of the rather limited s-range used in this investigation and the large number of nearly equal distances, the u-values are not very well determined by this electron-diffraction study. The conclusion drawn about the skeleton structure does not depend critically on the u-values as shown by carrying out a refinement keeping the mean amplitudes for all distances C2···C5 to C2···O6 in Table 1 equal to the values calculated from spectroscopic data (u_{calc}). The torsional angle refined then to $\phi = 4.8°$.

1. Ehrenberg, M. *Acta Cryst.* **19** (1966) 698.
2. Zeil, W., Haase, J. and Wegmann, L. *Z. Instrumentenk.* **74** (1966) 84.
3. Bastiansen, O., Graber, R. and Wegmann, L. *Balzers High Vacuum Report*, 1969, 1.
4. Andersen, B., Seip, H. M., Strand, T. G. and Stølevik, R. *Acta Chem. Scand.* **23** (1969) 3224.
5. Kuchitsu, K. and Cyvin, S. J. In Cyvin, S. J., Ed., *Molecular Structures and Vibrations*, Elsevier, Amsterdam 1972, Chapter 12.
6. Cyvin, S. J. *Molecular Vibrations and Mean Square Amplitudes*, Universitetsforlaget, Oslo, and Elsevier, Amsterdam 1968.
7. Seip, H. M. In Sim, G. A. and Sutton, L. E., Eds., *Molecular Structure by Diffraction Methods* (Specialist Periodical Reports), The Chemical Society, London 1973, Vol. 1, Part 1, Chapter 1.
8. Seip, H. M. and Stølevik, R. In Cyvin, S. J., Ed., *Molecular Structures and Vibrations*, Elsevier, Amsterdam 1972, Chapter 11.
9. Stølevik, R., Seip, H. M. and Cyvin, S. J. *Chem. Phys. Lett.* **15** (1972) 263.
10. diLauro, C., Califano, S. and Adembri, G. *J. Mol. Struct.* **2** (1968) 173.

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PMR Analysis of the *cis* and *trans* Isomers of 2-Chloro-4-methyl- and 2-Phenyl-4-methyl-1,3,2-dithiaphospholanes

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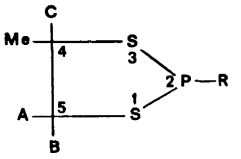
In the last two years the ring conformation of 1,3,2-dithiaphospholanes and 1,3,2-oxathiophospholanes have received some attention. The PMR spectra of 2-phenyl-[1,2] and 2-fluoro-1,3,2-dithiaphospholanes [3] have been completely analysed. The spin-system confirms the existence of pseudorotation with a pseudo-axial phenyl or fluor group. The PMR analysis of 2-chloro-, 2-phenyl-, and 2-phenoxy-1,3,2-oxathiaphospholanes [4] have shown that the five-membered oxathiaphospholane ring exists mainly in an equilibrium between two envelope conformations, with the carbon atom 5 out of the ring plane. This paper reports the PMR analysis of the *cis* and *trans* isomers of 2-chloro- and 2-phenyl-4-methyl-1,3,2-dithiaphospholanes, I and II.



I: R = Cl.
II: R = Ph.

The PMR spectra of I and II show that there are two kinds of methyl groups in magnetically different environments in the ratio approx. 1:3. The reasonable interpretation of the spectra is that the two kinds of methyl groups are *cis* and *trans* to the substituent attached to the phosphorus atom.

The 100 MHz spectrum of I (Fig. 1) consists of three main regions ($\delta = 4.7$ to 4.3, 4.2 to 3.9, and 3.8 to 2.8). The low field band is due to the methine proton at carbon 4 of the *cis* and *trans* isomers and the band in the region 4.2 to 3.9 is assigned to one of the protons at carbon 5 of the *cis* isomer. The complex high field region

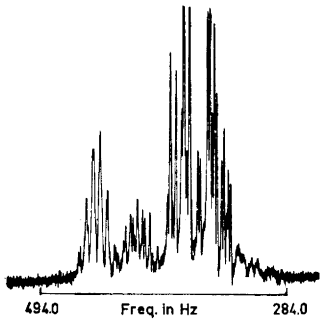


Fig. 1. 100 MHz PMR spectrum of 2-chloro-4-methyl-1,3,2-dithiaphospholane. The methyl group is not recorded.

of the spectrum is due to the two protons at carbon 5 of the *trans* isomer and one of the protons at carbon 5 of the *cis* isomer.

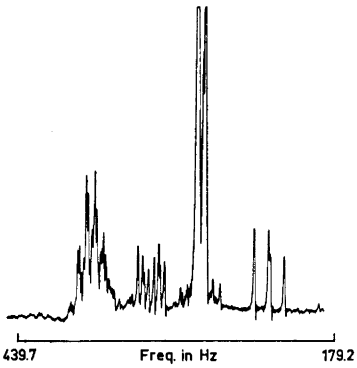


Fig. 2. 100 MHz PMR spectrum of 2-phenyl-4-methyl-1,3,2-dithiaphospholane. The phenyl group is not recorded.

The 100 MHz spectrum of II (Fig. 2) consists of four main regions ($\delta = 4.0$ to 3.5, 3.5 to 3.0, 2.8 to 2.6, and 2.4 to 2.1). The low field band is assigned to the methine proton at carbon 4 of the two isomers. One of the protons at carbon 5 of the *cis* isomer is found to resonance in the region 3.5 to 3.0, while the two protons at carbon 5 of the *trans* isomer resonance in the region 2.8 to 2.6. The high field band of the spectrum is due to one of the protons at carbon 5 of the *cis* isomer.

The detailed spectral analysis of the $CH_2-CH-CH_3$ protons was carried out successfully on the basis of an $ABCX_3P$ spin system for the *cis* and *trans* isomers of I and II, using the iterative computer program UEAITR.[5] The spectral parameters are listed in Table 1. The experimental and calculated spectra of some of the protons of the *cis* and *trans* isomers of II are shown in Figs. 3 and 4.

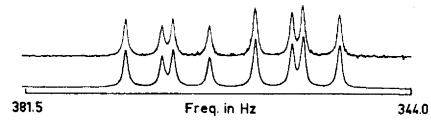


Fig. 3. 100 MHz PMR spectrum of the A proton in the *cis* isomer of 2-phenyl-4-methyl-1,3,2-dithiaphospholane. Upper: Observed spectrum. Lower: Calculated spectrum.

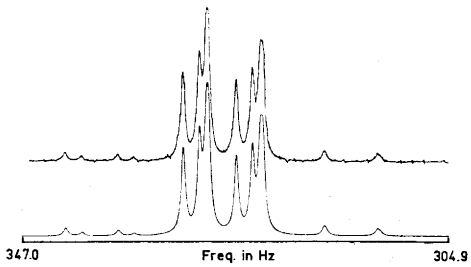


Fig. 4. 100 MHz PMR spectrum of the A and B protons in the *trans* isomer of 2-phenyl-4-methyl-1,3,2-dithiaphospholane. Upper: Observed spectrum. Lower: Calculated spectrum.

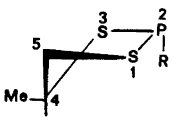
The succession of the chemical shifts for the methyl groups in the *cis* and *trans* isomers of I and II was found to be the reverse of that for the corresponding methine proton. It is believed that this effect must arise from the pseudo-axial position of the substituent attached to the phosphorus atom. The proton at carbon 5 which is *cis* to the methyl group, exhibits the same shielding as the axial protons at carbon 4 and 6 in 2-chloro-1,3,2-dioxaphosphorinane,[6] which are always found in the lower field as compared to the equatorial protons.

The magnitude of the geminal coupling constants in the isomers of I and II are in the expected range[1-3] (-11.0 to -12.5 Hz). These coupling constants are smaller (more negative) than those found in the 1,3,2-dioxaphospholanes.[7-8] This is ap-

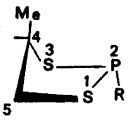
Table 1. Chemical shifts and coupling constants for the *cis* and *trans* isomers of I and II.

Compound	Isomer	δCH_3	δA	δB	δC	Coupling constants, Hz							
						J_{AB}	J_{AC}	J_{AP}	J_{BC}	J_{BP}	J_{CP}	J_{CH_3-C-H}	$J_{P-S-C-CH_3}$
I	*cis*	1.66	4.07	3.44	4.45	−12.07	4.40	6.50	10.44	−1.94	1.00	6.73	0.00
	trans	1.43	3.69	3.39	4.41	−11.82	5.05	0.15	5.64	2.30	1.36	6.58	0.00
II	*cis*	1.35	3.17	2.26	3.63	−12.48	3.52	4.59	11.11	−0.77	0.99	6.52	0.00
	trans	1.19	2.83	2.76	3.65	−11.61	5.47	2.34	5.03	−0.49	1.74	6.71	0.00

parently due to a combination of (a) a reduced H−C−H angle and (b) a smaller electron withdrawal effect of sulfur as compared to oxygen. The *cis* and *trans* coupling constants in the *trans* isomer of I and II are very similar in magnitude (Table 1), which probably implies that pseudo-rotation interconverts the non-polar forms at rates that are large on the PMR time scale, but that inversion at phosphorus is slow. Dreiding stereomodels of the *trans* isomer of I and II indicate that the ring exists mainly in an equilibrium between envelope conformations A and B with carbon atom 4 out of the ring plane.

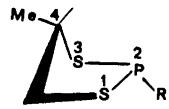


A



B

The large difference in the coupling constants between the *trans* and *cis* coupling in the *cis* isomer of I and II (Table 1) is probably due to a fixed conformation, C, with the carbon in position 4 out of the ring plane and with an equatorial position of both the methyl group and the substituent attached to the phosphorus atom.



C

This is also in agreement with that found from Dreiding models. Further detailed studies and analysis of proton magnetic resonance of other ring-substituted 1,3,2-dithiaphospholanes are in progress in this laboratory.

Experimental. *2-Chloro-4-methyl-1,3,2-dithiaphospholane (I)* was prepared from 1,2-propandithiol and phosphorus trichloride in benzene solution using triethylamine as base, b.p.$_1$ 104°C.

2-Phenyl-4-methyl-1,3,2-dithiaphospholane (II) was prepared from 1,2-propandithiol and dichlorophenylphosphine in benzene solution using triethylamine as base, b.p.$_{0.2}$ 118°C.

The PMR spectra of I and II were measured as 50 % solution in $CDCl_3$. The spectra were recorded on a 60 MHz JEOL, C-60 H and a 100 MHz Varian HA-100 spectrometer. The samples were degassed by the usual freezing and thawing procedure using a vacuum line, and the tubes were sealed in vacuum. Line positions were taken by averaging the data from five spectra using a frequency counter. The counter is accurate to 0.1 Hz for a 10 sec count. The computations were carried out on an IBM 360/50 computer and the graphical output was obtained using a Calcomp Plotter. The final error observed was 0.1 when all parameters were allowed to vary. The probable errors in the coupling constants are 0.02−0.03 Hz.

1. Peake, S. C., Field, M., Smutzler, R., Harris, R. K., Nichols, J. M. and Rees, R. G. *J. Chem. Soc. Perkin Trans. 2* **1972** 380.
2. Bergesen, K., Bjorøy, M. and Gramstad, T. *Acta Chem. Scand.* **26** (1972) 3037.
3. Albrand, J. P., Cogne, A., Gagnaire, D., Martin, J., Robert, J. B. and Venier, J. *Org. Magn. Resonance* **3** (1971) 75.
4. Bergesen, K., Bjorøy, M. and Gramstad, T. *Acta Chem. Scand.* **26** (1972) 2532.
5. Johannessen, R. B., Ferretti, J. A. and Harris, R. K. *J. Magn. Resonance* **3** (1970) 84.
6. Bergesen, K. and Albriktsen, P. *Acta Chem. Scand.* **25** (1971) 2257.
7. Bergesen, K. and Vikane, T. *Acta Chem. Scand.* **26** (1972) 2153.
8. Haake, P., McNeal, J. P. and Goldsmith, E. S. *J. Am. Chem. Soc.* **90** (1968) 715.

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Pseudomonas Cytochrome *c* Peroxidase

IX. Molecular Weight of the Enzyme in Dodecyl Sulfate-Polyacrylamide Gel Electrophoresis

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Cytochrome *c* peroxidase has been purified from *Pseudomonas aeruginosa* in a homogeneous form.[1,2] Minimum molecular weights of 21 600 and 24 400 were calculated for the enzyme from its iron and heme *c* content, respectively.[3] A molecular weight of 53 500 was obtained on the basis of the sedimentation and diffusion coefficients determined on the analytical ultracentrifuge and the experimentally measured partial specific volume.[3] This value indicates that the enzyme contains two heme groups per molecule. The molecular weights calculated for two iron atoms and two heme groups are 43 200 and 48 800, respectively. Further studies were necessary to establish the molecular weight of the enzyme and to clarify whether it consists of one or two polypeptide chains. The enzyme was therefore studied by electrophoresis in polyacrylamide gel in the presence of the ionic detergent sodium dodecyl sulfate (SDS) which dissociates oligomeric proteins to protomers. Under these conditions, the electrophoretic mobilities of proteins are related to the molecular weight of the protomer polypeptide chain.[4]

Experimental. Pseudomonas cytochrome *c* peroxidase (PsCCP) was prepared from the acetone-dried cells of *P. aeruginosa* as previously described.[1,2] The preparation was homogeneous in disc electrophoresis (performed according to Maurer,[5] pH 8.6, 7 % gel; staining according to Weber and Osborne [4]). SDS-polyacrylamide gel electrophoresis was carried out in 10 % gel as described by Weber and Osborne.[4] When preparing the samples for SDS-electrophoresis, the protein solutions were heated at 100°C for 5 min before incubation and dialysis to prevent possible proteolysis during these steps.[6] Bovine serum albumin (Fraction V, Armour), ovalbumin (Grade V, Sigma), pepsine (crystallized, Sigma) and horse

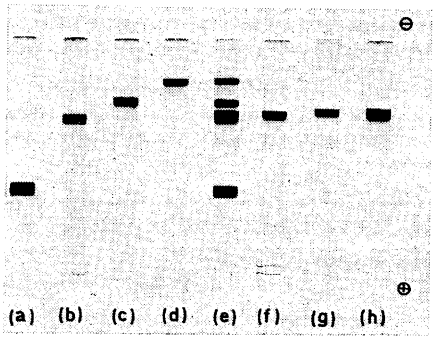

Fig. 1. SDS-polyacrylamide gel electrophoresis of *Pseudomonas* cytochrome *c* peroxidase and the molecular weight marker proteins in 10 % gel, according to Weber and Osborne.[4] (a) Horse heart cytochrome *c*, M.W. 12 400; (b) pepsin, M.W. 35 000; (c) ovalbumin, M.W. 45 000; (d) serum albumin, M.W. 67 000 (the marker proteins were treated with 1 % SDS and 1 % β-mercaptoethanol); (e) marker proteins *plus* PsCCP, treated with 1 % SDS and 1 % β-mercaptoethanol; (f) PsCCP, treated with 1 % SDS and 1 % β-mercaptoethanol; (g) PsCCP, treated with 1 % SDS; and (h) succinylated PsCCP, treated with 1 % SDS and 1 % β-mercaptoethanol.