has been related to changes in the immediate environment of the metal ion by a variety of spectroscopic methods.[10] The sigmoid pH functions describing these changes have been attributed to the ionization of either a coordinated water molecule or an adjacent amino acid side chain.[10a] For BCAB the apparent pK of the activity profile occurs at pH 6.9–7.4, while for HCAB the pK is 7.5–8.1, depending on substrate and conditions, for both the Zn(II) and Co(II) enzymes.[11] At pH 9.6 the resonance of ^{113}Cd(II) bound at the active site of HCAB appears as a relatively sharp line ($\delta\nu_{1/2} \simeq 28$ Hz) at −145.5 ppm. At pH 7.8 no resonance was detected under identical sampling conditions. The pH dependence of the ^{113}Cd resonance of HCAB thus appears in accord with the recently reported pH-rate profile for esterase activity of the Cd(II) enzyme (pK = 9.1) and the midpoint of the function describing the change in the nature of the Cd(II) coordination complex as detected by perturbed angular correlation of γ rays.[12] In contrast to ^{113}Cd(II) HCAB, a resonance from ^{113}Cd(II) bound to BCAB can be detected at pH 8.0 as a slightly broader line ($\delta\nu_{1/2} \simeq 40$ Hz) at −214 ppm. The variation in the relaxation of the ^{113}Cd nucleus at the active sites of the carbonic anhydrases with pH most likely reflects an alteration in the nature and exchange mechanisms of the monodentate ligand from solution. Broadening of the ^{113}Cd resonance by an exchangeable ligand (H_2O or ^-OH) from solution will be expected to be extremely sensitive to both the species of ligand and its exchange rate. ^{113}Cd NMR may prove to be a powerful tool for exploring the access of solvent or other ligands from solution to the metal binding site of Zn(II) metalloenzymes. Identification of the number and nature of the ligands contributing to the exchange phenomenon may in principle be derived from the solvent, temperature, and pH variation of the resonance and such studies are currently underway.

The appearance of a single sharp resonance ($\delta\nu_{1/2} \simeq 40$ Hz) at −172.2 ppm (Figure 1) for 2 equiv of ^{113}Cd(II) bound per AP dimer supports the postulated identity of the two metal binding sites in the absence of external ligands.[13] The effect on the ^{113}Cd(II) resonance of further metal ion addition and of alterations in the metal ion environment at one or both of the active sites arising as a consequence of allosteric interactions between the subunits accompanying phosphate binding is being determined.[14]

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The Use of ^{13}C Spin Lattice Relaxation Times for Determining the Position of the Proton in an Intramolecular Hydrogen Bond

Sir:

A particularly illuminating feature of the hydrogen bond is the location of the proton between the donor and acceptor atoms. Because of the difficulty of resolving hydrogen atoms by x-ray crystallography, the few data available have usually been obtained by neutron diffraction analysis.[1] We now describe a relatively simple method for locating the average position of the proton in the intramolecular hydrogen bond in 1-hydroxyfluorenone. This method appears widely applicable and involves measurements on liquids rather than single crystals.

Generally, the most important contribution to the spin lattice relaxation time of a ^{13}C nucleus is the dipole–dipole term (T_1^{DD}) association with neighboring protons. For a given proton, this term is proportional to $r_{C,H}^{-6}$, $r_{C,H}$ being the internuclear separation of the proton and the ^{13}C nucleus. For carbon bonded to hydrogen only the directly bound proton(s) contributes significantly to T_1^{DD}. For fully substituted carbon atoms, however, protons as far away as 3 Å may make a measurable contribution which can be used to estimate $r_{C,H}$. In general, the distances of a proton to three carbons will be necessary to define its location. In planar systems, such as 1-hydroxyfluorenone, two suffice.

The separation of the contribution of the hydrogen bonded proton to three fully substituted carbon atoms, 1, 1a, and 9, from the contributions of other protons is conveniently achieved by measuring the relaxation times in the normal molecule and the O-d species using samples prepared under identical conditions (simultaneous vacuum transfer of degassed solvent into two NMR tubes separately containing the same quantity of the two compounds). The contribution is then given by eq 1.[2]

$$1/T_1^{DD}(H) = [1/T_1^{obsd}(H) - 1/T_1^{obsd}(D)]/[1 - \gamma_D^2 I_D(I_D + 1)/\gamma_H^2 I_H(I_H + 1)]$$

$$1/T_1^{DD}(H) = 1.063[1/T_1^{obsd}(H) - 1/T_1^{obsd}(D)] \quad (1)$$

$$1/T_1^{DD}(H) = K/r_{C,H}^6 \quad (2)$$

Table I. Chemical Shifts (δ ppm: $CDCl_3$) and Spin Lattice Relaxation Times (s; $CDBr_3$) of Fluorenone and 1-Hydroxyfluorenone at 27 °C

	Fluorenone (1.1 M)		1-Hydroxyfluorenone (0.62 M)		
Position	δ	T_1	δ	T_1 (OH)	T_1 (OD)
1	124.2	1.32 ± 0.02	157.3	20.6 ± 0.7	33.8 ± 1.7
2	129.0	0.96 ± 0.01	118.2	1.00 ± 0.02	1.00 ± 0.01
3	134.6	1.14 ± 0.03	137.3	1.22 ± 0.01	1.25 ± 0.02
4	120.2	1.31 ± 0.01	112.7	1.29 ± 0.01	1.27 ± 0.01
5	120.2	1.31 ± 0.01	120.9	1.33 ± 0.01	1.35 ± 0.01
6	134.6	1.14 ± 0.03	134.5	1.10 ± 0.01	1.12 ± 0.01
7	129.0	0.96 ± 0.01	129.0	1.04 ± 0.01	1.03 ± 0.01
8	124.2	1.32 ± 0.02	123.9	1.32 ± 0.01	1.33 ± 0.02
1a	134.0		117.3	53.9 ± 2.4	89.2 ± 4.5
4a	144.3		143.7	35.7 ± 1.8	34.5 ± 0.6
5a	144.3		144.0	36.1 ± 0.9	35.9 ± 0.9
8a	134.0		134.1	37.2 ± 1.7	36.6 ± 2.0
9	193.7		185.4	53.1 ± 1.8	82.5 ± 4.2

Table II. Relaxation Times (s) and Internuclear Distances in 1-Hydroxyfluorenone

Carbon	$T_1^{DD}(H)$	$r_{C,H}{}^a$	$r_{C,H}{}^b$
1	49.6 ± 6.2	2.06 ± 0.06	2.01 ± 0.04
1a	128.1 ± 18.6	2.41 ± 0.07	2.36 ± 0.05
9	140.2 ± 19.5	2.45 ± 0.07	2.47 ± 0.05

[a] Based on an average value[6] of 1.22 s for T_1's of unsubstituted carbon atoms. [b] Values corrected for angular dependence.[6]

The estimation of $r_{C,H}$ requires the evaluation of the K in eq 2. The simplest approach is to assume isotropy of rotation diffusion and an *effective* aromatic carbon hydrogen internuclear distance (1.107 Å)[3] and use the relaxation times of the unsubstituted carbons to evaluate K. The necessary data are given in Table I, and the values of $r_{C,H}$ calculated from these data are listed in Table II.[6]

The propagated errors in $r_{C,H}$ include a significant contribution associated with the use of the average of the T_1's of the unsubstituted carbon atoms in the calculation of K. The variation in these T_1's is a consequence of anisotropy of rotational diffusion. In order to investigate this problem we have determined the relaxation times of the unsubstituted carbon atoms of fluorenone itself (Table I). Unequivocal assignments of the appropriate resonances were obtained from single frequency off-resonance and selective decoupling experiments on solutions containing the chemical shift reagent, tris(2,2,7,7-tetramethyl-4,6-octanedionato)europium. The relaxation times are recorded in Table I. The effect of anisotropy of rotational diffusion was investigated by means of eq 3[6,7] for an uncoupled planar rotor in which ψ_i is the angle which the CH internuclear vector for the ith carbon atom makes with the C_2 axis and a and b are constants which include the three principal rotational diffusion coefficients.

$$1/T_1^{DD}(H)_i = (a \cos^2 \psi_i + b \sin^2 \psi_i)/r_{C_iH}{}^6 \qquad (3)$$

The known geometry[8] of fluorenone has been used to obtain ψ_i. Equation 3 (with the least-squares coefficients $a = 1.16$ and $b = 1.65$) reproduces the observed T_1's within their standard deviations, indicating that the assumption of constant CH bond lengths is valid.[10] This treatment has been extended to 1-hydroxyfluorenone with the approximation that the same C_2 axis is present. The values of T_1's of the unsubstituted carbon atoms calculated by eq 3, with $a = 1.16$ and $b = 1.60$, agree well (standard deviation 3.5%) with the observed values. The above parameters were then used to correct the values of the approximate values r_{CH} in Table II for the angular dependences of the corresponding $T_1^{DD}(H)$'s. The results are also given in Table II.

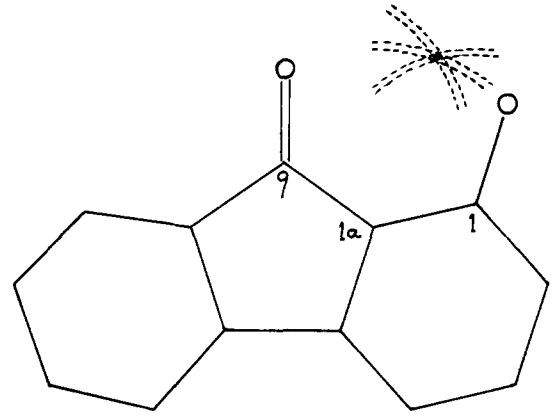

Figure 1. Location of the position of the hydroxyl proton in 1-hydroxyfluorenone. The pairs of concentric arcs indicate the propagated errors in r_{CH} and the shaded area is common to the three pairs.

The three internuclear separations[11] reported in Table II over-determine the location of the hydrogen bonded proton. This is depicted in the figure which corresponds to an O(1)H bond length of 1.13 ± 0.07 Å and C(1)O(1)H bond angle of 107 ± 2.5°. The relatively small propagated errors in these values are, of course, a result of their dependence on the sixth roots of the relaxation times. The internuclear distances recorded in Table II contain terms involving mean squared vibrational amplitudes.[3] For this system and for the distances involved, the appropriate corrections are expected to be an order of magnitude smaller than the experimental errors. Such considerations may, however, become important in systems in which the hydrogen bond is symmetric or is characterized by a very shallow double well potential, since the mean squared vibrational amplitudes might then be substantial.

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$$\langle 1/r^3 \rangle^{-1/3} = r_\alpha + (\langle \Delta x^2 \rangle + \langle \Delta y^2 \rangle - 4\langle \Delta z^2 \rangle)/2r_e$$

using the value 1.101 Å for r_α reported by Diehl and Niedenberger,[4] for benzene, the mean squared vibrational amplitudes determined by Brooks, Cyvin, and Kvande,[5] and the equilibrium CH bond length, r_e, equal to 1.1 Å. It is the appropriate value for the effective CH distance for dipole–dipole interactions in nonoriented systems.
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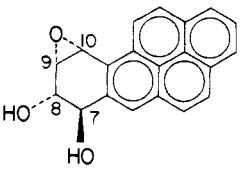
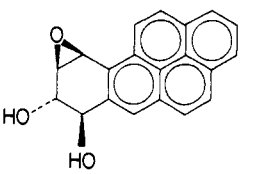
Benzo[*a*]pyrene–Nucleic Acid Derivative Found in Vivo: Structure of a Benzo[*a*]pyrenetetrahydrodiol Epoxide–Guanosine Adduct

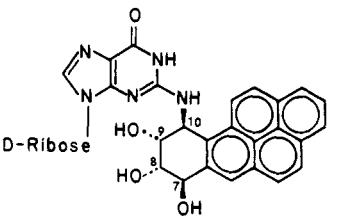
Sir:

Benzo[*a*]pyrene (BP) and other polycyclic aromatic hydrocarbon carcinogens are known to bind covalently to cellular nucleic acid in vivo. Several lines of evidence suggest that a 7,8,9,10-tetrahydro-7,8-diol 9,10-epoxide is involved.[1] Two isomeric forms of this compound (±)-I and (±)-II yield covalent complexes with poly(G) in vitro.[2] We have also recently found[2] that at least one of the forms of BP which becomes bound to nucleic acid in bovine bronchial explants during incubation of this tissue with ^{3}H-BP, and the product of reaction of isomer I with poly(G), are identical when hydrolyzed to ribonucleoside derivatives. Further chromatography of these derivatives as their diacetonides and diacetonide diacetates confirmed their identity and distinguished these derivatives from those obtained by in vitro reaction of poly(G) with isomer II. The structure of the major guanosine adduct formed with isomer I in vivo is now reported.

A pertinent finding was that the CD spectra of the two major in vitro components[3] were almost equal in shape but opposite in sign[4] and hence, except for the β-D-ribose moiety, the compounds were enantiomeric. The evidence presented below allows us to now assign structures **1** and **3** to these two compounds; the radioactive in vivo product corresponds to **3**.[2] Determination of the absolute configuration requires further studies.







1 (retention time, 21 min) and **3** (34 min)[3] (enantiomeric at 7, 8, 9 and 10)

Both **1** and **3** gave similar ^{1}H NMR spectra. However, the crucial regions of the original spectra (**1** shown in Figure 1 inset) were heavily overlaid by the solvent signals. Partially relaxed Fourier transform (PRFT) ^{1}H NMR measurements led to considerable improvement since the peaks due to protons with neighboring deuteriums, i.e., solvent signals, became in-

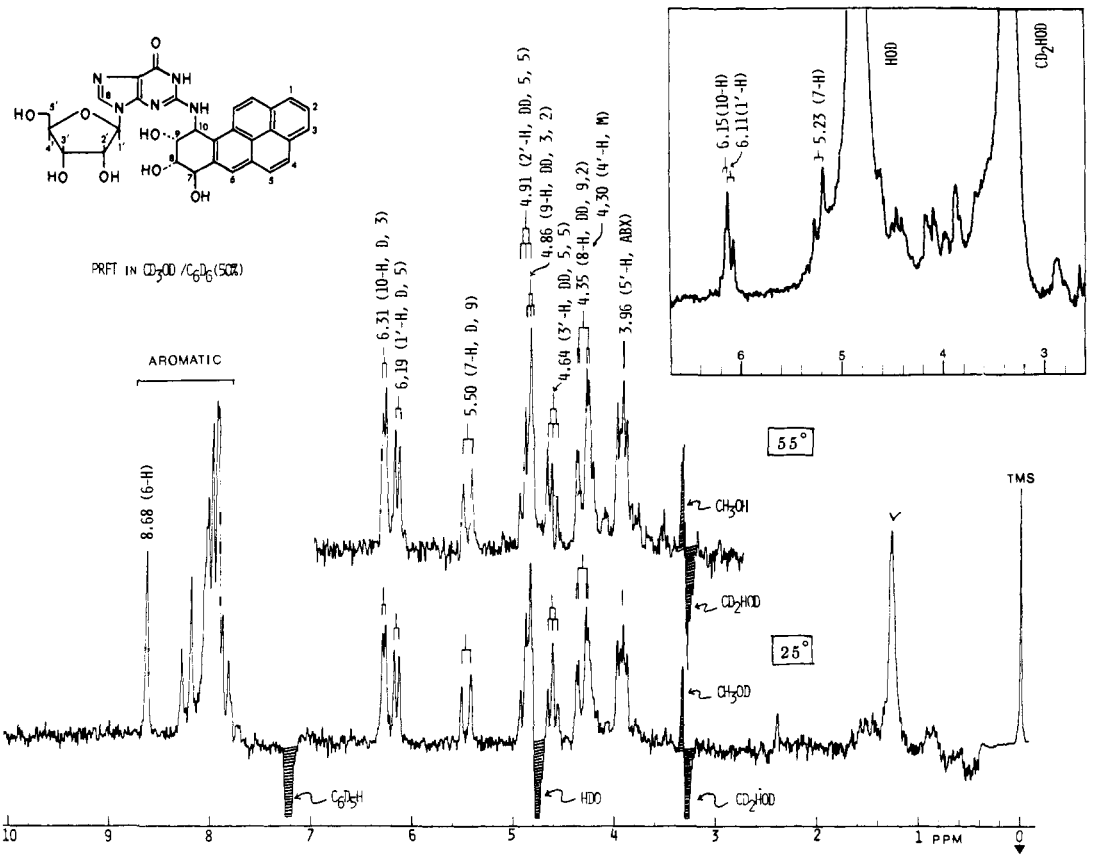


Figure 1. Inset: ^{1}H NMR of **1** in CD_3OD. Bottom and middle spectra: Partially relaxed Fourier transform spectra of **1** in CD_3OD:C_6D_6 (1:1) as measured by the inversion recovery method $(180\text{-}\tau\text{-}90\text{-}T)_n$, where τ = 2.2 s and T = 6.1 s, 4096 scans, JEOL PS-100. The sharp positive peaks at 3.35 ppm are due to CH_3OH present in the deuterated solvents.