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A solid-state ^{13}C NMR analysis of ambers

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Abstract

Colombian copalite samples (found at 73°5′W 6°19′N in the Cimitarra quadrangle), and commercial amber samples from Chiapas (Mexico), Dominican Republic and Poland (Baltic) were studied using solid-state ^{13}C CP-MAS Nuclear Magnetic Resonance (NMR). NMR spectra for Colombian amber are shown and compared to those reported for the resin from the genus of the African species *Hymenaea verrucosa* and other Hymenaea trees, for which a close resemblance is observed. NMR spectral features of the other studied ambers are also discussed. A physical aging experiment was performed on a Colombian sample at 65°C. Differences in the solid-state NMR and IR spectra are explained in terms of changes in chemical cross-linking, isomerization and morphology. © 1999 Elsevier Science Ltd. All rights reserved.

Keywords: Amber; High-resolution ^{13}C nuclear magnetic resonance; Physical aging

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1. Introduction

Amber is a fossilized form of terpenoid resins produced by both gymnosperms and angiosperms. Unlike other fossils, amber is completely organic in nature, and its chemical composition remains almost constant over millions of years [1,2]. The resinites (as amber has been called as a class by some authors [3], specially geochemists, based on their geologic and petrographic origin) derived from the African species *Hymenaea verrucosa* varied according to age, chemical composition and physical properties. From the knowledge of the geographic origin, maps of cultural and trade networks can be drawn.

The distinction of the different types of ambers is not trivial, and different characterization techniques such as IR [4] and NMR [5,6] have been proposed. By these methods, information on autenthicity, chemical structure, geographical and paleobotanic origin can be derived. However, these techniques do not allow a totally exclusive distinction, because spectral differences cannot be universally established for all the samples [7].

Amber may be as old as 200 million years, but some may be younger than one million years. In particular, there is a hardened non-fossilized version of amber denominated copal, which differ from fossilized amber in their physical properties such as color and resistance to heat and organic solvents [7,8]. Copals are young resins aged only for centuries or millennia. However, the polymeric character of ambers and copals are alike [8].

The main deposits of amber in America are found in southeast Mexico (Simojovel and Totolapa in Chiapas), Canada (Chemawinita), Alaska, Dominican Republic and Colombia. The deposits are generally found in sediments formed from ancient lagoons and river deltas [1,9,10] Dominican and Mexican amber were produced by a species of Hymenaea (Leguminosae) tree which is now extinct. There are some species that still produce resins in Central America, South America and in the Caribbean [1]. Baltic amber seems to be produced by pine trees of the genus Pseudolaris, which is currently found in Asia, Australia and Chile. It has been reported that Colombian amber is produced by a species of Hymenaea from deposits of the tertiary strata [8,11]. Other recently discovered deposits are in USA. The species found in these zones date from the Oligocene and Miocene periods, although there is some uncertainty about their real age.

Due to its insolubility, chemical characterization of amber has mainly relied in spectroscopy studies by IR and

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Fig. 1. The photograph of a Colombian sample.

solid-state NMR [12,13], and other techniques such as pyrolysis GC–MS [14,15] and Instrumental Neutron Activation Analysis (INAA) [16]. In this paper, copalite found at 73°5′W 6°19′N, in the Cimitarra quadrangle in Colombia, and samples of amber from Chiapas (Mexico), four samples from Dominican Republic and one from the Baltic region were examined by solid-state ^{13}C CP-MAS NMR.

2. Experimental section

2.1. Samples

The color of the three Colombian copalites studied in this work were yellowish, clear and transparent. Some amber pieces contained inclusions such as ants and mosquitoes (Fig. 1). Photographs of the four Dominican samples are shown in Fig. 2, from which one was a fake imitation, as the infrared spectrum of sample A shows the typical pattern of polystyrene. Untreated and extracted samples without inclusions were analyzed by solid-state NMR. Samples were subjected to Sohxlet extraction with ethyl acetate for 24 h. Solid-state NMR spectra for unextracted and extrated samples are similar.

2.2. Differential scanning calorimetry

Differential scanning calorimetry (DSC) thermograms were recorded using a DuPont 2100 thermal analyzer, at a heating rate of 5°C min^{-1} under nitrogen.

2.3. Fourier transform infrared

IR spectra were recorded from KBr pellets in the 4000–600 cm^{-1} range using a Perkin–Elmer FT 1600 spectrometer operating at a resolution of 4 cm^{-1}. Typically, 64 interferograms were collected.

2.4. NMR studies

Solid-state NMR spectra were recorded under proton decoupling on a Varian Unity Plus 300 NMR spectrometer operating at 75.47 MHz for ^{13}C. Approximately 200 mg of sample, as a fine powder, was packed into 7 mm diameter silicon nitride rotors with Kel-F packs. The spectra were obtained under Hartmann–Hann matching conditions with a contact time of 2.5 ms, a repetition time of 4 s and at a spinning rate of 4.25 kHz. The measurements were made using a spinlock power in radiofrequency units of 60 kHz and 4096 transients were recorded. Elimination of spinning side bands was accomplished by the TOSS sequence [17]. Spectra to identify non-protonated carbons were recorded using interrupted decoupling [18] for 60 and 150 μs after cross-polarization. Chemical shifts were referenced to the upfield peak of adamantane at 29.5 ppm (with respect to TMS) determined on a separate sample.

3. Results and discussion

Chemical characteristics of amber are a consequence of both biological origins and geological environment. Amber can be considered as a fossil resin, and classified according to their structural characteristics, as proposed by Anderson et al. Class I fossil resins include all materials in which the polymeric component is derived from labdanoid diterpenes (such as biformene, communic acid and communol, I) [19–21], whereas class II fossil resins are derived from bicyclic sesquiterpenoid hydrocarbons such as cadinene. Polylabdanoid resinites are mainly produced from trees of the families Araucariaceae and Leguminoseae [21]. Other chemical constituents present in ambers are mono-, sesqui-, di- and tri-terpenoids and other low-molecular weight compounds. The studies reported here correspond to class I amber and resins.

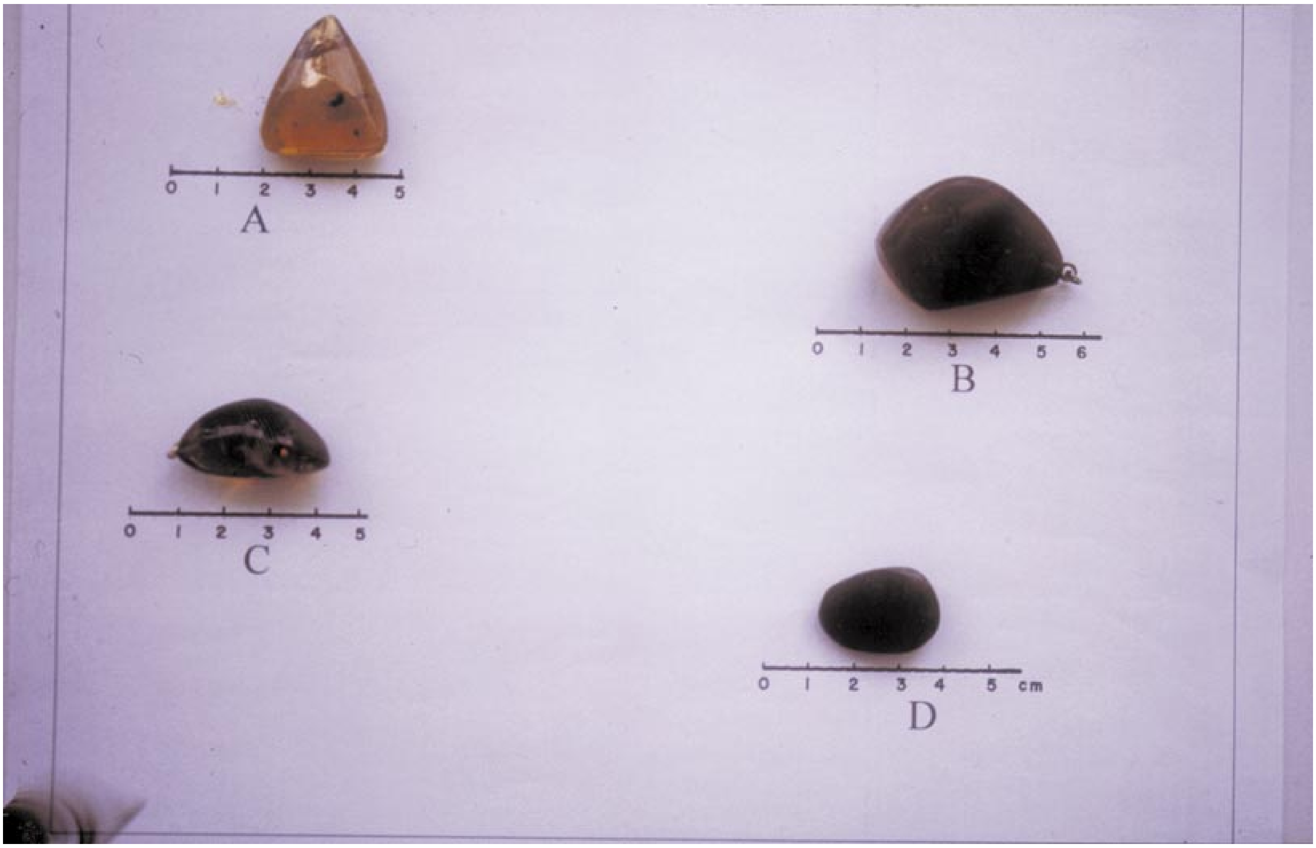
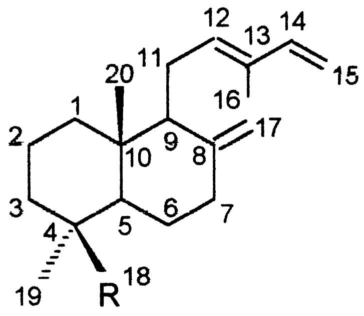


Fig. 2. The photographs of the four Dominican ambers.



$R = CH_3, CH_2OH$ or $COOH$

I

In general, the processes involved in fossilization seem to be progressive oxidation and polymerization via free-radical mechanisms [12,22–24]. Amber is not, however, resistant to structural alteration by geothermal processes, reactions which very recently have been proposed to hold potential as geothermal indicators [25,26]. Over the years, further cross-linking, partial isomerization and perhaps esterification can be observed by normal thermal reactions.

The CP-MAS spectra of amber depend on the origin of the sample. The spectra of extracted and unextracted samples are very alike; so, only extracted spectra are shown in this article. The general features include signals in the aliphatic (intense absorption centered around 39 ppm), olefinic and carbonyl regions. Fig. 3 shows the spectra of Colombian copalite samples, along with the interrupted decoupling spectra. Ten peaks can be clearly distinguished in the aliphatic region between 12 and 60 ppm. Two signals for methyl carbons (C-19 and C-20) at 15.7 and 19.5 ppm [26], three more signals for methyl and methylene groups at 25, 27 and 34 ppm, an intense peak centered at 39 ppm (that has been assigned to C-7 of the ladbane skeleton) and other four peaks in the region comprised between 46 and 62 ppm, due to carbons bonded to hydroxyl groups (CH_2OH and CHOH) and C-9 of polylabdatriene (at 58 ppm). There also exists a broad signal centered at 71 ppm that has been ascribed to polymer produced from a derivative of zanzibaric acid with a 6-β-hydroxyl group, which has been isolated from *H. verrucosa* pod resin [12]. Very small amounts of tertiary alcohol at 83 ppm (which is more evident in the protonated carbon suppressed spectra) are also seen [3]. In the unsaturated (carbon double bonds) region (100–150 ppm), four peaks assigned to carbons C-17 (δ 107.3), C-12 (δ 125.2), C-13 (δ 139.9) and C-8 (δ 148.1) of the labdane moiety can be distinguished. The intensity for

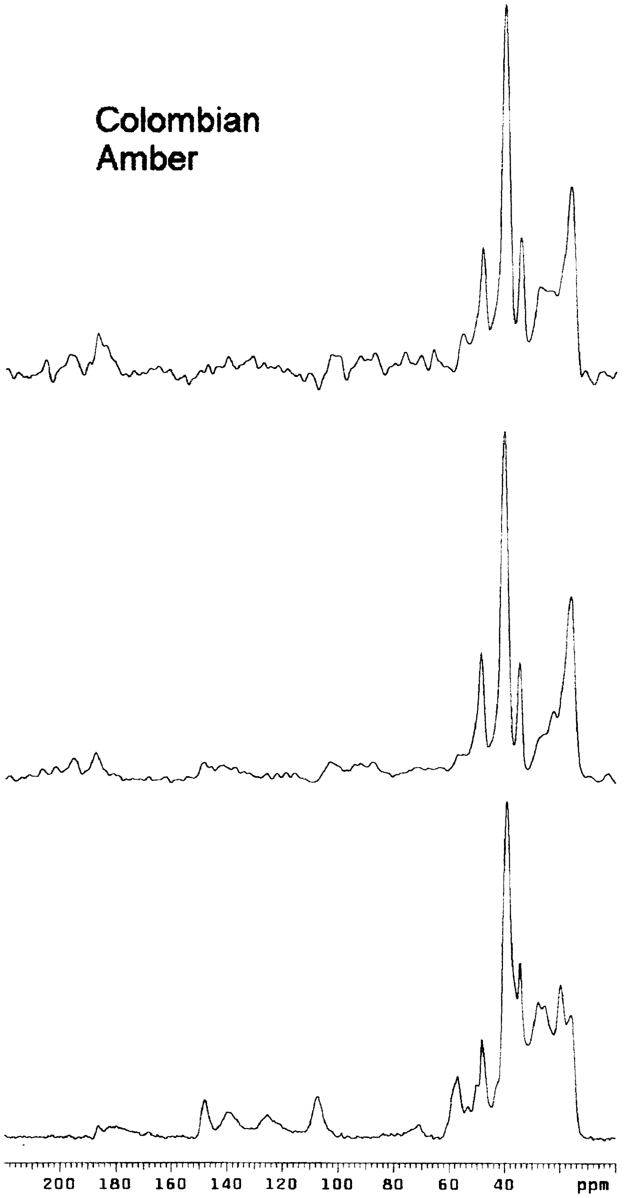


Fig. 3. A high-resolution CP-MAS ^{13}C NMR spectrum of Colombian amber (bottom) and protonated carbon suppressed by delaying decoupling spectra of 60 (middle) and 150 μs (top).

the exomethylene peaks has been interpreted as an indication that resins are very immature in contrast with the intensity of these peaks in the spectra of fossilized resins such as those from Mexico and the Baltic sea [27]. Finally, peaks due to carbonyl carbons of acids and esters are observed in the range of 170 and 190 ppm, being a broad peak at 187 ppm the most prominent in this region. The latter peak has been ascribed to the carboxylic acid group of neoabietic acid [21]. However, based on the analysis of soluble polylabdanoids by ^{13}C NMR solution spectra, it has been proposed by other authors [26] that multiple carbonyl peaks for acid groups in the CP-MAS spectra are mainly due to configurational effects, and not to the differences in chemical composition.

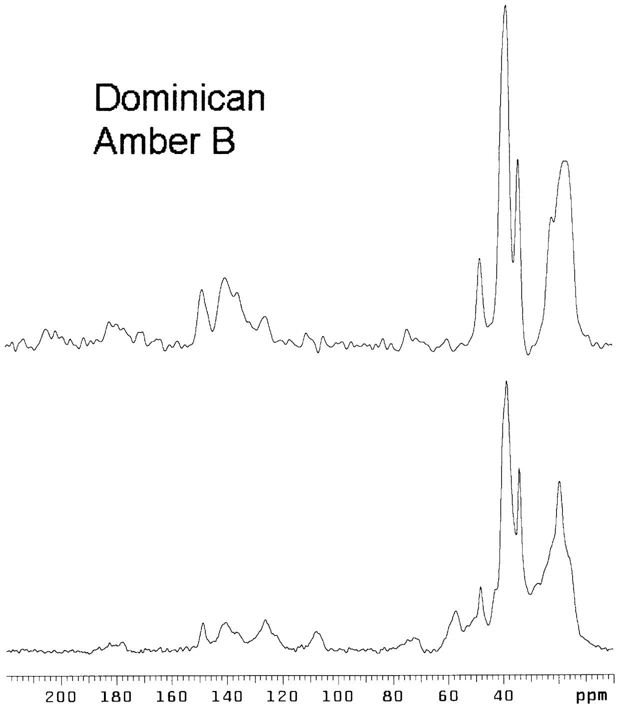


Fig. 4. A high-resolution CP-MAS ^{13}C NMR spectrum of Dominican amber B (bottom) and protonated carbon suppressed by delaying decoupling spectrum (60 μs).

CP-MAS spectra for Colombian samples using 60 and 150 μs for interrupted decoupling are also shown in Fig. 3. It can be seen that a delay of 150 μs is more effective to suppress signals from protonated carbons. As it was previously pointed out by other authors [12], the majority of the observed signals result from the superposition of different peaks with similar chemical shifts. Peaks due to C-20 methyls (14–30 ppm), quaternary carbons at C-10 and C-18 (32–44 ppm), quaternary carbon C-4 attached to a carbonyl group (48 ppm) of the labdane moiety, tertiary alcohol (83 ppm), and olefinic peak due to substituted C-8 (close to 147 ppm) are seen in this spectrum. The appearance of a series of peaks in the 182–220 ppm range due to carbonyl entities (and in particular that for the carboxylic acid of the neoabietic acid at 187 ppm) is very notorious. These peaks are not distinguished if lower delay decoupling times are used [12]. The spectrum resembles to that reported by Lambert et al. (with a small difference observed in the peak at 22 ppm) in the aliphatic region. However, the low intensity of the signals in the double bond region (at 139.8 and 148 ppm) in both spectra, contrast with the high intensity peaks observed for them in the decoupling spectrum. This indicates a large difference in the relaxation between the samples studied here and the commercial sample used in that study. Colombian samples were collected in places where Pliocene and Pleistocene deposits predominate [28], which indicates that they are young resins.

The spectrum of Colombian copalite most closely resembles in every peak to the CP-MAS spectrum for the resin of

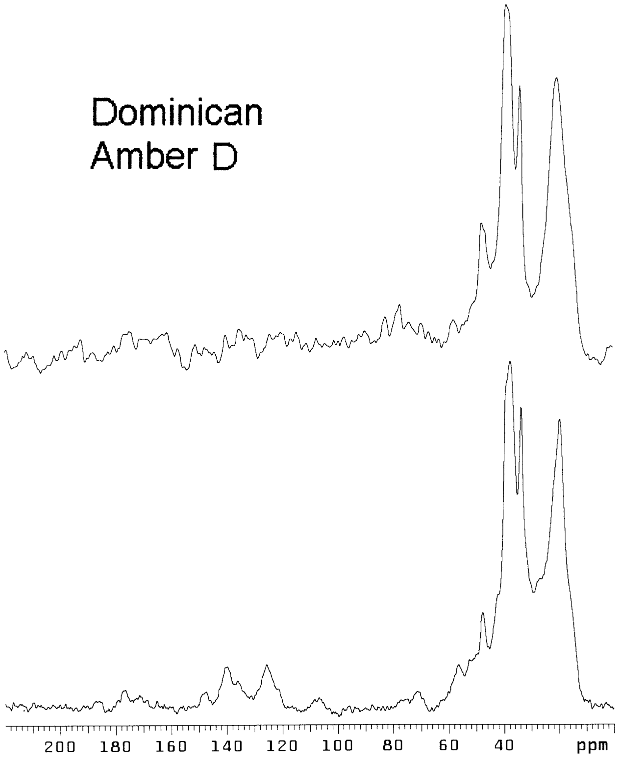


Fig. 5. A high-resolution CP-MAS ^{13}C NMR spectrum of Dominican amber D (bottom) and protonated carbon suppressed by delaying decoupling spectrum (60 μs).

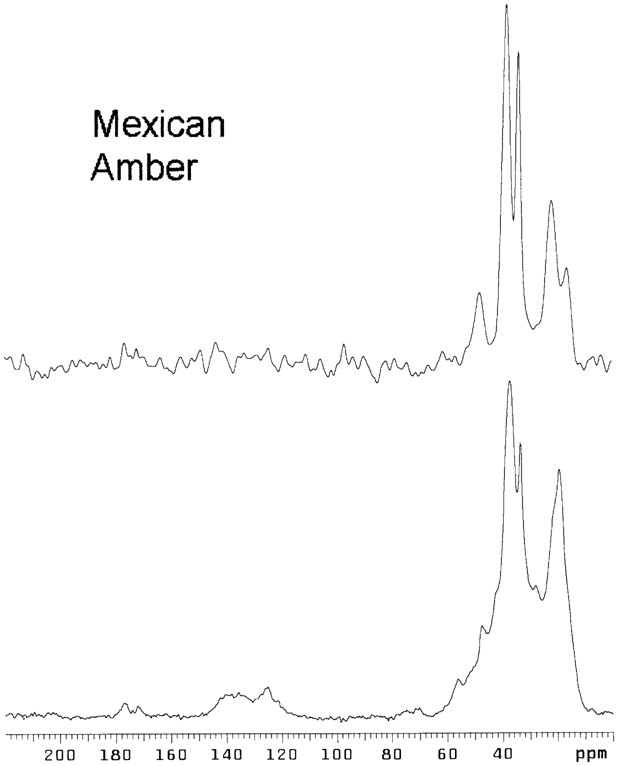


Fig. 6. A high-resolution CP-MAS ^{13}C NMR spectrum of Mexican amber (bottom) and protonated carbon suppressed by delaying decoupling spectrum (60 μs).

H. verrucosa [12], and to spectra of ambers from other Hymenaea species [26,27].

For Dominican and Mexican ambers, the total number of peaks in the aliphatic region is six. Spectra of two samples of Dominican Republic are practically identical (only one is depicted in Fig. 4); in the spectrum of the other sample (Fig. 5) additional peaks at 17.5 ppm (that appears as a shoulder) and in the carbonyl region can be distinguished. In the region comprised between 66 and 80 ppm, the broad band due to C-6 of zanzibaric acid can also be clearly observed. This signal has low intensity in the spectrum of Mexican amber (Fig. 6). In the unsaturated carbons region (105–155 ppm), at least four bands can be clearly distinguished for Dominican ambers, being the peak at a lower field (centered around 150 ppm) very broad for the Dominican amber D. Signals for Mexican amber are broader and less resolved in this region. Carbonyl carbons are also detected between 170 and 190 ppm for these ambers.

A very interesting feature is the large difference between the delayed decoupling spectra of both Dominican ambers in the unsaturated carbons and carbonyl regions. In the spectrum of Dominican amber B, at least four peaks in the olefinic region and various peaks in the carbonyl region are detected. Based on the relative intensity of the exocyclic double bond carbon signals at 108 and 148 ppm, it can be concluded that sample D is older than sample B. This fact creates a special environment to unsaturated carbons (olefinic and carbonyl) in such a way that they are not visible in the delayed spectrum of sample D, but are clearly seen in the spectrum of sample B.

The Baltic amber spectrum also shows six resolved bands in the aliphatic region (see Fig. 7). Two peaks for methyl groups at 15.7 and 20.3 ppm, one broad peak due to methyls and methylenes centered at 28.3 ppm, one broad signal centered at 38.1 ppm, two bands centered at 44.4 and 48.0 ppm, and various broad signals in the region comprised between 50 and 70 ppm (CH_2OH and CHOH peaks), are distinguished. In the olefinic region (100–150 ppm), several broad bands corresponding to C-17 (two peaks in the region 100–110 ppm), C-12 (125.5 ppm), C-13 (two peaks at 136.6 and 140.1 ppm) and C-8 (147.9 ppm) carbons of labdane moiety are seen. Carbonyl carbons that may be attributed to ester, acid and carboxylate (COO^-) are observed in the region between δ 170 and 190 ppm. All these peaks are more evident in the interrupted decoupling spectrum. One important feature of the interrupted decoupling CP-MAS spectrum, is the observation of a peak at around 195 ppm, that indicates the presence of ketone and/or aldehyde moieties.

The chemical composition of Baltic amber is very complex. Apart from labdene derivatives, other diterpenoid constituents such as pimarane, iso-pimarane and abietane derivatives (“resin acids”) are present [3]. Moreover, extensive succinylation of the hydroxyl group (which is completely absent in modern resins and other ambers) which provokes the presence of other chemical components,

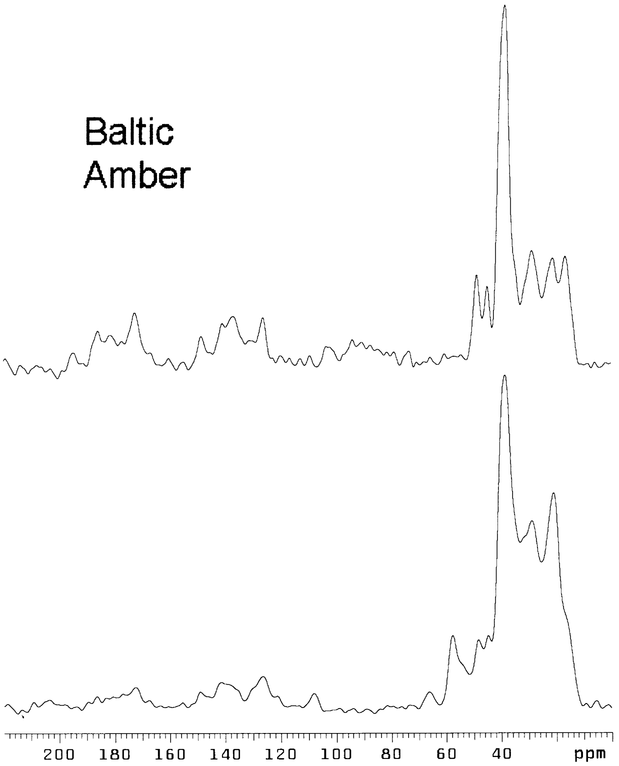


Fig. 7. A high-resolution CP-MAS ^{13}C NMR spectrum of Baltic amber (bottom) and protonated carbon suppressed by delaying decoupling spectrum (60 μs).

and further cross-linking contributes to a complex chemical composition [22]. This is also reflected in both spectra shown in Fig. 7, where a more complicated pattern is present when compared to the spectra of the other samples.

3.1. Aging studies

Physical aging affects the thermodynamic properties of amorphous polymers, altering the packing of the polymer and inducing chemical side reactions such as cross-linking [29].

In the DSC thermogram for a grounded sample of Colombian amber (see Fig. 8), upon heating a second-order transition at 40.5°C (that correspond to T_g), a first-order endothermic transition at 86°C, and a first-order exothermic transition at 155° are observed. After cooling and subsequent heating, only one second-order transition at 120°C is detected.

In an attempt to understand the chemical and morphological changes induced in the thermally treated amber, a sample of Colombian amber was placed in an oven at 65°C for 120 h. It is expected that amber composition includes a polylalbdanoid macromolecular structure, along with low-molecular weight terpenoids [25]. Based on ^{13}C NMR analysis, it has been determined that structural transformations of polylabdanoid resinites occurs mainly through the C8–C17 exocyclic double bond, and the high-molecular weight fraction of the polylabdanoids is only involved [4,12,21,26]. Some reactions have been proposed to occur during thermal treatment of amber, but it is still

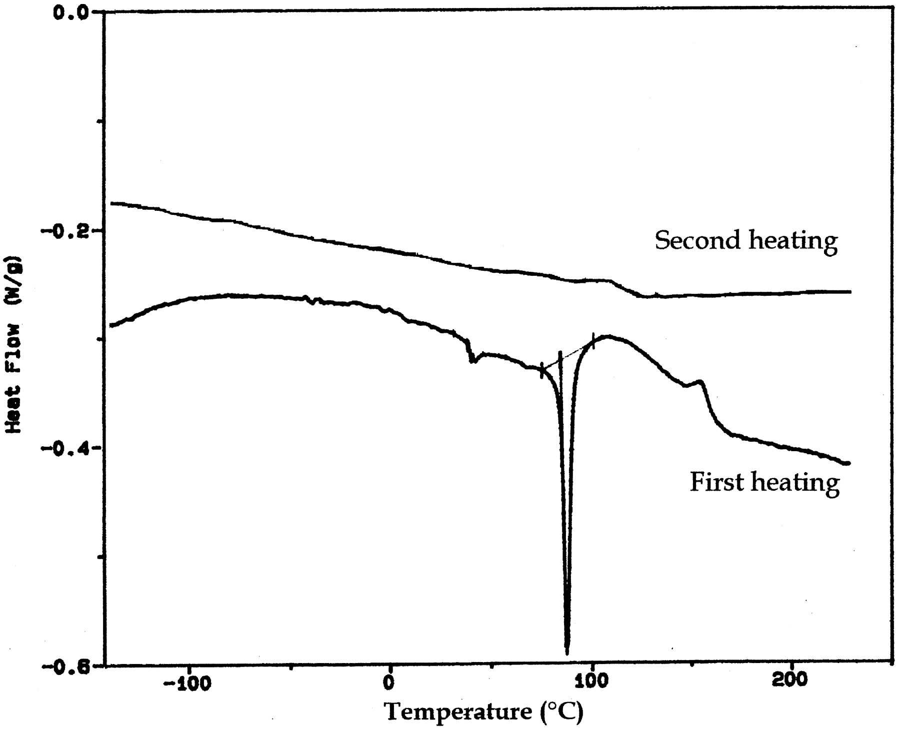


Fig. 8. Differential scanning calorimetry thermogram of Colombian amber.

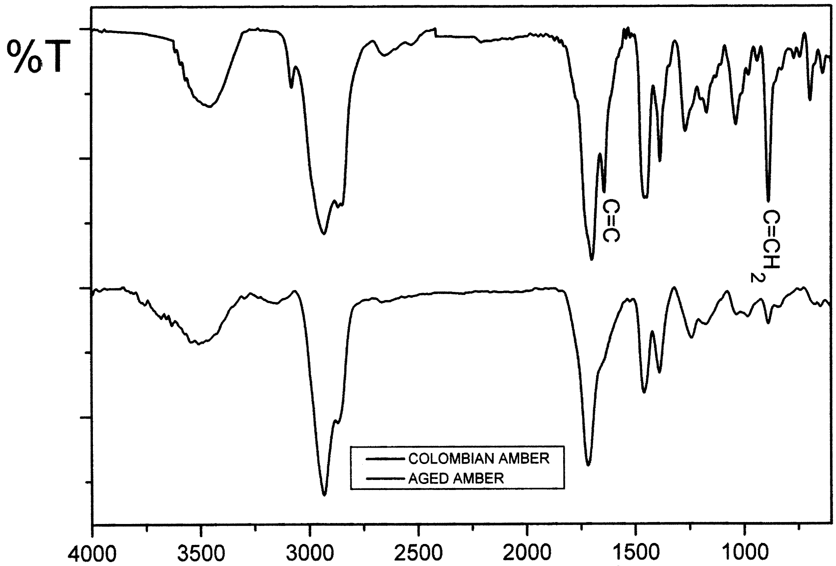


Fig. 9. A comparison of IR spectra of Colombian amber before (top) and after thermal treatment (bottom).

unclear which chemical transformations are involved in the resinite maturation [21].

This change is reflected in the FT-IR spectra (see Fig. 9): after thermal treatment, bands at 885 cm^{-1} (assigned to the C=CH$_2$ out-of-plane deformation) and at 3080 cm^{-1} (assigned to the double-bond carbon–hydrogen stretching band) are almost absent in the aged sample. The other bands corresponding to the double bond functionality (at 2950, 1640 and 1470 cm^{-1}) also decrease. More evident is the change observed in the prominent broad carbonyl band (1760–1660 cm^{-1}, mainly due to carboxylic acids and esters) [11,30], which become sharper with a minimum in transmittance at 1715 cm^{-1}. Another important difference is observed for the O–H stretching band, which becomes broader and less intense after aging. These observations can be due to the formation of ester linkages which have been proposed to occur by some authors [5].

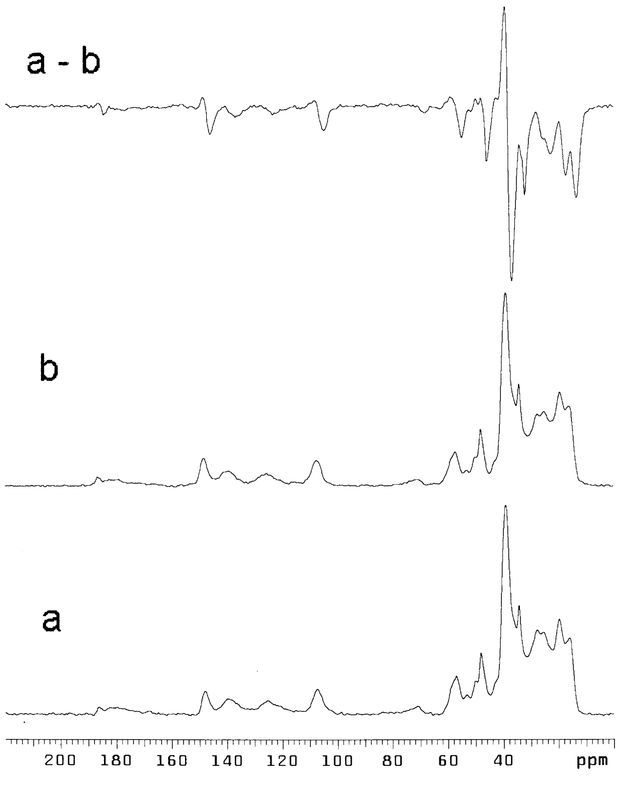


Fig. 10. A high-resolution CP-MAS ^{13}C NMR spectra of Colombian amber (spectrum a), aged sample (spectrum b) and the a − b difference spectrum.

Changes in the CP-MAS spectrum are reflected in different lineshapes, a shift toward lower field of some spectral bands, and also in the decrease of the relative intensity between olefinic (in the range of 102–156 ppm) and aliphatic carbons (in the range of 65–10 ppm) from 0.15 to 0.12. Major changes are observed for exomethylene carbon peaks at 107 and 148 ppm. These features are the consequence of the consumption of double bonds by chemical reactions (cross-linking), double-bonds isomerization, and morphological changes. In Fig. 10, the subtracted spectrum show that important differences are also present in the aliphatic region (with minima at 58, 32.0, 22.8, 17.3 and 13.53 ppm). Changes in the carbonyl region can be due to morphological features, but esterification of hydroxyl groups can be involved as discussed above. In fact, disappearance of 6-β-hydroxyl group is at least detected for the peak at 71 ppm, but this can also be due to chemical degradation and thermal oxidation. The observed decrease in the intensity of the peak at 58 ppm is also a consequence of the consumption of the exocyclic double bond [21].

4. Conclusion

Hymenaea is a leguminous resin-producing tree which belongs to the tribe Detarieae. Polymerization of labdatriene diterpenoids occurs just after they exude from the tree. Chemical evidence from IR, CP-MAS ^{13}C NMR and pyrolysis GC–MS indicates that species of the genus Hymenaea are the source of amber found in Mexico and Dominican Republic, and may be the source for that found in Brazil and Colombia [2]. There are 13 species in America and one in the eastern coastal of Africa. In particular, *H. verrucosa* produce copious masses of resin that has been extensively utilized commercially, and is thought to be a closest relative or ancestor to the tree that produces Tertiary Dominican amber [2]. In contrast, Brazilian copal from Amazonia are often attributed to *H. courbaril*, but probably it also comes from *H. oblongifolia* var. *palustris*. *H. intermedia* is considered the source tree of amber from Chiapas. However, there are no studies reported on the characteristics of the tree producer of Colombian amber. On the other hand, African “copal” is produced by different genera of Angiosperms, including Hymenaea.

In conclusion, ^{13}C CP-MAS spectra of ambers obtained in a 75 MHz spectrometer, yields valuable information on the overall chemical structure differences between samples. The spectrum of an authentic Colombian sample, herein described, is almost identical to that reported for an African species, a fact that is consistent with the knowledge that species of the genus Hymenaea are present both in South America and Africa.

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