shown in Figure 7C. Undoubtedly, more than one species is present, but the complete disappearance of the resonance due to the supported phosphine indicates the efficiency of the reaction. The major species exhibits a coupling to ^{195}Pt of 2850 Hz, indicative of a *trans*-$[PtCl_2L_2]$ moiety. A second species of unknown identity is also indicated by a resonance to slightly higher field. The complexity of the spectra we have obtained using this indirect route for immobilization cast considerable doubt upon the idealized structures often taken as representative of the nature of supported transition-metal phosphine complexes.[2]

Summary

These investigations demonstrate that CP/MAS ^{31}P NMR spectroscopy may be utilized to gain valuable information about surface-immobilized phosphine ligands and transition-metal complexes. Our studies of two of the common routes to supporting transition-metal complexes on silica and glass surfaces demonstrate that the simple schemes representing the course of these reactions, eq 1 and 2, are not sufficient to describe the chemistry that actually occurs. Equation 2 is misleading as the initial step results in substantial oxidation of the tertiary phosphine, while the product of the second step depends upon the exact reaction conditions and may well result in the production of more than one immobilized metal complex. The route described by eq 1 is more meaningful, but becomes invalid when the metal complex undergoes labile dissociative processes. In such cases, immobilization of the metal complex is accompanied by the formation of supported phosphine and phosphine oxide.

Currently we are expanding our work to include the study of rhodium(I) systems, using both inorganic and organic support materials, and to investigate the nature of supported catalysts before and after use in catalytic reactions.

Acknowledgment. Thanks are expressed to the Natural Science and Engineering Research Council of Canada for financial support in the form of operating grants (to H.C.C., C.A.F., R.E.W.) and to Johnson Matthey Limited for a very generous loan of platinum metal salts.

Registry No. *trans*-$[PtCl_2(PPr_3)_2]$, 15977-22-7; *cis*-$[PtCl_2(PMePh_2)_2]$, 16633-72-0; *cis*-$[PtCl_2(PPh_3)_2]$, 15604-36-1; *trans*-$[PtCl_2(PPh_3)_2]$, 14056-88-3; *cis*-$[PtCl_2(Ph_2P(CH_2)_2Si(OEt)_3)_2]$, 79919-60-1; *trans*-$[PtCl_2(Ph_2P(CH_2)_2Si(OEt)_3)_2]$, 79980-82-8; *cis*-$[PtCl_2(CO)(P(p\text{-}CH_3C_6H_4)_3)]$, 42958-15-6; *cis*-$[PtCl_2(CO)(P(p\text{-}FC_6H_4)_3)]$, 76705-01-6; *cis*-$[PtCl_2(CO)(P(c\text{-}C_6H_{11})_3)]$, 19618-81-6; *cis*-$[PtCl_2(CO)P(n\text{-}Bu)_3)]$, 73320-20-4; *trans*-$[NiCl_2(Ph_2PCH_2CH_2Si(OEt)_3)_2]$, 79919-61-2; *trans*-$[NiCl_2(PCy_3)_2]$, 55333-16-9; *trans*-$[NiCl_2(PPh_2Me)]$, 28665-20-5; *trans*-$[PdCl_2(Ph_2PCH_2CH_2Si(OEt)_3)_2]$, 79919-62-3; $[Pd((N)_2\text{-}(Ph_2PCH_2CH_2PPh_2)]$, 32288-97-4; $[Pd(PPh_3)_4]$, 14221-01-3; *cis*-$[PtCl_2(PEt_3)_2]$, 15692-07-6; $[PtCl_2(NCPh)_2]$, 14873-63-3; *cis*-$[PtMe_2\text{-}(PPh_3)_2]$, 17567-35-0; $[PtClMe(Ph_2P(CH_2)_2PPh_2)]$, 27711-50-8; PPh_3, 603-35-0; $P(p\text{-}CH_3C_6H_4)_3$, 1038-95-5; $P(p\text{-}FC_6H_4)_3$, 18437-78-0; $P(p\text{-}MeOC_6H_4)_3$, 855-38-9; $P(c\text{-}C_6H_{11})_3$, 2622-14-2; $Ph_2PCH_2PPh_2$, 2017-20-7; $Ph_2PCH_2CH_2PPh_2$, 1663-45-2; $Ph_2PCH_2CH_2AsPh_2$, 23582-06-1; $Ph_2PCH_2CH_2Si(OEt)_3$, 18586-39-5; $OPPh_3$, 791-28-6; $OP(C_{14}H_{29})_3$, 72931-37-4.

Effects of Mg(II) on the Conformation of Tetracycline in Me_2SO Solution

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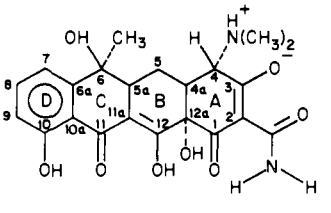
Abstract: Carbon-13 NMR and CD data are presented which show that tetracycline in Me_2SO solution undergoes a major conformation change upon addition of water. In Me_2SO/H_2O solvent mixtures, tetracycline is believed to be in a zwitterionic form. In addition, it is shown that Mg(II) is able to induce the same conformation change in Me_2SO in the absence of water, presumably by binding to the zwitterionic form of tetracycline. These results suggest that Mg(II) ions may effect the in vivo transport of tetracycline by their influence on the molecular conformation.

During the past decade there has been considerable interest in the tetracycline antibiotics with regard to their cation binding properties[1-4] and their molecular conformations in the crystalline[5-13] and solution phases.[14-15]

A recent paper from this laboratory reported the results of a ^{13}C NMR spin-lattice relaxation study of tetracycline, I (hereafter



I

abbreviated TC), in the presence of Mn(II) using an 80:20 (v/v) $Me_2SO\text{-}d_6$:D_2O solvent mixture in the pH range 7-8.[16] The

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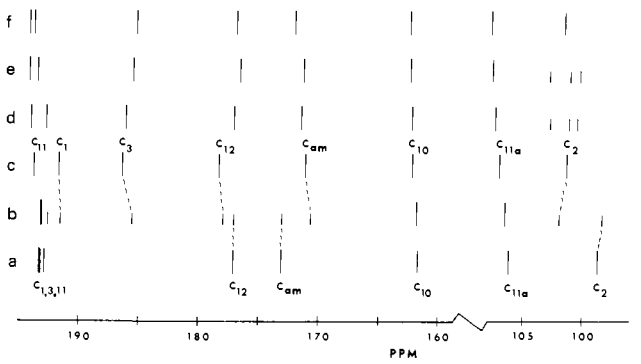


Figure 1. Diagram illustrating selected portions of carbon-13 NMR spectra of TC taken under the following conditions: (a) TC in Me_2SO; (b) TC in Me_2SO with $Mg(NO_3)_3$ at Mg/TC = 0.25; (c) TC in 80:20 (v/v) Me_2SO/D_2O; (d–f), TC in 80:20 (v/v) Me_2SO/D_2O with Mg/TC = 0.25 (d), 0.50 (e), and 1.0 (f).

Mn(II) ion was found to bind at three sites on TC—the two tricarbonylmethane sites on ring A and the $C_{12}O$–$C_{12a}O$ site—and the residence time of Mn(II) at any site is short on the NMR time scale. Mn(II) was used as a paramagnetic substitute for the biologically abundant but diamagnetic ion, Mg(II).

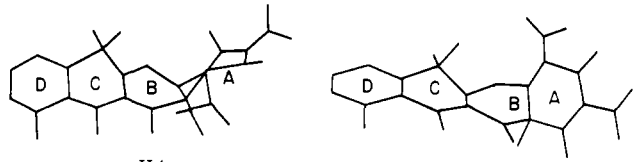
These findings, along with the recent study by Hughes, Stezowski, and Hughes[15] which showed that oxytetracycline undergoes a major conformation change on going from an ethanolic to an aqueous–ethanolic solvent, allow interpretation of NMR and CD data obtained earlier in this laboratory for TC in Me_2SO or Me_2SO/water solvents in the presence of Mg(II).[1-4] The data and our interpretation are presented here.

Experimental Section

Carbon-13 NMR spectra were recorded on a Bruker WP-80 FT spectrometer using solutions approximately 0.3 M in TC. 8 K data points were used over a 5000 Hz spectral width. CD spectra were taken on a Cary 60 spectropolarimeter with solutions approximately 1 mM in TC and a cell path length of 0.1 cm.[4] In Me_2SO/water solutions used for either NMR or CD spectra, the pH was adjusted to ~7 by addition of NaOH in D_2O. Anhydrous $Mg(NO_3)_2$ was prepared by the method of Ewing et al.[17]

Results and Discussion

According to Hughes et al.,[15] oxytetracycline in solution is involved in an equilibrium between a non-ionized form having the conformation represented schematically by IIB and a zwitterionic form having conformation IIA.[6] The conformational change involves rotation about the C_{12a}–C_{4a} bond.



IIA IIB

Selected portions of the ^{13}C NMR spectra[18] of TC taken in Me_2SO or Me_2SO/D_2O solvent mixtures and in the presence or absence of $Mg(NO_3)_2$ are shown schematically in Figure 1. Assuming the behavior of TC in Me_2SO/D_2O parallels that of oxytetracycline in $EtOH/H_2O$, one would expect the non-ionized form, IIB, to predominate in Me_2SO. Upon addition of ≥20% (v/v) of D_2O, however, the equilibrium should be displaced largely toward the zwitterionic form with conformation IIA. Thus, spectrum a in Figure 1 is believed to be representative of TC in conformation IIB, whereas spectrum c represents TC in conformation IIA. The largest changes in chemical shift occur for C_1, C_2, C_3, C_4, C_5, C_{amide}, and C_{12}. Consistent with the proposed

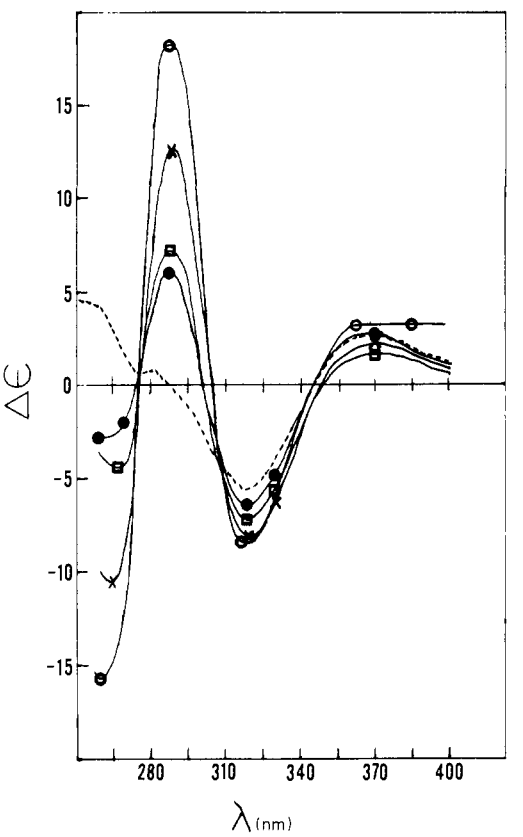


Figure 2. CD spectra of TC in Me_2SO: (---) no $Mg(NO_3)_2$; (●) Mg/TC = 0.25; (□) Mg/TC = 0.50; (×) Mg/TC = 1.0; (○) Mg/TC = 2.0.

H_2O-induced conformation change are large changes found in the CD spectra of TC on going from Me_2SO to Me_2SO/H_2O solvents (compare Figures 2 and 3). These CD changes are similar to those reported by Hughes et al. for oxytetracycline in $EtOH/H_2O$.[15]

If anhydrous $Mg(NO_3)_2$ is added to the Me_2SO solution of TC, significant changes in chemical shifts occur, and several of the ^{13}C (and proton[1,3]) signals appear doubled as shown in spectrum b. The "new" signals increase in intensity at the expense of the original signals as the Mg/TC mole ratio is increased and predominate when this ratio is >0.5.[4] These new signals have chemical shifts close to those found for TC in 80:20 $Me_2SO:D_2O$ (spectrum c). Thus in Me_2SO, Mg(II) appears to displace the IIA ⇄ IIB conformational equilibrium toward IIA, presumably by binding to TC in this conformation. Doubling is clearly observed for signals assigned to C_2, C_{amide}, and C_{12}. Only one of the two components may be identified readily for C_1 and C_3; the other components are not well resolved here. The occurrence of separate signals in spectrum b, attributed to the two conformations, demonstrates that interconversion is slow on the NMR time scale. The two species are believed to be IIB and Mg–IIA, and the rate of conformational interconversion may be limited by the rate of Mg^{2+} exchange between IIA and solvent.

The proposed Mg(II)-induced displacement of the conformational equilibrium is corroborated by CD spectra of TC in Me_2SO solutions containing anhydrous $Mg(NO_3)_2$. These are shown in Figure 2. As the Mg/TC mole ratio is increased, $\Delta\epsilon$ values at ~265 and ~290 nm change significantly.[20] These CD spectra bear a qualitative resemblance to that of TC alone in Me_2SO/H_2O (Figure 3) except for a 5–10-nm shift in wavelength which may result from Mg(II) binding. These changes cannot be attributed to water introduced via the $Mg(NO_3)_2$ for the following reasons.

(17) Ewing, W. W.; Klinger, E.; Brandner, J. D. *J. Am. Chem. Soc.* **1934**, *56*, 1053–1057.

(18) ^{13}C NMR signal assignments for TC and several of its derivatives in Me_2SO and in D_2O were made by Asleson and Frank[19] and are used in this paper.

(19) Asleson, G. L.; Frank, C. W. *J. Am. Chem. Soc.* **1975**, *97*, 6246–6248.

(20) Similar changes are observed upon addition of increasing amounts of anhydrous La(III) and Nd(III) nitrates.[4]

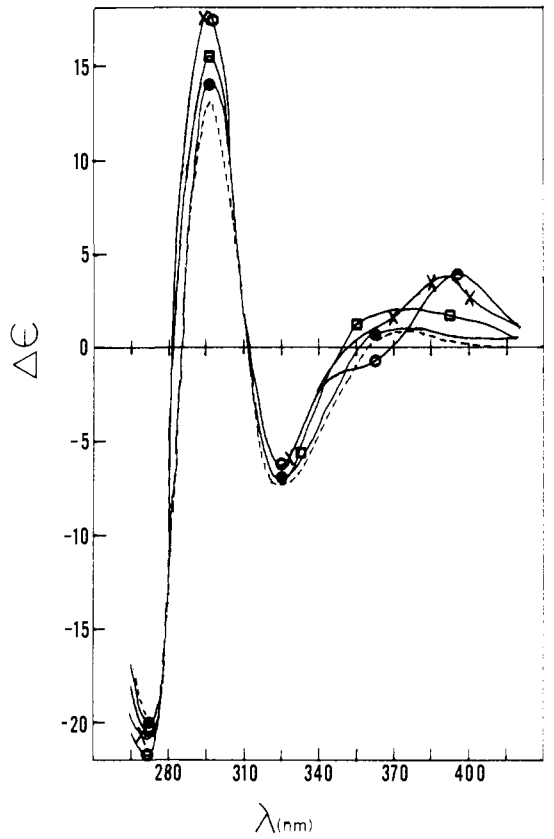


Figure 3. CD spectra of TC in 70:30 (v/v) Me_2SO/H_2O: (---) no $Mg(NO_3)_2$; (●) Mg/TC = 0.20; (□) Mg/TC = 0.75; (×) Mg/TC = 1.5; (○) Mg/TC = 2.0.

The CD spectrum of TC in 90:10 Me_2SO/H_2O shows a slight, positive maximum at 290 nm.[4] This solution is 5.5 M in H_2O. If it is assumed that the $Mg(NO_3)_2$ is not anhydrous and that six water molecules are introduced for each Mg(II) ion, the concentration of water would be only 0.012 M in the Me_2SO solution having a Mg/TC mole ratio of 2.0 for which a *large* CD at 290 nm is found.

The fact that the CD at 265 and 290 nm increases further at Mg/TC ratios greater than 1.0 implies that the equilibrium is not fully displaced toward conformation IIA at this ratio.[21] This could result from small K_{eq}'s for both equilibria, IIB $\rightleftarrows$ IIA $\rightleftarrows^{Mg^{2+}}$ Mg-IIA, so that high Mg(II) concentrations are needed to drive these processes toward the right. Mn(II) binds TC through oxygens at C_{12} and C_{12a} in addition to oxygens at the tricarbonylmethane moiety.[16] Chelation of a metal ion at $C_{12}O$–$C_{12a}O$ is possible only for TC in conformation IIA, and this may provide the driving force for the Mg(II)-induced conformation change. An alternative explanation is that binding at the tricarbonylmethane sites of IIB results in formation of the TC zwitterion which prefers conformation IIA.[6,15] Evidence that the Mg-bound conformer is IIA derives from the fact that the CD spectrum of crystalline TC hydrate, mulled in mineral oil, is very similar (except for a wavelength shift of 15–20 nm) to those of TC in Me_2SO in the presence of Mg^{2+}. Stezowski has shown by X-ray crystallography that TC hydrate has conformation IIA in the solid state.[6]

If the above arguments are correct, TC in Me_2SO/water solutions should be largely in the proper conformation for Mg(II) binding. Any changes in the NMR or CD spectra upon addition of Mg(II) should be due to (1) further displacement of the equilibrium toward conformation IIA and/or (2) intrinsic effects of bound Mg(II) on chemical shifts or chromophores responsible for the CD. Changes in the CD spectra of TC in Me_2SO/H_2O solution upon addition of $Mg(NO_3)_2$ are shown in Figure 3. Upon going from a Mg/TC mole ratio of 0 to 1.5, the 270 and 297 nm bands undergo intensity changes of 8% and 25%, respectively. No significant additional change occurs at higher Mg/TC ratios. Assuming these changes result predominately from (1) above, TC in Me_2SO/H_2O in the absence of Mg(II) is 75–92% in conformation IIA, and at Mg/TC ratios $\geq$1.5 TC is fully in this conformation. Separate NMR signals for the two conformations are not observed for Mg-free TC in Me_2SO/D_2O (spectrum c). This implies that either the conformational interconversion is rapid in Me_2SO/D_2O or that the signals of molecules in conformation IIB are too small to be detected above the base line noise.

Carbon-13 NMR spectra of TC in Me_2SO/D_2O with increasing Mg/TC ratios are shown schematically in Figure 1 d–f. The observed changes are best interpreted as resulting from both (1) and (2), above. At low Mg/TC ratios, it is expected that Mg(II) will bind at the same sites observed for Mn(II) under similar conditions.[16] Indeed, at a Mg/TC ratio of 0.25, chemical shift changes of 0.3–1.3 ppm are observed for C_1, C_2, C_3, C_{amide}, C_{12}, and C_{12a}. However, changes in signals assigned to C_4, C_{11}, and C_{11a} are also observed. The change at C_4 is attributed to a shift in the conformational equilibrium. The chemical shift changes (0.2–0.3 ppm) observed for C_{11} and C_{11a} may indicate binding at the C_{11}–C_{12} β-diketone group, a site which was *not* observed to bind Mn(II). In the Mn(II) study, the Mn/TC ratio was only 3×10^{-4}. At the relatively large Mg/TC ratios needed to produce observable NMR changes, Mg(II) may be forced to bind at additional sites. In this regard, it should be noted that at Mg/TC ratios >1.0, a new CD band appears at ~390 nm.

The reason for the apparent splitting of the C_2 signal into two or three poorly resolved components in spectra d and e of Figure 1 is not known at present. It may involve two orientations of the amide group related by 180° rotation about the C_2–C_{amide} bond. Both these orientations have been observed in the solid state by crystallography,[6] and both appear to be involved in Mn(II) binding in Me_2SO/D_2O solution.[16]

The most important conclusion to be drawn from this work is that Mg(II) is able to displace the IIA (zwitterion) $\rightleftarrows$ IIB (non-ionic) conformational equilibrium toward IIA. It has been proposed that this equilibrium plays an important role in the transport of tetracyclines through a sequence of polar and nonpolar media.[6,15] If this is correct, the present work suggests that Mg(II), and perhaps other biologically abundant cations, may affect in vivo TC transport through conformation changes induced by binding.

(21) The NMR spectra show no evidence of IIB at Mg/TC ratios greater than 0.5. This is likely a result of the fact that solutions used for NMR are 300× as concentrated as those used for CD.

Registry No. TC, 60-54-8; Me_2SO, 67-68-5; $Mg(NO_3)_3$, 10377-66-9.