Communications to the Editor

Cadmium Complexes of Dicysteinoethylenediaminetetraacetic Acid Exhibit ^{113}Cd NMR Shifts and ^{113}Cd–^{113}Cd Couplings Similar to Those of Metallothionein

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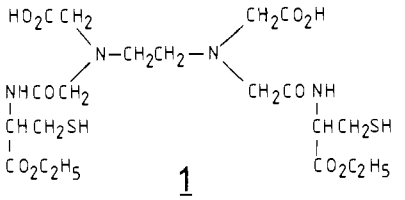
Metallothionein (MT) is thought to play an important role in vivo in both the detoxification of Cd and the storage of Zn and Cu.[1] Twenty of its 61 amino acid residues are Cys, and these bind to up to seven metal ions. An unusual feature in the ^{113}Cd NMR spectra of ^{113}Cd-labeled MT's is the presence of many resonances within the 600–700 ppm region (relative to 0.1 M $CdClO_4$) exhibiting ^{113}Cd–^{113}Cd spin–spin coupling.[2] Although the chemical shifts of cadmium thiolate complexes can occur in this range,[3] there appear to be no reports of model Cd complexes in which ^{113}Cd–^{113}Cd coupling has been observed.[4] The Cd EDTA-Cys_2 complexes described here provide the first examples of such couplings and involve cadmium nuclei with chemical shifts close to those of MT.

We chose to study cadmium complexes of the bis(ethyl cysteinate) amide of EDTA[5] **1** because the two cysteines are spaced



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similarly to those in Cys–X–Y–Cys sequences (where X and Y are amino acids) which occur 6 times in MT. It was also anticipated that the additional binding strength for Cd provided by the remnant of the EDTA skeleton might slow down Cd exchange rates.

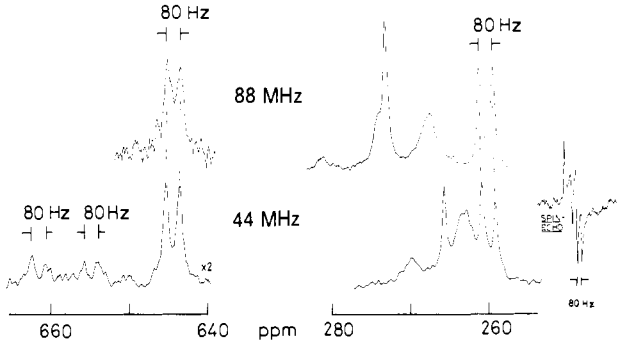

Figure 1. $\{^1H\}^{113}Cd$ NMR spectra of a solution containing 10 mM **1** and 10 mM $^{113}Cd^{2+}$ in D_2O at pH* 10.4, 298 K, are the result of overnight accumulations using 70° pulses at 2-s intervals. The digital resolution was 4 Hz and the broad-band ^1H irradiation was gated to avoid NOE's. The chemical shift scale applies only to the lower spectrum (44 MHz). The upper spectrum (88 MHz) has been plotted with the same hertz scale to show the constancy of splittings, and the 260 and 644 ppm peaks have been aligned. The inset shows the 250–280 ppm region of a 44 MHz spin–echo experiment, $90°-\tau-180°-\tau$–collect with $\tau = 6.25$ ms, and ^1H decoupling during collection of the echo. The inversion of the doublet at 260 ppm confirms the presence of homonuclear ^{113}Cd–^{113}Cd spin–spin coupling.[8] The additional doublets at 655 and 661 ppm can increase in intensity under certain conditions and may be coupled to peaks around 264 ppm.

First, $\{^1H\}^{113}Cd$ NMR spectra of an aqueous solution of **1** and $CdSO_4$ (containing ^{113}Cd in natural abundance, 12.8%) in a 1:1 molar ratio (0.2 M) were studied at 44 MHz over the pH* (meter reading) range 6–13.[6] Numerous resonances were observed from species in relatively slow exchange at 298 K on the NMR time scale. These appeared to fall into four distinct groups: δ 180–200, 260–275, 415–480, and 630–690. Significant intensities in the latter two regions were observed only at pH's greater than 9, presumably related to SH ionization.

In a second experiment, the $\{^1H\}^{113}Cd$ NMR spectrum of an equimolar solution of **1** and enriched (>95%) $^{113}CdCl_2$ (10 mM),[7] pH* 10.3, was studied. The most notable features were well-resolved doublets in the regions 260–270 ppm and 640–670 ppm (see Figure 1). Since the splittings of the most intense of these at 262 and 646 ppm remained the same (80 ± 4 Hz) at 44 and 88 MHz and were not present in spectra of unenriched samples, they were attributed to ^{113}Cd–^{113}Cd spin–spin couplings. In addition, the doublet at 262 ppm was clearly inverted in a Hahn spin–echo experiment with $\tau = 6.25$ ms (see Figure 1) indicative of homonuclear spin–spin coupling.[8]

To investigate further the nature of the cadmium cluster, a similar solution was subjected to gel filtration chromatography.[9] Seven species with molecular weights ranging from ca. 550 and 1500 were separated. The Cd and ligand contents of each were determined by atomic and electronic absorption spectroscopy,

(1) Kägi, J. H. R.; Nordberg, M. "Metallothionein"; Birkhauser Verlag: Basal, 1979.

(2) (a) For examples at 44 MHz and below, see: Otvos, J. D.; Armitage, I. M. In "Biochemical Structure Determination by NMR"; Bothener-By, A. A., Glickson, J. D., Sykes, B. D., Eds.; Marcel Dekker: New York, 1982; pp 65–96. (b) 88 MHz: Nicholson, J. K.; Sadler, P. J.; Cain, K.; Holt, D. E.; Webb, M.; Hawkes, G. E. *Biochem. J.* **1983**, *211*, 251–255.

(3) Carson, G. K.; Dean, P. A. W.; Stillman, M. J. *Inorg. Chim. Acta* **1981**, *56*, 59–71.

(4) For a recent review of ^{113}Cd NMR, see: Ellis, P. D. *Science (Washington, D.C)* **1983**, *221*, 1141–1146.

(5) **1** was synthesized from the bisanhydride of EDTA and ethyl L-cysteinate hydrochloride. Anal. ($C_{20}H_{34}N_4O_{10}$)C, H, N. ^1H NMR δ (D_2O, pH* 7.5) 1.28 (OCH_2CH_3), 3.05 (Cys CH_2 + NCH_2CH_2N), 3.43 (CH_2CONH), 3.65 (CH_2COO), 4.25 (OCH_2CH_3), 4.66 (Cys CH).

(6) All solutions used in this work were routinely purged with N_2.

(7) ^{113}CdO was dissolved in the minimum amount of 10 M DCl and added to the ligand in D_2O and the pH readjusted with 10 M NaOD.

(8) Following a Hahn spin–echo sequence, $90°_x-\tau-l80°_y-\tau$–echo, phase modulation of the resonances from homonuclear coupled spin systems is observed. If τ is chosen as $1/2J$, then coupled doublets are inverted (as in Figure 1) whereas triplets are upright; for a discussion, see: Rabenstein, D. L.; Nakashima, T. T. *Anal. Chem.* **1979**, *51*, 1465A–1474A. Since the T_2's of our ^{113}Cd resonances (ca. 7 ms as judged from line widths) are of the same order as τ, significant loss of signal to noise ratio occurs in the spin–echo experiment. This presumably explains why we did not observe the doublet at 644 ppm, although curiously the doublets at 655 and 661 ppm did appear to be inverted.

(9) The solution was applied to a calibrated column of Sephadex G15, eluted with 20 mM Tris base pH 10 and monitored at 280 nm.

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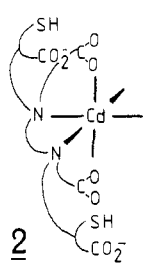
respectively. About 28% of the ligand that eluted from the column appeared to be unbound, the major complexes having MW's of ca. 1200 and Cd:ligand ratios of 2:2 and 3:2. That these contained Cd–S bonds was apparent from the high absorption at 254 nm characteristic of Cd–S charge-transfer bands,[1] which disappeared when the pH was lowered to 1.0.

^{1}H NMR spectra of similar solutions are unfortunately too complex for analysis at the present time (even at 500 MHz). However it was clear that in solutions giving rise to ^{113}Cd–^{113}Cd coupling, complete hydrolysis of the ethyl ester groups of the ligand had occurred.

Examination of space-filling models provided a useful insight into the structures of possible Cd complexes.[10] Coordination of 2N and $2CO_2^-$ from the EDTA remnant, as in **2**, allows further



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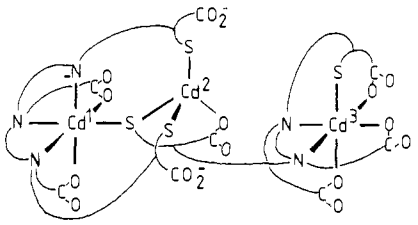

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binding of one S to the same Cd, but not two, due to steric constraints of the amide linkages. Indeed coordination of deprotonated amide NH's may be very favorable since five-membered rings (N/N$^-$) can be formed. Slow, downward drifts in pH are commonly observed with our solutions and may be related to this. The Cys arms in **2** could readily chelate (S/CO_2^-) to a second Cd, bringing it within 6 Å of the first, perhaps allowing through-space coupling. A structure that appears to account for much of our data is **3**, in which two hydrolyzed ligands provide coordination spheres of Cd(1)N_3O_2S, Cd(2)OS_3, and Cd(3)N_2O_3S. Cd(1) and Cd(2) are bridged by S and coupled. Removal of Cd(3) from the structure can lead to a 2:2 complex with Cd(1)N_3O_2S and Cd(2)S_4 coordinations that are again bridged and coupled.[11]

The resonances in the 600–670 ppm region of the ^{113}Cd NMR spectra of MT are usually assumed to arise from CdS_4 sites. However, Murphy et al.[12] have noted that CdS_3O, CdS_2O_2, and CdO_4 sites may give similar shifts. The present work appears to support this possibility. The ^{113}Cd–^{113}Cd couplings that we observe (80 Hz) are larger than those seen for MT's (20–50 Hz). This may be related to the nontetrahedral geometry of Cd(1) and to stronger bridge bonds.

These studies suggest that -Cys_2 EDTA and related ligands can add a new dimension to the study of metal cluster formation in aqueous media involving not only Cd but also a range of other metal ions. Further work along these lines is in progress.

Acknowledgment. We thank RTZ Services, the MRC, SERC, and University of London Intercollegiate Research Service for support.

Registry No. 1, 86004-61-7; **3**, 88441-26-3; $Cd_2(\mathbf{1})_2$, 88441-27-4; ^{113}Cd, 14336-66-4.

(10) The structures of Cd complexes have been reviewed recently by: Aylett, B. J. In "The Chemistry Biochemistry and Biology of Cadmium"; Webb, M., Ed.; Elsevier/North Holland: Amsterdam, 1979; pp 1–34. Cd complexes of amino acids and peptides isolated at near neutral pH's are often polymeric with bridging thiolates or carboxylates. An example close to the present work is (D-penicillaminato)cadmium(II) hydrate (Freeman, H. C.; Huq, F.; Stevens, G. N. *J. Chem. Soc., Chem. Commun.* **1976**, 90–91) in which S$^-$/CO_2^- six-membered rings are also present. Coordination numbers of 4, 5, 6, and 7 are common, and although no more than four RS$^-$ ligands are known to bind, additional (dodecahedral) capping by four carbonyls has now been observed: Dance, I. G.; Scudder, M. L.; Secomb, R. *Inorg. Chem.* **1983**, *22*, 1794–1797.

(11) The singlet at 266 ppm may be assignable to Cd(3).

(12) Murphy, P.; DuBois; Stevens, W. C.; Cheung, T. T. P.; Lacelle, S.; Gerstein, B.; Kurtz, M. *J. Am. Chem. Soc.* **1981**, *103*, 4400–4405.

2D Exchange Spectroscopy and Conformational Assignment in Macrocyclic Ring Natural Products: Cytochalasin B

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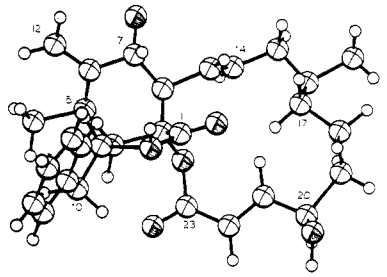
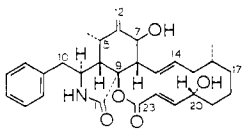
The structure assignment of medium- and large-ring (8–16 members) natural products is complicated by our limited knowledge of the conformational orientations these functionalized ring systems can adopt. Several studies[1,2] have shown that large carbocyclic systems minimize trans-annular nonbonded repulsions and high-energy torsional arrangements to the extent that at ambient temperatures only a small number of the many possible conformational orientations are appreciably populated. An a priori method of detecting these distant intramolecular interactions could greatly aid in establishing the conformational orientation of these systems and thus permit their structure assignment.

Two-dimensional (2D) exchange spectroscopy[3] has been established as a powerful technique for assigning internuclear proximities through cross relaxation, the same process that gives rise to nuclear Overhauser enhancements. This cross relaxation in macromolecules such as proteins is dominated by spin diffusion ($\omega\tau_c >> 1$) and results in intense cross peaks in the 2D experiment.[4] Little use of this experiment has been made with natural products that exist in the extreme narrowing limit ($\omega\tau_c << 1$).[5] In this communication we have utilized 2D exchange spectroscopy in the identification of distant internuclear interactions in the macrocyclic ring of the fungal metabolite cytochalasin B.[6]





Cytochalasin B

The ^{1}H NMR assignment[7] of cytochalasin B was greatly facilitated by the use of 2D homonuclear correlation spectroscopy (COSY).[8] A contour plot of the COSY map (Figure 1) identifies

(1) Borgen, G.; Dale, J. *J. Chem. Soc., Chem. Commun.* **1970**, 1340–1341. Dale, J. *Acta Chem. Scand.* **1973**, *27*, 1115–1129, 1130–1149, 1149–1158. Anet, F. A. L.; St. Jaques, M.; Hendrichs, P. M.; Cheng, A. K.; Krane, J.; Wong, L. *Tetrahedron* **1974**, *30*, 1629–1637. Anet, F. A. L.; Cheng, A. K. *J. Am. Chem. Soc.* **1975**, *97*, 2420–2424. Anet, F. A. L.; Rawdah, T. N. *Ibid.* **1978**, *100*, 7166–7171. Allinger, N. C.; Gorden, B.; Profeta, S., Jr. *Tetrahedron* **1980**, *36*, 859–864.

(2) Still, W. C.; Galynker, I. *Tetrahedron* **1981**, *23*, 3981–3996 and references therein.

(3) Jeener, J.; Meir, B. H.; Bachmann, P.; Ernst, R. R. *J. Chem. Phys.* **1979**, *71*, 4546–4553. Macura, S.; Huang, Y.; Suter, D.; Ernst, R. R. *J. Magn. Reson.* **1981**, *43*, 259–281. Macura, S.; Wüthrich, K.; Ernst, R. R. *Ibid.* **1982**, *47*, 351–357.

(4) Wagner, G.; Wüthrich, K. *J. Mol. Biol.* **1982**, *155*, 347–366 and references therein.

(5) Kotovych, G.; Aarts, G. H. M. *Can. J. Chem.* **1982**, *60*, 2617–2624. Hutton, W. C.; Phillips, N. J.; Graden, D. W.; Lynn, D. G. *J. Chem. Soc., Chem. Commun.* **1983**, 864–866.

(6) Rothweiler, W.; Tamm, C. H. *Experientia* **1966**, *22*, 750–752. Aldridge, D. C.; Armstrong, J. J.; Speake, R. N.; Turner, W. B. *J. Chem. Soc., Chem. Commun.* **1967**, 26–27.

(7) Tamm, C. H. *Front. Biol.* **1978**, *46*, Chapter 2.

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