera are represented in this study, one species by two samples. This subfamily until recently also was part of the Taxodiaceae, but was reclassified into the Cupressaceae. The three spectra are slightly different from each other. Figure 10 shows the spectrum of *Metasequoia glyptostrobiodes* (sample USNA). The saturated region is dominated by sharp singlets at δ 0.8, 1.0, 1.2, and 1.6, with smaller peaks at δ 0.6 and 2.2. The EWG, alkenic, and aromatic regions are all weak. The spectrum of *M. glyptostroboides* (sample NMNH) is similar, except for a series of small peaks from δ 3.4 to 5.8 (the aromatic region is empty). The spectrum of *Sequoiadendron giganteum* is quite different, as the saturated region is dominated by a set of singlets close to δ 1.2 (similar to *Widdringtonia nodiflora*), a weak alkenic region that also is similar to that of *W. nodiflora*, and a weak aromatic region with peaks at δ 6.6, 6.7, 6.8, 7.9, and 8.0 (thus, not pinacean). All three spectra exhibit a series of peaks in the EWG and alkenic regions, at δ 3.4 (d), 3.8 (d), 4.2 (d), 4.5, 4.9, 5.2, and 5.4, with variable intensity. The AX quartet at δ 3.4 and 3.8, presumably from diastereotopic protons attached to oxygen, as in $-CH_2-O-$ is present in all three spectra. Although the alkenic peaks also are seen in the Araucariaceae, the EWG peaks are not.

Family Cupressaceae, Subfamily Cunninghamioideae. This subfamily of one genus (also previously in the Taxodiaceae) is represented by the single species *Cunninghamia konishi* (Figure

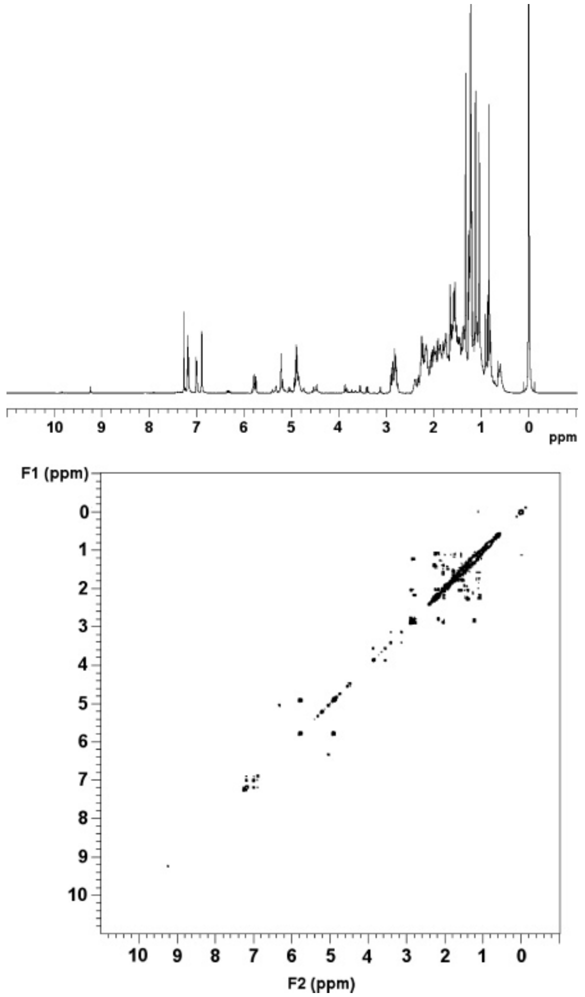

Figure 8. 500 MHz ^{1}H spectrum (top) and COSY spectrum (bottom) of *Callitris columellaria*.

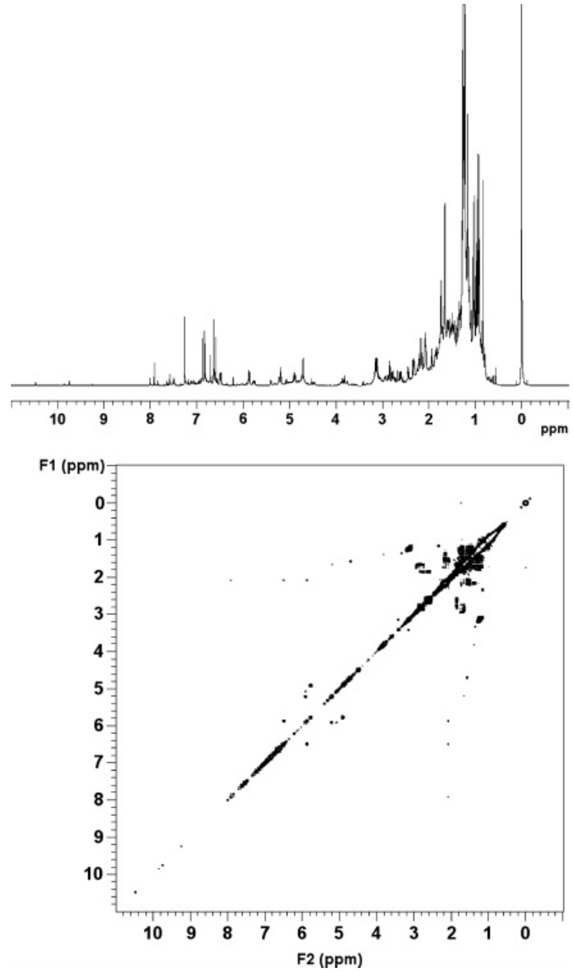

Figure 9. 500 MHz ^{1}H spectrum (top) and COSY spectrum (bottom) of *Taxodium distichum*.

11). This unique spectrum is dominated in the saturated region by two sharp singlets at δ 0.8 and 1.8. There are large peaks in the EWG region at δ 2.4, 3.1, and 3.4, the latter pair constituting an AX quartet that is different from the quartet seen in the subfamily Sequoioideae. The alkenic region has a series of strong peaks at δ 4.5, 4.8, 4.9 (m), 5.1 (d), 5.4, and 6.4 (dd). The aromatic region is empty, and there are weak aldehydic peaks at the unusual positions δ 9.2 and 9.3.

Family Cupressaceae, Subfamily Cupressoideae. This large subfamily is well represented by nine of the 11 genera and one intergeneric hybrid. We have 46 samples from 32 different species, many of which are represented by duplicate or triplicate samples. The largest genus is *Cupressus* (cypresses), with 14 samples from 11 species. There are many common, although not universal, elements to the spectra of these species, exemplified initially in Figure 12 by *C. forbesii*. The spectrum contains large, sharp singlets at δ 0.6, 0.8, 1.2 (d), and 1.6, several smaller singlets in this region up to δ 2.4, a multiplet in the EWG region at δ 3.7, two sharp alkenic singlets at δ 4.5 and 4.8, smaller alkenic resonances at δ 4.9 (m), 5.2 (d), and 5.8 (dd), and an almost empty aromatic region. Nearly identical spectra are seen for *C. bakeri* (two samples), *C. chengiana* (with an additional doublet at δ 5.1), *C. guadalupensis*, *C. macrocarpa* (sample SC of two), and *C. montana*. The same pattern is accompanied by additional peaks for *C. arizonica* [additional peaks at 4.2 (d), 4.4 (d), 5.1, 5.4, 6.3 (dd), and 6.8 (dd), all usually smaller, and lacking the peak at δ 3.7], *C. dupreziana* [two samples, δ 4.2, 4.6, 5.1, 5.4, an AX quartet at 6.5/7.0, 9.8, and 10.0 (d), most of these peaks being small], *C. glabra* [δ 0.6 (a second peak), 4.2, 5.1 (m), 6.3 (dd), 6.8 dd), 7.9, and 9.8], *C. sinensis* [δ 3.4, 5.1 (d), 5.9 (m), and 9.8], and *C. sempervirens* [δ 2.8, 3.0, 3.3, 4.2, 5.1 (d), 6.5 (d), and 7.0 (d)]. The spectrum of *C. macrocarpa* (sample SF) is significantly different. It lacks most of the common elements enumerated for *C. forbesii* and exhibits different peaks in both the saturated and alkenic regions. Moreover, it contains the distinctive pinacean peaks at δ 2.9, 6.9, 7.0, and 7.2 not usually seen in the families currently under study, as well as all seven of the other universal pinacean peaks. The unusual nature of this spectrum is reinforced by the fact that the other sample (SC) *C. macrocarpa* has a spectrum almost identical to that in Figure 12. Such evidence calls into question the identification of sample SF as *C. macrocarpa*. It likely is a species of the Pinaceae and not of the Cupressaceae. Although the samples from the subfamily Callitroideae also exhibit the three pinacean aromatic peaks, they do not resemble the Pinaceae in the saturated and alkenic regions.

From the genus *Juniperus* (the juniper family), we have 11 samples from six species and one without species designation. No spectrum exactly replicates the simple prototype of *Cupressus forbesii* seen in Figure 12, but there are many similarities. Three species, *J. deppeana* (three samples, Figure 13), *J. osteosperma*, and *J. virginiana* (two samples), have spectra almost identical to those of *C. arizonicus*, *C. dupreziana*, and *C. sinensis*. These are recognized as having the basic peaks of *C. forbesii*, but also a pair of double doublets at δ 6.3 and 6.8, additional doublets at δ 4.2 and 4.5, and multiplets at δ 5.1 and 5.4. The spectrum of *J. communis* has additional alkenic peaks in the region δ 5.0−5.4. The spectra of *J. chinensis* (two samples) have weak peaks in the alkenic/aromatic region δ 6.3−7.0. The spectrum of *J. scopulorum* contains the basic peaks of *C. forbesii*, but additionally has all the peaks seen in the spectrum of *C. sempervirens*. The near identity

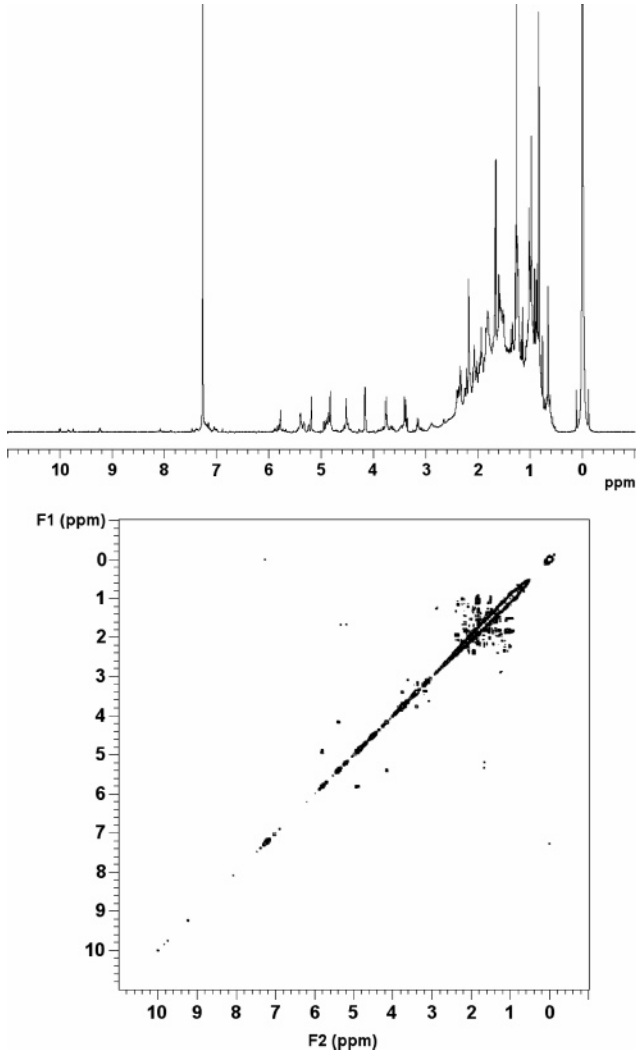

Figure 10. 500 MHz ^{1}H spectrum (top) and COSY spectrum (bottom) of *Metasequoia glyptostroboides* (sample USNA).

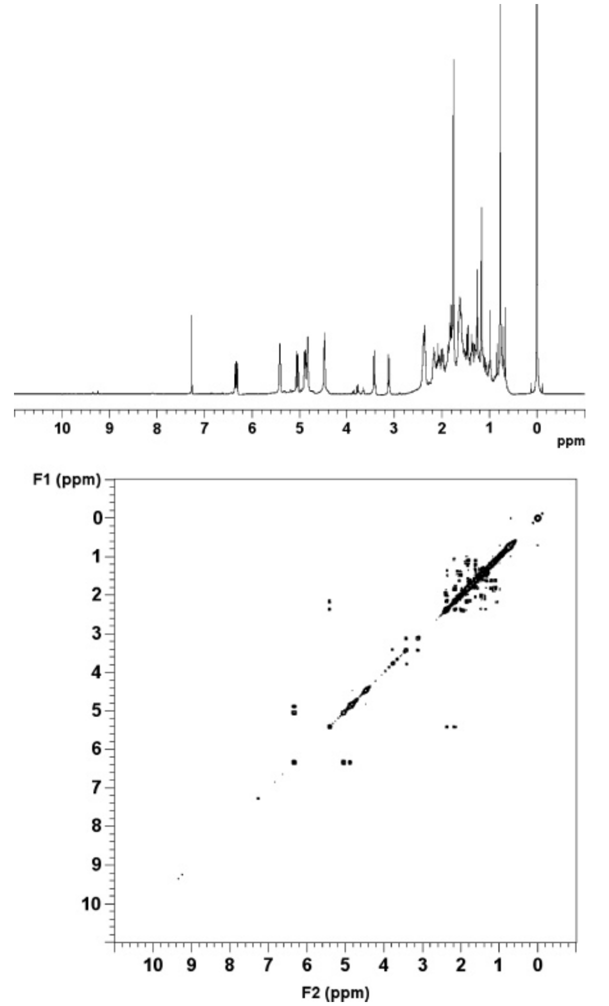

Figure 11. 500 MHz ^{1}H spectrum (top) and COSY spectrum (bottom) of *Cunninghamia konishi*.

of the spectra of *J. scopulorum* and *C. sempervirens* is very remarkable. The resins from these two species must be chemically identical. Finally, the spectrum of a sample identified only as *Juniperus* sp. is quite different from all the others, possibly suggesting another misidentification. It lacks most of the peaks of *C. forbesii* in the EWG and alkenic regions but exhibits a prominent AX quartet at δ 3.1 and 3.4 and a strong singlet at δ 5.8. This sample may not in fact be from the genus *Juniperus*, although we have not seen this particular pattern in other genera yet. Almost all the *Juniperus* spectra contain several small aldehydic peaks.

The genus *Chamaecyparis* (the false cypresses) is represented here by six samples from five species, which is the full complement of known species. In addition we include in this section two samples of the species until recently called *Chamaecyparis nootkatensis*, but now called *Callitropsis nootkatensis*.[8] None of the species has the simple spectrum of *Cupressus forbesii*, but they do resemble most of the richer spectra of *Cupressus* and *Juniperus* species. The spectra of *Chamaecyparis formosensis* and *C. lawsoniana* closely resemble those of *Cupressus arizonica* and others cited above. Two of the species, *Chamaecyparis obtusa* and *C. pisifera*, additionally have several new resonances in the region δ 5.0−5.4 and in the high-frequency region δ 6.3−7.0, which transitions from alkenic to aromatic. The pattern, however, is distinctly not pinacean, as it lacks all three resonances at δ 6.9, 7.0, and 7.2. We have two spectra of the species *Callitropsis nootkatensis*, one of poor quality (sample OH). The good-quality spectrum (Figure 14, sample PA) closely resembles those of *Cupressus glabra*, *Chamaecyparis obtusa*, *Chamaecyparis thyoides*, *Juniperus chinensis*, and *Juniperus communis*, all representing common elements of these closely related species.

The species X *Cupressocyparis leylandii* is a hybrid of *Cupressus* and *Chamaecyparis*. We have two separate samples of this species, and their common spectra resemble the more complex samples of both parent species with additional resonances close to or in the aromatic region, such as seen for *Cupressus glabra* and *Chamaecyparis obtusa*.

Although these four genera (*Callitropsis*, *Chamaecyparis*, *Cupressus*, and *Juniperus*) exhibit spectra with many common elements, we can discern four different groups. Group A represents the simple spectrum of *Cupressus forbesii* (Figure 12) and additionally includes *C. bakeri* (two samples), *C. chengiana* (with an extra peak at δ 5.1), *C. guadalupensis*, *C. macrocarpa* (sample SC), and *C. montana*. Group B possesses all the resonances of group A, save that at δ 3.7 sometimes, but exhibits additional peaks usually at δ 4.2 (d), 4.4 (d), 5.1, 5.4, 6.3 (dd), and 6.8 (dd). Members include *Cupressus arizonicus*, *C. dupreziana* (two samples), *C. sinensis*, *J. depeana* (three samples, Figure 13), *J. osteosperma*, *J. virginiana* (two samples), *Chamaecyparis formosensis*, and *Chamaecyparis lawsoniana* (Figure 13, two samples). Group C continues with increased complexity, including more alkenic peaks in the region δ 5.0−5.5 and the borderline alkenic/aromatic region δ 6.3−7.0. Members include *Cupressus glabra*, *Callitropsis nootkatensis*, *Chamaecyparis obtusa*, *Chamaecyparis pisifera*, *Chamaecyparis thyoides*, X *Cupressocyparis leylandii* (two samples), *J. chinensis* (two samples), and *J. communis*. Group D contains the two species *Cupressus sempervirens* and *J. scopulorum*, with additional peaks

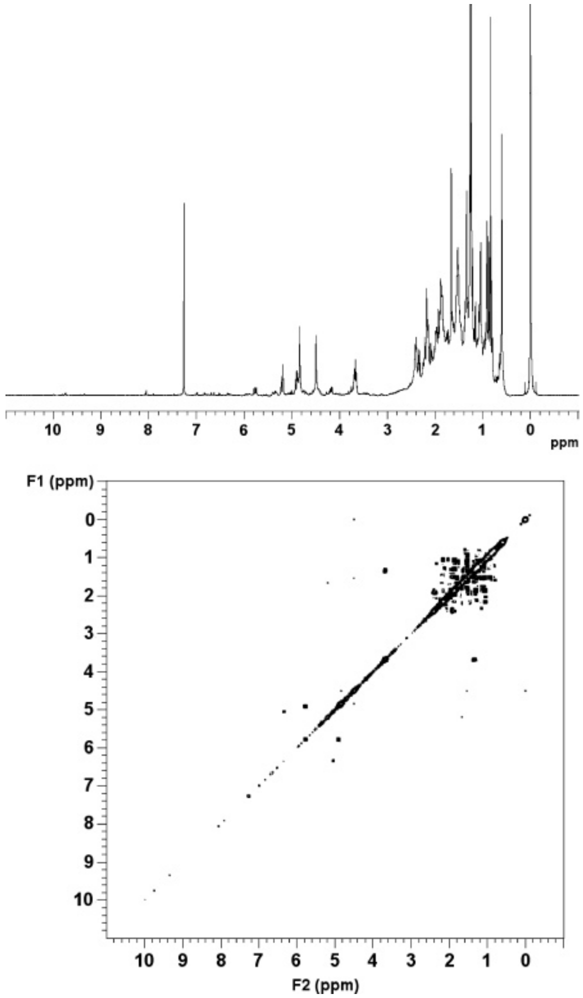

Figure 12. 500 MHz ^{1}H spectrum (top) and COSY spectrum (bottom) of *Cupressus forbesii*, representing the Cupressoideae group A.

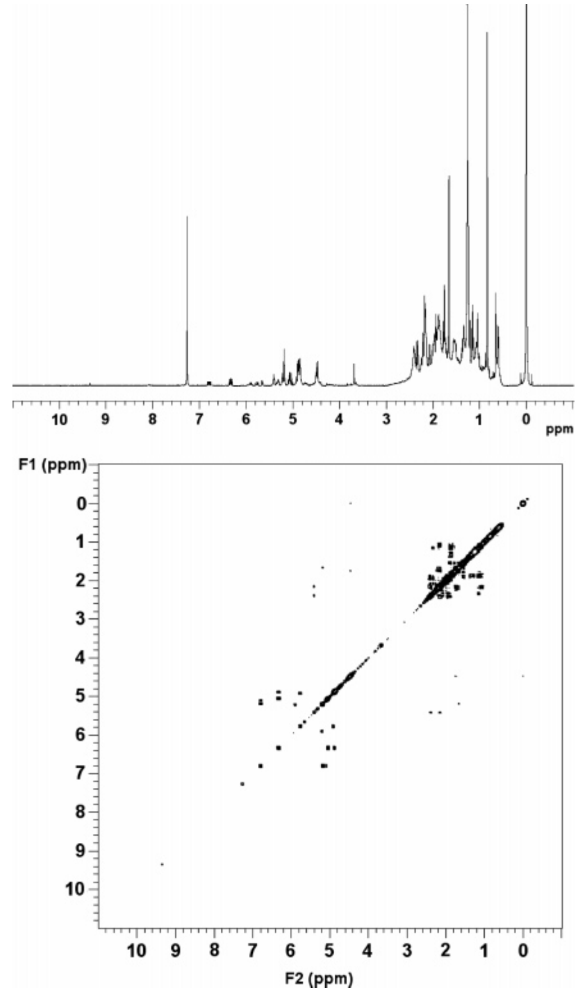

Figure 13. 500 MHz ^{1}H spectrum (top) and COSY spectrum (bottom) of *Juniperus deppeana* (sample SM), representing the Cupressoideae group B.

in the EWG region. Each of these groups overlaps with the adjacent groups to some extent because of commonalities throughout the three genera, so that classification is not clear-cut. Not included in any of these groups are *Cupressus macrocarpa* (sample SF) and *Juniperus* sp., which are suspected of misidentification.

We have several samples from the smaller genera of the Cupressoideae. The spectrum of *Tetraclinis articulata* (the sole species in this genus) follows the pattern of group C, with a richer alkenic region from δ 5.0 to 5.4 and some peaks in the region δ 6.3−7.0. The spectra of two samples of *Calocedrus decurrens* are nearly identical but bear no resemblance to those of the four Cupressoideae groups. They lack most of the peaks found in the simple spectrum of *Cupressus forbesii* but contain most of the basic pinacean elements: the EWG peak at δ 2.9, the trio of aromatic peaks at δ 6.9, 7.0, and 7.2, and the pair of weak aromatic peaks at δ 7.9 and 8.1. The saturated region, however, does not possess the traditional trio of peak sets characteristic of the family Pinaceae. In addition, both spectra possess singlets at δ 3.6, 6.3, and 7.4 that are rarely observed for the Pinaceae. We have three samples from *Platycladus orientalis* (the only species in this genus). The spectra are quite different from all the other Cupressoideae. Two of the samples (DC and OK) exhibit strong singlets at δ 7.2 and 7.4, a non-pinacean aromatic pattern. These two samples also show strong singlets at δ 3.6, 4.6, 4.9 (the strongest peak in the spectrum other than saturated resonances), and 6.3. Sample VA shows none of the singlets, but instead ones at δ 3.7, 3.8, 6.0, and 7.7. All three have strong resonances in the EWG region at δ 2.4. The spectrum of *Thujopsis dolabrata* (the sole species in this genus) is basically that of the Cupressoideae group B, including the doublet at δ 4.2 and the double doublets at δ 6.3 and 6.8. The genus *Thuja* is represented by three of its five species. The nearly identical spectra of samples of *T. standishii* and *T. plicata* are very similar to the basic spectrum of *Cupressus forbesii* (Cupressoideae group A), but they lack the small multiplet at δ 3.7 and have the doublet at δ 4.2, both characteristics of group B. One sample of *T. occidentalis* (sample OH) has the classic pattern of Cupressoideae group B, but a second (sample PA) has weak alkenic peaks and an AX quartet (or two doublets) at the alkenic/aromatic borderline δ 6.5 and 7.0, more reminiscent of group C.

Family Cupressaceae 1D Summary. The five (out of seven) represented subfamilies are quite different from each other. By far the largest subfamily, the Cupressoideae with the cypresses and junipers, exhibits four spectral groupings (see Figures 12−14) with many common peaks: δ 0.6, 0.8, 1.2, 1.6, 3.7, 4.5, 4.8, 4.9, 5.1 (d), and 5.8. All these groups lack aromatic resonances. The species that have only these peaks are called group A, and the other three groupings exhibit increasingly more complex patterns of additional peaks. There are only two prominent exceptions to this cupressean pattern. *Calocedrus decurrens* exhibits four important pinacean peaks at δ 2.9, 6.9, 7.0, and 7.2. The spectrum of *Platycladus orientalis* also contains aromatic resonances, but not at the pinacean positions. In addition, there are EWG peaks, such as at δ 2.4, not seen in other spectra. Examples from the other four subfamilies exhibit distinct patterns. Some of the Callitroideae exhibit the four pinacean peaks, and the alkenic region is decidedly non-cupressean. The Taxodioideae spectra exhibit non-pinacean aromatic resonances.

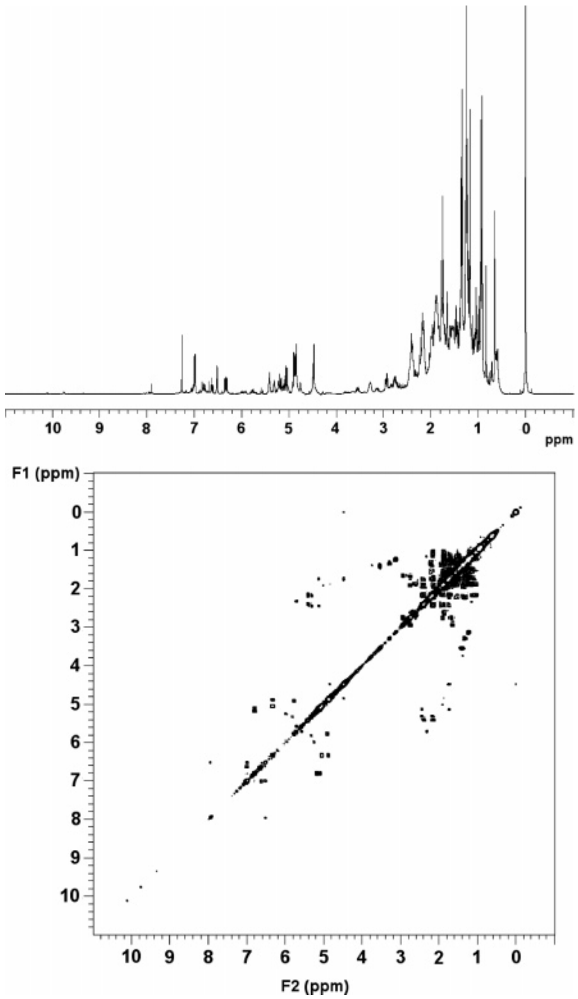

Figure 14. 500 MHz ^{1}H spectrum (top) and COSY spectrum (bottom) of *Chamaecyparis nootkatensis* (sample PA), representing the Cupressoideae group C.

The Sequoioideae and Cunninghamioideae lack aromatic resonances but contain EWG resonances, including for each subfamily a distinctive AX quartet. Thus many generic distinctions may be found within the Cupressaceae.

All Cupressaceae spectra may be distinguished from all *Agathis* and *Wollemia* spectra by the absence of peaks at δ 2.2 and 5.6−5.7, which are common to these genera of the Araucariaceae. Comparisons with the species of the *Araucaria* are more complex. The group C and D species of the Cupressoideae as well as all examples of the other four subfamilies of the Cupressaceae may be distinguished from all samples of the *Araucaria* species, as the latter lack aromatic and EWG resonances. Comparison of the group A (Figure 12) and B (Figure 13) spectra with the *Araucaria* spectra (Figures 4 and 5) shows numerous differences, in particular for the *Araucaria* species the more complex alkenic resonances between δ 5 and 6.

Last, it should be noted that small carboxylic acid peaks are observed at δ 13.1 in a few species: *Chamaecyparis lawsoniana* (both samples), *Chamaecyparis obtusa*, X *Cupressocyparis leylandii* (both samples), *Taxodium ascendens*, and *Taxodium distichum*. In addition, *Taxodium distichum* exhibits a second peak at δ 13.8. At the opposite extreme, peaks at around δ 0.2, which suggest cyclopropanes, are observed for *Cupressus sempervirens*, *Juniperus scopulorum*, and *Platycladus orientalis*.

Two-Dimensional Spectra. The COSY spectral data are listed in the Supporting Information, with peaks classified as large (L), medium (M), and small (S). All spectra have a dense box of cross-peaks in the saturated region between δ 1.0 and 2.5. We ignore these cross-peaks in our analysis, as we did for the Pinaceae, because many such peaks are universal in all species and there is considerable overlap of spectral frequencies.

Family Pinaceae. Again it is useful to summarize our results from the one previously studied conifer family, the Pinaceae.[6] These materials commonly exhibit 10 cross-peaks (see Figure 1). In the saturated/EWG region, medium to large cross-peaks were seen at 1.2/2.8, 1.5/2.9, 1.9/2.9, and 2.5/2.8 in almost all samples. In the alkene region, almost all samples showed a cross-peak at 4.9/5.8 and, in the aromatic region, at 7.0/7.2. Smaller peaks, seen somewhat less frequently, were observed commonly at 1.7/5.3, 2.1/4.8, 6.9/7.2, and 7.4/7.7. Many of the exceptions occurred with the genus *Abies*, which had somewhat divergent NMR properties.

Family Araucariaceae, Genus *Agathis*. Spectral data are given in the Supporting Information and are illustrated in Figures 2 and 3. Seven COSY cross-peaks are common to a majority of the spectra. Cross-peaks at 4.5/4.9 and 4.9/5.8 are found in six spectra out of seven. The former peak additionally is present with very small intensity in the spectrum of *A. robusta* (sample CA), and the latter peak with very small intensity in the spectrum of *A. moorei* (we have generally omitted from our discussion peaks classified as very small, VS). Cross-peaks at 2.1/5.7, 5.0/6.3, and 5.2/5.9 are present in five spectra. Found in four spectra are cross-peaks at 3.4/3.8 and 5.1/5.9. The peak at 4.9/5.8 also is common for the Pinaceae, but none of the others is. In fact, the other nine common pinacean peaks are rare for *Agathis*. Differences seen between the two *Agathis* groups in the 1D spectra are not so manifest in the 2D spectra.

Family Araucariaceae, Genus Araucaria. We highlight only cross-peaks found in at least seven of the 14 spectra (see Figures 4 and 5). The most populous cross-peak is that at 3.4/3.8, found in 12 samples. The next most populous is that at 5.2/5.9, found in 11 samples. Cross-peaks at 4.5/4.9 and 4.9/5.8 are found in 10 samples, at 1.5/4.5 in nine samples, and at 1.5/4.8 and 3.1/3.4 in seven samples.

Family Araucariaceae 2D Summary with Genus *Wollemia*. The single sample of *Wollemia nobilis* (Figure 6) from this new genus is best discussed in context of the other two genera of this family. There are only six cross-peaks in the spectrum in Figure 6: 1.2/2.9, 2.2/5.7, 3.4/3.8, 4.0/4.4, 4.6/5.3, and 4.9/5.8. The cross-peak at 4.9/5.8 is found in 17 of the 22 Araucariaceae samples and also is nearly universal for the Pinaceae. The cross-peak at 3.4/3.8 is found in 17 Araucariaceae samples but is not characteristic of pinaceans. The remaining cross-peaks are not common in the other two genera of this family, although that at 1.2/2.9 (found in only nine spectra of this family) is a common feature in pinacean spectra.

There are six cross-peaks found in at least half of the spectra of this family. In addition to the two mentioned above (3.4/3.8 and 4.9/5.8 in 17 spectra out of 22), the cross-peaks at 4.5/4.9 and 5.2/5.9 are found in 16 samples, 5.1/5.9 is found in 11 samples, and 1.5−1.6/4.5 is found in 12 samples (there are overlapping cross-peaks, as the actual frequencies of δ 1.53 and 1.56 are very close). There are no universal peaks. The cross-peak at 2.1/5.7 is most distinctive of *Agathis*, as it is found in five of the seven spectra of that genus and in none of the spectra of the other two genera. The cross-peak at 1.5/4.8 is most distinctive of *Araucaria*, as it is found in seven of 14 spectra of that genus and in none of the other two.

Family Podocarpaceae, Genus *Prumnopitys*. As with the 1D spectrum of *Prumnopitys andinus* (Figure 7), the COSY spectrum, with only about six major cross-peaks, sets this family and genus apart from the other conifers. Only the cross-peak at 4.9/5.8 is shared with the other families. Two large sets of peaks at about 1.6/2.8 and 1.8/2.8 may be associated with the pinacean cross-peaks at 1.5/2.9 and 1.9/2.9, but the resemblances end there. Thus *P. andinus* exhibits no aromatic cross-peaks. The other observed cross-peaks occur at 1.2/3.1, 3.1/3.4, and 6.6/6.7. Of these, only that at 3.1/3.4 is seen commonly in the genus *Araucaria* but not in the

Pinaceae or the other Araucariaceae. This single species thus may be distinguished by both 1D and 2D spectra from the other conifers discussed so far.

Family Cupressaceae, Subfamily Callitroideae. As was the case for the 1D spectra, the COSY spectra of *Callitris columellaria* (Figure 8) and of *C. preisii* resemble pinacean spectra. Both have five of the 10 common pinacean cross-peaks: 1.2/2.8−2.9, 1.9−2.0/2.9, 4.9/5.8, 6.9/7.2, and 7.0/7.2. In particular, the aromatic regions are very similar. Lacking, however, are three common pinacean cross-peaks involving EWG and alkenic protons. In addition, both species exhibit peaks at 2.2/2.7 and 5.0/6.3, which are not common to the Pinaceae. The latter peak, however, is common to the species *Agathis*. The only commonality between these two species and *Callitris* sp. and *Widdringtonia nodiflora* is the single, highly conserved, conifer cross-peak at 4.9/5.8. Neither of these latter two species exhibit pinacean characteristics, as also was the case for the 1D spectra, although they exhibit a common cross-peak at 5.2/6.8. In summary, *C. columellaria* and *C. preisii* give similar 2D spectra with pinacean characteristics, *Callitris* sp. and *W. cuppresoides* give unique, non-pinacean spectra, and all four are distinct from the Araucariaceae.

Family Cupressaceae, Subfamily Taxodioideae. The 2D spectra of the three available spectra of species from this subfamily are very similar (see Figure 9 for that of *Taxodium distichum*), dominated by five cross-peaks. In the saturated region, all three spectra have important peaks at 1.2/3.1, 1.7/2.7−2.9, and 1.9/2.6−2.8 (the latter two are multiple cross-peaks). All three have the common conifer peak at 4.9/5.8 and a smaller cross-peak at 4.9/6.5. Two of the spectra also have a smaller cross-peak at 3.4/3.8. Other than a coincidence with the 1.7/2.7−2.9 cross-peak, there are no pinacean cross-peaks.

Family Cupressaceae, Subfamily Sequoioideae. Two 2D spectra of *Metasequoia glyptostroboides* (see Figure 10) and one of *Sequoiadendron giganteum* are available. The former two are extremely similar and quite different from the latter. The dominant cross-peaks for *M. glyptostroboides* are 1.7/5.2, 3.1/3.4, 3.4/3.8, 4.2/5.4, and 4.9/5.8. For *S. giganteum*, the dominant peaks are 1.2/3.1, 1.8/2.6, 1.9/2.6, 3.4/3.8, 4.2/5.4, 4.6/5.3, 4.9/6.3, and 5.0/6.3. There are only two peak coincidences between the two genera. Notably, *S. giganteum* does not exhibit the usual large conifer cross-peak at 4.9/5.8.

Family Cupressaceae, Subfamily Cunninghamioideae. Six peaks constitute the COSY spectrum of *Cunninghamia konishi* (Figure 11) outside the saturated block: 2.2/5.4, 2.4/5.4, 3.4/3.8, 4.5/4.9, 4.9/6.3, and 5.0/6.3. It lacks the common conifer peak at 4.9/5.8.

Family Cupressaceae, Subfamily Cupressoideae. We have 14 samples from 11 species of the genus *Cupressus*, whose 1D spectra we divided into four classes. The 1D spectrum of *C. macrocarpa* (sample SF) has the appearance of a pinacean sample, and the 2D spectrum confirms this suspicion. It contains eight of the 10 common pinacean cross-peaks. Consequently, we exclude it from further comparisons within this genus and family. Seven of the remaining 13 samples fell into group A (see Figure 12). The most diagnostic cross-peak for this group is 1.6/4.5. All seven samples show this peak, and none of the other six does. Secondarily, five of the seven (and none from the other groups) exhibit a cross-peak at 1.6/3.7. No other peaks show strong distinguishing characteristics for any of the 1D groups. *C. sempervirens*, however, is the only one to show a cross-peak (M) at 1.3/3.3. The common cross-peaks for this genus (with the number out of 13 exhibiting them in parentheses) are 1.3−1.4/3.7 (9), 1.6/4.5 (9), 1.7/5.2 (9), 3.4/3.8 (8), 4.2/5.4 (10), 4.5/4.9 (13), 4.9/5.8 (10), and 5.0/6.3 (9). No other cross-peak is found in most of these samples.

We have 11 samples of the genus *Juniperus* from six known genera, plus one of unknown species, also divided into the four cupressean groups. This genus has no species with the characteristics of group A. Most fall into group B (see Figure 13). *J. scopulorum* exhibits a large peak at 1.3/3.3, as does the group D example *C. sempervirens*. The common cross-peaks for *Juniperus* (out of 11) are 1.6−1.7/4.5 (8), 1.7/5.2 (7), 2.1/5.4 (7), 2.4/5.4 (9), 4.2/5.4 (7), 4.9/5.8 (11), 4.9/6.3 (11), 5.0/6.3 (11), and 5.2/6.8 (10). In the 1D spectrum, *Juniperus* sp. appeared to be different from the others, whereas its COSY spectrum is relatively similar, having seven of these nine diagnostic peaks. There are some major differences between the *Cupressus* and *Juniperus* spectra. Only five of the 12 enumerated cross-peaks are common to both genera. In particular, *Juniperus* lacks the common peaks at 3.4/3.8 and 4.5/4.9.

We have six samples from five species of *Chamaecyparis*, plus two samples of the species *Callitropsis nootkatensis* (formerly *Chamaecyparis nootkatensis*,[8] Figure 14). The duplicate spectra in each case are very similar. The only cross-peaks common to all eight of these samples are 4.9/5.8 and 5.0/6.3. The COSY spectra of the two samples of *Callitropsis nootkatensis* are in fact somewhat different from the others. For example, they exhibit cross-peaks at 1.3/3.3 (L), 1.4/3.5 (L), and 6.6/7.0 (S), found in none of the *Chamaecyparis* spectra. Cross-peaks found in at least four of the six *Chamaecyparis* spectra (with the number given parenthetically first of *Chamaecyparis* spectra with that cross-peak, then of the two *Callitropsis nootkatensis* spectra, and finally of the two X *Cupressocyparis leylandii* discussed in the next paragraph) are 1.2/3.1 (5, 2, 2), 1.7/4.5 (4, 1, 1), 2.1/5.4 (4, 1, 2), 2.4/5.4 (4, 2, 2), 3.4/3.8 (4, 0, 2), 4.2/5.4 (5, 0, 2), 4.9/5.8 (6, 2, 2), 4.9/6.3 (5, 2, 2), 5.0/6.3 (6, 2, 2), 5.1/5.9 (5, 0, 0), and 5.2/5.9 (5, 0, 0). Additionally, 5.2/6.8 (1, 2, 2) is found in both spectra of *Callitropsis nootkatensis* but is absent in five of the six spectra of *Chamaecyparis*. These differences should suffice to distinguish a *Callitropsis nootkatensis* resin from any *Chamaecyparis* resin. All the spectra from these two genera were group B or group C according to the 1D spectra, but the COSY spectra show no particular distinctions between these two classes.

The species X *Cupressocyparis leylandii* is a hybrid of *Cupressus* and *Chamaecyparis*. There are 13 significant cross-peaks found in both samples, of which eight are in common with most of the *Chamaecyparis* species and six with both *Callitropsis nootkatensis* spectra. In addition, the spectra of X *Cupressocyparis leylandii* exhibit two cross-peaks found in both *Callitropsis nootkatensis* spectra but not in most of the *Chamaecyparis* spectra, at 2.3/5.7 and 6.6/7.0. A cross-peak at 5.1/6.8 appears in both spectra of X *Cupressocyparis leylandii* but in only one of the other eight spectra under comparison here. In general, these three groups of species exhibit their own patterns. Finally, an unusual cross-peak is found at 5.9/10.0 in three *Chamaecyparis* spectra, one of *Callitropsis nootkatensis*, and both of X *Cupressocyparis leylandii*, six of the 10 total spectra. Consequently, the aldehydic protons in these species are coupled to an alkenic proton, resulting in a doublet appearance in the 1D spectra. Such a coupling is expected in an α,β-unsaturated aldehyde, H(C=O)CH=CH−.

The less populated genera in the Cupressaceae generated a variety of COSY results. Two samples of the species *Calocedrus decurrens* give nearly identical, very pinacean spectra, as also was the case for the 1D spectra. They contain seven significant peaks, six of which are standard pinacean: 1.2/2.8, 1.5/2.9, 1.9/2.9, 4.9/5.8, 6.9/7.0, and 7.0/7.2. The additional peak is at 4.2/5.4. Thus the saturated and aromatic regions are strictly pinacean. The three samples of *Platycladus orientalis* give very different 2D spectra from each other and from the other Cupressaceae. The spectrum of sample DC is dominated by the general conifer peak at 4.9/5.8, whereas sample VA lacks the peak. Sample VA has several small peaks in the unusual unsaturated region near 6.5/6.7 (possibly phenolic). All three spectra are *sui generis* and unlike those from other genera. The spectrum of *Tetraclinis articulata* is dominated by a large number of cross-peaks within the unsaturated area, including 4.2/5.4, 4.9/5.8, 4.9/6.3, 5.0/6.3, 5.1/5.9, and 5.2/6.8 plus 3.4/3.8 in the EWG region, all of which are common in one or more of the three major genera of the Cupressaceae (*Cupressus*, *Juniperus*, and *Chamaecyparis*).

The spectra of the four *Thuja* species plus *Thujopsis dolabrata* are very similar, so they are treated together. The following cross-peaks are found in the majority of the five cases: 1.2/3.1−3.2 (4), 1.6−1.7/4.5 (4), 1.7/5.2 (3), 2.1/5.4 (3), 2.2/5.4 (3), 2.4/5.4 (3), 3.4/3.8 (3), 4.2/5.4 (4), 4.5/4.9 (3), 4.9/5.8 (5), 4.9/6.3 (5), 5.0/6.3 (5), 5.9/10.0 (3). When there are differences, the two samples of *Thuja occidentalis* generally are different from the other three.

Family Cupressaceae 2D Summary. This family is dominated by the many members of the subfamily Cupressoideae. We have enumerated eight cross-peaks found in the majority of *Cupressus* spectra, nine in the majority of *Juniperus* spectra, 11 in the majority of *Chymaecyparis* spectra, 10 in both *Callitropsi nootkatensis* spectra, 13 in both X *Cupressocyparis leylandii* spectra, and 13 in the combined genera of *Thuja* and *Thujopsis* (abbreviated as “Thuj*”). There is a total of 23 such cross-peaks, of which only two are found in the majority of all five groups: 4.9/5.8 and 5.0/6.3. Four cross-peaks out of a possible 15 are found in the majority of the three largest genera (*Cupressus*, *Juniperus*, and *Chymaecyparis*): 1.6−1.7/4.5, 4.2/5.4, 4.9/5.8, and 5.0/6.3. Other pairwise comparisons may be made: *Cupressus/Juniperus* (5 of 12), *Cupressus/Chymaecyparis* (5 of 14), *Juniperus/Chymaecyparis* (7 of 13), *Callitropsis/Chymaecyparis* (5 of the 11 *Chymaecyparis* cross-peaks), X *Cupressocyparis/Cupressus* (4 of the 8 *Cupressus* cross-peaks), X *Cupressocyparis/Chymaecyparis* (8 of the 11 *Chymaecyparis* cross-peaks), *Thuj*/Cupressus* (7 of 14), *Thuj*/Juniperus* (8 of 14), and *Thuj*/Chamaecyparis* (8 of 15). None of the three largest genera has strong overlap, and the hybrid species X *Cupressocyparis leylandii* resembles *Chymaecyparis* more than *Cupressus*.

When these six groups (*Cupressus*, *Juniperus*, *Chymaecyparis*, *Thuja/Thujopsis*, *Callitropsis nootkatensis*, and X *Cupressocyparis leylandii*) are examined together, there are 10 cross-peaks found in a majority of at least three of these groups: 1.2/3.2, 1.6−1.7/4.5, 1.7/5.2, 2.1/5.4, 2.4/5.4, 3.4/3.8, 4.2/5.4, 4.9/5.8, 4.9/6.3, and 5.0/6.3. We consider these cross-peaks to characterize the subfamily Cupressoideae. There are several genera with one or two species in this subfamily with little resemblance to the more populous genera. Two samples of *Calocedrus decurrens* give a very pinacean COSY spectrum. Three samples of *Platycladus orientales* give varied and unique spectra.

The subfamily Callitroideae includes two species from the genus *Callitris* that give the pinacean 2D pattern. The Taxodioideae samples exhibit five of the 10 Cupressoideae cross-peaks and additionally cross-peaks at 1.9/2.7−2.9, 3.1−3.4, and 5.9/6.5. The two species from the Sequoioideae give rather different patterns. *Metasequoia glyptostroboides* gives four Cupressoideae peaks plus 3.1/3.4, whereas *Sequoiadendron giganteum* gives five generally different Cupressoideae peaks plus 1.8/2.6. Finally, the Cunninghamioideae spectra show six of the Cupressoideae peaks and no distinctive peaks. Other than the Callitroideae, there is considerable overlap of the various Cupressaceae subfamilies.

Summary

The important question to answer is whether ^{1}H NMR spectroscopy can identify the family, genus, or species of a resinous exudate. We have enumerated specific cross-peaks that characterize families, as summarized in Table 1. The Podocarpaceae data are unique but are based on only a single species and will not be discussed further. The common peaks for the subfamily Cupressoideae are used to characterize the Cupressaceae not only because its members are the most numerous but also because several of the other subfamilies show considerable overlap. The cross-peak at 4.9/5.8 is found in all four families and clearly is distinctive of all conifers. Of the 15 possible frequent cross-peaks found for the Pinaceae and the Araucariaceae, the cross-peak at 4.9/5.8 is the only one that is common to both families. Of the 18 possible frequent cross-peaks found for the Pinaceae and the Cupressaceae, only those at 4.9/5.8 and 1.7/5.2−5.3 are common to both families. Of the 13 possible frequent cross-peaks found for the Araucariaceae and the Cupressaceae, only those at 1.5−1.7/4.5, 3.4/3.8, and 4.9/5.8 are common to both families. Thus 2D NMR provides a reliable method to characterize all three families. There are exceptions. A few of the cupressean species give a pinacean pattern. We are dealing with averages and probabilities, but 2D NMR spectroscopy generally accomplishes this task. We found, in contrast, that the solid state ^{13}C spectra gave a distinct pattern for the family Pinaceae but a distinct, single pattern for the two families Araucariaceae and Cupressaceae. Thus ^{1}H NMR spectroscopy is more discriminative than ^{13}C NMR spectroscopy.

Table 1. Coincidence of Frequent Cross-Peaks across Families

	Pinaceae	Araucariaceae	Cupressaceae	Podocarpaceae
1.2/2.8	×			
1.2/3.1			×	×
1.5−1.6/2.9	×			×
1.5−1.7/4.5		×	×	
1.7/5.2−5.3	×		×	
1.8/2.7−2.9	×			×
2.1/4.8	×			
2.1/5.4			×	
2.4/5.4			×	
2.5/2.8	×			
3.1/3.4				×
3.4/3.8		×	×	
4.2/5.4			×	
4.5/4.9		×		
4.9/5.8	×	×	×	×
4.9/6.3			×	
5.0/6.3			×	
5.1/5.9		×		
5.2/5.9		×		
6.6/6.7				×
6.9/7.2	×			
7.0/7.2	×			
7.4/7.7	×			

Confirmation of family, genus, or species often can be accomplished then by examination of the 1D spectrum. There are 13 peaks common to most species of the Pinaceae: δ 0.8−0.9, 1.2−1.3, 1.6−1.7, 2.9, 4.9, 5.1−5.2, 5.3−5.4, 5.8, 6.9, 7.0, 7.2, 7.9, and 8.1 There are nine peaks common to most species of the Cupressaceae: δ 0.6, 0.8, 1.2, 1.6, 3.7, 4.5, 4.8, 5.1 (d), and 5.8. The pinacean peaks at δ 2.9, 7.0, and 7.2 easily distinguish these two families, with some notable exceptions. All species of the Araucariaceae exhibit peaks at δ 0.6, 1.2, 3.7, 4.5, and 4.9. Again, they may be distinguished from the Pinaceae by the absence of peaks at δ 2.9, 7.0, and 7.2. The genera *Agathis* and *Wollemia* additionally show peaks at δ 2.2 and 5.7 invariably. The Cupressaceae may be distinguished by the absence of these peaks. The genus *Araucaria* usually shows peaks at δ 0.8, 1.0, 5.2, and 5.4. Those species may be distinguished from those of the Cupressaceae by comparisons within the alkenic and EWG regions.

The 1D and 2D NMR spectra of the resinous exudates from conifers provide independent methods to obtain taxonomic information about the source species. Resins almost always may be classified at the family level as Pinaceae, Araucariaceae, or Cupressaceae, and often genus and sometimes species identification as well may be made.

Experimental Section

Plant Materials. Samples were collected from or provided by major botanical gardens or arboreta with permission of the institutions, as listed in Table S1 (Supporting Information). These samples had been authenticated by the curators or plant taxonomists and always are confirmed at the time of collection. The exudates were removed from the plant surface by hand or with the help of a knife or other sharp object. This protocol does not produce incisions in plants. Small samples (1−5 g) were collected, of which the ^{1}H NMR experiment requires less than 50 mg. Although the material is dissolved in $CDCl_3$ for spectroscopic examination, the experiment is entirely nondestructive, and the exudate may be recovered by evaporation of the solvent. The materials will remain in the laboratory at Northwestern University for continued experiments but can be made available on request.

NMR Spectra. Data were obtained on a Varian INOVA-500 NMR spectrometer operating at 500 MHz. Approximately 50 mg of the pulverized exudate was dissolved in ca. 1 mL of $CDCl_3$ containing 1% TMS, the samples were filtered to remove any particulate matter, and the solutions were placed in 5 mm NMR tubes. Spectra were obtained at ambient temperatures without spinning. For 1D spectra one transient was recorded with a spectral width of 6000 and 12 000 Hz. A S/N of 4 normally was required to include 1D peaks. For 2D spectra four transients were recorded with 256 increments, spectral width 6000 Hz, 90° pulse width 11 μs, pre-delay time 1 s, and acquisition time 0.17 s. Cross-peaks less than 10% of the highest diagonal peak were ignored. Acceptable cross-peaks were classified qualitatively as small (S), medium (M), or large (L) by inspection.

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Supporting Information Available: Table S1 listing the species included in this study, along with their authorships, common names, and sources. Two Excel tables of the proton chemical shifts and COSY cross-peaks for all samples studied from the families Araucariaceae, Cupressaceae, and Podocarpaceae. This material is available free of charge via the Internet at http://pubs.acs.org.

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