NMR Studies on Cyclic Arsenites. Analysis of the NMR Spectra of *cis*- and *trans*-2-Phenyl-4,5-dimethyl-1,3,2-dioxarsolane

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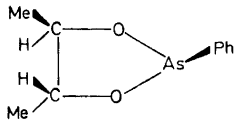
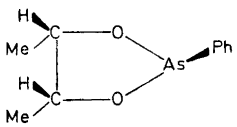
In a preceding paper [1] the present authors reported an analysis of the NMR spectra of *cis*- and *trans*-2-chloro-4,5-dimethyl-1,3,2-dioxarsolane. We have continued the previous work by preparing 2-phenyl-4,5-dimethyl-1,3,2-dioxarsolane and investigating the NMR spectra of its *cis* and *trans* isomers.

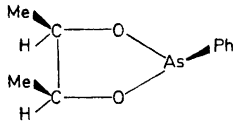
cis- and *trans*-2-Phenyl-4,5-dimethyl-1,3,2-dioxarsolane result from the reaction of racemic 2,3-butanediol with $AsPhCl_2$. The *trans* form (I) is unique



I



IIa



IIb

whereas the *cis* isomer may exist as *anti* (IIa) and *syn* (IIb) conformers which are interconvertible by inversion at arsenic.

The detailed spectral analysis of the $CHCH_3-CHCH_3$ protons was carried out successfully on the basis of $AA'X_3X_3'$ and ABX_3Y_3 spin systems for the *cis* and *trans* isomers, respectively.

By applying the composite particle technique these spin systems may be treated as 4-spin systems.[2] When $J_{XX'}=0$ all the spectral parameters of the $AA'X_3X_3'$ system may be obtained directly from the experimental spectrum with the limitation that $N=J_{AX}+J_{AX'}$ is found instead of the two individual coupling constants.[2]

The trial parameters of the *trans* molecule can be estimated directly from the experimental spectrum. The refined parameters listed in Tables 1 and 2 were all obtained by using iterative computations. The experimental and calculated spectra are shown in Figs. 1 and 2. The partial

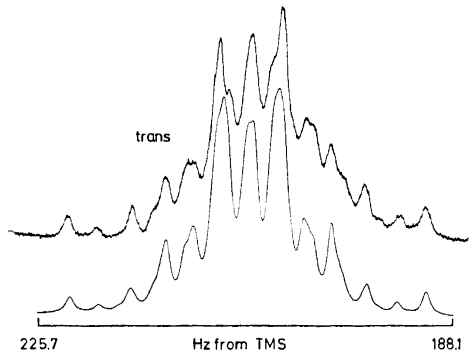


Fig. 1. Experimental (upper part) and calculated (lower part) methine proton spectra at 60 MHz of I.

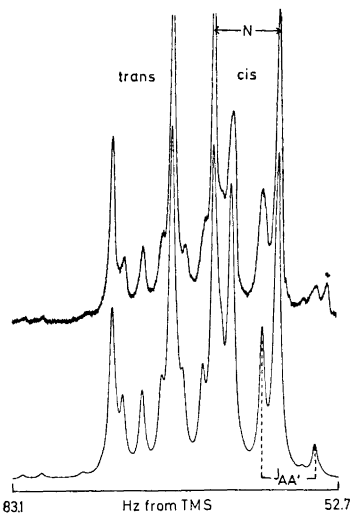


Fig. 2. Experimental (upper part) and calculated (lower part) methyl proton spectra at 60 MHz of I and II. The asterisk indicates an impurity.

Table 1. Chemical shifts in Hz from TMS measured at 60 MHz of neat 2-phenyl-4,5-dimethyl-1,3,2-dioxarsolane at 55°C.

Isomer	ν_A	ν_B	ν_X	ν_Y
trans	208.05	205.00	65.48	71.05
cis	247.66	–	61.42	–

Table 2. Spin-spin coupling constants in Hz measured at 60 MHz of neat 2-phenyl-4,5-dimethyl 1,3,2-dioxarsolane at 55°C.

Isomer	$J_{AA'}$	$J_{AX'}$	J_{AX}	J_{AB}	J_{AY}	J_{BX}	J_{BY}
trans	–	–	5.93	8.74	−0.33	−0.40	5.91
cis	4.90	−0.05	6.15	–	–	–	–

overlap between the methyl signals of the *cis* and *trans* forms has been taken into account when plotting this part of the spectrum (Fig. 2).

Integration of the methine proton signals of I and II showed that the examined sample contained about 58 % of the *trans* form and 42 % of the *cis* form. This is fairly close to the ratio reported for *cis*- and *trans*-2-chloro-4,5-dimethyl-1,3,2-dioxarsolane[1] as expected, since the same 2,3-butanediol was used when preparing these compounds.

Barriers to inversion at 3-coordinated arsenic of 25–42 kcal/mol have been observed.[3,4] Thus, pyrimidal inversion at arsenic is no doubt slow at moderate temperatures in the title compounds. The *anti* (IIa) and *syn* (IIb) conformers should then give rise to individual NMR spectra. However, the spectrum of the *cis* compound clearly shows that only one conformer is present to a measurable extent. In contrast, the *anti* and *syn* forms of *cis*-4,5-dimethyl ethylene sulfite with respect to the S=O group, have been observed to exist in the approximate ratio of 5 : 1.[5]

The type of spin system found for the *cis* molecule implies that the heterocyclic ring is planar or else undergoes rapid interconversions between equivalent nonplanar forms at a rate which is fast on the NMR time scale (Fig. 3).

The vicinal coupling constants in I and II, J_{AB} and $J_{AA'}$ respectively, are fairly close to the corresponding parameters in 2-chloro-4,5-dimethyl-1,3,2-dioxarsolane.[1] This indicates that the molecular conformations are similar in these molecules.

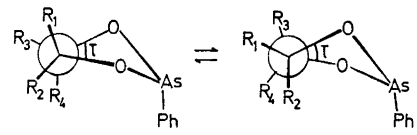


Fig. 3. Equivalent twist-envelope conformations for pseudo-axial phenyl. τ represents the dihedral angle. I: $R_1=R_4=H$, $R_2=R_3=CH_3$; IIa: $R_1=R_3=CH_3$, $R_2=R_4=H$; IIb: $R_1=R_3=H$, $R_2=R_4=CH_3$.

$J_{AA'}$ is considerably less than J_{AB}. The same observations have been made for the corresponding *cis* and *trans* isomers of 2,2,4,5-tetramethyl-1,3-dioxalane,[6] 2-chloro-4,5-dimethyl-1,3,2-dioxaphospholane,[7,8] and 4,5-dimethyl ethylene sulfite.[5] The reverse is, however, true for dioxaphospholanes [7,9,10] and ethylene sulfites.[9]

Even if allowance is made for stereospecific effects, the Karplus relationship [11] indicates that the *cis* form is considerably more twisted than the *trans* form. In order to minimize vicinal interactions of the methyl groups at the 4 and 5 positions, the ring probably adopts a twist-envelope conformation (Fig. 3). The $CHCH_3-CHCH_3$ fragment is presumably nearly staggered in conformation, with a dihedral angle, τ, approaching 60° for the *cis* molecule.

The data of Table 1 show a pronounced stereospecific anisotropy effect of the As-Ph group on the chemical shifts. Similar paramagnetic shifts caused by 2-phenyl substituents have been observed in 1,3-dioxolanes [12,13], 1,3-dithiolanes,[14] dioxaphospholanes,[9] and dithiophospholanes.[15] Owing to 1,3 non-bonded interactions in the *cis* molecule the *anti* conformer (IIa) is expected to be energetically favoured if the phenyl group occupies a pseudo-axial position. In analogy with previous assignments [5,8,9,14] the low field resonances at ν_A and ν_Y of I probably originate from methine and methyl protons *cis* to the phenyl group. It follows that nuclei B and X are *trans* to the phenyl group. This assignment is, however, also based on the assumption that the 2-substituent occupies a pseudo-axial position. Evidence for this assumption can be found from previously reported data on similar systems.[9,16-20] In a recent paper, however, Arbuzov *et al.* conclude from dipole moment measurements that the chair conformation of several 2,5,5-trisubstituted-1,3,2-dioxarsenanes is more stable with the As−X bond occupying the equatorial position (X = Cl, Ph, *etc.*).[21]

In a series of 2-alkyl-*cis*-4,5-dimethyl-1,3-dioxalanes the *syn* configuration in which the 2-substituent and one of the methyl groups at position 4 or 5 are pseudo-equatorial, is preferred [22] in analogy with the situation in 2,4-dialkyl-dioxalanes.[23]

It is hoped that further NMR studies on cyclic arsenites, at present being carried out in this laboratory, will clarify uncertainties concerning the conformations adopted by this type of ring system.

Experimental. Phenyl dichloroarsine was prepared according to the method of Michaelis and Reese.[24] 2-Phenyl-4,5-dimethyl-1,3,2-dioxarsolane was synthesized according to a method of Kamai *et al.* [1,19] $B.p._{10} = 122-124°C$, $n_D^{20} = 1.5589$.

The neat NMR sample was thoroughly degassed and sealed under vacuum after adding a small amount of tetramethylsilane. The NMR spectra were recorded at 55°C on a JEOL-C-60H spectrometer. Line positions were obtained by averaging the results of 4 scans at 54 Hz sweep width.

Computations were performed on an IBM/50H computer using the UEAITR[25] and KOMBIP [26] computer programs. 256 and 203 transitions were matched for the *cis* and *trans* isomers, respectively. The root-mean-square deviations for the experimental and calculated lines were 0.064 Hz or less. The calculated probable errors for the parameters were 0.01 Hz or less. The graphical output was obtained using a Calcomp Plotter.

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Pseudomonas Cytochrome *c* Peroxidase. VI. Large Scale Purification Procedure

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A method for the purification of cytochrome *c* peroxidase (PsCCP) from 25 g of acetone-dried cells of *Pseudomonas* has been previously reported.[1] The need for larger amounts of enzyme for further studies of its structural and enzymatic properties led to the development of a large-scale preparation method, described in this communication, starting with 125 g cells. The sequence of operations is basically as before, except that the final steps have been modified.

Experimental. Pseudomonas aeruginosa (previously reported as *P. fluorescens*, see Ref. 2) was cultivated and the acetone-dried cells were prepared as previously described.[1] CM-cellulose, DEAE-cellulose (Whatman DE-11), and Sephadex G-100 (Pharmacia) were used as previously described.[1] Peroxidase assays with *Pseudomonas* cytochrome c-551 (prepared from the same organism by the method of Ambler [3]) as substrate were performed as earlier.[1] Protein concentrations were determined according to Lowry *et al.*[4] with serum albumin (Finnish Red Cross) as standard. After the last step the protein concentration was determined spectrophotometrically using A_{280} (1 %, 1 cm) = 12.1 calculated on the basis of dry weight determinations of the purified preparation.[2] Disc electrophoresis in polyacrylamide gel was carried out according to Maurer [5] (pH 8.6, 7 % gel) and the protein bands were stained according to Weber and Osborne.[6] About 50 μg of protein was applied per gel.

Results. All the steps of the procedure were performed at 4°C unless otherwise stated. 125 g of acetone-dried cells were extracted with distilled water, treated with DNAase, and precipitated at pH 4.7 as previously described.[1] The precipitate was dissolved and the solution was left overnight before being centrifuged at 15 000 *g* for 15 min to remove a small amount of insoluble material.

The preparation was chromatographed on Sephadex G-100 (column 8.9 × 80 cm, eluent 0.1 M sodium phosphate buffer pH 6.5, hydrostatic pressure 17 cm, flow rate about 80 ml/h, 13 ml fractions). The elution pattern was similar to that of the small column.[1] The fractions containing PsCCP activity were pooled, those fractions forming the first part of the PsCCP peak but containing opalescent impurities being omitted, however. The pooled fractions were dialyzed against 0.02 M sodium phosphate buffer pH 6.5, overnight. The concentration step of the original method was omitted.

The dialyzed preparation was passed through a DEAE-cellulose column (6 × 40 cm, eluent 0.02 M sodium phosphate buffer pH 6.5, flow rate 60 ml/h, 10 ml fractions). All coloured fractions were pooled; the absorbance ratio A_{280}/A_{260} at this stage should be more than 1.2. The pooled fractions were dialyzed against distilled water overnight.

The pH of the preparation was adjusted to 6.5 with 0.005 M NaH_2PO_4 before it was fed onto a column of CM-cellulose (2.5 × 20 cm, equilibration buffer 0.005 M sodium phosphate pH 6.5, flow rates: sample 80 ml/h, eluent 23 ml/h, 5.8 ml fractions). The coloured proteins were adsorbed at the top of the column, forming a narrow band. The column was washed with 300 ml of the equilibration buffer. The band moved slightly down the column. Elution was performed with 0.01 M sodium phosphate buffer pH 6.8, and a red zone containing PsCCP was collected. Fractions with a spectral purity (A_{407}/A_{280}) greater than 3.1 were pooled. A green-red zone containing cytochrome oxidase moved down the column but remained separate from the PsCCP zone; cytochrome oxidase can be eluted with 0.05 M sodium phosphate buffer pH 6.8.