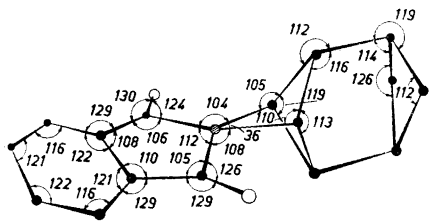

Fig. 3. Schematical drawing showing bond angles.

and 3) of the phthalimide part of the molecule are fairly resonable (σ(dist.)$\sim$0.01 Å, σ(angle)$\sim$1°), the r.m.s.-amplitudes of Table 1 and the distance $N-C_{9B}$ of 1.63 Å (Fig. 2), suggest that also this part is disordered (Fig. 1).

Several unsuccessful attempts have been made to refine disordered structures, starting with models satisfying the requirement of two equal N−C distances of about 1.49 Å. It is felt that the methods of calculations rather than wrong assumptions are responsible for the lack of convergence.

A list of observed structure factors is available by request to the author.

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Phosphorus Spin-Lattice Relaxation in Phosphorus Trioxide

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1. The liquid state. Recently Mowthorpe and Chapman [1] presented a spin-lattice relaxation study on phosphorus trioxide, P_4O_6. They concluded that the ^{31}P spin-lattice relaxation time is controlled by the spin-rotation interaction. This might well be the case; however, their conclusion rests on few experimental results, and apparently on erroneous formulae for the contribution to T_1 from the dipolar and spin-rotation interactions. Furthermore, the two reported T_1 values (11.3 and 11.7 s) measured at 'ambient' temperature, are certainly too small because we have measured T_1 values at 9 MHz by the $90°-\tau-90°$ pulse technique, from 17.0 to 20.5 s in supercooled P_4O_6 (see Fig. 1). The change in T_1 was small in the limited temperature range studied, but T_1 appeared to decrease slowly with temperature.

All possible care was taken in preparing the sample. In spite of this, it showed traces of red phosphorus after having been exposed to light for some time. An extremely pure sample of P_4O_6 might well be found to have even larger T_1 values.

The dipolar contributions to T_1 for P_4O_6 are given by [2]

$$\frac{1}{T_{1\,\mathrm{d\ intra}}} = \frac{9h^2\gamma_p^4}{8\pi^2 r_{p-p}^6}\,\tau_d \qquad (1)$$

$$\frac{1}{T_{1\,\mathrm{d\ inter}}} = \frac{6h^2\gamma_p^4 N\eta}{5kT} \qquad (2)$$

These equations are based on the assumptions of Brownian motion of molecules and incoherence of the various spin-spin interactions.

The correlation time, τ_d, for reorientation can be estimated from the Debye formula $\tau_d = 4\pi\eta a^3/3kT$. The 'radius', a, of the molecule is obtained from the melting point density assuming close packing. With $a^3 = 3.32\times10^{-23}$ cm^3 and $\eta_{21} = 0.025$ poise we find $\tau_d = 8.6\times10^{-11}$ s. Using eqns. (1) and (2) with $r_{p-p} = 2.98$ Å and $\varrho_{21} = 1.943$ we obtain the following contributions to T_1 at 21°C.

$$\frac{1}{T_{1\,\mathrm{d\ intra}}} = 8.4\times10^{-3}\ \mathrm{s}^{-1};\quad \frac{1}{T_{1\,\mathrm{d\ inter}}} = 2.4\times10^{-3}\ \mathrm{s}^{-1} \qquad (3)$$

The total dipolar contribution thus amounts to about 92.5 s at room temperature.

According to Hubbard [3] the spin-rotation contribution is given by

$$\frac{1}{T_{1\,\mathrm{sr}}} = \frac{8\pi^2 I_0 kT}{3\,h^2}\,(2c_\perp{}^2+c_{||}{}^2)\,\tau_{\mathrm{sr}} \qquad (4)$$

where the correlation time for the molecular angular velocity, τ_{sr}, is related to τ_{d} by the expression $\tau_{\mathrm{d}}\tau_{\mathrm{sr}}=I_0/6kT$. These equations are valid for a spherical molecule undergoing isotropic Brownian reorientation when $\tau_{\mathrm{d}}\gg\tau_{\mathrm{sr}}$. With $I_0=7.88\times10^{-38}$ cm^2 g which is the principal moment of inertia for P_4O_6, we get $\tau_{\mathrm{sr}}=3.8\times10^{-15}$s.

Assuming that the spin-lattice relaxation is controlled by the dipolar and spin-rotation interactions we obtain $T_{1\,\mathrm{sr}}=20.8$ s since $T_1=17$ s and $T_{1\,\mathrm{d}}=92.5$ s at 21°C. Eqn. (4) then gives 13.0 kHz for the spin-rotation interaction constant which is a rather small value.[4] It is often found that the Debye formula gives a maximum value for τ_{d}. In some cases values for τ_{d} which are from two to six times smaller than those obtained by the Debye expression have to be postulated.[4,5] If that is the case for P_4O_6 the spin-rotation interaction will be the dominant relaxing mechanism and thus yielding 14.4 kHz for the spin-rotation interaction constant. A few spin-lattice relaxation time measurements on liquid P_4 have been reported [6] and T_1 was found to fall with temperature. This was not explained satisfactorily; however, the possibility of a spin-rotation interaction was mentioned. No definite conclusion concerning P_4O_6 can be drawn, however, before T_1 measurements have been performed over an extended temperature range.

2. The solid state. The ^{31}P spin-lattice relaxation time of solid phosphorus trioxide has been measured at 9 MHz in the temperature range 21°C to −19°C (see Fig. 1). T_1 increases very rapidly from 40 s to 290 s for temperatures down to 14°C. Thereafter T_1 levels off gradually and reaches a value of about 600 s at −19°C. The steep rise in T_1 just below the melting point indicates that the first two T_1 values actually were measured in the premelt. The line width was broad and seemed to change only slightly in the temperature range studied. This indicates that the translational diffusion can be neglected and that the relaxation probably is caused by interactions modulated by molecular reorientation.

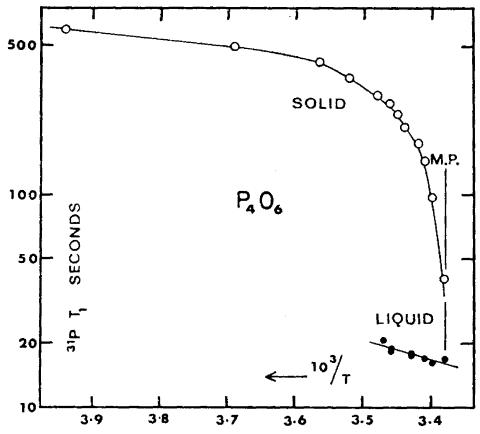

Fig. 1. The ^{31}P spin-lattice relaxation time in supercooled and solid P_4O_6 at 9 MHz. M.P. stands for melting point.

It is known that simple tetrahedral molecules exhibit nearly isotropic reorientation in the solid state.[7] This is also found to be the case for the P_4 tetrahedra.[6,7] The P_4O_6 molecules are reasonably spherical with a 'radius' of about 3.2 Å. The assumption of a fairly unhindered rotation of the molecules in the solid at temperatures not too far from the melting point, seems therefore plausible.

If an isotropic Brownian reorientation is assumed in the solid, the dipolar contribution to the spin lattice relaxation is easily found to be [2]

$$\frac{1}{T_1} = \frac{9\gamma_{\mathrm{p}}{}^4h^2}{20\pi^2 r_{\mathrm{p-p}}{}^6\omega_{\mathrm{p}}{}^2\tau_{\mathrm{d}}} = 1.3\times10^{-8}\ \tau_{\mathrm{d}}{}^{-1} \qquad (5)$$

at 9 MHz since $\omega_{\mathrm{p}}\tau_{\mathrm{d}}\gg1$ in the temperature region considered. This gives the correct temperature dependence for τ_{d}, and the graph of ln τ_{d} against $1/T$ could then consist of two straight lines of different activation energies. This sounds reasonable if the isotropic rotational motion of the molecules 'freezes out' gradually and becomes more restricted around 14°C. Using eqn. (5) τ_{d} is found to be of the order of 10^{-5} to 10^{-6} s in the measured temperature range. It would be interesting to measure T_1 at a different frequency to see if T_1 is frequency dependent as eqn. (5) predicts.

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Determination of the Rate Constants for Alkylation of DNA *in vitro* with Methanesulfonic Esters

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The mutagenic alkylating agents, methyl, ethyl, and isopropyl methanesulfonate (MMS, EMS, and iPMS, respectively) give quantitatively and qualitatively different effects both with biological materials [1,2] and with DNA *in vitro*.[3,4] The site of alkylation of DNA for these substances is supposed to be at different groups, *i.e.* unesterified phosphate oxygens and purine base nitrogens.[5,6]

As a basis for a quantitative comparison of the effects of treatment with different alkylating agents, the reaction rate constants with DNA *in vitro* were determined. The site of alkylation will be discussed.

Experimental. Calf thymus DNA was purchased from Sigma Chemical Co. Tritium labelled MMS, EMS and iPMS were synthesized according to Wachtmeister *et al.*[7] Unlabelled MMS and EMS from Eastman Organic Chemicals and iPMS from Koch-Light Laboratories were used.

The labelled alkylating agents were diluted with inactive substance to the following specific activities: 8 mCi/mmole for MMS, 50 mCi/mmole for EMS, and 70 mCi/mmole for iPMS. The concentration of MMS and EMS were 3 mM and for iPMS 0.5, 0.75, and 1.5 mM. DNA was alkylated at a concentration of 1 mg/ml in a 0.02 M phosphate buffer of pH 7.0 at 25°C. Samples of 1 ml were withdrawn at different times. iPMS, which has a half life of 1 h at 25°,[1] was incubated for 8 h with DNA, *i.e.* to practically complete consumption of the ester. DNA was precipitated by 2 volumes of 95 % ethanol and washed four times with 70 % ethanol. It was then redissolved and hydrolyzed in 1 N HCl for 20 min at 100°C in vacuum. The samples were mixed with a scintillation solution consisting of dioxane-naphthalene-PPO-POPOP [8] to which was added 0.5 ml 1 N HCl and 0.5 ml of hydroxide of hyamine 10X (Packard Co.). The liquid scintillation spectrometer used was a Nuclear Chicago Unilux model 6851 (Packard Co.).

Results. During incubation with DNA in a buffer solution an alkylating agent reacting according to S_N2 (in the present case MMS and EMS [1,5]) is used up mainly in additive reactions with the nucleophiles present. Besides water and DNA, the phosphate ions are alkylated. Thus, the alkylating agent disappears with the rate constant k'

$$k' = [H_2O] \cdot k_{H_2O} + [HPO_4^{2-}] \cdot k_{HPO_4^{2-}} + [H_2PO_4^-] \cdot k_{H_2PO_4^-} + [\text{DNA-P}] \cdot k_{\text{DNA}}$$

where [DNA-P] is the concentration of DNA in mole/l nucleotide phosphorus. Under the reaction conditions used the factors $[H_2PO_4^-] \cdot k_{H_2PO_4^-}$ and $[\text{DNA-P}] \cdot k_{\text{DNA}}$ are negligible. $k_{H_2PO_4^-}$ is *ca.* 17 times less than $k_{HPO_4^{2-}}$ and [DNA-P] is about ten times lower than $[HPO_4^{2-}]$.[9] The constants for secondary phosphate and water have been determined by Osterman-Golkar *et al.*[10]

iPMS reacts predominantly according to an S_N1 mechanism, especially with weak nucleophiles,[5,8,10] and its total decay rate is therefore uninfluenced by nucleophiles present, although it may be affected by salts.[9] In the absence of competing reactions and at low concentrations of a dissolved nucleophile, *i.e.* under conditions which apply to DNA in the present case, it is, however, possible to assign formally