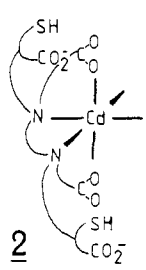
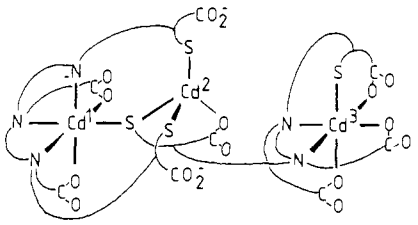
respectively. About 28% of the ligand that eluted from the column appeared to be unbound, the major complexes having MW's of ca. 1200 and Cd:ligand ratios of 2:2 and 3:2. That these contained Cd-S bonds was apparent from the high absorption at 254 nm characteristic of Cd-S charge-transfer bands,[1] which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



2



3

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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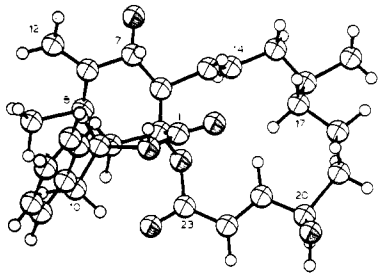
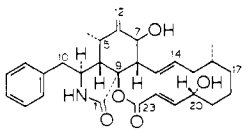
The structure assignment of medium- and large-ring (8-16 members) natural products is complicated by our limited knowledge of the conformational orientations these functionalized ring systems can adopt. Several studies[1,2] have shown that large carbocyclic systems minimize trans-annular nonbonded repulsions 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.

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(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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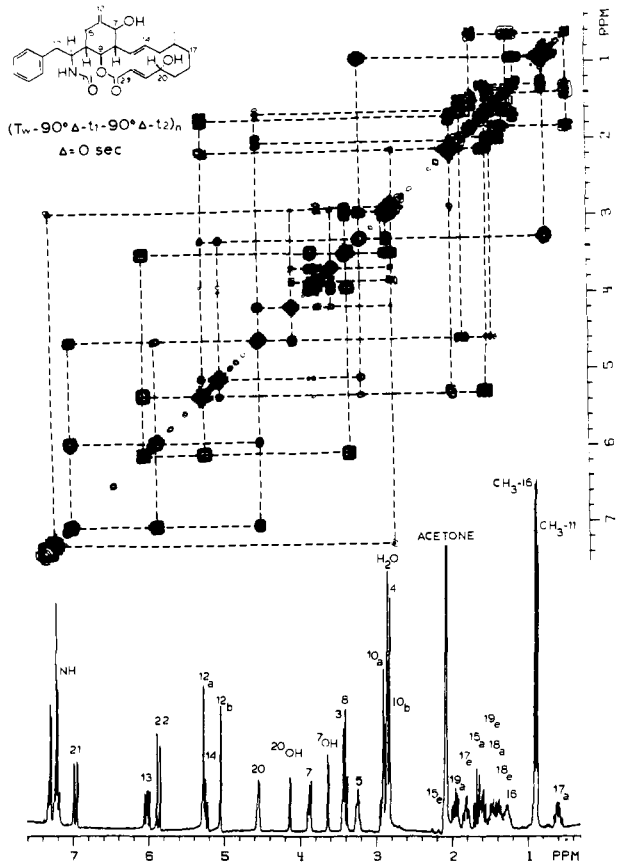

Figure 1. Contour plot of the COSY map of cytochalasin B (5 mg in 0.5 mL of acetone-d_6). A 128 × 512 data point spectrum was acquired (2.49 h) with quadrature detection in both dimensions. The entire data set was symmetrized.

Table I. Cytochalasin B Proton Coupling Constants (360 MHz)[a]

21:	20 - 4 Hz, 22 - 15 Hz
22:	21 - 15 Hz, 20 - 2 Hz
13:	14 - 15 Hz, 8 - 10 Hz, 15eq - 2 Hz
14:	13 - 15 Hz, 15ax - 10 Hz, 15eq - 4 Hz
12_A:	singlet
12_B:	singlet
20:	19ax - 8 Hz, 19eq - 3 Hz, 22 - 2 Hz, 21 - 4 Hz, 20_{OH} - 4 Hz
7:	8 - 10 Hz, 7_{OH} - 5 Hz
8:	7 - 10 Hz, 13 - 10 Hz
3:	10_A - 5 Hz, 10_B - 7 Hz, 4 - 2 Hz
5:	4 - 5 Hz, CH_{11} - 6 Hz
10_A:	10_B - 12 Hz, 3 - 5 Hz
10_B:	10_A - 12 Hz, 3 - 7 Hz
4:	5 - 5 Hz, 3 - 2 Hz
15eq:	15ax - 12 Hz, 13 - 2 Hz, 14 - 4 Hz, 16 - 2 Hz
19ax:	19eq - 14 Hz, 18ax - 8 Hz, 18eq - 1 Hz, 20 - 8 Hz
17eq:	17ax - 12 Hz, 16 - 2 Hz, 18ax - 12 Hz, 18eq - 6 Hz
15ax:	15eq - 12 Hz, 16 - 10 Hz, 14 - 10 Hz
19eq:	19ax - 14 Hz, 18ax - 1 Hz, 18eq - 12 Hz, 20 - 2 Hz
18ax:	18eq - 12 Hz, 17eq - 12 Hz, 17ax - 6 Hz, 19ax - 8 Hz, 19eq - 1 Hz
18eq:	18ax - 12 Hz, 17eq - 6 Hz, 17ax - 2 Hz, 19ax - 1 Hz, 19eq - 12 Hz
16:	15eq - 2 Hz, 15ax - 10 Hz, 17ax - 10 Hz, 17eq - 2 Hz
CH_3 11:	5 - 6 Hz
CH_3 16:	16 - 6 Hz,
17ax:	17eq - 12 Hz, 16 - 10 Hz, 18ax - 6 Hz, 18eq - 2 Hz

[a] The measurements were identical in acetone-d_6, dimethyl-d_6 sulfoxide, and chloroform-d. Pseudoaxial and equatorial designations are based on the magnitude of the coupling constants.

the spin coupling network of the individual proton signals.[9] The magnitude of the J couplings (Table I) provides information about the torsional arrangement of adjacent protons, but due to the double maxima of the angular dependence of three-bond coupling and the overlap of the high-field proton resonances, an unequivocal assignment of the orientation of the macrocyclic ring was not possible. However, the identification of spacial proximities offers another dimension in conformation assignment of these systems.

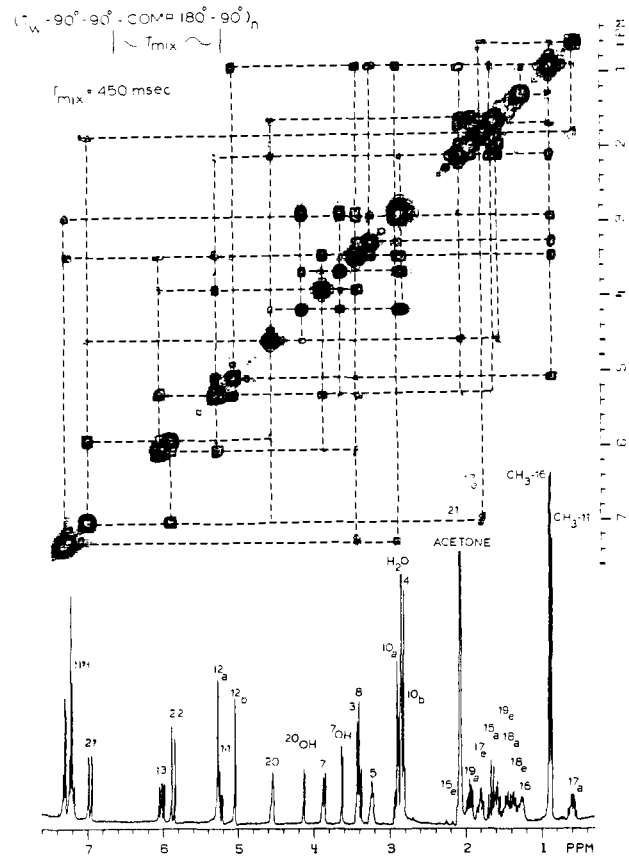

Figure 2. Contour plot of the 2D exchange map of cytochalasin B (5 mg in 0.5 mL of acetone-d_6). The 128 × 512 data set was acquired with a $(\pi/2\text{-}t_1\text{-}\pi/2\text{-}\tau_m/2\text{-}t_1/4\text{-}\pi\text{-}\tau_m/2\text{-}\pi/2\text{-}t_2)_n$ pulse sequence using a τ_m of 450 ms. It required 3 h of acquisition time.

Figure 2 shows the contour plot of the 2D exchange map of cytochalasin B. A comparison with Figure 1 reveals that the J coupling cross peaks have been completely suppressed by the indicated pulse sequence. The chosen mixing time allows for the detection of a number of cross peaks, the most striking of which connects H-21 to H-17e. This cross peak indicates a trans-annular interaction positioning these two protons within 2–3 Å of one another and rigidly restricts the possible orientations that the macrocyclic ring can adopt. Once these interactions have been mapped out, the more time-consuming NOE difference[10] experiments can be utilized to quantitate the Overhauser enhancements (Table II). With the combination of bonding orientation and spacial proximity, a detailed picture of the conformation of the macrocyclic lactone has emerged. Photographs of the Dreiding model constructed from this information[11] have provided the atom coordinates necessary to utilize the ORTEP[12] software and produce the three-dimensional drawing shown below.

The 2D exchange experiment provides a map of the internuclear spacial interactions present in the cytochalasin molecule. While internuclear distances are accessible through measurements of cross-peak intensity as a function of mixing time, NOE difference experiments can, in selective cases, access the same quantitative distance information in a shorter time. For the conformational assignment of the cytochalasin system, a qualitative picture of the proximities proved sufficient.

The assigned conformation of cytochalasin B is very similar to a previous conformation assigned to cytochalasin A (C-20

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Table II. Quantitative Nuclear Overhauser Enhancement Data[a]

IRR	OBS
Ortho	3 - 3%, 10_A - 3%
21	20 - 4%, 17eq - 4%, 22 - 10%
13	7 - 4%, 15ax - 5%
22	21 - 5%, 20 - 2%
12_A	12_B - 25%, 7 - 3%
14	13 - 3%, 8 - 3%
12_B	12_A - 25%, CH_3 11 - 1%
20	21 - 4%, 22 - 5%, 20_{OH} - 9%, 19ax - 1%, 19eq - 1%
7	13 - 4%, 12_A - 2%, 7_{OH} - 3%
3	10_A - 2%, CH_3 11 - 1%, Ortho - 2%, NH - 5%
8	14 - 4%, 5 - 9%, 4 - 3%
5	4 - 4%, CH_3 11 - 1%
4	5 - 4%, 8 - 3%, Ortho - 3%
10_A	Ortho - 3%, 3 - 3%, CH_3 11 -1%
10_B	Ortho - 2%, 3 - 3%, CH_3 11 - 1%
15eq	CH_3 16 - 10%, 15ax - 4%
19ax	20 - 4%, 19eq - 4%
17eq	17ax - 18%, 21 - 11%, 19eq - 3%, 16 - 1%
15ax	15eq - 15%, 13 - 4%
19eq	19ax - 15%, 20 - 5%, 21 - 2%
18ax	19ax - 7%, CH_3 16 - 2%, 17eq - 2%, 17ax - 2%
18eq	19ax - 3%, 17eq - 3%
16	14 - 2%, 15eq - 2%, CH_3 16 - 2%, 17eq - 2%
CH_3 16	15eq - 1%, 16 - 3%
CH_3 11	12_B - 8%, 3 - 7%, 5 - 9%, 4 - 3%
17ax	17eq - 12%, 18ax - 2%

[a] Nuclear Overhauser enhancements were determined by NOE difference at 360 MHz in acetone-d_6. Spectra were obtained with a 3-s irradiation, a 1-ms delay, and a 90° observe pulse.

ketone) from X-ray crystallographic data.[13] NMR data collected on cytochalasin A (data not shown) agrees very well with the solid-state conformation. The only X-ray data for cytochalasin B was collected on a $AgBF_4$ adduct,[14] and the conformation of the macrocyclic ring is significantly different in this case. The silver adduct, then, has resulted in distortions in the macrocyclic ring that are not present in solution. The solid-state conformation of cytochalasin B has been used to explain its high affinity for inhibition of the facilitated diffusion glucose transport system of human erythrocytes.[15] In light of these NMR results and the small energy difference (<2 kcal) between the dissociation constants of cytochalasin A and B, it seems more reasonable to assume that the two macrocyclic rings are of very similar conformation.[16]

The 2D exchange experiment provides access to data that can identify the trans-annular nonbonded and torsional arrangements that are inherently present in medium- and large-ring natural products. This information is critical in establishing the conformation and in assigning relative stereochemistry to such systems. The rapid mapping of these interactions should facilitate studies directed at understanding the chemical reactivity[2,17] and biological activity[18] of such systems.

Acknowledgment. Support from NIH through the Diabetes Research and Training Center at the University of Virginia is gratefully acknowledged.

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(13) Griffin, J. F.; Rampal, A. L.; Jung, C. Y. *Proc. Natl. Acad. Sci. U.S.A.* **1982**, *79*, 3759-3763.
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(16) It is noteworthy that these NMR studies were done in organic solvents rather than H_2O, but changes in solvent polarity from chloroform-*d*, to acetone-d_6, to dimethyl-d_6 sulfoxide showed no alteration of the cytochalasin B conformation.
(17) The stereospecific reduction of cytochalasin A to cytochalasin B is readily explained by the steric inaccessibility of the α-face of the C-20 ketone in cytochalasin A (see ORTEP drawing).
(18) For example, see: Egan, R. S.; Pesun, T. J.; Martin, J. R.; Mitscher, L. A. *Tetrahedron* **1973**, *29*, 2525-2538. Egan, R. S.; Freiberg, L. A.; Washburn, W. H. *J. Org. Chem.* **1974**, *39*, 2492-2494. Egan, R. S.; Martin, J. R.; Peran, T. J.; Mitschner, L. A. *J. Am. Chem. Soc.* **1975**, *97*, 4578-4583.

Reversible C-H Insertion/Reductive Elimination in (η^5-Pentamethylcyclopentadienyl)(trimethylphosphine)-iridium Complexes. Use in Determining Relative Metal-Carbon Bond Energies and Thermally Activating Methane

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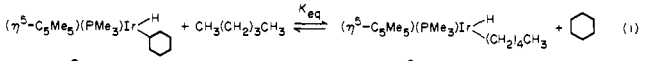
Heating causes reductive elimination of alkane from (η^5-pentamethylcyclopentadienyl)(trimethylphosphine)hydridoalkyliridium complexes, leading to an intermediate capable of undergoing oxidative addition to the C-H bonds in other alkanes.[1] We have used this property to achieve two goals in the C-H activation field that we wish to report: (a) establishment of reversible equilibrium between a pair of alkanes and hydridoalkyl complexes, allowing measurement of the equilibrium constant for this process and providing a method for determining relative metal-carbon bond energies, and (b) solution-phase thermal oxidative addition of methane[2] leading directly to a stable hydridoalkylmetal complex.

The equilibration studies began with the mixture of dihydride (**1**) and alkyl hydrides (**2-5**) formed on irradiation of **1** in *n*-pentane. As reported earlier and illustrated in Scheme I, heating this mixture to 110 °C in *n*-pentane caused disappearance of all the resonances in the ^1H NMR spectrum due to the secondary hydrides and a corresponding increase in the signal due to the primary hydride.[1] We judged from this observation that isomerization of secondary to primary hydridoalkyl complexes is possible by thermal activation at this temperature and that (as expected[3]) the primary complex is thermodynamically more stable. Evidence that this isomerization occurs by intermolecular reductive elimination/oxidative addition was obtained by carrying out the reaction in cyclohexane, rather than pentane, solvent. In this case, the amount of primary hydride remained constant, and the secondary hydrides were converted into hydridocyclohexyl complex **6** rather than primary hydridopentyl complex **2** (Scheme I).

This experiment also suggests that both the primary *n*-pentyl and secondary cyclohexyl complexes are stable to reductive elimination at 110 °C. In fact, higher temperatures are required to bring these materials into the reductive elimination/oxidative addition equilibrium illustrated in eq 1. The equilibrium constant



6 2 (1)

$$K_{eq} = \frac{[2][\text{cyclohexane}]}{[6][n\text{-pentane}]} \quad (2)$$

for this process (eq 2) can be conveniently measured by heating either the primary complex **2** or the hydridocyclohexyl complex **6** in a solvent containing 91.5% cyclohexane and 8.5% *n*-pentane. With either starting material, equilibrium is reached after 50 h at 140 °C; the ratio of **6** to **2** under these conditions[4] is 1.0 ± 0.1. This allows calculation of an equilibrium constant of 10.8, which

(1) Janowicz, A. H.; Bergman, R. G. *J. Am. Chem. Soc.* **1983**, *105*, 3929.
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(4) Slow decomposition of the hydridoalkylmetal complexes takes place under these conditions; however, the isomerization is rapid enough that the equilibrium concentrations of **2** and **6** are not significantly perturbed.

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