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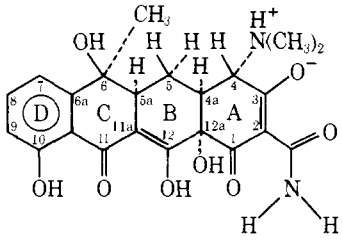
Effect of Added Electrolyte on the Binding of Tetracycline to Paramagnetic Ion Probes. A ^{13}C and ^{1}H Nuclear Magnetic Resonance Study

Sir:

Previous investigations of the site of metal binding in tetracycline base (I, abbreviated TC) and its derivatives by proton[1,2] and carbon-13[3] NMR, using Me_2SO-d_6 as a solvent, strongly indicate binding occurs primarily at the tricarbonylmethane function of ring A. However, these results are not in agreement with a number of binding site studies on tetracycline carried out by others. For example, results of a carbon-13 NMR study of TC·HCl by Asleson[4] in 50:50 (v/v) Me_2SO-d_6:D_2O in the pH range 8.0–9.5 suggests that binding involves the C_{11}–C_{12} β-diketone site. In Asleson's work TC·HCl was titrated with NaOH to the desired pH, thereby generating NaCl in a 1:1 mole ratio with TC. In an effort to resolve the apparent discrepancy, we have conducted experiments to determine what effect the solvent and/or the presence of electrolytes have on the nature of metal binding in TC.



I

Asleson and Frank have made complete assignments of the carbon-13 NMR spectra of TC and several of its derivatives in Me_2SO-d_6 and in D_2O.[5] Spectra in Me_2SO-d_6/D_2O mixtures show better signal separations than in either solvent alone, especially in the carbonyl region and in the aliphatic region at high field (see Figure 1A). In the present study a 70:30 (v/v) Me_2SO-d_6:D_2O solvent mixture was used. Solutions 0.3 M in TC can be prepared in this medium, thus enabling good spectra to be obtained in approximately 75 min.[6] The apparent pH was maintained in the 7.0–7.5 range throughout the experiments by addition of small amounts of NaOH dissolved in the same solvent mixture.[7] After recording the spectrum of TC, small portions of a 0.3 M solution of $Nd(NO_3)_3$ in the same solvent mixture were added to the sample tube such that Nd^{3+}/TC mole ratios were 0.018, 0.050, and 0.091. A spectrum was recorded at each of these ratios. Another series of spectra was recorded in the presence of $La(NO_3)_3$ at the same mole ratios to test for effects of a diamagnetic ion binding at the same site as Nd^{3+}.

As discussed previously,[3] short-range dipolar interactions between the paramagnetic Nd^{3+} ion and ^{13}C nuclei of TC cause selective broadening for nuclei near the binding site. Examination of the *differences* in spectra recorded at the same Nd^{3+}/TC and La^{3+}/TC mole ratios reveal those perturbations arising solely from the paramagnetism of Nd^{3+}. Figures 1B and 1C compare spectra taken in the presence of Nd^{3+} and La^{3+} at M^{3+}/TC = 0.050. Selective signal broadening in the presence of Nd^{3+} occurs for resonances assigned to C_3, the amide C, C_2, C_{12a}, and to a slight extent for C_4. The extent of broadening increases with increasing mole fraction of Nd^{3+}. Selective broadening of these signals may be shown quantitatively by comparing *differences* (Nd^{3+} vs. La^{3+}) in their T_2 values shown in Table I. These T_2 values were obtained from line-width measurements and include field inhomogeneity broadening. Signal shifts are all less than 0.3 ppm. Selective broadening of these same signals in the presence of Nd^{3+} occurs also for TC in 100% Me_2SO-d_6. It must be concluded that there is no significant solvent effect on the site of binding and that binding occurs primarily at the ring A tricarbonylmethane function, at least for lanthanide series ions under these conditions. The selective effect of Nd^{3+} on T_2 for C_{12a} is larger than expected for binding at this site and may indicate a contribution from scalar coupling. A more precise location of the metal ion would be possible using accurate T_1 values if contributions from ligand exchange and outer-sphere relaxation mechanisms could be determined.[8,9]

The effects of the presence of NaCl were determined by dissolving a sufficient amount of NaCl in the solution having a 0.050 mole ratio of Nd^{3+}/TC to give a 1:1 mole ratio of NaCl:TC. The carbon-13 NMR spectrum of this sample is shown in Figure 1D. Comparison of Figures 1C and 1D shows that the addition of NaCl has a remarkable effect on the spectrum of TC in the presence of Nd^{3+}. Signals which were selectively broadened by Nd^{3+} sharpen to line widths

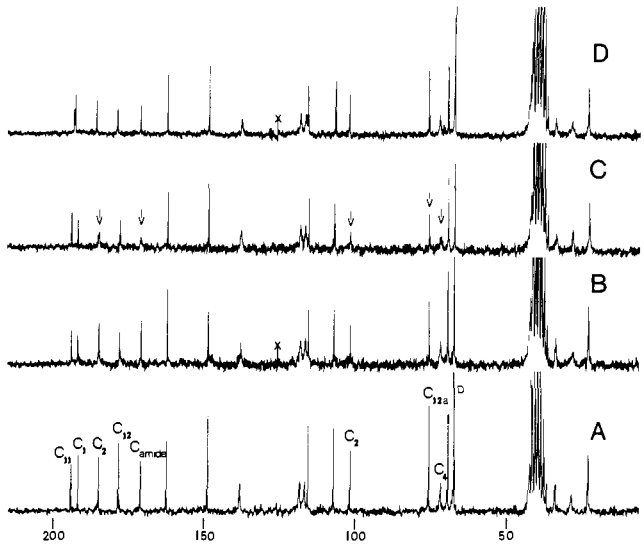


Figure 1. Carbon-13 NMR spectra of TC in 70:30 (v/v) Me_2SO-d_6:D_2O at pH 7.0–7.5. (A) No metal ion added; (B) La^{3+}/TC = 0.05; (C) Nd^{3+}/TC = 0.05; (D) Nd^{3+}/TC = 0.05 *and* Na^{+}/TC = 1.0. The scale is in ppm; the dioxane reference signal is labeled D; arrows point out signals selectively broadened.

Table I. Transverse Relaxation Times[a] for Carbon-13 Resonance Signals in the Presence of Nd^{3+} and La^{3+}

Signal	T_2, La^{3+}/TC = 0.050, s	T_2, Nd^{3+}/TC = 0.050, s
C_3	0.081	0.025
C_{amide}	0.11	0.016
C_2	0.078	0.043
C_{12a}	0.099	0.060
C_4	0.026	0.020

[a] Obtained from line width measurements on expanded-scale spectra. Errors are estimated to be 5–10%.

comparable with those found for TC alone. Upon comparison of Figures 1A and 1D, a slight broadening for C_{12} is apparent in addition to upfield and downfield shifts for C_{11} and C_1, respectively. Thus if $Nd(NO_3)_3$ were added to a solution of TC·HCl which had been titrated to pH ~7, the observed effects on ^{13}C NMR signals, *when compared to those of TC·HCl prior to addition of* Nd^{3+}, could be interpreted as indicating binding at the C_{11}-C_{12} β-diketone moiety. However when the solution containing a 0.050 mole ratio of La^{3+}/TC is treated likewise with NaCl, these same effects on C_{12}, C_{11}, and C_1 are observed. Thus there are no selective *paramagnetic* effects when NaCl is present at a 1:1 mole ratio with TC.

The above observations account for the conclusions drawn by Asleson from carbon-13 NMR studies regarding the site of binding of several metals to TC·HCl.[4] Indeed, in a number of previous investigations of metal binding in tetracyclines utilizing various techniques such as potentiometric titration, uv, CD, ir, and fluorescence spectroscopy, either the HCl salt of the antibiotic was used or the free base was used in combination with a relatively high concentration of buffers or other electrolytes.[10-20]

The effect of added electrolyte was further examined by proton NMR in Me_2SO-d_6. As reported earlier, the amide proton resonances show pronounced selective broadening in the presence of Nd^{3+} and other paramagnetic ions.[1,2] Upon addition of NaCl to a solution of TC and $Nd(NO_3)_3$ the broadened amide resonance signals sharpen considerably, and the upfield component shifts to a higher field as is observed for TC in the presence of diamagnetic ions such as Ca^{2+} or La^{3+}.[1] These same effects are observed upon addition of $NaNO_3$ or $NaClO_4$, but no significant changes occur upon adding Et_4NNO_3 or Et_4NClO_4. Only slight signal narrowing occurs when Et_4NCl is added. Thus it would appear that Na^+ is primarily responsible for the effects observed here. Presumably Na^+ competes successfully for the ring A binding site of TC by a mass action effect.

In conclusion, these experiments demonstrate that the long-standing controversy over the site at which metal binding occurs in tetracycline antibiotics may result, at least in part, from the presence of various electrolytes in the media at high molar concentrations relative to the drugs. Small cations from the electrolytes saturate the ring A binding site, forcing out cations of lower concentration. No NMR evidence of binding at other sites, under the conditions employed here, has been found.

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(7) Addition of metal ions to the TC solutions causes a drop in pH. Determination of the position of the acid–base equilibrium of TC here is complicated by the effects of D_2O on the pH meter reading and the combined effects of D_2O and DMSO on the p*Ka* values for TC. These effects are expected to cancel one another to a reasonable approximation. Thus in an effort to approximate physiological conditions, the pH range of 7.0–7.5, as read directly from a pH meter, was used for this work.
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Transition-Metal-Promoted Aldehyde–Alkene Addition Reactions

Sir:

The decarbonylation of aldehydes by chlorotris(triphenylphosphine)rhodium(I) (**1**) in solution under mild conditions (eq 1)[1] is one of many interesting and important examples of the utility of transition metal complexes in organic synthesis.

$$RCHO + \underset{\mathbf{1}}{RhCl(Ph_3P)_3} \rightarrow RH + RhCl(CO)(Ph_3P)_2 + PPh_3 \quad (1)$$

Although no evidence regarding the mechanism of this transformation has been reported, it has been suggested[1c] that the decarbonylation could proceed through an acylmetal hydride intermediate, i. The possible generation of metal

$$\underset{\mathbf{i}}{R{-}\overset{\overset{O}{\|}}{C}{-}Rh(H)(PPh_3)_2Cl}$$

complexes such as i via the oxidation additions of the aldehyde carbonyl carbon–hydrogen bonds to metal species is a fascinating concept. The potential utility of intermediates such as i in the synthesis of a variety of organocarbonyl compounds is great. Our continuing study of the role in catalysis of organotransition metal intermediates with unsaturated hydrocarbon ligands possessing C–M σ bonds[2] has led us to investigate the interaction of unsaturated aldehydes with transition metal compounds. We have sought to generate an acylmetal hydride intermediate and trap it, through the reaction of its M–C or M–H bond with an alkene ligand on the metal precursor, and also through reaction with unsaturation in the acyl ligand.

We wish to report that rhodium(I) complexes catalyze the addition of the aldehyde functional group in 4-pentenal to carbon–carbon double bonds to generate ketones. Both intermolecular and intramolecular reactions have been observed, the course of the reaction being dictated by the nature of the rhodium catalyst employed. Treatment of 4-pentenal with **1** in chloroform solution at room temperature afforded cyclopentanone (eq 2). When a 10:1 aldehyde:Rh mole ratio ([Rh] = 0.056 M) was employed, 32% of the