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β-Carbonylamides as Chelating Agents. N.M.R. Determination of the Equilibria Formation with Lanthanide Metal Ions

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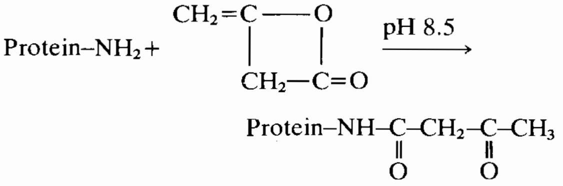
Complex formation of N-acetoacetylisopropylamide with europium(III) in methanol has been studied by N.M.R. spectroscopy. The stability constant indicates a fairly good binding power for such a ligand, and seems promising in view of the utilization of β-chetoamides as chelating agents for the study of the relationships between structure and function in biological systems.

Introduction

Recent investigations demonstrated that a β-carbonylamide moiety can be readily introduced into proteins by reaction with diketene.[1]



$$\text{Protein–NH–}\underset{\|}{\overset{}{C}}\text{–CH}_2\text{–}\underset{\|}{C}\text{–CH}_3 \quad (\text{C=O},\ \text{C=O})$$

While the reversibility of this modification is still under investigation,[2] the presence of such a functional group could provide a favourable site for the coordination of metal ions into specific regions of peptide and proteins.

Numerous investigations have been made on metal complexes with β-diketones[3–5], but only few data are available on binding of β-carbonylamide with metal ions, especially with lanthanides.

On the other hand, introduction of paramagnetic ions represents one of the most promising tools for the study of the relationships between structure and function in biological system.[6–10]

In this paper we report a preliminary proton N.M.R. study on the binding of europium(III) with a model β-carbonylamide ligand, namely N-acetoacetylisopropylamide (AIPA), in methanol at room temperature.

Results and Discussion

The induced upfield shift of the methyl protons in the ligand on increasing the lanthanide concentration, is reported in Figure 1.

Under conditions of fast chemical exchange for the ligand on the N.M.R. time scale, for the equilibrium $L + nS = LS_n$ in which one mol of metal ion combines with n moles of ligand, only a single signal is recorded for each nucleus, corresponding to weighted average of the free and associated environment. Denoting with Δ_B the shift of a given nucleus in the complexed species relative to its diamagnetic (uncoupled) position, the observed net shift Δ is given by: $\Delta = (1-[S]/[S_0])\Delta_B$. When $[S_0]$ of ligand is mixed with $[L_0]$ of metal ion, [S] represents the unbound ligand at equilibrium. Using material balance equations and introducing the variable Φ, defined as $[L_0]/[S_0]$, the pre-

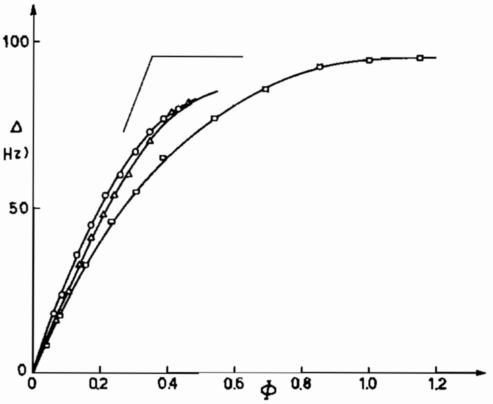


Figure 1. Plot of Δ (lanthanide induced shift for methyl protons of the isopropyl group) *vs.* the ratio between the total concentration of $EuCl_3$ $[L_0]$ and AIPA $[S_0]$ in methanol. $[S_0]$: O, 1.165 *M;* △, 1.366 *M;* □, 1.103 *M.* Measurements were carried out with a 90 MHz HF Brucker N.M.R. spectrometer. Chloroform was used as internal reference.

vious relation turns: $\Delta = \Delta_B\Phi([S_0]-[S])/[L_0]$. For large values of the binding constant K and for low values of Φ, this may be approximated by the straight line $\Delta = n\Delta_B\Phi$. For large values of Φ the relation $\Delta = \Delta_B$ holds. Therefore the point at which the two lines cross each other indicates the stoichiometry of the complex $n = 1/\Phi$. On the reasonable assumption that each ligand is equivalently bound to the metal, graphical approach, for the experimental data in the Figure, gives the 1 to 3 complex as the prevailing one.

The equilibrium constant K can be thus evaluated by the relation $K = \Delta\Delta_B^n/[S_0]^n\ (\Delta_B-\Delta)^n\ (n\Delta_B\Phi-\Delta)$.

A numerical fit of this equation has been performed independently in order to evaluate, on the basis of variables Δ and Φ, the parameters n, Δ_B and K. A 1/3 metal to ligand stoichiometry with a value Δ_B of 95 Hz has been confirmed, while a value of $(3 \pm .5)10^2$ has been found for the binding constant.

Investigations at lower initial concentrations of AIPA are underway. Preliminary data, suggest the presence in methanolic solution of different complexes with various stoichiometries.

Furthermore, a plot of $\bar{n}$* (average number of ligands per metal atom) *vs.* log[S], in the same conditions of the Figure, shows that $\bar{n}$ raises towards 3 on increasing log[S] for reasonable values of Δ_B. For different conditions, as low ligand concentrations, other chemical species may exist.

In conclusion, the binding constant of complexes between europium(III) and a model β-carbonylamide has been obtained by proton N.M.R. spectroscopy. The K value obtained indicates a fairly good binding power of the lanthanide for such ligands, at least in methanol.

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* $\bar{n} = \dfrac{[S_0]-[S]}{[L_0]}$; $[S] = \dfrac{[S_0][\Delta_B-\Delta]}{\Delta_B}$

Both $\bar{n}$ and log[S] are readily obtained from the N.M.R. measurements.