Table I

Compound	Buffer	ΔH, gross,[a] kcal mol^{-1}	ΔH, protonation of buffer,[b] kcal mol^{-1}	Net ΔH of hydrolysis, kcal mol^{-1}
Sodium ethylene phosphate	Pipes	-9.44 ± 0.04	-2.33 ± 0.3	-7.11 ± 0.3
	Tris	-16.6 ± 0.1	-10.1 ± 0.5	-6.5 ± 0.5
Sodium trimethylene phosphate	Pipes	-5.93 ± 0.13	-2.23 ± 0.3	-3.70 ± 0.3
	Tris	-13.0 ± 0.1	-9.6 ± 0.5	-3.40 ± 0.5
Sodium tetramethylene phosphate	Pipes	-4.69 ± 0.03	-2.07 ± 0.3	-2.62 ± 0.3
Sodium diethyl phosphate	Pipes	-4.81 ± 0.04[c,d]	-2.15 ± 0.3	-2.66 ± 0.3

[a] Gross heat of hydrolysis, measured by flow calorimetry, and corrected for the (minor) heats of viscous flow and dilution. The heat of dilution of Pipes buffer is unusually large and is under further investigation. However, since all solutions used in this work were 0.05 *M* in buffer, no dilution of the buffer is involved in these measurements. [b] Heat of protonation of the basic form of the buffer, calculated for pH 7.32 for Pipes and 7.30 for Tris on the basis of the experimentally determined p*K* values of the products, and heats of ionization. These are 11.34 kcal mol^{-1} for Tris (I. Grenthe, H. Ots, and O. Ginstrup, *Acta Chem. Scand.*, **24**, 1067 (1970)) and 2.59 ± 0.03 kcal mol^{-1} for Pipes, determined in this work [in fair agreement with the value reported by L. Beres and J. M. Sturtevant (*Biochemistry*, **10**, 2120 (1971))]. The major errors in this work arise because of an uncertainty of 3–5% in the protons released around pH 7.3; more refined measurements are planned. [c] Corrected by 0.32 kcal mol^{-1} for the exothermic heat of binding of product to enzyme. [d] Stopped-flow calorimetry.

Measurements of heats of hydrolysis for the cyclic phosphates were made with flow microcalorimeters[20] at several different flow rates, so as to establish that the reactions had proceeded to completion kinetically within the calorimeter. The heat of hydrolysis of diethyl phosphate was measured in the same instruments, used as stopped flow calorimeters. The concentration of diesters used was around 5×10^{-4} *M*, and that of the enzyme (subunit mol wt 29,000) around 2×10^{-5} *M* except for the experiments with diethyl phosphate, where the concentration was about 1×10^{-4} *M*. The kinetics of the hydrolysis of bis(*p*-nitrophenyl) phosphate with the diesterase shows that the enzyme is only slightly inhibited by the products at the concentrations produced in these experiments. However, because of the slow hydrolysis of diethyl phosphate, considerable enzyme (about 20 mol %) had to be used in this case, and therefore the data for diethyl phosphate had to be corrected for the (measured) heat of binding of product to enzyme. This and other standard corrections are shown in Table I. The enzyme does not cause hydrolysis of monoesters under the conditions of the thermochemical experiments, except for monoethyl phosphate, where the extent of hydrolysis is less than 2%. The reactions go to stoichiometric completion, within the experimental error of our techniques, as evidenced (a) by the fact that the heats of hydrolysis of trimethylene phosphate and of ethylene phosphate are about the same whether measured in Tris or Pipes buffer; in view of the corrections for heat of protonation of the buffer, which were calculated on the basis of complete reaction, such would not be so if the reactions were equilibrium limited, and (b) by analyses of product. These analyses were conducted by hydrolyzing the product of the action of the diesterase with alkaline phosphatase, and then analyzing for inorganic phosphate, and by measuring the concentrations of product by titration between appropriate end points.[1]

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[20] J. M. Sturtevant and P. A. Lyons, *J. Chem. Thermodyn.*, **1**, 201 (1969); J. M. Sturtevant, *Methods Enzymol.*, **26C**, 227 (1972).

Field Dependent Magnetic Susceptibilities of Diethylenetriammonium Chlorocuprate(II), $[(NH_3CH_2CH_2)_2NH_2][CuCl_4]Cl$

Sir:

Recent interest in the magnetism of two-dimensional, layer-like, Heisenberg systems and especially the effects of interlayer exchange on the magnetic properties of such systems[1,2] has prompted an investigation of the thermochromic compound $[(NH_3CH_2CH_2)_2NH_2][CuCl_4]Cl$. The magnetic susceptibility of this new Heisenberg, two-dimensional compound has revealed behavior which is surprisingly similar to $[C_2H_5NH_3]_2CuCl_4$ despite significant differences in the relative isolation of the corresponding two dimensional layers.

The crystal structure[3,4] of $[(NH_3CH_2CH_2)_2NH_2][CuCl_4]Cl$, which is illustrated in Figure 1, in exact analogy with $[C_2H_5NH_3]_2CuCl_4$, consists of a two-dimensional network of square planar tetrachlorocuprate ions. Two additional chlorides from neighboring $[CuCl_4]^{2-}$ ions complete a distorted octahedron about each copper. However, in addition to the protonated amine, the diethylenetriammonium ion, an additional chloride ion lies between these layers along the *b* axis. In $[C_2H_5NH_3]_2CuCl_4$ these adjacent layers are isolated by two layers of ethylammonium groups. The shortest copper–copper separation in $[(NH_3CH_2CH_2)_2NH_2][CuCl_4]Cl$ is 5.11 Å, and for the description of the magnetic properties the copper ions can be envisaged as forming a nearly quadratic lattice ($c/a = 1.03$), which

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[1] L. De Jongh, P. Bloembergen, and J. Colpa, *Physica* (*Utrecht*), **58**, 305 (1972).

[2] L. De Jongh, W. D. Van Amstel, and A. R. Miedema, *Physica* (*Utrecht*), **58**, 277 (1972).

[3] B. Zaslow and G. Ferguson, *Chem. Commun.*, 822 (1967).

[4] G. Ferguson and B. Zaslow, *Acta Crystallogr., Sect. B*, **27**, 849 (1971).

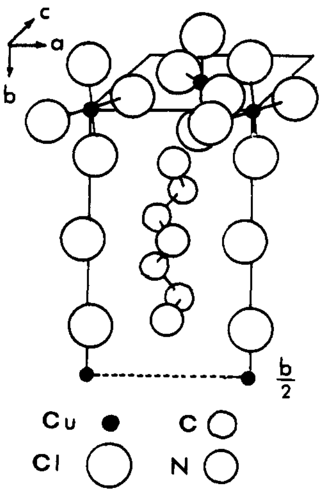

Figure 1. The crystal structure of $[(NH_3CH_2CH_2)_2NH_2][CuCl_4]Cl$ illustrating the two-dimensional network of chlorocuprate ions. For clarity only a segment of the unit cell has been included.

is isolated from the neighboring magnetic planes by more than 11.8 Å in the b direction.

Magnetic susceptibility measurements were performed using a vibrating sample magnetometer[5] at field strengths of 10 and 0.5 kG on a finely ground sample which weighed 0.09633 g and which was prepared according to published methods.[6] The temperatures were measured using a calibrated Ga/As diode,[7] a constant current source, and a digital voltmeter.[8] As can be seen from Figure 2, the measurements at 10 kG yield large susceptibilities which deviate markedly from simple Curie–Weiss behavior. Since it was not possible to explain this magnetic behavior using current models, a study was undertaken of the field dependence of the susceptibility.

The data from 40 to 60°K, when fit to a Curie–Weiss law of the form

$$\chi = \frac{Ng^2\beta^2S(S+1)}{3k(T-\theta)} \qquad (1)$$

result in $g = 2.26\ (\pm 0.02)$ and $\theta = 21.7\ (\pm 0.3)$°K. However, this fit is outside of the estimated precision for the measurements (*ca.* ±2%). In addition the g value is significantly larger than that of 2.14 which was reported[9] earlier from a powder epr study. Apparently very high temperature data are required for this compound in order to achieve a satisfactory Curie–Weiss fit.

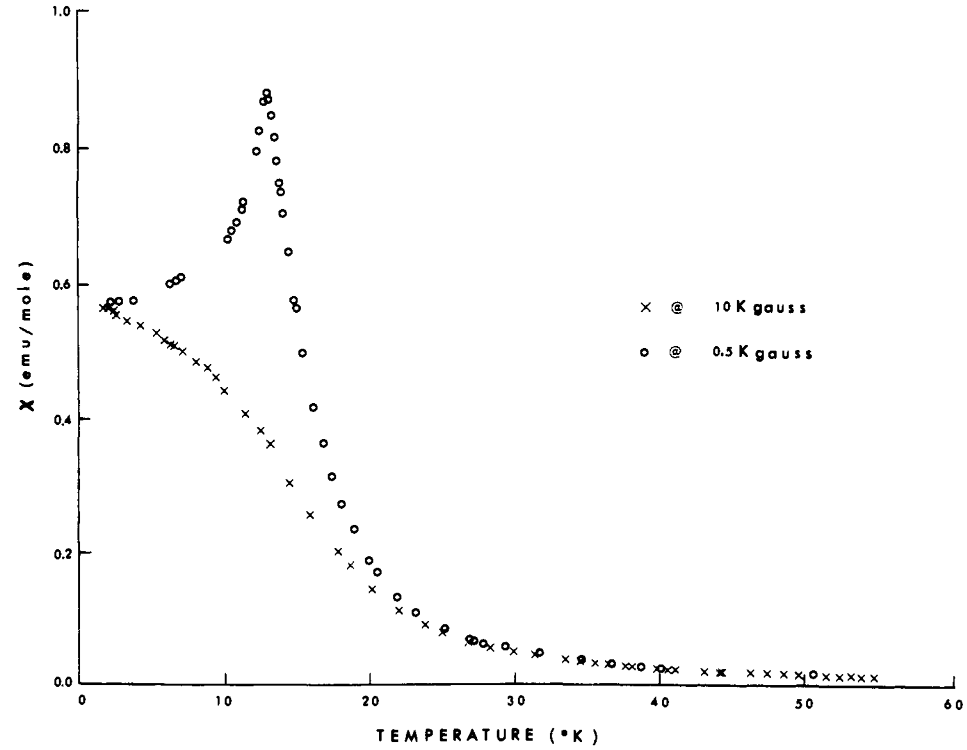

Figure 2. Powder magnetic susceptibility data collected at 10 kG and at 0.5 kG from 1.5 to 60°K.

As shown in Figure 2 when the measurements are made at 500 G, there is an abrupt change (corresponding to the maximum positive slope) in the susceptibility at 11.8 (±0.2)°K which suggests a phase transition to an antiferromagnetic state. It is apparent from Figure 2 that the susceptibility measurements become field independent above approximately 25°K. Anticipating that the two-dimensional nature of the crystal structure should be reflected in the magnetic properties, the data from 40 to 60°K were fit to the susceptibility series expansion result for a square Heisenberg lattice which was obtained by Baker, *et al.*[10] A preliminary fit to this equation

$$\chi = \frac{C}{T}\left[1 + 2\left(\frac{J}{kT}\right) + 2\left(\frac{J}{kT}\right)^2 + \frac{4}{3}\left(\frac{J}{kT}\right)^3 + \ldots\ldots\right]$$

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(5) Model 155, Princeton Applied Research Corp., Princeton, N. J.

(6) H. Jonassen, T. Crumpler, and T. O'Brien, *J. Amer. Chem. Soc.*, 67, 1709 (1945).

(7) Type TG-100FPA (special), no. 4277, Lake Shore Cryotronics, Inc., Eden, N. Y.

(8) Model 4700, Dana Laboratories, Inc., Irvine, Calif.

(9) M. Mori and S. Fujiwara, *Bull Chem. Soc. Jap.*, **36**, 1636 (1963).

(10) G. A. Baker, H. E. Gilbert, J. Eve, and G. S. Rushbrooke, *Phys. Lett. A*, 25, 207 (1967).

where $C = Ng^2\beta^2S(S + 1)/3k$, using ten terms in the series, results in $J/k = 18.5\ (\pm 0.7)$°K and $g = 2.11$. This fit is within the estimated error and is a considerable improvement over the Curie–Weiss fit, although deviations do occur at temperatures below 35°K. The satisfactory fit to a Heisenberg two-dimensional model for this compound along with the large values for the susceptibility close to the transition at low fields, as well as the field dependence of the susceptibility (decreasing the field increases the susceptibility above the transition), strongly suggests that spins are aligned ferromagnetically within the two-dimensional network of square-planar chlorocuprate(II) ions and at about 11.8°K order antiferromagnetically in three dimensions with these ferromagnetic planes aligned antiparallel to one another. As observed in $(C_2H_5NH_3)_2CuCl_4$ the ferromagnetic exchange is apparently significantly larger than the antiferromagnetic coupling between planes, for the susceptibility is dominated above the transition by this ferromagnetic coupling.

Preliminary single-crystal susceptibility and magnetization measurements confirm this general picture with the easy axis of magnetization (the *c* crystallographic axis) showing a possible spin flop or metamagnetic transition. Further investigation of these field induced transitions in this interesting, highly two-dimensional compound are in progress with an aim toward mapping out the various phase boundaries and comparing the anisotropy field to $[C_2H_5NH_3]_2CuCl_4$.

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Observation of *z*-Axis Anisotropic Motion of a Nitroxide Spin Label

Sir:

Lipid spin labels are providing useful information on the fluidity in the lipid phases of membrane model systems and biological membranes.[1,2] The long axis of the lipid usually corresponds to the nitroxide *x*, *y*, or *z* axis (see Figure 1), and studying anisotropic motion about these principal axes is an important step in understanding membrane spin labeling experiments. We recently obtained well-defined examples of *x*- and *y*-axis anisotropic motion by trapping spin labels in the tubular cavities of thiourea and β-cyclodextrin inclusion crystals.[3] We now report the first observation of *z*-axis anisotropic motion. This study relies on 35 GHz electron spin resonance measurements, where differences in *g* values produce a much larger effect than in the conventional 9.5 GHz esr spectra.

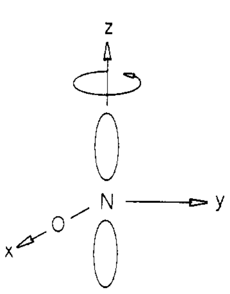


Figure 1. The nitroxide coordinate system.

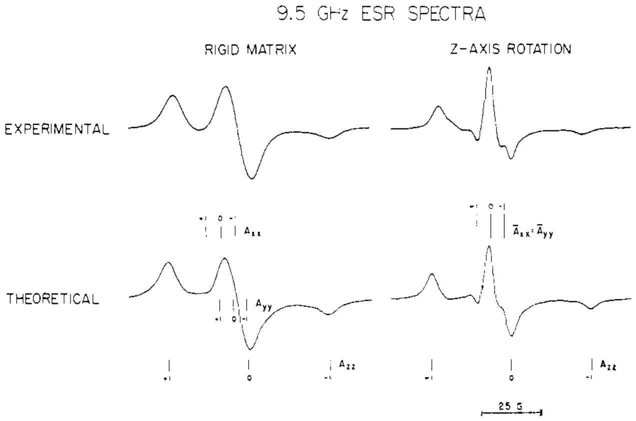
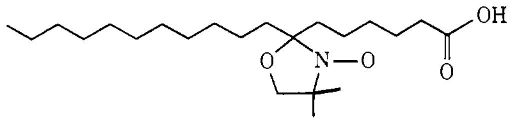


Figure 2. Experimental 9.5-GHz esr spectra of 7-doxylstearic acid trapped in γ-cyclodextrin at −196° (left spectrum), 24° (right spectrum), and the theoretical spectra for the rigid matrix and *z*-axis rotation. The principal values of the *A* and *g*-value matrix used in the simulations were those measured for 2-doxylpropane: $A_{xx} = 5.9$ G, $A_{yy} = 5.4$ G, $A_{zz} = 32.9$ G, $g_{xx} = 2.0088$, $g_{yy} = 2.0058$, and $g_{zz} = 2.0022$ (P. Jost, L. J. Libertini, V. C. Hebert, and O. H. Griffith, *J. Mol. Biol.*, **59**, 77 (1971)). To simulate *z*-axis rotation A_{xx}, A_{yy}, g_{xx}, and g_{yy} were replaced by $\overline{A_{xx}} = \overline{A_{yy}} = {}^1/_2(5.9 + 5.4) = 5.6$ G, and $\overline{g_{xx}} = \overline{g_{yy}} = {}^1/_2(2.0088 + 2.0058) = 2.0073$. The peak to peak widths of the Lorentzian lines were 3.8 and 6.0 G for the calculated *z*-axis motion and rigid matrix spectra, respectively. The indices +1, 0, and −1 refer to the *z* components of the nuclear spin angular momentum.



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The lipid spin label 7-doxylstearic acid (**1**) (the 4′,4′-dimethyloxazoladine-*N*-oxyl derivative of ketostearic acid) was obtained from Syva Associates and γ-cyclodextrin (**2**) (γ-Schardinger dextrin) was prepared as described previously.[4,5] The lipid spin label **1** was trapped in the host **2** by shaking together a solution of 0.5 mg of **1** in 0.8 ml of cyclohexane with a second solution of 40 mg of **2** in 0.8 ml of water. The resulting precipitate was washed with cyclohexane and then dried. It is well known that the nitroxide *z* axis of **1** is essentially parallel to the long axis of the extended stearic acid chain,[1,6] so that this molecule is a good candidate for the study of *z*-axis anisotropic motion. The 9.5- and 35-GHz esr spectra were recorded on

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(1) P. Jost, A. S. Waggoner, and O. H. Griffith, "Structure and Function of Biological Membranes," L. Rothfield, Ed., Academic Press, New York, N. Y., 1971, Chapter 3.

(2) H. M. McConnell and B. G. McFarland, *Quart. Rev. Biophys.*, 3, 91 (1970).

(3) G. B. Birrell, S. P. Van, and O. H. Griffith, *J. Amer. Chem. Soc.*, **95**, 2451 (1973).

(4) D. French, M. L. Levine, J. H. Pazur, and E. Norberg, *J. Amer. Chem. Soc.*, **71**, 353 (1949).

(5) D. French, *Advan. Carbohyd. Chem.*, **12**, 189 (1957).

(6) A. S. Waggoner, T. J. Kingzett, S. Rottschaefer, O. H. Griffith, and A. D. Keith, *Chem. Phys. Lipids*, **3**, 245 (1969).