Acknowledgment. We are grateful to the National Institutes of Health (GM24511 and GM28384) and the National Science Foundation (CHE77-14628) for financial support. Victor Martin thanks the Fundacion Juan March of Spain for a fellowship. We are indebted to our colleague Professor Daniel S. Kemp for several enlightening discussions.

Supplementary Material Available: Erythro–threo and absolute configuration correlations (5 pages). Ordering information is given on any current masthead page.

Restricted Gearing and Residual Stereoisomerism in Bis(1,4-dimethyl-9-triptycyl)methane

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In accord with predictions,[1] bis(2,3-dimethyl-9-triptycyl)methane (**1**) exhibits residual diastereoisomerism and a high barrier to gear slippage under the constraint of dynamic gearing.[2,3] Furthermore, empirical force-field (EFF) calculations show that correlated disrotation of the 2,3-dimethyl-9-triptycyl groups in **1** is virtually unhindered within each diastereomer [meso (**1a**) and DL (**1b**)].[4] *We now describe the first molecular gear system in which two experimentally observable barriers, one to gearing and the other to gear slippage, lead to residual stereoisomerism.*

The compound chosen for this study, bis(1,4-dimethyl-9-triptycyl)methane (**2**), was prepared by addition of an excess of 3,6-dimethylbenzyne (from 3,6-dimethylanthranilic acid[11] and isoamyl nitrite) to bis(9-anthryl)methane.[12] Purification by column chromatography on silica, eluant 99:1 pentane/ether, yielded as the only detectable product **2a**,[13] mp 351–354 °C.

Under conditions of rapid cogwheeling, and in the absence of gear slippage, two diastereomers (meso and DL) are expected[1] for **2**. EFF calculations[14] show that the perturbation due to the 1-(peri-)methyl groups results in drastic alteration of the energy hypersurface for gearing (Figure 1). The meso isomer consists of a *dl* pair of conformers with C_2 symmetry which interconvert through a C_s transition state 16.5 kcal mol^{-1} higher in energy. The D or L isomer consists of a pair of asymmetric homomers which interconvert through C_1 and C_2 transition states 3.5 and 22.8 kcal mol^{-1} higher in energy, respectively. These computational predictions are consistent with the NMR behavior exhibited by **2a**. At low temperature (−50 to −60 °C) the aromatic region of the ^{1}H NMR spectrum features 2 ABCD spin systems and the aromatic ^{13}C{^{1}H} NMR region 18 lines (Figure 2),[15a] whereas at high temperature (90–100 °C) 1 ABCD spin system and 12 lines[15b] are observed in the two spectra.[16] This site exchange phenomenon

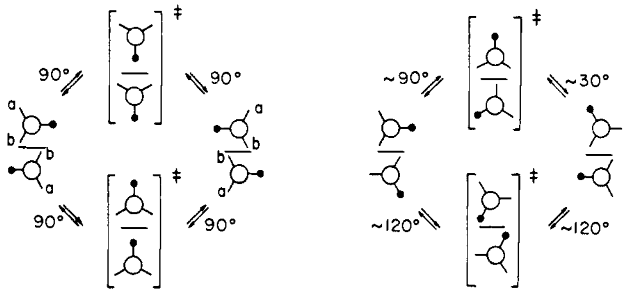

Figure 1. Conformational gearing circuits for the meso (*dl*) (left) and D or L (right) isomers of bis(1,4-dimethyl-9-triptycyl)methane (**2**). The schematic projections follow a previously adopted convention.[1,2] Structures in brackets are transition states. The diastereotopic benzene rings in the *dl* conformers are situated in sites a and b.

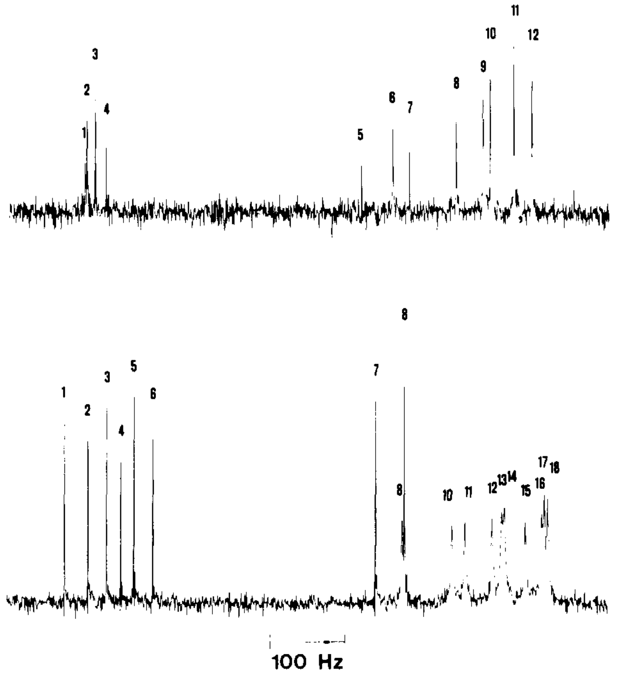

Figure 2. Aromatic ^{13}C{^{1}H} NMR region of the spectrum for **2a** at high temperature (top, 100 °C) and low temperature (bottom, −50 °C).

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(1) Hounshell, W. D.; Johnson, C. A.; Guenzi, A.; Cozzi, F.; Mislow, K. *Proc. Natl. Acad. Sci. U.S.A.* **1980**, *77*, 6961.

(2) Cozzi, F.; Guenzi, A.; Johnson, C. A.; Mislow, K.; Hounshell, W. D.; Blount, J. F. *J. Am. Chem. Soc.* **1981**, *103*, 957.

(3) Bis(3-chloro-9-triptycyl) ether exhibits similar behavior: Kawada, Y.; Iwamura, H. *J. Am. Chem. Soc.* **1981**, *103*, 958.

(4) Preliminary calculations had indicated a C_s ground state for bis(9-triptycyl)methane and a gearing transition state with C_2 symmetry lying ca. 1.0 kcal mol^{-1} above the ground state.[1] Using a different force field, a C_2 ground state was calculated, with a C_s transition state ca. 0.1 kcal mol^{-1} higher in energy.[5] However, these energy differences are well within the error limits of the EFF method, and it is now evident that the C_2 and C_s structures are essentially isoenergetic, i.e., the potential-energy hypersurface is virtually flat along the gearing coordinate.[8] Our calculations clearly predict that the barrier heights to gearing in bis(9-triptycyl)methane and related compounds are well below the limits of detection by NMR experiments; indeed, no line broadening has been observed in bis(9-triptycyl)methane and bis(9-triptycyl) ether (to −94 °C),[10] bis(9-triptycyl)carbinol (to −80 °C),[1] and **1a** (to −50 °C).

(5) The force field employed was that of Allinger's MM2 program (*QCPE* **1980**, *11*, 395) with two modifications designed for C_{ar}–C_{ar} bonds ($l^\circ = 1.3937$ Å and $k_s = 8.0667$ mdyn Å^{-1}).[6] Unconstrained geometry optimization was achieved with a modified version of BIGSTRN-2[7] utilizing analytical first and second derivatives of energy and full-matrix Newton–Raphson minimization. Examination of the eigenvalues of the force constant matrix of the final structures confirmed that the C_2 form of bis(9-triptycyl)methane was a minimum while the C_s form was a single partial maximum.

(6) For example, see: Ōsawa, E.; Ōnuki, Y.; Mislow, K. *J. Am. Chem. Soc.*, in press.

(7) Iverson, D. J.; Mislow, K. *QCPE* **1981**, *13*, 410.

(8) A $\pi/3$ disrotation of both 9-triptycyl moieties in the C_s or C_2 conformations leads to pseudorotation[9] of the whole molecule.

(9) Kilpatrick, J. E.; Pitzer, K. S.; Spitzer, R. *J. Am. Chem. Soc.* **1947**, *69*, 2483.

(10) Kawada, Y.; Iwamura, H. *J. Org. Chem.* **1980**, *45*, 2547.

(11) Newman, M. S.; Cella, J. A. *J. Org. Chem.* **1973**, *38*, 3482.

(12) Applequist, D. E.; Swart, D. J. *J. Org. Chem.* **1975**, *40*, 1800.

(13) NMR and high-resolution mass spectra were consistent with the assigned constitution.

(14) Calculations were carried out with use of BIGSTRN-2.[7]

(15) (a) ^{13}C{^{1}H} NMR (25.2 MHz) in [$^2H_2$]dichloromethane (−50 °C) δ 148.1, 146.8, 145.8, 145.1, 144.4, 143.4, 131.5, 130.1, 130.0, 127.4, 126.7, 125.3, 124.8, 124.6, 123.5, 122.7, 122.5, 122.3 (aromatic carbons); 57.1 (quaternary carbon); 50.8 (methine carbon); 28.0 (methylene carbon); 25.6, 19.4 (methyl carbons). (b) ^{13}C{^{1}H} NMR in [1,2-$^2H_2$]tetrachloroethane (100 °C) δ 147.00, 146.97, 146.5, 145.9, 132.3, 130.7, 129.8, 127.3, 125.8, 125.4, 124.2, 123.2 (aromatic carbons); 58.5 (quaternary carbon); 52.3 (methine carbon); 29.3 (methylene carbon); 26.0, 19.5 (methyl carbons).

(16) This behavior is unprecedented for this class of compounds and is traceable to the presence of the methyl groups in the 1-positions of the triptycyl moieties. As shown in pioneering studies by Ōki and co-workers,[17] bulky substituents in these positions are capable of slowing torsional processes at the 9-position of triptycene.

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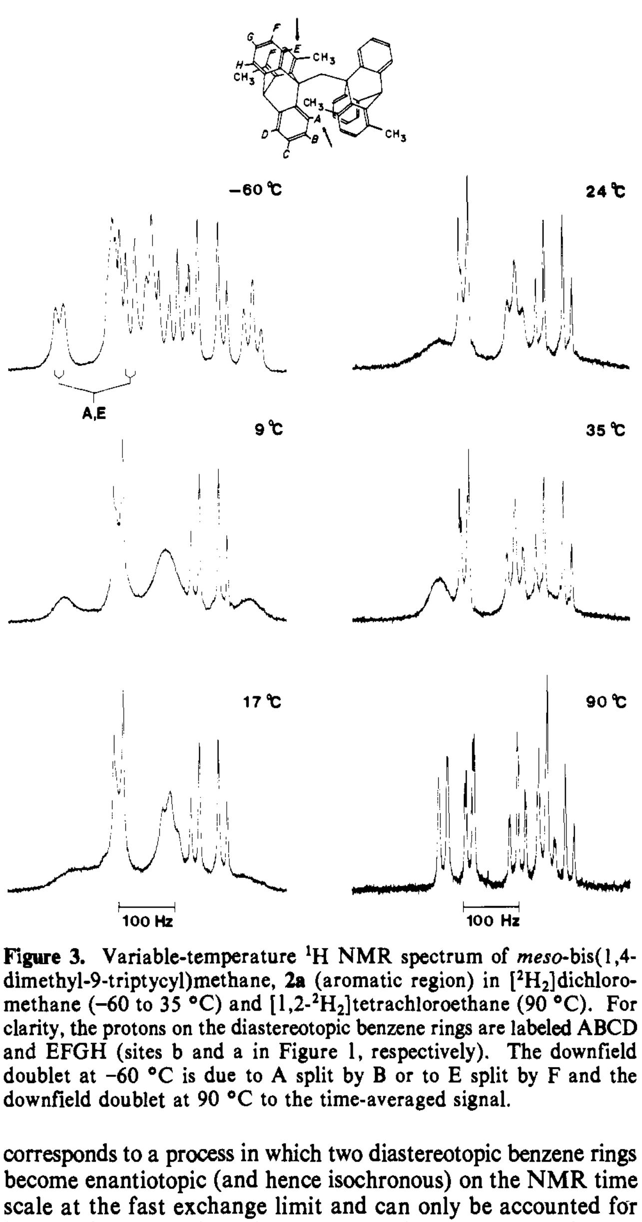

Figure 3. Variable-temperature ^1H NMR spectrum of *meso*-bis(1,4-dimethyl-9-triptycyl)methane, **2a** (aromatic region) in [$^2H_2$]dichloromethane (−60 to 35 °C) and [1,2-$^2H_2$]tetrachloroethane (90 °C). For clarity, the protons on the diastereotopic benzene rings are labeled ABCD and EFGH (sites b and a in Figure 1, respectively). The downfield doublet at −60 °C is due to A split by B or to E split by F and the downfield doublet at 90 °C to the time-averaged signal.

corresponds to a process in which two diastereotopic benzene rings become enantiotopic (and hence isochronous) on the NMR time scale at the fast exchange limit and can only be accounted for by one of two enantiomerization mechanisms. The first of these, enantiomerization of the DL pair by gear slippage, is ruled out because this process would require coalescence temperatures substantially higher than observed.[18] It follows that the observed site exchange results from enantiomerization of the *dl* pair by gearing. By the same token, **2a** is identified as the meso isomer.[19]

An experimental estimate of the enantiomerization barrier was obtained from the variable-temperature ^1H NMR spectrum of **2a** (Figure 3), most conveniently from the coalescence of the downfield doublets which originate in the proximal peri (A and E) protons of the diastereotopic benzene rings.[22] Application of the Gutowsky-Holm approximation yielded $\Delta G^{\ddagger} = 14.2 \pm 0.5$ kcal mol^{-1} (17–20 °C), in good agreement with the 16.5 kcal mol^{-1} estimated by EFF calculations.

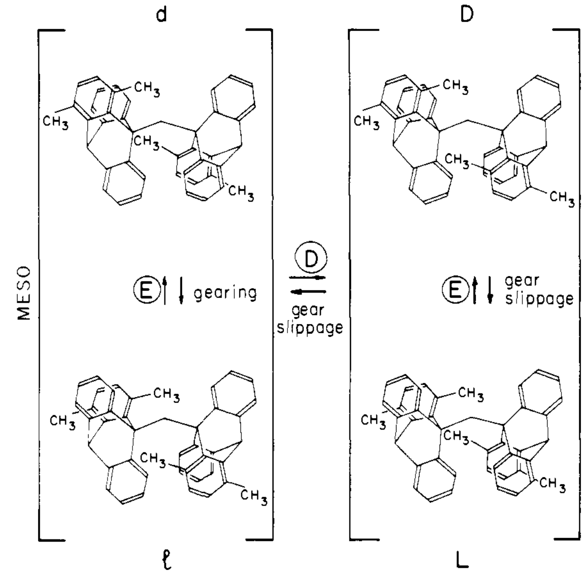

Figure 4. Representative conformations of stereoisomers of bis(1,4-dimethyl-9-triptycyl)methane (**2**). Enantiomers are paired in brackets. The mechanism of stereomutation is indicated next to the circled E (enantiomerization) and D (diastereomerization).

As in the case of **1**,[2] gear slippage becomes observable only at elevated temperatures. In [$^2H_5$]nitrobenzene at 215 °C (sealed tube under argon), **2a** is slowly converted to a mixture containing the DL isomer (**2b**).[24] The **2a** → **2b** diastereomerization barrier, $\Delta G^{\ddagger} = 39.4 \pm 0.5$ kcal mol^{-1},[25] is substantially higher than the corresponding barrier ($\Delta G^{\ddagger} = 33.6$ kcal mol^{-1}) for **1a** → **1b**,[2] as might be expected from the steric effect of the 1-methyl groups in **2**.

Our findings are summarized in Figure 4. At elevated temperatures, i.e., on the time scale of rapid gearing and gear slippage, **2** is a single, achiral species. As the temperature is lowered and gear slippage—but not gearing—is frozen out, enantiomerization of the D or L forms and diastereomerization of the meso and DL forms is slowed, and three stereoisomers (meso, D, and L) are formed. Finally, as the temperature is lowered further and gearing is also frozen out, the meso isomer is partitioned into a pair of enantiomers (*dl*). Thus, on the time scale of slow gearing and gear slippage there are four stereoisomers: *d*, *l*, D, and L. Under these conditions, the *d* and *l* conformers are virtually immobilized,

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(17) Ōki, M. *Angew. Chem., Int. Ed. Engl.* **1976**, *15*, 87 and references therein. See also: Yamamoto, G.; Ōki, M. *Bull. Chem. Soc. Jpn.* **1981**, *54*, 473, 481.

(18) No line broadening was observed for **1b** in [1,2-$^2H_2$]tetrachloroethane up to 150 °C. In conjunction with previous work,[2] this finding indicates that $34 > \Delta G^{\ddagger}_{rac} > 22$ kcal mol^{-1}. The steric effect of the 1-methyl groups in **2** is certain to increase the height of the racemization barrier even further.

(19) This ad hoc nomenclature, which follows a previously adopted convention,[1,2] describes stereoisomers according to symmetry properties which are appropriate to the time scale of observation.[20] Thus there is no contradiction in the designation of the same species as "meso" or "*dl*", depending on circumstances. We have previously drawn attention to the difficulty of using standard stereochemical nomenclature in systems consisting of "conformers-within-conformers".[21]

(20) Mislow, K.; Bickart, P. *Isr. J. Chem.* **1976/1977**, *15*, 1.

(21) Finocchiaro, P.; Gust, D.; Mislow, K. *J. Am. Chem. Soc.* **1974**, *96*, 3198.

(22) Assignments are based on an analysis of proton chemical shifts for a set of related bis(9-triptycyl)methane derivatives. In every case, the proximal peri protons in the unsubstituted rings are shifted downfield relative to the other aromatic protons. For $CDCl_3$ solutions of **1a**,[2] bis(9-triptycyl)methane,[10] bis(9-triptycyl)carbinol,[1] bis(9-triptycyl) ketone,[23] and **2a** at ambient temperature, δ (H_A, H_B, H_C, H_D) = (7.64, 6.81, 6.99, 7.44), (7.57, 6.78, 6.99, 7.46), (7.85, 6.71, 6.91, 7.41), (7.58, 6.75, 6.99, 7.50), and (7.68, 6.70, 6.99, and 7.44), respectively. For triptycene itself, δ ($H_{A/D}$, $H_{B/C}$) = (7.29, 6.88). In further support of this assignment, we note that the signals of the downfield diastereotopic protons are separated by a much larger chemical shift [$\Delta\delta$(A,E) = 0.63 ppm] than the comparable pair of diastereotopic protons in the distal peri positions [$\Delta\delta$(D,H) = 0.17 ppm].

(23) The preparation and molecular structure of this compound will be the subject of a separate paper.

(24) The two NMR signals for the 1- and 4-methyl protons appear at δ 2.33 and 2.55 in **2a** and δ 2.95 and 2.55 in **2b** (nitrobenzene solvent). The ratio of **2a** and **2b** was determined from the relative peak areas of the signals at 2.33 and 2.95 ppm and was monitored as a function of time. The accidental isochrony of one set of signals (presumably ascribable to the diastereotopic methyl groups in the 4-position) at 2.55 ppm is reminiscent of the accidental isochrony at 2.07 ppm previously observed[2] in the ^1H NMR spectra of **1a** and **1b**. The relative chemical shifts of the other two signals are consistent with our assignment of ground-state conformations to **2a** and **2b**.

(25) This value was obtained from a kinetic treatment[26] of the data.[24] The equilibrium constant $K(\mathbf{2a}/\mathbf{2b}) = 1.0 \pm 0.1$ at 215 °C.

(26) Glaser, R.; Blount, J. F.; Mislow, K. *J. Am. Chem. Soc.* **1980**, *102*, 2777.

but topomerization of the D or L homomers still takes place quite rapidly through the low-energy transition state (Figure 1).

Acknowledgment. We thank the National Science Foundation (CHE-8009670) for support of this work, Mary Baum for valuable technical assistance, and Dr. Robert B. Nachbar, Jr., for helpful discussions and the force-field calculation described in ref 5.

Fractionation Factor for Hydrogen Isotopes at the Aqueous Ligand of Cobalt in Co(II)-Substituted Bovine Carbonic Anhydrase

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The accurate interpretation of the solvent deuterium isotope effect in the hydration of CO_2 catalyzed by carbonic anhydrase requires a knowledge of the fractionation factor of the reactant state of the enzyme's active site,[1] which is a species of water bound to the metal of carbonic anhydrase.[2] The NMR method of Gold[3] and Kresge and Allred[4] for the determination of reactant state fractionation factors is based on the chemical shift which results from the rapid exchange of protons between solute and solvent. When this exchange is sufficiently rapid, a single resonance is observed with chemical shift that is the weighted average of the chemical shift at the solute site and the solvent site in the absence of exchange, information from which a fractionation factor can be obtained. This method has not been applied to proteins because of the complexity of the proton NMR envelope and also because the concentration of protein required to give detectable changes in chemical shifts is so large as to exceed protein solubility or at least to introduce errors from solute-solute interactions. This report demonstrates that these difficulties can be overcome by modifying the method of Gold[3] and Kresge and Allred[4] to use the relaxation rate, $1/T_1$, of the exchanging ligands of a paramagnetic metal in a metalloenzyme, such as cobalt in Co(II)-substituted carbonic anhydrase. Previous studies of Fabry et al.[5] have determined that the proton relaxation of water in the presence of Co(II)-carbonic anhydrase occurs in the rapid exchange limit for which $1/T_1$ observed for water protons is a weighted average between solvent water and the species of water at the inner coordination sphere of cobalt in the enzyme's active site. Furthermore, the relaxation rate of protons in bulk solvent is near 0.3 s^{-1} and that of the species of water bound to the cobalt is near 6×10^4 s^{-1}.[5] This means that the effect of the cobalt-substituted enzyme on the observed $1/T_1$ occurs at concentrations of enzyme (10^{-4}–10^{-3} M) which are practicable. I report here that the fractionation factor is unity, within experimental error, for the species of water bound to the metal at the active site of Co(II)-substituted bovine, red-cell carbonic anhydrase.

Bovine, red-cell carbonic anhydrase, purchased from Sigma Chemical Co., was purified by the affinity chromatography procedure of Khalifah et al.[6] The apoenzyme was prepared by dialysis using dipicolinic acid[7] followed by thorough dialysis. The amount of apoenzyme present was estimated from the absorbance at 280 nm, and the cobalt-substituted enzyme was obtained by adding an equimolar amount of $CoSO_4$. The enzyme was dialyzed further for 3 days against five changes of a large volume of distilled water. Enzyme samples were prepared by using glassware rinsed in EDTA and adding lyophilized Co(II)-carbonic anhydrase to solutions containing 0.033 M of Na_2SO_4 and 50 mM of [tris(hydroxymethyl)amino]methane adjusted to pH(D) 8.2 with H_2SO_4 (D_2SO_4) and NaOH (NaOD). This value of pH(D) is the uncorrected pH meter reading. Experiments were performed at 23 ± 1 °C on samples in equilibrium with air using a Jeol Co. FX100 spectrometer at a resonance frequency of 100 MHz. Each relaxation time of water protons was determined by a three-parameter, nonlinear least-squares fit of 11 measurements of relaxation recovery after a 180–90° pulse sequence.

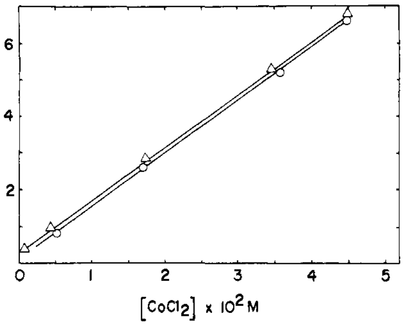

Figure 1. Proton magnetic relaxation rate $1/T_1$ at 100 MHz of solvent water as a function of concentration of $CoCl_2$ in aqueous solutions with atom fraction of deuterium in solvent $n = 0.10$ (△) and $n = 0.75$ (○). Samples were unbuffered solutions in equilibrium with air and measured at 23 ± 1 °C.

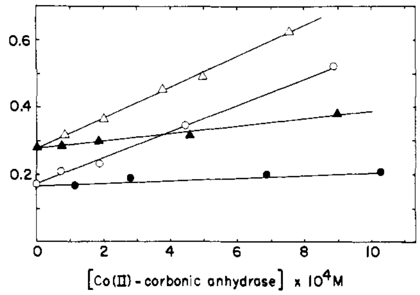

Figure 2. Proton magnetic relaxation rate $1/T_1$ at 100 MHz and 23 ± 1 °C of solvent water as a function of concentration of Co(II)-substituted, bovine, red-cell carbonic anhydrase. All solutions contained 0.033 M of Na_2SO_4 and 50 mM [tris(hydroxymethyl)amino]methane sulfate at pH(D) 8.2 and were in equilibrium with air. pH(D) is the uncorrected pH meter reading. Samples with $n = 0.10$ contained no methazolamide (△) and 4.9×10^{-3} M methazolamide (▲). Samples with $n = 0.75$ contained no methazolamide (○) and 4.9×10^{-3} M methazolamide (●).

Figure 1 demonstrates the linear dependence of the relaxation rate $1/T_1$ of solvent water protons on the concentration of hexaaquo, paramagnetic Co^{2+} ions, an effect which is interpreted in terms of an electron-nuclear dipole–dipole interaction.[8] For concentrations of $CoCl_2$ up to 0.045 M, this effect was the same in solutions with atom fraction of deuterium in solvent, n, of 0.10 and 0.75, based on the identical slopes in Figure 1. There was also a linear dependence of $1/T_1$ for water protons on the concentration up to 1 mM of cobalt(II)-carbonic anhydrase at pH(D) of 8.2 (Figure 2). These data show that this concentration dependence is nearly the same for two solvent compositions, $n = 0.10$ (least-squares slope in Figure 2 is 4.6×10^2 M^{-1} s^{-1}) and $n = 0.75$ (slope is 3.9×10^2 M^{-1} s^{-1}). That this effect is in large part due to the paramagnetic cobalt at the active site is demonstrated by the relaxation rates measured in the presence of 1.0 mg/mL (4.9×10^{-3} M) of methazolamide, a specific inhibitor of carbonic anhydrase ($K_I \sim 2 \times 10^{-8}$ M), the sulfonamide group of which binds to the metal at the active site displacing the species of water bound there.[2] This inhibition does not completely abolish the paramagnetic contribution of cobalt to the relaxation rate; the paramagnetic component of the relaxivity is reduced by about

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