Conformational analysis of some aromatic copolyesters in solution by means of ^1H–^1H nuclear Overhauser effect experiments

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Conformational analysis was performed on some aromatic copolyesters in solution by means of ^1H–^1H nuclear Overhauser effect (NOE) measurements, using the cyclic dimer of isophthalic acid and ethylene glycol (DIMIS) as the model compound. From the observed NOE, a folding of the ethylene glycol towards the aromatic ring can be assumed for all the polyesters containing an isophthalic ring. In addition, the spatial interaction between adjacent aromatic rings, put forward in order to explain the chemical shift distribution observed in the ^1H n.m.r. spectra of these copolymers, has been demonstrated with steady-state NOE experiments. The average distance between protons on adjacent aromatic rings has been calculated by means of dynamic NOE measurements. The most probable conformation, giving rise to these NOE effects, has been postulated on the basis of a formalism introduced in a previous work.

(Keywords: ^1H n.m.r.; conformational analysis; nuclear Overhauser effect enhancement; aromatic polyesters)

INTRODUCTION

The importance of ^1H–^1H nuclear Overhauser effect (NOE) measurements as a tool for conformational analysis in organic chemistry and biochemistry is very well known. With the introduction of two-dimensional n.m.r. and with the increase in magnetic field strength this method has been greatly improved, making it possible to determine the three-dimensional structure even of small proteins in solution[1,2]. Surprisingly no equivalent studies can be found for synthetic polymers. For this class of compounds, many papers have appeared, where ^1H–^{13}C NOE measurements were used to probe motion distribution in the macromolecular chain in terms of correlation times[3–6]; others reported ^1H–^1H intermolecular NOEs as proof of the existence of polymer–polymer interactions in concentrated solutions of polymeric blends[7–10], but only very few papers have been concerned with the study of the macromolecular chain conformation.

Only Heffner *et al.*[11] reported a new ^{13}C and ^1H n.m.r. analysis, based mainly on 2D-NOESY, of the local conformation of poly(methyl methacrylate)–polystyrene copolymers. In our opinion, the difficulty in using ^1H–^1H NOEs for conformational analysis in polymers stems from the following. In high molecular weight structures like proteins, relaxation is dominated by slow molecular tumbling and consistent negative NOEs are found. In polymers, because of the lack of highly polar groups, the significant mobility of small segments of molecular chain, due to conformational jumps, shortens the correlation times. For this reason, polymers are usually situated on the low side of the motional narrowing region, where small NOEs are expected. From the variety of possible segmental motions in random coil polymers, a large distribution of correlation times is also expected that further decreases the observed NOEs.

In addition, the ^1H chemical shift dispersion is often inadequate to allow selective irradiation of the relevant peaks.

For the last few years we have been involved in microstructural studies, by means of ^1H n.m.r., of some aromatic polyesters formed by condensation of aliphatic dialcohols with different aromatic diacids[12]. The ^1H n.m.r. spectra of these copolymers show a chemical shift sensitivity to the sequence distribution of aromatic moieties, which can only be due to a ring-current shielding, caused by spatial interaction between two next neighbour aromatic units. In an attempt to directly prove this hypothesis, we performed ^1H–^1H NOE measurements in systems like poly(ethylene isophthalate-*co*-terephthalate) (PEIT, **1**) (see *Figure 1*), poly(ethylene isophthalate) (PEI, **2**), poly(ethylene terephthalate) (PET, **3**) and poly(oxydiethylene isophthalate-*co*-terephthalate) (PDIT, **5**) to be compared with the cyclic dimer of isophthalic acid and ethylene glycol (DIMIS, **4**), the reference model.

EXPERIMENTAL

Copolyesters were synthesized by melt polycondensation of ethylene glycol (PET, PEIT, PEI) or diethylene glycol (PDIT) with aromatic diesters in the presence of manganese acetate and antimony trioxide as catalysts, following a previously reported procedure[12].

DIMIS is a by-product of the PEI synthesis, collected as a white powder, sublimated on the cold part of the polymerization apparatus.

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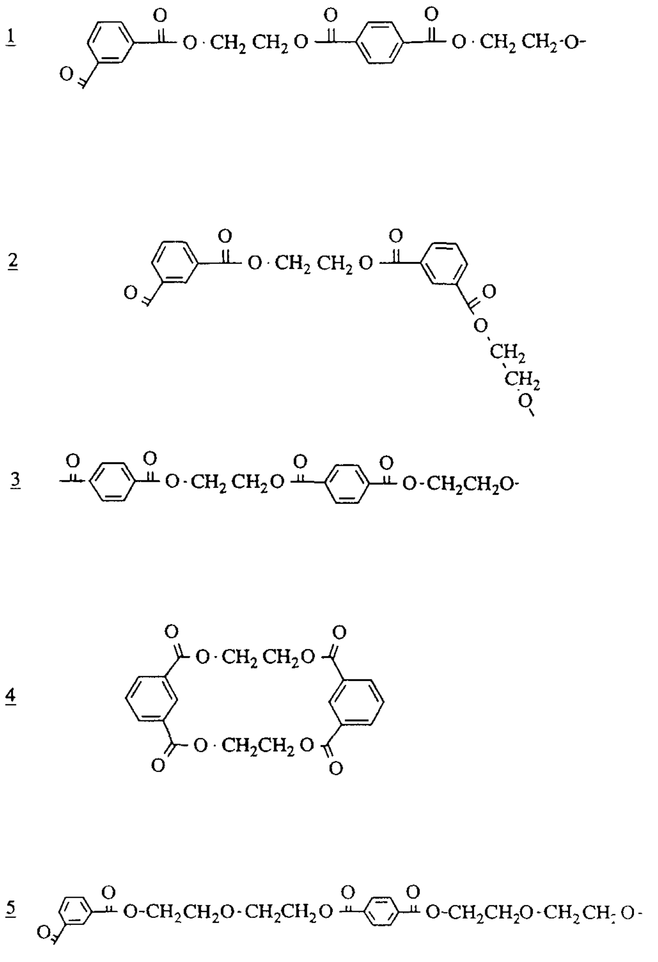

Figure 1 Aromatic copolyesters studied and DIMIS (**4**)

^{1}H n.m.r. spectra of **1**, **2**, **3**, **4** and **5** were obtained from 5% wt/v solutions in 99.5% deuterated 1,1,2,2-tetrachloroethane on a Bruker AM 300 spectrometer. Chemical shifts were referred to the solvent proton signal, taken at 6.0 ppm. ^{1}H spin-lattice relaxation times were measured with the standard inversion–recovery pulse sequence (180°–τ–90°) with a delay of 15 s between scans at 70°C. ^{1}H–^{1}H steady-state NOEs were measured, at 70°C, by presaturation of each resonance (every individual transition in the H_b and H_c multiplets), subtraction of the off-resonance spectrum from the presaturated spectrum and automatic integration of the difference peaks. NOE enhancements for each signal were calculated according to the formula:

$$f(I)=\frac{I-I_0}{I_0}\times 100$$

where $I-I_0$ is the difference peak area and I_0 is the off-resonance peak area. The f(I)s obtained for the different irradiation frequencies in the H_c and H_b multiplets were averaged to a single value.

The experiment was repeated three times for each sample. Experimental conditions were: spectral width 3000 Hz; pulse width 13 μs (90°); delay time 5 s; and low-power (35 dB of attenuation for a 0.2 W decoupler power) irradiation time 3 s. A total of 160 scans (in 10 cycles) was acquired for each irradiated resonance.

Driven truncated NOE experiments (TOE) were performed by selective irradiation of the signals H_a, H_c and H_d and mixing time ranging from 0.5 to 3 s.

RESULTS

^{1}H n.m.r. spectra

Figure 2 shows the ^{1}H n.m.r. spectra of all the compounds studied. The $-CH_2-$ signals fall in the region between 4.00 ppm and 3.00 ppm for **1**, **2**, **3** and **4**. In PDIT two $-CH_2-$ signals are observed: the signal at 4.53 ppm is assigned to the outer CH_2, the other (3.90 ppm) is assigned to the inner ones. In the region between 9.00 ppm and 7.00 ppm all the aromatic signals are observed.

As already noted[12], the ^{1}H n.m.r. spectra of **1** and **5** show an unexpected multiplicity of signals, explicable only in terms of sensitivity to the sequence distribution of aromatic residues: the H_c triplet ($J_{H_c,H_b}=7.0$ Hz) is split into three resonances, shifted 1.7 Hz apart in PEIT (*Figure 3*) and 2.7 Hz apart in PDIT, and corresponding to the TIT, IIT and III triads, respectively, where T stands for terephthalic and I for isophthalic acid. Smaller shifts are also observed for H_a and H_b.

As already pointed out in the Introduction, this chemical shift sensitivity, noticeable also in PDIT where next neighbour aromatic units are very far apart, can only be ascribed to a direct spatial interaction of aromatic rings, a consequence of folding around the aliphatic spacer.

A transmission of long-range effects through the π-electron system, as observed by other authors[13,14], can be neglected in these cases, because of the number of saturated carbons between the observed aromatic proton and the next neighbouring aromatic unit (the iso- or tere- aromatic ring).

In order to prove the existence of this aromatic interaction, causing a ring-current shielding, ^{1}H–^{1}H

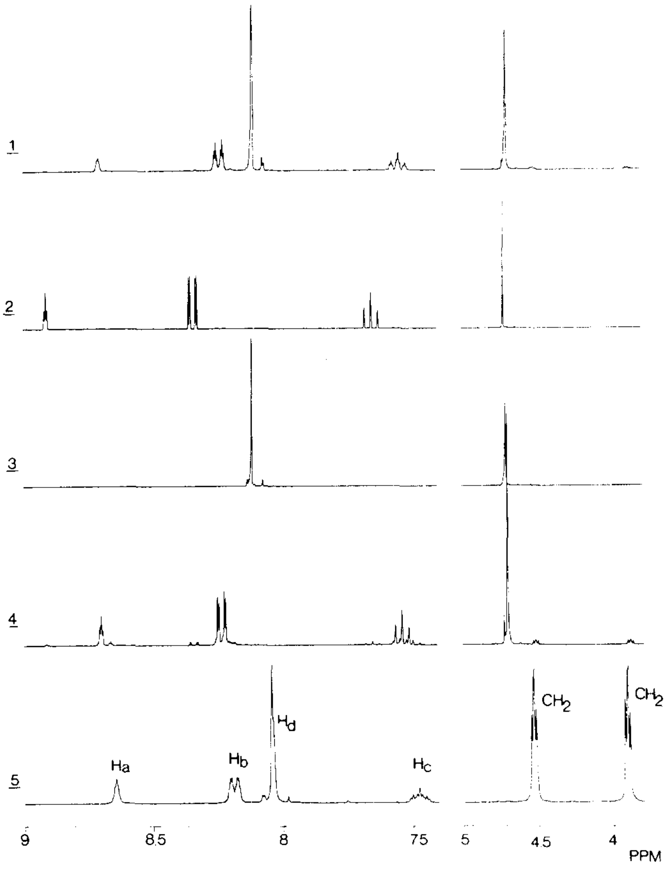

Figure 2 ^{1}H n.m.r. spectra of: (**1**) PEIT; (**2**) PEI; (**3**) PET; (**4**) DIMIS; (**5**) PDIT

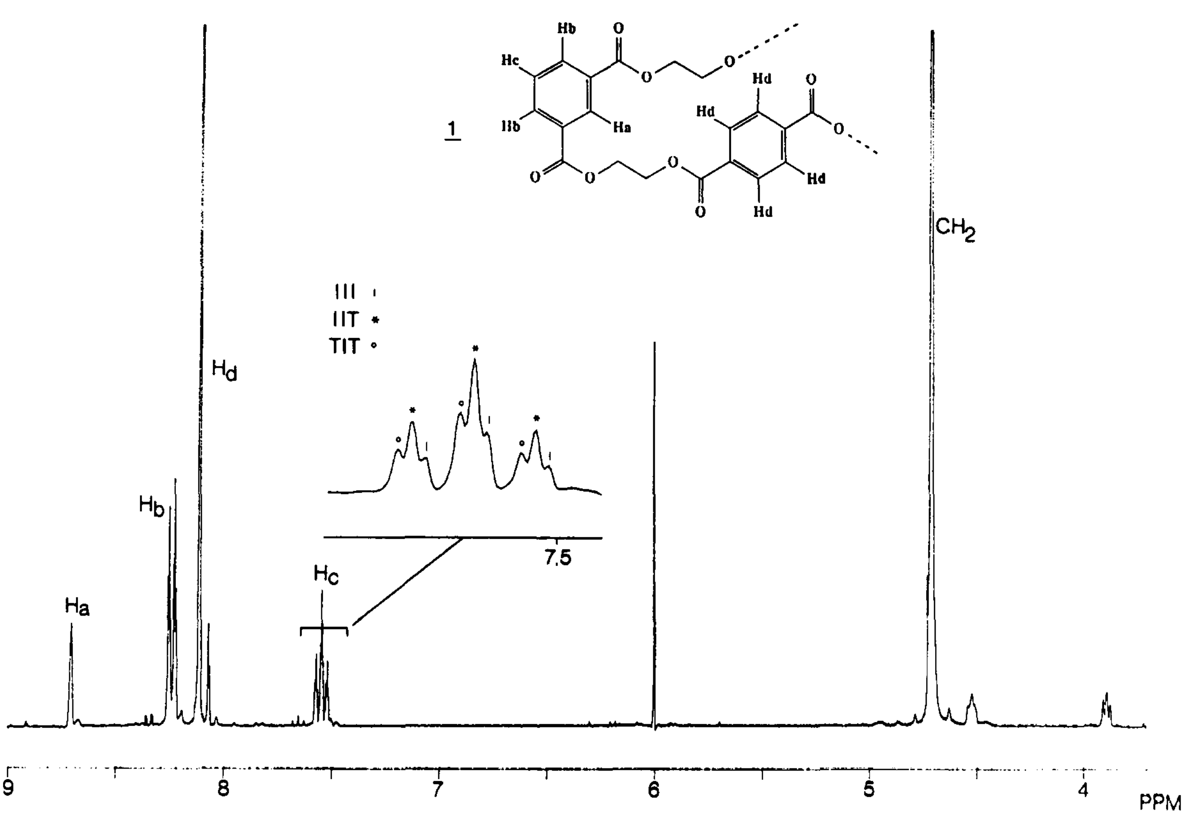

Figure 3 ^1H n.m.r. spectrum of PEIT (**1**) — the assignment is shown on the chemical structure. The insert shows the enlarged area of the H_c signal

NOE enhancements were evaluated in all the polyester samples.

^1H-^1H steady-state NOE

Figure 4 shows a steady-state NOE experiment, performed on PEIT: the standard spectrum (aromatic region) is shown in *Figure 4a*; in the other parts difference spectra obtained by subtracting the off-resonance spectrum from the pre-irradiated one are reported, with irradiation frequency, respectively, on H_a (*Figure 4b*), H_b (*Figures 4c′* and *c″*, two irradiated resonances), H_d (*Figure 4d*), H_c (*Figure 4e*) and $-CH_2-$ (*Figure 4f*).

Observed NOEs between interacting nuclei are always positive, a consequence of the fact that the correlation times of the investigated structures meet the extreme narrowing conditions ($w\tau_c < 1$), and that mainly dipolar relaxation occurs[15].

Table 1 gives all the NOEs observed in samples **1**, **2**, **3**, **4** and **5**. By presaturation of H_a, a NOE enhancement on $-CH_2-$ is observed in all the compounds containing the isophthalic residue, decreasing by presaturation of H_b and H_c.

In DIMIS, the cyclic structure leads to a folding of the ethylene glycol towards the aromatic ring on the H_a side. This NOE enhancement is therefore expected. But the same conformation (*Figure 5*) can be assumed also for polymers **1**, **2** and **5**, as the same behaviour, in terms of NOE contacts, is observed.

In PET (**3**), the lower NOE between H_d and the $-CH_2-$ group (0.9) is consistent with an average conformation where the aromatic ring and the glycolic portion are more distant.

By presaturation of H_b, an expected very strong NOE enhancement is observed on the *ortho* proton, H_c, in all the compounds; a weaker NOE is also observed on H_a (*meta* proton).

What is more important is the NOE observed in PEIT on H_a by presaturation of H_d: this is a direct indication of the spatial proximity of next neighbour aromatic rings.

It should be noted that the NOE enhancement on H_a from H_d is visible while the symmetric one (from H_a to H_d) is not. This is not surprising because, while H_a is an isolated proton and therefore a long-range NOE on it can be easily detected (if sufficiently high), protons H_d have other close protons (the other H_d) with which the cross-relaxation is much more efficient.

In PEIT by presaturation of H_a, a significant NOE on H_b is observed (1.3). In DIMIS this same NOE is slightly lower (1.1). Because of its small dimensions, DIMIS should have shorter correlation times than PEIT. By assuming NOEs only stemming from direct interactions between protons on the same aromatic ring, higher values of NOE should be expected in DIMIS than in PEIT. Therefore, additional enhancements arising from dipolar interactions between H_a and H_b belonging to adjacent isophthalic rings in the polymer, can be assumed (intermolecular NOEs can be excluded because of the low concentration).

The NOE from H_a to H_c is weak in any case, thus excluding any close interaction between these protons, including one between different aromatic rings.

Kinetic NOE measurements on PEIT

In order to evaluate the average distance between the aromatic protons, H_a and H_d, a TOE (truncated driven experiment) was performed. In the TOE experiment[15], the presaturating magnetic field is gradually increased; consequently, the NOE increases to reach its steady-state value. At short times, only the cross-relaxation rate

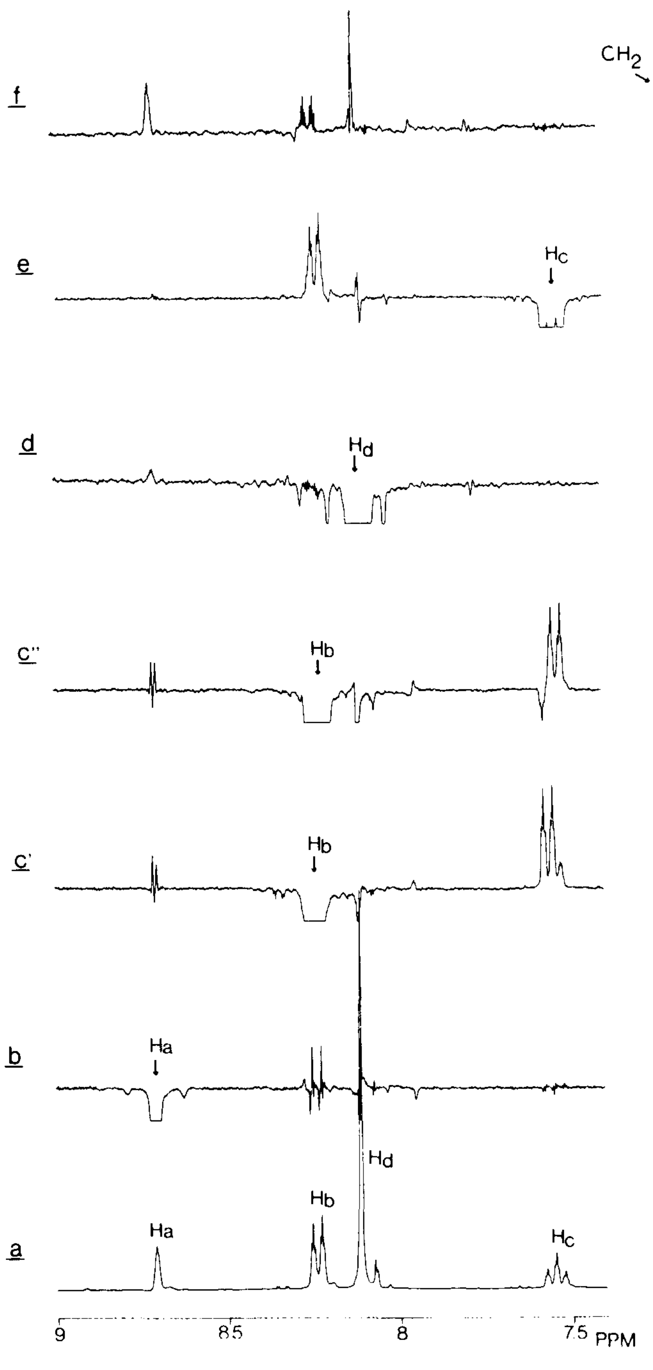

Figure 4 ^{1}H steady-state NOE experiment of PEIT (**1**): (a) normal spectrum; (b) NOE difference spectrum with pre-irradiation of H_a; (c′, c″) pre-irradiation of H_b (the two lines of the doublet); (d) pre-irradiation of H_d; (e) pre-irradiation of H_c; (f) pre-irradiation of $-CH_2-$

between the protons controls the size of the enhancement, while at longer times other relaxation sources also start to become relevant, in opposition to the enhancement.

Therefore, in the early stages of the build-up of the NOE, an unknown interatomic distance can be directly obtained with the following formula, providing that, in the same structure, another NOE effect is observable between two protons whose distance is known and fixed, and providing that the correlation times for the two pairs of protons are the same:

$$\frac{f_a\{b\}}{f_c\{d\}}=\frac{(r_{ab})^{-6}}{(r_{cd})^{-6}}$$

where r_{ab} is the unknown distance, r_{cd} the reference distance, and $f_a\{b\}$ and $f_c\{d\}$ are the two corresponding enhancements.

Our molecular structure is suitable for this kind of calculation, because there is another clearly observable NOE effect between two aromatic *ortho* protons, which is one of the most widely used reference systems.

Regarding the correlation times, the relaxation of the aromatic protons of the terephthalic unit can also be affected by internal rotation of the aromatic ring about the 1,4-phenylene axis, while a reduction of this phenyl group mobility can reasonably be supposed for the isophthalic unit. For this reason the correlation times of the two aromatic units could also be different.

In order to perform only a qualitative examination of the correlation times, $T_1(^{13}C)$ were measured for all the protonated carbons of PEIT at 70°C (*Table 2*). Although

Table 1 NOE enhancements[a]

Saturated proton	Compound	H_a	H_b	H_d	H_c	$-CH_2-$	$-CH_2-$
H_a	**1**		1.3	0.0	0.0	2.6	
	2		0.4	–	0.1	2.5	
	3		–	–	–	–	
	4		1.1	–	0.2	3.7	
	5		0.8	0.0	0.6	1.7	1.6
H_b	**1**	0.8		[b]	7.9	0.7	
	2	0.1		–	6.5	0.2	
	3	–		–	–	–	
	4	0.2		–	6.0	0.7	
	5	0.0		[b]	6.8	1.0	1.1
H_d	**1**	0.7	[b]		0.0	0.8	
	2	–	–		–	–	
	3	–	–		–	0.9	
	4	–	–		–	–	
	5	0.0	[b]		0.0	0.9	0.6
H_c	**1**	0.2	11.1	0.1		0.1	
	2	0.0	10.5	–		0.2	
	3	–	–	–		–	
	4	0.0	13.4	–		0.1	
	5	0.1	13.0	0.0		0.0	0.0
$-CH_2-$	**1**	1.5	0.7	0.6	0.0		
	2	0.4	0.2	–	0.1		
	3	–	–	−0.4	–		
	4	0.1	0.1	–	0.0		
	5	0.2	0.1	0.2	0.2		4.1
		0.1	0.2	0.2	0.1	4.9	

[a] The enhancement values are not corrected for incomplete suppression

[b] Enhancement values affected by partial presaturation of the signals, too close to the irradiation sites. Their intensity is decreased by ~10%

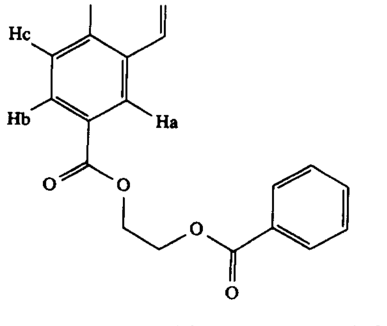

Figure 5 Conformation of the ArCO–O–CH_2–CH_2–O fragment in DIMIS

Table 2 T_1 relaxation times (s) for ^{13}C in PEIT

Compound	C_b	C_a	C_d	C_c	CH_2
1	0.31	0.46	0.53	0.47	0.20

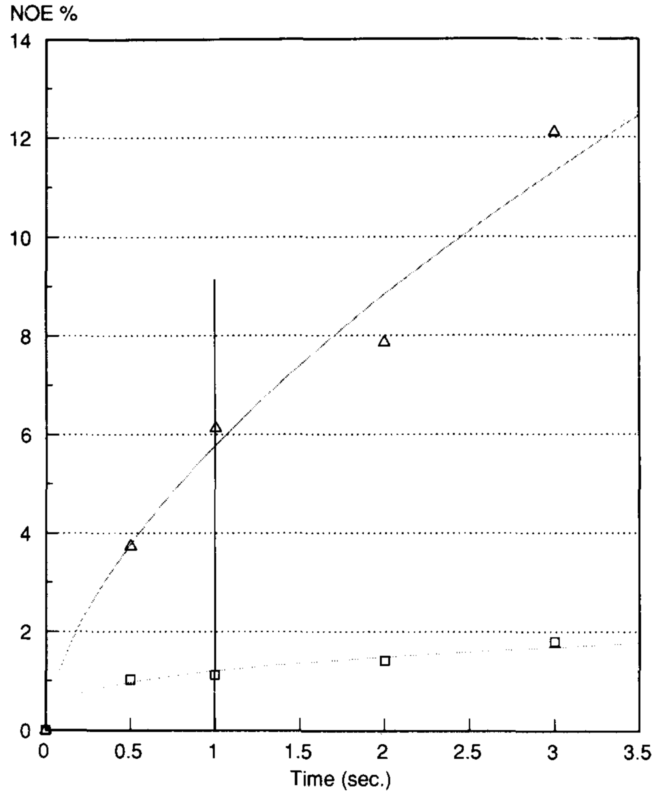

Figure 6 Truncated driven NOE experiment on $H_a\{H_d\}$ (□) and $H_c\{H_b\}$ (△). At 1 s the 'initial rate approximation' can be applied (sharply increasing part of the curve)

it is confirmed that the T_1 relaxation times for the carbons of the terephthalic aromatic ring (C_d) are slightly higher than the others, and therefore an additional motional process can be assumed to contribute to the relaxation of these nuclei, the difference is not so substantial as to void the assumption that the motion of the different interatomic vectors can be described by a single correlation time.

In *Figure 6* the NOE build-up for $H_a\{H_d\}$ and for $H_c\{H_b\}$ is reported. At a presaturation time of 1 s, we are in the sharply increasing part of the curve and the 'initial rate approximation' can be applied. An average distance between 3.25 Å and 3.45 Å can therefore be derived for the H_a–H_d pair of protons, assuming that the reference distance between the *ortho* protons, H_c–H_b, is 2.80 Å. Obviously this is the average distance over all the different conformations that the molecular structure assumes during the course of the n.m.r. observation time.

With the same kind of procedure, the expected NOE between the *meta* protons, H_a and H_b, can be calculated, assuming a distance of 4.3 Å between them. The significant difference between the calculated value of 0.23, and the experimental enhancement value, in the approximation conditions, of 0.89, further supports the hypothesis of an interaction between next neighbour isophthalic units, analogous to that found between iso- and terephthalic rings.

One-dimensional transient NOE and 2D-NOESY were also performed on PEIT. These experiments are known to be less sensitive than TOE[15] and in fact in the 2D-NOESY reported in *Figure 7* (mixing time of 500 ms) only the cross-peaks due to the expected NOEs between protons in the same aromatic ring are visible. *Table 3* gives the detected T_1 for each kind of proton. They follow the expected values.

DISCUSSION

Conformational analysis on solid PET, mainly made by means of Fourier transform infra-red spectroscopy, Raman spectroscopy and X-ray diffraction, in the amorphous or crystalline state, has been previously reported[16,17]. The generally accepted conclusions are:

1. in amorphous PET, the ethylene glycol unit has a *gauche* conformation, while in crystalline PET it has a mostly *trans* structure;
2. the phenylene/carboxylate conformation is essentially planar, in order to gain the maximum stabilization through π-orbital overlap;
3. the carboxylic groups on either side of the phenylene rings are roughly 50% in the *cis* conformation, in unoriented PET.

The conformational structure of PET in solution was derived by Štokr *et al.* on the basis of i.r. and ^{13}C n.m.r. studies[18]. They described, besides the configuration of

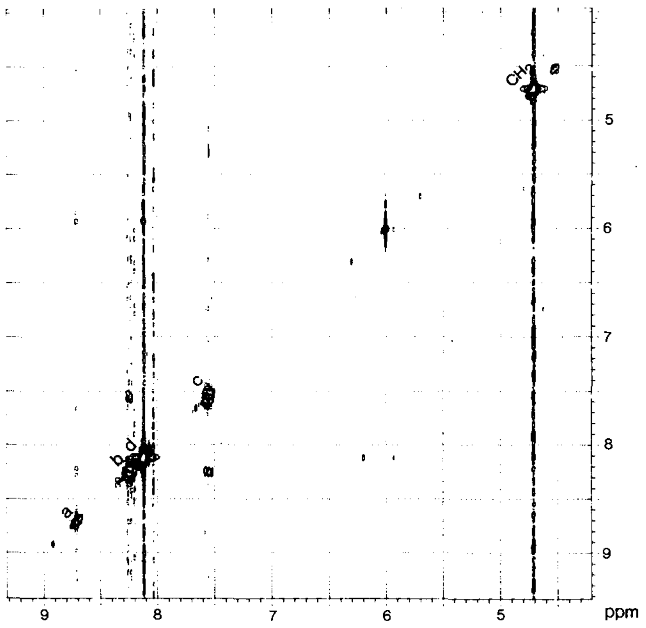

Figure 7 2D-NOESY on PEIT (mixing time 500 ms; spectral width 3000 Hz; 128 scans acquired for each T_1 value)

Table 3 T_1 relaxation times (s) for ^1H

Compound	H_a	H_b	H_d	H_c	CH_2	CH_2
1	7.9	2.7	2.2	1.5	0.4	
2	5.5	4.1	–	2.4	0.8	
3	–	–	1.9	–	0.4	
4	5.7	2.3	–	1.3	0.4	
5	4.4	2.1	1.8	1.3	0.6	0.5

the aromatic ring (*Figure 8a*), the conformational distribution of the CO–O–CH_2–CH_2–O–CO fragment in terms of *gauche* or *trans* positions of the substituents relative to two different bonds: the COO–CH_2 (*Figure 8b*) and the OCH_2–CH_2O (*Figure 8c*).

In particular, they concluded that PET in solution is ~63% in the form tGt, 26% in the form gGt and 7% in the form tTt, where t and g stand for a *trans* or a *gauche* conformation around the COO–CH_2 bond, and G and T refer to the glycolic bond OCH_2–CH_2O (predominantly in a *gauche* conformation) (*Figure 9*). No information was given on the relative position of the aromatic and the –CH_2– groups around the CO–O bond.

From our studies we can add that the modest NOE enhancement observed between aromatic and glycolic protons in PET can be ascribed to the existence of conformations where these groups are *cis* around the –CO–O– bond, irrespective of the gGt or tGt conformation of the glycolic portion (*Figure 10*).

The same conclusions can also be drawn for polymers containing an isophthalic ring: only a *cis* conformation around the CO–O bond explains the detected NOE between the glycolic fragment and the aromatic proton H_a, this being valid either for a tGt conformer or for a gGt one. But, since the NOE between the aromatic protons and the CH_2 is higher in the isophthalic residue than in the terephthalic one, we suggest a higher population of the *cis* conformation in the first case than in the second one.

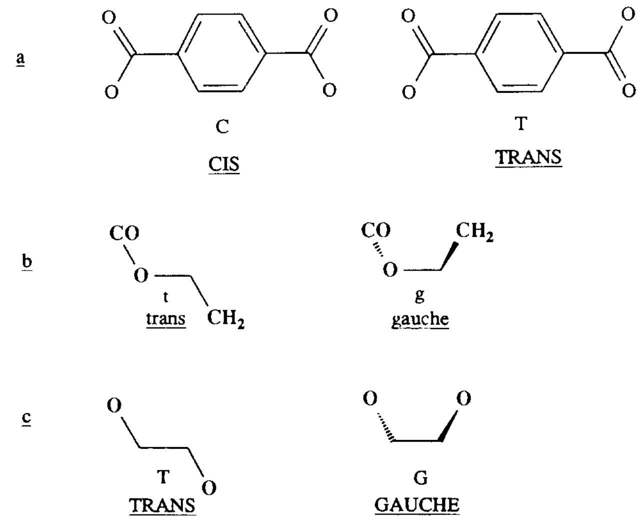

Figure 8 Degrees of conformational freedom in PET, as reported by Štokr *et al.*[18]

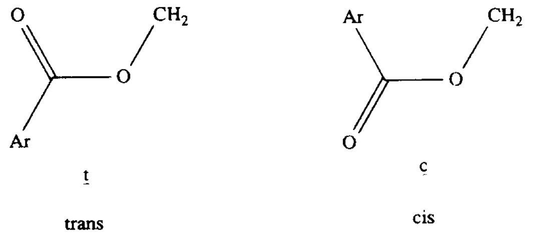

Figure 10 Additional conformational freedom in PET about the ArCO–O bond

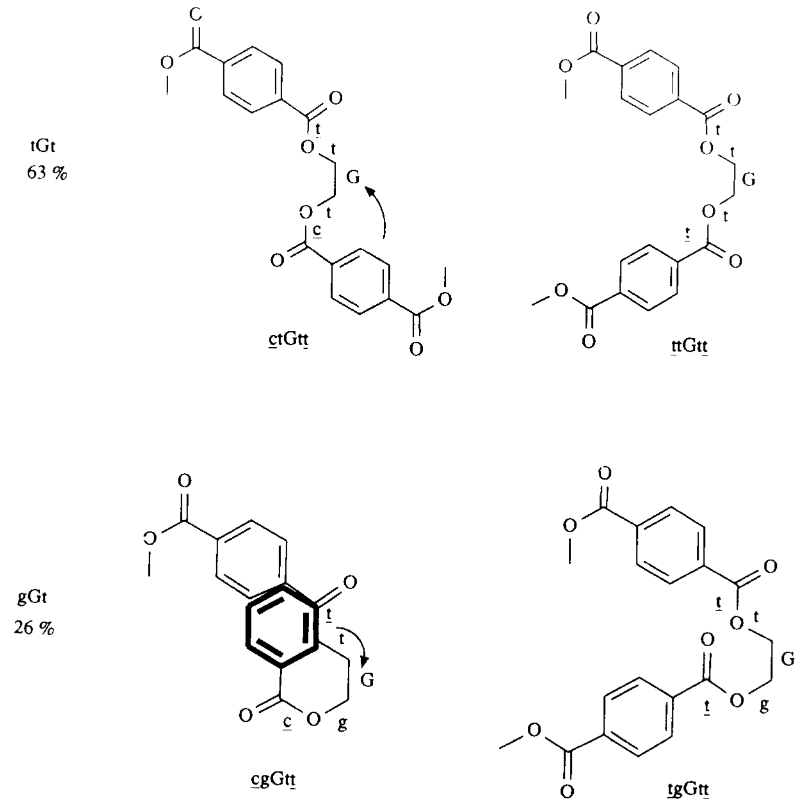

Figure 9 The tGt and gGt conformations of PET. In the upper part of the figure the two possible conformations around the ArCO–O bond are indicated, only for one of the two ArCO–O bonds; the other ArCO–O bond is always kept *trans* for simplicity. In the lower part of the figure the arrows indicate where the $CH_2\{H_a\}$ NOEs are produced. The only conformation where the two aromatic rings are very close to each other is the cgGtt

Conversely, in order to detect a NOE between adjacent aromatic rings, the presence of the cgGtt or cgGtc conformations is required, since in the tGt conformation (*Figure 9*) the aromatic protons are too far away. Of course, the tGt is always the most probable conformation but, not producing NOE effects between aromatic protons, its presence cannot be evaluated.

CONCLUSIONS

Although only a few papers have been published on this subject, ^1H–^1H NOE measurements on aromatic polyesters proved to be useful in adding a new insight into the conformation of macromolecular segments.

In fact steady-state NOE experiments on PEIT and PDIT point to a greater presence of a *cis* conformation around the ArCO–O bond for the isophthalic residue than for the terephthalic one. Furthermore, the probable spatial interaction between adjacent aromatic rings, postulated in order to explain the sensitivity to the sequence distribution observed in the ^1H n.m.r. spectra of these copolymers, has been demonstrated with NOE steady-state experiments and quantified with TOE experiments (3.25–3.45 Å, on average). These results, together with the previously reported ones on PET, suggest that cgGtt and cgGtc are the conformations responsible for the detected NOEs between aromatic protons.

From the ^1H n.m.r. spectra, it can be observed that the displacement of chemical shift due to the sequence distribution is more evident for H_c than for H_a. Conversely, the strongest NOE enhancement is between H_d and H_a.

According to Heffner *et al.*[11], this is not contradictory because ring-current shielding requires specific geometric conditions (position in the shielding cone) which can be more properly satisfied by H_c than by H_a. In addition, the H_a–H_d distance is shorter, according to the NOE data.

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