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LANTHANIDE INDUCED SHIFTS IN THE NMR SPECTRA OF METHYL METHOXYBENZOATES AND METHOXYBENZENES

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Abstract—Aromatic methyl ethers appear to bind strongly to the NMR shift reagent Eu(fod)$_3$ only when there are at least two ether groups *ortho* to each other. Isolated or lone methyl ethers are very weakly bound to shift reagent as compared to an ester group. It is proposed that europium is involved in strong bidentate binding to *ortho* oxygens in veratrole, 1,2,3-trimethoxybenzene, methyl veratrate, methyl 3,4,5-trimethoxybenzoate and reserpine. The resultant shifts in molecules with several sites of comparable binding ability are subjected to population analysis using shifts from model compounds. Populations of individual substrate-lanthanide complexes are calculated and demonstrate the additivity of lanthanide induced shifts in these systems.

INTRODUCTION

We had examined in connection with a previous study[1] the effects of the shift reagent Eu(fod)$_3$ on the NMR spectrum of reserpine (**1**). As shown in Fig 1, the greatest downfield shifts were experienced by the *p*-OMe protons on the trimethoxybenzoyl (TMB) ring. In view of the large number of potential binding sites available in reserpine and the unknown binding ability of aromatic ethers and esters to shift reagents, it was surprising that the TMB ring appeared to be the major site of binding. Other detectable shifts fell off rapidly with distance from this part of the molecule. We, therefore, set out to determine the factors which lead to strong binding of lanthanide to the ether group(s) with a view toward the application of shift reagents to structural studies of complex natural products.

RESULTS

Methyl methoxybenzoates As model compounds, a series of methyl methoxybenzoates was prepared from the corresponding methoxybenzoic acids and their lanthanide induced shifts (LIS) were determined by extrapolation of induced shifts to 1:1 mole ratio. These are presented in Fig 1 in brackets with the corresponding chemical shifts (δ) of esters alone in deuterochloroform in parentheses. Initially, in the case of methyl 3,4,5-trimethoxybenzoate (**2**), it was uncertain whether the methyl ester or the *p*-OMe protons were shifted to the greater extent. Consequently, the methyl-d$_3$ ester (**3**) was prepared and its LIS's determined, confirming the shift assignments given for **2**. Methyl-d$_3$ esters were also prepared for all other methoxyesters shown and the LIS of the remaining protons were identical (± 5%) to those in the normal esters. Since association constants for binding of Eu(fod)$_3$ to a number of ligands tend to be indeterminate (> 100),[2] we consider that the LIS in Fig 1 (extrapolated to 1:1 mole ratio of shift reagent to substrate) are comparable in magnitude with the bound chemical shifts of the complexes. In fact, it was impossible to obtain consistent association constants for these compounds. In one instance, where bound chemical shifts were determined, i.e. for methyl veratrate (**4**), the calculated bound chemical shifts were consistently only 25% greater than the LIS values obtained by extrapolation.

Several features of the data in Fig 1 are noteworthy. An ester group by itself, as in methyl benzoate (**8**), is a relatively good ligand for europium as evidenced by the large LIS of the methyl ester and *ortho* ring protons. Even with one methoxy group in the ring (**6**, **7**), ester group is still the primary site of binding. However, in methyl veratrate (**4**), the two *ortho* OMe groups bind strongly to the shift reagent and appear to compete with the ester group for the reagent. Indeed, this binding to two adjacent ether oxygens is thought to be responsible for the LIS pattern observed in **2** and in reserpine (**1**), although in these compounds there are two equivalent sites for such binding. This explanation is further corroborated by the low shifts of the OMe protons compared to the methyl ester protons in methyl 3,5-dimethoxybenzoate (**5**) where the two ethers are *meta* to one another.

Methoxybenzenes. In order to elucidate the shift patterns arising from lanthanide complexation only to OMe groups, i.e., in the absence of an ester group, a series of methoxybenzenes was examined. The chemical shift and LIS data for these compounds are shown in Fig 2. As expected, only **9** and

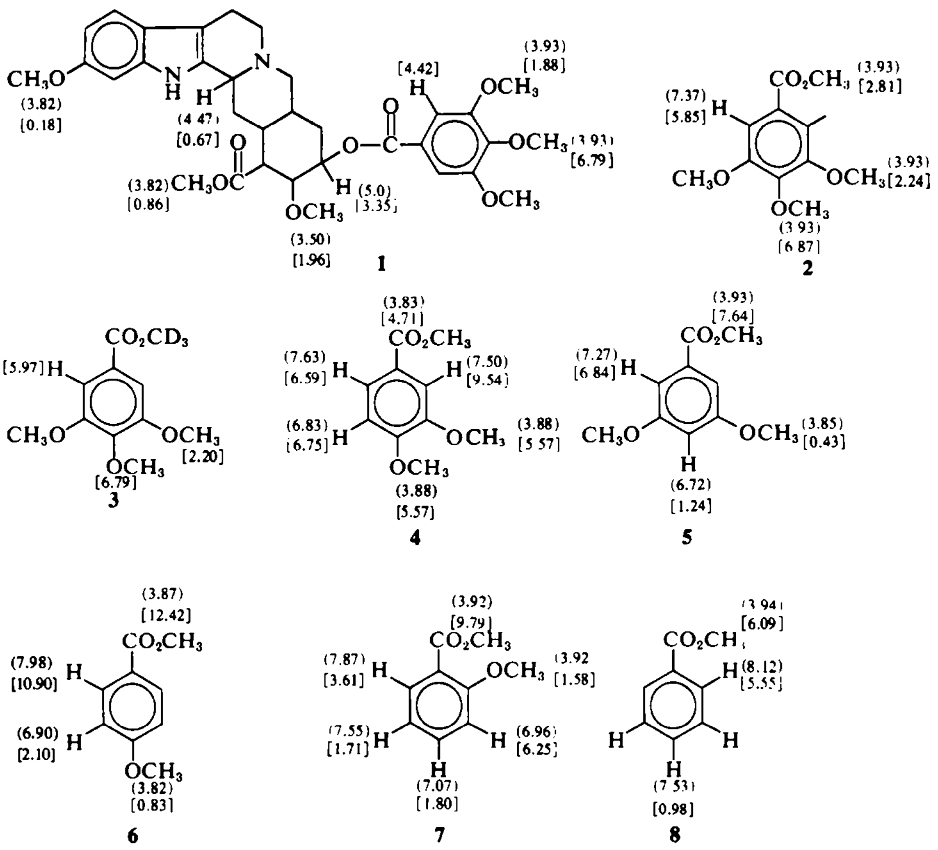


Fig 1. NMR Chemical Shifts (ppm) and Lanthanide Induced Shifts [ppm] of Methoxybenzoates in $CDCl_3$.

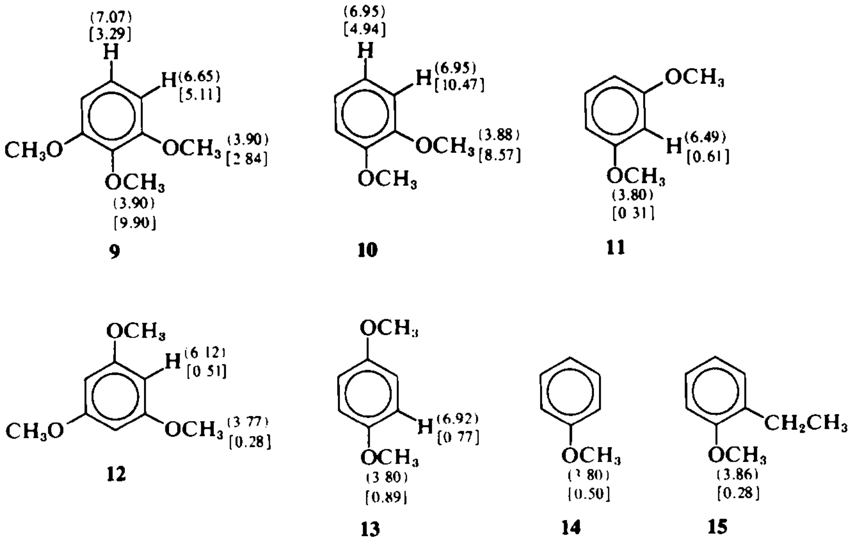


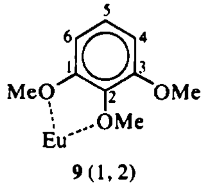
Fig 2. NMR Chemical Shifts (ppm) and Lanthanide Induced Shifts [ppm] of Methoxybenzenes in $CDCl_3$.

10 with *ortho* OMe groups show large LIS values, confirming that strong binding occurs between the *ortho* ether oxygens. Anisole itself (**14**) shows a small shift of the ether protons, while the shifts of the ring protons were too small to be determined. Even lower induced shifts are observed in **11** and **12**, where two and three OMe groups are *meta* to each other, respectively. This is partially due to the presence of two and three binding sites where the fraction of any one complex is only 1/2 or 1/3 for **11** and **12**, respectively. *p*-Dimethoxybenzene (**13**) appears to be anomalous in this regard in that the OMe shift is larger than in anisole. The electron releasing resonance effect of a *p*-OMe group may serve to increase the binding ability of the second OMe oxygen over anisole itself. Thus, the net *observed* shifts of the OMe protons are very small. These results do show, however, that ring protons *ortho* to a OMe group are shifted very nearly the same amount as the ether protons themselves. Alternate mechanisms exist for the occurrence of large shifts of OMe protons in **9** and **10**. One possibility is that OMe protons *ortho* to the complexation site (assuming monodentate binding) actually get a large shift contribution, while the protons on the complexed group receive a very small shift, as in anisole itself. This possibility was ruled out by determining LIS's for *o*-ethyl anisole (**15**), where the Et group takes the place of the second OMe. The shifts for **15** show that the C-Me group does not get a large LIS based on complexation of shift reagent to the ether. Steric influence of an *ortho* OMe also should not give a dramatic increase in LIS of a bound OMe: in **15** where the *o*-Et group is isosteric with a OMe group of veratrole (**10**), the OMe proton LIS is actually lower than that in anisole. It is interesting that almost imperceptible shifts of Et protons in **15** were observed. In fact, it would appear that the C-Me protons of **15** were shifted slightly *upfield* by the shift reagent, although the extent of the shift was too small to be determined accurately. The concept of this shielding effect is seen to be important later (*Population analyses*).

The mechanism which we propose for the large LIS of compounds with at least two OMe groups *ortho* to each other involves bidentate binding of lanthanide to *ortho* oxygens. The geometry of such a complex will be dependent on deviations of the Me groups from coplanarity with the aromatic ring. Moreover, the lanthanide ligand bond axis needed for pseudocontact correlations of observed shifts is unknown.

Population analyses. If it can be assumed that the LIS values observed for a compound with only one strong binding site represent intrinsic shifts for protons in such a complex, then the resultant shifts in a molecule with two or more good binding sites might be additive functions of intrinsic shifts and complex populations (fractions). A further assumption is that the mechanism of complexation of shift reagent to *ortho* diethers is as proposed above. We are interested both in calculating complex populations and in using such populations to calculate unknown shifts.

(1) 1,2,3-*Trimethoxybenzene.* For 1,2,3-trimethoxybenzene (**9**) two equivalent complexes with Eu(fod)$_3$ can be formed, designated (1,2) and (2,3), implying complexation to each *ortho* pair of OMe groups, as indicated by structure **9** (1,2). The



9 (1, 2)

observed LIS (Δ_B) for any proton i in the molecule, assuming very large K, will be a weighted average of the shift contributions from each complex. Thus,

$$\Delta_{B,i} = p(1,2)\,\Delta_{i(1,2)} + p(2,3)\,\Delta_{i(2,3)} \qquad (1)$$

where $p(1,2) = p(2,3) = 0{\cdot}5$, and $\Delta_{i(1,2)}$ represents the LIS for proton i in complex (1,2) as determined from the model compound veratrole. As shown in the structure of complex **9** (1,2), ring proton 6 should have a shift contribution corresponding to the shift of protons 3,6 of veratrole, 10·47 ppm. Similarly, the induced shifts of protons 4 and 5 will be 4·94 ppm. In the (2,3) complex, however, proton 4 will experience a 10·47 ppm shift while the 5 and 6 proton shifts will be 4·94 ppm. The LIS of **9** calculated with Eq 1 are:

$$\Delta_{B,4} = \Delta_{B,6} = 7{\cdot}70 \text{ ppm and } \Delta_{B,5} = 4{\cdot}94 \text{ ppm}$$

The observed LIS for **9** are $\Delta_{B,4(6)} = 5{\cdot}11$ ppm and $\Delta_{B,5} = 3{\cdot}29$ ppm, smaller than those calculated above. However, the ratios of the shifts are identical: $\Delta_{4,6}/\Delta_{5(\text{calcd.})} = 1{\cdot}56$; (obs) = 1·55. The larger calculated shifts may represent the unknown effect (steric, electronic) of the third OMe group in each complex.

Turning to the OMe groups, the calculated LIS of the 2-OMe protons is 8·57 ppm, somewhat smaller than the observed value of 9·90 ppm. Calculation of the 1,3-OMe shift would require a knowledge of the shift of the third, uncomplexed OMe group protons in each complex. Since this value is unknown, equation 1 can be solved for this shift using the observed value of the 1,3-OMe shift:

$$\Delta_{3(1,2)} = \Delta_{1(2,3)} = \frac{2{\cdot}84 - 0{\cdot}5(8{\cdot}57)}{0{\cdot}5} = -2{\cdot}87 \text{ ppm.}$$

It would appear that the unbound OMe protons are actually shielded in each complex and experience an upfield shift contribution. This would arise from a negative pseudocontact geometric factor, $(3\cos^2\theta-1)r^{-3}$, for this group. Indeed, preliminary calculations for an assumed geometry of each complex (unbound OMe protons *trans* to bound OMe's) reveal a large angle θ for the unbound OMe protons and, consequently, a shielding effect by the europium atom. A similar shielding was alluded to in reference to *o*-ethylanisole (**15**) where the C-Me protons (C-Me *trans* to O-Me) could also be at a large angle θ with respect to the lanthanide-oxygen bond axis.

(2) *Methyl veratrate.* Bound LIS for an ester-europium complex could be taken from the observed shifts in methyl benzoate. However, it is noticed that the LIS values for methyl anisate (**6**) are larger than for methyl benzoate, probably relating to the electron releasing effect of the *p*-OMe group in **6**, and the formation of a stronger complex. Since methyl veratrate (**4**) also possesses a *p*-OMe group, it seems reasonable to use the LIS values for **6** as the contributions to the ester-europium complex (1) in **4**, i.e., $\Delta_{Me(1)} = 12{\cdot}42$ ppm, $\Delta_{2,6(1)} = 10{\cdot}90$ ppm and $\Delta_{3,5(1)} = 2{\cdot}10$ ppm. The contributions to the complex involving the 3,4-OMe groups (3,4) will be the shifts of veratrole itself.

The observed LIS of proton(s) i in methyl veratrate are given by Eq 2.

$$\Delta_{B,i} = p(1)\,\Delta_{i(1)} + p(3,4)\,\Delta_{i(3,4)} \tag{2}$$

In this case, $p(1) \neq p(3,4)$. Rearranging and solving for p(1) gives

$$p(1) = \frac{\Delta_{B,i} - \Delta_{i(3,4)}}{\Delta_{i(1)} - \Delta_{i(3,4)}} \text{ and } p(3,4) = 1 - p(1)$$

Calculations of p(1) and p(3,4) for each proton in IV give average values $p(1) = 0{\cdot}376$ and $p(3,4) = 0{\cdot}623$, indicating stronger binding of europium to the *ortho* OMe's. If Eq 2 is solved for p(3,4), the complex populations are $p(1) = 0{\cdot}359$ and $p(3,4) = 0{\cdot}641$, in close agreement with the above values.

(3) *Methyl 3,4,5-trimethoxybenzoate.* The most serious test of the assumptions made so far is in calculating complex populations in methyl 3,4,5-trimethoxybenzoate (**2**). This ester has three binding sites, (1), $(3,4) \equiv (4,5)$. The net LIS of a proton is:

$$\Delta_{B,i} = p(1)\,\Delta_{i(1)} + p(3,4)\,\Delta_{i(3,4)} + p(4,5)\,\Delta_{i(4,5)} \tag{3}$$

Since $\rho(3,4) = p(4,5)$ but $\Delta_{(3,4)} \neq \Delta_{(4,5)}$, Eq 3 can be solved for either p(1) or p(3,4):

$$p(1) = \frac{\Delta_{B,i} - \frac{1}{2}(\Delta_{i(3,4)} + \Delta_{i(4,5)})}{\Delta_{i(1)} - \frac{1}{2}(\Delta_{i(3,4)} + \Delta_{i(4,5)})}$$

and

$$p(3,4) = p(4,5) = \tfrac{1}{2}(1 - p(1))$$

Again, LIS contributions from methyl anisate will be used for complex (1). Calculated shift contributions for complexes (3,4) and (4,5) are obtained from 1,2,3-trimethoxybenzene: $\Delta_{2(3,4)} = \Delta_{6(4,5)} = 6{\cdot}93$ ppm; $\Delta_{6(3,4)} = \Delta_{2(4,5)} = 3{\cdot}29$ ppm; $\Delta_{5(3,4)} = \Delta_{3(4,5)} = -4{\cdot}22$ ppm, and; $\Delta_{4(3,4)} = \Delta_{4(4,5)} = 9{\cdot}90$ ppm.

Solving Eq 3 for p(1) gives an average value of $p(1) = 0{\cdot}239$ and $p(3,4) = p(4,5) = 0{\cdot}380$ for all protons in **2**. When equation 3 is solved for p(3,4), the values obtained are $p(1) = 0{\cdot}262$ and $p(3,4) = p(4,5) = 0{\cdot}369$.

It is interesting to calculate "theoretical" populations for the complexes of **2** based on the calculated populations for methyl veratrate, assuming that each binding site maintains its relative binding ability. The "theoretical" ratio $p(3,4) + p(4,5)/p(1)$ based on average values obtained for comparable binding sites in methyl veratrate is $0{\cdot}637 + 0{\cdot}637/0{\cdot}363 = 3{\cdot}51$, whereas the actual ratio using average populations for **2** is 3·00. This represents, in our opinion, a fairly good agreement and justification for the population analysis method in these systems.

DISCUSSION

Several important conclusions can be drawn from the above results. It has been shown that aromatic ethers are very poor ligands for binding of the lanthanide shift reagent, $Eu(fod)_3$, at least as compared with an ester group. However, when two ether groups are *ortho* to one another, surprisingly strong binding occurs with europium. From the results of the population analysis discussed below, it appears that the *ortho* diether group has nearly twice the affinity for shift reagent as an ester group. Furthermore, in a 1,2,3-triether substitution pattern, where the lanthanide atom can complex to two *ortho* oxygens, the third, uncomplexed ether protons are shielded by the paramagnetic atom, resulting in low *net* downfield shifts. The apparently high binding ability of an *ortho* diether is attributed to bidentate binding of the lanthanide to the oxygens, in which case the europium-ligand bond vector would be directed between the O atoms. Use of this model in analyzing solution conformations of these compounds according to the pseudocontact equation is in progress. It should, however, be pointed out that the pseudocontact approach to conformational analysis is being seriously questioned. Several studies[3,4] have shown that LIS: substrate geometry correlations based on pseudocontact interaction do not lead to satisfactory results on solution conformations. The assumption of magnetic axiality of the electronic g tensors in shift reagent:substrate complexes has been criticized,[5] although a recent report[6] indicates that complexes of borneol and isoborneol with $Eu(fod)_3$ and $Pr(fod)_3$ are essentially axially symmetric. The uncertainty in the position of the magnetic axis in complexes where bidentate binding of substrate to shift reagent is proposed may render pseudocontact correlations impossible.

There was no indication in this work that the shift reagent: substrate complexes possessed other than 1:1 stoichiometry. In the event that 1:1 and/or 1:2 complexes are found, there should be a direct relationship between induced and "bound" shifts if the ratio of induced shifts is independent of shift reagent concentration.[7] Such was found to be the case in these studies.

It has been demonstrated that, for a molecule with multiple binding sites of roughly comparable binding ability, population analysis of the observed LIS based on induced shifts from suitable model compounds show good internal consistency, and provide evidence for the additivity of shifts. The agreements obtained depend, in large part, on the similarities of molecular and complex geometries, and steric and resonance factors between model compounds and the polyfunctional compound of interest.

EXPERIMENTAL

Methoxybenzoic acids and methoxybenzenes were purchased from Aldrich Chemical Co. Methyl esters were prepared in MeOH containing a catalytic amount of conc H_2SO_4. Methyl-d_3 esters were prepared in methanol-d_3 (Norell Chemical Corp). All m.ps corresponded to literature values. Solids were sublimed *in vacuo* twice prior to NMR analyses, and liquids were distilled and stored over P_2O_5.

Shift studies were carried out by stepwise addition of known amounts of $Eu(fod)_3$ (Norell Chemical Corp.), stored over P_2O_5 to *ca* 0·15 M solns of substrate in $CDCl_3$. The LIS data were obtained by least squares extrapolation of observed shifts to 1:1 shift reagent: substrate mole ratio on a Hewlett-Packard Model 9100A calculator. Correlation coefficients were >0·999 for most analyses, but were somewhat lower (>0·99) for weakly binding ethers, i.e., **11-15**. NMR spectra were determined with a JEOL C60-HL spectrometer, external lock mode, at a probe temperature of *ca* 20°. The chemical shifts and LIS reported are considered to be accurate to ±5%.

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