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Article

Study of the Binding of Eu^{3+} and Tb^{3+} to L-phenylalanine and L-tryptophan

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Os íons európio e térbio trivalentes possuem raios iônicos semelhantes àquele do Ca^{2+}. Em função disto, eles são usados como sondas de sítios de ligação de cálcio em moléculas de seres vivos. Tais íons possuem características espectroscópicas muito úteis ao seu estudo, particularmente uma luminescência intensa. Em proteínas contendo estes íons lantanídios a elas ligados, emissão de luz pode ser observada quando radiação no UV é absorvida por seus resíduos de aminoácidos aromáticos, indicando que há transferência de energia para o íon lantanídio. O presente trabalho foi feito com o objetivo de definir os sítios de ligação do Eu^{3+} e do Tb^{3+} nos complexos com os aminoácidos aromáticos L-fenilalanina e L-triptofano. As técnicas utilizadas foram as espectroscopias no infravermelho e de ressonância magnética nuclear de ^{13}C. Foi verificado que európio e térbio trivalentes interagem com o grupo carboxilato de ambos os aminoácidos. Com o L-triptofano o grupo imino do anel indólico também se liga ao lantanídio, representando um segundo ponto de coordenação.

Trivalent europium and terbium ions have ionic radii similar to that of Ca^{2+}. So they are employed as probes of calcium binding sites in biological molecules. These ions exhibit very useful spectroscopic characteristics, chiefly a pronounced luminescence. In protein bound lanthanide, visible light emission from the lanthanide excited states can be observed when UV light is absorbed by aromatic amino acids. Subsequently, the energy is transferred to the lanthanide ion. The present work was carried out to define the binding sites of Eu^{3+} and Tb^{3+} in complexes with the aromatic amino acids L-phenylalanine and L-tryptophan. The techniques utilized were infrared and ^{13}C nuclear magnetic resonance spectroscopies. It was found that trivalent europium and terbium interact with the carboxylate group of both amino acids. With L-tryptophan, the imino group of the indole ring is also involved representing another coordination site.

Keywords: *aromatic amino acids, lanthanides*

Introduction

About a third of all proteins in their native state contain bound metal ions, or require metal ions for a variety of metabolic pathways[1]. In many cases, Ca^{2+} either constitutes protein metal center or is needed for cell biological activity. This metal ion exhibits no satisfactory spectroscopic properties, so the study of calcium depending proteins by spectroscopic techniques is extremely difficult. To overcome this problem, it is often possible to substitute a trivalent lanthanide (Ln^{3+}) for Ca^{2+} ions within those proteins. No serious structural changes or loss of specific functions are observed after such substitution. Among the lanthanide ions, Tb^{3+} and Eu^{3+} generally show luminescent emission that is enhanced when these ions are bound to a protein. The luminescence excitation may be performed by irradiation in the range of aromatic spectral absorption bands. As a rule, tryptophan is responsible for energy transfer to Ln^{3+} ions, although tyrosine and phenylalanine may be involved in this process[2].

So, it seems profitable, in view of eventual biochemical and biophysical applications, to study the interaction between aromatic amino acids and lanthanide ions, whose luminescence may be considered as specific to prove their presence. No paper has been published on this issue in

recent years. The last work published is that by Aizawa *et al.*, in 1987, where the interaction of tryphophan with Tb^{3+} ions had been studied using fluorescence and ^{1}H-NMR spectroscopies[3]. These authors had concluded that terbium interacts with α-amino and imine groups, the latter belonging to the indole ring. No interaction with the carboxylate group was reported. Such conclusions, based mainly on the ^{1}H-NMR measurements, seem unconvincing, since they are in contradiction with the coordination chemistry of the lanthanides. These hard acids according to Pearson's theory[4], should interact more strongly with oxygen than with nitrogen. Especially when the latter has a positive charge, this being the case of the α-amino group in the zwitterion form of the amino acid. In aqueous solution even a neutral amine cannot compete with water molecules for Ln^{3+} coordination[5].

This disagreement has been the reason to re-open the issue, resulting in the present work. Herein, we describe the interaction of Eu^{3+} and Tb^{3+} with the aromatic amino acids L-tryptophan and L-phenylalanine, by nuclear magnetic resonance with ^{13}C-NMR and infrared spectroscopies.

Experimental

Infrared spectra were obtained using a Nicolet 730 FT-IR spectrometer, using the samples in the form of KBr pellets. A Bruker AC200 spectrometer was utilized for recording the ^{13}C-NMR spectra (field of 4.7 T and frequence of 50 MHz), the samples were dissolved in D_2O.

The coordination compounds of lanthanides (Eu^{3+} and Tb^{3+}) and the ligands (L-phenylalanine and L-tryptophan) were obtained by reaction of the lanthanide perchlorates with the corresponding amino acids, in 1:1 water-ethanol mixtures. These were concentrated by solvent evaporation and the water content reduced by successive additions of absolute ethanol, until the remaining water was minimal. After that, some drops of benzene were added to them, and the solutions were placed into a refrigerator and left there for some days until precipitation started. After completed the precipitation, the solutions were filtered, and the solid residue washed with absolute ethanol and dried under vacuum. The stoichiometry of the complex compounds was confirmed by CHN elemental analysis, while the metal content was determined by complexometric titrations with EDTA. The lanthanide perchlorates were prepared heating the lanthanide oxides with 1.0 M perchloric acid solution, in stoichiometric quantities.

All the reagents utilized were of analytical grade, the *L*-amino acids were supplied by Sigma and the lanthanides oxides from Aldrich.

Results and Discussion

The elemental analyses (Table 1) were compatible with the following compound formulae: $Tb(L\text{-phe})_3(ClO_4)_3.2H_2O$; $Eu(L\text{-phe})_3(ClO_4)_3.4H_2O$; $Tb(L\text{-trp})_3(ClO_4)_3.H_2O$ and $Eu(L\text{-trp})_3(ClO_4)_3.4H_2O$. The infrared spectra of the compounds (Figs. 1 and 2) have shown changes in the position and profiles of some bands, as compared to that of the free amino acids, suggesting the participation of the groups that produce these bands in the coordination bon with the lanthanides. Major changes, in all IR patterns, are related to the carboxylate bands. In the case of L-phenylalanine (Fig. 1), the bands at 1410 cm^{-1} and 1565 cm^{-1}, corresponding to the carboxylate symmetrical and asymmetrical stretchings, respectively, are shifted to higher wavenumbers after complexation with Tb^{3+} and Eu^{3+}. Thus indicating coordination through that group. The remaining

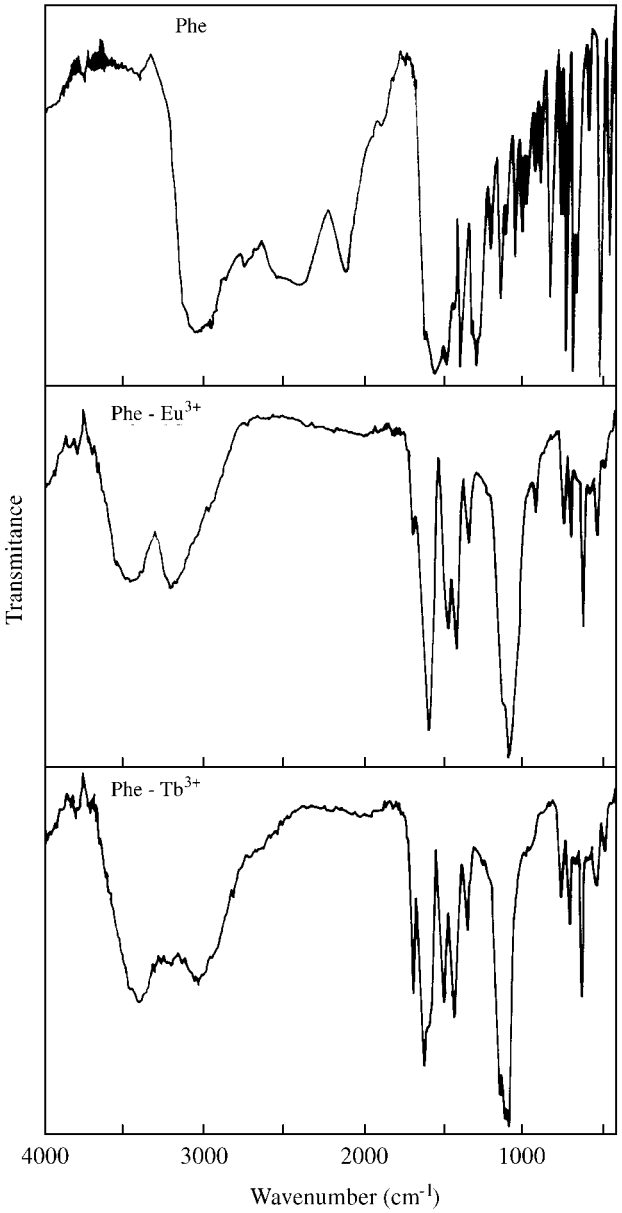

Figure 1. Infrared spectra. From top to bottom: Phenylalanine, Phenylalanine complexed with Eu^{3+}, and Phenylalanine complexed with Tb^{3+}.

carboxylate bands, namely γCOO^-, ωCOO^- and ρCOO^-, formerly at 780, 681 and 526 cm^{-1}, respectively, also showed changes as a result of the coordination process.

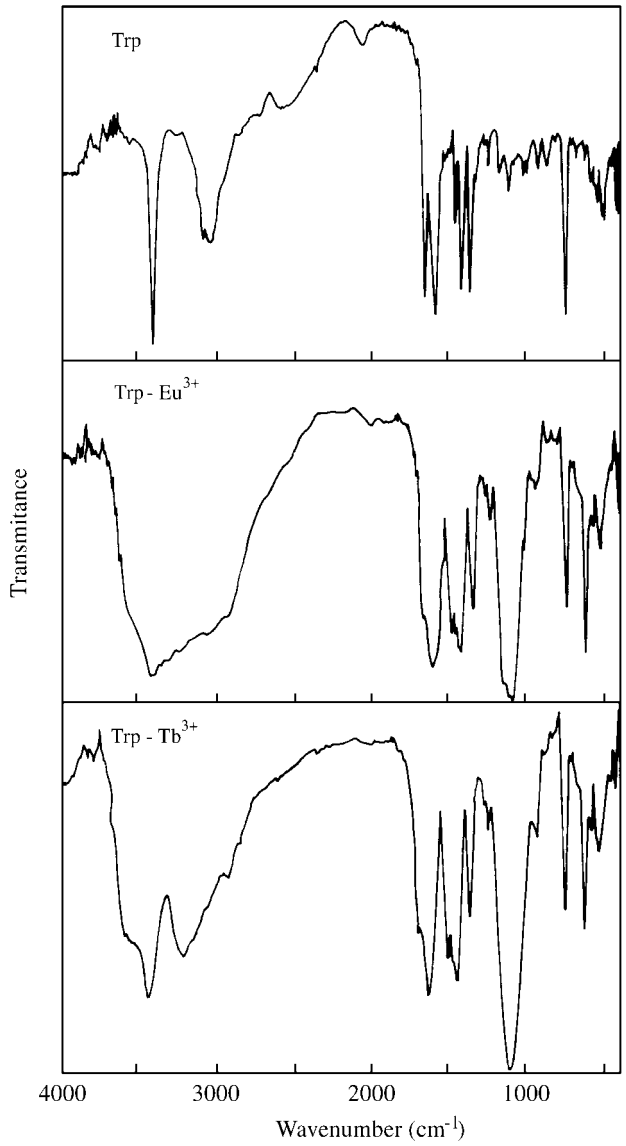

Figure 2. Infrared spectra. From top to bottom: Tryptophan, Tryptophan complexed with Eu^{3+}, and Tryptophan complexed with Tb^{3+}.

L-tryptophan (Fig. 2) behaved similarly with respect to the carboxylate bands: the asymmetric stretch at 1592 cm^{-1}, the symmetric stretch at 1413 cm^{-1} and the deformation vibrations in the region between 700 and 520 cm^{-1}. The former, stretching vibration, was displaced to 1619 and 1623 cm^{-1} (compounds with Eu^{3+} and Tb^{3+} respectively) and the latter to 1425 and 1429 cm^{-1}, with respect to Eu^{+3} and Tb^{+3}. The indolic nitrogen contributes to the bands at 1413 and 533 cm^{-1} , and their displacement after the coordination suggests that this group is a second binding center for the lanthanide ions.

The ^{13}C-NMR spectrum of L-phenylalanine (Fig. 3) shows no carboxylate carbon signal at 174.3 ppm after the complexation with Eu^{3+}, and it was shifted to lower field (197.1 ppm) in the Tb^{3+} compound. The signal of the carboxylate neighbouring carbon was shifted to higher field in both complexes. These results are compatible with the lanthanide complexation by the carboxylate, since it causes a decrease of the electron density around the corresponding carbon atom with its consequent striping in relation to the magnetic field. On the contrary, the neighbouring carbon enriches its electron density and this increase in shielding displaces the NMR signal toward a higher field.

The L-tryptophan ^{13}C-NMR spectrum (Fig. 4) shows a similar behaviour of the C-carboxylate and α-C resonance peaks after complexation with $Eu^{3+}$and Tb^{3+}: the shift of the former to lower fields and of the latter to higher fields. The interpretation is the same, thus indicating that carboxylate is a coordination site. The most interesting feature of the spectra of the coordination compounds with tryptophan is the appearence of two additional signals, at 136 and 128 ppm. These peaks may be associated with quaternary like carbon atoms, located close to the indole nitrogen. These signals are absent in the spectrum of free L-tryptophan and their presence in the lanthanide complexes also suggests coordination by the nitrogen atom of the indole group. The nitrogen interaction with the lanthanide ion shortens the nuclear spin relaxation time of the two neighbouring carbons, resulting in the appearence of the above signals.

Table 1. Elemental analysis results.

Compound	% Metal		% C		% H		% N	
	E*	C#	E	C	E	C	E	C
$Eu(L\text{-}phe)_3(ClO_4).\ 4H_2O$	16,20	16,07	31,13	31,80	4,04	4,02	4,17	4,12
$Tb(L\text{-}phe)_3(ClO_4)_3.2H_2O$	16,72	16,68	32,54	32,80	4,07	3,46	4,51	4,25
$Eu(L\text{-}trp)_3(ClO_4)_3.4H_2O$	14,64	14,29	36,31	37,25	5,43	5,08	7,58	7,90
$Tb(L\text{-}trp)_3(ClO_4)_3.H_2O$	14,54	14,85	36,51	36,42	3,94	3,49	7,74	7,72

* Experimental value.
# Calculated from formula.

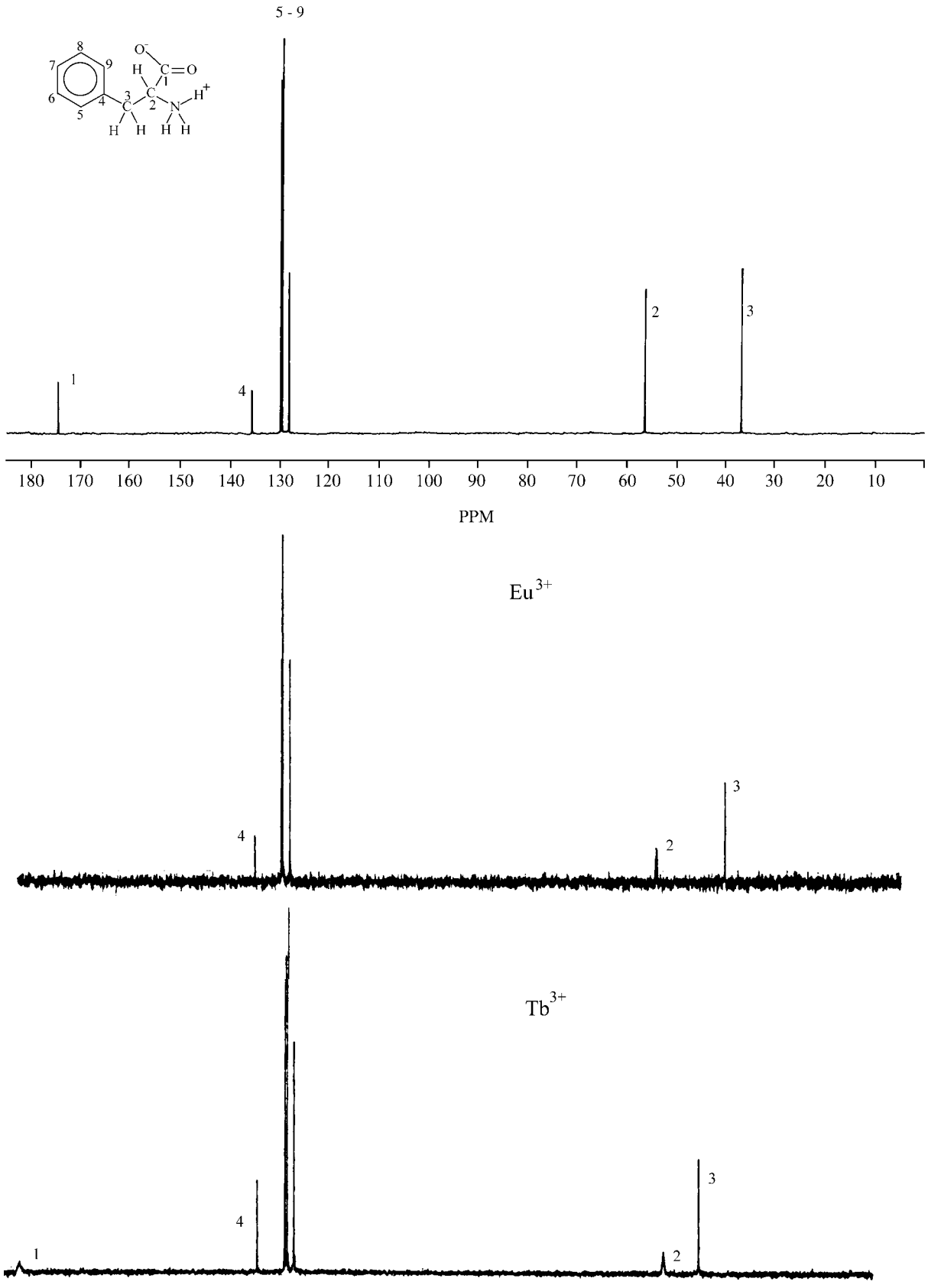

Figure 3. ^{13}C Nuclear Magnetic Resonance spectra of Phenylalanine, Phenylalanine-Eu^{3+} and Phenylalanine-Tb^{3+}, with signal assignment.

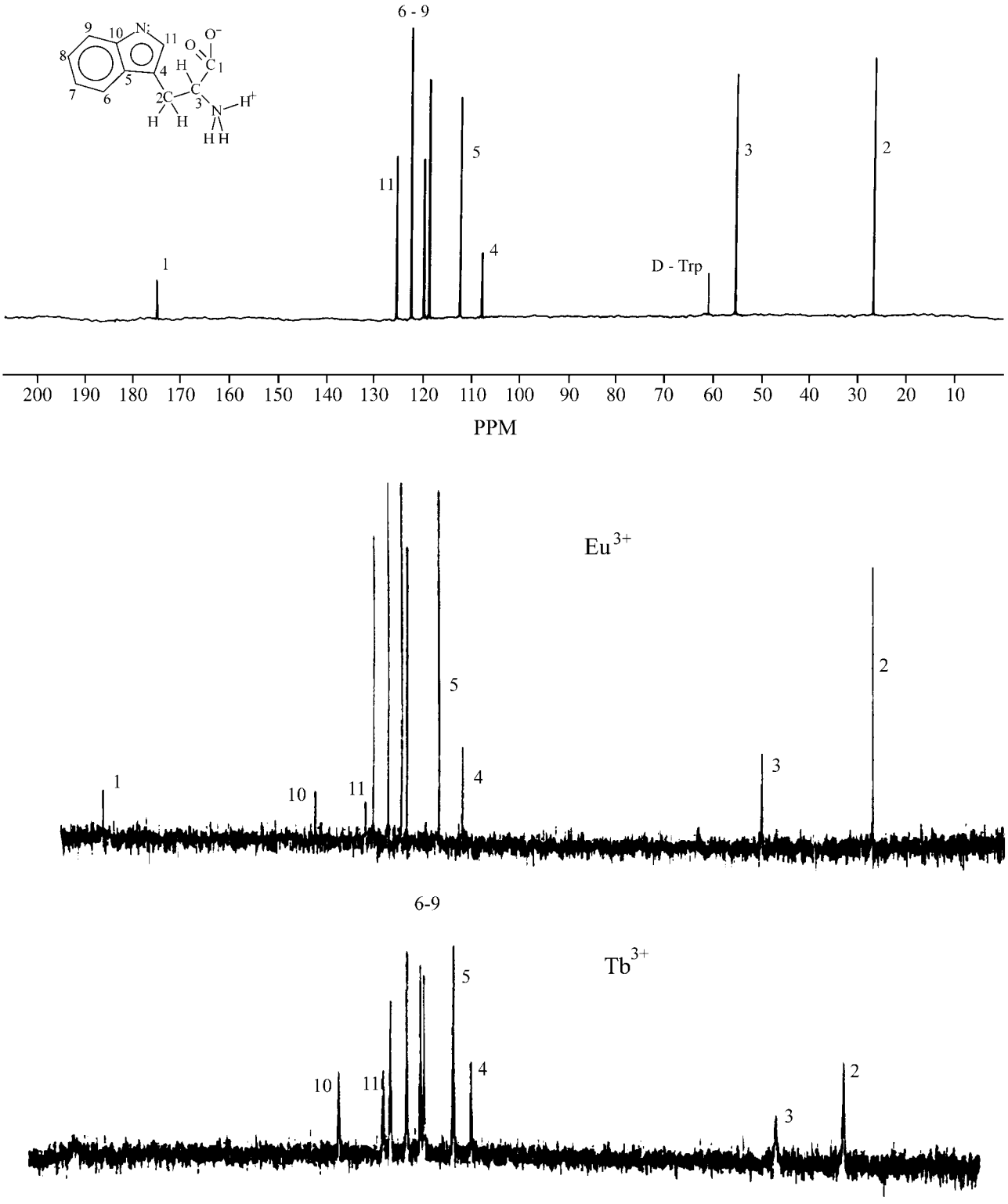

Figure 4. ^{13}C NuclearMagnetic Resonance spectra of Tryptophan,Tryptophan-Eu^{3+} and Tryptophan-Tb^{3+}, with signas assignment.

In conclusion, the IR and ^{13}C-NMR results suggest that the interactions between Tb^{3+} and Eu^{3+} and the ligand L-phenylalanine are due to an arrangement via the carboxylate group, while in the case of L-tryptophan both the carboxylate and the nitrogen of the indole group are involved. The protonated amine group does not participate in the coordination, as would be suggested by lanthanide chemistry and by the presence of a strong positive charge on this group.

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