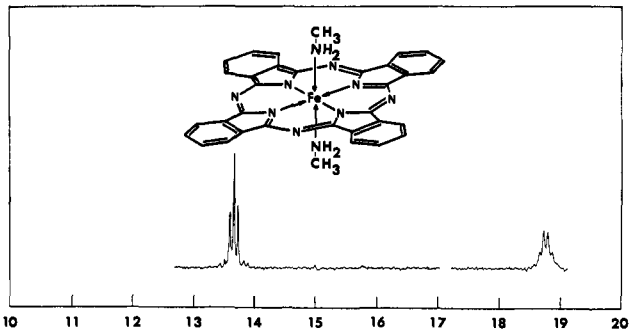

Figure 1. The 100-MHz Fourier transform spectrum of the bis-methylamine complex of iron(II) phthalocyanine in deuteriobenzene.

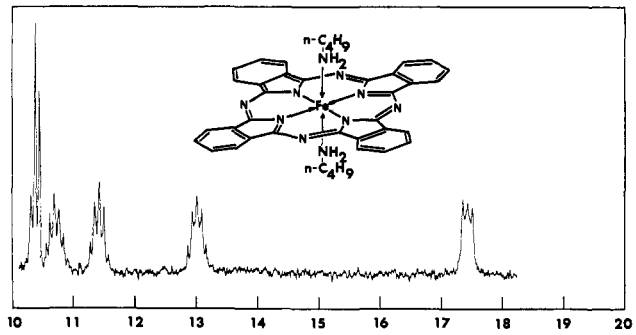

Figure 2. The 100-MHz continuous wave spectrum of the bis-*n*-butylamine complex of iron(II) phthalocyanine in deuteriochloroform.

To find out whether or not the type of data provided by these shift reagents could also be used for quantitative purposes, several trial structure parameter calculations were carried out. The data used in these calculations were those for the bis-4-methylpyridine adduct of iron(II) phthalocyanine and for free 4-methylpyridine ($CDCl_3$). The ring current equation used was that developed earlier for silicon phthalocyanines.[2,17] This equation, shown in graphical form in Figure 3, was not modified for these calculations because the separation between the 3,6 and the 4,5 phthalocyanine multiplets for silicon phthalocyanines, ~1.34 ppm, was found to be very similar to that for iron(II) phthalocyanine amine complexes, ~1.35 ppm.[18]

In one calculation the differences between the resonance positions of the three amine protons for the free and complexed amine were used to determine the amine Fe–N bond length in the complex. The value obtained, 1.94 Å, Table I, is in good agreement with the value predicted for this type of bond, 1.92 Å,[19] and the value found for the iron–amine nitrogen bond length in the tetrakis-4-methylpyridine complex of iron(II) phthalocyanine, 2.00 Å.[20] Of particular importance to this use of these shift reagents is the concentration independence of the incremental shifts, the consistency of the ring current effect of the phthalocyanine ring, and the required octahedral geometry at the iron.

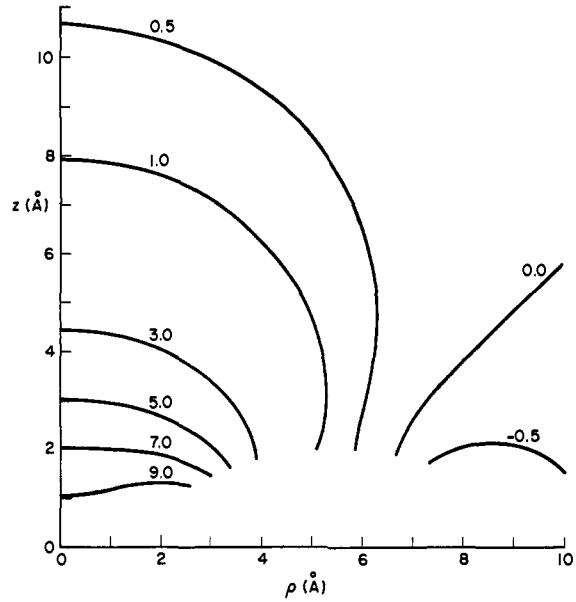

Figure 3. The average isoshielding lines of the phthalocyanine ring in parts per million as developed for the silicon phthalocyanine ring. The ordinate represents the distance from the ring center along the fourfold axis and the abscissa represents the distance from the ring center in the ring plane.[17]

Table I

	Incremental shifts, ppm			Bond length, Å
	$H_{2,6}$	$H_{3,5}$	CH_3	Fe–N
Obsd	6.47	2.30	1.30	
Calcd	6.41	2.44	1.41	1.94[a]

[a] Esd 0.04.

Acknowledgment. The contributions of Professor Kerro Knox and Dr. Thomas R. Janson to the program used in making the calculations are gratefully acknowledged.

(20) T. Kobayashi, F. Kurokawa, T. Ashida, N. Uyeda, and E. Suito, *Chem. Commun.*, 1631 (1971).

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iron(II) phthalocyanine and can also act as a shift reagent for amines.[16]

(15) P. C. Krueger and M. E. Kenney, *J. Inorg. Nucl. Chem.*, **25**, 303 (1963).

(16) J. E. Maskasky, Ph.D. Thesis, Case Western Reserve University, Cleveland, Ohio, 1972, p 111.

(17) T. R. Janson, Ph.D. Thesis, Case Western Reserve University, Cleveland, Ohio, 1971; University Microfilms T.R. 72-00051, p 8.

(18) This separation is assumed to be sensitive to changes in the ring current of phthalocyanines.

(19) J. L. Hoard, "Structural Chemistry and Molecular Biology," A. Rich and N. Davidson, Ed., W. H. Freeman, San Francisco, Calif., 1968, p 579.

Laser Raman Spectra of Aqueous Lysozyme Denatured by Lithium Bromide

Sir:

Investigation by Raman spectroscopy of the structural changes in the lysozyme molecule denatured by thermal means is difficult because of the optical inhomogeneity produced by heating concentrated solutions of this enzyme.[1] Although chemical denatura-

(1) R. C. Lord and N. T. Yu, *J. Mol. Biol.*, **50**, 509 (1970).

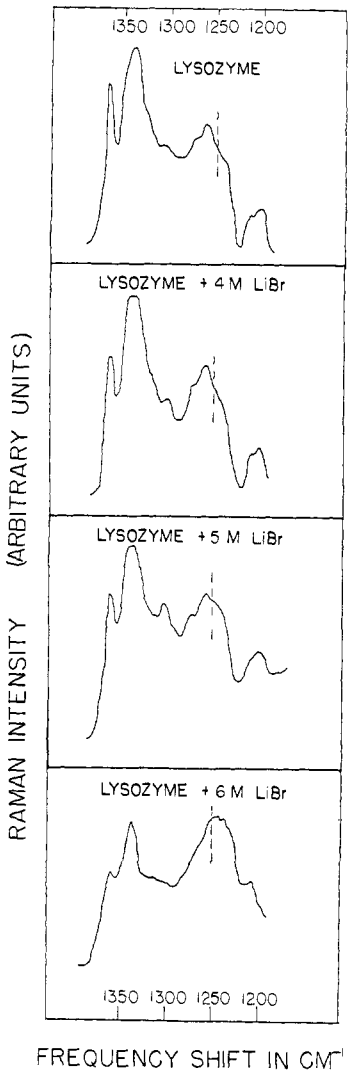

Figure 1. Raman spectra of 5% aqueous lysozyme in LiBr solution (1200–1400 cm^{-1}). Excitation by 200 mW of 4880-Å radiation, pH 4.2, $t = 25°$. The vertical dashed line indicates 1250 cm^{-1}.

tion by 6 *M* guanidine hydrochloride or 8 *M* urea does not lead to optically unsatisfactory samples, the tremendous excess of these denaturing agents masks the spectrum of the protein at its far more dilute concentrations (<0.02 *M*). We have now found that 6 *M* LiBr, whose ions in solution affect the Raman spectrum of water relatively little, can be used to denature lysozyme without obscuring the enzyme's Raman spectrum. Raman spectra in the range 1200–1500 cm^{-1} of lysozyme in solutions of LiBr of several concentrations at pH 4.2 and 25° are plotted in Figure 1. The changes in the spectrum that occur between 4 and 8 *M* parallel those found by other techniques such as ultraviolet[2] and nmr spectroscopy,[3] optical rotatory dispersion,[2] and viscosity.[2]

In the Raman spectrum of native lysozyme in H_2O the observed frequencies are mostly those of the amino acid side chains.[1] Two bands characteristic of the peptide backbone, however, are centered at 1660 (so-called amide I) and 1262 cm^{-1} (amide III). The former arises from the peptide carbonyl and the latter from a vibration that is mainly an in-plane bending vibration of the amide NH group mixed with some C–N stretching. The amide III band in lysozyme contains at least three components that appear as broad maxima at 1274, 1262, and 1240 cm^{-1} (Figure 1, top), the whole collection shifting to about 940 cm^{-1} upon deuteration. These peaks have been ascribed by Lord and Yu[1] to amide III vibrations in segments of the backbone in the α-helical, β-pleated sheet, and random-coil conformations, though they did not attempt to identify which frequency is associated with which conformation. They pointed out that a study of progressive denaturation, such as that given in Figure 1, should enable this to be done.

Studies of the Raman spectra of model polypeptides and of various proteins of known structure[4] indicate that the ordered forms of polypeptide conformation, *i.e.*, α-helical and β-pleated sheet forms, have most of their Raman intensity from the amide III vibrational modes concentrated above 1260 cm^{-1}, while the random-coil conformation shows this frequency below 1250 cm^{-1}. On this basis the skeletal structure of lysozyme can be seen to change little when the concentration of LiBr varies from 0 to 4 *M*. However, in the range 4–6 *M* the center of gravity of the amide III band shifts from above 1260 cm^{-1} to about 1245 cm^{-1}. We interpret this shift to show that the ordered structure in the protein backbone is removed and only a random-coil structure remains (Figure 1, bottom spectrum).

A more detailed interpretation of the spectra of lysozyme denatured by LiBr will be deferred to a later publication. At present we wish to mention that the changes in structure shown by the amide III region are accompanied by interesting changes elsewhere in the spectrum. The disulfide frequency at 509 cm^{-1} broadens and weakens. We interpret this to mean that the dihedral angles around the four S–S bonds vary considerably more than in native lysozyme. However, the approximate constancy of the area under the peak at 509 cm^{-1} indicates that the number of disulfide bonds has not changed.

We have also examined the denaturation of lysozyme by temperature and by chemical reagents that cleave the S–S bonds. At pH 5 and 75°, under which conditions lysozyme denatures reversibly,[5] the amide III region of the spectrum is closely similar to the top spectrum of Figure 1. From this we conclude that under these conditions the peptide backbone of lysozyme retains essentially the same amount and kind of ordered structure as at room temperature. On the other hand, when the disulfide bonds are ruptured chemically and the resultant sulfhydryl groups blocked with acrylonitrile, the amide III region is almost identical with the bottom spectrum of Figure 1, that is, the ordered structure of lysozyme is destroyed when the disulfide bonds are broken,[4,6] as is to be expected. It may also be of interest to remark that the Raman spectrum of solid native lysozyme agrees closely with the spectra of aqueous solutions in the 1–10% range.

(2) K. Hamaguchi, A. Kurono, and S. Goto, *J. Biochem.*, **54**, 259 (1963).
(3) W. D. Phillips, private communication.
(4) R. Mendelsohn, Ph.D. Thesis, Massachusetts Institute of Technology, Jan 1972.
(5) C. C. McDonald and W. D. Phillips, *J. Amer. Chem. Soc.*, **91**, 1513 (1969).
(6) R. C. Lord, *XXIIIrd Int. Congr. Pure Appl. Chem., Pure Appl. Chem., Suppl.*, 7, 179 (1971).

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Metal Ion–Aromatic Complexes. XIV. Structural Evidence for the $Sn_2Cl_2^{2+}$ Species in $Ar \cdot SnCl(AlCl_4)$

Sir:

We report here the surprising and previously unknown dimer, $Sn_2Cl_2^{2+}$, in $Ar \cdot SnCl(AlCl_4)$ where Ar = C_6H_6 (I) or *p*-xylene (II). These complexes also include an axially symmetric aromatic–Sn(II) bond.

In an earlier communication[1] we reported the structure of $C_6H_6 \cdot Sn(AlCl_4)_2(C_6H_6)$ which consists of a pentagonal-bipyramidal Sn(II) entity (6Cl, $1C_6H_6$) wherein the metal ion lies along the sixfold axis of the benzene ring. In this structure the second aromatic molecule is not coordinated to the metal but resides in a cleft in the $Sn(AlCl_4)_2$ chain. Very recently we have completed the crystal structure determination of $C_6H_6 \cdot Pb(AlCl_4)_2(C_6H_6)$[2] which is structurally similar to the analogous Sn compound.

For a variety of experimental reasons we have been unsuccessful in isolating crystalline aromatic Sn(II) complexes of the type $Ar \cdot Sn(AlCl_4)_2$ under a wide variety of experimental conditions. However, we have succeeded in isolating interesting compounds of the type $Ar \cdot SnCl(AlCl_4)$ which contain a $Sn_2Cl_2^{2+}$ moiety. We report on two of these structures at this time.

$Ar \cdot SnCl(AlCl_4)$ was prepared by placing purified $SnCl_2$ (0.044 mol) and $AlCl_3$ (0.075 mol) in one arm of an "H" tube.[3] Dry, degassed aromatic was sublimed into the system and allowed to come to room temperature and then heated to ~50° for 1 hr. While the reaction was still warm it was filtered *in vacuo* and the excess solvent removed by immersion of one arm in a liquid nitrogen trap until crystals formed. The crystals could be repeatedly recrystallized from benzene.

Compounds I and II exhibited the following crystal data.

$C_6H_6 \cdot SnCl(AlCl_4)$ (I): $P2_1/n$; $Z = 4$; $\rho_c = 2.02$ g cm^{-3}, $\rho_o = 2.1$ g cm^{-3}; $a = 19.624$ (6) Å, $b = 9.531$ (1) Å, $c = 7.099$ (1) Å; $\beta = 93.65$ (1)°; number of observations used in solution and refinement of structure = 2446; $\mu = 27.3$ cm^{-1}; crystal size 0.33 × 0.43 × 0.70 mm; transmission coefficient variation,[4] 0.47–0.50; $\lambda = 0.71068$ Å.

***p*-$C_8H_{10} \cdot SnCl(AlCl_4)$ (II):** $I2/c$; $Z = 8$; $\rho_c = 1.86$ g cm^{-3}, $\rho_o \sim 1.7$ g cm^{-3}; $a = 18.970$ (7) Å, $b = 10.903$ (4) Å, $c = 15.470$ (4) Å; $\beta = 107.33$ (1)°; number of observations used in solution and refinement of structure = 2476; $\mu = 25.5$ cm^{-1}; crystal size 0.30 × 0.15 × 0.60 mm; transmission coefficient variation,[4] 0.47–0.71; $\lambda = 0.71068$ Å.

(1) H. Lüth and E. L. Amma, *J. Amer. Chem. Soc.*, **91**, 7515 (1969).
(2) A. G. Gash, P. F. Rodesiler, and E. L. Amma, to be published.
(3) R. W. Turner and E. L. Amma, *J. Amer. Chem. Soc.*, **88**, 1877 (1966).
(4) Absorption corrections were made with a local variation of program GONO9 originally written by W. C. Hamilton, Brookhaven National Laboratory, Upton, N. Y.

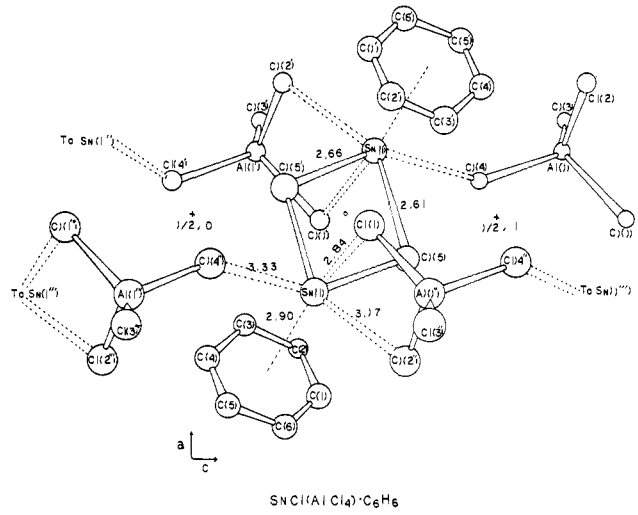

Figure 1. Perspective view of the structure of $C_6H_6 \cdot SnCl(AlCl_4)$ down the *b* axis. The $Sn_2Cl_2^{2+}$ dimer is composed of atoms Sn(1), Cl(5), Cl(5′), and Sn(1′). The $Sn(AlCl_4)^+$ chain goes from Sn(1‴) at the lower far left through Sn(1) to Sn(1‴) at the far right. Esd's for interatomic distances are Sn–Cl ±0.005 Å, Al–Cl ±0.007 Å, and Sn–C ±0.02 Å. The angle between the normal to the ring and Sn center of the ring is 7 ± 1°.

Single crystal intensity data were collected on a Picker automated diffractometer by standard techniques.[5] The structures were solved by standard heavy atom techniques and refined by full-matrix least squares.[5] I refined to a final conventional *R* of 0.070 and II to a final *R* of 0.049. Although the crystals of I and II are not isomorphous, the structures are quite similar in spite of the differing steric requirements of the aromatic moieties. The most prominent and unexpected feature of the structures is the presence of the lozenge-shaped dimer (Figure 1) with Sn–Cl distances of ~2.6 Å. These structures are the first reports of such species which may be formally considered as $Sn_2Cl_2^{2+}$ units. The coordination polyhedron of each Sn is completed by three longer Sn–Cl interactions ranging from 2.8 to 3.3 Å and an axially symmetric Sn–aromatic bond to produce a distorted octahedron (5Cl, $1C_6H_6$) (Figure 2). One might ask the question whether the dimer is indeed the structural building block as we maintain, since the Sn–Cl distances vary between 2.61 and 3.33 Å in these two structures. We reason as follows. (1) In I the Sn–Cl lozenge distances are 2.61 and 2.66 Å with the next shortest distance at 2.84 Å (Sn–Cl axial), and in II the lozenge Sn–Cl distances are 2.62 and 2.68 Å with the next shortest Sn–Cl distance at 2.92 Å. Hence, the Sn–Cl distances within the dimer are always the shortest, and the next shortest Sn–Cl distance is at least 0.18 Å longer. Furthermore, the next shortest distances are associated with chlorine atoms bound to $AlCl_4^-$ units and are expected to be weaker. (2) These 2.61–2.68-Å bridging Sn–Cl distances are essentially

(5) For data collection methods, weighting scheme, computer programs, and source of scattering factors, see R. L. Girling and E. L. Amma, *Inorg. Chem.*, **10**, 335 (1971). Data for I and II were collected on a Picker card-controlled diffractometer. Listings of structure factors, coordinates, and anisotropic temperature factors will appear following these pages in the microfilm edition of this volume of the journal. Single copies may be obtained from the Business Operations Office, Books and Journals Division, American Chemical Society, 1155 Sixteenth St., N.W., Washington, D. C. 20036, by referring to code number JACS-72-2135. Remit check or money order for $4.00 for photocopy or $2.00 for microfiche.