plexation and preliminary results indicate that certain carbon signals are preferentially broadened in the presence of paramagnetic metal ions.[22,23]

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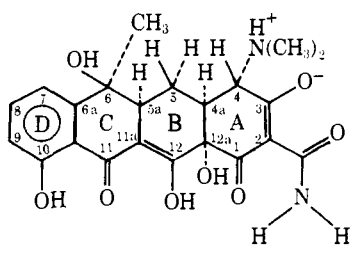
A ^{13}C Nuclear Magnetic Resonance Analysis of the Metal Binding Site in Tetracycline

Sir:

Recently a paper from this laboratory reported results of a proton NMR investigation directed toward establishing the site or sites of metal binding in tetracycline(I), abbreviated TC.[1] The study involved adding small amounts of



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paramagnetic and diamagnetic metal salts to DMSO-d_6 solutions of TC free base and observing selective effects of these salts on the proton NMR signals of TC. The results

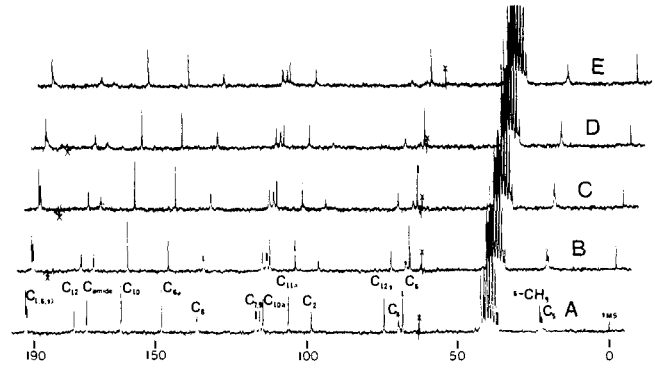

Figure 1. Natural abundance ^{13}C NMR spectra of tetracycline free base in DMSO-d_6 at Nd^{3+}/TC mole ratios of 0 (A), 0.017 (B), 0.034 (C), 0.050 (D), and 0.067 (E).

strongly indicate that binding occurs through ring A functional groups for each metal ion investigated. These conclusions have now been corroborated by a carbon-13 NMR study using lanthanide series ions as binding site probes. Results of the carbon-13 work are presented here.

The carbon-13 NMR spectrum[2] of TC (0.25 *M*) in DMSO-d_6 shows 18 of the possible 21 signals (Figure 1A). Asleson and Frank have made complete carbon-13 NMR signal assignments for TC in DMSO utilizing a series of TC derivatives.[3] Their assignments are used here. The solvent resonances (37–42 ppm) obscure signals assigned to carbons at positions 4a and 5a and in the dimethylamine group.

After recording the spectrum of TC free base, small amounts of a 0.21 *M* solution of anhydrous $Nd(NO_3)_3$ in DMSO-d_6 were added to the TC solution such that Nd^{3+}/TC mole ratios were 0.017, 0.034, 0.050, and 0.067. A spectrum was recorded after each addition of $Nd(NO_3)_3$. Similarly, another series of spectra containing increasing amounts of anhydrous $La(NO_3)_3$ was recorded. The paramagnetic Nd^{3+} ion was found previously to be particularly effective in causing selective broadening and shifting of proton resonance signals of TC.[1] As the mole fraction of Nd^{3+} is increased, a selective broadening occurs for signals assigned to C_{12}, the amide-C, C_2, C_{12a}, C_4 and two carbonyl signals in the group[4] containing C_1, C_3, and C_{11} as shown in Figure 1. Signal shifts in the presence of Nd^{3+} are no more than a few hertz. The largest shift occurs for C_5 which is about 10 Hz downfield of the free TC resonance at a Nd^{3+}/TC mole ratio of 0.067. These effects do not arise entirely from interaction of unparied electrons on Nd^{3+} with ^{13}C nuclei, however, since broadening and shifting of some of the same ^{13}C NMR signals of TC are also observed in the presence of the diamagnetic La^{3+} ion. With increasing La^{3+}/TC ratio, signals attributed to C_{12}, the amide-C, C_2, and *one* of the C_1, C_3, C_{11} carbonyls broaden, and the C_5 signal shifts downfield. Signal shifts in the presence of La^{3+} and Nd^{3+} are of the same magnitude and sign. Broadening is more pronounced for Nd^{3+} than for La^{3+} in most cases. Signal broadening in the presence of La^{3+} must arise from environmental averaging between bound and free TC.

In principle the location of the bound paramagnetic ion can be determined by analyzing the relative relaxation times and isotropic shifts of the perturbed NMR signals. However, one must first correct for signal perturbations caused by a diamagnetic ion binding at the same site. In the previous proton NMR study, this was done by examining *differences* in the spectra of TC in the presence of Nd^{3+} and in the presence of the same mole fraction of La^{3+}. When this procedure is employed for the carbon-13 spectra, the following conclusions emerge. (1) The effects of Nd^{3+}

and La^{3+} on all ^{13}C resonances unambiguously associated with rings C and D are the same within error. This absence of paramagnetic effects indicates binding is not significant in the immediate vicinity of rings C and D. (2) Paramagnetic effects of significance for rings A and B carbon signals include *marked* broadening for C_{12a}, C_4, at least one of the carbonyls C_1, C_3, or C_{11}, and less easily assessed broadening for C_2 and the amide carbon. Also a paramagnetic shift of a few hertz to lower field is observed for the amide carbon resonance. It s unlikely that the observed paramagnetic broadening in the C_1, C_3, C_{11} group occurs for C_{11}, since paramagnetic effects on neighboring signals are absent. These results then lead to the conclusion that metal binding (at least for rare earth ions in DMSO) involves ring A functional groups.

The proton NMR work strongly indicated that the ring A tricarbonylmethane group is responsible for metal binding in DMSO.[1] If such is the case, the paramagnetic broadening for C_2 and the amide carbon should be more pronounced than that of C_{12a} and C_4 using a simple $1/r^6$ dipolar broadening mechanism. The La^{3+}-induced broadening of C_2 and the amide carbon is appreciable, and it is difficult to evaluate differences between La^{3+}- and Nd^{3+}-induced broadening of these signals.[5] However, at high M^{3+}/TC ratios, broadening is definitely more severe in the presence of Nd^{3+} for both these signals. For example, the C_2 signal is clearly visible at La^{3+}/TC = 0.067 but is lost in the baseline at this mole ratio of Nd^{3+} (see Figure 1E). Although the present data cannot be used quantitatively to locate the metal ion, the conclusions drawn from proton NMR data appear to be substantiated.

The effects of Mg^{2+} on the carbon-13 NMR spectrum of TC in DMSO-d_6 were also investigated. The results parallel those of the proton NMR work in that (1) signals which broaden in the presence of Nd^{3+} or La^{3+} remain sharp at even higher Mg^{2+}/TC ratios, and (2) at high Mg^{2+}/TC ratios several new NMR signals appear concomitant with reduction in intensity of the original signals nearby.[6] The appearance of new proton NMR signals for TC in the presence of Mg^{2+} was interpreted earlier as evidence for a Mg^{2+}-induced conformational change of TC.[1] Here the new ^{13}C signals could arise from a Mg-bound conformer, but further work will be necessary to establish this.

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(2) All ^{13}C NMR spectra were recorded on a Bruker HX-90-E spectrometer located at the University of Iowa. Normally 2500–3000 scans were recorded for each spectrum using a 4 μsec pulse and a 2 sec repetition time. Internal TMS was used as the reference in each case.
(3) G. L. Asleson and C. W. Frank, *J. Am. Chem. Soc.*, preceding paper in this issue.
(4) Detailed assignments for the C_1, C_3, and C_{11} signals have been made for TC·HCl in DMSO,[3] but assignments of these signals are less certain for TC free base in DMSO due to their small chemical shift differences.
(5) The C_2 and amide-C signals are broadened by La^{3+} to a larger extent than any other signals of TC. La^{3+}-induced broadening of the C_4 and C_{12a} signals is negligible.
(6) This occurs for signals assigned to the amide carbon, C_{12}, C_{11a}, and C_2.

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The Vitamin D–3,5-Cyclovitamin D Rearrangement

Sir:

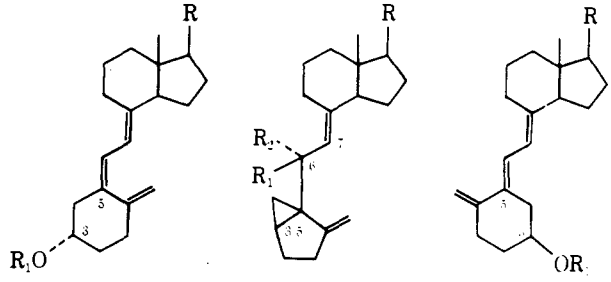
The pronounced chemical reactivity of the 1,3,5-triene system of vitamin D has considerably hampered investigation of its chemistry. The recently discovered high biological activity of vitamin D derivatives[1] induced us to search for a method to functionalize vitamin D, while protecting its reactive triene system. This report described the conversion of vitamin D_3 (cholecalciferol) to 3,5-cyclovitamins D_3 containing such a protecting system, and their stereoselective reconversion to the starting vitamin.

Heating (55°, 12 hr) of a methanol:acetone (4:1) solution of cholecalciferyl tosylate, **1b**[2,3] (1 mmol/cm^3), in the presence of NaOAc (8 equiv) yields in ca. 65% three methyl ethers, **2a, 2b,** and **1c,** in a ratio of 4.5:1:1.[4,5] The structure of the C_3-methyl ether of cholecalciferol, **1c,** was established by its uv and ^1H NMR spectra which were characteristic for the (*EZZ*)-hexa-1,3,5-triene system of cholecalciferol (**1a**) (uv, λ_{max} 264 nm, ϵ 17.000 in C_6H_{12} and in the presence of I_2, λ_{max} 272 nm;[6] ^1H NMR δ 5.08, 4.85 (=CH_2), 6.3, 6.1 ppm, AB quartet, J = 11 Hz (=C*H*—C*H*=)). The structure of (6*R*)-methyl ether, **2a,** was assigned to the major product of the solvolysis of **1b,** based on the absence in its uv spectrum of an absorption of a conjugated double bond system and on ^1H NMR evidence for the presence of two isolated exocyclic double bonds, one having two methylene protons (δ 4.89, 5.04 ppm) and the other, one methine proton vicinal to a proton on a methoxy bearing C atom (δ 4.98, 4.15 ppm, AB quartet, J = 9.7 Hz).

This structure assignment was corroborated by the off-resonance decoupled ^{13}C NMR spectrum which indicated four vinylic C atoms: two of them quaternary, one tertiary, and one secondary (δ 152.3, 143.4, 119.3, and 103.9 ppm, respectively). The configuration of C_6 was inferred both from its preponderance in the solvolysis products and from its highly stereoselective reconversion to cholecalciferol, **1a** (see below).

The third methyl ether, **2b,** is the C_6-epimer of **2a;** it shows an identical mass spectral fragmentation pattern and similar ^1H NMR spectrum to **2a** (δ 4.98, 4.81 (=CH_2), 4.69, 4.45 ppm, AB quartet, J = 8.5 Hz (OCH_3)C*H*—C*H*=)).

On acid solvolysis the cyclovitamins D_3 are reconverted to the vitamins. Thus heating (2 hr, 55°) of 75% aqueous dioxane solution of **2a** (2 mmol/cm^3) with *p*-toluenesulfonic acid (0.3 equiv) results in 80% of a mixture of cholecalciferol (**1a**) and *trans*-cholecalciferol[6] (**3a**) in 13:1 ratio.[7,8] Analogous treatment of **2b** gives also 80% of **1a** and **3a** but in a 2:1 ratio.



1a, R_1 = H
b, R_1 = Ts
c, R_1 = CH_3

2a, R_1 = H; R_2 = OCH_3
b, R_1 = OCH_3; R_2 = H

3a, R_1 = H
b, R_1 = Ts
c, R_1 = CH_3

The marked stereoselectivity in the solvolysis of cholecalciferyl tosylate **1b** (the ratio of **2a:2b** being 4.5:1) and the solvolysis of the (6*R*)-methyl ether **2a** (the ratio of **1a:3a** being 13:1) leads to the conclusion that the formation of the