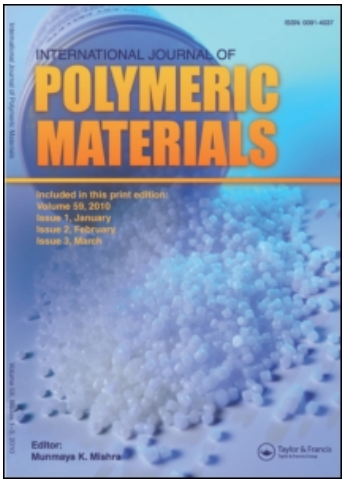

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NMR Assignment of Carbonyl and Olefinic Regions of Amescla Resin

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The structural assignment of amescla resin was carried out using a methodology already established in a previous work published by Tavares and Silva. From the methodology, three structural fragments were determined, allowing us to conclude that this natural resin was polymerized from the double bond localized in the middle of the aliphatic chain.

Keywords: amescla resin, assignment, NMR

INTRODUCTION

The great majority of synthetic polymers today are obtained from a nonrenewable source like petroleum. Polymers obtained from renewable material, such as natural resins, became an alternative resource since they can be used together with synthetic polymers and/or substitute for them in some applications like food packaging, for example. Natural resins are becoming a promising raw material, because of their potential to develop integrated resin systems to protect wood and act as alternatives to conventional toxic biocides [1].

Resins produced from certain trees are known as typical natural resins. These are products of resinous secretions (also called exudates),

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which drip out of the tree when the bark or root is wounded by nature or man. Upon injury, higher plants exude resins as a thick, viscous fluid to protect any wounds or lacerations from atmospheric pathogenic as well as from herbivorous and omnivorous animals. After exudation and in contact with air these viscous materials quickly, polymerize, forming a solid protective layer encompassing exposed plant tissues [1].

The chemical structure of the polymer fractions of the natural resins needs to be studied. The natural resins are mixtures of several complex organic compounds, especially polyterpenoids. Usually, these polyterpenoids are formed from constitutional units that contain at least four isoprene units, i.e., when they are diterpenoids (C_{20}) or higher [2]. Moreover, in many cases, a variety of related terpenoid-precursors are incorporated into the developing polymeric structure, resulting in a final copolymeric structure incorporating terpenoids, carboxylic acids, alcohols and hydrocarbons [3]. Thus, the elucidation of their structure is very difficult. Many techniques have been developed to study the molecular structure of ambers (a fossilized form of terpenoid resins) [4–8], however, the molecular structure of the compounds of modern resins remains obscure, in spite of some efforts [9]. Some natural resins such as the amescla resins (a resin exuded from *Trattinnickia burseraefolia*) have no characterization study in the scientific literature.

According to the previous work [9] in which a methodology was developed to study the natural resins, in the present work the amescla resin was first characterized by solid state nuclear magnetic resonance (NMR). After that a solubility study, thermal analysis (DSC), X-ray diffraction, X-ray fluorescence and solution nuclear magnetic resonance spectroscopy were carried out. In this work the main objective was to investigate in detail the carbonyl and olefinic regions using solution NMR spectroscopy. This technique allows us to evaluate the chemical structure and microstructure of polymer samples due to the good spectral resolution, which allows the use of diverse pulse sequences in one and two dimensions [10–12].

EXPERIMENTAL

Sample

The raw amescla resin was collected in the Amazon forest zone of Sinop in Mato Grosso state, Brazil, three years ago, during a prolonged dry season in which the stressed trees produce more resin.

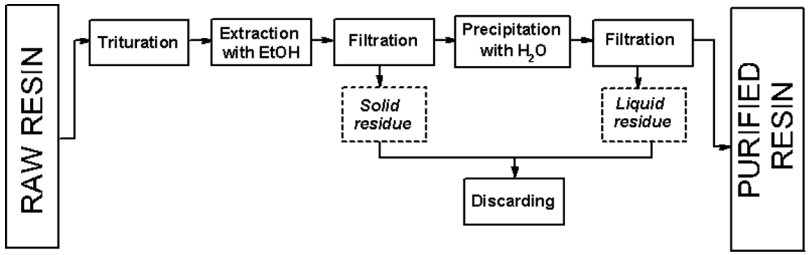


FIGURE 1 Scheme of raw resin purification.

Purification

The raw resin was submitted to a purification process, according to the scheme showed in Figure 1. After the solubility test, the sample was purified by extraction with ethanol (solvent) and precipitated with water (non-solvent). The purification procedure was designed to remove impurities and non-polymeric terpenoids from the sample. The raw resin was then exhaustively extracted with ethanol at room temperature. The ethanolic extract was precipitated in water and after that dried.

NMR Measurements

All solid NMR spectra, on a VARIAN INOVA 300 spectrometer operating at 75.4 MHz for ^{13}C, were obtained at ambient probe temperature and were performed using gated high decoupling. A zirconium oxide rotor of 7 mm diameter was used to acquire the NMR spectra at rates of 5.8 kHz. The ^{13}C NMR spectra were carried out in the magic angle spinning (MAS) using 60.000 Hz of spectral width and 0.023 s of acquisition time; cross-polarization magic angle spinning (CPMAS) with 2 s of recycle delay, 30.000 Hz of spectral width and 0.04 s of acquisition time. For the variable contact-time (VCT) the recycle delay was 2 s and a range of contact time was established from 0.2 to 8 ms. Proton $T_1\rho$ was measured from the decay of all resolved carbon with increasing the contact time.

The solution NMR spectra were obtained on VARIAN MERCURY 300, operating at 75.4 MHz for ^{13}C. The sample concentration for ^{13}C analysis was about 20% of resin in 3.5 ml of chloroform-d, using a 10 mm NMR tube at 20°C. The sample concentration for ^{1}H analysis was about 5% of resin in 0.8 ml of chloroform-d, using a 5 mm NMR tube at 20°C. The qualitative ^{13}C NMR spectrum was acquired using 16 k data points, spectral width 220 ppm, acquisition time 1.59 s,

recycle delay 1 s, pulse width 90° and 30,000 scans. The NOE effects were removed by gating the decoupling. The hydrogen analyses were acquired using 32 k data points, spectral width 4500 Hz, acquisition time 3.3 s, recycle delay 1 s, pulse width 45°C and 128 scans. The pulse sequences APT and DEPT were acquired in standard conditions.

The COSY experiment was acquired using a pulse of 90°, recycle delay = 1 s, number of transients = 16, number of increments = 160, Fourier number 1 = 1024, and Fourier number 2 = 1024, in ambient temperature.

The HMQC experiment was acquired using a pulse of 90°, recycle delay = 1 s, number of transients = 32, number of increments = 256, Fourier number 1 = 1024, Fourier number 2 = 2048, and Gaussian function = 0.071, at 30°C.

X-rays

The X-ray analysis was carried out on a Rigaku miniflex diffractometer, operating at the Cu K α wavelength (1.542Å).

X-ray Fluorescence

The X-ray fluorescence analyses were carried out on a Rigaku Rix 3100 diffractometer.

RESULTS AND DISCUSSION

The development of analytical methods that could lead to the identification of natural materials are of great importance. However, much cautions must be used. One procedure involves solid state NMR studies, X-ray diffraction, X-ray fluorescence, solubility test and solution NMR.

Solid State NMR

The solid state NMR analyses were carried out to obtain information on resin molecular mobility. The total ^{13}C CPMAS NMR spectrum (Figure 2) showed signals in distinct regions: olefinic (δ 100–140) and aliphatic carbons (δ 10–80). The ^{13}C MAS NMR spectrum (Figure 3) was collected with short recycle delay between 90° pulses to record the mobile fraction of the resin and it showed intense signals located from δ 15 to 30, and three other signals with minor intensity located at δ 39.5, δ 47.8 and δ 78.1. These signals belong to long aliphatic chains, which show high mobility compared to the aromatic/double bonds. The ^{13}C CPMAS NMR with dipolar dephasing spectrum (Figure 4) was also registered to obtain information on rigid carbons,

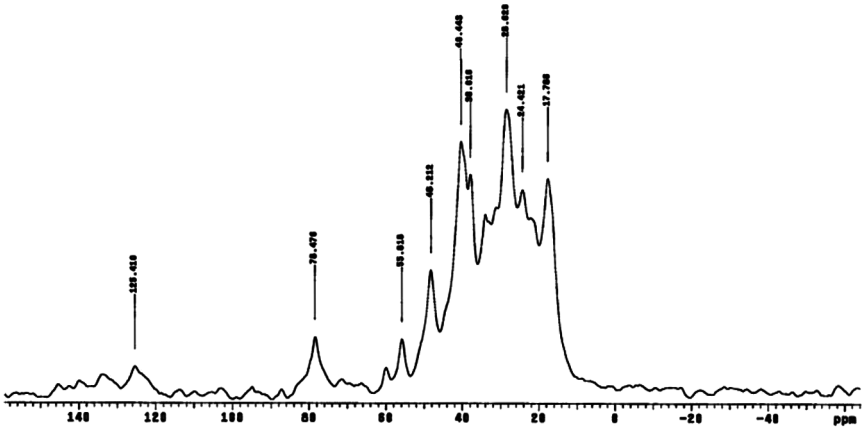


FIGURE 2 Total ^{13}C CPMAS NMR spectrum of amescla resin.

especially that belonging to the entanglements regions. From this spectrum only carbons belonging to the aliphatic region δ 10–40 were assigned and the signal width was very large, which suggests that this resin has some entangled bonds.

The solid state NMR ^{13}C spectra of this natural resin were compared to the literature [13] and it was observed that they exhibit a typical spectra profile of a triterpenoid resin.

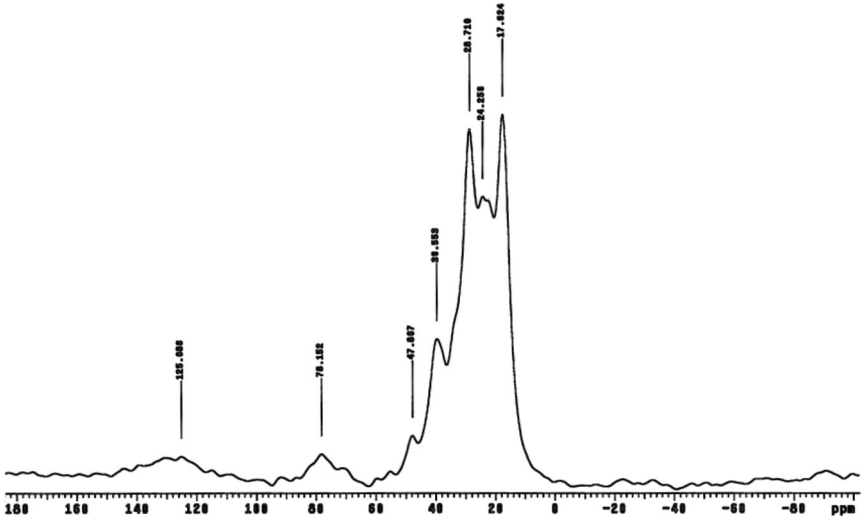


FIGURE 3 Total ^{13}C MAS NMR spectrum of amescla resin.

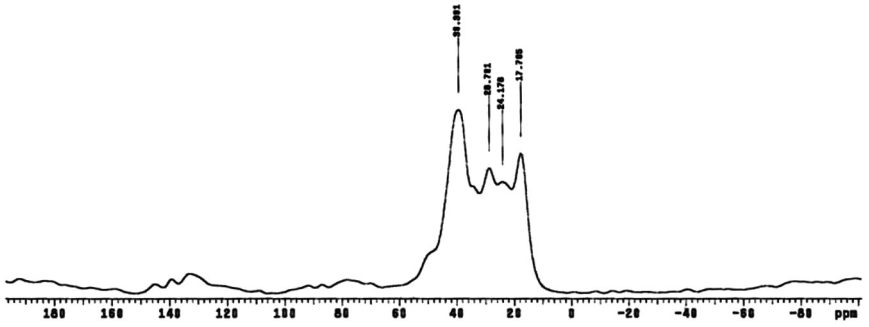


FIGURE 4 Total ^{13}C CPMAS/DD NMR spectrum of amescla resin.

The proton $T_1\rho$ was measured from the variable contact time experiment and the values are listed in Table 1.

The relaxation data confirm that the resin is heterogeneous, and at least two domains with different molecular mobilities were detected. These results corroborate the ^{13}C CPMAS, MAS and CPMAS with dipolar dephasing spectra.

X-ray Diffraction and Fluorescence

The X-ray diffraction was recorded to observe the crystallinity profile of the amescla resin. Figure 5 shows the X-ray pattern and according to the obtained spectrum this resin is an amorphous sample, due to the fact that during its natural polymerization process chains with different molecular size are formed, hindering the crystallization process.

The chemical composition was determined from the X-ray fluorescence and the results are listed in Table 2. We were surprised by the iron concentration in the amescla resin. But it did not interfere in

TABLE 1 Proton $T_1\rho$ Values for the Resolved Carbons of the Amescla Resin, as a Function of Chemical Shifts, Measured by Variable Contact Time

($\delta^{13}C$)	$T_{1\rho}^{H}$(ms)
78.5	9
48.2	10
40.4	13
28.5	15
24.4	17
17.9	24

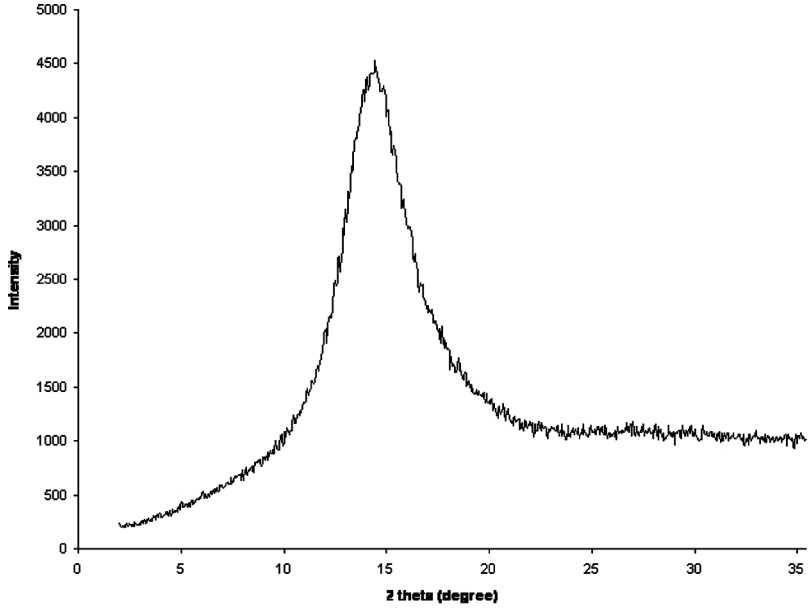


FIGURE 5 X-ray diffraction spectrum of amescla resin.

the solid state analyses, leading us to conclude that this element is inside the resin structure, and not in the surface area.

The X-ray diffraction and fluorescence data obtained corroborate the solid state NMR measurements.

Solubility Test

Samples of 100 mg of purified natural resin have their solubility tested in water, ethanol, acetone, chloroform and cyclohexanone, at room

TABLE 2 Chemical Composition Obtained by X-ray Fluorescence

Atomic element	Mass %
Al	0.031
S	0.003
K	0.007
Fe	1.850
Cr	0.488
C	96.318

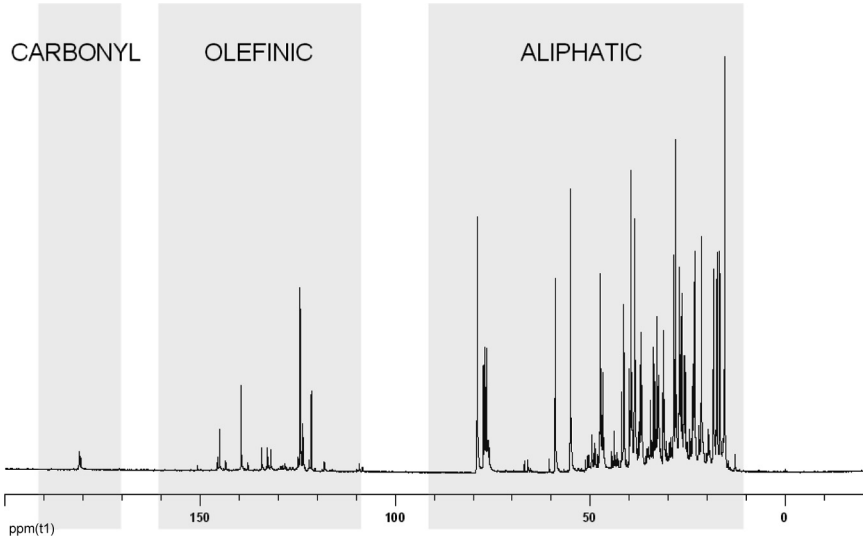


FIGURE 6 Solution state ^{13}C NMR spectrum from the amescla resin.

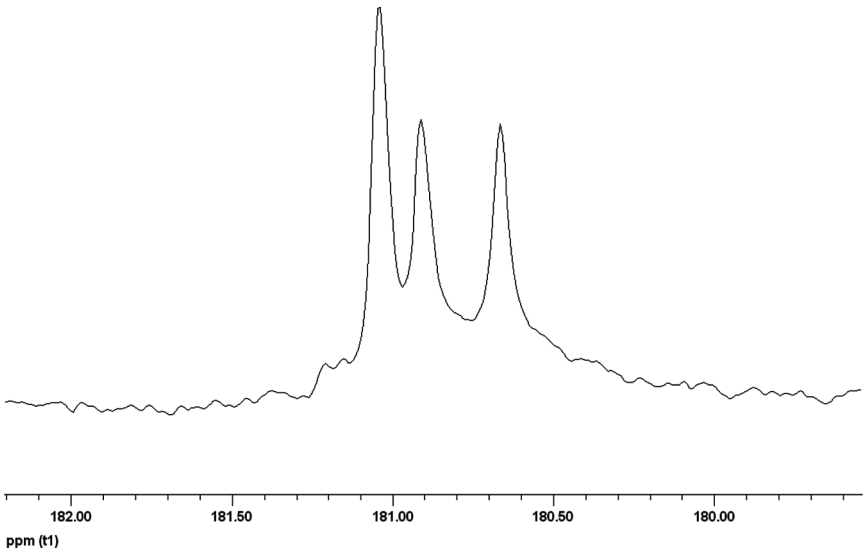


FIGURE 7 Expanded ^{13}C NMR spectrum of carbonyl region of amescla resin.

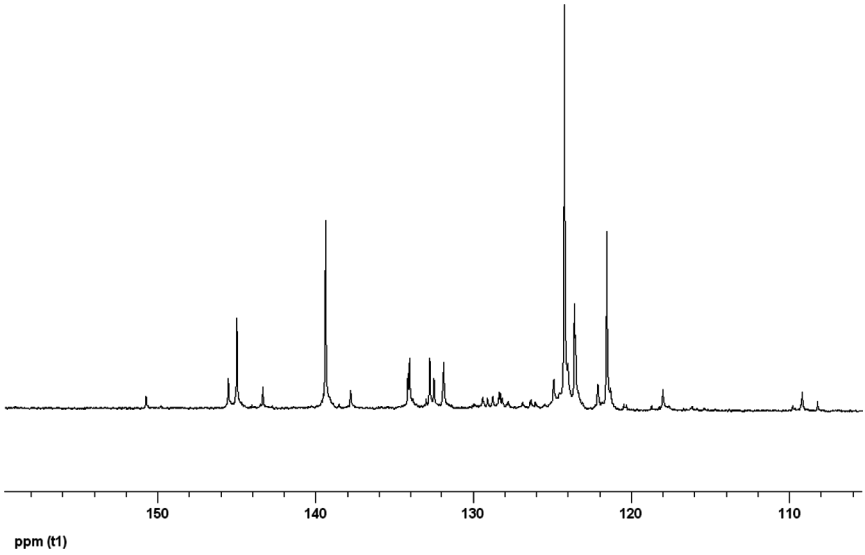


FIGURE 8 Expanded ^{13}C NMR spectrum of olefinic region of amescla resin.

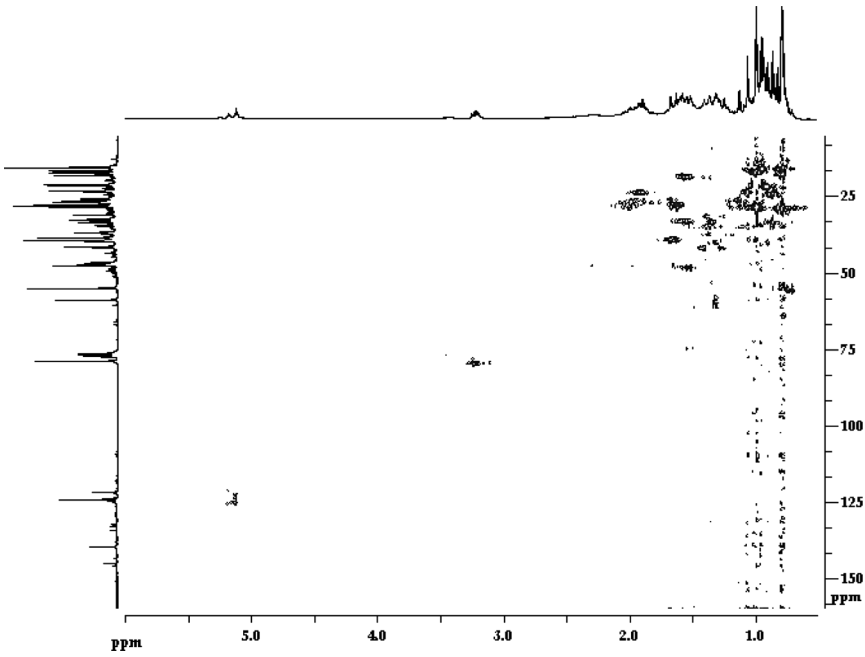
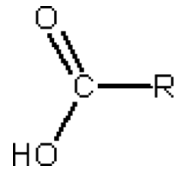


FIGURE 9 HMQC spectrum of natural resin from amescla.



SCHEME 1 Carboxylic acid structural fragment.

temperature using 1.0 ml of each solvent. The resin was soluble in all solvent tested, except for water.

Solution NMR

In the total ^{13}C solution NMR spectrum (Figure 6) three distinct regions were assigned: carbonyl (δ 170–180), olefinic (δ 110–160), and aliphatic (δ 10–80). In this present work an emphasis was given to the carbonyl and olefinic regions.

In the carbonyl region (Figure 7), three signals were detected: δ 181.0, 180.9 and 180.6. From chemical shift considerations, these

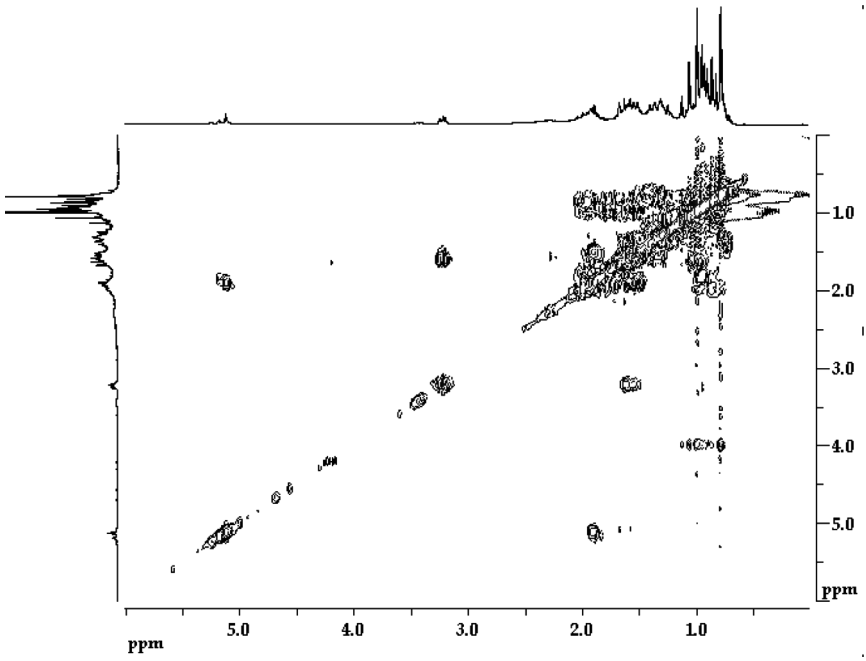


FIGURE 10 COSY spectrum of natural resin from amescla.

signals are attributed to carboxylic acid carbons. The multiplicity of signals indicates that there are, at least, three different constitutional units that form different polymers or copolymers. In addition to that, this multiplicity can be due to configuration effects and/or to different chain sizes. This observation suggests that in this resin there is a fragment of carboxylic acid, according to Scheme 1.

TABLE 3 APT and DEPT Assignments and HMQC and COSY Correlations

δ ^{13}C	APT and DEPT (Type of carbon)	HMQC correlations ^{13}C–^{1}H (δ ^{1}H)	COSY correlations ^{1}H–^{1}H (δ ^{1}H)
150.7	C	–	–
145.5	C	–	–
145.0	C	–	–
143.3	C	–	–
139.4	C	–	–
137.8	C	–	–
134.2	C	–	–
134.1	C	–	–
132.7	C	–	–
132.5	C	–	–
131.9	C	–	–
129.4	CH	nd	nd
129.1	CH	nd	nd
128.8	CH	nd	nd
128.7	CH	nd	nd
128.3	CH	nd	nd
128.2	CH	nd	nd
127.8	CH	nd	nd
126.9	CH	nd	nd
126.4	CH	nd	nd
126.3	CH	nd	nd
126.1	CH	nd	nd
126.0	CH	nd	nd
125.5	CH	nd	nd
124.9	CH	nd	nd
124.2	CH	nd	nd
124.0	CH	5.11	1.91
123.6	CH	nd	nd
123.5	CH	nd	nd
122.1	CH	nd	nd
121.6	CH	5.17	1.84
121.3	CH	nd	nd
118.0	CH	nd	nd
109.2	CH	nd	nd
108.2	CH	nd	nd

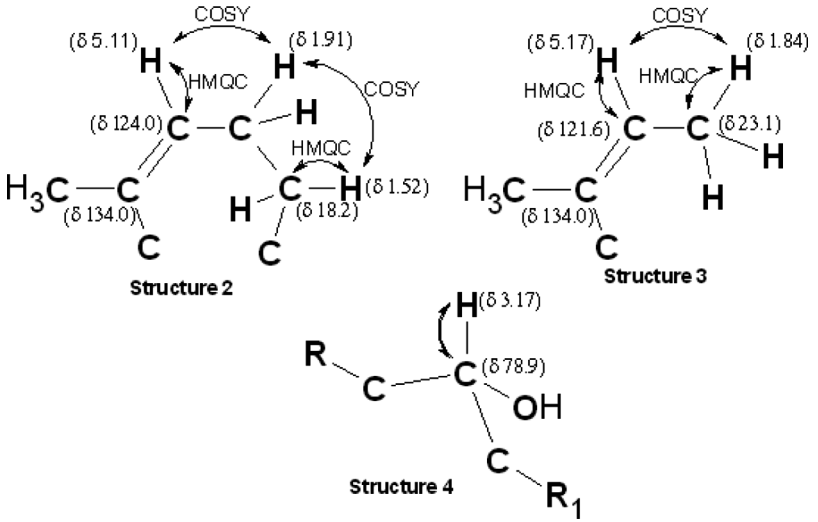


FIGURE 11 Structural fragments of amescla resin assigned from the ^{13}C NMR solution spectra.

The R segment (Scheme 1) was investigated initially from the olefinic region (Figure 8), which presented many signals due to structural complexity and heterogeneity of amescla resin. Similar to the carbonyl region, the olefinic region showed signals with multiplicity of signals, formed by groups of three signals. The assignments of olefinic region were established based on APT, DEPT, HMQC (Figure 9) and COSY (Figure 10) techniques. The assignments of all techniques are summarized in Table 3.

The key point of this strategy was the correlation between HMQC and COSY data. The HMQC spectra show the correlation between carbon and hydrogen atoms, while the COSY spectra show the correlation between hydrogen atoms. Then, the correlation carbon–carbon is found indirectly and the structural fragments can be elucidated. From Table 3, according to the assignments, three structural fragments, 1, 2, and 3 are proposed (Figure 11).

The assignment of the $=CH_2$, (δ 108.1) confirmed by APT and DEPT, makes us conclude that this natural resin was not polymerized from the double bond located at the chain ends, but from the double bond located in the middle of the aliphatic chain.

CONCLUSIONS

According to the main objective of this work a detailed investigation of the carbonyl and olefinic regions, using the solution NMR techniques,

allowed us to evaluate the three structural fragments of the amescla resin. These fragments were also detected in other natural resins, already published.

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