

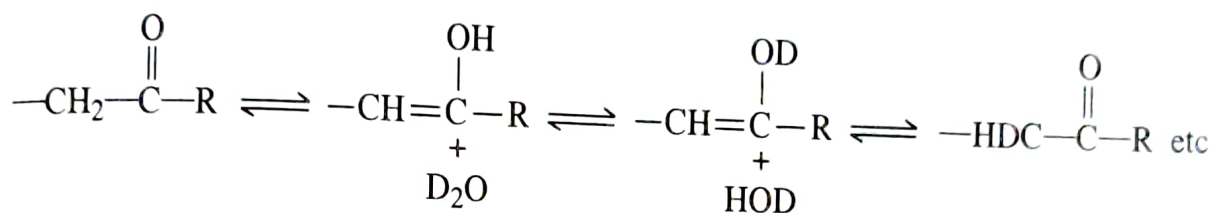
4.12 TECHNIQUES FOR SIMPLIFICATION OF SPECTRA

(a) CHEMICAL TECHNIQUES

4.12.1 Deuteration

Deuteration is the most elementary and very useful technique for assigning exchangeable protons and for locating exchangeable masked by other signals in the spectrum.

A sufficiently acidic proton is prone to undergo deuterium exchange. Methylene protons α to a carbonyl group, for example, may exchange if the solution is kept standing with D_2O for any length of time, as they can exchange via the keto-enol route i.e.



The general procedure is to add a few drops of D_2O into the solution, shake vigorously for a few seconds and filter through sodium sulphate filter to remove as much emulsified D_2O as possible. Now rerun and reintegrate the spectrum of the sample and detect any drop in integral intensity which will indicate the presence of an exchangeable proton. If the exchange of protons is slow, like amides, a solution of sodium bicarbonate in D_2O or $NaOD$ may be used. Deuterated samples can be back-exchanged by shaking with an excess of water.

4.12.2 Basification and acidification

Salts generally give sub-standard spectra. The actual degree of deterioration can vary from merely slight broadening to total "unassignability". However, in some cases, the basification of the salt solution (free base extraction) with a few drops of sodium bicarbonate or hydroxide in D_2O on acidification with a few drops of DCl in D_2O can clarify certain features of a spectrum.

Figure 4.61 shows a simple example of how free base extraction can help clarify the spectrum of a salt by comparing with that of the base. In the spectrum of the salt, the proton 'b' is obscured by the HOD peak but it is clearly visible in the spectrum of the free base. The upfield shift of the 'c' protons in the free base (due to the removal of the large β deshielding effect of $-\text{NH}_3^+$) also clarifies the broad multiplet for 'd' and 'e' in the salt by separating them with greater certainty of their assignment. There is also an upfield shift of 'c' in the free base, though it is not quite as large as usual.

Conversely, of course, acidification can be used to identify protons α and β to nitrogen.

4.12.3 Solvent effects

Specific solvent effects have been observed and used with advantage, especially to separate and differentiate proton resonance signals of individual methyl groups in steroids. Benzene, because of its high magnetic anisotropy and its tendency to form specific complexes with the solute, is particularly used to differentiate among the proton resonance signals of methyl groups, when the PMR spectrum of 4,4-dimethyl-5- α -androstanone is run in $C_6H_6-d_6$ and $CDCl_3$ (Fig. 4.62).

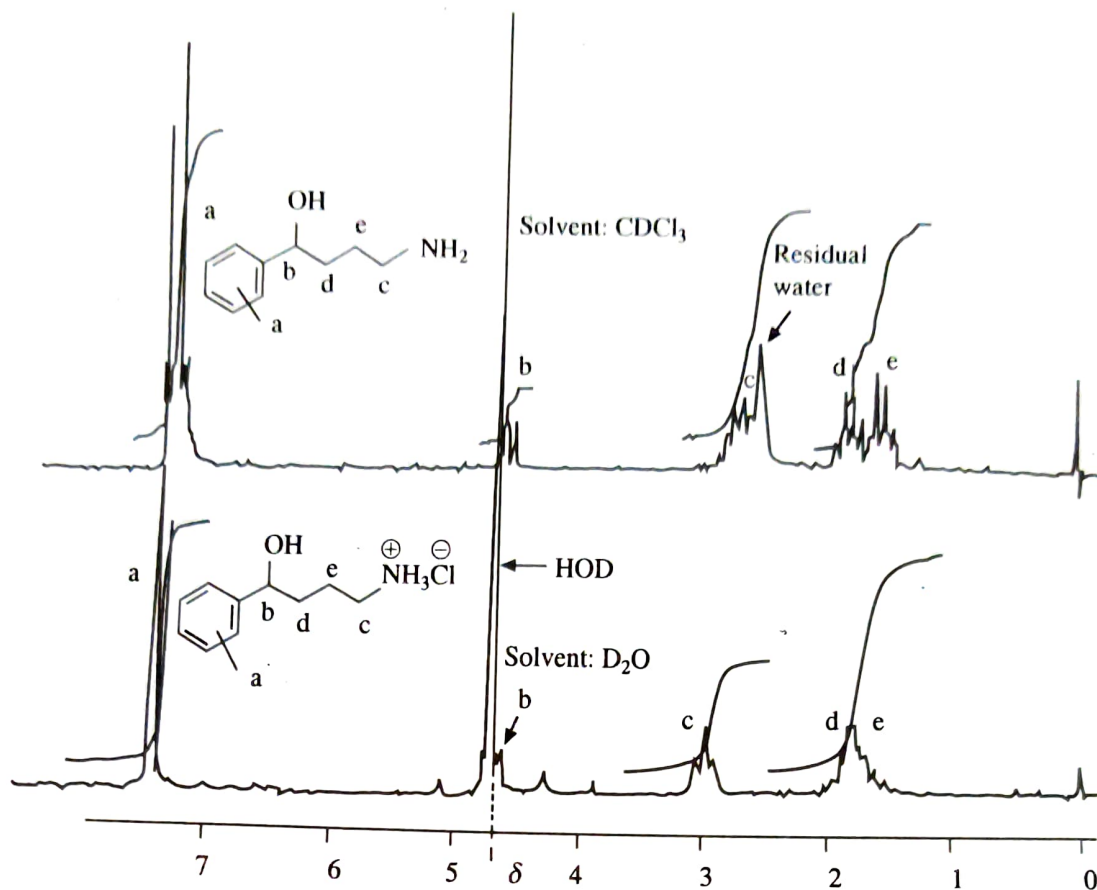


Fig. 4.61 Simplification of PMR spectrum of 4-Amino-1-phenylbutanol hydrochloride on basification.

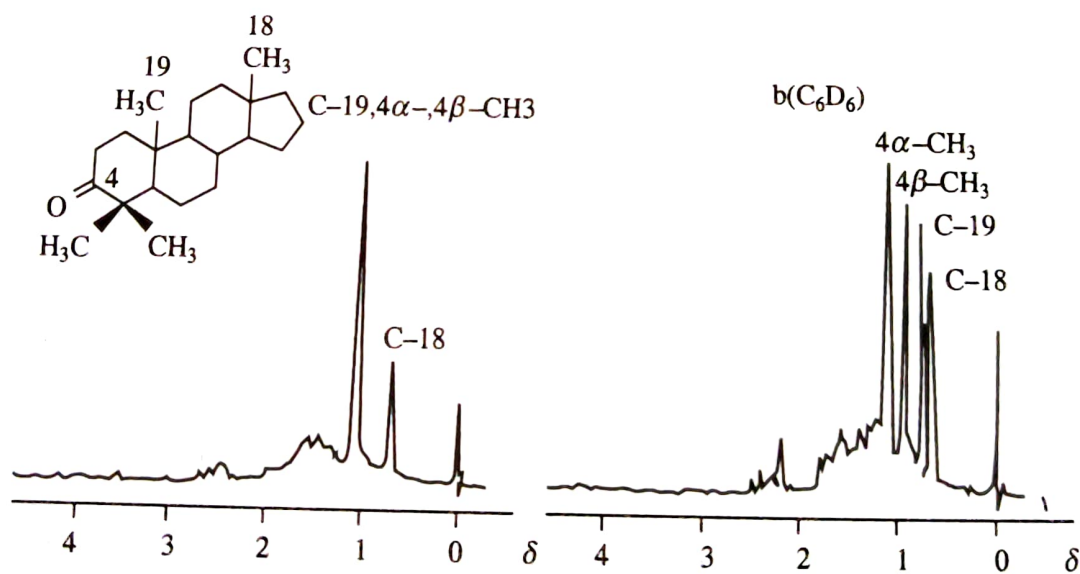


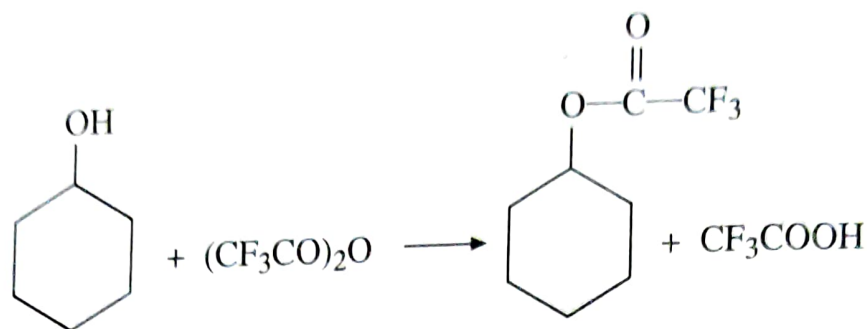
Fig. 4.62 The PMR spectrum of 4,4-Dimethyl-5- α -androstan-3-one taken in (a) CDCl_3 and (b) C_6D_6 .

Solvent effects and coupling constants

The solvent dependence of spin-spin coupling is, in general, less marked than that of the resonance frequency, but in polar solvents variations in even this PMR parameter have been observed. For example, the variations in geminal and vicinal coupling constants over two and three bonds respectively in formaldoxime (I) and 1-chloro-2-ethoxyethylene (II) have been measured in the range of 7.6–9.9 and 4.2–6.3 Hz.

compared with the inherent accuracy of integration by continuous wave spectrometers. Also the trace of water in CDCl_3 or residual water in the compound will absorb in this region.

However, the OH group in cyclohexanol may be identified by carrying out trifluoroacetylation by shaking it for a few seconds with a few drops of trifluoroacetic anhydride.* The following reaction occurs.



If the reaction is a little slow as is often the case with phenolic $-\text{OH}$ group, it can be speeded up a little by gentle warming, more shaking and even adding a drop of pyridine- d_5 to base catalyse the reaction. Dry the solution through sodium sulphate before rerunning.

The resultant spectrum is clearly very different from the alcohol, as the trifluoroacetic ester function is far more deshielding with respect to proton 'a' than is the $-\text{OH}$ group—well over 1 ppm downfield shift has occurred. Thus, this technique clearly identifies the $-\text{OH}$ group in cyclohexanol.

The trifluoroacetylated samples can be deacetylated by base hydrolysis.

4.12.5 Lanthanide shift reagents

Identification of organic compounds using PMR spectral studies reveals that in some compounds, overlapping of proton signals makes it inconvenient either to correlate specific signals with appropriate protons or to extract coupling information from them. The complexity of the spectrum of *n*-pentanol, for example, is due to the very similar chemical shifts of protons on carbons b, c and d (Fig. 4.66) leading to the phenomenon of virtual coupling. Addition of small amount of shift reagent results in complete separation of the signals, i.e. converts a second order spectrum to that of the first order spectrum.

The lanthanide shift reagents are β -diketonate complexes of europium and praseodymium (paramagnetic lanthanides), the most commonly used in practice are Tris-(1,1,1,2,2,3,3, -heptafluoro-7, 7-dimethyloctane-4,6-dionato) europium, $\text{Eu}(\text{fod})_3$ (I), Tris-(dipivaloylmethanato) europium, $\text{Eu}(\text{dpm})_3$ (II) and Tris-(3-trifluoroacetyl-d-camphorato) praseodymium (III) which can be abbreviated Pr (facam) $_3$ (Fig. 4.64).

4.12.5.1 SIMPLIFICATION OF COMPLICATED PMR SPECTRA BY THE USE OF LANTHANIDE SHIFT REAGENT

Simplification of complex PMR spectra by the use of lanthanide shift reagent was first introduced by Hinckley⁹ who discovered that the addition of small amounts of certain lanthanide shift reagents to

*This reagent can only be used in solvents which do not react with it (MeOH- d_4 and D_2O obviously are not suitable) or contain a lot of water i.e. $\text{DMSO-}d_6$.

9. C.C. Hinckley, J. Am. Chem. Soc., **91**, 5160 (1969).

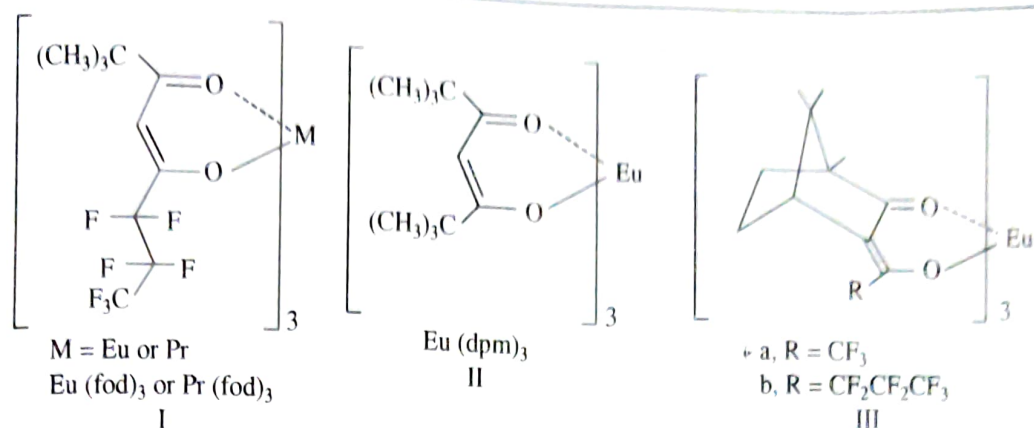


Fig. 4.64 Commonly used lanthanide shift reagents.

particular compounds induced large changes in the chemical shifts without line broadening, thus resulting in the simplification of complex second order spectrum to first order.

Lanthanide shift reagents are mild Lewis acids which attach themselves to basic sites such as amino, hydroxy and keto-groups. The purpose of the shift reagent is to incorporate a powerful little magnet into the molecule which stretches out the spectrum, thus, separating the overlapping signals. The large shifts produced by these reagents are due to the co-ordination of molecules under study (substrate) to the paramagnetic lanthanide ion. The magnetic field experienced by the bound substrate changes under the influence of the magnetic field induced by the unpaired electrons of the lanthanide ion. The shift is generally downfield with europium and upfield with praseodymium.

The lanthanide shift reagents, thus, attach to the sites which have lone pairs, the more available the lone pair the greater the affinity. The strength of the complex formation is in the following order:

NH₂ > OH > C = O > - O - > -COOR > CN, provided the complexation site of the substrate molecule is not sterically hindered.

4.12.5.2 CALCULATION OF INDUCED SHIFT OF A PROTON BY MCCONNELL AND ROBERTSON¹⁰

The through space interaction of the magnetic field with the substrate nuclei is called pseudo contact to distinguish it through bond interaction of nuclei, called Fermi-contact interaction.

The induced chemical shift of any proton is inversely proportional to the cube of its average distance from the lanthanide atom. There is also a geometric term to be considered which is important in rigid molecules. Both these factors are combined in McConnell and Robertson equation (13) for calculating the change in the chemical shift of various protons

$$\Delta\nu = X(3 \cos^2 \theta - 1)/r_1^3 \quad (13)$$

where $\Delta\nu$ = lanthanide induced shift of the proton
 X = a constant
 r_1 = average distance of the proton from the lanthanide atom
 θ = the angle between r_1 (the distance vector which joins the proton and the lanthanide atom) and the line joining the lanthanide atom with the atom bearing the lone pair.

10. H.M. McConnell and R.E. Robertson, J. Chem. Phys., 29, 1361 (1958).

That is, the induced chemical shift of ortho proton in the complex formed by adding $\text{Eu}(\text{dpm})_3$ into benzyl alcohol will depend upon the distance r_1 (the distance from the center of the lanthanide atom to the ortho proton in the complex) and the angle θ as shown in Fig. 4.65.

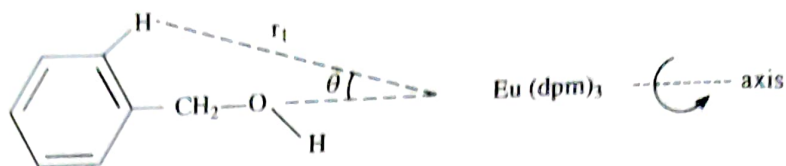


Fig. 4.65 The distance r_1 and the angle θ as used by McConnell and Robertson.

The use of $\text{Eu}(\text{fod})_3$ in the simplification of complex spectra is illustrated by running the PMR spectra of *n*-pentanol before and after adding $\text{Eu}(\text{fod})_3$ (Fig. 4.66). The bottom trace exhibits the ordinary spectrum of *n*-pentanol in CDCl_3 (about 25 mg in 0.5 ml). The methylene alkyl signals 'c' and 'd' overlap and even 'b' is not well separated. The methylene protons 'a' are broad because of partial -OH coupling.

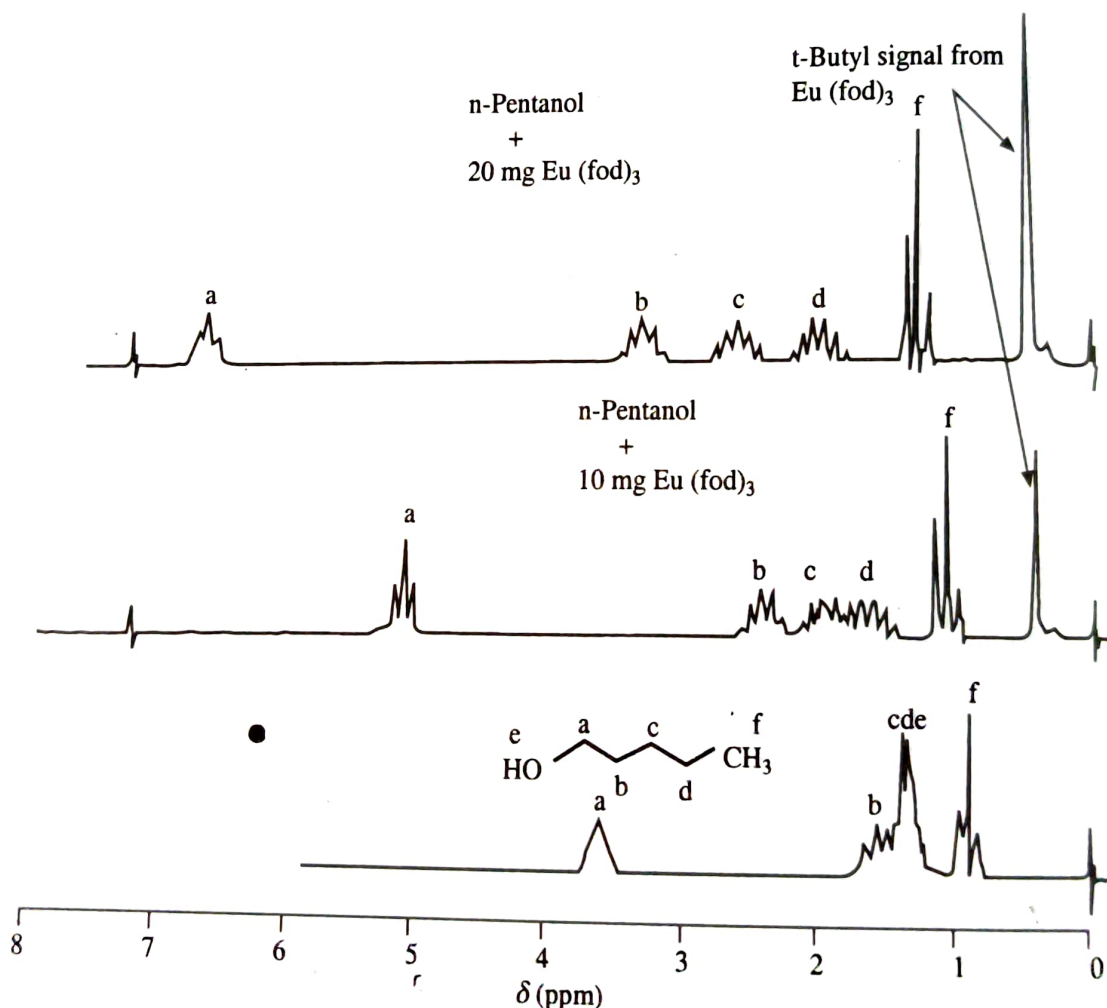


Fig. 4.66 Simplification of PMR spectrum of *n*-Pentanol by the use of $\text{Eu}(\text{fod})_3$.

The middle trace displays the result of adding 10 mg of $\text{Eu}(\text{fod})_3$. The signals begin to stretch out downfield: the closer the protons are to the site of complexation, the greater is the downfield shift.