JOURNAL OF Pharmaceutical Sciences

September 1967 volume 56, number 9

Review Article

Applications of Nuclear Magnetic Resonance Spectroscopy in Medicinal and Pharmaceutical Chemistry

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NUCLEAR MAGNETIC RESONANCE (NMR) spectroscopy is a physical method which enables chemists to study certain atomic nuclei within molecules. One phase of this area, proton magnetic resonance (PMR) spectroscopy, is of particular interest to the organic chemist. The spectroscopic technique was first applied to organic compounds soon after 1950 and its use has grown enormously over the last decade. It is probable that most medicinal and pharmaceutical chemists have encountered the method and are aware of its physical basis. Several textbooks written with the requirements of the organic chemist in mind have been published (1–5) since the pioneer account of Jackman (6). One of the more recent publications is a comprehensive two-volume work by Emsley, Feeney, and Sutcliffe (7).

Numerous reviews are available dealing with the application of NMR spectroscopy to specific topics, such as the structure elucidation of natural products (8, 9) and biochemistry (10). (Other reviews are mentioned throughout this paper.) However, no particular emphasis has been given to the potentialities for application of the NMR technique to problems of interest to medicinal chemists, and the present review has been written to this end. A comprehensive account is not intended—rather, a selection of the literature has been made, and the topics therein outlined and discussed in some detail so that the value and versatility of NMR spectroscopy in the field of medicinal chemistry may be demonstrated.

A general outline of the basic principles of NMR spectroscopy is not included here because of space limitations and the large number of introductory accounts now available. Apart from the textbooks referred to previously, several texts describing the use of physical methods in organic chemistry include useful chapters on NMR spectroscopy (11–13).

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): (a) the frequencies of the absorbed radiation (ν) expressed as the *chemical shift* relative to an arbitrary standard absorption line; (b) the multiplicity of the lines originating from a given group of nuclei and described by the appropriate *coupling constants* (J values); and (c) 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 number of protons within each absorption band to be determined. Of the above parameters, the first two are the most widely applied to chemical

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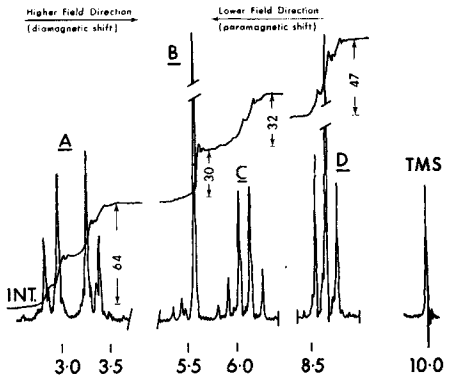


Fig. 1—PMR spectrum of 4-ethoxybenzyl chloride ($CH_3CH_2OC_6H_4$p-CH_2Cl) in $CDCl_3$ (60 Mc./sec.). Key: A, *aryl protons* (A_2B_2 *system*), *symmetrical about 413 c.p.s.;* B, $ArCH_2Cl$ *singlets*, ν *262 c.p.s.;* δ *4.37 p.p.m.;* τ *5.63 p.p.m.;* C, CH_3CH_2O *quartet* (J *7 c.p.s.*), ν *231 c.p.s.;* δ *3.85 p.p.m.;* τ *6.15 p.p.m.;* D, CH_3CH_2O *triplet* (J *7 c.p.s.*), ν *79 c.p.s.;* δ *1.32 p.p.m.;* τ *8.68 p.p.m.*

problems, although more attention is now being given to relaxation times because of their value in the study of molecular interactions (14). NMR spectra are displayed as plots of a detector signal (ordinate) against a magnetic field (abscissas) calibrated in cycles per second (c.p.s.) since H is proportional to ν. In many cases chemical shifts and coupling constants may be read directly from a spectrum (first-order treatment);[1] an example is the spectrum of 4-ethoxybenzyl chloride (Fig. 1). In this spectrum chemical shifts are measured relative to tetramethylsilane (TMS), the most commonly employed internal standard [in deuterium oxide solutions the water-soluble analog, sodium 2,2-dimethyl-2-silapentane-5-sulfonate (DSS) may be employed (16)]. Three scales are used to express the chemical shift: (*a*) a frequency scale (ν) given in c.p.s. for TMS set at zero (coupling constants are also measured in these units); (*b*) parts per million (δ), obtained from the expression:

$$\delta = \frac{\text{c.p.s.} \times 10^6}{\text{oscillator frequency in c.p.s.}}$$

TMS at zero; and (*c*) the τ (or tor) scale where:

$$\tau = 10 - \delta(\text{TMS at } 10)$$

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. (This is not the serious drawback it first appears because the great majority of reported spectra are recorded at a frequency of 60 Mc./sec.) All three systems are in common use [their relative merits have been discussed by Bible (4)]; therefore, in studying NMR literature, care must be taken to ascertain the chemical shift scale and the standard employed. In this review, no attempt has been made to standardize chemical shift values, and examples of all three scales are included.

[1] Sometimes nonmagnetically equivalent nuclei give rise to spectra that appear to be susceptible to first-order treatment, but are really not; such "deceptively simple" spectra are considered in a review by Becker (15).

USE IN ANALYSIS

Integral data may be used for the analysis of mixtures when overlap between signals due to individual components does not occur (or may be corrected for), and NMR methods for the quantitative assay of mixtures of aspirin, phenacetin, and caffeine (17) and of barbiturates (18) have been described. The spectrum of an APC tablet in $CDCl_3$ (binders such as starch and lactose are insoluble and do not interfere) is shown in Fig. 2. The signal at 2.3 p.p.m. is due to OCO*Me* of aspirin, and its integral value (compared with that of Me in a standard solution of caffeine) enables the aspirin content of the mixture to be determined. The signals at 3.4 and 3.6 p.p.m. (both due to Me) give the caffeine content, and the integral of the quartet centered near 4 p.p.m., due to OCH_2Me (and corrected for the overlapping N^7-Me signal of caffeine), gives the phenacetin content. The method is claimed to have the advantage of speed and to be of an accuracy sufficient for quality control. An example in the field of organic synthesis is the determination of isomeric ratios obtained in alkylations employing 2-chloro-1-dimethylaminopropane (these proceed *via* ethyleniminium ions and hence lead to mixtures of products). Thus, it is found that alkylation of diphenylacetonitrile leads to an approximately 1:1 mixture of the cyanide precursors of methadone and isomethadone (Fig. 3), while that of 2-benzylbenzimidazole gives a mixture in which one isomer shows a marked preponderance (19) (Fig. 4). A molecular weight

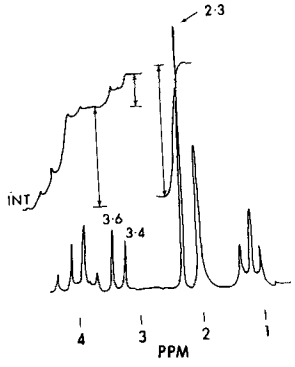


Fig. 2—Part of the PMR spectrum of an aspirin, phenacetin, and caffeine mixture in $CDCl_3$. [After Hollis (17) and reproduced with permission from Anal. Chem., **35,** *1682 (1963).]*

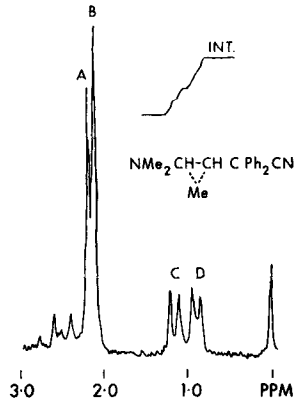


Fig. 3—Part of the PMR spectrum of mixed methadone-isomethadone cyanides in $CDCl_3$. A (N-Me) and C(sec Me) are due to $NMe_2CH_2CHMeCPh_2CN$(iso), and B (N-Me) and D (sec Me) to $NMe_2CHMeCH_2CPh_2CN$.

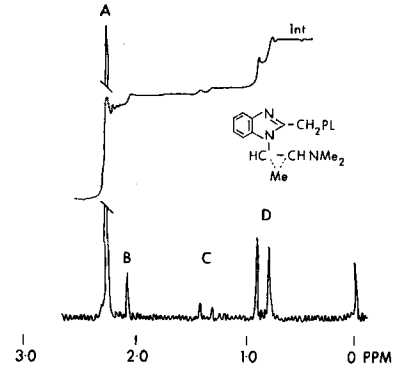


Fig. 4—Part of the PMR spectrum of product of alkylating 2-benzylbenzimidazole with 2-chloro-1-dimethylaminopropane (solvent $CDCl_3$); A(NMe_2) and D (sec Me) are due to isomer with $CH_2CHMeNMe_2$ N-1 side chain, and B (NMe_2) and C (sec Me) are due to isomer with $CHMeCH_2NMe_2$ side chain.

determination method based on PMR integrals has been described (20). It consists of comparing the integrated intensities of an added standard and a recognizable peak or group of peaks of the unknown in a solution containing known weights of standard and unknown. Molyneux, Rhodes, and Swarbrick (21) used a PMR method to check the homogeneity of the alkyl chain in some betaines (I). With D_2O as solvent, distinct signals for the proton groups a–e were observed and, using the integral of the Me signal (a) as standard, the number of protons in the other groups were calculated.

$$\underset{a}{Me}\ \underset{b}{(CH_2)_{n-2}}\underset{c}{CH_2}\overset{+}{N}\underset{d}{Me_2}\underset{e}{CH_2CO_2^-}$$

I

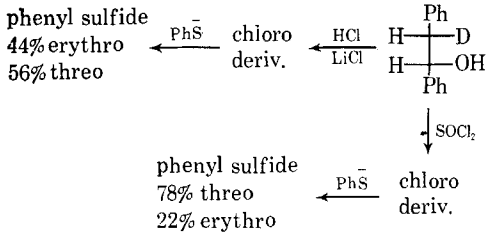
Diastereoisomeric mixtures may be analyzed by NMR spectroscopy provided the spectrum exhibits one or more signals, due to each isomer, which do not overlap. Raban and Mislow (22) have pointed out the principles involved but give, as an example, a case in which a clear separation of isomer signals is not observed. We have used this method to analyze isomeric 4-phenyl-3-methyl-4-piperidinols obtained by treating a 3-methyl-4-piperidone with lithium phenyl, and find that mixtures of two parts of the *trans* (3-Me/4-Ph) and one part of the *cis* isomer result (23); the analyses were based on the secondary Me signals which do not overlap and are particularly well separated in the base hydrochlorides, as discussed later. The isomeric mixture of alcohols (II)—the propionate ester of the α-isomer is propoxyphene—may be analyzed similarly.[2]

$$Me_2NCH_2CHMeCPh(OH)CH_2Ph$$

II

Chemical shifts of CH*Me* (c.p.s. from TMS in $CDCl_3$) are: α-(base) 50, β-(base) 66, α-(HCl) 75, and β-(HCl) 97.

Kingsbury and Thornton (25) have obtained evidence of the stereochemistry of displacement reactions using diastereoisomeric substrates, reaction mixtures being analyzed by measurement of NMR integrals (Scheme I). The advantage of the method is that the amount of retention and displacement product can be directly observed in favorable cases.



Scheme I

Mixtures of structurally isomeric alkenes are also amenable to assay by the integration technique. Figure 5 shows the spectrum of the product of dehydration of 3-methyl-4-phenyl-4-piperidinol. The 3-methyltetrahydropyridine content of the mixture is obtained from the 3-Me integral (broad singlet at 92 c.p.s.), and the 5-methyl isomer from integrals of the secondary Me (doublet at 59 c.p.s.) and vinylic proton (triplet at 357 c.p.s.) signals (26). By this analytical procedure, a study of the relative stabilities of isomeric 3- and 5-methyltetrahydropyridines and of the influence of structure and stereochemistry upon the direction of elimina-

[2] Treatment of $NMe_2CH_2CHMeCOPh$ with benzyl magnesium bromide gives the alcohols (II) in the ratio of 5(α) to 1(β) from integral data derived from the spectrum of the total base (24).

tion of 4-aryl-3-methyl-4-piperidinols has been made (27).

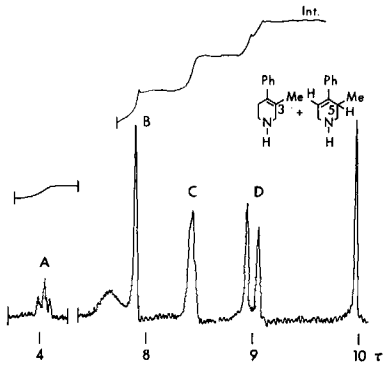
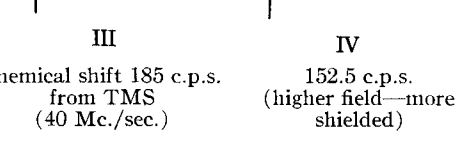


Fig. 5—Part of the PMR spectrum of the dehydration product of 3-methyl-4-phenyl-4-piperidinol in $CDCl_3$. C (tert Me) is due to 3-methyl-4-phenyl-1,2,5,6-tetrahydropyridine, and A (vinylic) and D (sec Me) to the 5-methyl isomer; B (N-H) is due to both isomers.

CONFIGURATIONAL AND CONFORMATIONAL STUDIES IN CYCLIC MOLECULES

In cyclohexane derivatives, an equatorial proton is generally less shielded than the corresponding axial one [*e.g.*, 4-H in *cis*(III) and *trans*(IV) 4-bromo-*tert*-butylcyclohexane (28)] and much use has been made of this observation



III — chemical shift 185 c.p.s. from TMS (40 Mc./sec.)

IV — 152.5 c.p.s. (higher field—more shielded)

in calculating the conformational preferences of substituent groups (29). A valuable relationship for assigning configuration and conformation in rigid systems is the dependence of coupling constants between protons on adajcent carbon atoms (vicinal coupling) upon the dihedral angle (ϕ) between the protons. A number of formulas have been developed to express this relationship (30, 31), and the plot of J_{vic} against ϕ for Williamson and Johnson's formula (31) is shown in Fig. 6. From this, J_{vic} should have a value of about 16 c.p.s. when $\phi = 180°$ (as in axially related protons) and 2.5 c.p.s. when $\phi = 60°$ (as in a/e or e/e related protons). In practice, J_{aa}, J_{ae}, and J_{ee} values deviate from these values as a result of distortion of the ideal dihedral angles

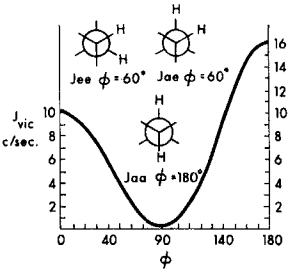


Fig. 6—Plot showing the dihedral angle dependence of vicinal coupling constants from Williamson and Johnson (31).

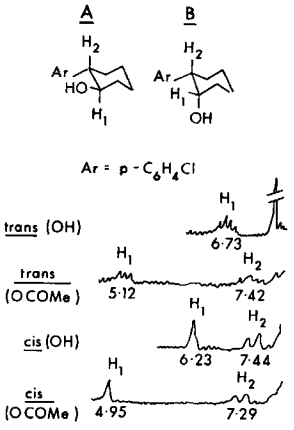
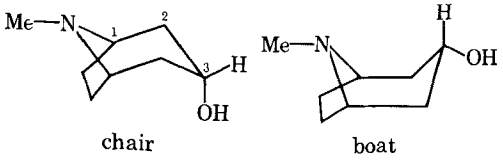


Fig. 7—Part of the PMR spectra of trans *and* cis *2-(p-chlorophenyl) cyclohexanol and corresponding acetate esters; chemical shifts in τ values from TMS, solvent CCl_4. [Adapted with permission from* J. Org. Chem., **27**, *715(1962).]*

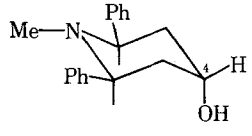
and the influence of substituent electronegativities upon J values, but it is clear that J_{aa} coupling constants exceed J_{ae} and J_{ee} values. Thus, large J_{vic} coupling constants between protons in a cyclic structure may be associated with an approximately diaxial orientation of atoms, and smaller separations with a–e or e–e interactions. Much use has been made of these principles in establishing the stereochemistry of biologically active cyclic compounds. A typical example, taken from Huitric's extensive study of aryl substituted cyclohexanols (32), is the investigation of *cis*- and *trans*-2-(chlorophenyl)cyclohexanol (33). Signals of the 1- and 2-protons are shown in Fig. 7; these are readily differentiated by the large downfield shift of the 1-hydrogen upon acetylation (6, 34). [Use of the upfield shift of the methine proton which follows methylation of *sec* alcohols has recently been proposed as an alternative method of identifying protons on carbon bearing a hydroxyl group (35).] The broad unresolved multiplets for the 1 and 2-hydrogens in A (OH and acetate) are consistent with their *trans* configuration; in the geometry depicted in A, both are axial and each is adjacent to two nonidentical axial hydrogens. In the *cis* geometry, B, the 1-hydrogen is equatorial and has little spin–spin coupling with adjacent hydrogens (hence an approximate singlet results), while the

2-hydrogen is axial and adjacent to only one other axial hydrogen giving rise to what is essentially a doublet with $J_{2,3}$ of about 11.5 c.p.s. PMR evidence of the same nature has been used to settle the controversial problem of whether the piperidine ring of tropane derivatives exists in a chair or a boat conformation (36, 37). The most valuable information in this respect derives from the approximate triplet due to the 3-proton of tropine (V)—the small value of its base width shows that it cannot involve a *trans* (or near *trans*) coupling (as in the boat form) and establishes that the 3-proton must be attached equatorially to a six-membered ring. Furthermore, the characteristics of this signal are very similar to those of the 4-proton in the β-isomer of 1-methyl-2,6-diphenylpiperidin-4-ol (VI) of known conformation (38).



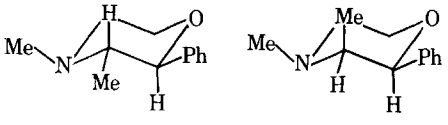
V

base-width 3-H, 16.8 c.p.s.



VI

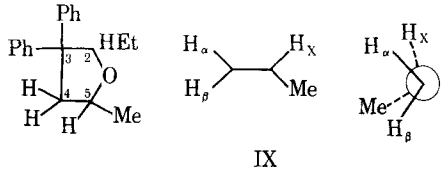
4-H, 16.0 c.p.s.



VII VIII

Acid-catalyzed cyclization of *N*-(β-hydroxyethyl)-(−)-ephedrine (erythro configuration) yields the appetite depressing drug (+)-3,4-dimethyl-2-phenylmorpholine (VII, phendimetrazine) while cyclization of *N*-chloroacetyl ephedrine and reduction of the product with lithium aluminium hydride gives the corresponding diastereoisomer (VIII) (without influence upon appetite). From the $J_{2,3}$ coupling constants, phendimetrazine is assigned a threo ($J_{2,3}$ 8.8 c.p.s., a value indicative of *trans* coupling) and its isomer an erythro ($J_{2,3}$ 2.7 c.p.s.) configuration (39). Hence, the acid-catalyzed process must proceed with inversion of configuration.

Knowledge of the stereochemistry of the α- and β-2-ethyl-5-methyl-3,3-diphenyltetrahydrofurans (IX) derived from the diastereoisomeric analgesics α- and β-methadol, respectively (40, 41), is of importance in that it provides evidence of the configurations of the precursor amino alcohols themselves (41). The PMR spectra of the isomeric cyclic ethers differ markedly in the respect to the 4-methylene signals (40), which form the AB part of an ABX system (Fig. 8). Assignments to H_α and H_β (*cis* and *trans* to the methine proton, H_x) may be made by use of the dihedral angle dependence of coupling constants [the reasonable assumption is made that the C-4 and C-5 substituents are staggered (29) with the $H_\alpha H_x$ and $H_\beta H_x$ dihedral angles intermediate between 0° and 60°, and 120° and 180°, respectively, leading to the conclusion that $J_{\beta x}$ should exceed $J_{\alpha x}$], and it is apparent, from comparisons with the AB signals of a series of related 5-methyl-3,3-diphenyltetrahydrofurans, that the H_α proton has an abnormally high field position in the α-isomer (IX). This is a result to be expected if the same isomer has *cis*-2-Et/5-Me geometry, since a consideration of the probable conformations of the *gem* diphenyl groups in this molecule (that of phenyl *trans* to 2-Et will be determined largely by the orientation of the *cis*-phenyl group, itself influenced by the bulky 2-Et substituent) leads to a conformer in which the α-C-4 methylene proton falls within the screening zone of the adjacent phenyl group (B in Fig. 9). In the β-isomer (*trans*-2-Et/5-Me) interactions between 2-Et and a flanking phenyl group will likewise be an important factor in determining the preferred conformation of phenyl *trans* to Et. Here, however, the methylene proton *cis* to 5-methyl (H_β) will be screened and, as a result, the H_α-H_β chemical shift difference will increase because H has the higher field position in the absence of differential screening effects; these arguments account for the well separated H_α and H_β signals and the unusually high field position of H_β in the β-isomer,



IX

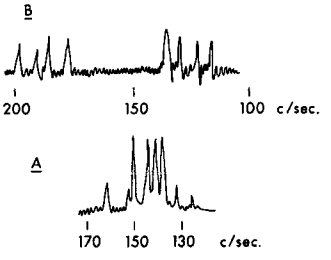


Fig. 8—C-4 Methylene protons PMR signal of A, *α-2-ethyl-5-methyl-3,3-diphenyltetrahydrofuran and* B, *the β-isomer. Chemical shifts in c.p.s. from TMS.*

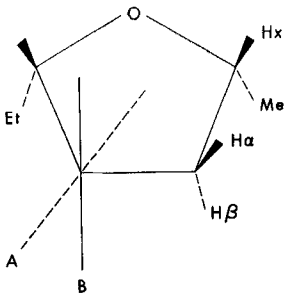


Fig. 9—Diagram of Dreiding model of cis-*2-ethyl-5-methyl-3,3-diphenyltetrahydrofuran viewed from above. Heavy lines lie above, and dotted lines below the plane of the tetrahydrofuran ring;* A *and* B *denote the planes of the aromatic rings* cis *and* trans *to 2-Et, respectively.*

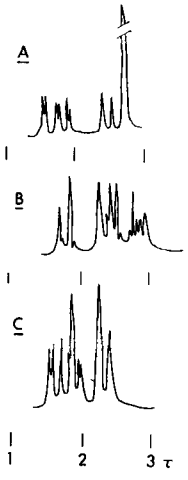
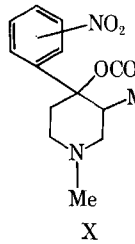


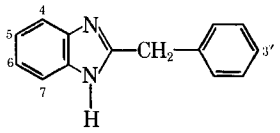
Fig. 10—Aromatic PMR signals of some nitro-2-benzylbenzimidazoles in dimethylsulfoxide. Key: A, *5-nitro;* B, *3′-nitro;* C, *3′5-dinitro. Chemical shifts in τ values from TMS.*

PROBLEMS OF AROMATIC SUBSTITUTION

The position taken by an aromatic substituent can often be identified by PMR spectroscopy from considerations based upon a combination of chemical shift, spin–spin couplings (*ortho* 5–9, *meta* 2–3, and *para* coupling 0–1 c.p.s.), and integration data. A simple example is provided by the nitration product of alphaprodine (X) (42). If the nitro group enters the *para* position,

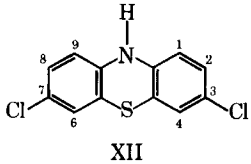


X



XI

the aromatic protons will comprise an A_2X_2 (or A_2B_2) system, and their signal will be a pair of superimposed doublets with J values typical of *ortho* coupling. If the nitro group enters the *ortho* or *meta* positions, the aromatic protons will comprise nonsymmetrical groups, and their resonance signal will be correspondingly more complex. In fact, the aromatic signal of nitro alphaprodine consists of two doublets that both integrate for two protons and have a J value of 9 c.p.s.; hence X is a *p*-nitro derivative. Orientation in the case of the dinitro product obtained from 2-benzylbenzimidazole (XI) was assisted by comparison of its PMR spectrum with that of 3′- and 5-nitro-2-benzylbenzimidazole (Fig. 10) (43). The A_2B_2-4 proton quartet of the 3′-nitro derivative and signals due to protons of the benzimidazole nucleus of the 5-nitro isomer may both be discerned (in spite of the signal overlap) in the spectrum of the dinitro derivative. In the 5-nitro isomer, assignment of the lowest field doublet (511 c.p.s.) to the 4-proton (on the basis of its being flanked by electronegative, deshielding groups) is confirmed by the fact that it exhibits a coupling constant (2 c.p.s.) typical of *meta* placed protons. The doublet of doublets (center 490 c.p.s. J 2 and 9) must arise from the 6-proton since this is *meta* coupled to the 4- and *ortho* coupled to the 7-proton, the latter giving a signal of the expected multiplicity and J value (doublet at 464.5 c.p.s. J 9 c.p.s.), *ortho* coupling alone being involved in this case. Craig and others (44) have reported chemical shift data for a series of mono- and di- derivatives (mostly chloro and methoxy) of phenothiazine and have demonstrated the feasibility of deducing orientation in substituted phenothiazines by analysis of the aromatic proton resonance signals. When substitution is 3,7, for example, two doublets, both having J = 9 and one further split by *meta* coupling [due to the 1 (9) and 2 (8) protons, respectively], should be differentiated —as in the spectrum of 3,7-dichlorophenothiazine



XII

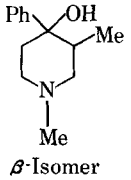
(XII). The problem of orientation has particular significance in characterizing hydroxylated phenothiazines (formed in the metabolism of pharmacologically active derivatives) since the necessary synthetic reference compounds are not yet available. Warren *et al.* (45) have published the PMR spectra of a collection of commercially available phenothiazines for use in their identification.

HYDROGEN BONDING STUDIES

The resonance position of a hydroxylic proton moves to lower fields when it is hydrogen bonded

(2, 6, 7, 46), a phenomenon which has made PMR spectroscopy a valuable tool for the study of hydrogen bond formation. The dependence of the OH resonance position (a time average of a weighted average of the various hydrogen-bonded species present) upon the extent of hydrogen bonding accounts for the changes in the OH chemical shift which result when the concentration of the hydroxyl compound or the temperature are varied. Concentration independent (or largely so) OH resonance signals are obtained when solvents capable of forming strong hydrogen bonds such as dimethylsulfoxide and pyridine (47, 48) are employed; solvent–substrate bonding (unaffected by substrate concentration) then becomes the predominant hydrogen-bonded species because of the large excess of solvent over solute molecules. $CDCl_3$ is also a solvent of this nature as seen from the OH chemical shifts of β-1,3-dimethyl-4-phenylpiperidin-4-ol, which are far more sensitive to concentration changes in CCl_4 than in $CDCl_3$ (49) as the data in Table I demonstrate.

TABLE I—CHEMICAL SHIFTS OF



β-Isomer

Solvent	Concn., mg./ml.	Chemical Shift (from TMS), c.p.s.
CCl_4	70	122
CCl_4	23	95.5
$CDCl_3$	70	133
$CDCl_3$	23	122

The chemical shift of the hydroxyl proton has been shown to be linearly related to concentration in CCl_4 in the 0.015 to 0.002 mole fraction range and, by extrapolating to infinite dilution, the chemical shift of the monomeric OH proton can be determined (48). Comparisons of such values in triads of compounds such as those shown in Fig. 11 have enabled the conformation preferences of cyclohexane substituents to be calculated (48). It is probable (on steric grounds) that an axial OH group is less hydrogen bonded than one in the equatorial positon; hence, the OH chemical shift of an axial alcohol should be less concentration-dependent than that of the epimer. From these arguments evidence of stereochemistry may be derived from the limiting slopes of chemical shift–concentration plots (48). Thus, from the data of Fig. 11, the slope for *cis*-4-*tert*-butylcyclo-

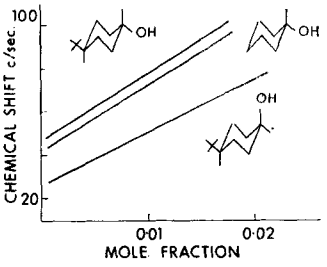


Fig. 11—Chemical shift dependence of hydroxyl proton with concentration at 40°. Solvent CCl_4. [Adapted with permission from J. Am. Chem. Soc., **86**, *4378 (1964).]*

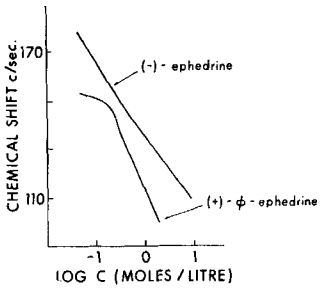


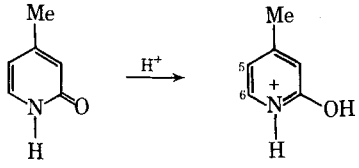
Fig. 12—Chemical shift dependence of NH/OH protons with concentration in $CHCl_3$. [Adapted with permission from Can. J. Chem., **38**, *125 (1960).]*

hexanol (axial OH) is less than that of the *trans* isomer (equatorial OH). A more subtle use of PMR spectroscopy in these studies is the detection of intra- in distinction from intermolecular hydrogen bonding, as may occur in diols and amino alcohols. Inter- is more sensitive than intramolecular hydrogen bonding to dilution, and this difference should be reflected in OH chemical shift–concentration plots. It is found, for example, that the concentration dependence of the *o*-chlorophenol OH resonance frequency reaches a maximum at low concentrations, while that of the *m*- and *p*-isomers continues to increase throughout the dilution range studied (intramolecular hydrogen bonding is only possible in the *o*-chloroisomer) (50). Figure 12 shows the concentration dependence of resonance frequencies of the NH and OH protons of ephedrine and pseudoephedrine (these appear as a single line because of rapid exchange). At low concentrations the pseudoephedrine signals are less sensitive to concentration changes and Hyne (51) advances this fact as evidence for intramolecular hydrogen bonding (N---H—O—) in the pseudoisomer. The position of the OH resonance lines in chloramphenicol and its isomers in acetone at low fields compared with those of the OH of simple monohydric alcohols at infinite dilution in CCl_4, indicates that both OH groups of the antibiotic are involved in hydrogen bonds. These bonds are considered to be of an intra- rather than intermolecular nature because of the comparative concentration independence of the OH chemical shifts, while bonding to the solvent

was thought unlikely on account of evidence that inter- and intramolecular hydrogen bonds are stronger than those formed between hydroxyl groups and acetone (the chemical shifts of monohydric alcohols at infinite dilution in acetone are at higher fields than those of OH in dihydric alcohols capable of intramolecular bonding) (52).

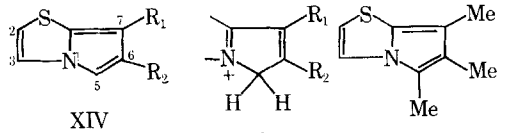
SITE OF PROTONATION

The problem of identifying the site of protonation in a molecule containing more than one basic center, often encountered in medicinal chemistry, may frequently be solved by studying the effect of proton uptake on chemical shifts and the spin–spin coupling patterns of protons in the vicinity of the various possible sites. Some examples follow. The C-6 proton peak of 4-methyl-2-pyridone (XIII) in sulfuric acid appears as a triplet indicating approximately equal coupling with the C-5 proton and *one* proton on the nitrogen atom (53). This result demonstrates the



XIII

O-protonation of the pyridone (XIII); if *N*-protonation had occurred, the C-6 proton signal would have been a quartet (two protons on nitrogen). Similar evidence supports sulfur and ring nitrogen as protonation sites in thiopyridones and aminopyridines, respectively (54). Protonation of the pyrrolo[2,1-*b*]thiazoles (XIV*a–c*) is considered to occur at a carbon atom because



XIV salt XV

a, $R_1 = R_2 = H$
b, $R_1 = H$, $R_2 = Me$
c, $R_1 = R_2 = Me$

PMR spectra of the salts display a two proton singlet that has a position characteristic of a methylene group (55). A low field AB system (two doublets), which is common to spectra of all the salts of XIV*a–c*, and XV can only arise from the C-2 and 3 protons; hence protonation must occur in the pyrrole ring. C-5 must be the site since, in XIV*c*, a methylene group can only develop at this position; the same site is postulated for the other derivatives since the methylene signals of the spectra (XIV*a–c*), all have similar chemical shifts. In the salt of XV, the C-5 proton signal is the anticipated quartet. In the next example evidence for the protonation site is obtained by identifying the proton most deshielded when a base is converted to its salt. The aromatic proton signals of the 2-benzylbenzimidazole shown in Fig. 13 are readily analyzed. The lowest field signal is assigned to the C-4 proton (it is flanked by electronegative groups and exhibits *meta* coupling only), the quartet (center τ 1.8) to the C-5 proton (it exhibits *ortho* and *meta* coupling), and the high field doublet (*ortho* coupled) to the C-6 proton. In the monohydrochloride, this pattern of signals, although disturbed, is maintained, and the downfield shifts may be measured. These are C-4 (7.2 c.p.s.), C-5 (13.2 c.p.s.), and C-6 (24.5 c.p.s.). The fact that the C-6 proton suffers the greatest shift indicates that N-1, rather than N-3, is the protonation site (56).

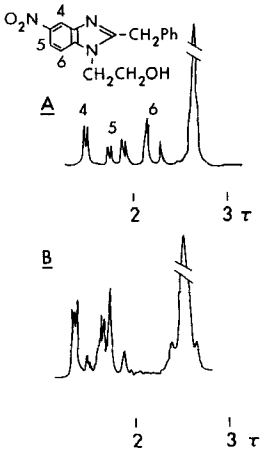


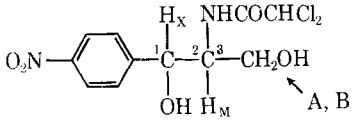
Fig. 13—Aromatic PMR signals of 2-benzyl-1-(2-hydroxyethyl)-5-nitrobenzimidazole in dimethylsulfoxide. Key: A, free base; B, hydrochloride. Chemical shifts in τ values from TMS.

Appropriate to this section is mention of the potentiality of PMR spectroscopy in determining pKa values of acids and bases. When an acid ionizes, the chemical shifts of protons near the acidic function change and if the PMR spectra of the unionized acid (HA) alone, and the anion (A^-) alone are known, the ratio HA/A^- for mixtures may be derived from chemical shift values [the spectral characteristics resulting from two species undergoing rapid chemical exchange depend only upon the PMR parameters of the individual species and their relative amounts (2)]. The same arguments apply in the case of a base and its conjugate acid. These principles have been applied by Birchall and Jolly (57) to the determination of the relative acidities of weak acids in liquid ammonia.

CONFORMATION OF PHARMACOLOGICALLY ACTIVE MOLECULES

Knowledge of the probable conformations of pharmacologically active molecules in solution, particularly as solutes in water, is of great importance to medicinal chemists because of the value of such information in extending the understanding of receptor sites. Some of the relatively few examples in which NMR spectroscopy has been employed in this connection are discussed below.

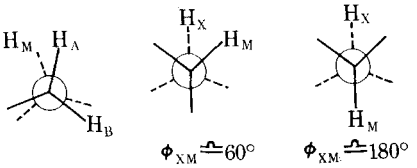
Jardetzky (52) has made a detailed analysis of the PMR spectra of chloramphenicol (XVI) and its erythro isomer. Signals arising from the



J_{AB} = 10–11 c.p.s
$J_{AM} = J_{BM}$ = 5–6 c.p.s
J_{MX} = 2–3 c.p.s

XVI

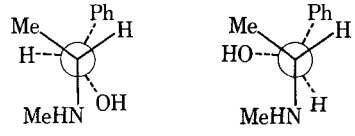
proton group attached to carbons 1, 2, and 3 of chloramphenicol could be analyzed by the ABMX procedure, and it was concluded (*a*) from the smallness of the J_{MX} value (2.6 c.p.s.) and (*b*) the nonequivalence of the two hydrogens on C-3, that rotation about the single C—C bonds of the propanol side chain is restricted. In principle, methylene protons (AB) adjacent to an asymmetric center must be nonequivalent because of differences in the averaging of the A and B shifts by rapid rotation (rotational nonequivalence); however, nonequivalence of this type is not observed in the PMR spectra of many asymmetrically substituted molecules in which "free" rotation can be inferred from the magnitude of the coupling constants [*e.g.*, catecholamines (58)], and for it to be observed, differences in conformation populations are an additional requirement. The preferred conformation (XVII) of the C_2 and C_3 portion of the molecule (with



XVII $\phi_{XM} \doteq 60°$ XVIII $\phi_{XM} \doteq 180°$ XIX

the AM and BM dihedral angles close to 0° and 180°, respectively) is based upon the values and equality of the J_{AM} and J_{BM} coupling constants (see plot of J_{vic}/dihedral angle, Fig. 6), while the low value of the J_{MX} constant (2–3 c.p.s.) shows the X and M protons to have a gauche conformation as in XVIII. In the erythro isomer the larger J_{MX} value (6 c.p.s.) shows the X and M protons to be *trans* as in XIX. A choice of the most likely conformations, influenced also by the assumption of an intramolecular hydrogen bond (evidence discussed elsewhere in this review) led to a structure which bore a striking resemblance to a pyrimidine ribonucleotide. Any alteration of the geometry of the propanol side chain (as in erythro isomers) destroys the similarity to the ribose moiety; hence this relationship is considered to have a possible bearing upon the stereochemical features of structure-activity relationships in the chloramphenicol series (52).

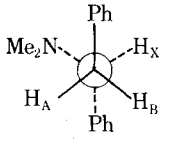
Hyne (59) has studied the conformation of a related diastereoisomeric pair, ephedrine and pseudoephedrine, in chloroform. The preferred "off staggered" conformations he proposes for these molecules (XX and XXI) are based upon



pseudoephedrine XX ephedrine XXI

$Ph^1CH(OH)^2CH(NHMe)Me$

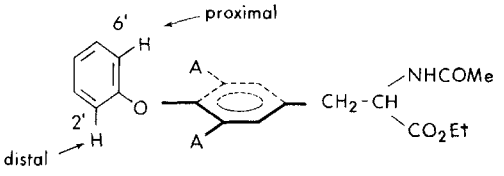
PMR evidence for similar methyl environments in the two isomers and for a greater C_1-H/C_2-H dihedral angle in the case of pseudoephedrine (J_{vic} 8.2 for pseudo- and 4.0 c.p.s. for ephedrine). The occurrence of intramolecular hydrogen bonding in the pseudoisomer (see previous discussion) is also accounted for by conformation XX; in the ephedrine conformation (XXI) the tendency for hydrogen bonding should be less because of the great OH-NH distance. Sasaki *et al.* (60), using similar arguments, have advanced a favored conformation for (−)-*N*,*N*-dimethyl-1,2-diphenylethylamine (XXII), an analgesic one-half to one-third as potent as morphine (61).



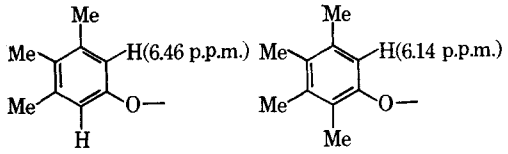
J_{AB} 13.9
J_{BX} 6.4
J_{AX} 9.3
(hydrochloride in D_2O)

XXII

Lehman and Jorgensen (62) have presented PMR evidence in support of a preferred skewed conformation for *O*,*O*-disubstituted diphenyl ethers (XXIII) related to thyroxine; this work is

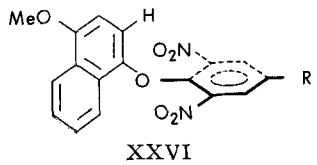


XXIII



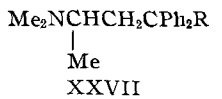
XXIV XXV

related to Jorgensen's proposed receptor for the thyroid hormone (63). Study of models shows that the proximal 6′-proton (but not the distal 2′-proton) lies well within the region in which its magnetic resonance would be shielded by the π-cloud as estimated by Johnson and Bovey (64). In compounds substituted at the 3- and 5-positions (A in XXIII) by iodine or nitro but lacking a 2′-substituent, signals due to the aromatic protons in the outer ring ranged from 6.36 to 8.2 p.p.m. However, in trisubstituted derivatives the 6′-protons resonated at unusually high fields (6.22 p.p.m. in the dinitro- and 6.07 p.p.m. in the diiodo series) as should be the case if the skewed conformation (XXIII) is preferred in these compounds. A consistent difference between the chemical shifts of the 6′-proton and other protons in the same ring was seen throughout the series which included a variety of substituents attached at various points; hence, the possibility of the observed shifts being due to substituent effects is unlikely. Furthermore, the chemical shifts of the 6′-protons in XXIV and XXV may be compared; the upfield shift suffered by this proton when the 2′-proton is replaced by methyl is unlikely to be a substituent effect (the 2′-methyl group is too far removed) and is more reasonably attributed to a steric positioning of the outer ring as in XXIII. Evidence for positioning was found for 2,6-dinitro and diiodo ethers with cyclohexyl, isopropyl, allyl, trifluoromethyl, and methyl C-2′ substituents and also in the case of α-naphthyl derivatives such as XXVI.



XXVI

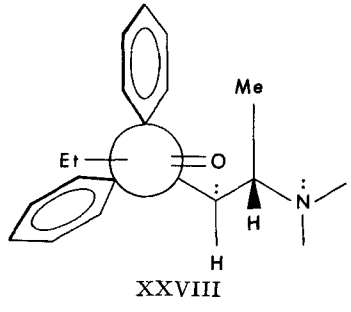
In the case of methadone (XXVII*a*), evidence for probable conformations has been derived by comparing the PMR spectrum of this analgesic with those of related 1-amino-3,3-diphenyl-



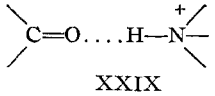
XXVII

R = *a*, COMe; *b*, H; *c*, OH; *d*, CN; *e*, C(NH)Et, *f*, CO_2Et; *g*, CH(OCOMe)Et

propane derivatives (65). It is found that the chemical shift of the secondary methyl group in methadone (XXVII*a*) (29 c.p.s.) is at an abnormally high field position compared with those of the derivatives (XXVII*b*, *c*, and *d*) (all near 60 c.p.s.). Unusually high field *sec* Me signals are also observed in the ketimine (XXVII*e*, 20 c.p.s.) and analogs of methadone bearing different basic groups. When the basic derivatives (XXVII) are protonated, the *sec* Me signals move downfield as a result of the greater deshielding influence of positively charged nitrogen; however, downfield shifts are low (13 compared with 20–25 c.p.s.) in the amino ketones. These results are taken to indicate that in favored conformations of methadone and related ketones, the *sec* Me group lies close to and above the plane of one of the aromatic rings in the molecule, as is the case in crystalline methadone hydrobromide whose conformation has been established by X-ray crystallography (66). The model (XXVIII), based upon the structure of the crystalline salt, is therefore taken to represent a favored conformation for methadone hydrochloride as solute in $CDCl_3$ or water (an unusually high field *sec* Me signal is also observed when D_2O is solvent). The conformation (XXVIII) could be stabilized by an interaction of the type XXIX and the PMR demonstration of the nonequivalence of the two *N*-methyl groups

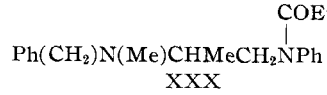


XXVIII



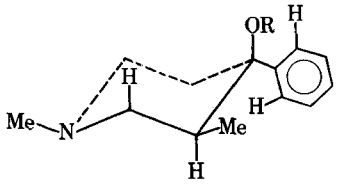
XXIX

(in $CDCl_3$, the NMe_2 signal is a doublet of doublets collapsing to a singlet when D_2O is added) provides evidence for such an interaction; an association involving the protonated basic group may be expected, if sufficiently strong, to lead to restricted rotation about the nitrogen atom (65, 67). High field *sec* Me chemical shifts are also observed in nonketonic analgesically active 3-amino-1,1-diphenylpropane derivatives such as XXVII*f* and *g* (68). In diampromide (XXX,



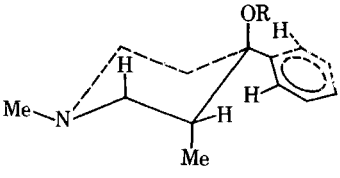
XXX

regarded as a methadone analog in which the quaternary carbon and one phenyl group of the parent are replaced by nitrogen), the *sec* Me group is not especially shielded by the aromatic groups, this and other spectroscopic evidence (69) indicating that the phenyl-*sec* Me-basic groups orientations in the two analgesics differ—a possible reason for their differing stereochemical and basic group features.



XXXI

trans 3-Me/4-Ph



XXXII

cis 3-Me/4-Ph

R = H, COMe, or COEt

The configuration of the two diastereoisomeric propionate esters of 1,3-dimethyl-4-phenylpiperidin-4-ol (α- and β-prodine) has been the subject of several papers (70) and is now firmly established as *trans* (3-Me/4-Ph) for α- and *cis* for β-prodine by X-ray crystallography (71). Differences in the PMR spectra of the two esters and of the alcohols from which they are derived provide evidence of the conformation of these molecules as solutes in $CDCl_3$ and support structures XXXI and XXXII as the most probable conformations for the α- and β-isomers, respectively (49). Signals of greatest value in this

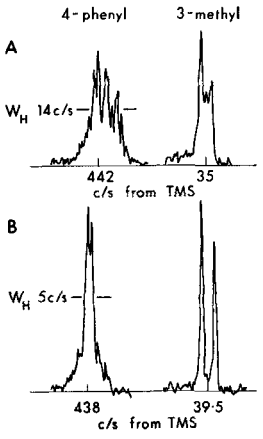
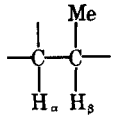


Fig. 14—Part of the PMR spectra of α-1,3-dimethyl-4-phenyl-4-piperidinol (A) *and of the corresponding β-isomer* (B) *in CCl_4. [Reproduced with permission from* Tetrahedron, **22,** *2711 (1966).]*

interpretation are those of the 4-aryl and 3-methyl groups. It is postulated that chemical shift differences among the aryl protons should be greater in the α-diastereoisomer with the result that the *trans*- aromatic signal should be more complex than that of the *cis* isomer; this is confirmed experimentally (Fig. 14). Dreiding models of XXXI and XXXII show that both *sec* Me groups fall within the diamagnetic screening zone of the benzene nucleus; this fact accounts for their high field resonance positions (relative to *sec* Me in compounds such as 3,4-dimethyl-1-phenethyl-4-piperidinol, *sec* Me chemical shift 54.5 c.p.s. in $CDCl_3$) but does now allow a stereochemical differentiation to be made (the α/β-*sec* Me chemical shift difference is small with the α-signal at higher field by 5–6 c.p.s.). However, axial Me (in XXXII) is closer than equatorial Me (in XXXI) to the ring nitrogen atom; hence, when the deshielding influence of this atom is enhanced by protonation the β-Me signal should suffer the greater downfield shift. Again this is found to be the case, shifts being 2 c.p.s. for α-Me and 16 c.p.s. for β-Me. The nature of the α- and β-Me signals also provides evidence of conformation. The distorted appearance of the α-signal (Fig. 14) is a typical result of virtual coupling which occurs in the system:



when the coupling constant between H_α and H_β is large and of the same order as the chemical shift difference between the two protons (72). Such conditions are more likely to prevail in XXXI than in XXXII since the former contains an axial proton at C-3 which will be strongly coupled to the C-2 axial proton. In propionate

esters of the piperidinols the $COCH_2Me$ and $CO\mathit{CH_2}Me$ signals are at higher field in the β-isomer. These results are also consistent with the *cis* ester having a preferred conformation akin to XXXII because the acyl portion of the ester group will spend some of its time above the plane of the phenyl group (*i.e.*, well within the aromatic shielding zone) if the planes of the C—O bond and the aromatic ring are approximately perpendicular. The conformation of drug molecules in water is of greater importance from a biological point of view and PMR studies of the prodine esters and alcohols (as hydrochlorides) in D_2O show that replacement of $CDCl_3$ by D_2O brings about significant changes in conformational equilibria. Thus, while the downfield shifts of the aromatic and *N*-methyl signals (resulting from this solvent change) are similar in α- and β-1,3-dimethyl-4-phenylpiperidin-4-ol hydrochlorides, that of the β-*sec*-Me group is almost 11 c.p.s. less than that observed for the corresponding α-signal. In addition, the chemical shift difference between the α- and β-*sec* Me groups is much less in D_2O than in $CDCl_3$. These results indicate an increase in the population of equatorial *sec* Me (chair) and/or skew-boat conformations at the expense of axial-*sec* Me conformers in the β-isomer when $CDCl_3$ is replaced by D_2O. Such an increase would reduce the deshielding influence of protonated nitrogen upon *sec* Me, the chemical shift of this group moving nearer to that of *sec* Me in the α-isomer in consequence. Radical conformational changes through solvent variation must also occur in esters of the piperidinols as is evident from the *sec* Me chemical shifts recorded in Table II. The zero or near-zero chemical shift differences between the α- and β-*sec* Me signals observed with D_2O as solvent are particularly noteworthy in this respect (24). When $CDCl_3$ is replaced by D_2O as solvent, a considerable increase in the degree of solvation of both the protonated basic center and the oxygen function of the piperidinol derivatives is probable and the larger destabilizing Me/H and OR/H 1,3-diaxial interactions obtaining in the solvated molecules are considered responsible for the decrease in the conformational preference for axial 3-Me conformers (49).

TABLE II—SOLVENT EFFECTS UPON *sec* Me CHEMICAL SHIFTS IN ESTERS OF α- AND β-1,3-DIMETHYL-4-PHENYLPIPERIDIN-4-OL HYDROCHLORIDES

4-Function	Solvent	Chemical Shift (c.p.s.) [a] α-Me	β-Me	Difference (β − α)
OCOMe	$CDCl_3$	44	61	+17
	D_2O	44	41	− 3
OCOEt	$CDCl_3$	44	62	+18
	D_2O	43.5	43.5	zero
$OCOC_3H_7$-*n*	$CDCl_3$	44	61	+17
	D_2O	40	42.5	− 2.5

[a] From TMS, internal in $CDCl_3$ and external in D_2O (60 Mc./sec. spectra).

RELAXATION TIME STUDIES

The extreme sensitivity of spin–spin (T_2) and spin–lattice (T_1) relaxation processes to variations in the molecular environment of the proton species concerned provide an elegant method for the study of the interactions of drugs with macromolecules of a type liable to be encountered in the body (14). In this technique, relaxation constants are measured for the drug alone and for the drug in the presence of a macromolecule. (T_2 is obtained from measurements of line widths at half the maximum peak height, and T_1 by measuring the recovery time of a signal which has been saturated by the application of a strong R.F. field.) Relative changes in T_1 and T_2 for each proton group that occur in the presence of the added substance are then derived from the data. Differing values indicate that a specific binding mechanism is involved (a non-specific mechanism such as an increase in viscosity would shorten all relaxation times by the same factor), the groups implicated being identified as those which exhibit the greatest decreases in relaxation times. In a study of the binding of sulfonamides to albumin (73), it was found that addition of this protein to the sulfonamide solution generally produced a larger increment in the relaxation rate of the *p*-aminobenzenesulfonamide aromatic protons than for any protons of the N-1 substituent. Results for sulfacetamide (Fig. 15), for example, showed that the aromatic moiety is preferentially stabilized by the interaction of the drug molecule with albumin, whereas the acetyl group retains much greater freedom of motion; in this molecule it is probable, therefore, that the aromatic ring is the primary binding site. A similar study of the binding of benzyl penicillin to serum albumin identifies the benzyl group as the binding site (74), while, in the case of the interaction between epinephrine and adenosine triphosphate (a complex of this nature is thought to be the storage form of catecholamines in the chromaffin granules of the adrenal medulla), results indicate that the propanol side-chain, but not the aromatic ring, is involved in complex formation (14). Chapman and Penkett (75) have studied the interaction of phospholipids with cholesterol by relaxation studies and have obtained evidence for the direct interaction of a portion of the phospholipid (particularly the hydrocarbon chains, the major

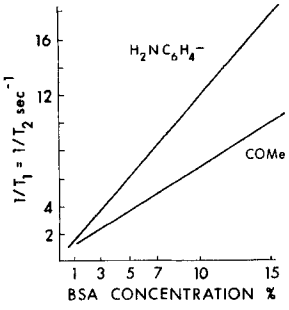
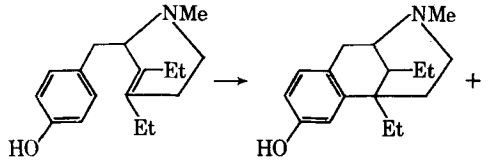


Fig. 15—Relaxation rates of 0.1 M sulfacetamide peaks, pH 8.8, as a function of bovine serum albumin (BSA) concentration. [Adapted with permission from Mol. Pharmacol., **1**, *214 (1965).]*

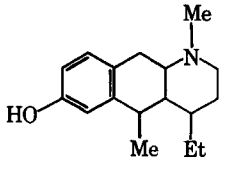
PMR peak arising from protons associated with the hydrocarbon chain of lecithin virtually disappears when an equimolar proportion of cholesterol is present) with the cholesterol molecule. It is obvious that work of this nature has great potentialities in the study of drug–enzyme and drug–receptor interactions.

MISCELLANEOUS

The routine use of PMR data as an aid to structure determination in natural products and in organic synthesis is self-evident. To quote but one example, cyclization of the tetrahydropyridine (XXXIII) by aluminum bromide gives an analgesically active benzo[*g*]quinoline (XXXV) as well as the expected benzomorphan derivatives (XXXIV), the former being characterized



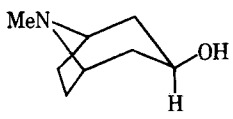
XXXIII *cis* and *trans*-Et/Et XXXIV



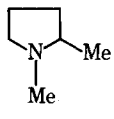
XXXV

by a *sec* Me doublet and a single CH_2Me triplet in its PMR spectrum (76).

Systems in which protons may exchange their environment within the same, or in a different, molecule are also studied conveniently by PMR spectroscopy. If the exchange rate is slow, absorption lines for each environment are seen, fast rates yield single, sharp, lines (corresponding to a weighted average of the separate environmental chemical shifts) while intermediate rates give rise to broad signals. [See Delpuech (77) for a recent review of this topic.] The kinetics of proton exchange in salts of organic bases may be studied in this way. In cyclic bases such as pseudotropine (XXXVI) and 1,2-dimethylpyrrolidine (XXXVII), the separate existence of isomers formed as a result of the protonated basic



XXXVI



XXXVII

HCl N—Me doublets ($J5$)	
130.5 c.p.s. (minor)	6.97 (major)
138.5 c.p.s. (major)	7.27 (minor)
from reference in H_2O, pH 1.0	from TMS in $CHCl_3$

center having two possible configurations is made evident by the appearance of two N-Me signals in the PMR spectra of their salts (78, 79) when solvents unfavorable to proton exchange are employed. The signals differ in intensity, and the more pronounced is assumed to be due to the more stable configuration (equatorial N-Me for XXXVI, *trans* 1,2-diMe for XXXVII). When the rate of proton exchange is accelerated, single N-Me signals are obtained. Protonated N-epimers are also observed in 1,5-dimethyl-4-phenyl-1,2,5,6-tetrahydropyridine (XXXVIII) hydrochloride and related compounds (80).

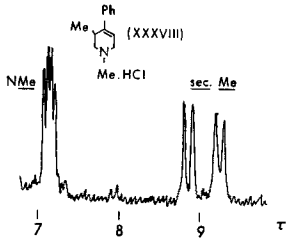
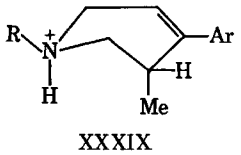
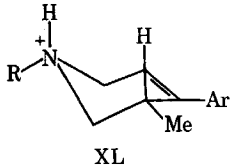


Fig. 16—Part of the PMR spectrum of 1,5-dimethyl-4-phenyl-1, 2, 5, 6-tetrahydropyridine hydrochloride in $CDCl_3$.

Thus, the spectrum of XXXVIII hydrochloride in $CDCl_3$ displays a doublet of doublets N-Me signal and two well-separated *sec* Me doublets (Fig. 16) which collapse to a singlet and a doublet, respectively, when D_2O is added. In these cases, there is evidence that the epimers differ in their 5-methyl rather than N-methyl configurations, XXXIX and XL being the proposed preferred conformations.



XXXIX



XL

Interesting developments in the differentiation of optical enantiomorphs by PMR spectroscopy

$PhCH(OH)CF_3$ $PhCH(OH)CHMe_2$
XLI XLII

have recently been reported. Magnetic equivalence of the two methyl groups of isopropanol no longer obtains when the alcohol is esterified with the acid chloride derived from (R)-*O*-methylmandelic acid; the now diastereoisomeric methyl groups in the resultant ester give rise to two doublets which differ in chemical shift by 5 c.p.s. at 60 Mc./sec. (81). The optical purity of propan-2-ol-1-d_3 may then be checked by conversion to the *O*-methylmandelate and examination of the PMR spectrum of the ester; if the material is a single enantiomorph, only one of the *sec* Me doublets of the nondeuterated ester should appear. It has now been shown that optical enantiomorphs themselves may have different PMR characteristics when examined in an optically active solvent as a result of diastereoisomeric interactions (82). Thus, the fluorine resonance signal of racemic 2,2,2-trifluoro-1-phenylethanol (XLI) is a doublet in racemic α-phenethylamine, but a doublet of doublets (of equal intensity) in the levorotatory base. (Each doublet is further split by long-range coupling with aromatic protons.) Partially resolved XLI in the latter solvent gives a doublet pair of unequal intensity. Similarly, the signal of the carbinyl proton of racemic phenylisopropylcarbinol (XLII), a doublet in racemic α-(1-naphthyl)ethylamine, forms two equally intense doublets in the dextrorotatory base (separation 1.6 c.p.s. at 60 Mc. and 2.5 c.p.s. at 100 Mc./sec.).

In conclusion, attention is drawn to some useful reviews. Surveys of the whole field are published periodically in *Analytical Chemistry* (83) and *Annual Reports of the Chemical Society* (for last review, see *Reference 84*). Kinetic studies by NMR (77), NMR of fluorine (85), of ^{13}C (86), of organometallic compounds (87), spin–spin coupling and double resonance (88) [see also Baldeschweiler and Randall (89) for the latter topic], and NMR and electronegativity (90), have all been recently reviewed. Useful accounts of long-range spin–spin coupling (91), geminal coupling constants in methylene groups (92), and conformational analysis of cyclohexane derivatives by NMR spectroscopy (93) are also available.

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Research Articles

Effect of Deuterium Oxide on Culturing of *Penicillium janczewskii* I

Growth, Nutritional Factors, and Antibiotic Production

By D. A. NONA, M. I. BLAKE, and J. J. KATZ*

Penicillium janczewskii, **a mold which produces the antifungal antibiotic griseofulvin, was cultured in media containing 50, 75, and 99.6 per cent deuterium oxide. Protio- and deuterio-carbohydrates were used as carbon sources. Griseofulvin production decreased markedly with increase in deuterium content of the medium. There was also a marked reduction in mycelial dry weights with increase in deuterium oxide in the nutrient. The surface culture technique proved more effective than the shake method for both tissue growth and antibiotic formation.**

A VARIETY OF organisms, including bacteria, fungi, and algae, have been successfully cultured in media containing pure heavy water. The growth of the heterotroph *Euglena gracilis* in a fully deuteriated form has also been recently reported (1). Higher plants, including peppermint (2, 3), belladonna (4, 5), and duckweed (6), have been grown in nutrient solutions containing as high as 70% deuterium oxide. These studies have been summarized by Flaumenhaft (7) and by Katz (8), who have reviewed extensively the biological effects of deuterium.

Deuteriated organisms provide a practical source of metabolites containing deuterium in all

Received April 14, 1967, from the Department of Pharmacy, University of Illinois at the Medical Center, Chicago, IL 60612, and *the Chemistry Division, Argonne National Laboratory, Argonne, IL 60439
Accepted for publication May 25, 1967.
Presented to the Pharmacognosy and Natural Products Section, A.PH.A. Academy of Pharmaceutical Sciences, Las Vegas meeting, April 1967.
This investigation was supported by grant AI 06825-01 from the National Institutes of Health, U. S. Public Health Service, Bethesda, Md., and was performed in part under the auspices of the U. S. Atomic Energy Commission, Washington, D.C.