Determination of the Chemical Structure of Poly-β (-)-pinene by NMR Spectroscopy

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This paper is dedicated to the memory of our friend and colleague Annalaura Segre.

The chemical structure of a series of β(-)-pinene polymers (PBP) obtained by radiation-induced polymerization, free radical initiation, cationic polymerization over a Friedel-Craft catalyst and by coordinative polymerization over a Ziegler-Natta catalyst has been fully elucidated by ^{1}H and ^{13}C-NMR spectroscopy. 2D NMR techniques have been applied in order to assign all the NMR resonances to the structures of the PBP investigated. The NMR spectra show that the most regular PBP structure is obtained by radiation-induced polymerization followed by the free radical initiated polymerization. The most defective structure has been observed in the case of PBP prepared by cationic mechanism over a Friedel-Crafts catalyst. The discussion accounts for different types of defects and cross-links present in the PBPs investigated whose fundamental structure is based on the p-menthene repeating unit.

NMR self-diffusion measurements have been performed to evaluate the molecular weight of all the PBP investigated. The highest molecular weight (2600 Dalton) was found in the case of PBP prepared by Ziegler-Natta catalyst, while the lowest molecular weight was found in the case of PBP prepared by radiation-induced polymerization (about 1000 Dalton).

Keywords: Poly-β(-)-pinene, ^{1}H-NMR spectroscopy, ^{13}C-NMR spectroscopy, chemical structure, sequence regularity, defective structures, optical activity, molecular weight

1. Introduction

In our studies on the effects of high energy radiation on chiral molecules (1, 2) and on the research of the origin of homochirality in the biological world (3–5) we came across an unusual phenomenon when β(-)-pinene was irradiated. In general, chiral molecules undergo a radioracemization when irradiated with γ radiation (1, 2) and this phenomenon can be easily measured by polarimetry or by optical rotatory dispersion (6), but the case of β(-)-pinene is unusual: when irradiated with γ-rays, its specific optical rotation undergoes a strong enhancement as measured by polarimetry (7–9) or by optical rotatory dispersion (6). In contrast with other chiral molecules, β(-)-pinene polymerizes in a γ radiation field producing a polymer, poly-β-pinene (PBP), whose specific optical rotation is unusually higher than that of the starting monomer.

In the previous papers (7–9), we have reported the FT-IR and ^{13}C CP-MAS NMR spectra of PBP obtained by irradiation in comparison to the spectra of PBP synthesized by a free radical initiator or by a Friedel-Crafts catalyst through a cationic mechanism. It was immediately evident that the spectra of PBP synthesized with radiation or with a free radical initiator were much more defined, much richer in sharp bands than the spectra recorded on the PBP prepared by cationic polymerization. There was a clear correlation between the specific optical rotation of the PBP and their infrared or NMR spectra, being much higher than the starting monomer for the samples prepared with γ-radiation or with a free radical initiator and being much lower than the monomer for the PBP prepared by a Friedel-Crafts catalyst.

However, till now no detailed chemical elucidation has been attempted in order to explain the structural differences between the PBP prepared through different routes and the resulting optical activity. It has simply been stated (7–9) that higher ordered PBP structures are obtained by a free radical mechanism leading to higher optical activity

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than the one of the starting monomer, while PBP obtained by cationic polymerization is characterized by a much less ordered chemical structure and therefore, displays a lower level of specific optical rotation than the starting monomer.

The present paper is dedicated to a complete explanation of the structures of various PBP obtained through different synthetic routes including also a synthesis over a Ziegler-Natta catalyst which is reported in detail.

2. Experimental

2.1. *Materials*

The details on the preparation of Poly-β-pinene (PBP) by radiation-induced polymerization and by a free radical initiator starting from β(-)pinene have been reported elsewhere (8). Similarly, also the preparation of PBP by cationic polymerization over a Friedel-Crafts catalyst has been reported previously (8).

2.2. *Materials for Ziegler-Natta polymerization of β(-)pinene*

Manipulations of air and/or moisture sensitive materials were carried out under inert atmosphere using a dual vacuum/nitrogen line and standard Schlenk techniques or in a drybox under nitrogen atmosphere (<10 ppm oxygen, <20 ppm water).

β(-)-pinene used in this study (90 ml) was purchased from Fluka (purum, >99.0%) dried on CaH_2 and distilled twice under vacuum (P = 0.1 mm), collecting 80 ml of the product.

Methylaluminoxane (MAO) (Witco, 10 wt% solution in toluene) was used after drying in vacuum to remove the solvent and the unreacted trimethylaluminum (TMA) and was stored under nitrogen.

Toluene was dried by distillation from sodium under nitrogen atmosphere. (*Rac*-(EBI)ZrCl$_2$ (*rac*-(ethylenebis(1-indenyl) zirconium dichloride) catalyst was provided by Witco and used without further purification. [Rac-(EBTHI)ZrCl$_2$] (*rac*-(ethylenebis(tetrahydroindenyl)-zirconium dichloride) catalyst was provided by Basell Poliolefine Italia S.r.l. and used without further purification. Nitrogen was purified by passage through columns of BASF RS-11 (Fluka) and Linde 4 A molecular sieves. Absolute ethanol (>99.8%, from Fluka) and hydrogen chloride (37%, from Riedel De Haen) were used without further purification.

2.3. *Polymerization of β(-)pinene on Ziegler-Natta catalyst*

The β(-)pinene polymerizations were performed at 30°C in a 100 ml glass reactor, equipped with a magnetic stirrer, according to the following general procedure: 40 ml of toluene freshly distilled, 5 ml (32 mmols) of β(-)pinene twice distilled, 5.1–5.5 μmols of catalyst and 5.1–5.5 mmols

(295–320 mg) of MAO were added in the stated order. The molar ratio MAO/Zr was 1000.

The polymerizations were terminated after 142–162 h with a small amount of water, and the whole reaction mixture was poured into water (200 ml) and hydrogen chloride 37% (10 ml).

The polymerization mixture was then placed in a separating funnel, shaken, and the organic layer was separated from the aqueous phase. The organic phase was washed with dilute Na_2CO_3, with water, then concentrated under vacuum until a volume of 10 ml and treated with 100 ml of absolute ethanol. A further concentration under vacuum was required to eliminate the residual toluene. Thus, the addition of absolute ethanol and the concentration under vacuum were repeated three times. When all toluene was removed, the addition of 100 ml of absolute ethanol caused the precipitation of the solid PBP which was collected by filtration and dried under vacuum at 70°C. The PBP polymer yield was 52 mg.

An oligomeric fraction was collected from the filtrate after evaporating to dryness. The PBP oligomer yield was 37–45 mg.

2.4. *NMR spectroscopy*

Samples, $\approx$4 mg, were solubilized in 0.8 ml of d$_2$-tetrachloroethane (TCE).

^{1}H and ^{13}C-NMR experiments were recorded at 363 K on a Bruker Avance 600 spectrometer operating at 600.13 and 150.92 MHz, respectively, and equipped with a Bruker multinuclear, Z-gradient probehead.

^{1}H and ^{13}C assignments were obtained with 2D ^{1}H-^{1}H COSY (Correlation Spectroscopy), ^{1}H-^{1}H TOCSY (Total Correlation Spectroscopy), ^{1}H-^{13}C HSQC (Heteronuclear Single Quantum Coherences), and ^{1}H-^{13}C HMBC (Heteronuclear Multiple Bond Coherences) (10). All 2D experiments were carried out using 1024 data points in the f2 dimension and 512 data points in the f1 dimension. A recycle time of 2s was used. The ^{1}H-^{1}H TOCSY experiments were recorded with a spin lock time of 80 ms. The HSQC experiments were performed using a ^{1}J$_{C-H}$ coupling constant of 150 Hz. The HMBC experiments were optimized for a long range coupling constant of 5 Hz. ^{1}H-^{1}H TOCSY, ^{1}H-^{13}C HSQC and ^{1}H-^{13}C HMBC were processed in the phase sensitive mode with 512 × 512 data points. ^{1}H-^{1}H COSY experiments were processed in the magnitude mode with 512 × 512 data points. Pulsed gradient spin-echo (PGSE) experiments (11) were performed with a pulsed field gradient unit capable of producing a magnetic field gradient in the Z direction with a strength of 55.3 G cm^{-1}. The strength of the sinusoidal gradient pulses was logarithmically incremented in 32 steps from 2% up to 95% of the maximum gradient strength. Diffusion times between 300 and 1000 ms and gradient pulse lengths between 1.7 and 2.5 ms were applied. To avoid any overheating of the probehead, the diffusion experiments were performed at 300 K.

At this temperature, even though some ^{1}H resonances of the polymer are rather broad and overlapped, accurate self-diffusion coefficients can be measured. After Fourier transformation and baseline correction, the diffusion dimension was processed by means of the DOSY (Diffusion Ordered SpectroscopY) (12) subroutine of the Bruker Topspin software package (version 1.3).

Samples used for obtaining the calibration curve of molecular weights: dilute solutions (0.8 mg/0.7 ml) of PEG (polyethylene glycol) with known molecular weights were used for the calibration, in particular: PEG200 (Mw = 200 D), PEG300 (Mw = 300 D), PEG750 (Mw = 750 D), PEG1000 (Mw = 1000 D), PEG2025, (Mw = 2025), PEG4000 (Mw = 4000), PEG6000 (Mw = 6000 D), PEG8000 (Mw = 8000 D), PEG26000 (Mw = 26000 D), PEG 46000 (Mw = 46000 D). Typically, 0.8 mg of sample solubilized in 0.7 mL of d$_2$-TCE were used for DOSY experiments. In the case of PEG200 and PEG8000, DOSY experiments were also performed on more diluted samples, that is, 0.2 mg/0.7 ml. The same values of the self-diffusion coefficients were obtained with both solutions.

The deconvolution of ^{1}H-NMR spectra was performed using the dm2006 software (13). Applying the deconvolution procedure, the chemical shift and the integral of each signal resonating between 0.8 and 1 ppm was obtained.

3. Results and discussion

3.1. *Poly-β-pinene: structural aspects*

The polymerization of β(-)pinene involves the ring opening reaction of its cyclobutane ring (14–16). Because of the ring opening reaction, the β(-)pinene molecule is transformed into the p-menthene moiety which, in fact, is the repeating unit of the resulting poly-β-pinene (PBP). The general structure of PBP is shown in Scheme 1. Such a structure is consistent with the spectroscopic data available (7–9). In the following sections, it will be shown that a careful analysis and interpretation of the NMR spectra of PBP obtained by radiation-induced polymerization unequivocally confirms the chemical structure based on the p-menthene repetitive unit.

3.2. *NMR spectroscopy resonance assignments*

The ^{1}H-NMR spectrum of PBP polymerized by irradiation with a dose of 300 kGy is shown in Figure 1 along with
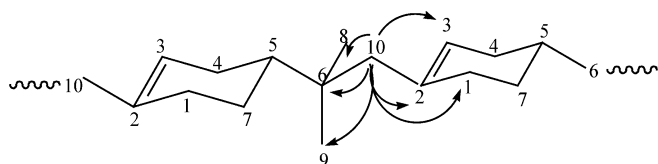
Sch. 1. Structure of PBP obtained by irradiation. The arrows show the long-range couplings relative to H10 found analyzing the HMBC 2D map.

Table 1. ^{1}H and ^{13}C assignment of PBP in d$_2$-TCE (T = 363 K) obtained by irradiation with a dose of 300 kGy

^1H ppm	Position	^{13}C ppm
2.037; 2.068	**1**	33.50
–	**2**	136.14
5.377	**3**	125.61
1.846; 2.048	**4**	27.48
1.333	**5**	43.99
–	**6**	36.35
1.178; 1.802	**7**	25.03
0.825	**8**	27.43
0.862	**9**	25.60
1.913	**10**	47.88

the resonances assignment, the labelling of resonances was performed according to Scheme 1. The ^{1}H and ^{13}C resonances assignment obtained by COSY and TOCSY (data not shown), HSQC (Fig. 2a) and HMBC (Fig. 2b) experiments is reported in Table 1. The slice of the 2D TOCSY map corresponding to the resonance of methine 3 at 5.377 ppm allows the resonances of methylene 4, of methine 5 and of methylene 7 to be assigned. The slice of the HMBC map corresponding to the proton resonances of methyl groups 8 and 9 at 0.825 and 0.862 ppm, respectively, allows the resonances of the quaternary carbon C6, of the methylene carbon C10, and of the methine carbon C5 to be assigned. Finally, the slice of the HMBC map corresponding to the proton resonance of methylene 10 allows the quaternary carbon C2 and the methylene carbon C1 to be assigned and to further confirm the assignment of methine C3. Arrows reported in Scheme 1 represent the long range contacts relative to methylene 10 obtained analyzing the HMBC map.

The ^{1}H spectra of PBP polymerized by irradiation with a dose of 300 kGy (a), of PBP polymerized through a free radical initiator (b), of PBP obtained by cationic polymerization (c), and of PBP prepared by Ziegler-Natta catalyst (d), are compared in Figure 3. The resonance of methine 3, is also shown in an expanded vertical an horizontal scale. The spectrum of PBP prepared by cationic polymerization, as well as the spectrum of PBP prepared by Ziegler-Natta catalyst, show broad resonances suggesting a less ordered structure on respect the ordered structure found in the case of PBP polymerized by irradiation and in the case of PBP polymerized by free radical initiator. In particular, at least three rather broad resonances due to methine 3 are observed in the spectrum of PBP by cationic polymerisation and in the spectrum of PBP obtained by Ziegler-Natta catalyst, see Figures 3(c and d), suggesting the occurrence of a more disordered structure. The spectrum of the radiopolymer is very similar to the one of PBP obtained by radical polymerization, with one sharp resonance due to methine 3 in both cases, (Figure 3(a and b)). This observation may account for the high optical activity of both PBP radiopolymer and PBP polymerized by free radical
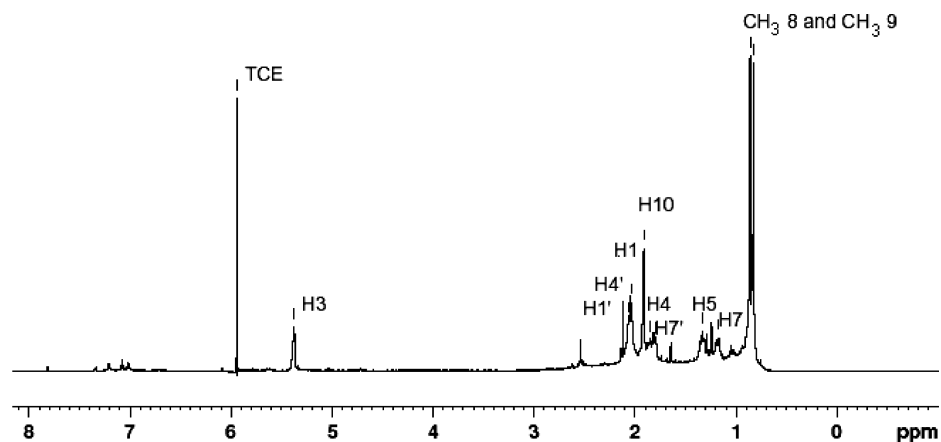
Fig. 1. ^{1}H-NMR spectrum at 600.13 MHz of PBP polymerized by irradiation with a dose of 300 kGy. The labelling of resonances was performed according to Scheme 1.
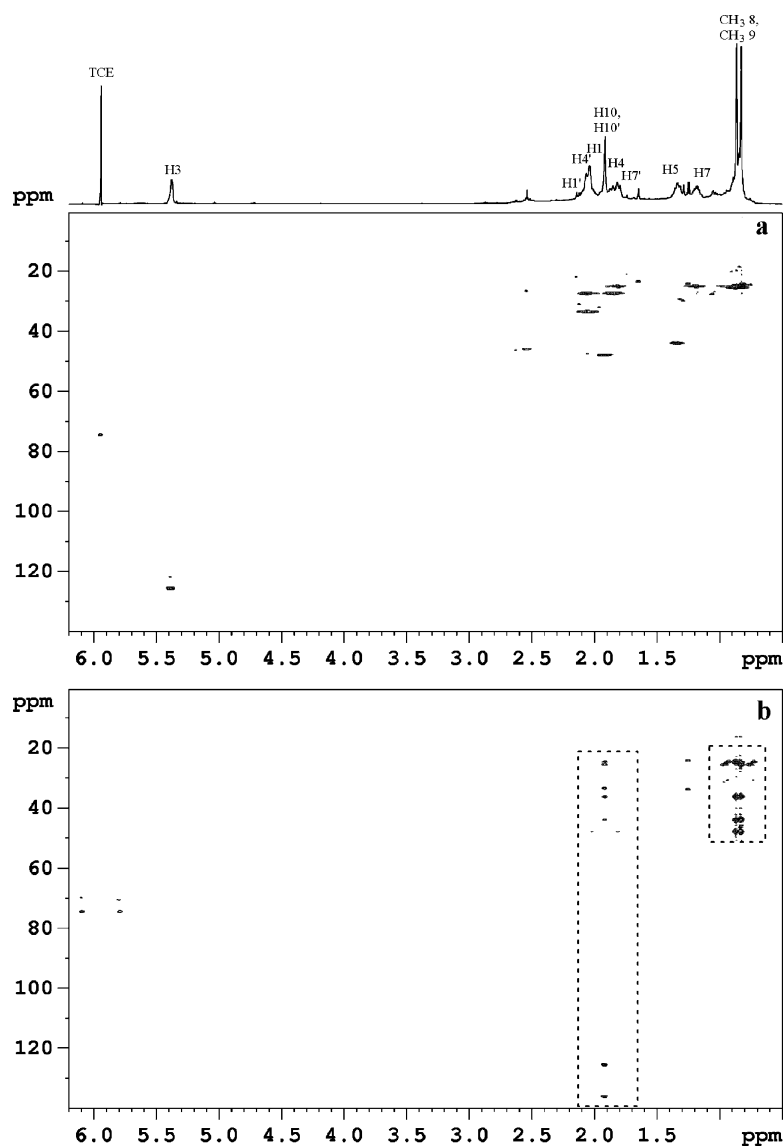

Fig. 2. (a) HSQC map of PBP polymerized by irradiation with a dose of 300 kGy; (b) HMBC map of PBP polymerized by irradiation with a dose of 300 kGy, the slice corresponding to the chemical shift of proton H10 and the slice corresponding to the geminal methyl groups have been evidenced with dashed lines.
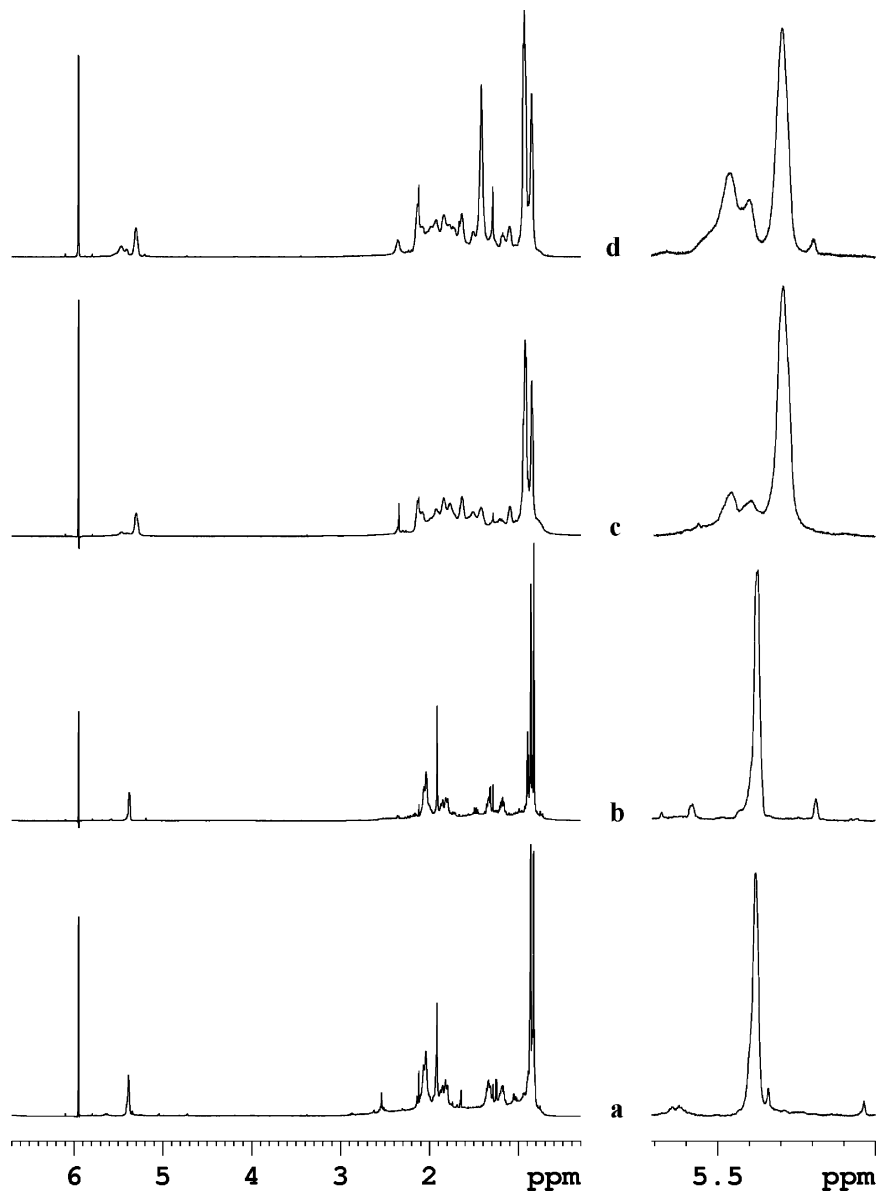
Fig. 3. ^{1}H-NMR spectra at 600.13 MHz (T = 363 K) of PBP polymerized by irradiation with a dose of 300 kGy (a), PBP polymerized through a free radical initiator (b), PBP obtained by cationic polymerization (c), and PBP prepared by Ziegler-Natta catalyst (d). The region of methine 3 is also reported in an expanded vertical and horizontal scale.

initiator and, consequently, the very poor optical activity of PBP obtained by cationic polymerization (7–9). This observation led us to state (7–9) that the radiation-induced polymerization of β(-)pinene (at a dose rate of ≈2 kGy/h) occurs by a free radical mechanism rather than a cationic mechanism, as proposed by earlier investigators (17, 18), otherwise the chemical structure of PBP obtained by irradiation should be more similar to that of PBP obtained by cationic polymerization with the consequently low optical activity. However, the situation is more involved, since we have shown (8) that PBP obtained through irradiation or through the free radical initiator can be easily racemized in the presence of an acid catalyst suggesting that the chiral

centre present in the p-menthene units of PBP is sensitive to protonation and undergoes an inversion of its configuration leading to racemization.

3.3. *Structural differences of various pbp obtained by different synthetic approaches*

Other aspects affecting the PBP regularity and hence, influencing its optical activity are the regularity of the monomeric unit sequence and the uniqueness of the p-menthene units. In the previous section 3.2, it has been shown that this is essentially true for the PBP radiopolymer where the NMR spectra show a sequence of p-menthene
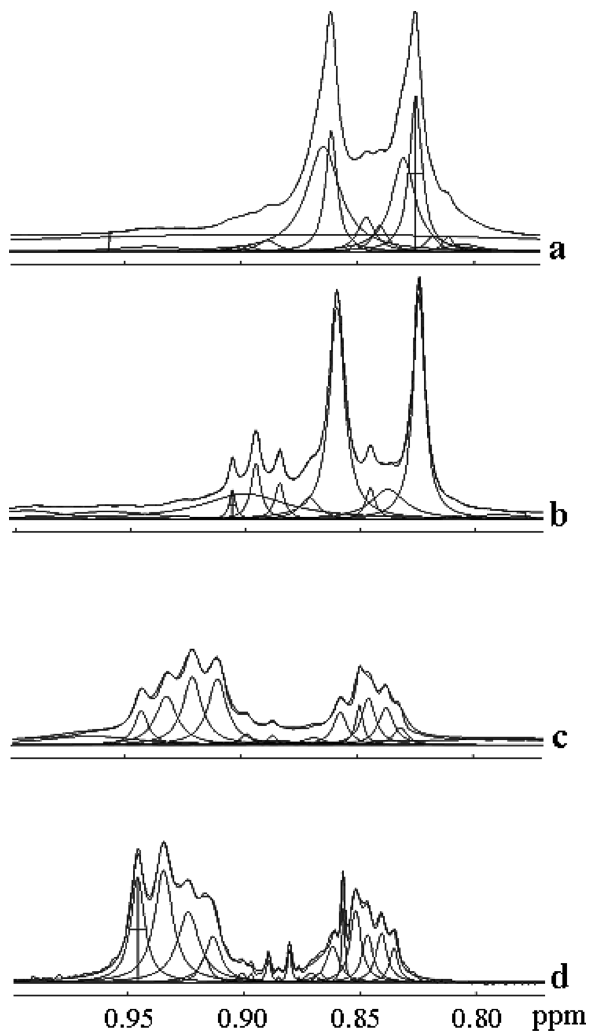
Fig. 4. ^{1}H-NMR spectral pattern of the geminal methyl groups in the 0.8–1 ppm region. The deconvoluted spectra have been superimposed on the experimental ones. (a) PBP polymerized by irradiation with a dose of 300 kGy, (b) PBP polymerized through a free radical initiator, (c) PBP obtained by cationic polymerization, (d) PBP prepared by Ziegler-Natta catalyst.

units connected head-to-tail (Scheme 1). This observation is further corroborated by the assignments of the ^{1}H-NMR resonances pertaining to the methyl groups (position 8 and 9 in Scheme 1). Such an assignment is not straightforward due to the presence of many overlapping signals. Therefore, a deconvolution of the ^{1}H-NMR spectra was applied in the region between 0.8 to 1.0 ppm, not only in the case

of the PBP radiopolymer (Fig. 4a), but also in the cases of PBP obtained by free radical (Fig. 4b), cationic (Fig. 4c) and Ziegler Natta catalysts (Fig. 4d), respectively. From the position of the resonances of the geminal methyl groups, it is possible to obtain important information regarding the enchainment of the monomeric units. The deconvolution of the resonances of the methyl groups reported in Figure 4a confirms that in the case of PBP radiopolymer the p-menthene units are regularly connected according to the head-to-tail sequence, moreover it is also possible to observe such a regularity even to the resolution of triadic sequences of monomeric units like that shown in Scheme 2. Such a structure has been defined as $(2,5–2,5–2,5)_n$ and the assignment of the resonances is shown in Table 1. The high level of regularity is still observable in the case of PBP prepared by a free radical initiator, (Fig. 4b), and it is possible to affirm that about 90% of the triads is of the type $(2,5–2,5–2,5)_n$. It is worth to note that the percentage of triads is obtained using the integrals obtained from the deconvolution procedure.

The topic becomes much more complex in the case of PBP prepared over a Friedel-Crafts catalyst or over a Ziegler-Natta catalyst. In both cases, the spectral pattern of the geminal methyls region suggests a significant level of irregularity, (Figures 4 (c and d)). Two different phenomena contribute to such a disorder. The first aspect to be considered is the acidity of the Friedel-Crafts and the Ziegler-Natta catalysts. Both catalysts act as Lewis acids over the monomer and if the Friedel-Crafts catalyst promotes the cationic polymerization of $\beta(-)$pinene, it is foreseen that also certain Ziegler-Natta catalysts like the zirconocene type used in the present work, act with a cationic and coordinative mechanism as well (19, 20). One of the drawbacks of these two catalysts is the fact that they cause the rearrangement of $\beta(-)$pinene (and of certain other cyclic hydrocarbons) into other moieties prior or during the polymerization. The rearrangement we are talking about is well known as the Wagner-Meerwein reaction (21–23), known also as transposition camphenique of the first type. The carbocation formed on $\beta(-)$pinene causes rearrangements, i.e., the opening of cyclobutane ring with the formation of p-menthene moiety or the much less common and less known formation of the o-menthene structure (Scheme 3). Other rearrangements are also possible with the formation of derivatives with camphene or other terpenes skeleton (15, 21). Thus, due to the cationic mechanism, the partial racemization of $\beta(-)$pinene is granted before and during the
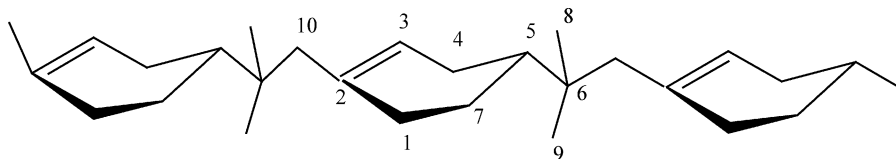
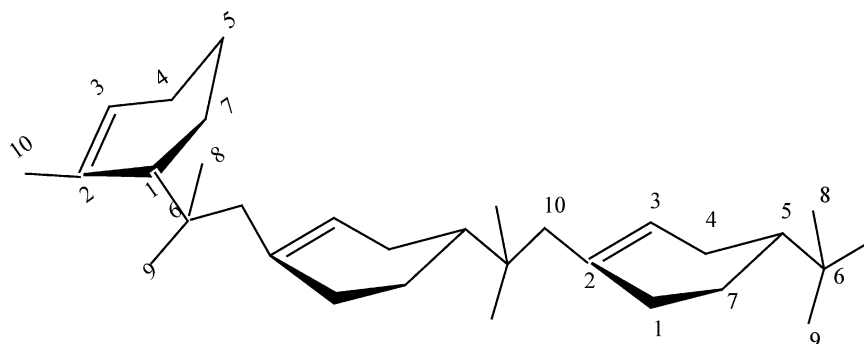**Sch. 2.** "2,5–2,5–2,5" triad structure of PBP.
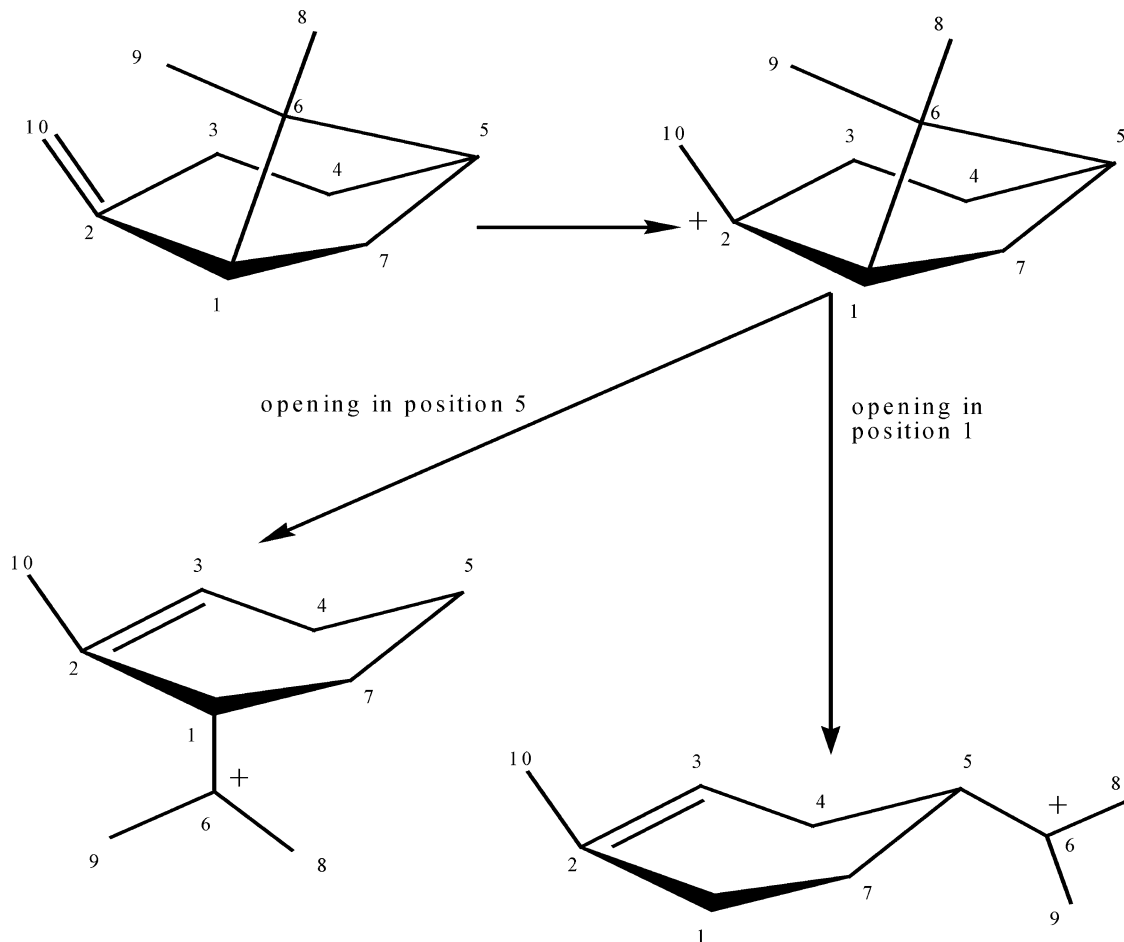
Sch. 3. The reaction scheme of polymerization of *β* (-)Pinene.

cationic polymerization and even after the polymerization due to the contact of the polymer with the acidic catalyst. Consequently, it is not surprising to find out an irregular, disordered structure in the case of PBP prepared by Friedel-Crafts and Ziegler-Natta catalysts as observed in Figure 4(c and d), respectively. Such a structural irregularity can be quantified with a certain degree of simplification by introducing the triad $(2,5–2,1–2,5)_n$ that can be also written as $(2,1–2,5–2,5)_n$; this triad is shown in Scheme 4. The as-

signments of the 2,1 and 2,5 units in this triad are shown in Table 2 and 3, respectively. It is worth to note that two consecutive units 2,1 have never been found in any investigated polymer.

The spectral pattern of the geminal methyl region, (Fig. 4c), and the corresponding resonance integrals permit us to ascribe to PBP prepared by cationic catalyst about 30% of irregular structure (Table 4), and the regular sequence of triads $(2,5–2,5–2,5)_n$ represent less than 5%

Sch. 4. "2,1–2,5–2,5" triad structure of PBP

Table 2. [1]H and ^{13}C assignment (T = 363 K) of 2,1 unit in the "2,5–2,1–2,5" triad in PBP obtained by cationic polymerisation and in PBP obtained by Ziegler-Natta catalysis

[1]H ppm	Position	[13]C ppm
2.130	**1**	36.43
—	**2**	—
5.291	**3**	127.40
2.193	**4**	48.47
1.102	**5**	18.40
—	**6**	—
1.091; 1.802	**7**	43.69
0.944	**8**	21.77
0.939	**9**	21.77
1.838; 2.080	**10**	44.05

Table 4. Percentage of 2,1 and 2,5 units, percentage of crosslink and type of structure obtained for all investigated PBP samples.

Sample	%2.1–%2.5	% crosslink	Structure (excluded the crosslinked forms)
PBP 300 kGy	0–100	4%	(2,5)n
PBP 600 kGy	0–100	9%	(2,5)n
PBP 1000 kGy	0–100	13%	(2,5)n
PBP 2000 kGy	0–100	12%	(2,5)n
PBP free radical initiator	10–90	23%	(2,5)n
PBP cationic catalysis	30–70	30%	irregular, (2,5)n < 5%
PBP Ziegler Natta	35–65	15%	(2,1–2,5–2,5)n or (2,5–2,1–2,5)n

of the total structure. The PBP prepared by Ziegler-Natta catalyst, (Figure 4d), resembles that obtained by cationic polymerization, since the level of irregular units is about 35% (Table 4). The triad sequence (2,5–2,5–2,5) found in all PBP radiopolymers is absent in PBP obtained by Ziegler-Natta catalysis, while it is barely present (<5%) in PBP obtained by cationic catalysis. In the case of PBP synthesized over a Ziegler-Natta catalyst, most units are of the type (2,1–2,5–2,5)$_n$ or (2,5–2,1–2,5)$_n$.

Another source of defective structure in PBP is the amount of crosslink due to the formation of bonds between adjacent chains. The percentage of crosslink can be easily obtained by integrating the signal of methine 3, (Schemes 1, 2 and 4), with respect to the signals resonating in the geminal methyl region. In fact, in the absence of crosslink, the ratio between the integral of methine 3 and the integral of geminal methyl groups is 1/6, whereas, under the occurrence of crosslink, this ratio decreases due to the opening of the double bond between C2 and C3. Such signal ratio reduction quantitatively reflects the percentage of crosslink. The percentage of crosslink found in all investigated PBP samples is reported in Table 4. In the case of PBP radiopolymers, the percentage of crosslink increases with the irradi-

ation dose from about 4% up to 12–13%. This behavior is easily foreseen since it is well known that the irradiation of polymers causes crosslinking reactions (24). Nevertheless, all PBP samples obtained by irradiation are definitely less affected by crosslink than PBP prepared by a free radical initiator or PBP obtained by cationic catalysis. As expected, the maximum level of this type of defect is found in the case of the PBP prepared by Friedel-Crafts catalyst and hence, by cationic mechanism, (Table 4). In fact, this polymer contains a rather large amount of crosslinked forms, about 30%, and there is evidence that the cyclohexene rings of the monomeric units are opened either in position 1 or in position 5, (Schemes 1,2, 4). This observation is fully consistent with the Wagner-Meerwein rearrangement undergone by β(-)pinene in contact with the Friedel-Crafts catalysts as discussed above.

PBP prepared by Ziegler-Natta catalyst shows a percentage of crosslink, about 15%, definitely lower than that found in the case of PBP prepared by free radical initiator, about 23%, and of PBP prepared by cationic catalysis, about 30%, (Table 4).

3.4. *Molecular weight determination of PBP by NMR spectroscopy*

In a previous paper (25) NMR self-diffusion measurements were shown to be a suitable tool for determining the average molecular weight of linear water soluble uncharged polysaccharides. In that case, the technique achieved results which were in very good agreement with the average molecular weight obtained with other conventional methods. Moreover, it was possible to achieve the determination of the molecular weight of the same samples used for the NMR structural analysis without any need of further manipulation which could affect the sample. The good results obtained in the case of uncharged water soluble polysaccharides encouraged us to apply this methodology to polymer solubilized in organic solvent. In particular, we were interested in determining the

Table 3. [1]H and ^{13}C assignment (T = 363 K) of 2,5 unit in the "2,1–2,5–2,5" triad of PBP obtained by cationic polymerisation and in PBP obtained by Ziegler-Natta catalysis

[1]H ppm	Position	[13]C ppm
1.710; 1.768	**1**	33.83
—	**2**	135.80
5.314	**3**	124.07
1.782; 1.870	**4**	28.67
1.093	**5**	44.42
—	**6**	—
1.180	**7**	25.03
0.922; 0.912	**8**	21.20
0.859; 0.850; 0.849; 0.838; 0.834	**9**	18.49
1.837; 1.800	**10**	44.02

molecular weight of all investigated PBP samples. Because we performed the NMR structural determination on PBP solubilized in d_2-TCE, we built a calibration curve for the molecular weight determination using dilute solution of PEG, with known molecular weight, in d_2-TCE.

Molecular self-diffusion can be encoded into NMR data sets by means of pulsed magnetic field gradients. As first proposed by Morris and Johnson (26), the diffusion information may be conveniently represented in the form of a two dimensional map showing chemical shifts and self-diffusion coefficients along the horizontal and vertical axis, respectively, according to the so-called Diffusion Ordered SpectroscopY (DOSY) scheme. Basically, DOSY allows compounds to be distinguished according to their size. This method has been used in many applications (27–31).

For instance, the 2D DOSY spectra of three PEG samples with different molecular weight, namely $M_w = 200$ D (a), $M_w = 6000$ D (b) and $M_w = 26000$ D (c), are compared in Figure 5. The resulting 2D maps clearly show that the self-diffusion coefficient of d_2-TCE remains constant in all samples, whereas the self-diffusion coefficient of PEG samples decreases with the increase of the molecular weight. The values of the self-diffusion coefficient are more precisely obtained by fitting the exponential decay of the resonance signal intensity against the gradient strength. The diffusion coefficients measured for ten PEG samples used as standards, are reported in Table 5. According to a method previously reported (32), the double-logarithmic plot of the self-diffusion coefficient D of standard PEG samples in dilute solution against the molecular weight M_w is shown in Figure 6. In this plot, the M_w values of the PEG samples lie on a straight line. A least-squared fitting of the

Table 5. Self-diffusion coefficients of PEG samples obtained performing NMR self-diffusion measurements and then fitting the decay of the resonance signal intensity against the gradient strength

Standard samples	D (m^2/s)
PEG200	$(4.31 \pm 0.02)10^{-10}$
PEG300	$(3.30 \pm 0.01)10^{-10}$
PEG750	$(1.878 \pm 0.008)10^{-10}$
PEG1000	$(1.79 \pm 0.01)10^{-10}$
PEG4000	$(6.83 \pm 0.03)10^{-11}$
PEG2025	$(1.23 \pm 0.01)10^{-10}$
PEG6000	$(5.37 \pm 0.03)10^{-11}$
PEG8000	$(4.91 \pm 0.02)10^{-11}$
PEG26000	$(2.57 \pm 0.02)10^{-11}$
PEG46000	$(1.79 \pm 0.05)10^{-11}$

data leads to the relationship (Equation 1):

$$D = 1.1 \times 10^{-8} M_w^{-0.60} \, (m^2 s^{-1}) \qquad (1)$$

The exponent value of 0.60 ± 0.01 is in very good agreement with the Zimm model (33), which predicts an exponent value of 0.6 for flexible polymers in a good solvent.

Note that in some cases the values of self-diffusion coefficients were also measured on more diluted solutions (0.4 mg/0.7 ml and 0.2 mg/0.7 ml), however, these values remain constant within the experimental error. This observation confirms that the solutions used for measuring the diffusion coefficient were sufficiently diluted.

It is noteworthy that for all PEG samples, the self-diffusion coefficient of d_2-TCE remains equal within the
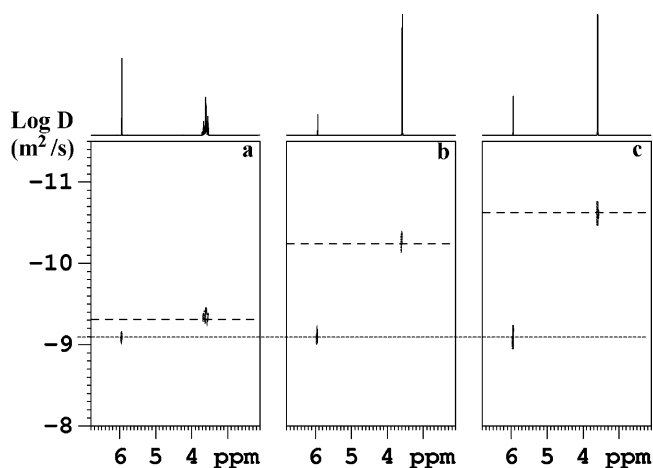

Fig. 5. 2D DOSY map of three PEG samples with different molecular weight, (a) $M_w = 200$ D, (b) $M_w = 6000$ D and (c) $M_w = 26000$. The diffusion coefficient of d_2-TCE remains constant in all samples (dotted line), whereas the diffusion coefficient of PEG decreases with the increase of the molecular weight (dashed lines).
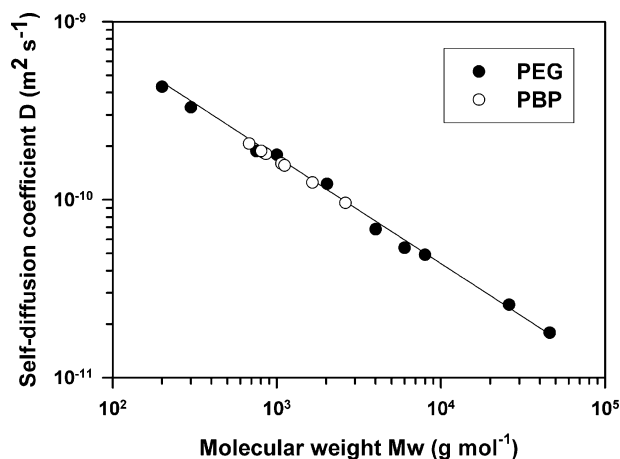

Fig. 6. Double-logarithmic plot of the self-diffusion coefficient D against M_w for ten samples of PEG in dilute solutions of d_2-TCE, as determined by NMR diffusion measurements. All data points (filled symbols) are located on a straight line. The straight line is the calibration curve determined. The empty symbols represent the self diffusion coefficients determined for the investigated PBP samples.

Table 6. Self-diffusion coefficients, calculated molecular weights and number of monomeric units of all investigated PBP samples. The error on the calculated molecular weights was found to be between 10% and 12% of the nominal value in all samples

Samples	$D\ (m^2/s)$.	Calculated MW	Monomeric units
PBP 160 kGy	$(2.07 \pm 0.05)10^{-10}$	678	5
PBP 300 kGy	$(1.84 \pm 0.06)10^{-10}$	834	6
PBP 600 kGy	$(1.81 \pm 0.06)10^{-10}$	859	6
PBP 1000 kGy	$(1.60 \pm 0.03)10^{-10}$	1066	8
PBP 2000 kGy	$(1.56 \pm 0.04)10^{-10}$	1115	8
PBP cationic catalysis	$(1.25 \pm 0.03)10^{-10}$	1646	12
PBP free radical initiator	$(1.88 \pm 0.04)10^{-10}$	803	6
PBP Ziegler Natta	$(9.6 \pm 0.2)10^{-11}$	2619	19
PBP cationic catalysis 0.4 mg/0.7 ml	$(1.24 \pm 0.03)10^{-10}$	1670	12
PBP cationic catalysis. 0.2 mg/0.7 ml	$(1.25 \pm 0.03)10^{-10}$	1646	12

experimental error. This means that no retarding effects on the solvent diffusion are detectable, further confirming that the solutions we used were sufficiently diluted.

The next issue to be addressed is whether the obtained calibration curve might be applied to evaluate the molecular weights of samples of PBP obtained by different doses of irradiation, as well as of PBP by cationic, radical and Ziegler-Natta polymerization. Toward this aim, dilute solutions of PBP (0.8 mg/0.7 ml) samples were analyzed by DOSY, and the results were compared with the calibration curve derived from the study of PEG samples. The measured self-diffusion coefficients, the corresponding molecular weights, and the corresponding number of repetitive units are reported in Table 6. Note that the calculated molecular weights were found to be affected by an error between 10–12% of their nominal value. In the case of PBP obtained by cationic polymerization, the self-diffusion measurements were also performed on more dilute solutions (0.4 mg/0.7 ml and 0.2 mg/0.7 ml), however, also in this case, the values of the self-diffusion coefficients were the same, within the experimental error, to those measured at a concentration of 0.8 g/0.7 ml.

It is worth noticing that, by increasing the irradiation dose, a slight increase of the length of the polymeric chain from 5 to 8 repetitive units is observed. The molecular weight found in the case of PBP obtained by cationic polymerization accounts for 12 repetitive units, whereas in the case of PBP obtained by free radical initiator, the molecular weight accounts for 6 repetitive units. The PBP obtained by Ziegler-Natta polymerization shows the highest molecular weight, which accounts for 19 repetitive units, (Table 6).

The molecular weight values measured on PBP prepared with Friedel-Crafts catalyst reported in the literature (11, 12) are comprised in the range from between 800 and 3000 Daltons, depending on the synthesis conditions and on the purification of the PBP. Thus, the molecular weights measured by the NMR diffusion technique are in full agreement with those reported in the literature.

The calibration curve obtained with PEG samples solubilized in d_2-TCE will be further extended using other standard samples with higher molecular weights (34). Accordingly, it should be possible to extract the unknown molecular weight of samples over a very wide range.

4. Conclusions

The full ^{13}C and ^{1}H assignment of NMR spectra of PBP prepared with different synthetic routes and conditions has been achieved. The PBP prepared by radiation-induced polymerization of β(-)pinene has been shown to be the most regular polymer with a structure based on p-menthene repetitive unit. PBP prepared by Ziegler-Natta catalyst shows a rather high amount of irregular units, thus appearing, to the same extent, more similar to PBP synthesized over a Friedel-Crafts catalyst which is characterized by the most disordered or defective chemical structure which is the result of the Wagner-Meerwein rearrangement of β(-) pinene exerted by the Lewis acid activity of the Friedel-Crafts catalysts. Moreover, the triad sequence found in the case of PBP radiopolymer is barely present in PBP obtained by Ziegler-Natta catalysts, where most units show a different triad sequence.

The highly ordered structure of PBP obtained by radiation-induced polymerization or by the free radical initiation is corroborated by the very high optical activity measured on these polymers (8). Conversely, the low optical activity correlates with the disorder and defective action of PBP synthesized by a cationic mechanism.

The molecular weight of all PBP samples studied in the present paper has been determined by NMR self-diffusion measurements. For all samples, the molecular weight was found in the range comprised between 700 and 2600 Dalton. The highest molecular weight was measured on PBP prepared by cationic and Ziegler-Natta catalysts. On the other side, PBP prepared by radiation induced polymerization and by free radical initiation are characterized by a lower molecular weight.

Acknowledgements

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