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Application of the Lanthanide-Induced Shifts to the Nuclear Magnetic Resonance Determination of the Helical Conformation of Poly(β-hydroxybutyrate) in Solution

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ABSTRACT: The method of shift reagents is applied quantitatively to the determination of the internal rotation angles in the helical structure of poly(β-hydroxybutyrate) in solution and results in fair agreement with those deduced from conformational energy and X-ray structure factors calculations.

The minimum energy crystalline conformation of a stereoregular polymer is helical. X-Ray fiber diagrams or electron diffraction patterns of ultra thin (100–200 Å) and often imperfect crystals give very easily the number of residues per turn and the pitch of the molecular helix.[1] They are however not rich enough for a direct determination of the atomic positions. When the repeat unit has several internal rotation angles, there is a large number of conformations compatible with the two helical parameters. Generally a few conformations specified by a set of internal angles are selected on the basis of steric hindrance (space-filling model building) or more refined conformational energy calculations and the intensities of the diffraction patterns are recalculated with the additional parameters coming from the relative positions of the helices in the lattice. Such a process can generally be refined to values of the reliability factor of the order of 0.15. Such a work has recently been performed on poly(β-hydroxybutyrate) (PHB) a natural polyester forming a 2_1 helix with a pitch of 5.96 Å (Figure 1).[2-4]

Since it has been recently shown[5,6] that PHB in solution in $CDCl_3$ and TFE retains essentially its local helical structure, but with enough flexibility to give a well-resolved high-resolution nmr spectrum[7] (Figure 2), we have attempted a more direct and eventually more precise determination of the four internal angles defining the local conformation. Our method rests on the relative pseudocontact shifts experienced by the four types of protons upon binding of a lanthanide chelate to the carbonyl oxygen. This method of conformational analysis has recently been applied quantitatively to small molecules or protein with a single chelating site[8] but has never been applied quantitatively to stereoregular homopolymers where, on one hand, the rapid jump of the lanthanide between equivalent sites must be considered (precluding the simultaneous use of relative broadening) but where, on the other hand, the helical condition reduces considerably the number of conformations.

Nmr Spectrum of PHB and the Determination of Possible Values of the Angle ψ. A first piece of structural information can be derived from the coupling constants J_{AC} and J_{BC} obtained from analysis of the nmr spectrum.

The results are given in Table I where the index *A* has arbitrarily been given to the one of the nonequivalent CH_2 protons with the higher chemical shift and higher coupling constant. Recalculated intensities of the CH_2 signal are shown in Figures 3 and 4. Despite the uncertainties in the use of Karplus relations,[9] one can use the difference between J_{AC} and J_{BC} to derive from the dihedral angle an interval of possible ψ values. Owing to the arbitrariness in the choice of H_A there are two such intervals $0 < \psi < 20°$ and $120° < \psi < 140°$ (Figure 5). It must be remarked that these values are incompatible with the model proposed by Okamura[2] ($\psi = 218°$) and this disagreement has given some impetus to the reexamination of the data.[3]

Principle of Lanthanide-Induced Pseudo-Contact Shift and Its Application to Polymers. It is now well established[10,11] that the shift $\Delta\delta$ experienced by a proton close to lanthanide complexed to a lone pair bearing atom is almost entirely due to pseudo contact and therefore proportional to the product of a magnetic factor *M* related to the anisotropy of the magnetic susceptibility by a geometrical factor

$$F_g = 3 \cos^2 \theta_i - 1/r_i^3$$

where θ_i and r_i specify the position of the proton relative to the ion (Figure 6). The exact origin of the magnetic factor *M* and the conditions for a proper application of the method for conformational analysis have been recently stressed.[10,11] The actual shift observed at a given lanthanide molar ratio depends upon the equilibrium be-

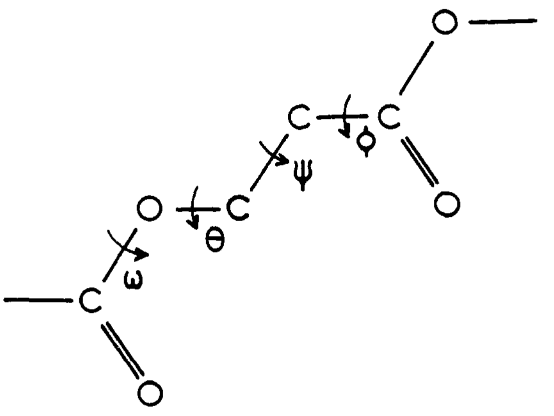

Figure 1. Repeat unit in PHB. Internal rotation angles ω, θ, ψ, and ϕ are taken as zero for the all trans structure.

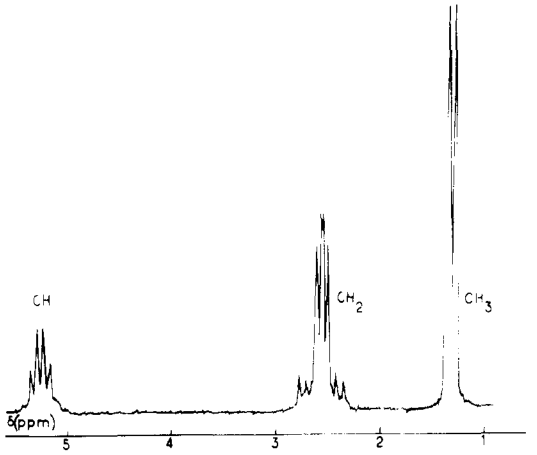

Figure 2. Proton magnetic resonance spectrum of PHB in $CDCl_3$.

tween the free and complexed species. But when several protons can be observed, the ratio of their shifts will be equal to the ratio of their geometrical factors. This ratio should not depend on the lanthanide if pure pseudo-contact and axial symmetry are involved. Slight differences can however arise from change of the distance h between the lanthanide and the oxygen ligand (Figure 6) when passing from one lanthanide or chelate to the other. Experiments on small rigid molecules lead to values of h between 2.8 and 3.6 Å.[12]

Case of Homopolymers. For homopolymers, the number of complexing sites is large and the lanthanide ion exchanges rapidly from site to site as revealed by the exchange narrowed single signal.[13] However, it is reasonable to suppose that the ion spends most of its time at the chelating site so that the shift becomes an average over all possible distributions of n ions among N sites of the polymer. The geometrical factor becomes

$$F_g = \frac{1}{N}\left\langle \sum_{j=1}^{N}\sum_{k=1}^{n} \langle 3\cos^2\theta_{jk} - 1/r_{jk}^3\rangle \right\rangle \tag{1}$$

where θ_{jk} and r_{jk} specify the position of a proton in the jth unit with respect to the ion complexed on the kth unit, the double averaging being over the position of the ion at the site and over the distribution along the polymer.

For lanthanide chelates with axial symmetry it is reasonable to locate the ion in average on the C=O axis and to define its position by the single parameter h (Figure 6). If $N \gg n$, one can moreover assume that any proton will only feel the influence of one nearest ion which leads to the replacement of (1) by

$$F_g = \frac{n}{N}\sum_{k=j-(N/2n)}^{j+(N/2n)} 3\cos^2\theta_{jk} - 1/r_{jk}^3 \tag{2}$$

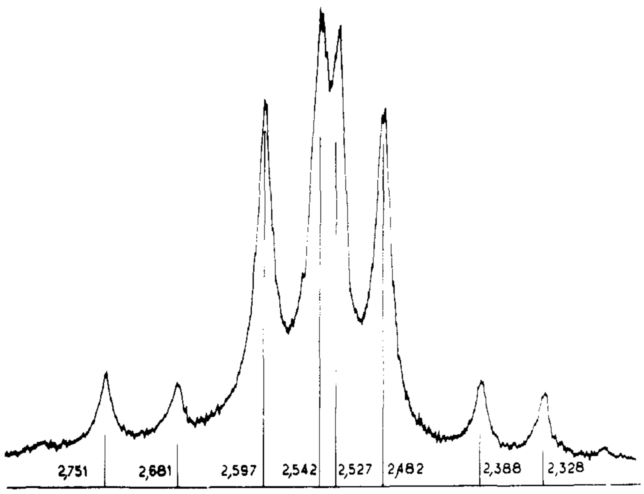

Figure 3. Analysis of the methylene signal of PHB in $CDCl_3$.

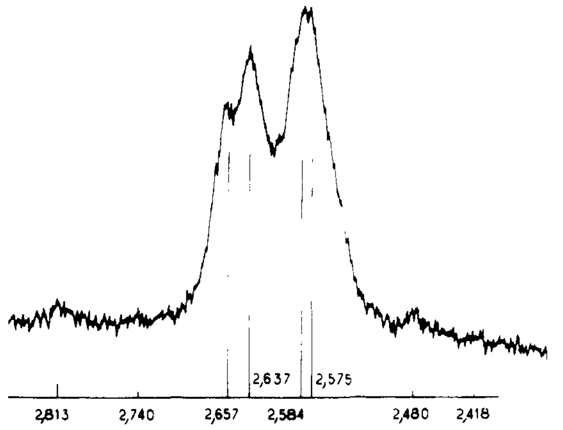

Figure 4. Analysis of the methylene signal of PHB in TFE.

If the complexation is not quantitative n is unknown but, as the resonances of the different protons differ by a few parts per million, the ratio of the shifts experienced by two types of protons i and i' becomes

$$\frac{\Delta\delta_i}{\Delta\delta_{i'}} = \frac{F_{g_i}}{F_{g_{i'}}} = \frac{\Sigma 3\cos^2\theta_{ik} - 1/r_{ik}^3}{\Sigma 3\cos^2\theta_{i'k} - 1/r_{i'k}^3} \tag{3}$$

where the sum is extended over a sufficient number of monomer units on both sides of the one bearing i and i' to ensure convergence. This calculation is easy to perform numerically for comparison with the experiments. Four monomers on each side have been found sufficient for convergence in our calculations.

Nmr Spectra of PHB in the Presence of Shift Reagents. The nmr spectra of solutions of PHB in $CDCl_3$ at concentrations between 2 and 3% have been recorded on a Varian HA-100 nmr spectrometer with Me_4Si as a lock and reference signal. Small weights of the solid shift reagents were successively added to known quantities of the solution in the nmr tube.

Three different shift reagents from Pierce Chemicals have been used: $Eu(Fod)_3$, $Eu(thd)_3$, and $Pr(Fod)_3$. All resonances were shifted in the same direction, toward low fields for Eu derivatives and high field for the Pr chelate. Low-field shifts have been found more interesting since they increase the separation of H_A and H_B signals. $Eu(Fod)_3$ has given the best results due to higher solubility and restricted line broadening. A typical example of the shifts of the H_A and H_B proton is given in Figure 7. For all the three chelates analysis of the shifted spectrum (Table II) fails to reveal significant changes in J_{AC} and

Table I
Chemical Shifts in ppm and Coupling Constants in Hz for the Protons of PHB

	δ_{HA}	δ_{HB}	δ_{HC}	δ_{CH_3}	J_{AC}	J_{BC}	J_{AB}
$CDCl_3$	2.608	2.466	5.254	1.282	7.1	5.9	15.4
TFE	2.650	2.577	5.258	1.30	7.8	5.6	15.7

Table II
Coupling Constants for PHB in $CDCL_3$ in the Presence of Shift Reagents

Molar	$Eu(Fod)_3$				$Eu(thd)_3$	
Ratio:	1:17	1:9	1:6	1:4	1:6	1:4.5
J_{AB}	15.5	15.5	15.6	15.3	15.5	15.6
J_{AC}	7.3	7.2	7.0	7–7.3	7.2	7.4
J_{BC}	5.6	5.1	5.0	4.3–4.9	5.5	5.6

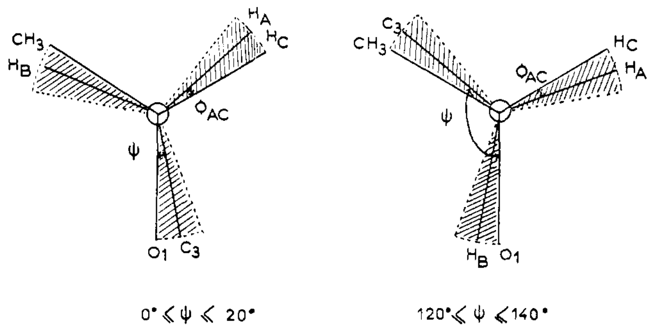

Figure 5. Possible conformation around the CH—CH_2 bond from the vicinal coupling constants.

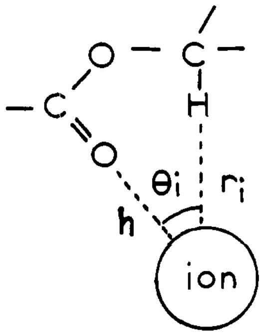

Figure 6. Position of the lanthanide ion with respect to the molecule.

J_{BC} which would be observed as a result of helix breaking upon complexation. Figure 8 gives a typical example of the shift of the four protons compared to a pure PHB solution as a function of the total lanthanide to carbonyl ratio. The curves are not linear, a fact which can be accounted both by a small equilibrium constant for complexation and by the break of the $N \gg n$ assumption. Since the ratios of the shifts do not vary appreciably over the whole range of relative concentrations, including the nonlinear part, no effort has been made to study separately the binding equilibrium. The ratios for the three shift reagents are given in Table III and found to vary only slightly with its nature, a necessary prerequisite for assuming pure pseudo-contact shift and axial symetry.[11]

Calculation of the Geometrical Factors for 2_1 Helices of Pitch 5.96 Å. A program has been written for a Univac 1108 computer which generates from bond lengths, bond angles, and a set of internal rotation angles ω, θ, ϕ, and ψ a sequence of PHB units. Selection of those set of angles leading to a 2_1 helix with a pitch close of 5.96 Å is made through calculation of $\mathbf{r}_{j,j-2}$ and $\mathbf{r}_{j,j-2}\cdot\mathbf{r}_{j,j-2}/(\mathbf{r}_{j,j-2})^2$, where $\mathbf{r}_{j,j-2}$ is the vector connecting two identical atoms in units j and $j-2$. A tolerance is chosen to keep with the possible distorsion of bond angles and bond lengths from their classical values and with the finite angular step in the span of the set of internal angles. For each selected set of atomic coordinates, averages of the geometric factors and their ratios are calculated according to relation (3). In the refinement stages an additional constraint concerning the

Table III
Relative Ratios of the Lanthanide-Induced Chemical Shifts of PHB Protons

	$\Delta\delta_{HC}/\Delta\delta_{HA}$	$\Delta\delta_{HC}/\Delta\delta_{HB}$	$\Delta\delta_{HC}/\Delta\delta_{CH_3}$
$Eu(Fod)_3$	1.43 ∓ 0.02	1.65 ∓ 0.02	2.94 ∓ 0.06
$Eu(thd)_3$	1.38 ∓ 0.02	1.39 ∓ 0.02	2.49 ∓ 0.04
$Pr(Fod)_3$	1.34 ∓ 0.03	1.65 ∓ 0.03	2.58 ∓ 0.03

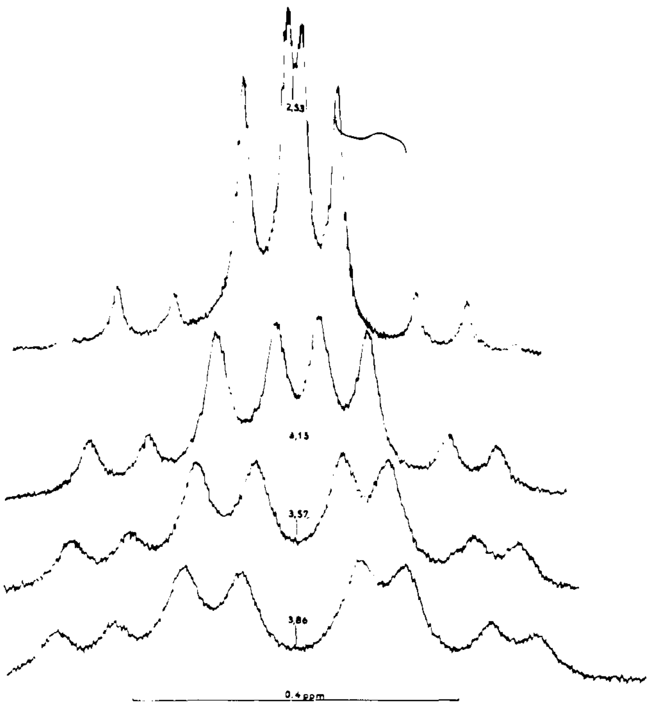

Figure 7. Nmr methylene signal with increasing additions of $Eu(fod)_3$.

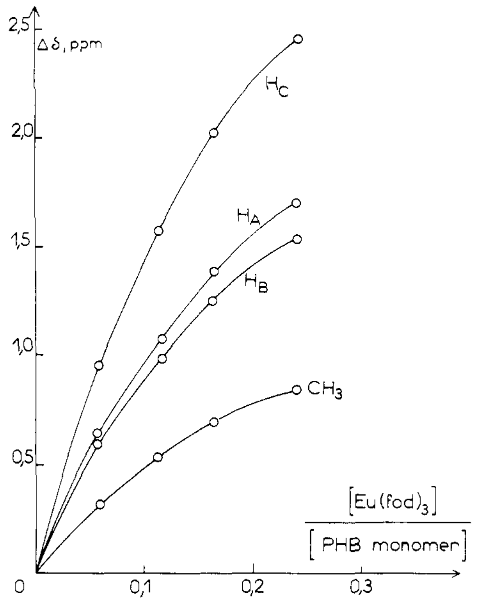

Figure 8. Shift of the PHB protons as a function of added $Eu(fod)_3$.

Table IV
Calculated Geometric Factor Ratios for Previously Proposed[2,3] Helical Models

	ω	θ	ψ	ϕ	$F_{\mathrm{gHC}}/F_{\mathrm{gHA}}$	$F_{\mathrm{gHC}}/F_{\mathrm{gHB}}$	$F_{\mathrm{gHC}}/F_{\mathrm{gCH_3}}$
I	0	322	123	149	1.842	2.018	2.925
II	4	240	211	41	1.224	0.702	3.117
III	36	318	220	247	1.512	0.978	2.319
IV	36	293	218	266	1.021	0.445	−0.313
Experiment Eu(Fod)$_3$					1.65	1.43	2.94

Table V
Sets of Internal Rotation Angles Giving Geometric Ratios Within 10% of Experiment

ω	θ	ψ	ϕ	$F_{\mathrm{gHC}}/F_{\mathrm{gHA}}$	$F_{\mathrm{gHC}}/F_{\mathrm{gHB}}$	$F_{\mathrm{gHC}}/F_{\mathrm{gCH_3}}$	h
16	330	116	152	1.684	1.438	3.098	3
24	322	116	148	1.654	1.472	2.911	3
24	322	120	144	1.624	1.435	2.879	3
12	330	124	144	1.696	1.425	2.880	3
12	330	128	140	1.672	1.400	2.802	3
20	314	136	128	1.617	1.453	2.807	3
20	302	144	116	1.599	1.502	2.917	3
Experiment Eu(Fod)$_3$				1.65	1.43	2.94	

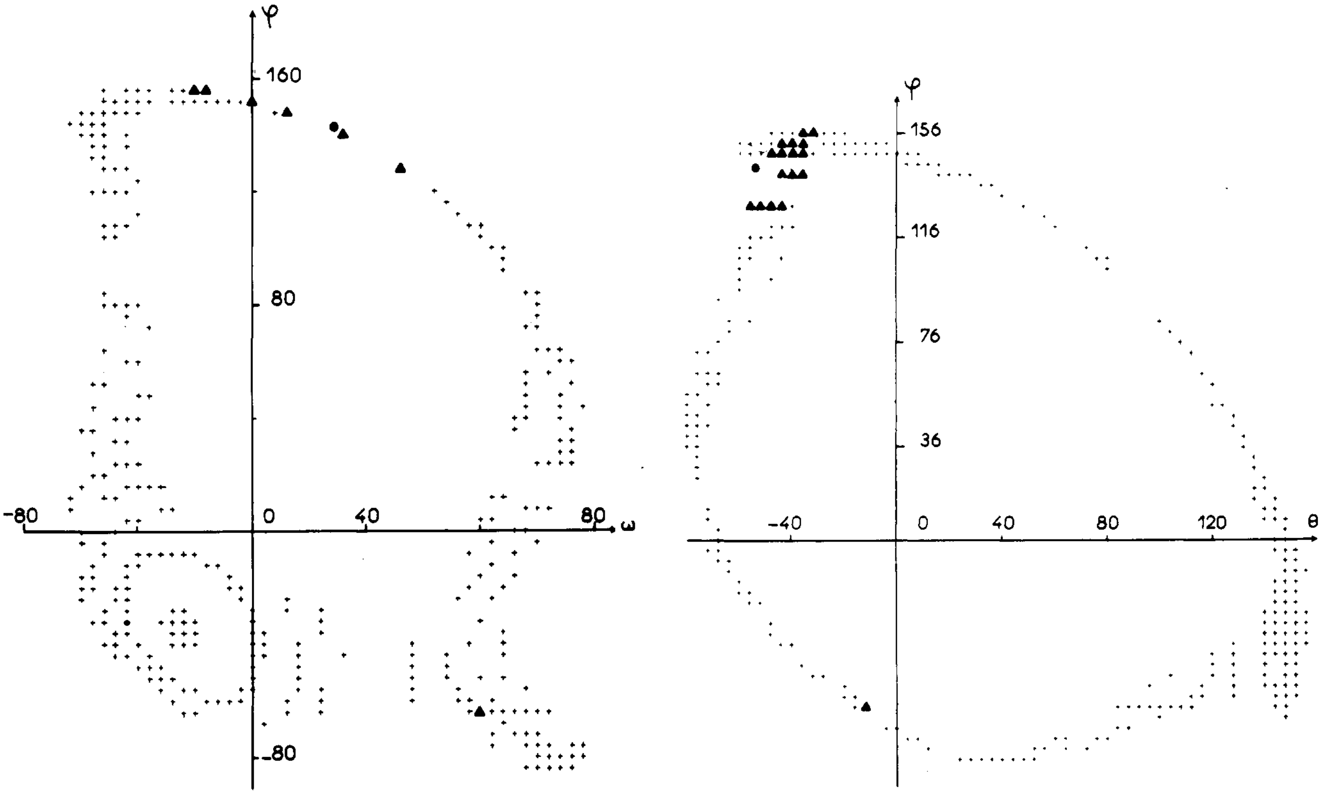

Figure 9. Regions of possible ω, θ, ϕ values for a 2_1 helix of pitch 5.96 Å for ψ = 124°.

distance between nonbonded and the sum of their Van der Waals radii can be introduced. The bond lengths and bond angles are the same as given by Cornibert.[2] For the methyl group the geometric factor is calculated assuming free rotation (average over 36 positions).

A first run with a value of ψ chosen in the two intervals predecently defined (ψ = 4° and ψ = 124°), a 4° angular step and a tolerance of 0.02 Å and 0.01 rad leads to the following results. (i) For ψ = 4°, it is impossible to build a 2_1 helix with the right pitch. (ii) For ψ = 124°, 395 solutions have been retained out of 72,000 sets of internal angles (computing time 2 hr). Each solution defines a point in the space ω, θ, ϕ. The two projections in Figure 9 show the density of points confined in toroidal space which is the geometrical transcription of the helical closure relation. Eighteen solutions for which all the calculated geometrical ratios fall within ±25% of the experimental values have been indicated on Figure 9. They define a narrow angular range

$$-20 < \omega < 50°$$
$$290 < \theta < 330°$$
$$120 < \phi < 160°$$

with the exception of one solution with

$$\omega = 74°$$
$$\theta = 352°$$
$$\phi = 298°$$

which can be rejected due to a methyl—oxygen distance of 2.98 Å, compared to a sum of Van der Waals' radii of

Table VI
Refinement of the Internal Rotation Angles

N	ω	θ	ψ	ϕ	h	$F_{\mathrm{gHC}}/F_{\mathrm{gHA}}$	$F_{\mathrm{gHC}}/F_{\mathrm{gHB}}$	$F_{\mathrm{gHC}}/F_{\mathrm{gCH_3}}$
1	24	322	116	148	3.0	1.654	1.472	2.911
					3.3	1.635	1.453	2.540
2	24	326	116	148	3.0	1.615	1.407	2.229
					3.2	1.606	1.401	2.638
3	24	322	120	144	3.0	1.624	1.435	2.879
					3.2	1.614	1.429	2.622
4	12	326	128	140	3.0	1.714	1.457	2.828
					3.2	1.700	1.454	2.599
5	12	330	128	140	3.0	1.672	1.400	2.802
6	20	318	128	136	2.9	1.665	1.474	2.950
					3.3	1.639	1.468	2.517
7	12	326	132	136	2.9	1.688	1.425	2.904
					3.1	1.680	1.430	2.657
8	20	318	132	132	2.9	1.627	1.434	2.922
					3.2	1.613	1.438	2.585
9	20	314	136	128	2.9	1.621	1.449	2.923
					3.2	1.603	1.455	2.608
10	20	318	136	128	2.9	1.591	1.400	2.902
					3.2	1.578	1.411	2.566
11	16	310	144	120	2.8	1.667	1.475	3.014
					3.2	1.628	1.487	2.612
Experiment Eu(fod)$_3$						1.65	1.43	2.94
Experiment Pr(fod)$_3$						1.65	1.34	2.58

Table VII
Final Set of Internal Rotation Angles (in deg)

	RMN in Soln This Work	Energy and Structure Factor Calcn Cornibert[2]	Mol Model and Structure Factor Calcn Yokouchi[3]
ω	$+20 \mp 4$	0	5
θ	$+318 \mp 4$	322	322
ψ	$+132 \mp 4$	123	128
ϕ	$+132 \mp 4$	149	133

3.35 Å, and to the high energy involved in such a distorsion of the ester group from planearity corresponding to $\omega = 0$.

A second run with no *a priori* limitation on ψ but a limitation in the range (−60°, +60°) for ω, based on energy considerations and (210°, 360°) for θ based on steric hindrance in dimers[2] selects 186 conformations out of 233, 280 (angular step 10°). None of them has values of ψ outside the interval (40°, 260°). Among them one finds conformations very close to all four conformations considered by Cornibert.[2] The geometrical ratios for these conformations have been computed and are presented in Table IV. Only conformation I with ψ in the range 120–140° which correspond to Cornibert's final choice leads to values within 25% of experiment. Retaining this level of accuracy a new restricted interval

$$0 < \omega < 40°$$
$$290° < \theta < 350°$$
$$112° < \psi < 144°$$
$$120° < \phi < 160°$$

has been spanned with an angular step of 4° and values of h of 3, 3.3, and 3.6 Å. Seven conformations with geometrical ratios within 10% of experiment are given in Table V. It must be remarked that no conformation with $\omega = 0$ leads to satisfactory agreement.

In the last interval defined by extreme values of Table V a final calculation has been made with h varying from 2.8 to 3.6 Å by 0.1 step assuming that experimental differences among shift reagents can be accounted for by small changes in the ion oxygen distance. Results are given in Table VI for the eleven best sets of internal angles. Conformations 5–8 are selected on the basis of Van der Waal's radii, leading to the final set of Table VII where the structures selected by Cornibert[2] and Yokouchi[3] have also been reported.[14]

Discussion and Conclusions

But for a rather large distorsion from ester group planearity our structure determination in solution falls very close to the results obtained from energy calculations and structure-factor calculations. This simultaneity brings further proof for PHB retaining largely its helical conformation in solution and establish the validity of the method for the study of local conformation in stereoregular polymers with a lone pair bearing atom. Among them there is a large number of linear polyesters which, as PHB, seem to present structures with a set of angles ω, θ, ψ, and ϕ very close to a TTGG conformation.[3] PHB happened to be a nearly ideal molecule due to the presence of its four magnetically different protons and of the methine—methylene protons coupling. For polyesters with a smaller number of distinguishable protons, there is however some hope to use the same technique, since our results can be used to derive the magnetic factor M and to interpret the absolute shifts.

An obvious improvement would be the use of ^{13}C resonance which would increase the number of topological points where the geometrical factor can be tested. Preliminary measurements both on PHB and methyl acetate show that the ^{13}C carbonyl shift is dominated by the contact interaction. There is however some hope to minimize the contact shift for more distant carbons, especially using Yb derivatives.[15]

The method seems therefore to deserve further study. In the case of our optically active polymer PHB, the more exact knowledge of the conformation in solution open new possibilities for the calculation of the rotatory strength associated with the n–π^* and π–π^* transitions in the helical form of PHB.[16]

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^{13}C Nuclear Magnetic Resonance Spectroscopy of Polydienes, Microstructure of Polybutadiene

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ABSTRACT: ^{13}C nmr spectra have been recorded of several polybutadienes and their hydrogenated analogs, with various amounts of 1,2 structure, but with a roughly constant ratio of cis-1,4 to trans-1,4 structures. From an assignment of these spectra a detailed picture has emerged of the sequence distribution of the cis- and trans-1,4 and the 1,2 units in the polybutadiene chain. It is shown that these units are distributed in an essentially random manner and that 1,2 units are head to tail incorporated.

The use of ^{13}C nmr as a tool for investigating the microstructure of polybutadiene was first reported by Duch and Grant.[1] Their initial observation was that *cis-* and *trans*-1,4-butadiene incorporation could be distinguished from the spectra since there is a difference of 5.4 ppm between cis and trans oriented methylene carbons and a difference of 0.7 ppm between the olefinic carbons. More recently Mochel[2] observed that 1,2-polybutadiene displays a set of chemical shifts completely different from that of 1,4-polybutadiene. This indicates the sensitivity of the ^{13}C chemical shift to differences in the polymer microstructure.

We have studied a number of polybutadienes with varied 1,2-butadiene contents in order to obtain more detailed information on the microstructure.

I. Experimental Section

Polybutadiene samples with various amounts of 1,2 structure were prepared by polymerization in hydrocarbon solvents or ethers (for a higher 1,2 content) with *sec*-butyllithium as initiator. Three of these polymers were completely hydrogenated.

For nmr purposes the polybutadienes were dissolved in CCl_4 and the hydrogenated products in a 1:1 mixture of $CHCl_3$ and CCl_4. ^1H nmr spectra were recorded on a Varian XL-100 and an HR-220 spectrometer. The ^{13}C measurements were carried out in part in a Varian HA-100 equipped with a Fourier transform accessory and in part on a Varian XL-100, using a CAT for spectrum accumulation. ^{13}C-enriched CS_2 was employed as an external ^{13}C lock for the HA-100 and deuterated methylene chloride provided the internal ^2H lock for the XL-100. The carbon spectra were all noise decoupled except when spectral analysis required partial decoupling of the protons.

II. Experimental Results and Analysis

A. Experimental Results. First of all the constitution of the polymers in terms of percentages of cis, trans, and vinyl structures was established with the aid of nmr. The vinyl content was readily calculated from 100-MHz ^1H nmr spectra; the cis and trans contents could be determined with 220-MHz ^1H nmr, ir, or ^{13}C nmr. The results of these measurements are summarized in Table I.

For the analysis of the microstructure both pulsed FT ^{13}C spectra and CW ^{13}C spectra were recorded. Although FT spectra have the advantage of a high signal-to-noise ratio (see *e.g.*, Figure 3), we often recorded CAT spectra to obtain the best possible spectral resolution, avoiding at the same time the dependence of the signal intensity on the pulse repetition rate (Figures 1, 2, and 4).

B. Spectral Analysis. In view of the complexity of the spectra at high vinyl content and the need for an unambiguous interpretation we carried out the analysis in three phases.

First, we eliminated the distinction between cis and trans structures by completely hydrogenating the polybutadienes. This resulted in a substantial reduction in the number of ^{13}C nmr lines to be identified (see Figure 1). Besides the fact that the resultant polymers were alkanes, for which abundant models studies and shift prediction formulae[3,4] are available, elucidation of their structure led to an understanding of the incorporation of 1,2 and 1,4 units in the original polybutadiene. To obtain a clear picture of cis-trans ordering in alkyllithium-based polybutadienes without interfering vinyl features, we reduced the vinyl content as far as possible. For this purpose we synthesized two polymers having a very low vinyl content (*ca.* 4%) and different cis:trans ratios (samples F and G in Table I). Their ^{13}C nmr spectra (Figure 2) could readily be interpreted in terms of cis-trans polyads.

Making use of the experience thus gained we attacked the more complicated problem of analysing the spectra shown in Figures 3 and 4. This analysis should throw some light on the cis-trans vinyl ordering in alkyllithium-based polybutadienes.