PMR Spectroscopy in Medicinal and Biological Chemistry

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Preface

There is a good selection of books which give accounts of the fundamental principles of NMR spectroscopy appropriate to organic chemists (see selective bibliography, p. 403). Within the text of such books many examples of the applications of the technique to various problems are given, but descriptions of basic theory and data tabulations take precedence. I feel, therefore, that there is a need for an NMR text that, by assuming knowledge of essential principles, is left free to concentrate on applications, i.e. on what NMR can be used for and what problems it can solve.

The fields of medicinal and biological chemistry, embracing as they do the disciplines of organic chemistry, biology and pharmacology, present a rich variety of problems that are amenable to solution by NMR spectroscopy (and in some cases *only* by NMR spectroscopy). I came to appreciate this through personal research experience over the last 10 years and also through literature surveys that I carried out while preparing a review article on the topic for the *Journal of Pharmaceutical Sciences* [*J. Pharm. Sci.* (1967), **56**, 1049]. For this reason I decided that the NMR book I had in mind could be specially directed towards the interests of medicinal chemists (i.e. chemists in pharmaceutical industry, chemists teaching pharmaceutical and medicinal chemistry in schools of pharmacy plus graduate students in such departments), biochemists (two chapters deal exclusively with biochemical problems), and pharmacologists interested in drug-receptor interactions and structure-activity relationships. Since so much of the material described falls in the realm of organic chemistry, I hope that organic chemists not specifically working in a biological field will also find the book of value (e.g. for stereochemical problems).

The majority of NMR studies in fields connected directly and indirectly with life sciences concern protons and I have, therefore, restricted the book, with a few exceptions, to examples of proton magnetic resonance (PMR) as is reflected in the title. Rapid advances in studies of other nuclei (especially carbon-13), now in progress, will probably alter this balance during the next few years.

In the book's presentation, I have assumed an empirical knowledge (first order approach) of the fundamentals of NMR spectroscopy, and have adopted an explanatory rather than review style. I have attempted to give

full accounts of all aspects discussed apart from basic principles. My choice of topics is largely a personal one and comprehensive accounts have not been attempted except in a few cases. Literature references up to 1970 have been included.

A few words about the content of the book are given below. Analytical applications of PMR spectroscopy are described in Chapter 1. These include procedures of a degree of accuracy appropriate to the assay of pharmaceuticals and also more approximate methods useful for the analysis of reaction product mixtures. Studies of alcohols and aromatic derivatives provide examples of the value of PMR spectroscopy as a tool in qualitative analysis. Chapter 2 is devoted to the NMR aspects of organic nitrogen derivatives, a topic included because of the wide-spread occurrence of nitrogen-containing biochemicals and drugs. The stereochemical utility of PMR data is described in Chapter 3. The fairly detailed account given is warranted by the increasing demand for knowledge of the specific molecular geometry of biologically active molecules, particularly in the solute condition. There is sufficient interest in the stereochemistry of cyclic bases to justify their separate discussion and this is provided in Chapter 4. Widespread observation of radical differences in the biological activities of enantiomorphic forms of chiral molecules has greatly stimulated study of optically active molecules over the past 10 to 20 years. The power of PMR spectroscopy in the resolution and configurational assignment of optically active molecules is described in Chapter 5. Specific examples of the use of the PMR technique in the study of compounds of pharmacological interest are given in Chapter 6 (narcotic analgesics, cholinergic agonists, histamine, and antihistamines) and Chapter 7 (tropanes, sympathomimetic amines, antibiotics and steroids). Some of these applications illustrate principles described in earlier chapters. The use of PMR spectroscopy in biochemistry is described in the last two chapters. Chapter 8 concerns proteins and their simpler components while studies of carbohydrates, nucleosides, nucleotides and nucleic acids are described in Chapter 9. Relaxation-time investigations, of particular importance to the study of protein conformation and the uptake of small molecules by macromolecules, are also included in Chapter 8. The book concludes with an appendix on some solvent and hydrogen bonding effects. The routine use of PMR spectroscopy in structure elucidation as encountered in synthetic and natural product work is not dealt with specifically in this book.

Of the many colleagues who have fostered my interest in PMR spectroscopy, I wish to thank Dr Alain Huitric (University of Washington) in particular. I also thank Drs H. Booth (University of Nottingham), R. U. Lemieux and G. Kotowycz (University of Alberta) for reading chapters of this book. I am indebted to the Medical Research Council of Canada for

support of my own studies in this field and for the provision of an NMR spectrometer. Many of the spectra reproduced here were recorded on this machine and I acknowledge the able assistance of Mrs Sining Lee in this work. I owe much, in addition, to the willing co-operation of my graduate students and post-doctoral associates. Finally I wish to record my gratitude to the staff of the School of Chemical Sciences, University of East Anglia, for their kind hospitality during my completion of the manuscript.

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October, 1971

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The author is grateful to authors and publishers for permission to reproduce figures. The source is given in the legend and the full reference is listed in the reference list at the end of each chapter.

Foreword

The advent of proton magnetic resonance spectroscopy has, over the last 15 years, revolutionized organic chemistry. Initially an exciting new technique, which in the hands of forward-looking pioneer chemists provided elegant solutions to old problems, and enabled the solution of many new ones, NMR has now progressed to the single most useful and most used physical method in organic chemistry. It is now unthinkable for a strong research laboratory in organic chemistry to be without NMR facilities, or for an active organic chemist to be unskilled in its use.

The applications of NMR far outrange the boundaries of pure organic chemistry, and its potential in the medicinal and biological fields is enormous. Although such applications have in fact been numerous, the *routine* use of NMR by biologists, biochemists, pharmacologists and medicinal chemists is not yet general. Such routine use has been hindered by the lack of a suitable text book. It is to fill this gap that Professor Casy has prepared the present text. His book discusses a series of topics pertinent to the life sciences as he is well qualified to do.

It is to be hoped that this book will widely stimulate the application of NMR to chemical problems in the life science area, and it is commended to all workers in this area. In addition the book will be of interest to many pure organic chemists, particularly those concerned with nitrogen compounds and/or stereochemical problems. The work deserves wide dissemination.

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October, 1971

Introduction

Nuclear magnetic resonance (NMR) spectroscopy is a physical technique which enables chemists to study the environment of certain atomic nuclei within molecules. Protons (^1H) have been, and still are, the atomic species most commonly studied, and applications of proton magnetic resonance (PMR) form the specific topic of this book, except for a few cases in which reference is made to ^{13}C and ^{19}F spectra. PMR spectroscopy was first applied to organic compounds soon after 1950 and its use has grown enormously over the last 20 years. A general account of the basic principles of NMR spectroscopy is not included in this book; a reference list of texts providing this information is given in the bibliography at the end of the book (other books are mentioned throughout the text). Several elementary accounts of the use of physical methods in organic chemistry include useful chapters on NMR spectroscopy and these are also listed together with certain review series, journals and source books.

The interpretation of NMR spectra is based on three sets of parameters which characterize the absorption of radiofrequency radiation by atomic nuclei placed in a magnetic field (H):

1. The frequences of the absorbed radiation (ν) expressed as the *chemical shift* relative to an arbitrarily standard absorption line;
2. The multiplicity of the lines originating from a given group of nuclei and described by the appropriate *coupling constants* (J values); and
3. The decay times characterizing the return of the nuclei excited by the absorption of radiation to a lower energy state, referred to as *relaxation times*.

Additional information is derived from the integration curve which enables the relative numbers of protons within each absorption band to be determined. Of the above parameters, the first two are the most widely applied to chemical problems, although more attention is now being given to relaxation times because of their value in the study of molecular interactions (Chapter 8).

NMR spectra are displayed as plots of a detector signal (ordinate) against the magnetic field strength (abscissa). Three scales are in current use for expressing ^1H chemical shifts all of which are related to a standard

(preferably internal) which is normally tetramethylsilane (TMS). The scales are:

1. Hertz (Hz) from TMS set at zero; use of Hz has now largely replaced that of the equivalent term cycles per second (cyc/sec).
2. Parts per million (ppm), obtained by multiplying the Hz value by the term 10^6/oscillator frequency in Hz. For most protons, values obtained fall in the range 0–10 ppm with TMS at zero. This scale is denoted by the Greek letter δ followed by the numerical value, e.g. δ 1·5 (the factor ppm is omitted since it is understood). The reverse form, e.g. 1·5 δ, is occasionally used in the literature.
3. The τ (tau or tor) scale where

$$\tau = 10 - \delta \text{ with TMS at 10, e.g., } \tau\ 5{\cdot}5\ (\equiv \delta\ 4{\cdot}5).$$

The last two scales are dimensionless, while the first depends on the operating frequency and must always be used in conjunction with the frequency value. Relative merits of the three systems have been discussed by Bible (1965).

Attempts are being made to encourage the presentation of NMR data in a uniform manner by the international Union of Pure and Applied Chemistry and other bodies, and it is probable that the δ scale will eventually gain the approval of the majority of NMR spectroscopists. Meanwhile, however, chemists employing the technique must be familiar with all three variants, and for this reason no attempt has been made to standardize chemical shift values in the examples discussed in this book. Coupling constants and the dimensions of a resonance peak (usually its width at half maximum height, W_H) are expressed in Hz (replacing cyc/sec). It is important to remember that an increase in the numerical value of a chemical shift expressed in Hz or on the δ scale implies a move to *lower* field (deshielding), while an increase on the τ scale means a move to *higher* field (shielding).

Most of the examples given in this book involve first order spectral analyses in which chemical shifts and coupling constants are read directly from spectra. Such an approach usually gives only an approximation of the true values but provides data which are adequate for the solution of many problems concerning molecular constitution and even stereochemistry. Garbisch (1968), in commenting upon this question, states that "the key to the solution may be simply recognition of the gross multiplicities of the bands (neglecting higher-order splittings which are often undetectable under the conditions which the spectra were determined) or the observation that a coupling constant is large, say 16 Hz, or small, say 3 Hz. The reliability of the solution of the problem will rarely rest on knowledge that the

correct coupling constant is 16·87 or 3·44 Hz". Nevertheless, in the first order approach, care must be taken to guard against the possibility of the spectra being "deceptively simple", i.e. spectra which appear susceptible to first order treatment but which in fact are not (Becker, 1965). Guides to the second order analysis of complex NMR spectra have been provided by Garbisch (1968), Bible (1965) and Mathieson (1967).

Abbreviations

$CDCl_3$	deuterochloroform
D_2O	deuterium oxide
DMSO-d_6	deuterated dimethylsulphoxide
TMS	*te*tra*m*ethyl*s*ilane
DSS	sodium 2,2-*d*imethyl-2-*s*ilapentane-5-*s*ulphonate
$J_{gem}(^2J)$	a geminal coupling constant
$J_{vic}(^3J)$	a vicinal coupling constant
W_H	width at half maximum height
Me	methyl
Et	ethyl
Ph	phenyl (and other common radical abbreviations)
ν	chemical shift
$\Delta\nu$	difference in chemical shift

NOTE: In most representations of asymmetric molecules, only one of the two possible enantiomorphic forms is shown.

Contents

CHAPTER 1

Analytical Aspects

QUANTITATIVE ANALYSIS

The application of PMR to quantitative analysis depends on the fact that the area beneath a particular PMR signal is directly proportional to the number of protons from which the signal is derived. This area may be obtained from the corresponding integral trace (an integrator is now incorporated within most commercial instruments) or by means of a planimeter provided the signal is removed from nearby resonances which may distort or overlap the non-horizontal portion of the integral line. The accuracy of area measurements and the degree to which an area truly represents the number of protons giving rise to the integrated signal depends critically upon the instrumental operating conditions. Some of the factors affecting PMR integration accuracy are listed below (J. N. Shoolery, 1969, private communication).

(i) The signal-to-noise ratio must be as high as possible to ensure that the background noise contribution to the integration trace is negligible. A sensitive spectrometer should be used (modern instruments operated under optimal conditions provide signal-to-noise ratios near 20:1 with reference to a standard 1% ethylbenzene sample) and fairly concentrated (~10%) solutions analysed.

(ii) A non-saturating mode of operation must be employed. Saturation effects, i.e. the decrease in the population difference between nuclei in ground and excited states which occurs as a peak is being swept, lead to deceptively low areas, the extents of reduction varying from signal to signal. Corrections may be applied but require knowledge of the relaxation times T_1 and T_2 for each peak, and the avoidance of saturation is considered a more satisfactory general procedure (Paulsen and Cooke, 1964a). This can be achieved by the proper choice of r.f. power level (H_1) and sweep rate (dH/dt). Low H_1 and high dH/dt values minimize differences in integrated

intensities due to saturation, but at the same time the integral is decreased and the signal-to-noise ratio degraded, so a compromise must be chosen. Paulsen and Cooke (1964a) identify the best conditions by measuring the ratio of areas of the sample and an internal standard at progressively increasing power rates (or decreasing sweep rates). The optimum area ratio is that obtained at the r.f. power level just below that at which a change in the ratio is detected.

(iii) The use of rapid sweeps allows a large number of independent measurements to be made per unit time and these can be averaged and statistically analysed to give an indication of the precision of the measurement.

(iv) The settings of both the baseline zero and the r.f. phase control are critical. Maladjustment of the latter leads to recording the integral of a mixture of absorption and dispersion mode signals. The dispersion mode is positive on one side of the centre and negative on the other, and this introduces baseline slopes near the integral which are of opposite sign (Fig. 1.1). On the other hand, correct phasing but incorrect detector zero introduces a constant slope on both sides of the integral. Correct setting of the controls gives a square step-function integral which can be measured accurately (Fig. 1.1).

(v) By careful adjustment of magnetic field homogeneity and use of high-precision sample tubes spinning at an optimal rate, it should be possible to reduce spinning side bands to a point where they may be neglected. If their area is significant, however, it should be added to that of the main peak to obtain the true area representative of the protons giving rise to the signal. Corrections for ^{13}C satellite lines are described on p. 13.

Further useful information on accuracy and optimum operating conditions in quantitative PMR work is available in papers by Alexander and Koch (1967) of the U.S. Food and Drug Administration.

In quantitative PMR procedures a particular integral value is related to a specific number of protons by the inclusion of an internal standard in the solution to be analysed. The reference and analytical peak should, if possible, have comparable areas to minimize errors caused by non-linearity of the electronic measuring circuits. The assay of meprobamate (Turczan and Kram, 1967), described below, illustrates the principles involved. The PMR spectrum of meprobamate (Fig. 1.2) displays sharp singlets for methylene (4 protons) and *t*-methyl protons; either could be used for analytical purposes but the former is preferable since it is well isolated, making impurity detection more easy (impurities give an erroneous integral

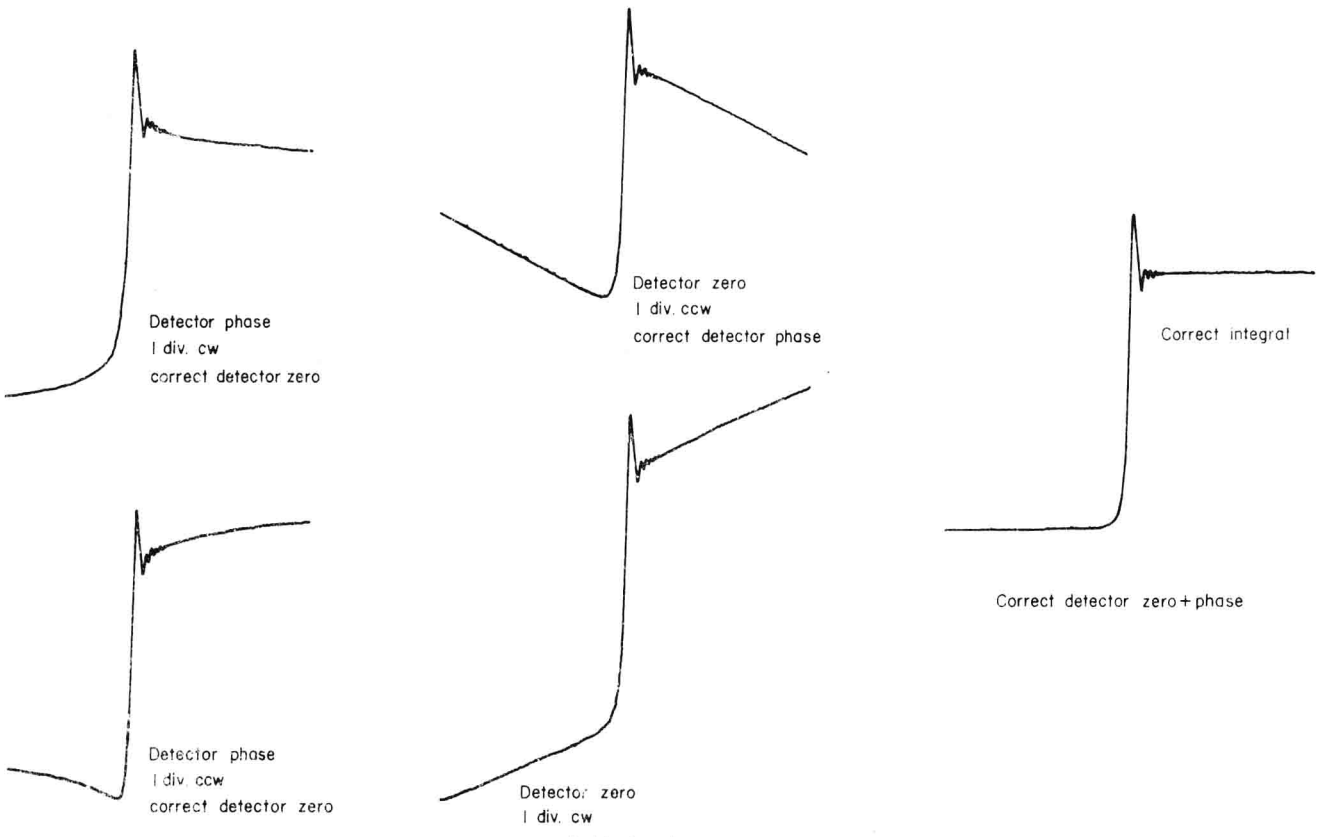

FIG. 1.1. Varian integrals. Integral traces recorded for 6% TMS in $CHCl_3$ under various settings of detector phase and zero (cw denotes clockwise and ccw counter clockwise).