DECLARATION

I declare that all the **SPECTRA** and calculated chemical shifts data presented is my own work, and all the sources I have used or quoted have been acknowledged by means of references.

Signed by candidate

Zolani Dyosi

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Abstract

Ab-initio, semi-empirical, and empirical calculations are used in computer programs separately or in conjunction with one another to calculate predicted chemical shifts for a wide range of chemical compounds.

^{1}H-spectra for several flexible and rigid organic compounds were acquired on Oxford-Bruker spectrometer and interpreted using two-dimensional NMR techniques. ACD-V4 and CHARGE-V7 programs were used to predict ^{1}H chemical shifts for these compounds. ^{1}H and ^{13}C-spectra for alkaloids were also collected using a Varian spectrometer and also interpreted with the use of multipulse techniques. CHARGE-V7, ACD-V4 (^{1}H and ^{13}C) predictor programs together with SpecInfo-V3.2 (^{1}H and ^{13}C) predictor program were used in the prediction of chemical shifts for alkaloids.

The results obtained for flexible and rigid organic compounds indicate good calculation of ^{1}H-chemical shifts by both CHARGE-V7 and ACD-V4 programs. These results are contrary to the poor chemical shifts generated by these programs for alkaloids. The poor prediction of alkaloids could be due to the absence of similar fragments in ACD-V4 and SpecInfo-V3.2 program's databases. Poor parameterisation of CHARGE-V7 program coupled with complex spectra of alkaloids could be contributing to the inaccurate predicted chemical shifts. Increasing the size of the user database for SpecInfo and ACD programs would improve the accuracy in prediction of both ^{1}H and ^{13}C chemical shifts. Improvement in the accuracy of parameterisation of CHARGE-V7 to cater for rare compounds such as natural products is also required.

Abbreviations and Symbols

NMR	Nuclear magnetic resonance
^{1}H	Proton
^{13}C	Carbon-13
σ	Shielding constant
δ	Chemical shift
Φ	Dihedral angle
TMS	Trimethylsilane
COSY	Correlation Spectroscopy
DFQ	Double Quantum Filterd
HMQC	Heteronuclear Multiple Quantum Correlation
HSQC	Heteronuclear Single Quantum Correlation
HMBC	Homonuclear Multiple Bond Correlation
TOCSY	Total Correlation Spectroscopy

Other abrreviations and symbols are defined in the chapter(s) in which they are used.

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OBJECTIVE AND MOTIVATIONS OF THE PRESENT STUDY

Several NMR techniques used for structural elucidation of chemical compounds exist [**1-3**]. The limitation of these techniques is the amount of time required to collect and analyse spectral data manually. In most cases the two dimensional spectra is required in the interpretation of unresolved proton and carbon-13 spectra. The development of computer technology gave rise to the development of chemical shift prediction programs using empirical [**4**], semi-empirical [**5**] and quantum mechanical calculations [**6**] to obtain NMR parameters. These programs are applicable to a large variety of chemical compounds, including biological compounds. The available programs are also capable of predicting homonucleic and heteronucleic chemical shifts as well as coupling constants.

The objective of this study is to determine whether the existing chemical shift prediction programs are capable of predicting ^{1}H and ^{13}C chemical shifts of most compounds used in the pharmaceutical industry for medicinal purposes. Compounds (e.g substituted benzenes) were selected to represent a wide range of known compounds even those used in combinatorial libraries. The alkaloids were selected due the complexity of their spectra. The programs chosen are based on semiempirical (CHARGE) and empirical calculations (SpecInfo and ACD programs). The accuracy of these programs would indicate easier, quicker and cheaper ways of interpreting the spectra of existing and in some cases new compounds.

REFERENCES

1. E.Breitmaeir, carbon-13 NMR Spectroscopy 3rd ed., VCH Verlagsgesellschaft New York, (1987).
2. George C. Levy, NMR spectroscopy: New methods and applications, American chemical society, 1982.
3. Pierre Laszlo, NMR of Newly accessible nuclei, **1**, Academic press, (1983).
4. C. Pascual, J. Meier and W. Simon, Helv. Chim. Acta, **48**, (1969), 164.
5. N. J. B. green, Quantum mechanics, Oxford University press New York, (1997).
6. A. C Bizzard and D. P Santry, J. Am. Chem. Phys., **55**, (1971), 950.

CHAPTER 1

NMR-SPECTROSCOPY

1.1 Introduction

Nuclear magnetic signals were independently observed for the first time in 1946 [1-2]. The chemical shift effect was then observed in 1949 [3]. Purcell's experience in radio frequency electronics aided in the development of his NMR instrument, which was originally much like optical spectrometers [4]. The NMR spectroscopists use NMR in:

(i) obtaining spectra and developing the techniques;
(ii) evaluating the nuclear magnetic parameters from the spectra;
(iii) interpreting or predicting these parameters using physical or theoretical models; and
(iv) applying the knowledge gained to problems of chemical interest such as structural determination, evaluation of kinetic or thermodynamic data, etc [5].

The necessary NMR information is obtained by measuring, analyzing and interpreting high-resolution NMR spectroscopy recorded on liquids of low viscosity, solids and sometimes gases.

Liquids or solution systems are often studied by NMR, but gas phase NMR is uncommon due to low sensitivity and resonance lines that are usually broader than those of liquids [6]. The nuclides of special interest are proton (^{1}H) and carbon-13 (^{13}C) as they are very important in the structural determination of organic compounds. ^{31}P, ^{15}N, ^{17}O, ^{19}F, etc, can also be used in structural determination of compounds containing these nuclides in their structures [7].

X-ray crystallography is the method of choice in many cases of chemical structural identification. However crystallography has many limitations beyond its obvious need for crystals which are often difficult to obtain. Crystallography cannot yield any information about the solutions, conformational equilibria, and complex mixtures or reaction kinetics.

Difficulties of assignment of signals in ^{1}H and ^{13}C spectra are encountered when the spectral peaks are very close to one another. The availability of two-dimensional NMR techniques such as COSY, HSQC, NOESY, HMBC etc, makes it easier to resolve these structural assignment difficulties [**8**].

1.2 THEORY OF NMR CHEMICAL SHIFTS

1.2.1 Origin of chemical shifts

The field B in Fig 1.1 experienced by a nucleus in an atom or molecule, gives rise to the chemical shift. This field is slightly different from the external field B_0 experienced by a nucleus without electrons. The value of B is smaller than that of B_0 in an atom, due to the external field that causes the electrons to circulate within their atomic orbitals, and therefore generates a small field B' in the opposite direction to B_0 [9].

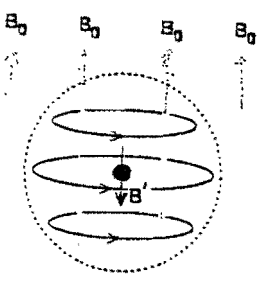


Figure.1.1: An applied magnetic field B_0 causes the electrons in an atom to circulate within their orbitals.

B' is proportional to B_0 and approximately 10^4 times smaller. The field at the nucleus is represented by the following equation:

$$B = B_0 - B' = B_0 (1-\sigma) \qquad \text{equation 1.1}$$

Where σ (shielding constant) is the constant of proportionality between B' and B_0. The electronic structure of the molecule in the vicinity of the nucleus determines the size and the sign of the shielding constant.

Using chemical shifts (δ) to measure the shielding constant, the frequency difference ($\nu-\nu_{ref}$) is divided by ν_{ref} so that the chemical shift is a molecular property, independent of the magnetic field used to measure it. This is shown in equation 1.2.

$$\delta = \frac{(\nu - \nu_{ref})}{\nu_{ref}}$$

equation 1.2

The factor 10^6 scales the numeric value of δ to a more appropriate size, leading to a measure of δ in parts per million (ppm). The relation of δ to shielding constant is

$$\delta = \frac{\sigma ref - \sigma ref}{1 - \sigma ref}$$

equation 1.3

where $\sigma_{ref} << 1$ is used.

This shows that an increase in σ leads to a decrease in chemical shift. A small amount of suitable compound is added to the NMR sample for obtaining a reference signal. Tetramethylsilane, $(CH_3)_4Si$ or TMS, is normally used as standard in ^1H and ^{13}C spectra [9]. TMS is soluble in most organic solvents. The residual solvent peak can also act as a secondary, internal reference.

In general the chemical shifts in a spectrum are represented by

$$\delta = \frac{\text{shift of sample(in Hertz)-shift of std(in Hertz)}}{\text{frequency of the spectrometer(MHz)}}$$

equation 1.4

1.2.2 Effects of neighboring groups on NMR chemical shifts

The equation for shielding constant is represented by:

$$\sigma = \sigma_{para} + \sigma_{dia} + \sigma_N + \sigma_R + \sigma_e + \sigma_I$$

equation 1.5

Where, σ_R -the ring current effect in arenes;

σ_e-the electric field effect;

σ_I-effects of intermolecular interactions.

The shielding constants in this equation besides the two shielding terms σ_{para} and σ_{dia} (for paramagnetic and diamagnetic shielding), are important to account for intermolecular contributions [**10**].

1.2.3 Local diamagnetic shifts (σ_{dia})

Chemical shifts from local diamagnetic currents are dependent on the electron density around the nucleus, the larger the electron density the greater the shielding, and therefore the decrease in chemical shift [**10**]. There is a linear correlation in electronegativities [11] of halogens; these increase from iodine to fluorine [**12**].

1.2.4 Local paramagnetic shifts (σ_{para})

The local paramagnetic effect depends on the excitation energies of electrons primarily associated with the nucleus under consideration. The induced magnetic moment, which leads to deshielding, is produced [**13**].

Ligands such as carbonate, oxalate, and acetylacetonate, which produce small ligand fields, give large paramagnetic de-shielding.

Electron donating groups delocalize their lone pairs into the ring and increase the electron density at the ortho and para carbons. The increased electron repulsion causes the orbitals around these atoms to expand, reducing $\sigma_{para,}$ hence the chemical shift decreases. Local paramagnetic shifts make only modest contributions to ^{1}H shielding because of low electron densities and high electronic excitation associated with hydrogen atoms [**14**].

1.2.5 Ring currents

Ring currents arise when a molecule with delocalized π-electrons is placed in a magnetic field. The ring current in turn generates an additional magnetic field, whose lines of force at the centre of the arene ring are in opposite direction to the external

magnetic field B_0 [15], (Fig. 1.2). The ring current effect is greater when the plane of a benzene ring is perpendicular to the field direction. It is zero when the magnetic field lines do not pass through the ring. Hence during isotropic rotation the effect does not average to zero [16].

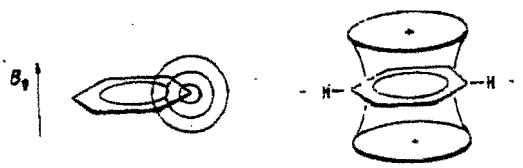


Figure 1.2:Ring currents in arenes, with zones of increased (+) and reduced (-) shielding.

1.2.6 Electric field effects

Intra-molecular electric fields exist in molecules containing polar groups such as carbonyls or nitro groups. The electric field influences the electron density distribution in the molecule and therefore the magnetic shielding of the nuclei. The electric field effect is usually present in protonated amines. A comprehensive investigation of the substituent group effects on distant protons was given by Zurcher [17].

1.3 ^{1}H AND ^{13}C NMR-CHEMICAL SHIFTS OF ORGANIC COMPOUNDS

^{1}H and ^{13}C chemical shifts can be used for the structural elucidation and conformation of most organic compounds. Factors affecting some common organic groups (alkanes, alkenes, aldehydes etc.) will be discussed here. Some of these factors were also employed in the assignment of ^{1}H and ^{13}C chemical shifts of alkaloids used in this study.

1.3.1 ^{1}H CHEMICAL SHIFTS

1.3.1.1 ^{1}H Chemical shifts of alkanes and cyclo-alkanes

The substituents in organic compounds have a major influence on the chemical shifts produced relative to the original compound [**18**]. The chemical shift signals of substituted alkanes are distributed over a wide range. The chemical shifts as well as the electronegativities of some of the substituents are given in Table 1.1. A relationship is observed between the electronegativities of the substituents and the chemical shifts [**19**].

Table 1.1: ^{1}H chemical shifts of methyl protons for different substituents X

X	$\delta(X\text{-}CH_3)$	$E_x^{(a)}$
H	0.4	2.1
CH_3	0.8	2.5
NH_2	2.36	3.0
OH	3.38	3.5

(a): Ex is electronegativities according to Pauling [20].
Table taken from Horst Friebolin, Basic one and two-dimensional NMR spectroscopy,VCH Publishers, New York,(1993), 52.

Values of chemical shifts for -C-CH_3, -O-CH_3 and -N-CH_3 chemical shifts increase with the decrease in shielding.

In cycloalkanes the chemical shifts of the protons depend on:

(i) the size of the ring;

(ii) the conformation mobility; and

(iii)steric effects [**21**].

Table 1.2 shows chemical shifts of some well-known cyclo-alkanes.

Table 1.2: ^{1}H chemical shifts of cycloalkanes

Compound	δ(ppm)
cyclopropane	0.22
cyclobutane	1.94
cyclopentane	1.51
cyclohexane	1.44
cycloheptane	1.54
cyclooctane	1.54

Table taken from Horst Friebolin, Basic one and two-dimensional NMR spectroscopy,VCH Publishers, New York,(1993), 53.

Cyclopropane shows high shielding of protons with a value of δ = 0.22ppm. This is accounted for by the diamagnetic anisotropy of the cyclopropane ring. From cycloheptane to other larger rings the values are not very different, varying by tenths of a ppm [22].

1.3.1.2 ^{1}H Chemical shifts of alkenes and aldehydes Inductive and mesomeric effects are the major contributors of the substituent effects in alkenes [22].

The aldehydic protons RCHO can be recognized at δ between ~ 9 to 11ppm. A triplet at δ ~ 9.8ppm is observed for propionaldehyde. The signal for crotonaldehyde is at δ ~ 9.48ppm and that of benzaldehyde is found at δ ~ 10.0ppm [23].

1.3.1.3 ^{1}H Chemical shifts of OH, SH, NH groups

The hydrogen atoms of these groups can form hydrogen bonds, they can undergo exchange, and they have varying degrees of acidic character. Their chemical shifts are further influenced by concentration, temperature, solvent, and by impurities such as water [21].

1.3.2 ^{13}C CHEMICAL SHIFT

1.3.2.1 ^{13}C Chemical shifts of Alkanes and cylcloakanes

In alkanes, the chemical shift of a particular ^{13}C nucleus depends on the number of neighboring carbon atoms at the alpha (α) and beta (β) positions and on the degree of branching.

Chemical shift values of a few commonly used cyclo alkanes are presented in Table 1.3. The ^{13}C shielding in cyclo propane is similar to that found in ^{1}H-NMR spectroscopy. Substituent effects have been studied in particular detail for the cyclo hexane system, especially alkyl substitution [**24**].

Table 1.3: ^{13}C chemical shifts (δ-ppm) of cycloalkanes

Compound	δ
cyclopropane	-2.8
cyclobutane	22.4
cyclopentane	25.8
cyclohexane	27.0
cycloheptane	28.7

Table taken from Horst Friebolin, Basic one and two-dimensional NMR spectroscopy,VCH Publishers, New York,(1993), 60.

1.3.2.2 ^{13}C Chemical shift of Alkenes

^{13}C chemical shift values of alkenes fall in the range $\delta \sim 100$ to 150ppm. In alkyl-substituted ethylenes, the olefinic ^{13}C nuclei carrying the substituent are less deshielded ($\delta \sim 120$ to 140ppm) than those at the terminal position ($\delta \sim 105$ to 120ppm) [**25**].
Some δ values are given in Table 1.4.

Table 1.4: ^{13}C chemical shifts(δ-ppm) of alkenes

Compound	$\delta(C^1)$	$\delta(C^2)$	$\delta(C^3)$
$H_2C^1 = C^2H_2$	123.5		
$H_3C^3C^1 = C^2H_2$	133.4	115.9	19.9
$(H_3C)_2C = CH_2$	141.8	111.3	24.2

Table taken from Horst Friebolin, Basic one and two-dimensional NMR spectroscopy,VCH Publishers, New York,(1993),p 61.

1.3.2.3 ^{13}C Chemical shifts of aldehydes and ketones

The shielding values of the ^{13}C nuclei in the carbonyl groups of aldehydes and ketones are among the largest found, being in the range 190 to 220ppm. The shielding is increased where there is conjugation with an unsaturated moiety such as a vinyl or phenyl group [**26**].

1.3.2.4 ^{13}C Chemical shift effects of carboxylic acid derivatives

The shielding of carboxylic group ^{13}C nuclei in mono-carboxylic acids are greater than those for the carbonyl group in ketones and aldehydes. These resonances are found in the region δ ~ 160 to 180ppm [**27**].

1.4 COUPLING CONSTANTS

NMR chemical shifts give information about the numbers and types of electronic environment present in a molecule. However the analysis of coupling between nuclei is also important in providing structural information. These interactions give rise to another element in NMR spectroscopy known as scalar coupling constants. The methods used to determine scalar coupling constant, are discussed elsewhere. H,H and C,H coupling constants have been extensively investigated by spectroscopists.

1.4.1 H, H Coupling Constants

H,H coupling constants comprise two categories viz. geminal and vicinal coupling constants.

1.4.1.1 Geminal couplings ^2J (H, H)

This type of coupling is observed between the protons of CH_2 groups, which are chemically unequivalent. The unequivalency happens when the CH_2 group forms part of a rigid molecular structure, or the two protons are diastereotopic. Geminal coupling constants are usually negative, that is $^2J(H, H) < 0$. Geminal H,H couplings are much dependent on the bond angle between the coupled nuclei, and they are greatly affected by the neighboring π electrons [**4**].

1.4.1.1(a) Dependence on Bond Angle

The greater the bond angle between the coupled nuclei, the more positive is ^2J (H, H). The coupling constant of -4.5 Hz for cyclopropane lies between the values for methane and ethylene [**21**].

A positive contribution to the geminal coupling is introduced by saturated compounds and electronegative substitution at the α-position. In three-membered rings ^2J (H, H) becomes more positive with increasing electronegativity of the substituent. The geminal coupling constants of cycloalkanes vary from -10 to -15Hz, except in cyclopropane where a value of -4.5 Hz is observed. A coupling constant of +41 Hz is observed for formaldehyde. The high value in the formaldehyde case is due to the

contribution of hybridization, the electronegativity of the oxygen substituent, the proximity to a π-bond, and the electron lone pair on the oxygen atom [**28**].

1.4.1.1 (b) Effect of the neighboring π-electrons

A negative contribution to the geminal coupling is also caused by neighboring π electrons. A comparison of methane, cyclopenten-1, 4-dione, and toluene shows that a geminal coupling constant of -14.4 Hz for methyl is observed in toluene and the value is 2Hz more negative than that found in methane [**9**].

1.4.1.2 Vicinal couplings ^3J(H, H)

The magnitude of proton-proton coupling is influenced by the following factors [**29**].

(i) the torsion or dihedral angle;
(ii) the substituents; and
(iii) the H-C-C bond angle.

1.4.1.2(a) Dependence on the Dihedral Angle, Φ

Karplus [**30**] derived an empirical equation that described the dihedral angular dependence of the vicinal coupling constant. The curve in Fig.1.3 corresponds to the Karplus curve. The coupling constants are largest for Φ = 0° or 180°, and smallest for Φ = 90°. The most important use of the Karplus relationship is in determining the conformations and configurations of ethane derivatives and saturated six-member rings [**31**].

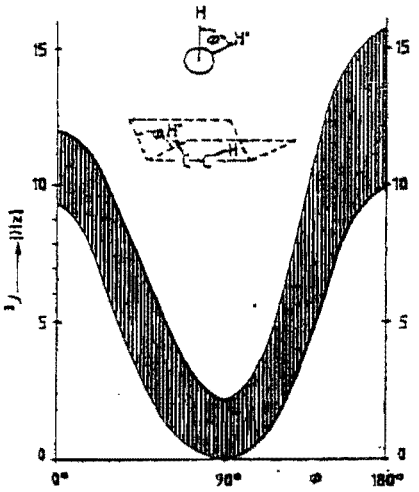


Figure 1.3:Range of observed vicinal coupling constants for different values of the dihedral angle (Dihedral angle).

1.4.1.2(b) H, H Coupling in Aromatic Compounds

The ortho, meta, and para couplings in benzene derivatives are different. First order methods have been used to analyze the spectrum at high magnetic field strength. The differences between ortho couplings may be due to different bond lengths. In heteroaromatic compounds, the coupling constants depend on the electronegativity of the heteroatom, the bond length, and the charge distribution in the molecule. In pyridine and its derivatives, the ortho coupling is as in benzene, considerably larger than the meta and para couplings. In five membered heterocycles, such as furan, thiophene and pyrrole, there are smaller differences between the ortho, meta and para couplings [32].

1.4.2 C, H Coupling constants

1.4.2.1 C,H couplings through one bond ^{1}J

Substituent effects have considerable influence on C, H coupling constants. The changes in ^{1}J (C, H) with high polar substituents are due to their inductive effects [**33**].

1.4.2.2 C,H coupling

Organic molecules are also affected by geminal and vicinal couplings. Long range couplings are also important especially in aromatic rings as well as in saturated organic molecules.

1.4.2.2(a) Geminal couplings

The structural element H-C-^{13}C can undergo many different possible variations in making up a molecule such as substitution at one or both carbon atoms, incorporation into carbon chains or rings, single or double bonding between the carbon atoms, etc. The coupling constant is affected by all these variations and the couplings are known as geminal [**34**].

1.4.2.2 (b) Vicinal couplings

These couplings occur between vicinal protons and they are very important for elucidating the stereochemistry of most organic molecules. Theoretical studies on propane lead to the observation of a relationship between the vicinal coupling constants and the dihedral angle.

1.4.2.2(c) Long range couplings

These couplings are often observed in aromatic systems and unsaturated compounds [**35**]. In benzene there are four different C, H couplings: ^{1}J (C, H), ^{2}J(C, H), ^{3}J(C, H),

^4J(C, H). Coupling constants for benzene, toluene, chlorobenzene and fluorobenzene are listed in Table 1.5.

Table1.5: C,H couplings for benzene, toluene, chlorobenzene and fluorobenzene

X	$^1J(C^1,H^1)$	$^2J(C^1,H^2)$	$^3J(C^1,H^3)$	$^4J(C^1,H^4)$
H	158	+1.1	+7.6	-1.3
CH_3		+0.5	+7.6	-1.4
Cl		-3.4	+10.9	-1.8
F		-4.9	+11.0	-1.7

Table taken from Horst Friebolin, Basic one and two-dimensional NMR spectroscopy,VCH Publishers, New York,(1993), 98.

1.5 COMPUTATIONAL METHODS USED IN THE CALCULATION OF CHEMICAL SHIFTS

The prediction of ^{13}C NMR chemical shifts has been performed using incremental estimations based on empirical calculations for decades. The problem of accuracy and amount of time required for this process was overcome by the development of computers with high processing power. These empirical calculations were then coupled with other methods such as *ab-initio,* and semiempirical methods for optimal calculation of NMR parameters.

1.5.1 Empirical calculations

1.5.1.1 Introduction

Empirical calculations are used to make approximate calculations of chemical shifts if the spectra are known. In calculating ^{1}H chemical shifts approximations have been made [**36-38**] with limited success due to the complexity of the chemical shifts and the presence of through space interactions in chemical compounds.

Empirical estimates of ^{13}C chemical shift have been successful due to the larger range of chemical shift of this nucleus. The availability of a large amount of reliable ^{13}C chemical shift data has made the derivation of empirical equations possible [**39-40**].

Chemical shifts for the elucidation of structure can be summarized in terms of empirical relationships. Unstrained alkanes were discussed by Grant and Paul [**41**]. Their equation:

$$\delta_i = -2.3 + 9.1n_\alpha + 9.4n_\beta - 2.5n_\lambda + 0.1n_\varepsilon + \sum S_{ij}$$

equation 1.6

Where,

δ_i= chemical shift of carbon nucleus of interest,

n=numbers of carbon atoms,

ε= position relative to this nucleus,

S_{ij}=steric correction terms taking account of branching.

Activity schemes of a simpler nature are generally used for substituted benzenes. The empirical additions to the effect of chemical shift for substituents at various positions, taken from the compilation of Wehrli and Wirthilin [**42**], are presented in Table 1.6.

Table 1.6: Empirical substituent effects (ppm) on ^{13}C chemical shifts for benzene derivatives.

δ OF SUBSTITUENT	IPSO	ORTHO	META	PARA
CH_3	+9.3	+0.8	~0	-2.9
CO_2H	+2.1	+1.5	~0	+5.1
CHO	+8.6	+1.3	+0.6	+5.5
CN	-15.4	3.6	+0.6	+3.9
OH	+26.7	-12.7	+1.4	-7.3
NH_2	+18.0	-13.3	+0.9	-9.8
NO_2	+20.0	14.8	+0.9	+5.8

Table taken from Carbon-13 NMR Spectroscopy, New York, Wiley, 1988, H.O Kalinowski, S.Burger and S.Brauni.

1.5.2 Semi-empirical methods

1.5.2.1 Introduction

Semi-empirical methods were used before *ab-initio* methods were known. Semi-empirical techniques are based on the initial calculation of geometry, initial calculation for energy and initial calculation for thermochemistry. Approximate molecular orbital theories are by nature semi-empirical, in that molecular properties are no longer derived directly from the principle of quantum mechanics, but rather from experimental data [**43**].

Several semi-empirical methods exist namely:

(i) CNDO (Complete Neglect of Differential Overlap) was introduced by Pople et.al [**44**]. This method has been frequently used in independent electronic calculations.

(ii) INDO (Intermediate Neglect of Differential Overlap), was also developed by Pople and co-workers [**45**], and has been used to study many molecular properties, particularly the spin densities of radicals [**46**].

(iii) NDDO (Neglect Diatomic Differential Overlap), was another method developed by Pople and his co-workers [**47**]. An extra feature at this level of approximation is the retention of dipole-dipole interactions.

(iv) MNDO (Modified Neglect of Diatomic Differential Overlap) was introduced by Dewar and Thield [**48**] for applications in organic research.

(v) MINDO, is a modified INDO, which was introduced by Baird and Dewar and modified by Dewar and Hasselbach [**49**]. This method is applicable over a wide range of compounds.

The advantage of semi-empirical calculations is that they are able to deal with large molecular systems at reasonable cost. The disadvantage of semi-empirical calculations is that they are inaccurate compared to *ab-initio* calculations.

1.5.3 *Ab-initio* methods

1.5.3.1 Introduction

The term *ab-initio* is Latin, meaning from the beginning. The *ab-initio* calculation implies a non-parameterized molecular orbital treatment derived from first principles. The term is employed for computations, which are derived directly from theoretical principles, with no inclusion of experimental data. Mathematical approximations such as using a simpler functional form or getting an approximate solution to a differential equation are usually made [**50**].

The Hartree Fock method [**51**] is the most common type of *ab-initio* calculation. The primary approximation used in the Hatree Fock method is called the central field of approximation. The functions used mostly are linear combinations of Slater type orbitals or Gaussian type orbitals abbreviated STO and GTO.

Ab-initio calculations also make use of the Born-Oppenheimer approximation [**52**] that the nucleus remains fixed on the time scale of electron movement, meaning that the electronic wave function is unaffected by nuclear motion.

The first stage of all *ab-initio* calculations to be considered is the single-determinant Local Atomic Orbital Self Consistent Field (LCAO-SCF) calculation. Almost all ab-*initio* calculations employ GAUSSIAN type orbital (GTO) basis set, in which each atomic orbital is made up from GAUSSIAN probability [**53**].

The advantage of a*b-initio* calculations is that they eventually converge to the exact solution. However this convergence is not monotonic. Sometimes, the smallest calculation gives the best results for a given property.

The worst disadvantage of *ab-initio* methods is that they are expensive. The *ab-initio* methods often take much computer central processing unit (cpu) time, memory and disk space. In practice, extremely accurate solutions are obtainable only when the molecule contains half a dozen atoms or less [**54**].

1.6 INTRODUCTION TO TWO DIMENSIONAL NMR

The basic experiment leading to a 2D-exchange spectrum can be considered as a modulation transfer experiment with the initial magnetization frequency modulated by the resonance frequency of each site [**55**]. Owing to the frequency labeling of the magnetization components, it is possible to follow them through the complex exchange process. The frequency modulated z magnetization is created as shown in Fig 1.4, by a $\pi/2$ pulse; transverse magnetization is generated for all involved nuclei. After precession for a time t_1, the x components, modulated by the angles are rotated back along the z-axis by a second $\pi/2$ pulse. The amplitude modulated z magnetization from the initial condition for the exchange-process, takes place during the following exchange period of length t. At the end of the exchange period, the z components are converted into observable transverse magnetization and the resulting free induction decay is recorded as a function of t_2 [**56**].

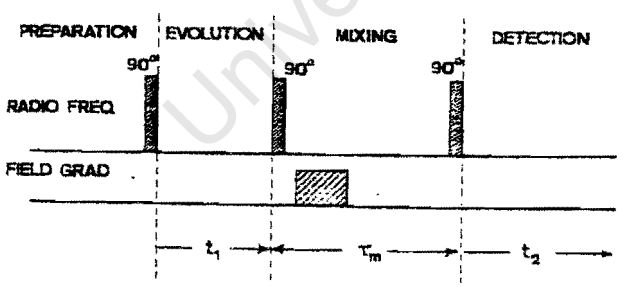


Figure 1.4: Experimental scheme of 2D-exchange spectroscopy.

1.6.1 Correlation Spectroscopy (COSY)

Correlated spectroscopy originated from a proposition by Jeener in 1971 [**57**]. This technique was developed further by Barthold and Ernst [**58**]. COSY was the first two-dimensional experiment to be proposed. It is widely used for the elucidation of structures of simple chemical compounds as well as proteins and nucleic acids [**59**]. The pulse sequence for the basic COSY experiment (Fig 1.5) requires the application of two 90° proton pulses separated by a systematically evolution period t_1, and an acquisition period t_2 [**60**].

The pulse sequence for the COSY experiment is perceived to be simple, but several density matrix treatments [**61**] are employed in this technique. Biopolymers [**62**] have been studied using the COSY pulse sequence due to its ability to allow all connectivity pathways to be exploited without regard to overlap.

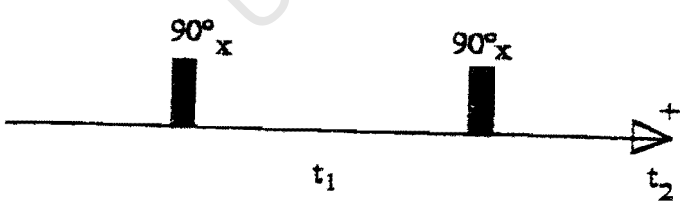


Figure 1.5: Pulse sequence for COSY.

1.6.2 Double quantum filtered COSY (DQF-COSY)

There is some sensitivity loss in the DQF-COSY experiment relative to a conventional COSY experiment [**63**], and this is compensated for by improvement in the dynamic range of the experiment provided by the suppression of the singlets and undesired

diagonal elements. Double quantum filtered COSY has a wide range of applications, and spectra of sesquiterpenes [**64**], peptides, oligomers of DNA and oligosaccharides, and polynuclear aromatics [**65**] have been assigned using this technique. DaBrowski and co-workers [**66**] and Rance and Wright [**67**] have successfully exploited this method. Double quantum filter (DFQ-COSY) involves a third 90° pulse and can be illustrated by the following pulse sequence:

$$\phi(\pi/2)_{\phi}\text{-}t_1\text{-}(\pi/2)_{\phi}\text{-}\Delta\text{-}(\pi/2)_{\chi}\text{-}t_2\rightarrow(\text{Acquire})$$

A schematic representation of DFQ-COSY is in Fig 1.6 [**68**].

Figure 1.6: Pulse sequence for Double Quantum Fitered COSY.

1.6.3 Heteronuclear Multiple Quantum Correlation (HMQC) and Heteronuclear Single Quantum Correlation (HSQC).

The transverse proton magnetization in HMQC is excited by the first pulse and is present during the whole sequence. In the HSQC technique, heteronuclear decoupling can be applied during t_2 (Fig 1.7) [**69**] to gain sensitivity due to the reduction of the multiplet structure from a doublet to a singlet. A 2DJ spectrum would result without the heteronuclear pulses [**70**].

HSQC is derived from HMQC by rotating the transverse proton magnetization to the longitudinal plane at the beginning of t_1(Fig 1.8) [**71**]. There are no homonuclear couplings of the active spins H to the passive spins Hj(J_{HH}), because the proton magnetization is longitudinal during t_1 [**72**].

HSQC is more sensitive than HMQC, because the fast decay due to proton transfer relaxation during t_1 is absent [**73**]. Extraction of traces from HSQC spectra at the carbon frequency allows the collection of proton multiplets with higher resolution, making this method clearly superior for the extraction of coupling constants. In contrast, in the HMQC technique multiplets are complicated both by the presence of homonuclear couplings in the indirect dimension and by impure phases.

HSQC has sensitivity and resolution advantages over HMQC.
However, the sensitivity advantages of HSQC over HMQC, are variable, depending upon ^{1}H multiplet width and the extent of linear prediction.

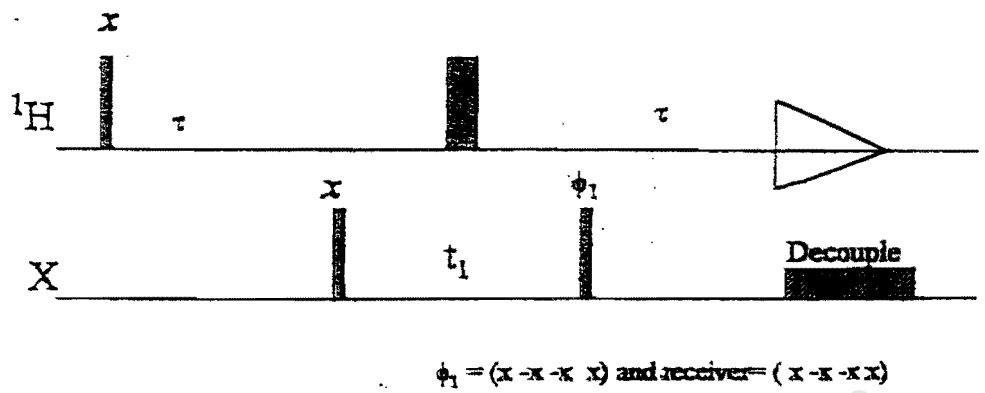


Figure 1.7: Conventional HMQC experiment

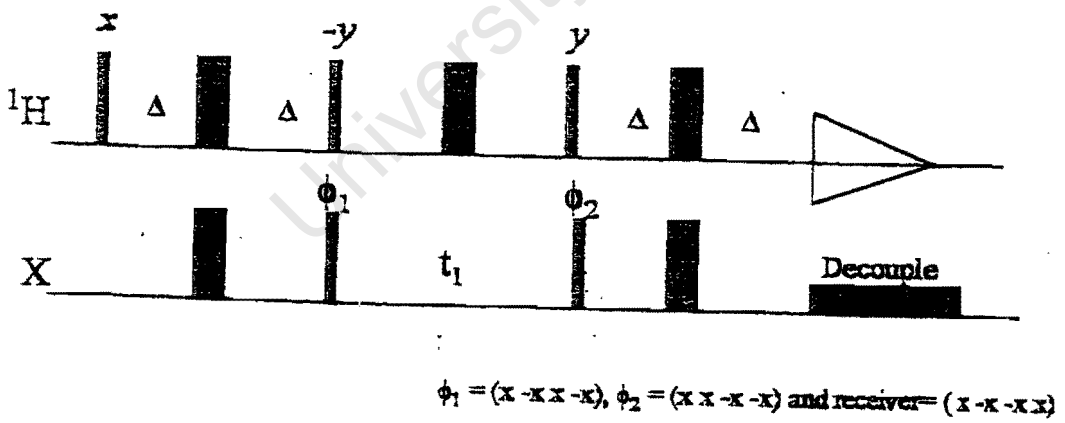


Figure 1.8: Conventional HSQC experiment

1.6.4 Homonuclear Multiple Bond Correlation (HMBC)

Heteronuclear long range coupling constants are important parameters for the structural elucidation and conformational analysis of organic compounds. Homonuclear couplings alone are not sufficient for unequivocal estimation of segmental conformations. This is only possible in combination with complementary information from vicinal and long range H,X coupling constants; where X= (C13, N15 or P31) [**74**].

The HMBC experiment is used to measure long range H,X coupling constants by stepwise incrementation of the coupling evolution time in a constant time manner. HMBC long range correlations are accessible, even those to unprotonated heteronuclei. The HMBC experiment was successfully used by Bermel et.al. [**74**], who compared the total multiplet width of an HMBC signal, including the long range couplings with that from a COSY type experiment without the long range coupling [**75**].

Long-range ^{1}H-^{13}C connectivity provides a wealth of structural and assignment information, but the 2D Correlation Spectroscopy via Long Range Couplings (COLOC) [**76**] experiment proposed for this purpose suffers from low sensitivity.

The disadvantage of the HMBC experiment in the phase-cycled version is intense t1 noise, but magnetogyric field gradients are used to select coherent pathways. HMBC is in principle the most suitable experiment to obtain information on heteronuclear long range couplings, because all correlations are accessible, even those to unprotonated nuclei.

1.6.5 Total Correlation Spectroscopy (TOCSY)

The TOCSY experiment allows identification of cross peaks between spins that are not directly coupled but share a coupling partner. An initial 90° pulse, which is phase cycled to suppress axial responses and quadrature phase images, is followed by an evolution time, t1, incremented in the usual fashion to digitize the second frequency domain [77]. TOCSY differs from COSY in that the second 90° pulse of the COSY sequence is replaced by a mixing interval during which isotropic mixing and coherence transfer take place.

The isotropic mixing sequence, based on the work of Braunschweiler and Ernst [**78**], has the sequences:

(i) a repetitive sequence of 180° pulse with constant phase x;

(ii) a repetitive sequence of the phase altered 180° pulse which compensates for radio frequency inhomogeneity; and

(iii) a cycle consisting of the minimum number of pulses for isotropic mixing comprising eight 90° pulses distributed equally among the four possible phases [**79**] are shown in Fig 1.9.

The representation of isotropic mixing process employed in TOCSY, which employ transverse magnetisation [MLEV] [**80**] and that which operates with z magnetisation [DIPSI-z]. The introduction of delays in MLEV-17 pulse sequence allows for the suppression of contributions from cross- relaxation.

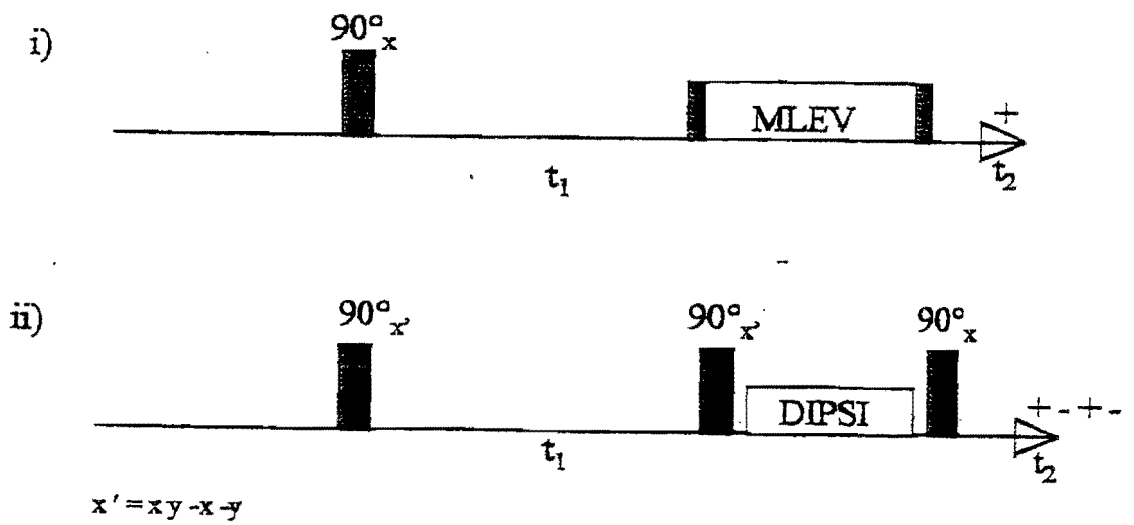


Figure 1.9: TOCSY pulse sequences with isotropic mixing schemes(i) MLEV and (ii) DIPSI-2

REFERENCES

1. E.M Purcell, H. C. Torrey, R. V. Pound, Phys. Rev., **69**, (1964), 37.
2. F. Bloch, W.W. Hansen, M. E. Packard, Phys. Rev., **69**, (1946), 127.
3. W.G. Procter and F.C Yu, Phys. Rev., **77**, (1950), 717.
4. Robin K. Harris: Nuclear Magnetic Resonance. Spectroscopy, A physicochemical view, Pitman Publishing Inc. London, (1983).
5. W. Dickinson, Physic. Rev., **77**, (1950), 736.
6. J.B. Stothers: Carbon-13 NMR spectroscopy, Academic Press Inc. New York, (1982).
7. T. Alecenrod, G. A. Webb: Nuclear magnetic resonance Spectroscopy of nuclei other than proton, Wiley Interscience Publication New York, (1974).
8. R. R. Ernst, Chimica Acta, **29**, (1975), 179.
9. P. J. Hore: Nuclear Magnetic resonance, Oxford University Press London, (1995), 9.
10. Edwin D. Becker: High Resolution NMR theory and Chemical applications, 2nd edition, Academic press. Inc. New York, (1980), 54.
11. Darrell D. Ebbing and Mark S. Wrighton: General Chemistry, 4th edition, Houghton Mifflin Company Boston, (1993), 354.
12. George C.Levy: Topics in carbon 13 spectroscopy, J. Wiley and Sons New York, **2** (1976).
13. K.A.K. Ebraheem, G. A. Webb and M. Witenowski, Org. Magn. Reson., **8**, (1976), 317.
14. G.N. La Mar, W. D. Horrock, and R. H. Holin: NMR of paramagnetic molecules, Academic Press New York, (1973).
15. C. W. Haigh R. B. Mallion: Ring Current Theories in NMR, Prog. Nucl. Magn. Res. Spectrosc., **13**, (1980), 303.
16. S. E. Holkev and T.J. Simpson, J. Chem. Soc. Perkin Trans. I, (1981), 1397.
17. R. F.Zurcher, Prog. Nucl. Magn. Reson. Spectrosc., **2**, (1976), 205.
18. D. Daneels, M. A Anteunis, Org. Magn. Reson., **11**, (1974), 617.
19. L. Pretsch, W. Simon, Helv. Chim. Acta., **52**, (1969), 2133.
20. L. Pauling: The nature of chemical bonding and structure of molecules and crystals 3rd ed., Cornell University Press New York, (1960).

21. Horst Friebolin: Basic one and two-dimensional NMR spectroscopy, VCH Publishers New York, (1993).
22. A. A Bothner-By, Discussions Faraday Soc., **3**, (1962), 66.
23. Y. Yong, T. Haino, S. Usui, Y. Fukuzawa, Tetrahedron, **32**, (1996), 2325.
24. H. O. Kalinowski, S. Berger, S. Brauni: Carbon-13 NMR spectroscopy, J.Wiley and Sons New York, (1988).
25. R. A Friedel, H.L Retcovsky, J. Am. Chem. Soc, **85**, (1963), 1300.
26. L. M Jackman, D.P. Kelly, J. Chem. Soc. B, (1970), 102
27. R. D. Roberts, J. Am. Chem. Soc, **91,** (1969), 4504.
28. H. Gunther, Encyclopaedia of NMR, **8,** (1996), 4923.
29. M. Karplus, J. Chem. Phys., **30**, (1959), 11.
30. M. Karplus, J. Amer. Chem. Soc., **85**, (1963), 2870.
31. K. Takahash, T. Sone and K. Fujieda, J. Phys. Chem., **74** (1970), 2765.
32. E. Breitmaier and W. Voelter: ^{13}C-NMR-Spectroscopy. 3rd edition, Weiheim: VCH Verlagsgesellschaft New York, (1989).
33. R. Wasylishen and T. Scafer, Can. J. Chem., **50,** (1972), 2710.
34. L. B. Krivdin, E. W. Della, Progress in NMR Spectroscopy, **23,** (1991), 301.
35. J. N. Shoolery, Technichal Information Bulletin, Varian Associates California, (1959).
36. L. M. Jackman and S. Sternhell: Applications of NMR Spectroscopy in Organic Chemistry, 2nd edition, Pergamon Press Oxford, (1969).
37. H. Primas, R. Arnodt, and R. Ernst: Advances in Molecular Spectroscopy, Pergamon Press Oxford, (1962), 1246.
38. D. M. Gramt and E.G Paul, J.Am. Chem. Soc., **86,** (1964), 2984.
39. L. P. Lindaman and J. Q. Adams, J. Anal. Chem., **43,** (1971), 1245.
40. G. C. Levy and G. L. Nelson: ^{13}CNMR for Organic Chemists, Wiley Interscience New York, (1972).
41. D. M. Grant and E.G. Paul, J. Amer. Chem. Soc., **86,** (1964), 4532.
42. F. W. Wherli and T. Wirthlin: Interpretation of C-13 NMR Spectra, London: Heyden, (1976), 1071.
43. J. A. Pople and David L. Beveridge: Approximate Molecular Orbital Theory, Macgraw Hill Inc. New York, (1970), 57.
44. J. A. Pople, D. P. Santry and G. A Segal, J. Chem. Phys., **43,** (1965), S136.
45. J. A. Pople, D. L. Beveridge, P. A Dobosch, J. Chem. Phys., **47,** (1967), 2026.

46. R. N. Dixon, Mol. Phys., **12,** (1967), 83.
47. J. A Pople, D. P. Santry, G. A Segal, J. Chem. Phys., **43** (1965), S139.
48. J. A. Pople, N.C. Baird and M. J. Dewar, J. Chem. Phys, **50,** (1969), 1262.
49. M. J. S. Dewar and E. Hasselbach, J. Amer. Chem. Soc., **92,** (1970), 590.
50. D. B. Chesnut, G. A. Webb: Annual Reports on NMR Spectroscopy, Academic Press, London, **29**, (1994), 71.
51. W. N. Lipcomb, J. S. Waugh: Advances in Magnetic Resonance, Academic Press New York, **62**, (1966), 137.
52. E. Cliffords Dykstra: Ab initio calculation of the structures and properties of molecules, Elsevier New York, **58,** (1988).
53. W. J. Hehre, L. Radom, P. V. R. Schafer, and J. A. Pople: Ab Initio Molecular Orbital Theory, Wiley Interscience Publications New York, (1986).
54. Tim Clarke: A handbook of computational chemistry, John Wilely and Sons New York, (1985), 7.
55. A. Bax and R. Freeman, J. Am. Chem. Soc., **104,** (1982) 1099.
56. T. Jippo, O. Kawo and K. Nageyan, J. Magn. Reson., **66,**(1986), 344.
57. Jeener, J. Ampere International Summer School, Busko Polje, Yugoslavia, (1971).
58. W. P. Anne, E. Bartholdi and R.R Ernst, J. Chem. Phys., **64,** (1976), 2226
59. K. Nagayama, Adv. Biophys., **14,** (1981), 139.
60. A. Bax, R. Freeman and G. A. Morris, J. Magn. Reson., **42,** (1981), 164.
61. R. R. Ernst, G. Bodenhausen and A. Wokaun: Principles of Nuclear Magnetic Resonance in One and Two Dimension, Claredon Press Oxford, (1987).
62. K. Wurthrich: NMR of Proteins and Nucleic acids, Wiley New York, (1986).
63. O. W. Sorensen, G. Eich, M.H. Levitt, G. Bodenhausen and R.R. Ernst, Prog. Nucl. Magn. Reson. Spectrsc., **16,** (1983), 163.
64. M. Pais, C. Fontaine, L. Dominique, S. La Barre and E. Guittet, Tetrahedron Lett.,(1987), 1409.
65. D.S. Williamson, P. Cromonesi, E. Cavalier, D. L. Nagel, R. S. Markin and S.M. Cohen, J. Org. Chem., **51,** (1986), 5210.
66. J. Dabrowski, A. Ejchart, M. Kordowwicz and P. Hafland, J. Magn. Reson., **25,** (1987), 338.
67. M. Rance and P. E. Wright, J. Magn. Reson., **66,** (1986), 372.
68. A. Bax, R. J. Griffey, B. L. Hawkins, J. Magn. Reson., **55,** (1983), 301.
69. W. P. Aue, J. Karhan, R. R. Ernst, J. Chem. Phys., **64,** (1976), 4226.

70. D. Bruhwiler, G. J. Wagner, J. Magn. Reson., **69,** (1986), 564.

71. A. Bax, M. Ikura, Li Kay, D. A. Torchia, R. J. Tschudin, J. Magn. Reson., **86,** (1990), 304.

72. A. Bax, M. F. Summers, J. Amer. Soc., **108,** (1986), 2093.

73. W. Bermel, C. Griesmiger, H. Kessler, K. Wagner, Magn. Reson. Chem, **25,** (1987), 325.

74. D. G. Davis, J. Magn. Reson., **90,** (1990), 584.

75. T.J. Nowood, H. E. Heritage, N. Soffe, I.D. Campbell, J. Magn. Reson., **87,** (1990), 488.

76. A. Bax and D. G. Davies, J. Magn. Reson., **65,** (1985), 355.

77. L. Braunsweiler and R.R. Ernst, Mol.Phys., **38,**(1979), 963.

78. M. Rance, G. Wagner, O. W. Sorensen, K. Wuthrich and R. R. Ernst, J. Magn. Reson., **59,** (1984), 250.

79. C. Grissinger, G. Otting, K. Wuthrich, R. R. Ernst, J. Am. Chem. Soc., **110,** (1988), 7870.

80. S. P. Rucker and S. J. Shaka, Mol Phys., **68,** (1989), 509.

CHAPTER 2

EXPERIMENTAL

2.1. ONE DIMENSIONAL ^{1}H NMR EXPERIMETS

2.1.1 One Dimensional Spectra of other chemical compounds

The following compounds were obtained from Smithkline Beechams Pharmaceuticals: phenyl acetate, 2-nitrobenzyl alcohol, ethyl 4-iodobenzoate, ethyl benzoate, 2-phenyl-1,3-dithiane, 4-(3-phenylpropyl)-pyridine, 1-(4-nitrophenyl)-piperazine, 2-phenylbutyric acid, 3,4-methylenedioxy)-benzyl alcohol, benzyl acetate, 2-thiophenecarboxaldehyde, 2,3-pyridine dicarboxylic acid, quinaldic acid, 2,3-benzofuran, 1-methyl indole-3-carboxylic acid and 3-indolylacetonitrile. ~3mg of the above-mentioned compounds was dissolved in adequate a volume of chloroform. A Bruker 400MHz spectrometer was used to obtain ^{1}H NMR spectra of all these compounds. These samples were run at 303.1 °K.

2.1.2 Alkaloids

~ 3mg of *lupinine*, *scopoline*, *eseridine*, *piperine* and *conhydrine* were separately dissolved into sample tubes. The spectra were recorded on a Varian 300MHz spectrometer for all the alkaloids except *scopoline*, which spectra was recorded on a Varian 400MHz spectrometer. The samples were run at 303.1 °K. All data processing and integration was conducted on a Sun data workstation employing VNMR software (Varian Associates). These alkaloids were supplied by Merck.

2.2 TWO DIMENSIONAL (2D) NMR SPECTRA

All the two dimensional spectra obtained for the alkaloids were collected using a Varian Mercury 300 NMR spectrometer at 303.1 °K.

The 2D data for all other compounds used in this study, were obtained using the Bruker AMX 400 NMR spectrometer at 303.1 °K. The details used to acquire 2-D spectra could be found in the spectra presented in the appendix.

2.3. METHODS USED FOR THE PREDICTION OF CHEMICAL SHIFTS

2.3.1 Charge-V7 program

PC model [1] was used to draw structures of all compounds mentioned in section 2.1.1 and 2.1.2. The individual structures were minimized using the GMMX routine [2] and saved in a PC model file (.pcm) and Mopac Input file (.inp). The Mopac input files were opened in Notepad and were copied to the Charge data file (.dat) in Notepad. The CHARGE-V7 data file was then used by the CHARGE-V7 program to predict the corresponding proton chemical shifts.

2.3.2 ACD-V4 program

All compounds mentioned in section 2.1.1 and 2.1.2 were drawn in the ACD-V4 program using ACD/chemsketch. The resultant structures were used by ACD-V4 ^{1}H δ predictor program to generate chemical shift tables. The corresponding chemical shifts were compared with the results from the CHARGE-V7 programs and experimentally obtained results. The proton chemical shifts were also compared with those predicted by the SpecInfo-V3.2 program for all the alkaloids. ACD/^{13}C-NMR was also used to generate corresponding chemical shifts for all the alkaloids, and these were compared with ^{13}C chemical shifts generated by the SpecInfo-V3.2 program.

2.3.3 SpecInfo-V3.2 program

All the alkaloids mentioned in section 2.1.1 and 2.1.2 were drawn using the SpecInfo structure editor. The SpecInfo assignment editor was used to calculate and display

corresponding ^{1}H and ^{13}C chemical shifts for the structures. The ^{1}H chemical shifts obtained from SpecInfo-V3.2 were then compared to those from the Charge program, those predicted by ACD-V4, and those obtained experimentally. The ^{13}C chemical shifts were compared with the chemical shifts predicted by ACD/^{13}C-NMR predictor and with those obtained experimentally.

REFERENCES

1. PC model version 5, Serena Software Ltd., P. O. Box 3076, Bloomington, IN, (1994).
2. Raymond J. Abraham, B. Hudson, J. Comput. Chem., **6**, (1984), 175.

CHAPTER 3

3. NMR-CHEMICAL SHIFTS OF ALKALOIDS

3.1 Assignments of chemical shifts

The alkaloid compounds are used in this experiment due to the complexity of their spectra, therefore providing a very good test of the programs used to predict chemical shifts.

The spectra of alkaloids used in the chemical shift prediction of the CHARGE-V7, ACD-V4 and SpecInfo-V3.2 programs will be assigned in this chapter. The ^{1}H and ^{13}C spectra of the alkaloids were assigned using the COSY, HMQC, TOCSY, and HMBC pulse sequences. The assignment of the spectra of the other compounds studied will be discussed in the next chapter.

3.1.1 Scopoline (Oscine)

3.1.1(i) Background

Scopoline was first examined by Hesse [1] and later by Luboldt [2]. It forms colorless, hygroscopic prismatic crystals from ether or light petroleum. King resolved *dl*-Oscine into *d* and *l* forms by crystallization with *d*-hydrogen tartrates. In 1915 it was already known that it was a tertiary base, and that the second atom was present in an ether-like linkage.

Scopoline is mostly used as a cerebral sedative, especially in the treatment of mania, hysteria, and drug addiction, while in the treatment of insomnia and epilepsy its uses increases the effects of other drugs, such as morphine and bromides [3]. It is also used to allay sexual excitement. In 1900 the use of a combination of morphine and scopolamine was introduced as a means of producing anaesthesia, under the name of 'Twilight Sleep'. Its peculiar effect in large doses is to cause loss of memory [4].

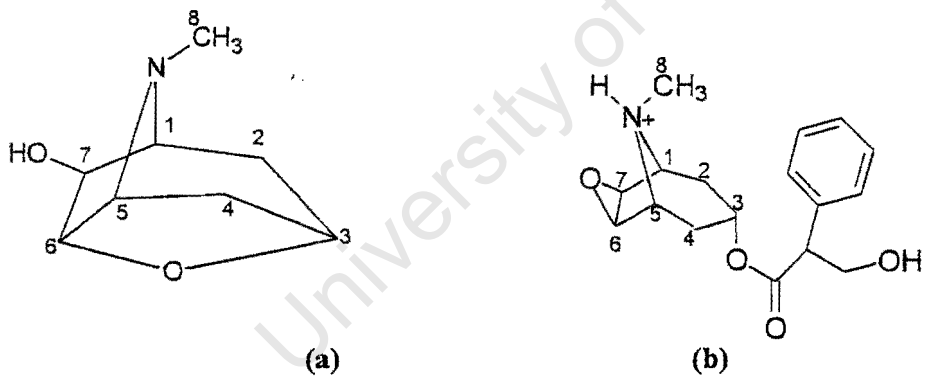


Figure 3.1: Structure of (a) *scopoline*; (b) *scopolamine*.

3.1.1(ii) Spectral chemical shifts (δ)

The experimental ^{1}H and ^{13}C NMR chemical shifts of *scopoline* are presented in Table 3.1. ^{13}C chemical shifts for *scopoline* will be compared with those found in the literature for scopolamine [5], because the *scopoline* literature chemical shifts could not be found.

Table 3.1: ^{1}H and ^{13}C experimental NMR chemical shifts[a] (δ-ppm) for *scopoline.*

At No.	*Experimental*		*Literature (scopolamine)*
	^{13}C	^{1}H	^{13}C
1	64.20	2.94	53.8
2	30.40	1.22; 2.33	25.2
3	74.30	4.22	64.4
4	27.70	1.40; 1.87	25.2
5	61.50	3.65	53.8
6	83.60	4.47	58.1
7	75.40	3.86	58.3
8	33.80	2.56	53.4

[a] *chloroform solvent used.*

3.1.1(iii) Assignment of *scopoline* proton spectrum

The similar chemical environment for H7 and H6 led to difficulty in the assignment of in the ^{1}H spectrum. The peak at 3.86ppm in the proton spectrum presented in Fig 3.2 does not show any coupling with any proton in the COSY spectrum given in Fig 3.3. This peak corresponds to H7, which is 90° to H6 and 60° to H1, compared to H6 which should have a large coupling to H5 due to a torsion angle of 0° observed between the two protons. H6 was therefore assigned to be the most downfield peak at 4.47ppm. Correlation between H6 and H5 is observed in the COSY spectrum. The ^{1}H spectrum shows a triplet at 3.65ppm, which is assigned to H5; H5 then couples to H4. H3 couples to only one of the H4 protons and then couples to the beta H2 proton, forming a triplet in the ^{1}H spectrum. Correlation of H1 with both H2 protons is observed in the COSY spectrum. A small cross peak is observed between H1 and H6 in the COSY spectrum as a result of W-coupling.

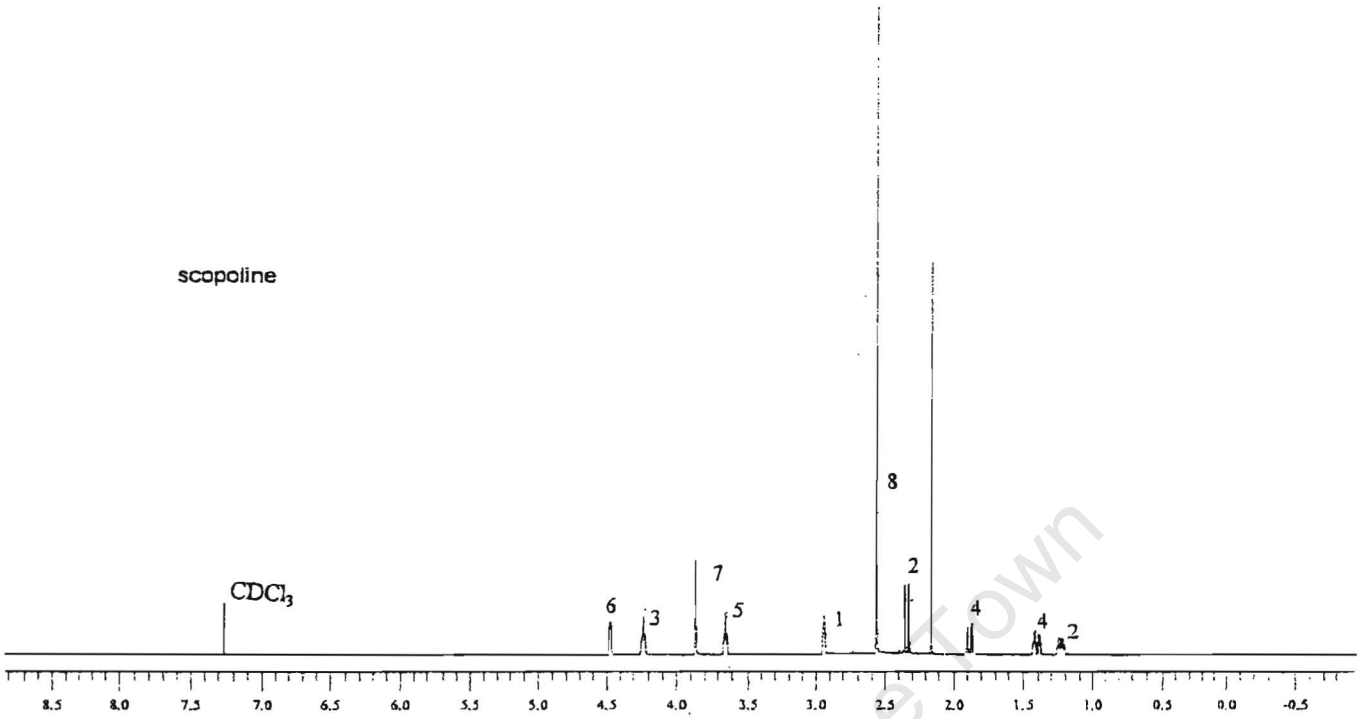


Figure 3.2: Proton spectrum of *scopoline*

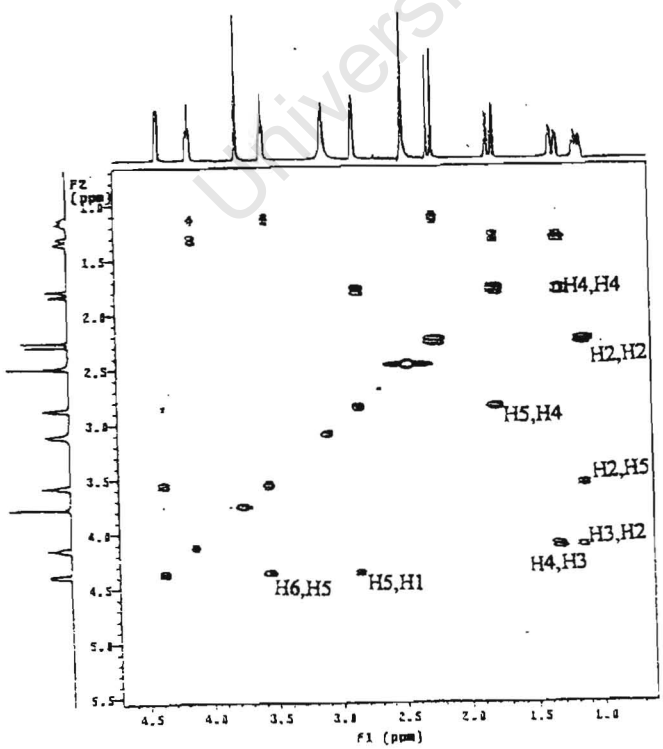


Figure 3.3: COSY spectrum of *scopoline*

3.1.1(iv) Assignments of ^{13}C spectrum

The ^{13}C and HSQC spectra are given in Fig 3.4 and Fig 3.5 respectively. All the ^{13}C assignments followed directly from the ^{1}H assignments using the HSQC spectrum. The experimental ^{13}C chemical shifts for *scopoline* are different from those presented in Table 3.1 for scopolamine, but similar trends in chemical shift values are observed.

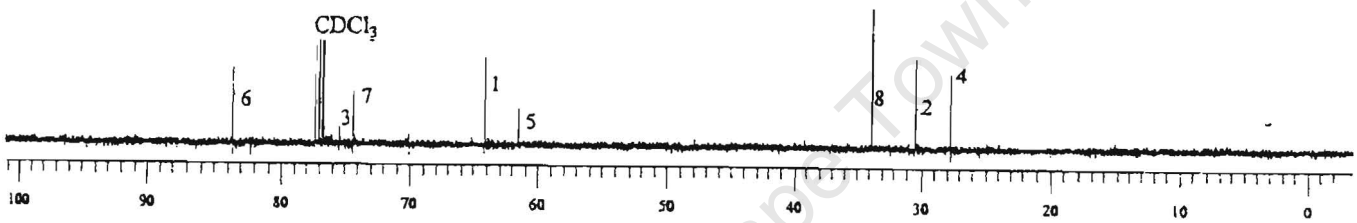


Figure 3.4: Carbon-13 spectrum of *scopoline*

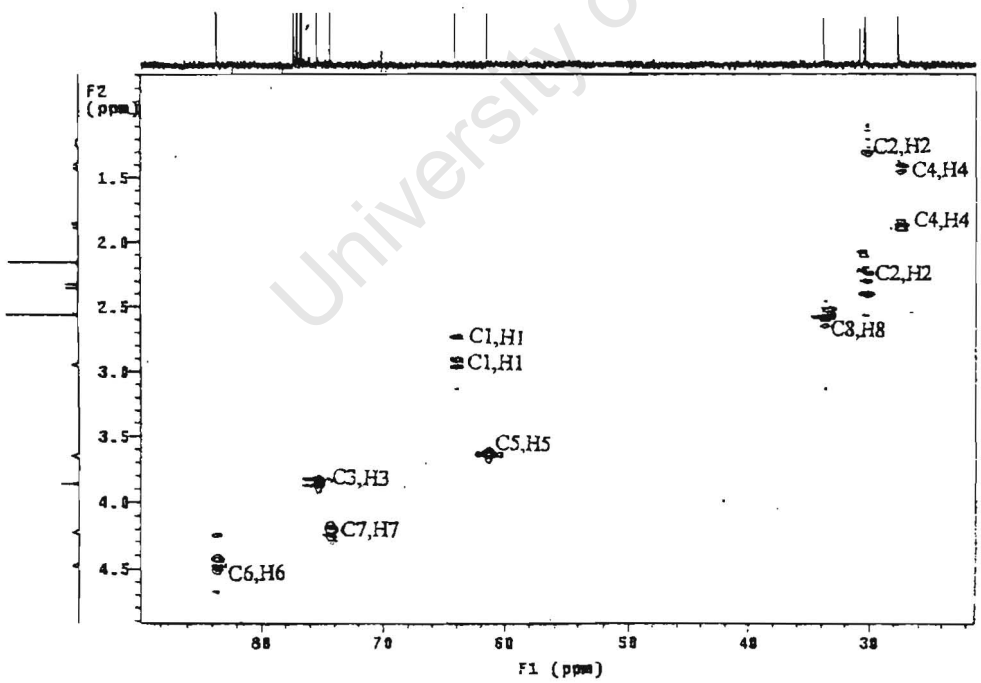


Figure 3.5:HSQC spectrum of *scopoline*

3.1.2 Lupinine (bluebonnet)

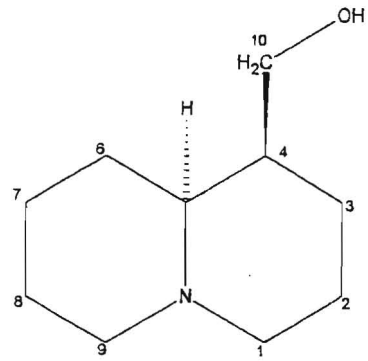


Figure 3.6: Structure of *lupinine* alkaloid

3.1.2(i) Background

Baumert [**6**] was the first person to isolate lupinine, and it was characterised by Schimdt and Berend [7] and other workers. Cassola isolated the lupinine alkaloid from lupin seed [**8**]. A process of isolation of this compound is described by Karre et.al. [**9**], and a method of separating lupinine from other compounds has been developed by Sadykov and Sasokukotski [**9**].

Evidence of the presence of a primary alcohol group in lupinine is provided by the benzoyl derivative (needles, mp.49-50°) the phenylcarbimide addition product, $C_{10}H_{18}N.O.CONHPh$ (prism, m.p.94-5°), the oxidation of the alkaloid to lupininic acid, $C_9H_{16}N.COOH$ (long needles, m.p.255°), and the dehydration to anhydrolupinine. Anhydrolupinine is optically inactive, but a laevorotatory isomer has been obtained by Clemo and Raper [**10**].

Lupinine, a quinolizidine alkaloid, induces nicotinic effects in animals. Leaves, seeds and fruits all contain lupinine, which is retained in dried plants. Pods may concentrate the toxin, becoming a source of poisoning during the winter season when livestock are moved through infested areas or fed contaminated hay. Lupinine poisoning results in depressed heart and nervous systems, and a consequent sensation of numbness, especially in the hands and feet [**11**].

3.1.2(ii) Spectral chemical shifts (δ) of *lupinine*

^{1}H and ^{13}C NMR chemical shifts of *lupinine* are presented in Table 3.2. The experimental ^{13}C NMR chemical shifts were compared with those observed from literature [12].

Table 3.2: ^{1}H and ^{13}C experimental NMR chemical shifts[a] (δ-ppm) for *lupinine*.

At No.	*Experimental*		*Literature*
	^{13}C	^{1}H	^{13}C
1	57.1	1.86; 2.03	57.2
2	22.9	1.51; 1.83	22.6
3	30.8	1.55; 1.83	30.5
4	38.2	1.51	38.8
5	65.1	2.13	65.0
6	29.7	1.52; 1.73	29.6
7	24.6	1.24; 1.75	24.8
8	25.6	1.56	25.6
9	57.1	2.86	57.2
10	65.9	3.68; 4.14	64.7

[a] *chloroform solvent used.*

3.1.2 (iii) Assignment of ^{1}H spectrum

All the protons adjacent to electron withdrawing atoms nitrogen and oxygen, are expected to be deshielded downfield in the ^{1}H spectrum given in Fig 3.7. The C10 methylene protons are not equivalent because of the bonding to the chiral centre, C4. These protons are deshielded by the -OH group and assigned at 4.14 and 3.68ppm in the ^{1}H spectrum. These could also be observed in the HSQC spectrum in Fig 3.8.

The assignment of H9 methylene protons in similar environment to H1 led to the assignment of the not equivalent methylene protons at 1.86 and 2.03ppm to H1.

The HSQC spectrum shows that the C1 methylene protons are not equivalent and are assigned at 1.86 and 2.03 ppm. The remaining equivalent methylene protons observed in the HSQC spectrum were assigned to H9 at 2.86ppm. Irradiation of H9 in the TOCSY spectrum given in Fig 3.9 led to the assignment of methylene protons H8, H7, H6 and H5. The remaining -CH peak observed in the HSQC spectrum at 1.58ppm was then assigned to H4.

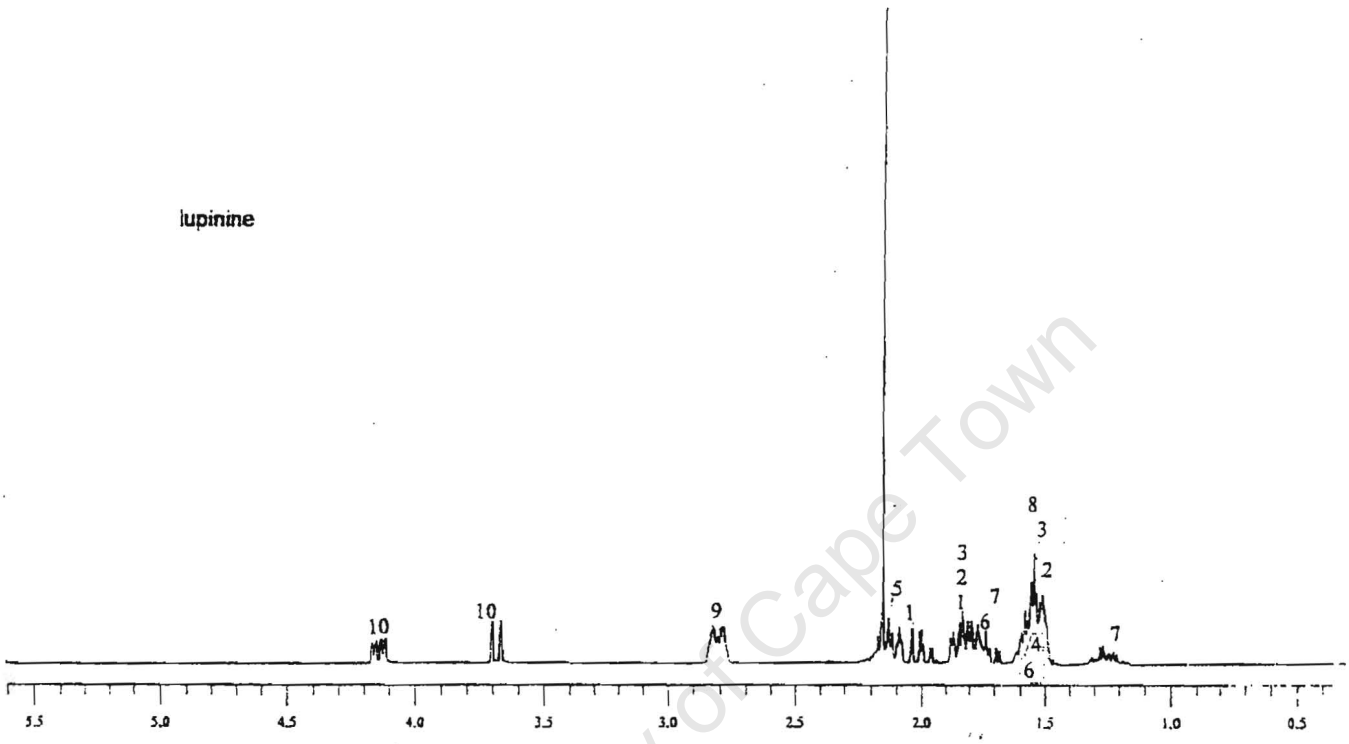


Figure 3.7: Proton spectrum of *lupinine*

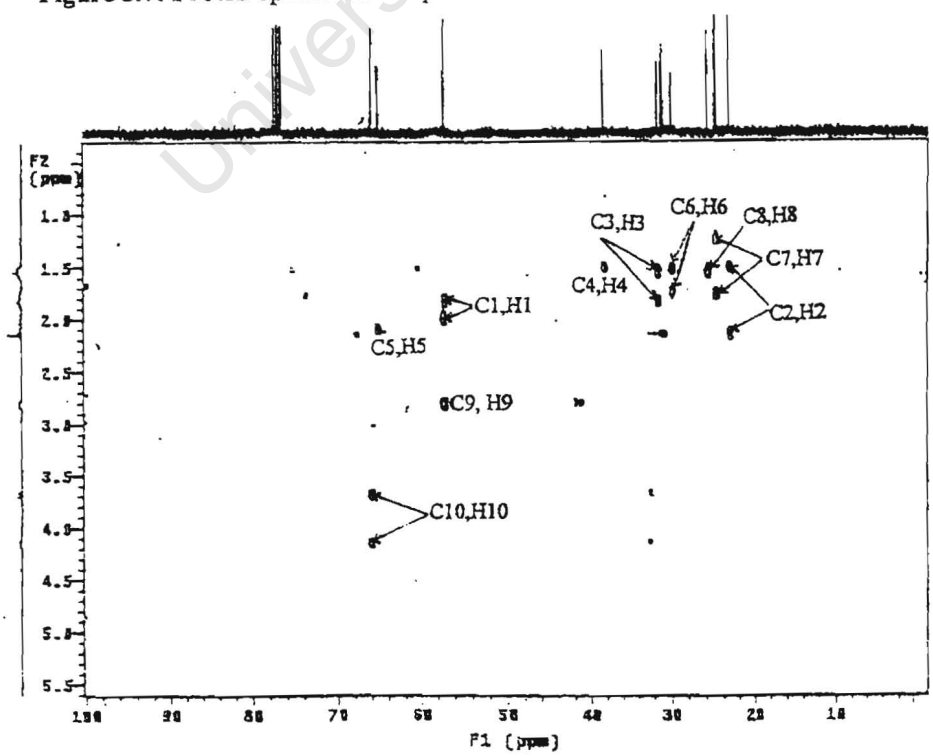


Figure 3.8: HSQC spectrum of *lupinine*

3.1.2(iv) Assignments of ^{13}C spectrum

The ^{13}C spectrum is given in Fig 3.10 and its assignment followed directly from the ^{1}H assignments using the HSQC spectrum. The experimental chemical shifts for *lupinine* are in good agreement with those from the literature, included in Table 3.2.

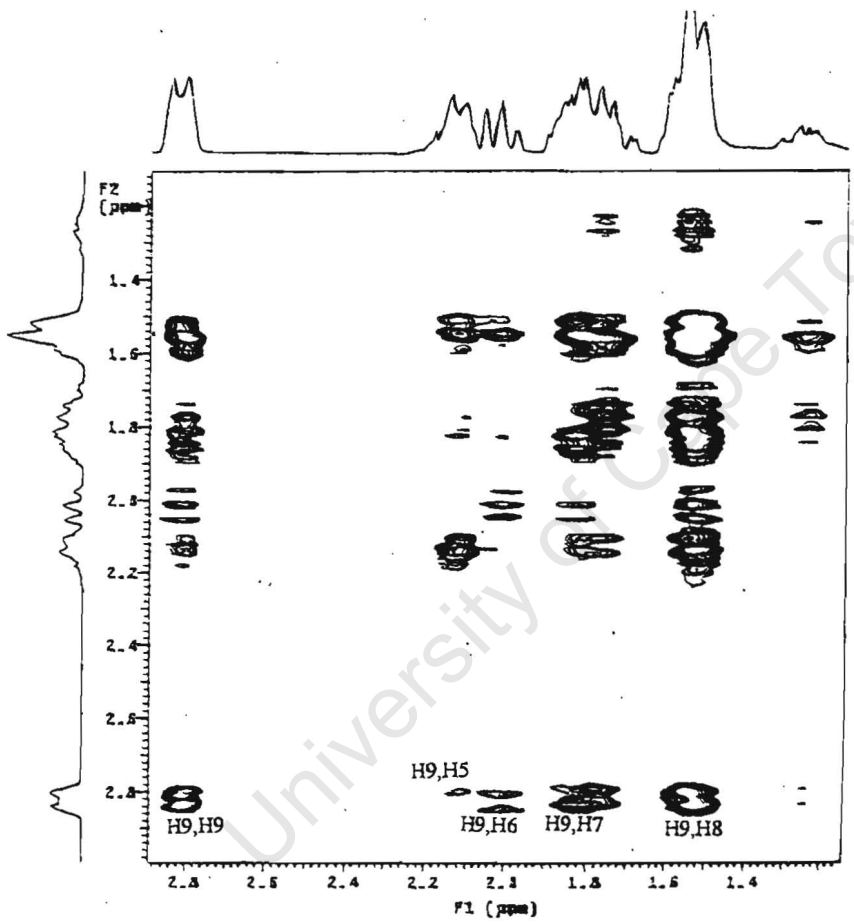


Figure 3.9: TOCSY spectrum of *lupinine*

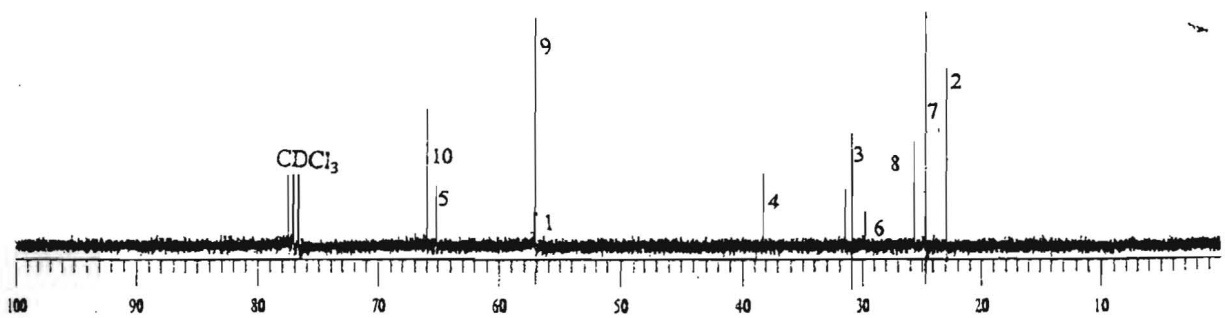


Figure 3.10: Carbon-13 spectrum of *lupinine*

3.1.3 Piperine (piperoylpiperidine)

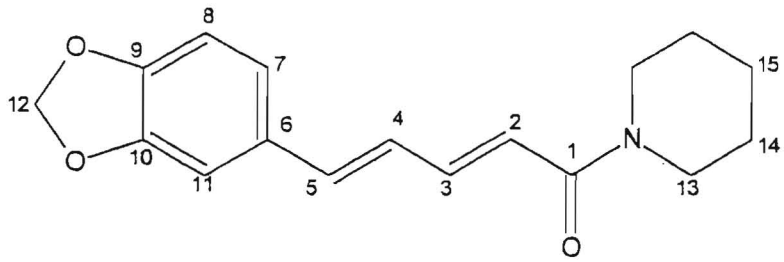


Figure 3.11: Structure of *piperine* alkaloid

3.1.3(i) Background

Piperine (piperoylpiperidine), $C_{17}H_{19}O_3N$, occurs in several black peppers and was isolated from the fruits of *Piper nigrum*, which furnishes the black and the white pepper of commerce [**13**]. Ifluckekinger and Hanbury [**14**] obtained *piperine* from long pepper (*P. longum)* and *P. officinarum*. Stenhouse found it in Ashanti black pepper (*P.clussi*), and Sabetay and Trabaund recently obtained it in Kissi pepper (*P.farnechoni*) [**15**].

The amount of piperine varies from 1-2 % in long pepper, to 5-9% in the white and black pepper which is sold commercially. Piperine is hydrolysed by alkali into a base and an acid, and these are known as piperidine and piperie [**16**].

Piperine's most important use is perhaps in the medical field. For thousand of years people have been using piperine to cure many minor ailments. In recent years it has been a very popular medication and is prescribed for dyspepsia, flatulence and diarrhoea, and used as a gargle for sore throats [**17**].

3.1.3(ii) Spectral chemical shifts (δ) of *piperine*

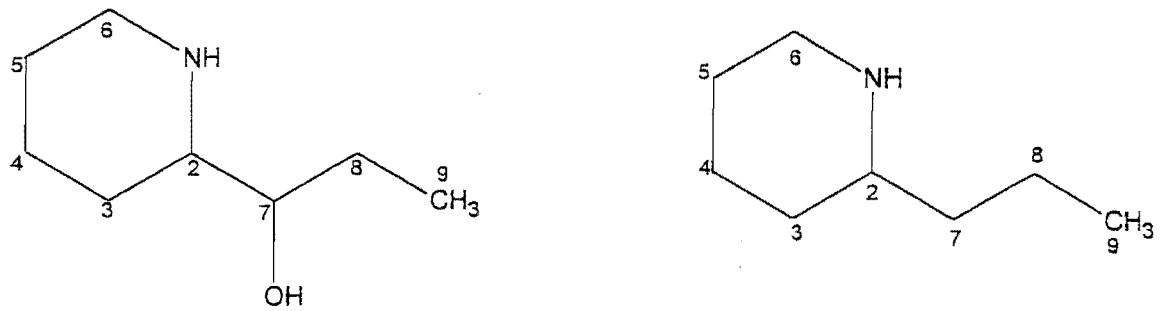
In an analogous manner to *scopoline* and *lupinine*, COSY and HSQC spectra were used to assign the ^{1}H and ^{13}C spectra of *piperine* presented in the appendix. The results are given in Table 3.3 along with literature [**18**].

Table 3.3: ^{1}H and ^{13}C experimental and literature NMR chemical shifts[a] (δ-ppm) for *piperine*.

At no.	*Experimental*		*Literature*
	^{1}H	^{13}C	^{13}C
1	-	165.40	165.1
2	6.44	120.10	120.0
3	7.38	142.40	142.1
4	6.74	125.40	125.1
5	6.75	138.10	137.9
6	-	131.00	130.9
7	6.88	122.40	122.3
8	6.78	108.40	108.2
9	-	148.20	148.0
10	-	148.20	148.0
11	6.96	105.70	105.5
12	5.95	101.20	101.1
13	3.58	46.80	46.3
14	1.57	26.70	25.8
15	1.63	25.60	24.4

[a] *chloroform solvent used.*

3.1.4 Conhydrine (Conium maculatum)



(a) **(b)**

Figure 3.12: Structure of *conhydrine* (a) and *coniine* (b) alkaloids.

3.1.4(i) Background

Conhydrine alkaloid that was isolated by Wertheim [**19**], is soluble in ethanol and chloroform and moderately soluble in water and diethyl ether. It is converted into *l*-coniine by reduction of the iodo-derivative (iodoconiine), $C_8H_{16}NI$, [**20**].

Spath and Adler [**21**] have studied the degradation of conhydrine by exhaustive methylation to trimethylamine and a mixture of two products, oil ($C_8H_{14}O$) and a crystalline substance, $C_8H_{16}O_2$ was obtained. The oil, when heated with water at 170° is converted by addition of a molecule of water into a crystalline substance, which leads to the formation of valeric acid.

Piperidine (nicotinic) alkaloids in conium include coniceine, coniine, N-methyl coniine, conhydrine, and pseudoconhydrine. The alkaloid content is variable with the stage of development and the stage of reproduction of the plant. During the first year of growth, the plant alkaloid content tends to be low. Plants in the second year, however, have alkaloid contents of approximately 1% in all plant parts. Conium is used as a sedative for pain and to correct excessive motility [**22**].

3.1.4(ii) Spectral chemical shifts (δ) of conhydrine

The ^{1}H and ^{13}C spectra of *conhydrine* presented in the appendix were assigned using COSY and HSQC spectra in analogous manner to *scopoline* and *lupinine*. The results of experimental

chemical shifts for *conhydrine* are given in Table 3.4 along with literature ^{13}C chemical shifts of *coniine* [23].

Table 3.4: ^{1}H and ^{13}C experimental NMR chemical shifts[a] (δ-ppm) for *conhydrine* and literature chemical shifts for *coniine.*

At No.	*Experimental (conhydrine)(ppm)*		*Literature (Coniine)(ppm)*
	^{13}C	^{1}H	^{13}C
2	60.2	2.55	57.1
3	24.4	1.81	33.7
4	25.2	1.27	25.7
5	26.6	1.28; 1.44	27.4
6	47.0	2.67; 3.08	47.6
7	75.9	3.38	40.2
8	25.3	1.44; 1.57	19.4
9	10.4	0.95	14.5

[a] *chloroform solvent used.*

3.1.5 Eseridine (Eserine oxide)

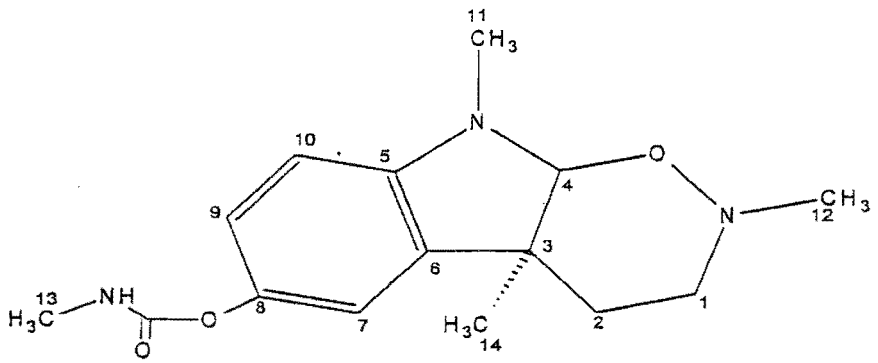


Figure 3.13: Structure of *eseridine* alkaloid.

3.1.5(i) Background

Eseridine ($C_{15}H_{23}$ O_3N_3) was first isolated by Bohringer and Sohne [**24**], and subsequently examined by Eber [**25**]. It is stated to melt at 132°C and to be converted into physostigmine when heated with diluted mineral acids. Salway [**26**] was unable to obtain evidence of its presence in calabar beans. It has been stated that geneserine and eseridine are identical. Geneserine ($C_{15}H_{23}O_3N_3$) was obtained by Polonovski and Nitzberg [27] by extracting with ether calabar beans previously soaked in a 2 % sodium hydroxide solution. Geneserine differs from physostigmine by one atom of oxygen, and is regarded as an amine oxide of physostigmine.

Eseridine is used in the treatment of epilepsy, progressive paralysis, tonic convulsions, gastralgia, strychnine and atropine poisoning and constipation (combined with belladonna and nux vomica). The poisonous effects are nausea, giddiness, abdominal pain, indistinct vision, diminished heart action, muscular tremors and weakness, then complete relaxation, retarded respiration, motor paralysis contractions of the sphincters, cold extremities and skin covered with cholera-like sweat [**27**].

3.1.5(ii) Spectral chemical shifts (δ) of *eseridine.*

COSY and HMBC spectrum were used to assign the ^{1}H and ^{13}C spectra of *eseridine* presented in the appendix. The results are given in Table 3.5

Table 3.5: ^{1}H and ^{13}C experimental NMR chemical shifts[a] (δ-ppm) for *eseridine*.

At no.	*Experimental*	
	^{1}H	^{13}C
1	2.63;2.45	53.3
2	2.10;1.94	32.5
3	-	40.2
4	4.70	101.7
5	-	147.3
6	-	136.2
7	6.80	115.4
8	-	143.5
9	6.76	120.0
10	6.24	106.5
11	2.83	31.5
12	2.51	46.0
13	2.79	27.5
14	1.17	25.3

[a] *chloroform solvent used.*

REFERENCES

1. L. Hesse, Annalen, **271**, (1892), 114.
2. J. Luboldt, Arch. Pharm., **236**, (1898), 11.
3. T.L Stedman, Steadman Shorter's Medical Dictionary, (1942) Poisons & Antidotes.
4. M.A. Fliniauxi: Unit Regulation of Cellular Metabolism Vegetal - EA 2085 Laboratory of Phytotechnologie, FACULTE OF PHARMACY, 1 street Louvel. - 80037 AMIENS Cedex .
5. L. Simeral, G. E. Marciel, J. Magn. Reson., **6**, (1974), 226.
6. G. Baumert, K. Landw. Versuchs. Stat., **27**, (1881), 15.
7. W. Smith, F. Berend, Arch. Pharm., **235**, (1897), 263.
8. S. Cassola, Annalen, **13**, (1835), 308.
9. C. Karre, K. Clemo, S. Raper, Helv. Chim. Acta, **11**, (1928), 1062.
10. K. Clemo and S. Raper, J. Chem Soc., (1929), 1927.
11. B. Alice, L. Russell, J. Hardin, North Carolina State University USA.
12. F. Bohlmann, R.Zeiberg, Chem. Ber., **108,** (1975), 1043
13. H. Kisned, Scheigger's Journal, **29**, (1819), 80.
14. T. Iflukekinger, S. Hanbury, Pharmacographia London: Mcmillan & Co., (1879), 584.
15. S. Sabetay and B. Trabaund, Pharm. J., **14**, (1855), 383.
16. H. Sohne, J. Am. Pharm. Assoc., **32**, (1943), 234.
17. K. H. Shin, W. S Woo, Korean Biochem J., **18**; (1985), 95.
18. E. Wenkert, J. Am. Chem. Soc., **93**, (1971), 6271
19. F. Werthin, Chem. Ber., **67**, (1934), 1011
20. L. Hofmann, Chem. Ber., **18**, (1885), 5.
21. G. Spath, H. Adler, Monats, **63,** (1933), 127.
22. T. A. Lopez, C. Bianchini, M. L. Toxicon, J. Am. Chem. Soc., **37**, (1999), 841-865.
23. S. Korre, J. Am. Chem. Soc., **123**, (2001), 11829-11830.
24. L. Bohringer, Pharm. Post, **21,** (1888), 663.
25. G. Eber, L. Apath. Zeit., **19,** (1904), 891.
26. M. Salway, J. Chem. Soc., **99**, (1911), 2148.
27. D. Polonovski and S. Nitzberg, Merck's Berichte, **40**, (1926), 37.

CHAPTER 4

4. ^{1}H AND ^{13}C CHEMICAL SHIFTS OF RIGID AND FLEXIBLE CHEMICAL COMPOUNDS

4.1 INTRODUCTION

Many computer programs have been developed which use quantum mechanical or empirical rules to predict NMR parameters. The problem with some of these programs is that they often do not give accurate results due to the complexity of the calculation. This could be due to the fact that for newly synthesized products or rare compounds, retrieval systems may fail because the libraries may not always contain the reference spectra. A few such programs are briefly described below.

(i) Spectool [1] was designed by Chemical Concepts is a hyperlinked toolbook that includes interpretation tools, correlation tables, interpretation data, and spectra for MS, ^{1}H-NMR, ^{13}C-NMR, IR, and UV spectroscopy. This is a good resource to help in interpreting unknown spectra and to assign shifts for known compounds.

(ii) HyperNMR [2] was designed by Hypercube Inc. The user selects the NMR active atoms in the structure. The program calculates the NMR chemical shifts and coupling constants from the electronic structure of the molecule. The information is presented in tables or as a simulated spectrum. It is important to note that these spectra are calculated from the electronic structure, not from tables of chemical shifts. As a result this software is very computationally intensive.

(iii) Carbon-13 NMR module [3] was designed by Softshell International. This program assigns ^{13}C chemical shift tables to structures drawn with ChemWindows.

There are many other programs available commercially which can be used to predict NMR chemical shifts. SpecInfo-V3.2 and ACD-V4, which predict chemical shifts by

using knowledge-based systems, are introduced below. The CHARGE-V7 program, uses quantum mechanical calculations in its prediction, and will also be introduced.

4.2 SPECINFO-V3.2 NMR CHEMICAL SHIFT PREDICTION PROGRAM

4.2.1 Introduction

The SpecInfo program was designed for structural elucidation purposes, using cycles of spectrum searching and spectrum prediction. The SpecInfo database system at Daresbury (UK), is a database managed system from Chemical Concepts, designed to store, retrieve, and manipulate NMR, IR and mass spectra [**4**]. It comprises 9900 ^{13}C NMR datasets. In estimation and prediction of structures used for calculation of spectra, a statistical method based on the contents of the database is used for prediction. A novel feature of the program is the ability to predict the NMR spectrum for an analytical structure using statistical information taken from spectra stored in the database [**5**].

SpecInfo prediction programs use data generation packages such as:

(i) CHESS: used to search for chemical structure similar to a structural query;

(ii) COUPCAL: used for estimation of coupling constants;

(iii) SPECAL: used for estimation of spectral information from a structural query;

(iv) EDSPEC: which is a spectrum editor used to modify existing spectra or create new spectra;

(v) GETSPEC used to search for similar spectra to structural query with STN (standard) Messenger command language [**6**].

The estimation is an increment based spectrum calculation, which does not depend on the database [7]. For example alkanes ^{13}C chemical shift values can be calculated using the following equation [10]:

$$\delta = -2.3 + A + B \qquad \textbf{equation 4.1}$$

where, A is the sum increment allowed for various substituents depending on their positions as α, β or γ to the ^{13}C atom in question. B is the sum of branching.

For alkenes

$$\delta = 22.8 + A + B \qquad \textbf{equation 4.2}$$

where, 22.8 is the value for ethene,

and for alkynes:

$$\delta = 71.9 + A \qquad \textbf{equation 4.3}$$

where, 71.9 is the chemical shift value for ethyne. SpecInfo supports data exchange formats of most spectrometers, including an efficient import batch mode. An incorporated commercial relation database management system ensures high levels of data security and integrity for multiuser applications. The client server of SpecInfo offers a high degree of installation flexibility, hence existing hardware configurations are taken into account [**8**].

4.3 CHARGE-V7 program

4.3.1 Introduction

The CHARGE-V7 program is a fast, easy to use and well-parameterized program for the calculation of the atomic charges and proton chemical shifts in molecules. The calculation uses atomic electronegativities and polarizabilities, which are incorporated, in the parameter file PARAM.DAT. It is suitable for the calculation of molecular and inter-molecular electrostatic energies and interactions, electrostatic potentials, π charges, dipole moments etc. CHARGE-V7 will accept a variety of input formats, including the z-matrix (GAUSSIAN or MOPAC format), Cambridge CSSR format and MOL2 (SYBIL) [**9**].

The program is dimensioned to handle 99 atoms and is easily re-dimensioned for larger molecules. A wide range of functional groups may be accepted; these include all the groups commonly encountered in drug design and biological research, including charged species. CHARGE-V7 is written in FORTRAN 77 and can be used with PC and compatible computers. CHARGE-V7 program requires information from the input file for its execution of chemical shifts. The CHARGE.exe file executes the CHARGE-V7 program calculation, and the output is displayed in a Notepad file [**10**].

In a four atom I-J-K-L, partial atomic charge on I is influenced by α effect from atom J, β effect from K and γ effect from L [**11**]. The α effect depends on relative electronegativities of two adjacent atoms; the β effect is a function of both electronegativity of the substituent and the polarizability of atom being considered; the γ effect is non-orientational and a function of the polarizability of two atoms involved [**11**].

4.3.2 Electronegativity and polarizability on CHARGE

The α charge effect is represent by

$$qi(\alpha)=(E_j-E_i)/A[I,J] \qquad \textbf{equation 4.4}$$

where, A(I,J) is a constant dependent on the exchange and the overlap integrals for the bond I-J. The β charge effect is represented by

$$qi(\beta) = (E_k - E_h)Pi/c \qquad \textbf{equation 4.5}$$

where, c is a constant and E_k electronegativity of atom K E_h of hydrogen and Pi is the polarizability of atom i.

The gamma effect on charge is represented by

$$qi(\gamma) = \beta_{il}/10.0 \qquad \textbf{equation 4.6}$$

where, l is an atom in a four atom fragment I-J-K-L.

Values of electronegativity and polarizability are required for an atom to be included into the CHARGE scheme [**12**].

4.3.3 Inductive and resonance effect on CHARGE

Inductive and resonance calculations are used to calculate partial atomic charges and molecular dipole moments. Proton chemical shift for alkanes is related to electronegativity difference (Ec-Eh) for α and β atoms. Orbital electronegativity compiled by Hinze and Jeffe [**13**] are applied.

4.3.4 Electric field effect on CHARGE

Abraham et.al. [**14**], attempted two models for calculating electric field in ether oxygen. Oxygen atom effects were also investigated, and the divalent nature of the oxygen atom leads to an additional degree of freedom compared to monovalent substituents [**15**]. The electric fields due to the charge on the oxygen atom, and of both attached carbon atoms were calculated at the proton in question. A second CHARGE model, which gave better results, was based on the calculation of the electric field due to the other oxygen atom and a dummy atom, midway between the attached carbon atom with an equal but opposite charge [**14**].

4.3.5 Effect of Halogens on CHARGE

Calculations of ^{1}H NMR chemical shifts in halo-substituted alkanes are based on semi-classical calculations of atomic charges including contributions of polar, steric and anisotropic effects [**16**].

In addition to chlorine, fluorine is also catered for in the CHARGE-V7 program, and the vector components of the electric field are calculated from the fluorine and from the carbon to the proton and summed to give the component of the total field along the C-H bond [**17**].

Bromine and iodine were compared in cyclohexane and long range effects due to electric field effects were used for calculating chemical shifts. β(CH)(X= halogen) proton chemical shifts for both axial and equatorial substituents decrease in the order F>I>Br>Cl which does not follow the electronegativity trend. The de-shielding effect of the halogen at the γ protons increases in the sequence from F<Cl<Br<I [**18**].

4.4 ACD-V4 PROGRAM

4.4.1 Introduction

The ACD-V4 program is most valuable to those working in the pharmaceutical industries or other biological orientated industries, and other chemists needing spectral predicting programs. The ACD-V4 program predict NMR chemical shifts for H1, C13, F19 and P31 nuclei. A database of molecular structures is used by this program [**19**].

One approach of detecting structural fragments is to:

(i) search for the structures having spectra similar to the specified experimental one, and rank them in order of the decreasing degree of spectral similarity;

(ii) select a structure bearing the closest similarity to the structure of the analysed compound, determine the maximum number of common fragments with a high probability of being in the structure of the unknown compound. [**20**]

Hierarchical Organisation of Spherical Environment (HOSE) codes are also utilised in creating large NMR spectral databases, Substructure sub-spectrum databases are generated and used for structural elucidation [**21**].

ACD/structure Elucidator is used when fragment combination alone cannot resolve the structure in question. The methodology employed is used in systems such as X-PERT [**22**] and CHEMICS [**23**]. The chemical structure used in ACD-V4 prediction software can be drawn in the integrated chemical structure drawing package (ACD/Chemsketch) or imported from other drawing packages such as ISIS Draw or ChemDraw.

ACD Chemsketch is equipped with capabilities to

(i) draw structures in a chemistry orientated interface;

(ii) instantly calculate the chemical formula, molecular weight and percentage composition;

(iii) customize display properties e.g atomic numbering, chemical symbols, and valence;

(iv) work with structures, text and graphics simultaneously, and transfer to any OLE-supported software [**24**].

The program ACD/NMR, which has a knowledge base, gives best results, and the greatest advantage of this program is the possibility of training this knowledge base by the user [**25**].

The creation of the user databases and optimisation of molecular structures to 3D format allows proton-proton coupling constants to be predicted according to Karplus angles extracted from the 3D-optimised structure.

NMR predictor in the ACD-V4 program is designed to quantitate intramolecular interactions in new organic structures and to predict their chemical shifts. It also uses a unique algorithm based on the intramolecular inter-atomic parameters for over 3000 structural fragments, which have been derived from the experimental data using the specially developed ^{13}C NMR data processing approach. The ^{1}H NMR predictor includes an internal DAT file with over 800 000 experimental chemical shifts and 180 000 experimental coupling constants to quantify intermolecular interactions in a new organic structure and to predict their chemical shifts. The ACD-V4 program has the capability of recognizing differences between diastereotopic protons, *cis-trans* isomers of alkenes, and *syn-anti* isomers of amides, oximes, hydrazones and nitrosamines [**26**].

^{1}H NMR prediction algorithms allow predicted spectra to be generated for each of the suggested structures and displayed on the screen for the combinatorial plate data contained within the database. This method can be used to search spectra of starting material as well as spectral responses associated with particular functional groups that can be detected. In order to allow prediction algorithms to aid in the assignment process the NMR processing software is directly integrated with the prediction modules for both ^{1}H and ^{13}C NMR [**27**].

Advanced Chemistry Development (ACD) laboratories investigated ways for manipulation and visualisation of spectra and chemical structures, and a set of Java applets were developed for viewing peak assignments to the corresponding atoms in a molecule. Approaches for the prediction of NMR chemical shifts and coupling constants have varied from standard tables of increments through basic rule sets, to the approaches of software packages.

The most accurate spectra are obtained for solutes in non-polar non-aromatic solvents, and calculated chemical shifts and coupling constants are provided with 95% accurate intervals to indicate the reliability of the calculated values [**28**].

4.5 Prediction of simple chemical compounds

The CHARGE-V7 and ACD-V4 ^{1}H δ predictor programs were used in predicting ^{1}H-NMR chemical shifts for selected chemical compounds. ^{13}C prediction of simple chemical compounds was not performed due to the accuracy of a number of available programs, some highlighted in the introduction. Compounds with flexible chemical structures like phenyl acetate, 2-nitrobenzyl alcohol, and compounds with rigid structures such as quinaldic acid, 3- indolacetonitrile etc., were selected due to their extensive use in the pharmaceutical industry for making new medicinal products (i.e they are intermediates of existing compounds). CHARGE-V7 was specifically re-parameterized [**29**] to cater for the heteroatoms in some of the compounds used below. The structure with the lowest minimized energy conformation (3-dimensional), is used by the CHARGE-V7 program to calculate chemical shifts of simple compounds and these structures are also presented in this section. The R-factor presented in equation 4.1 was used to estimate the accuracy of the prediction programs used. It also gave some indication of the differences and similarities of the shifts given by these programs relative to the experimental chemical shifts.

Formula used for calculation of shift difference between the experimental and predicted chemical shifts is presented as follows:

Shift diff = experimental - calculated chemical shifts.

R-factor = SQRT [SUM (exp shifts - calc shifts)/n] **equation 4.1**

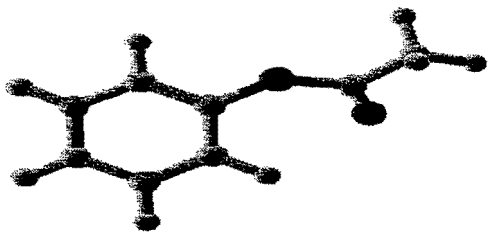
Where, n is the number of atoms with predicted chemical shifts in a molecule.

Criteria used for good agreement:

shift difference of < 0.20;

R-factor < 0.2.

4.5.1 *Phenyl acetate*



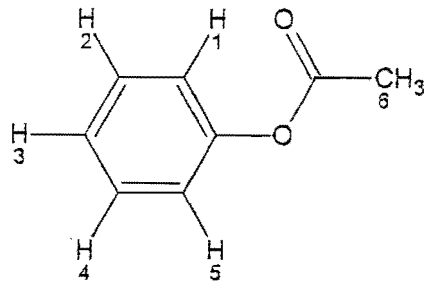


Figure 4.1: Structure of *phenyl acetate*.

The differences between the chemical shift given by programs used for the prediction of *phenyl acetate* chemical shifts and the experimental values are presented in Table 4.1.

Table 4.1: Predicted ^{1}H Chemical shift[a] (ppm) differences of *phenyl acetate* relative to experimental values.

At.No.	*Exp Shifts*	*CHARGE-^{1}H*	*Shift Diff*	*ACD-^{1}H*	*Shift Diff*
1,5	7.07	7.13	0.06	7.07	0.00
2,4	7.35	7.27	0.08	7.32	0.03
3	7.20	7.13	0.07	7.16	0.04
6	2.25	2.46	0.21	2.23	0.02
R-factor			**0.1732**		**0.1500**

[a] *chloroform solvent used.*

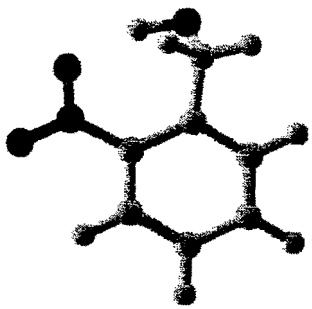
(a) CHARGE-V7 program predicted ^{1}H-chemical shifts.

Accuracy in prediction of chemical shifts for all protons of phenyl acetate except H6 is observed. The divalent nature of the oxygen atom was found to lead to an additional degree of freedom compared to a monovalent substituent [**30**]. It could also be observed that the chemical shift predicted for methyl group 6 is inaccurate. Nevertheless, the predicted chemical shifts could be used successfully in the assignment of the phenyl acetate spectrum.

(b) ACD-V4 program-predicted ^{1}H-chemical shifts.

The chemical shifts predicted by the ACD-V4 ^{1}H δ predictor program for phenyl acetate are better than those predicted by CHARGE-V7. The largest error observed is 0.04, this proves the accuracy of this program in predicting ^{1}H chemical shifts for phenyl acetate. The predicted chemical shifts could be used without any ambiguity in the assignment of the phenyl acetate spectrum. The R-factor calculated for the ^{1}H-chemical shifts predicted by ACD-V4 program is slightly less than that of ACD-V4 program.

4.5.2. *2-nitrobenzyl alcohol*



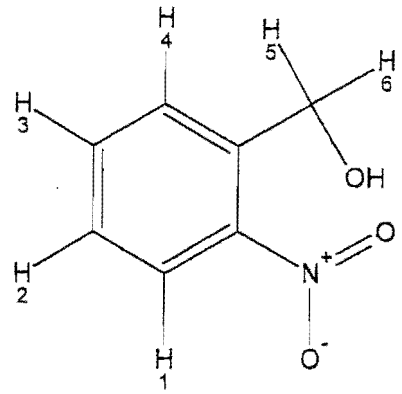


Figure 4.2: Structure of *2-nitrobenzyl alcohol.*

The differences between the chemical shifts given by programs used for the prediction of *2-nitrobenzyl alcohol* chemical shifts and the experimental values are presented in Table 4.2.

Table 4.2: Predicted ^{1}H Chemical shift[a] (ppm) differences of 2-nitrobenzyl alcohol relative to experimental values.

At.No	*Exp Shifts*	*CHARGE-^{1}H*	*Shift Diff*	*ACD-^{1}H*	*Shift Diff*
1	7.75	7.34	0.41	7.46	0.29
2	7.47	7.46	0.01	7.64	0.17
3	7.67	7.37	0.30	7.69	0.02
4	8.10	7.98	0.12	7.75	0.35
5,6	4.98	4.96	0.02	5.24	0.26
R-factor			**0.41**		**0.19**

[a] *chloroform solvent used.*

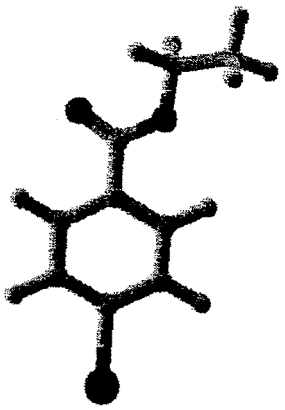
(a) CHARGE-V7 program predicted ^{1}H-chemical shifts.

The ^{1}H-chemical shifts predicted by the CHARGE-V7 program are accurate with the exception of H1 and H3. The chemical shift predicted for H3 is influenced by the mesomeric effect [31] caused by the presence of electron withdrawing NO_2 group. The calculated R-factor indicates the accuracy of chemical shifts calculated by CHARGE-V7.

(b) ACD-V4 program-predicted ^{1}H-chemical shifts.

Good prediction for H3 chemical shift is observed from the ACD-V4 program, which was calculated inaccurately by CHARGE-V7. However, ACD-V4 predicted the H1 chemical shift to be less than that for H2; this is contrary to the trend of the chemical shifts obtained experimentally. The large errors in predicted ^{1}H-chemical shift indicate poor adaptation of the program to cater for the $-CH_2OH$ as well as the $-NO_2$ groups.

4.5.3. *Ethyl-4-iodobenzoate*



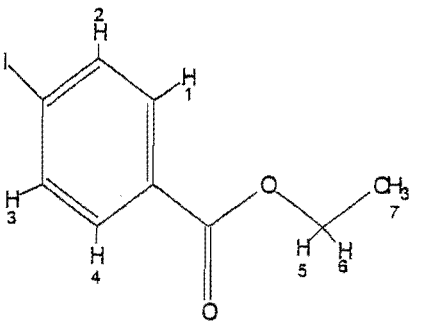


Figure 4.3: Structure of *ethyl-4-iodobenzoate.*

The differences between the chemical shifts given by programs used for the prediction of *ethyl-4-iodobenzoate* chemical shifts and the experimental values are presented in Table 4.3.

Table 4.3: Predicted ^{1}H Chemical shift[a] (ppm) differences *of ethyl-4-iodobenzoate* relative to experimental values.

At.No	*Exp Shifts*	*CHARGE-^{1}H V7*	*Shift Diff*	*ACD-^{1}H*	*Shift Diff*
1,4	7.82	7.73	0.09	8.16	0.34
2,3	7.78	7.59	0.19	7.82	0.04
5,6	4.38	4.12	0.26	4.17	0.21
7	1.40	1.32	0.08	1.33	0.07
R-factor			**0.39**		**0.16**

[a] *chloroform solvent used.*

(a) CHARGE-V7 program predicted ^{1}H-chemical shifts.

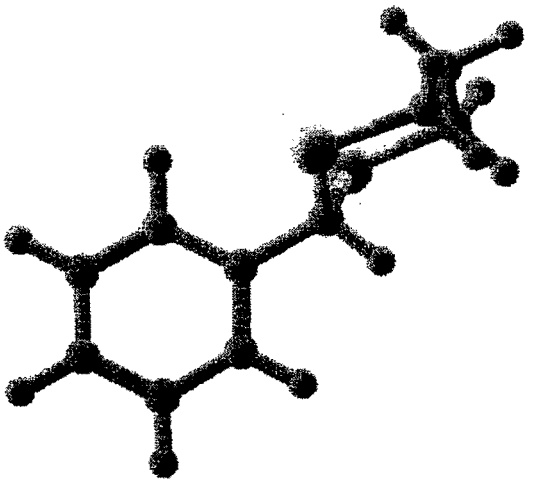
The chemical shifts predicted by CHARGE-V7 for the benzyl ring are similar to the experimental values. Large error (0.26) was seen for the ethylene protons of the substituent -$COOCH_2CH_3$ group. Inaccurate prediction of these chemical shifts indicates poor parameterization of the -$COOCH_2CH_3$ group when attached to the phenyl ring. The presence of the acetate group on the phenyl ring did not have a negative effect on chemical shifts produced by CHARGE-V7. Although the effect on esters, had not been fully investigated to be incorporated into the CHARGE-V7

program, the methyl effect, the long range effects, and electric field effects could have contributed to the large chemical shift errors observed for some protons *of ethyl-4-iodobenzoate*. Accurate calculation of the H1,4 chemical shifts were obtained from CHARGE-V7. Even though both programs predicted these protons with high error, it could be observed that the H-H interactions between H1,4 and H in the OH group of the bulky -$COOCH_2CH_3$ group could have contributed to the prediction of H3. Good prediction of chemical shifts for the protons attached to carbons alpha to the iodine group could be attributed to the inclusion of the steric and electric field effects into CHARGE [**32**].

(b) ACD-V4 program-predicted ^{1}H-chemical shifts.

ACD-V4 program predicted the chemical shifts of the H5, 6 protons better than the CHARGE-V7 program. The overall accuracy indicated by the R-factors showed that the ACD-V4 program performed better than CHARGE-V7.

4.5.4 *2-Phenyl dithiane*



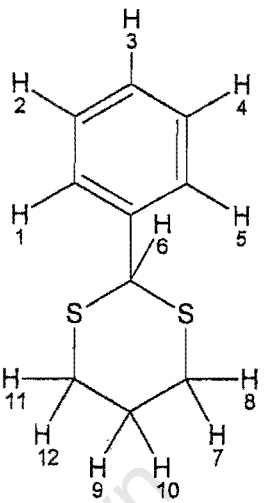


Figure 4.4: Structure of *2-phenyl dithiane.*

The differences between the chemical shifts given by the programs used for the prediction of *2-phenyl diathiane* chemical shifts and the experimental shifts are presented in Table 4.4.

Table 4.4: Predicted ^{1}H Chemical shift[a] (ppm) differences of *2-phenyl diathiane* relative to experimental values.

At.No	*Exp Shifts*	*CHARGE-^{1}H*	*Shift Diff*	*ACD-^{1}H*	*Shift Diff*
1,5	7.34	7.05	0.29	7.07	0.27
2,4	7.47	7.27	0.20	7.26	0.21
3	7.31	7.19	0.12	7.30	0.01
6	5.16	4.63	0.53	5.10	0.06
7	3.05	3.06	0.01	2.92	0.13
8	2.92	2.46	0.46	2.76	0.16
9	2.15	2.14	0.01	1.98	0.17
10	1.94	1.98	0.04	1.82	0.12
11	2.92	2.46	0.46	2.76	0.16
12	3.05	3.06	0.01	2.92	0.13
R-factor			**0.45**		**0.38**

[a] *chloroform solvent used.*

(a) CHARGE-V7 program predicted ^{1}H-chemical shifts.

Equivalent ^{1}H-chemical shifts are observed for H7 ≡ H11, H8 ≡ H12 in the experimental results, but these are not equivalent in the chemical shift results produced by CHARGE-V7. The poor parameterization of the S-atoms in the dithiane ring contributes to poor predicted ^{1}H-chemical shifts. The presence of the dithiane group had a negative effect on the shifts for H1, 5 predicted by CHARGE-V7. The contribution of C-C bond anisotropy in cycloalkanes was included in CHARGE [**33**], but the presence of the S-atoms of the dithiane required reparameterization for accurate prediction of ^{1}H-chemical shifts. The phenyl dithiane structure was re-parameterized on CHARGE-V7, but no improvement was observed. The overall accuracy of predicted chemical shifts by CHARGE-V7 indicated by the high R-factor, show the incapability of CHARGE-V7 to calculate ^{1}H-chemical shifts for *2-phenyl dithiane* because of the dithiane ring effects.

(b) ACD V4 program-predicted ^{1}H-chemical shifts.

The ACD-V4 ^{1}H δ predictor program gave an accurate chemical shift for H3. However, shifts for H1, 5 of the phenyl ring are predicted inaccurately, as are those for all the dithiane protons except H6. Poor prediction of the dithiane ring is a consequence of the absence for the dithiane ring in the structural database used by the ACD-V4 ^{1}H δ predictor program. The high shift difference calculated for most protons led to a high R-factor calculated for ACD-V7 predicted chemical shifts but it is lower than that predicted by the CHARGE-V7 program.

4.5.5 *4-(3-phenylpropyl)-pyridine*

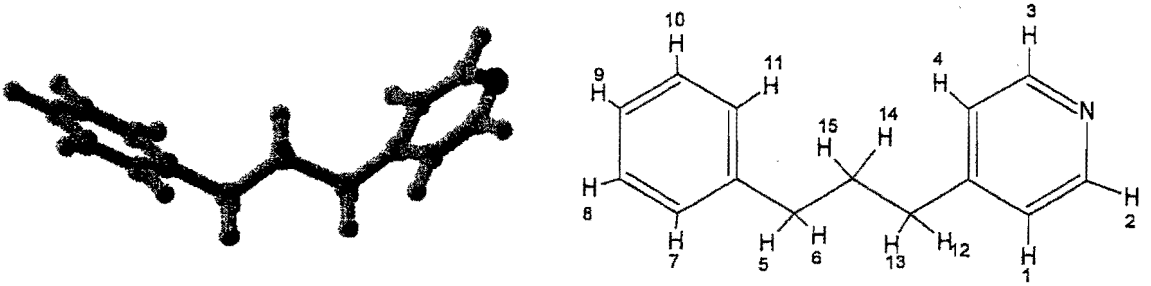


Figure 4.5: Structure of *4-(3-phenyl propyl)-pyridine.*

The differences between the chemical shifts given by the programs used for the prediction of *4-(3-phenyl propyl)-pyridine* chemical shifts and experimental values are presented in Table 4.5.

Table 4.5: Predicted ^{1}H Chemical shift[a] (ppm) differences of *4-(3-phenyl propyl)-pyridine* relative to experimental values.

At.No	*Exp Shifts*	*CHARGE-^{1}H*	*Shift Diff*	*ACD-^{1}H*	*Shift Diff*
1,4	7.05	7.15	0.10	7.10	0.05
2,3	8.47	8.64	0.17	8.32	0.15
5,6	2.59	2.42	0.17	2.58	0.01
7,11	7.15	6.89	0.26	7.09	0.06
8,10	7.27	7.07	0.20	7.09	0.18
9	7.17	6.95	0.22	7.09	0.08
12,13	2.59	2.47	0.12	2.60	0.01
14,15	1.95	2.17	0.22	1.60	0.35
R-factor			**0.25**		**0.31**

[a] *chloroform solvent used.*

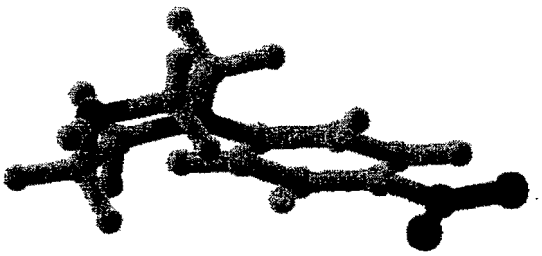
(a) CHARGE-V7 program predicted ^{1}H-chemical shifts.

The chemical shift for the H7, 11 protons, which are attached to carbons alpha to the propyl pyridine group are predicted inaccurately by CHARGE-V7. For H14, 15 of the propyl group there are also large errors in the predicted chemical shift relative to the experimental results. This is despite a thorough investigation of H-H and C-H interactions that was performed on the straight chain hydrocarbons [**34**]. The C-C bond anisotropy in substituted alkanes was introduced into CHARGE-V7. The error in chemical shift of the propyl group is therefore due mainly to the effects of the phenyl and pyridine substituents.

(b) ACD-V4 program-predicted ^{1}H chemical shifts.

Good predictions of ^{1}H-chemical shifts by the ACD-V4 ^{1}H δ predictor program are observed for all protons except H14, 15 of the propyl group. The large error in these protons increased the resultant R-factor to be more than that of CHARGE although individual errors are less. Equal chemical shifts for all the protons of the phenyl ring are predicted by the ACD-V4 ^{1}H δ predictor program, this is contrary to experimental results. It would be difficult to assign the predicted chemical shifts of the phenyl propyl pyridine spectrum using the predicted ^{1}H-chemical shifts, due to this error.

4.5.6 *1-(4-nitrophenyl)-piperazine*



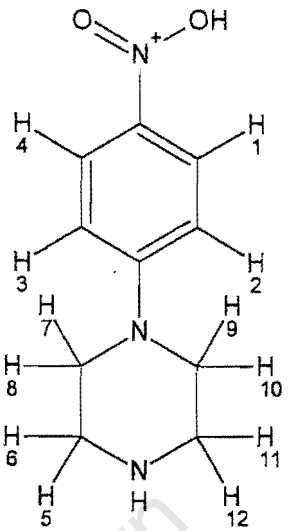


Figure 4.6: Structure of *1-(4-nitrophenyl)-piperazine.*

The differences between the chemical shifts given by programs used for the prediction of *1-(4-nitrophenyl)-piperazine* chemical shifts and the experimental shifts are presented in Table 4.6.

Table 4.6: Predicted ^{1}H Chemical shifts[a] (ppm) differences of 1-(4-nitrophenyl)-piperazine relative to experimental values.

At.No	*Exp Shifts*	*CHARGE-^{1}H*	*Shift Diff*	*ACD-^{1}H*	*Shift Diff*
1,4	8.14	7.93	0.21	8.04	0.10
2,3	6.82	7.24	0.42	6.57	0.25
5	3.02	3.04	0.02	3.26	0.24
6	3.02	3.23	0.21	3.01	0.01
7	3.39	3.53	0.14	3.28	0.11
8	3.39	3.50	0.11	2.82	0.57
9	3.39	3.40	0.01	3.28	0.11
10	3.39	3.51	0.12	2.82	0.57
11	3.02	3.10	0.08	3.01	0.01
12	3.02	3.26	0.24	3.26	0.24
R- factor			**0.34**		**0.35**

[a] *chloroform solvent used.*

(a) CHARGE-V7 program predicted ^{1}H-chemical shifts.

Fairly accurate prediction of the chemical shift for H1, and H4 is observed with the CHARGE-V7 program, despite the effects of the -NO_2 group. The ^{1}H-chemical shifts predicted for the atoms attached to the piperazine ring are unequal, but these are equal in the experimental results. The equivalency of H7, H8, H9 and H10 is also observed in the experimental chemical shifts, these are unequal in the calculated chemical shifts. A high error observed for H2,3 adjacent to the piperazine ring which might not be well parameterized in CHARGE-V7. These results show that CHARGE-V7 has difficulty in predicting the equivalent protons accurately.

(b) ACD-V4 program-predicted ^{1}H-chemical shifts.

The ACD-V4 ^{1}H δ predictor program produced better prediction of the shifts for H1,4 than did CHARGE-V7. Equal chemical shifts results predicted by ACD-V4 ^{1}H δ predictor program for H7 and H9, and also for H8 and H10. The R-factor calculated for ACD-V4 is similar to that calculated from CHARGE and the inaccuracy is also indicated by most protons having high chemical shift differences.

4.5.7 *2-phenylbutyric acid*

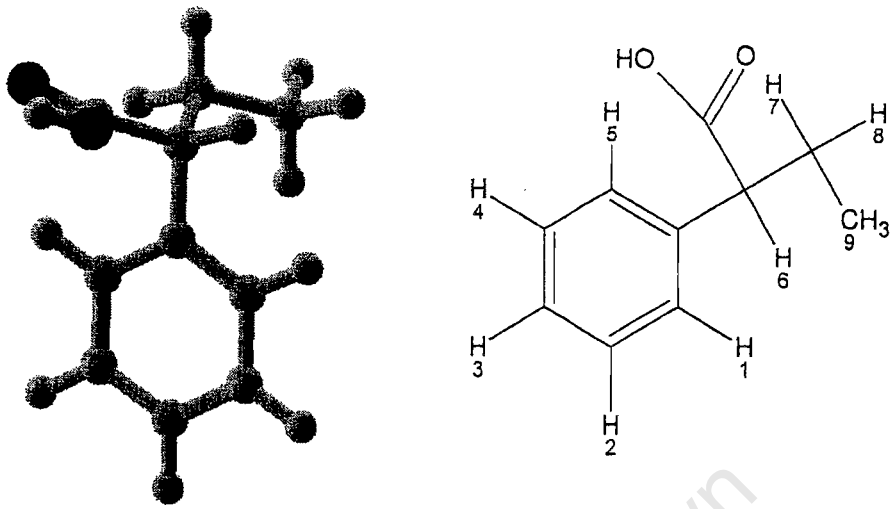


Figure 4.7: Structure of *2-phenylbutyric acid.*

The differences between the chemical shifts given by programs used for the prediction of *2-phenylbutyric acid* chemical shifts and the experimental values are presented in Table 4.7.

Table 4.7: Predicted ^{1}H Chemical shift[a] (ppm) differences of 2-phenylbutyric acid relative to experimental values.

At.No	*Exp Shifts*	*CHARGE-^{1}H*	*Shift Diff*	*ACD-^{1}H*	*Shift Diff*
1,5	7.27	7.24	0.03	7.53	0.26
2,4	7.27	7.31	0.04	7.26	0.01
3	7.27	7.20	0.07	7.27	0.00
6	3.45	3.66	0.21	3.55	0.10
7	1.81	1.64	0.17	1.84	0.03
8	2.10	2.08	0.02	2.17	0.07
9	0.90	0.79	0.11	0.99	0.09
R-factor			**0.15**		**0.28**

[a] *chloroform solvent used.*

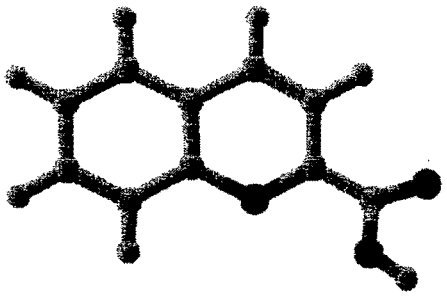
(a) CHARGE-V7 program predicted ^{1}H-chemical shifts.

Good ^{1}H-chemical shift prediction is observed for *phenyl butyric acid* protons, except for H6, H7 and H9. These protons are close to the -COOH group, which could have an effect on the calculated chemical shifts if not parameterized accurately. The magnetic anisotropy effects and electric field effects have been investigated and incorporated into CHARGE-V7, but a thorough investigation of these effects on the -C-O bond also need to be performed. The R-factor calculated for this molecule shows good ^{1}H-chemical shift prediction by CHARGE-V7.

(b) ACD-V4 program-predicted ^{1}H-chemical shifts.

The ACD-V4 ^{1}H δ predictor program also produced good chemical shifts for most protons of phenyl butyric acid. For protons of the branched chain H8 and H9, inaccurate chemical shifts were produced. It could be observed that in the experimental results the shifts for H1, H2 and H3 are equal, but these are unequal in the ^{1}H chemical shifts produced by the ACD-V4 program. This could make it difficult to assign the predicted ^{1}H-chemical shifts in the spectrum of *phenyl butyric acid.*

4.5.8 *Quinaldic acid*



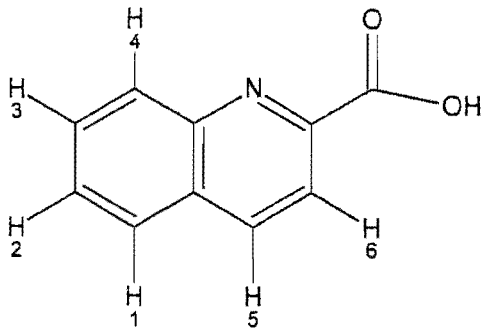


Figure 4.8: Structure of *quinaldic acid.*

The differences between the chemical shifts given by programs used for the prediction of *quinaldic acid* chemical shifts and the experimental values are presented in Table 4.8.

Table 4.8: Predicted ^{1}H Chemical shift[a] (ppm) differences of *quinaldic* acid relative to experimental values.

At.No	*Exp Shifts*	*CHARGE-^{1}H*	*Shift Diff*	*ACD-^{1}H*	*Shift Diff*
1	7.95	7.91	0.04	8.08	0.13
2	7.73	7.57	0.16	7.92	0.19
3	7.85	7.65	0.20	7.83	0.02
4	8.17	7.88	0.29	8.54	0.37
5	8.42	8.39	0.03	8.25	0.17
6	8.28	7.92	0.36	8.17	0.11
R-factor			**0.42**		**0.26**

[a] *chloroform solvent used.*

(a) CHARGE-V7 program-predicted ^{1}H-chemical shifts.

Accurate predictions are observed for H1 and H5. The H-H interactions of H6 and H atom of the hydroxyl group could have contributed to a high error observed for H6. The re-parameterization of *quinaldic acid* did not improve the predicted ^{1}H-chemical shifts results produced by CHARGE-V7.

(b) ACD-V4 program-predicted ^{1}H-chemical shifts.

Large errors in predicted ^{1}H chemical shifts are observed for H2, H4 and H5. H4 experimental chemical shift is upfield from H6, but the opposite is observed for the predicted chemical shifts. These differences would make it difficult to assign the ^{1}H-spectrum of *quinaldic acid* using predicted ^{1}H-chemical shifts. The overall accuracy of predicted chemical shifts for the ACD-V4 is better than that of CHARGE.

4.5.9 *2,3-benzofuran*

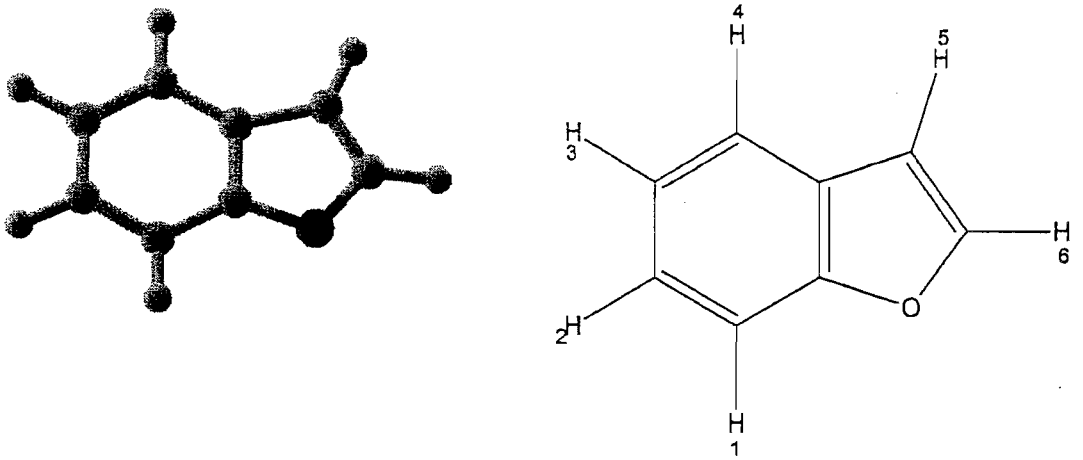


Figure 4.9: Structure of *2,3-benzofuran.*

The differences between the chemical shifts given by programs used for the prediction of *2,3-benzofuran* chemical shifts and the experimental values are presented in Table 4.9.

Table 4.9: Predicted ^{1}H Chemical shift[a] (ppm) differences of *2,3-benzofuran* relative to experimental values.

At.No	*Exp Shifts*	*CHARGE-^{1}H*	*Shift Diff*	*ACD-^{1}H*	*Shift Diff*
1	7.50	7.48	0.02	7.46	0.04
2	7.25	7.46	0.21	7.20	0.05
3	7.25	7.42	0.17	7.15	0.10
4	7.58	7.62	0.04	7.51	0.07
5	7.58	8.12	0.54	7.53	0.05
6	6.73	6.80	0.07	6.61	0.12
R-factor			**0.41**		**0.27**

[a] *chloroform solvent used.*

(a) CHARGE-V7 program predicted ^{1}H-chemical shifts.

Equivalent experimental chemical shifts are observed for H2 and H3 as well as for H4 and H5, whereby these are predicted differently by CHARGE-V7. This would make it difficult to assign ^{1}H-chemical shifts of the *benzofuran* spectrum using ^{1}H-chemical shifts predicted by CHARGE-V7. Furthermore a large error in the predicted^{1}H-

chemical shift is observed for H5, which is meta to the oxygen atom of the furan ring. The large errors observed in some predicted ^{1}H-chemical shifts indicate the need for the electron withdrawing effect of the heteroatom on the ring protons to be thoroughly investigated and incorporated into CHARGE-V7.

(b) ACD-V4 program-predicted ^{1}H-chemical shifts.

Unequivalent ^{1}H-chemical shifts for H2 and H3 are also predicted, and also those for H4 and H5, but this is contrary to those obtained experimentally. This could lead to uncertainty in the assignment of the *benzofuran* spectrum using predicted ^{1}H- chemical shifts. The overall prediction indicated by the R-factor is better than that of CHARGE.

4.5.10 *1-methylindole-3-carboxylic acid*

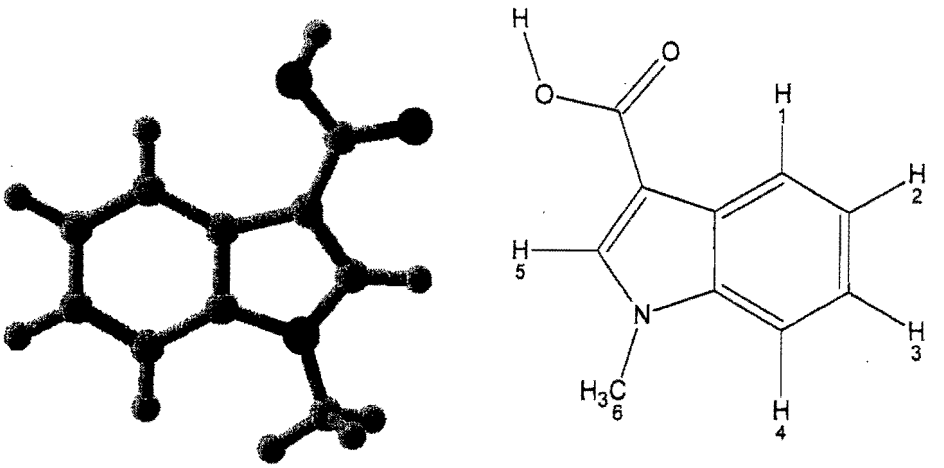


Figure 4.10: Structure of *1-methylindole-3-carboxylic acid.*

The differences between the chemical shifts given by programs used for the prediction of *1-methylindole-3-carboxylic acid* chemical shifts and the experimental values are presented in Table 4.10.

Table 4.10: Predicted ^{1}H Chemical shift[a] (ppm) differences *of 1-methylindole-3-carboxylic acid* relative to experimental values.

At.No	*Exp Shifts*	*CHARGE -^{1}H*	*Shift Diff*	*ACD -^{1}H*	*Shift Diff*
1	8.19	8.29	0.10	7.90	0.29
2	7.28	7.45	0.17	7.38	0.10
3	7.28	7.41	0.13	7.31	0.03
4	7.41	7.68	0.27	7.55	0.14
5	7.92	8.92	1.00	7.64	0.28
6	3.92	4.23	0.31	3.77	0.15
R-factor			**0.57**		**0.27**

[a] *chloroform solvent used.*

(a) CHARGE-V7 program predicted ^{1}H-chemical shifts.

Inaccurate prediction of H4, H5 and H6 chemical shifts are observed from CHARGE-V7. The poor chemical shift calculations for H5 could be due to the electron withdrawing effects of the carboxylic acid group adjacent to it. On the other hand the

prediction of the H1 chemical shift is not affected by the presence of the bulky carboxylic acid group in a gamma position to it. The experimental H2 and H3 chemical shifts are equal but this is contrary to the predicted ^{1}H-chemical shifts. It could be difficult to assign these protons in the spectrum of *1-methyindole-3-carboxylate*.

(b) ACD-V4 program-predicted ^{1}H-chemical shifts.

The ACD-V4 ^{1}H δ predictor program produced a large chemical shift error for H1 in a gamma position to the carboxylic acid group. Inaccurate predictions of the shifts for H5 and H6 are also observed. However, the overall accuracy of prediction indicated by the R-factor is better than that for CHARGE-V7.

4.5.11 *3-Indol-acetonitrile*

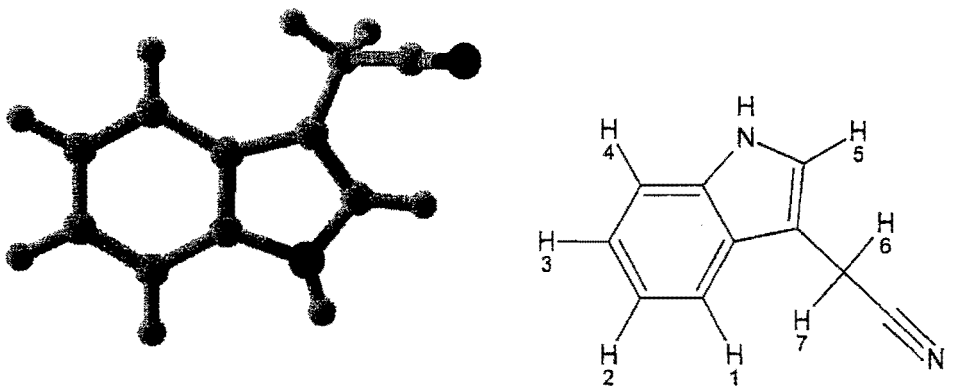


Figure 4.11: Structure of *3-Indol-acetonitrile.*

The differences between the chemical shift given by programs used for the prediction of *3-Indol-acetonitrile* chemical shifts and the experimental values are presented in Table 4.11.

Table 4.11: Predicted ^{1}H Chemical shift[a] (ppm) differences of *3-Indol-acetonitrile* relative to experimental values.

At.No	*Exp Shifts*	*CHARGE -^{1}H*	*Shift Diff*	*ACD -^{1}H*	*Shift Diff*
1	7.59	7.49	0.10	7.48	0.11
2	7.19	7.28	0.09	7.20	0.01
3	7.24	7.31	0.07	7.20	0.04
4	7.38	7.44	0.06	7.48	0.10
5	7.19	7.85	0.66	7.01	0.18
6,7	3.82	3.89	0.07	3.66	0.16
R-factor			**0.38**		**0.25**

[a] *chloroform solvent used.*

(a) CHARGE-V7 program predicted ^{1}H-chemical shifts.

Good CHARGE-V7 prediction of ^{1}H-chemical shift is observed for most protons of 3-*indole-acetonitrile*. The large chemical shift error for H5 confirms the inability of CHARGE-V7 to predict the ^{1}H-chemical shift for an -H atom adjacent to the -N atom of the *indole* group.

(b) ACD-V4 program-predicted ^{1}H-chemical shifts.

The chemical shifts predicted by the ACD-V4 program are accurate for most protons of *indol-acetonitrile,* with the exception of H1, H5, H6,7. H5 chemical shift prediction could be influenced by the effects of the bulky acetonitrile group adjacent to it.

4.5.12 CHARGE-V7 and ACD-V4 ^{1}H programs on simple chemical compounds.

Even though the accuracy of the chemical shifts predicted for most of the chemical compounds used are good, large errors are common in predicting the shifts for protons alpha to the heteroatom in the fused heteroaromatics. In the case of methyl indole-3-carboxylic acid this error may be partially reduced by an opposing error due to carboxylic acid group. In pyridine substituted compounds, there is improvement of results in the newly parameterised CHARGE.

The calculated chemical shifts of rigid compounds such as *quinaldic acid* and *2,3 benzofuran* showed similar R-factors to that of compounds with bulky substituents, for example in *2-phenyl butyric acid* and *1-(4-nitrophenyl)piperazine.*

Good chemical shifts for the methyl protons no.9 of *2-phenyl butyric acid*, and no.6 of *phenylacetate* is observed for both CHARGE-V7 and ACD-V4. ACD results have been found to be better than CHARGE-V7 in most of compounds investigated except those showing a high R-factor.

REFERENCES

1. Stephen R. Heller, J. Chem. Inf. Comput. Sci., **34**, (1994), 1026.
2. WWW: http://www.hyper.com/.
3. http://www.bruker.com/nmr/.
4. J. Schuur: Nachr. Chem. Tech. Lab., **45**, (1997), 401.
5. M.G. Weller: On-line Inf., **15**, (1991), 61.
6. J. Chem. Ed, **62**, (1985), 4456.
7. D. Redeker, M. Ballabio, D. Borghi, E. Martinelli: Chemom. Intell. Lab. Syst., **19**, (1993), 233.
8. D Canzler, M. Hellenbrandt: Fresenius, J.Anal.Chem., **344** , (1992), 167.
9. Raymond J. Abraham, Progress in Nuclear Resonance spectroscopy, **35**, (1999), 85-152.
10. Raymond J. Abraham, Marcos Canton, M. Reid, J. Chem. Soc. Perkin Trans.2, **3**, (1999), 7.
11. R. J. Abraham, Mark Edgar, Robert P. Glover, Mark A. Warne, Lee Griffiths, Substituted chemical shifts in spectroscopy, J. Chem. Soc. Perkin Trans 2, (1997), 33.
12. R.J.Abrahams , Mark A. Warne, Lee Grifiths, Magn. Reson. Chem., **36**, (1998), 180.
13. J.Hinze, H. H. Jaffe, J. Phys. Chem, **67**, (1963), 1501.
14. R. J. Abraham, L. Griffith, M. A. Warne, J. Chem. Soc. Perkin Trans II, (1998), 1751.
15. C.P. Rader, J. Am. Chem. Soc., **91**, (1969), 3248.
16. R. J. Abrahams , Mark A. Warne and Lee Griffiths, J. Chem. Soc. Perkin Trans II, (1997), 2151.
17. R. J. Abraham, M. Edgar and L. Griffiths, R. J. Powell, J. Chem. Soc. Perkin Trans II, (1995) 561.
18. A. A. Bothner-by, C. Naar-Colin, J. Am. Chem. Soc., **80**, (1958), 1728.
19. http:/lasher.vei.co.uk/ve/nmrec3/exhibits/Advanced Chemistry Development_prid.html.
20. www.acdlabs.com/pblish/publ_press98.html.
21. W. Bremser, Anal. Chim. Acta, **103**, (1978), 85-365.

22. M.E. Elyashberg, E. R. Mortirosian, Yu Z. Karasev, H. Thiele and H. Sonberg, Anal. Chim. Acta, **337,** (1997), 265.
23. K. Funatsu and S. Sasaki, J. Chem. Inf. Comp. Sci, **36**, (1996), 190.
24. Fred A. Turner, The Chemical Bulletin, (1996).
25. T. Williams, J. Chem Inf. Comput.Sci., **37**, (1997), 726.
26. Scott van Bramer, Concepts in Magn. Reson., **9** (4), (1997), 271.
27. Antony Williams, S. Golotvin, V. Lasin, S. Burkulin: High throughput Screening Software Tools for Analytical Spectroscopy, 39th Eastern Analytical Symposium and Exposition, 29 Oct-3 Nov 2000.
28. www.acdlabs.com/products/spec_lab/predict_nmr/hnmr.
29. Raymond J. Abraham, Chemistry department, The University of Liverpool, Crown Street, P. O. Box 147,L69 3BX, UK.
30. H. J. Scheider, U. Buchheit, N. Becker, G. Schmidt, J. Chem Soc., **107**, (1985), 7027.
31. L.D.Hall, Tet Lett., **23**, (1964), 1457.
32. R.J. Abrahams, L. Griffiths, M.A Warne and Lee Griffiths, J. Chem. Soc. Perkin Trans II, (1998), 1751.
33. R. J. Abrahams, L. Griffiths, M.A Warne, J. Chem. Soc. Perkin Trans II, (1997), 2151.
34. R. J. Abrahams, L. Griffiths, M. A Warne and Lee Griffiths, J. Chem. Soc. Perkin Trans II, (1996), 333.

CHAPTER 5

5. Discussion of Chemical shifts Differences for Alkaloids

5.1 Introduction

CHARGE-V7, SpecInfo-V3.2 and ACD-V4 ^{1}H chemical shift predictor programs were also used to calculate the ^{1}H chemical shifts for the alkaloids introduced in Chapter 3. SpecInfoV3.2 introduced in chapter 1, was also used for the evaluation of alkaloids due to the complexity of alkaloid ^{1}H spectra . The accuracy of the predicted chemical shifts was evaluated and the programs were compared with one other. ACD-V4 and SpecInfo-V3.2 ^{13}C chemical shift predictor programs were also used to calculate ^{13}C chemical shifts for the selected alkaloids. ^{13}C chemical shifts generated by the programs were also compared to one another and to those found in literature. The R-factor explained in chapter 4 was also used to evaluate the accuracy of these programs. The same criteria for the evaluation of chemical shift difference and R-factor used in simple chemical compounds was also used to evaluate the accuracy of these programs on alkaloids.

5.1.1 Scopoline

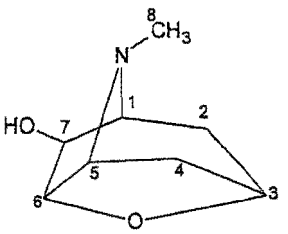


Figure 5.1: structure of *scopoline.*

The differences between the chemical shifts given by programs used for the prediction of *scopoline* chemical shifts and the experimental values are presented in Table 5.1.

Table 5.1: Predicted ^{1}H and ^{13}C Chemical shift[a] differences of *scopoline* relative to experimental values.

At No.	*CHARGE-^{1}H*	*ACD-^{1}H*	*SpecInfo-^{1}H*	*ACD-^{13}C*	*SpecInfo-^{13}C*
1	0.60	0.07	0.09	1.30	3.50
2	0.24	0.10	0.16	4.40	8.40
3	0.25	0.07	0.48	1.50	7.70
4	0.10	0.73	0.23	6.70	11.00
5	0.33	0.56	0.47	2.10	1.50
6	0.50	0.89	0.19	4.00	14.90
7	0.19	2.27	0.42	50.30	23.10
8	0.16	0.89	0.29	26.60	2.30
R-factor	**0.16**	**0.84**	**0.52**	**3.48**	**1.54**

[a] *chloroform solvent used.*

(a) CHARGE-V7 program predicted ^{1}H-chemical shifts.

The CHARGE-V7 program did not accurately predict the chemical shifts of *scopoline* H1, H2, H5 and H6. The program did predict the H7 chemical shift accurately even though the carbon of this proton is directly attached to an electron withdrawing OH group. The overall predicted ^{1}H-chemical shifts for the *scopoline* structure, indicated by the R-factor, is observed to be more accurate than that predicted by the ACD-V4 ^{1}H δ predictor program and also better than that of SpecInfo-V3.2 ^{1}H predictor program. The results obtained from the calculated chemical shifts by CHARGE-V7 program

indicate that the program can be used successfully in the assignment of the *scopoline* ^{1}H-NMR spectrum.

(b) ACD-V4 program-predicted ^{1}H-chemical shifts.

The ACD-V4 ^{1}H δ predictor program gave mainly good ^{1}H-chemical shift predictions. However, there was a large error in the calculated H7 chemical shift. This is contrary to what was obtained from the CHARGE-V7, showing that the effect of the de-shielding OH group was not adequately taken into account by this program. Large errors in ^{1}H-NMR chemical shift predictions are also observed for H4, H5 and H8. The R-factor obtained from the ACD-V4 ^{1}H δ predictor program is higher than that from the CHARGE-V7. There would be some ambiguity in the assignment of H2 and H4 methylene protons using the predicted chemical shifts. These protons are calculated to have the same chemical shift by the ACD-V4 program. Experimentally H2 is split into a doublet of doublets at 2.3ppm and a doublet of multiplets at 1.2ppm, while H4 is also split to a doublet of doublets, but at 1.8ppm and a doublet of multiplets at 1.4ppm.

(c) SpecInfo-V3.2 program-predicted ^{1}H-chemical shifts.

SpecInfo ^{1}H predictor program's predicted chemical shifts were found to be in general better than those calculated by CHARGE-V7 and the ACD-V4. However, the overall R-factor was larger than that obtained using CHARGE. In this case, the major contributor to the R-factor was the inaccuracy of the calculated H3, 4, 5, 7 and 8 chemical shifts. The error in H3 and H5 is understandable as these are protons on bridgehead carbons. However, H4 are ring methylene protons and H8 are N-methyl protons. The calculation of H6, a proton attached to a bridgehead carbon with an electron-withdrawing substituent, is within experimental error. There would be some confusion in the assignment of chemical shifts of the spectrum of *scopoline* using the SpecInfo program. The predicted H2 chemical shift is very close to the value for H4 measured experimentally and vice versa. Also, the same ^{1}H-chemical shifts are calculated for H6 and H7, which was not observed in the experimental results.

(d) ACD-V4 program-predicted ^{13}C -chemical shifts.

The ACD-V4 ^{13}C chemical shift predictor program generally gave poor results in the prediction of chemical shifts of *scopoline*. In fact the R-factor is dominated by the relatively large errors in the calculated chemical shifts of C7 and C8. This is surprising as the chemical shifts of the other carbons with electron withdrawing substituents are faithfully reproduced.

(e) SpecInfo-V3.2 program-predicted ^{13}C-chemical shifts.

The SpecInfo-V3.2 ^{13}C chemical shift predictor program also gave poor ^{13}C-chemical shifts predictions for most carbons of *scopoline,* especially C2, C3, C4, C6 and C7 which have large errors in the calculated chemical shift. The R-factor indicates that the overall chemical shift prediction was better than that obtained from predictions performed by the ACD-V4 ^{13}C δ predictor program. However, equal chemical shifts are predicted for C6 and C7. This would be confusing in the assignment of ^{13}C-chemical shifts based on this program, as this is not the case in the experimentally observed chemical shift.

(f) Comparison of CHARGE-V7, SpecInfo-V3.2 and ACD-V4 programs.

The ACD-V4 ^{1}H and SpecInfo-V3.2 ^{1}H δ predictor programs predicted the chemical shifts of the H4 methylene protons inaccurately, whereas CHARGE-V7 gave good chemical shift values for these protons. A similar trend is observed in the prediction of H5 and H8 in all three programs used to calculate proton chemical shifts of *scopoline*. Poor predictions are obtained from the ACD-V4 ^{1}H and ^{13}C δ predictor programs, indicating that the ACD-V4 program is not capable of predicting the N-methylene protons. The ACD-V4 ^{13}C δ predictor program gave the worst chemical shift results compared to all other programs. The R-factors indicate that the CHARGE-V7 chemical shift results are better than those of the other programs used. Thus the chemical shifts for sc*opoline* are predicted inaccurately by all ^{1}H and ^{13}C programs used except the ^{1}H chemical shifts predicted by CHARGE-V7. This is shown in Table 5.1 by the R-factors, which are all greater than 0.1.

5.1.2 Lupinine

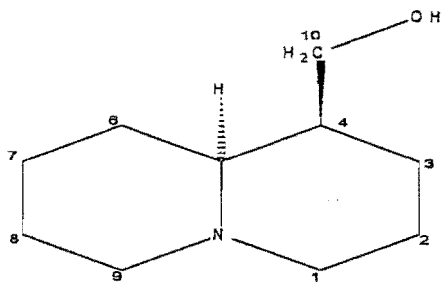


Figure 5.2: structure of *lupinine*.

The differences between the chemical shifts given by programs used for the prediction of *lupinine* chemical shifts and the experimental values are presented in Table 5.2.

Table 5.2: Predicted ^{1}H and ^{13}C-Chemical shift[a] differences of lupinine relative to experimental values.

At No.	*CHARGE-^{1}H*	*ACD-^{1}H*	*SpecInfo-^{1}H*	*ACD-^{13}C*	*SpecInfo-^{13}C*
1	0.87	0.89	0.29	0.20	1.40
2	0.08	0.14	0.17	0.50	3.20
3	0.32	0.19	0.23	0.50	2.50
4	0.03	0.23	0.06	2.20	0.10
5	0.15	0.16	0.09	0.40	6.90
6	0.10	1.06	0.17	0.60	4.50
7	0.03	0.14	0.00	0.10	2.30
8	0.02	0.08	0.06	0.00	1.10
9	0.23	0.11	0.62	0.20	4.50
10	2.41	0.31	0.51	1.30	1.30
R-factor	**0.46**	**0.37**	**0.36**	**0.20**	**1.17**

[a] *chloroform solvent used.*

(a) CHARGE V7 program predicted ^{1}H-chemical shifts.

Inaccurate prediction of H1, H3 and H10 chemical shifts for *lupinine* are observed. The H10 calculated ^{1}H-chemical shift is very low compared to the experimental value. This could be due to the effect of its attachment to the electron withdrawing OH-group. This is contrary to the good prediction observed in 2-nitrobenzyl alcohol due to the

structure of 2-nitrobenzyl alcohol being specifically catered for in CHARGE-V7 program by reparameterizing the molecule. The R-factor also indicates poor prediction, mainly due to large errors in the chemical shifts predicted for H10. The ^{1}H-chemical shifts calculated by the CHARGE-V7 program could be used without any confusion in the assignment of the *lupinine* spectrum, but the closeness in chemical shifts predicted for H4 and H6 would make it difficult to distinguish between them.

(b) ACD-V4 program-predicted ^{1}H-chemical shifts.

The ^{1}H chemical shift is not predicted accurately by ACD-V4 ^{1}H chemical shift predictor program. The difference in chemical shift errors for H1 and H9 show inconsistency in their predicted chemical shifts even though they are in similar chemical environment in the structure of *lupinine*. Particularly poor prediction of the H6 chemical shift is observed. The overall accuracy of prediction of chemical shifts by the ACD-V4 ^{1}H δ program is better than that given by the CHARGE-V7 program. The order of increasing chemical shifts calculated for H2, H3 and H4 is different to that observed in the experimental results. This would lead to difficulties in the assignment of the *lupinine* spectrum using these predicted chemical shifts.

(c) SpecInfo-V3.2 program-predicted ^{1}H-chemical shifts.

Accurate predictions are observed for H4, H7, H8 and H9. However, H2, H7 and H8 are predicted to have the same chemical shifts. This could lead to confusion in the correct assignment of these protons in the *lupinine* spectrum. Nevertheless, the overall accuracy of prediction indicated by the R-factor is similar to that of ACD-V4 program.

(d) ACD-V4 program-predicted ^{13}C-chemical shifts.

The prediction of ^{13}C-chemical shifts by the ACD-V4 is inaccurate for most carbons of *lupinine*. The R-factor showed even better accuracy of results compared to that given by ACD-V4 program for the ^{1}H shifts. The predicted ^{13}C-chemical shifts could be used without any confusion in the assignment of the *lupinine* spectrum.

(e) SpecInfo-V3.2 program-predicted ^{13}C-chemical shifts.

Prediction of ^{13}C-chemical shifts by the SpecInfo program is inaccurate for all carbon atoms except C4. The large error in the chemical shifts of these carbons led to a greater R-factor being obtained from the SpecInfo program than that from ACD-V4. This shows inaccuracy in the overall ^{13}C-chemical shift prediction. Notwithstanding the poor agreement between the calculated and the observed ^{13}C chemical shifts, the correct order of chemical shifts was obtained. Therefore the SpecInfo-V3.2 program could have been used to assign *lupinine* spectrum unambiguously.

(f) Comparison of CHARGE-V7, SpecInfo-V3.2 and ACD-V4 programs.

Good chemical shift prediction is observed from the SpecInfo ^{1}H predictor program, ACD-V4 ^{1}H, and ACD-V4 ^{13}C δ predictor programs. Although the R-factor for CHARGE is large, this is not a true indication of the overall accuracy of results because only H10 has a large error. The individual CHARGE results are actually good.

5.1.3 *Piperine*

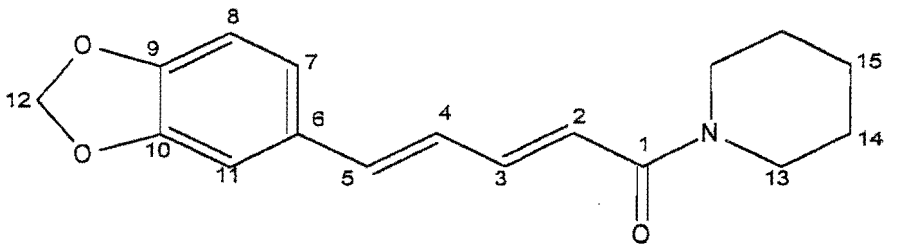


Figure 5.3: structure of *piperine.*

The differences between the chemical shifts given by programs used for the prediction of *piperine* chemical shifts and the experimental values are presented in Table 5.3.

Table 5.3: Predicted ^{1}H and ^{13}C-Chemical shift[a] differences of piperine relative to experimental values.

At no.	*CHARGE-^{1}H*	*ACD-^{1}H*	*SpecInfo-^{1}H*	*ACD-C13*	*SpecInfo-C13*
1	-	-	-	0.90	1.00
2	0.26	0.05	0.20	0.10	1.90
3	0.49	0.06	0.02	0.30	0.70
4	2.64	0.23	0.11	0.20	2.00
5	0.73	0.20	0.10	0.40	6.90
6	-	-	-	0.10	2.00
7	5.44	0.04	0.13	0.30	7.60
8	2.94	0.02	0.17	0.20	0.20
9	-	-	-	0.30	0.70
10	-	-	-	0.30	0.70
11	4.38	0.05	0.26	0.20	5.90
12	0.43	0.44	0.05	0.20	0.20
13	0.53	0.02	0.24	3.50	2.40
14	0.08	0.01	0.07	0.90	0.60
15	0.11	0.07	0.13	0.90	1.10
16	0.06	0.01	0.07	0.60	0.60
17	0.37	0.10	0.24	2.90	1.10
R-factor	**0.61**	**0.14**	**0.27**	**0.62**	**0.39**

[a] *chloroform solvent used.*

(a) CHARGE-V7 program predicted ^{1}H-chemical shifts.

CHARGE-V7 produced large chemical shift errors for several protons of *piperine*. The effects of alkenes have been investigated for their incorporation into CHARGE-V7 but H2 is the only proton of the unsaturated chain, which was predicted accurately by the program. Although H12 is directly bonded to two oxygen atoms, these do not have much effect on the predicted ^{1}H-chemical shift. The protons of the piperidine ring are predicted more accurately than most protons of *piperine*. However, although H14 and H15 of the piperidine ring experimental chemical shifts are not equivalent, the calculated chemical shifts are not equal. Although CHARGE-V7 usually calculates the ^{1}H-chemical shifts of the aromatic ring accurately, H7, H8 and H11 are out of order compared to the experimental values. The high error in chemical shift calculation for several protons of the *piperine* structure shows that CHARGE-V7 is not the program of choice for calculating ^{1}H-chemical shifts for *piperine*.

(b) ACD-V4 program-predicted ^{1}H-chemical shifts.

The ACD-V4 program predicted most of the *piperine* ^{1}H-chemical shifts accurately with the exception of H4 and H12. These chemical shift results are more accurate than those obtained from CHARGE-V7; this is also confirmed by the low R-factor. Although good predictions are obtained from ACD -V4 predicted ^{1}H-chemical shifts, H14, H15 and H16 have equal chemical shift values, which would make it difficult to identify H15 in the spectrum of *piperine* using the predicted chemical shifts.

(c) SpecInfo-V3.2 program-predicted ^{1}H-chemical shifts.

The SpecInfo program also produced good results for most protons of piperine. The exceptions were H11, H13 to H17, which indicates difficulty of using SpecInfo-V3.2 to predict chemical shifts for the piperidine ring. H7 is found to be more de-shielded than H8 in the predicted chemical shifts, whereas the opposite trend is observed in the experimental results. Notwithstanding, the overall results produced for *piperine* are very good as can be seen from the low R-factor. The accuracy of the chemical shifts

predicted by the SpecInfo-V3.2 program is better than that obtained from the CHARGE-V7 and worse than from the ACD-V4 ^{1}H program.

(d) ACD-V4 program-predicted ^{13}C-chemical shifts.

Good ^{13}C-chemical shift predictions were obtained by the ACD-V4 ^{13}C δ predictor program in the case of *piperine*. Inaccurate chemical shifts predictions are observed, however for C13 to C17 of the piperidine ring. The R-factor indicates good overall accuracy of prediction of ^{13}C-chemical shifts. Unequivalent ^{13}C experimental chemical shifts are observed experimentally for C14, C15 and C16. This is contrary to the equivalent chemical shifts observed from the ACD-V4 ^{13}C program.

(e) SpecInfo program-predicted ^{13}C-chemical shifts.

^{13}C-chemical shifts for C2, C7, C11, C13 and C15 are predicted inaccurately compared to the rest of the carbons of *piperine*. Good prediction of ^{13}C-chemical shifts was observed for C1, C3, C8, C9, C10 and C12. The chemical shifts predicted for the carbon atoms of piperidine are more accurate than those predicted by the ACD-V4 ^{13}C δ predictor program. Good prediction of the equivalent C14 and C16 carbon atoms is also observed.

(f) Comparison of CHARGE-V7, SpecInfo-V3.2 and ACD-V4 programs.

The shifts for the tertiary carbons 6, 9 and 10 are predicted accurately by both ACD-V4 ^{13}C and SpecInfo ^{13}C δ predictor programs. Similarities are observed in the ^{1}H-NMR chemical shifts for H12 as predicted by CHARGE-V7 and ACD-V4 ^{1}H δ predictor programs. The CHARGE-V7 program produced the worst prediction of ^{1}H chemical shift compared to all other programs used in the prediction of ^{1}H-NMR chemical shift for *piperine*. This was mainly due to the large error obtained for the aromatic region. The ACD-V4 ^{1}H program gave the best results, even though the shifts of the piperidine ring were not predicted accurately.

5.1.4 *Conhydrine*

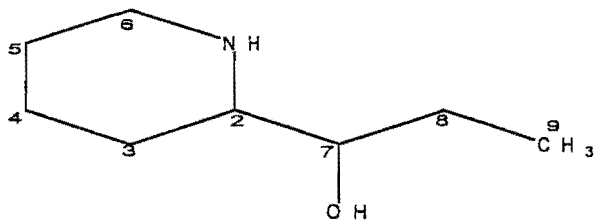


Figure 5.4: structure of *conhydrine.*

The differences between the chemical shifts given by programs used for the prediction of *conhydrine* chemical shifts and the experimental values are presented in Table 5.4.

Table 5.4: Predicted ^{1}H and ^{13}C-Chemical shift[a] differences of conhydrine relative to experimental values.

At No.	*CHARGE-^{1}H*	*ACD-^{1}H*	*SpecInfo-^{1}H*	*ACD-^{13}C*	*SpecInfo-^{13}C*
2	0.07	0.35	0.26	10.70	1.50
3	0.17	0.50	0.35	3.10	3.10
4	0.19	0.02	0.23	1.50	0.20
5	0.21	0.39	0.14	1.10	0.30
6	0.00	0.13	0.13	0.70	0.60
7	0.52	0.09	0.06	1.90	7.20
8	0.03	0.02	0.02	2.10	0.10
9	0.07	0.02	0.01	0.30	0.30
R-factor	**0.28**	**0.23**	**0.16**	**1.14**	**0.72**

[a] *chloroform solvent used.*

(a) CHARGE-V7 program predicted ^{1}H-chemical shifts.

H4, H5 and H7 protons are calculated inaccurately by CHARGE-V7. H7 is directly bonded to an OH group, and the electron withdrawing effects of the oxygen atom could contribute to the inaccurate calculation of protons closer to it. Good chemical shift prediction is observed for H2, H6, H8 and H9. Precise calculation of H6 chemical shift is observed; this is equal to the experimental value (2.87ppm). The low R-factor obtained shows that CHARGE-V7 calculated chemical shifts are similar to the experimental ^{1}H-chemical shifts obtained. The ^{1}H-chemical shifts produced could be

used without any difficulty in the assignment of chemical shifts for the *conhydrine* spectrum.

(b) ACD-V4 program-predicted ^{1}H-chemical shifts.

The ACD-V4 program showed large errors for H2, H3 and H5 ^{1}H-chemical shifts. The directly bonding of the H2 to the N atom could have any effect on the high error observed. However, accuracy in prediction of ^{1}H-chemical shifts is observed for the other protons of *conhydrine* especially H4, H7, H8 and H9. The relatively low R-factor obtained indicates the accuracy of the ACD- V4 ^{1}H δ predictor program to produce accurate chemical shifts for this structure. These predicted chemical shifts could be used without any ambiguity in the assignment of the *conhydrine* spectrum.

(c) SpecInfo-V3.2 program-predicted ^{1}H-chemical shifts.

Accurate ^{1}H-chemical shifts were also produced by the SpecInfo program, with the exception of those for H2 and H3 and H4. H2 is directly bonded to a NH group and this could have contributed to the large error in chemical shift obtained for this proton. The overall accuracy of predicted ^{1}H-chemical shifts indicated by the R-factor is in trend with that of the CHARGE-V7 and ACD-V4. There would be some confusion in the assignment of the *conhydrine* spectrum using SpecInfo-V3.2 predicted chemical shifts, because two protons, H4 and H5 are predicted to have the same chemical shifts, while in fact they are experimentally different.

(d) ACD-V4 program-predicted ^{13}C-chemical shifts.

The ACD-V4 ^{13}C δ predictor program accurately predicted the carbon chemical shifts for *conhydrine, with* the exception of C2, C3 and C8. The effects of the OH group could explain the poor prediction of the C2 chemical shifts. The overall accuracy of predicted ^{13}C chemical shifts, indicated by the R-factor value showed that ACD-V4 ^{13}C δ predictor program was capable of predicting ^{13}C chemical shifts for *conhydrine*.

(e) SpecInfo-V3.2 program-predicted ^{13}C-chemical shifts.

The SpecInfo-V3.2 ^{13}C program gave large errors in the prediction of C1, C2 and C7 in *conhydrine*. However, the program gave reasonable ^{13}C-chemical shifts for the piperidine ring. Confusion in the assignment of C3, C4 and C5 would be experienced if the predicted ^{13}C chemical shifts were used in the assignment of the spectrum. The predicted C3 chemical shift is higher than those for C4 and C5, whereas in the experimental results, the C3 chemical shift is less than C4 and C5. The R-factor obtained from the SpecInfo-V3.2 ^{13}C predictor program indicates good prediction of ^{13}C-chemical shifts for *conhydrine*.

(f) Comparison of CHARGE-V7, SpecInfo-V3.2 and ACD-V4 programs.

A common trend of poor prediction of H3/C3 chemical shifts was observed from all programs. The SpecInfo ^{13}C program provided the best overall results compared to all the other programs. The ACD-V4 ^{1}H δ predictor program gave worse chemical shift results than all the programs used to predict chemical shifts of *conhydrine*. Large chemical shift errors are observed for the *piperidine* ring protons in all programs except the SpecInfo-V3.2 ^{13}C program. The R-factors indicate that all programs used were capable of predicting chemical shifts for *conhydrine*, with an acceptable degree of accuracy.

5.1.5 *Eseridine*

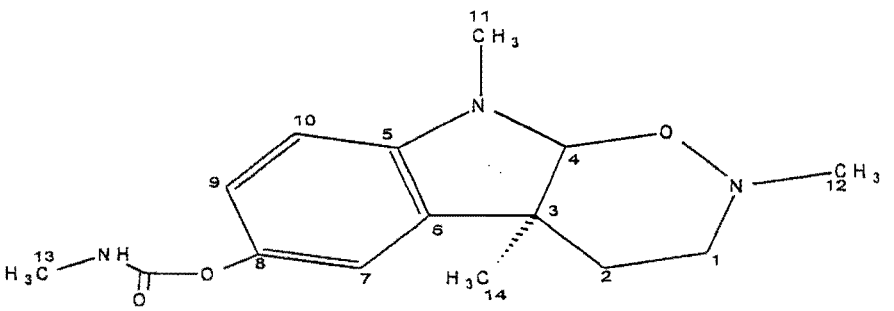


Figure 5.5: structure of *eseridine*.

The differences between the chemical shift given by programs used for the prediction of *eseridine* chemical shifts and experimental shift values are presented in Table 5.5.

Table 5.5: Predicted ^{1}H and ^{13}C-Chemical shift[a] differences of *eseridine* relative to experimental values.

At no.	CHARGE - ^{1}H	ACD-^{1}H	SpecInfo-^{1}H	ACD-^{13}C	SpecInfo-^{13}C
1	0.25	0.63	0.01	0.20	0.80
2	0.44	0.19	0.23	1.50	6.30
3	-	-	-	10.00	4.70
4	0.69	1.04	0.05	2.50	2.00
5	-	-	-	5.70	4.70
6	-	-	-	0.70	2.40
7	1.83	0.60	1.04	3.50	3.40
8	-	-	-	6.20	12.90
9	0.35	0.13	0.41	9.60	2.80
10	0.93	0.51	0.15	0.20	7.60
11	0.18	0.34	0.02	0.50	4.00
12	0.11	0.04	0.04	0.10	6.30
13	0.27	0.81	0.60	35.50	24.60
14	0.17	0.24	0.27	0.20	2.60
R-factor	**0.34**	**0.54**	**0.25**	**1.47**	**1.46**

[a] *chloroform solvent used.*

(a) CHARGE-V7 program predicted ^{1}H-chemical shifts.

The CHARGE-V7 program predicted ^{1}H-chemical shifts for *eseridine* accurately except in the case of H2, H4, H7, H9 and H10. The direct bonding of C4 to oxygen and nitrogen atoms, could contribute to a large error in the chemical shift value obtained for the attached proton. Anisotropic effect of the -COOH group could also be contributing to the large error obtained in the chemical shift calculated for H7 and H9. The prediction of shifts for the methyl groups bonded to the N-groups no.11 and 12 is good showing the capability of CHARGE-V7 to predict shifts for methyl groups attached to a N atom. The ^{1}H-chemical shifts predicted by CHARGE-V7 could be used in the assignment of *eseridine* spectra without any ambiguity. The R-factor for *eseridine* ^{1}H-chemical shifts is comparable to that obtained from the ACD-V4 ^{1}H δ predictor program, but worse than that from the SpecInfo-V3.2 ^{1}H program.

(b) ACD-V4 program-predicted ^{1}H-chemical shifts.

The ACD-V4 ^{1}H program produced very good results for the H9 and H12 protons. The shifts for the H1, H4, H7, H11 and H13 protons on the other hand are predicted with an error of > 0.5, indicating inaccuracy in the predicted chemical shifts. The R-factor shows poor overall accuracy of prediction for *eseridine* by the ACD-V4 ^{1}H δ predictor program compared to CHARGE-V7. It can be observed from Table 5.5 that all the poorly predicted ^{1}H-chemical shifts are for environments where carbons are directly attached to the electron withdrawing nitrogen atom. The methyl protons no. 11 and methylene protons no. 1 are predicted to be equivalent. This could lead to difficulty in assigning predicted chemical shifts in the *eseridine* spectrum.

(c) SpecInfo-V3.2 program-predicted ^{1}H-chemical shifts.

Good chemical shift prediction is observed for all protons of *eseridine* except the aromatic ring protons H7, H9 and H10. The high error observed in these protons could be due to the presence of the electron withdrawing CH_3NHCOO- group of the benzyl ring attached to C8. Although the overall chemical shift prediction indicated by the R-factor is better than that obtained from CHARGE-V7, the magnitude of this factor also show poor prediction in general.

(d) ACD-V4 program-predicted ^{13}C-chemical shifts.

C13 has the largest chemical shift error compared to all other chemical shift predictions performed on *eseridine*. This chemical shift error could be due to the effect of its direct bonding to the N atom of -NHCOO- group attached to the aromatic ring. The ^{13}C-chemical shifts predicted by the ACD-V4 ^{13}C δ predictor program could be used without any ambiguity in the assignment of the *eseridine*. The presence of the -$OCONHCH_3$ group in the aromatic ring was not observed to have an effect on the prediction of the ^{13}C-chemical shifts of carbons close to it (i.e C7, C9 and C10), as these are comparable to the experimental values. The overall accuracy of prediction indicated by the R-factor showed a similar chemical shift prediction to SpecInfo-V3.2 ^{13}C.

(e) SpecInfo-V3.2 program-predicted ^{13}C-chemical shifts.

SpecInfo also showed a large chemical shift error in ^{13}C-chemical shift prediction for *eseridine* although the predicted are better than those obtained from the ACD-V4 program. Although C3 and C11, are in different chemical environment in the structure of *eseridine*, these carbon atoms are predicted to have equal ^{13}C-chemical shifts. This could lead to difficulty in assigning the *eseridine* spectrum.

(f) Comparison of CHARGE-V7, SpecInfo-V3.2 and ACD-V4 programs.

Inconsistency in the prediction of shifts for the CH_3 groups 11, 12, 13, and 14 is observed from the different programs. Very good chemical shift results are observed from ACD-V4 ^{13}C for C12, but this program also gave the worst results for C13, showing further inconsistency. CHARGE-V7 also did not give good results for these methyl protons. The large chemical shift error observed from these programs show that they have difficulty in dealing with the effects of the N-atom attached to the methyl group. There is not much difference in the predicted chemical shifts for atoms of the aromatic ring, although better predictions are observed for the ^{13}C-chemical shifts than the ^{1}H-chemical shifts.

5.1.6 Comparison of the performance of CHARGE-V7, SpecInfo-V3.2 and ACD-V4 programs on Alkaloids.

In general the programs used in the prediction of chemical shifts for alkaloids produced poor results for both ^{1}H and ^{13}C-chemical shifts. ACD-V4 ^{1}H δ predictor program gave the worst results for *lupinine* compared to all the other programs used. The *lupinine* structure consists of several $-CH_2$ groups in a ring, which could lead to large chemical shift errors in programs using structural fragments if the interactions between these methylenes are not well catered for. The poor prediction of methylenes by the ACD-V4 ^{1}H δ predictor program is also observed in the chemical shifts predicted for *conhydrine* and *scopoline.*

SpecInfo-V3.2 ^{13}C and ACD-V4 ^{13}C δ predictor program showed good chemical shift predictions for rigid structures and especially the equivalent tertiary carbons 9 and 10 in *piperine*. Although the shifts for the tertiary carbons are predicted accurately in most compounds, carbons directly bonded to a N-atom were in general, poorly simulated.

Inconsistency in the prediction of ^{1}H and ^{13}C chemical shifts of the benzene ring is observed in *eseridine* and *piperine*. The prediction of benzene rings protons in simple compounds produced good results due to the reparameterization of *piperidine* and *eseridine* in CHARGE-V7, and their presence in the structural database of ACD-V4 program.

^{1}H and ^{13}C-atoms adjacent to heteroatoms (i.e oxygen and nitrogen) produced inconsistent chemical shifts depending on which program is used. The same trend is observed in SpecInfo ^{1}H program, ACD-V4 ^{13}C-chemical shift predictor and SpecInfo-V3.2 ^{13}C δ predictor programs. The inconsistency in predicting the methylene protons was observed.

The R-factor observed from CHARGE-V7 showed poor prediction for alkaloids. This indicates a need for accurate parameterization of these molecules or those with similar structures.

ACD-V4 ^{1}H-chemical shift predictor program also showed poor overall prediction of all the alkaloids used except *piperine* (i.e R-factor >2). The absence of similar structures in ACD-V4 database might have contributed to poor predictions. ACD ^{13}C program predicted all the alkaloids inaccurately (R > 0.5).

SpecInfo ^{1}H chemical shift predictor program produced poor results for all alkaloids used (R>0.2) except for *conhydrine*. SpecInfo ^{13}C program was also very poor for all alkaloids used(R>0,5) except *piperine*.

In general, these program show inconsistency in predicting structures which have not been used in them before. CHARGE -V7 would need to be reparameterized to produce good predicted chemical shifts, and ACD-V4 and SpecIfo-V3.2 programs would require structural fragment similar to the predicted structures to be present in their database.

CHAPTER 6
CONCLUSION

The programs used in this investigation were selected due to their capability of predicting chemical shifts for different type of chemical compounds using different methods of calculations. ACD-V4 program, and SpecInfo-V3.2 use *empirical* calculations, whereby CHARGE-V7 program *use ab-initio* and *semi-empirical* calculations.

^{1}H and ^{13}C-NMR spectra could not be used alone for the assignment of chemical shifts due to the complexity of some compounds especially alkaloids. Spectroscopists have developed many multipulse techniques to keep up with the demand of faster and less time consuming interpretation of spectra. Two-dimensional NMR techniques namely HSQC, COSY, TOCSY and HMBC used in this thesis were sufficient in providing relevant coupling data required for the assignment of ^{1}H and ^{13}C spectra of all chemical compounds used. The extensive knowledge of the interpretation of the two-dimensional experiment is important for accurate assignment of complex spectra, for example the symmetry of *scopoline* structure could lead to incorrect assignment if its angles were not taken into account during assignments.

Accurate chemical shift results obtained for the simple chemical compounds indicate the effectiveness of re-parameterization of CHARGE to cater for the effects of certain atoms in them. CHARGE program showed an inability to predict ^{1}H-chemical shifts for protons adjacent to the heteroatoms, even after the re-parameterization was performed. Although CHARGE-V7 re-parameterization led to the improvement in the calculated ^{1}H-chemical shifts for simple chemical compounds, this process is expensive and also takes a lot of time, hence it does not guarantee the success of chemical shift calculation thereafter.

ACD-V4 and SpecInfo-V3.2 program's capability of using internal as well as user databases to automatically generate chemical shifts was mentioned earlier. Poor prediction of ^{1}H-chemical shifts produced by CHARGE- V7 and ACD-V4 programs is observed for all alkaloids except *conhydrine*. All programs used commonly predicted

methyl protons and carbons inaccurately. The poor calculation of the methyl groups might be due to the miscalculation of the electronic effects between the hydrogen atoms.

In general, the overall calculated ^{1}H-chemical shifts of the alkaloids indicate that ACD-V4 program is more accurate than SpecInfo-V3.2, which is more accurate than CHARGE-V7 program. ACD-V4 and SpecInfo programs similarly calculated ^{13}C-chemical shifts although ACD-V4 database is larger than that of SpecInfo-V3.2 program. The overall results produced for simple chemical compounds used indicated that CHARGE-V7 is capable of producing good chemical shift results if parameterization is thoroughly performed for new groups.

The chemical shift results obtained in this experiment also show that more work need to be performed on CHARGE-V7 program to deal with flexible compounds and compounds populated with more than one significant conformers. The results of most cyclic structures containing heteroatoms were poor, this shows a need for thorough adjustments of the electron withdrawing effects of these heteroatoms on the hydrogen atoms adjacent to them. Improvement of SpecInfo-V3.2 database and ACD-V4 to cater for bicyclo compounds such as *scopoline* and hetero-aromatic compounds is also required to increase the accuracy of these programs in the prediction of chemical shifts. SpecInfo-V3.2 and ACD-V4 could be more accurate if the user updates the database for new compounds that were not used in these programs before. The problem associated with this process would be the high cost involved in buying the program other than using it at a cheaper cost over the internet.

APPENDICES: A CHARGE PROGRAM

Examples of z-matricex

1-(4-nitrophenyl)-piperazine

GEOMETRY (Z-MATRIX)4

NO	AN		BL		ALPHA		BETA		CHARGE	PIEXS	SHIFT
1	55	0	0.0000	0	0.0000	0	0.0000	0	0.0428	-0.0207	154.143
2	55	1	1.4162	0	0.0000	0	0.0000	0	-0.0315	0.0107	142.262
3	55	2	1.3932	1	121.6749	0	0.0000	0	-0.1206	-0.0728	128.001
4	55	3	1.4178	2	121.6642	1	-0.5036	0	0.0891	0.1050	161.557
5	55	4	1.4177	3	116.5841	2	0.4898	0	-0.1206	-0.0728	128.001
6	7	4	1.4144	3	121.9172	2	-179.6221	0	-0.1068	0.1367	0.000
7	55	5	1.3935	4	121.7093	3	-0.0862	0	-0.0315	0.0107	142.262
8	6	6	1.4667	4	122.3541	3	-160.8263	0	-0.0330	0.0000	58.883
9	6	6	1.4672	4	122.6976	3	29.5140	0	-0.0330	0.0000	58.889
10	6	9	1.5334	6	110.7613	4	-137.7449	0	-0.0420	0.0000	54.945
11	7	10	1.4499	9	110.7363	6	-54.9961	0	-0.4227	0.0000	0.000
12	57	1	1.4462	2	121.6175	3	-179.8587	0	0.5141	0.3796	0.000
13	6	11	1.4496	10	111.2905	9	58.5950	0	-0.0420	0.0000	54.940
14	1	2	1.1000	3	118.3226	1	-180.0000	0	0.0779	0.0000	7.952
15	1	3	1.1000	2	118.2702	1	179.4964	0	0.0780	0.0000	7.321
16	1	5	1.1000	4	120.0285	3	179.9138	0	0.0780	0.0000	7.294
17	1	7	1.1000	5	118.3754	4	179.6804	0	0.0779	0.0000	7.950
18	60	12	1.2202	1	119.8896	2	-179.1952	0	-0.3726	-0.2382	0.000
19	60	12	1.2204	1	119.8239	2	0.8325	0	-0.3726	-0.2382	0.000
20	1	11	1.0168	10	109.6255	9	-179.9441	0	0.2738	0.0000	1.096
21	1	13	1.1101	11	108.5522	10	65.6885	0	0.0608	0.0000	3.049
22	1	13	1.1101	11	108.5522	10	179.3028	0	0.0617	0.0000	3.240
23	1	8	1.1101	6	109.2093	4	-97.6909	0	0.0626	0.0000	3.548
24	1	8	1.1101	6	109.2092	4	16.6151	0	0.0634	0.0000	3.432
25	1	9	1.1101	6	109.2485	4	-14.9191	0	0.0634	0.0000	3.321
26	1	9	1.1101	6	109.2485	4	99.4293	0	0.0626	0.0000	3.508
27	1	10	1.1101	9	111.4672	6	66.5207	0	0.0608	0.0000	3.095
28	1	10	1.1101	9	111.4673	6	-176.5130	0	0.0617	0.0000	3.259

DIPOLE MOMENT = 7.697 DEBYES

GEOMETRY (Z-MATRIX)

phenyl propy pyridine

NO	AN		BL		ALPHA		BETA		CHARGE	PIEXS	SHIFT
1	55	0	0.0000	0	0.0000	0	0.0000	0	0.0383	0.1168	153.428
2	55	1	1.3434	0	0.0000	0	0.0000	0	-0.0822	-0.0290	134.152
3	55	2	1.3426	1	120.0149	0	0.0000	0	0.0492	0.0572	155.174
4	58	3	1.2773	2	122.2499	1	0.1342	0	-0.3512	-0.1732	0.000
5	55	4	1.2772	3	119.0218	2	-0.1948	0	0.0492	0.0572	155.174
6	55	5	1.3427	4	122.2688	3	0.0198	0	-0.0822	-0.0290	134.152
7	6	1	1.5110	2	121.5542	3	-179.9184	0	-0.0618	0.0000	46.277
8	6	7	1.5367	1	111.2466	2	-80.5949	0	-0.0852	0.0000	36.035
9	6	8	1.5369	7	111.8590	1	-179.4132	0	-0.0618	0.0000	46.264
10	55	9	1.5125	8	111.0506	7	-178.8089	0	-0.0076	0.0718	146.091
11	55	10	1.3456	9	120.6756	8	-85.4688	0	-0.1002	-0.0284	131.276
12	55	10	1.3457	9	120.8632	8	94.4076	0	-0.1002	-0.0284	131.276

13	55	11	1.3422	10	120.9325	9	-179.9720	0	-0.0708	0.0017	135.964
14	55	13	1.3419	11	120.0020	10	-0.0280	0	-0.0952	-0.0184	132.072
15	55	14	1.3418	13	119.6708	11	-0.0766	0	-0.0708	0.0017	135.964
16	1	2	1.1035	3	119.7826	1	179.9604	0	0.0763	0.0000	7.153
17	1	3	1.1050	2	119.0273	1	-179.9072	0	0.0829	0.0000	8.639
18	1	5	1.1051	4	118.7158	3	-179.8673	0	0.0829	0.0000	8.648
19	1	6	1.1033	5	119.6273	4	-179.7259	0	0.0763	0.0000	7.161
20	1	9	1.1155	8	110.0230	7	59.5198	0	0.0554	0.0000	2.418
21	1	9	1.1154	8	109.8853	7	-56.8347	0	0.0554	0.0000	2.399
22	1	11	1.1038	10	119.8867	9	0.1009	0	0.0763	0.0000	6.889
23	1	13	1.1044	11	120.0253	10	179.9477	0	0.0772	0.0000	7.069
24	1	14	1.1041	13	120.1792	11	179.9720	0	0.0772	0.0000	6.952
25	1	15	1.1044	14	119.9806	13	-179.8629	0	0.0772	0.0000	7.079
26	1	12	1.1037	10	119.9830	9	-0.1918	0	0.0763	0.0000	6.907
27	1	7	1.1154	1	109.3502	2	41.1169	0	0.0554	0.0000	2.490
28	1	7	1.1153	1	110.1588	2	157.4503	0	0.0554	0.0000	2.462
29	1	8	1.1164	7	109.3064	1	-57.9939	0	0.0539	0.0000	2.166
30	1	8	1.1163	7	109.5024	1	59.3233	0	0.0539	0.0000	2.173

DIPOLE MOMENT = 2.460 DEBYES

GEOMETRY (Z-MATRIX)

phenyl dithiane

NO	AN		BL		ALPHA		BETA		CHARGE	PIEXS	SHIFT
1	55	0	0.0000	0	0.0000	0	0.0000	0	0.0119	0.0463	149.212
2	55	1	1.4042	0	0.0000	0	0.0000	0	-0.0834	-0.0183	133.959
3	55	2	1.3999	1	120.7751	0	0.0000	0	-0.0711	0.0011	135.921
4	55	3	1.3987	2	120.0780	1	0.0198	0	-0.0886	-0.0118	133.125
5	55	4	1.3989	3	119.7009	2	-0.0198	0	-0.0711	0.0011	135.921
6	55	5	1.3997	4	120.0699	3	0.0198	0	-0.0834	-0.0183	133.959
7	6	1	1.5132	2	120.3566	3	180.0000	0	0.0674	0.0000	102.764
8	16	7	1.8256	1	108.2039	2	117.8919	0	-0.1881	0.0000	0.000
9	16	7	1.8255	1	108.1936	2	-117.8187	0	-0.1881	0.0000	0.000
10	6	8	1.8233	7	97.6534	1	-176.3760	0	-0.0223	0.0000	63.540
11	6	10	1.5376	8	110.7349	7	-60.2849	0	-0.0572	0.0000	48.286
12	6	11	1.5376	10	114.4363	8	70.8993	0	-0.0223	0.0000	63.540
13	1	2	1.1025	3	118.7996	1	-180.0000	0	0.0763	0.0000	7.030
14	1	3	1.1032	2	120.0319	1	179.9720	0	0.0772	0.0000	7.274
15	1	4	1.1031	3	120.1590	2	179.9802	0	0.0772	0.0000	7.190
16	1	5	1.1032	4	119.9308	3	-179.9802	0	0.0772	0.0000	7.267
17	1	6	1.1028	5	118.7766	4	-180.0000	0	0.0763	0.0000	7.084
18	1	7	1.1139	1	109.1300	2	0.0343	0	0.0698	0.0000	4.627
19	1	10	1.1146	8	111.0997	7	62.5379	0	0.0578	0.0000	2.461
20	1	10	1.1150	8	108.5693	7	179.6737	0	0.0598	0.0000	3.061
21	1	11	1.1169	10	110.8896	8	-55.4430	0	0.0535	0.0000	2.137
22	1	11	1.1173	10	107.2559	8	-170.2632	0	0.0535	0.0000	1.979
23	1	12	1.1146	11	110.2140	10	52.4357	0	0.0578	0.0000	2.461
24	1	12	1.1150	11	109.3358	10	169.5170	0	0.0598	0.0000	3.061

DIPOLE MOMENT = 2.508 DEBYES

GEOMETRY (Z-MATRIX)
thiophencarboxaldehyde

NO	AN		BL		ALPHA		BETA		CHARGE	PIEXS	SHIFT
1	55	0	0.0000	0	0.0000	0	0.0000	0	-0.0947	-0.0405	132.147
2	55	1	1.4674	0	0.0000	0	0.0000	0	-0.0315	0.0201	142.256
3	55	2	1.3592	1	111.8100	0	0.0000	0	0.0180	-0.0339	150.179
4	16	3	1.7822	2	113.8869	1	0.0000	0	-0.0771	0.1097	0.000
5	55	3	1.4660	2	124.2112	1	-180.0000	0	0.1667	0.1090	173.965
6	55	4	1.7850	3	88.1100	2	0.0000	0	0.0445	0.0394	154.419
7	1	1	1.1023	2	123.6982	3	180.0000	0	0.0794	0.0000	7.501
8	1	2	1.1023	3	125.0568	1	180.0000	0	0.0796	0.0000	7.974
9	1	6	1.1015	4	121.3966	3	-180.0000	0	0.0776	0.0000	8.022
10	60	5	1.2217	3	121.8399	2	0.0000	0	-0.3553	-0.2037	0.000
11	1	5	1.1162	3	119.0322	2	180.0000	0	0.0930	0.0000	9.805

DIPOLE MOMENT = 3.379 DEBYES

Examples of GMMX files

thiophenecarboxaldehyde

GMMX Conformation Search Output Results
Temperature for Boltzmann Calculations: 300.00
Average Energy: -0.284 Kcal/mol
Average Heat of Formation: 5.265 Kcal/mol
Average Dipole Moment: 2.56
Dipole Moment Squared: 6.68
Pop conf 1 = 94.43 E = -0.378 DE = 0.000 HF = 5.141 DHF = 0.000 DPM = 2.480
Pop conf 2 = 5.57 E = 1.310 DE = 1.688 HF = 6.527 DHF = 1.386 DPM = 3.948

Entropy of mixing is: 0.43 cal/mol-K
At 300.00 deg K, TdS = 0.13 Kcal/mol

Phenyldithiane

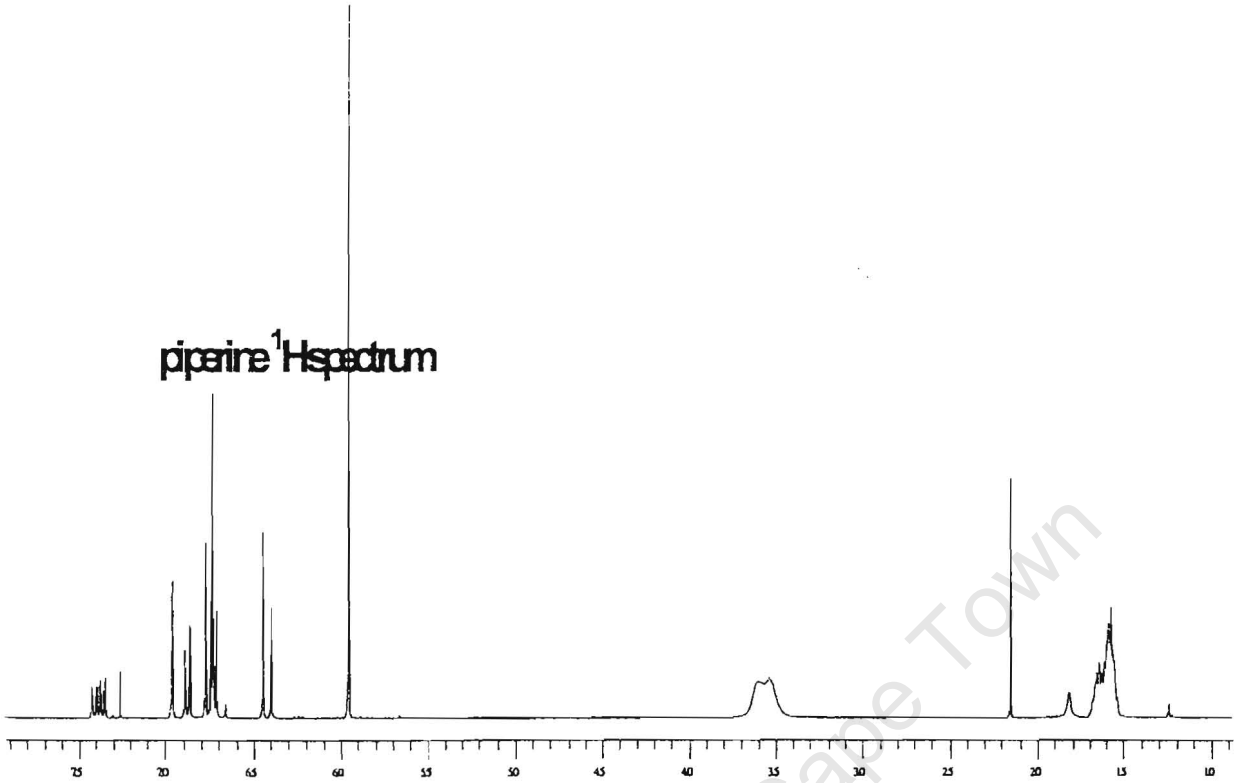
GMMX Conformation Search Output Results
Temperature for Boltzmann Calculations: 300.00
Average Energy: 12.023 Kcal/mol
Average Heat of Formation: 12.281 Kcal/mol
Average Dipole Moment: 2.57
Dipole Moment Squared: 6.61
Pop conf 1 = 50.29 E = 12.020 DE = 0.000 HF = 12.272 DHF = 0.000 DPM = 2.571
Pop conf 2 = 49.71 E = 12.027 DE = 0.007 HF = 12.291 DHF = 0.019 DPM = 2.572
Entropy of mixing is: 1.38 cal/mol-K
At 300.00 deg K, TdS = 0.41 Kcal/mol

phenyl propyl pyridine

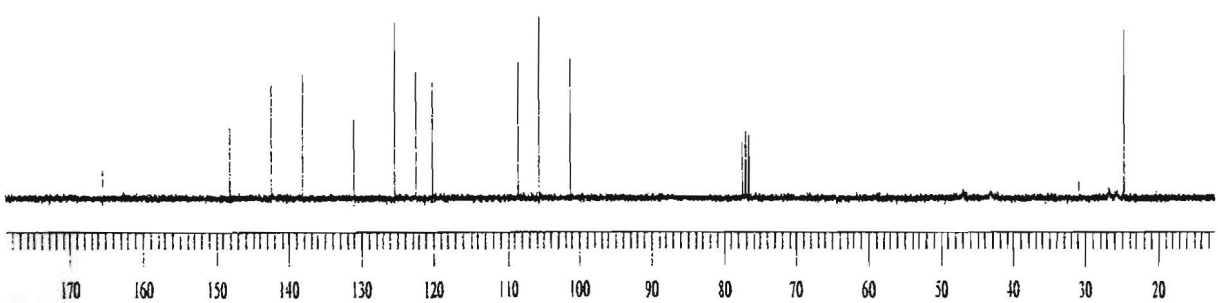
GMMX Conformation Search Output Results
Temperature for Boltzmann Calculations: 300.00
Average Energy: 26.846 Kcal/mol
Average Heat of Formation: 262.343 Kcal/mol
Average Dipole Moment: 2.30
Dipole Moment Squared: 5.28
Pop conf 1 = 4.46 E = 26.549 DE = 0.000 HF = 262.046 DHF = 0.000 DPM = 2.302
Pop conf 2 = 3.97 E = 26.618 DE = 0.069 HF = 262.115 DHF = 0.069 DPM = 2.295
Pop conf 3 = 3.75 E = 26.652 DE = 0.103 HF = 262.149 DHF = 0.103 DPM = 2.303
Pop conf 4 = 3.75 E = 26.652 DE = 0.103 HF = 262.149 DHF = 0.103 DPM = 2.303

Pop conf 5 = 3.75 E = 26.652 DE = 0.103 HF = 262.149 DHF = 0.103 DPM = 2.302
Pop conf 6 = 3.75 E = 26.652 DE = 0.103 HF = 262.149 DHF = 0.103 DPM = 2.303
Pop conf 7 = 3.74 E = 26.653 DE = 0.104 HF = 262.150 DHF = 0.104 DPM = 2.295
Pop conf 8 = 3.74 E = 26.653 DE = 0.104 HF = 262.150 DHF = 0.104 DPM = 2.295
Pop conf 9 = 3.74 E = 26.653 DE = 0.104 HF = 262.150 DHF = 0.104 DPM = 2.295
Pop conf 10 = 3.74 E = 26.653 DE = 0.104 HF = 262.150 DHF = 0.104 DPM = 2.295
Pop conf 11 = 3.60 E = 26.676 DE = 0.127 HF = 262.173 DHF = 0.127 DPM = 2.295
Pop conf 12 = 3.56 E = 26.683 DE = 0.134 HF = 262.180 DHF = 0.134 DPM = 2.295
Pop conf 13 = 3.15 E = 26.756 DE = 0.207 HF = 262.253 DHF = 0.207 DPM = 2.295
Pop conf 14 = 3.11 E = 26.763 DE = 0.214 HF = 262.260 DHF = 0.214 DPM = 2.301
Pop conf 15 = 2.64 E = 26.860 DE = 0.311 HF = 262.357 DHF = 0.311 DPM = 2.293
Pop conf 16 = 2.53 E = 26.886 DE = 0.337 HF = 262.383 DHF = 0.337 DPM = 2.296
Pop conf 17 = 2.53 E = 26.886 DE = 0.337 HF = 262.383 DHF = 0.337 DPM = 2.296
Pop conf 18 = 2.53 E = 26.886 DE = 0.337 HF = 262.383 DHF = 0.337 DPM = 2.296
Pop conf 19 = 2.53 E = 26.886 DE = 0.337 HF = 262.383 DHF = 0.337 DPM = 2.296
Pop conf 20 = 2.53 E = 26.886 DE = 0.337 HF = 262.383 DHF = 0.337 DPM = 2.296
Pop conf 21 = 2.53 E = 26.886 DE = 0.337 HF = 262.383 DHF = 0.337 DPM = 2.296
Pop conf 22 = 2.29 E = 26.947 DE = 0.398 HF = 262.444 DHF = 0.398 DPM = 2.303
Pop conf 23 = 2.06 E = 27.009 DE = 0.460 HF = 262.506 DHF = 0.460 DPM = 2.295
Pop conf 24 = 2.06 E = 27.009 DE = 0.460 HF = 262.506 DHF = 0.460 DPM = 2.299
Pop conf 25 = 2.06 E = 27.009 DE = 0.460 HF = 262.506 DHF = 0.460 DPM = 2.293
Pop conf 26 = 2.05 E = 27.011 DE = 0.462 HF = 262.508 DHF = 0.462 DPM = 2.304
Pop conf 27 = 1.85 E = 27.072 DE = 0.523 HF = 262.569 DHF = 0.523 DPM = 2.302
Pop conf 28 = 1.80 E = 27.088 DE = 0.539 HF = 262.585 DHF = 0.539 DPM = 2.302
Pop conf 29 = 1.63 E = 27.149 DE = 0.600 HF = 262.646 DHF = 0.600 DPM = 2.302
Pop conf 30 = 1.56 E = 27.176 DE = 0.627 HF = 262.673 DHF = 0.627 DPM = 2.304
Pop conf 31 = 1.56 E = 27.176 DE = 0.627 HF = 262.673 DHF = 0.627 DPM = 2.304
Pop conf 32 = 1.56 E = 27.176 DE = 0.627 HF = 262.673 DHF = 0.627 DPM = 2.304
Pop conf 33 = 1.56 E = 27.176 DE = 0.627 HF = 262.673 DHF = 0.627 DPM = 2.304
Pop conf 34 = 1.55 E = 27.177 DE = 0.628 HF = 262.674 DHF = 0.628 DPM = 2.304
Pop conf 35 = 1.55 E = 27.177 DE = 0.628 HF = 262.674 DHF = 0.628 DPM = 2.304
Pop conf 36 = 1.55 E = 27.177 DE = 0.628 HF = 262.674 DHF = 0.628 DPM = 2.304
Pop conf 37 = 1.40 E = 27.238 DE = 0.689 HF = 262.735 DHF = 0.689 DPM = 2.306
Pop conf 38 = 0.86 E = 27.532 DE = 0.983 HF = 263.029 DHF = 0.983 DPM = 2.292
Pop conf 39 = 0.61 E = 27.730 DE = 1.181 HF = 263.227 DHF = 1.181 DPM = 2.289
Pop conf 40 = 0.39 E = 27.999 DE = 1.450 HF = 263.496 DHF = 1.450 DPM = 2.288
Pop conf 41 = 0.39 E = 27.999 DE = 1.450 HF = 263.496 DHF = 1.450 DPM = 2.288
Entropy of mixing is: 7.17 cal/mol-K
At 300.00 deg K, TdS = 2.15 Kcal/mol

piperine ^{1}H spectrum



piperine C13 spectrum

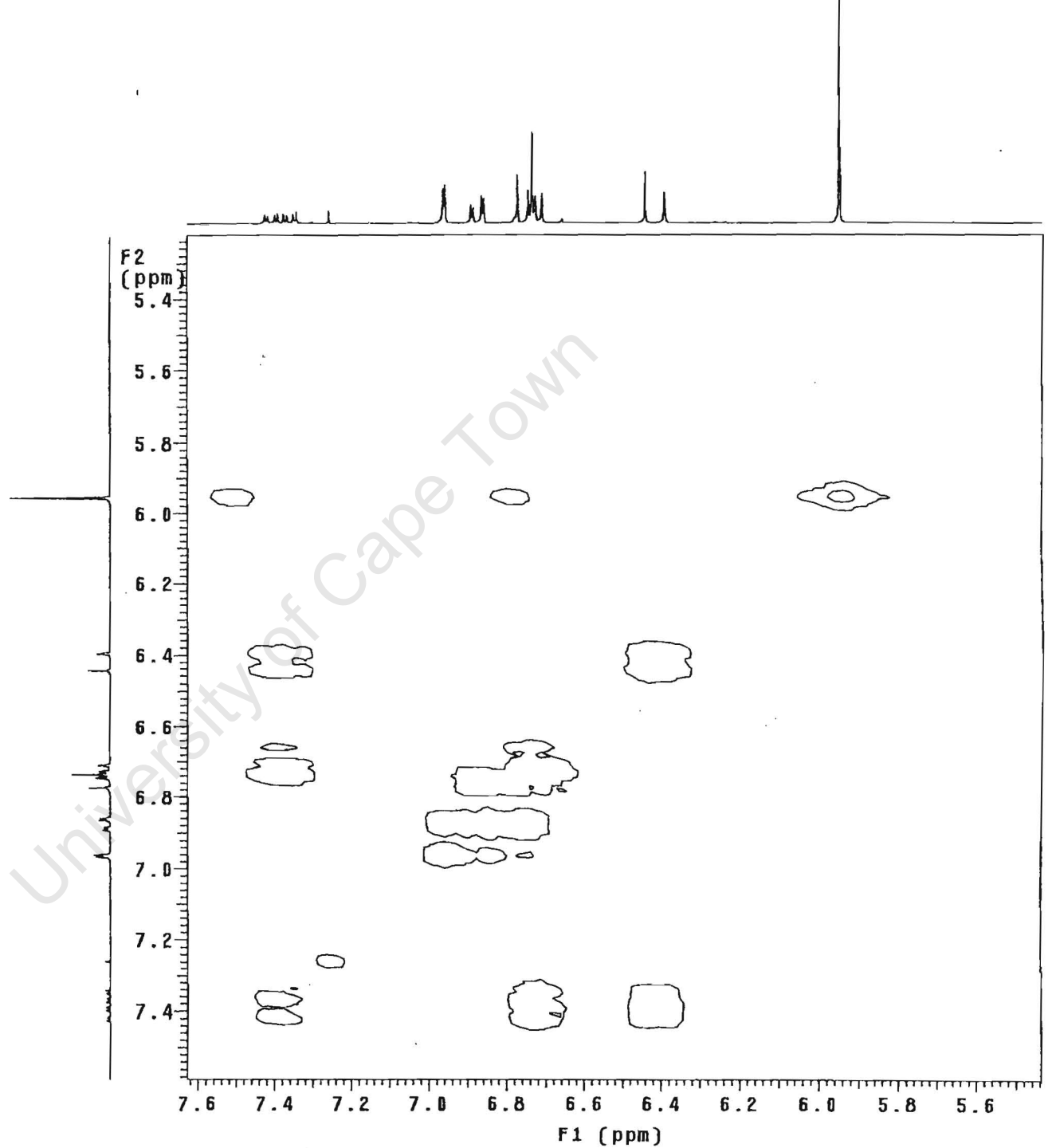


Zolani-54_1h

Pulse Sequence: relayh

Solvent: CDCl3
Temp. 30.0 C / 303.1 K
File: Zolani-54_cosy
INOVA-500 "eland"

PULSE SEQUENCE: relayh
Relax. delay 1.000 sec
COSY 90-90
Acq. time 0.242 sec
Width 2118.6 Hz
2D Width 2118.6 Hz
64 repetitions
256 increments
OBSERVE H1, 300.0742464 MHz
DATA PROCESSING
Sq. sine bell 0.121 sec
F1 DATA PROCESSING
Sq. sine bell 0.060 sec
FT size 1024 x 512
Total time 6 hr, 9 min, 30 sec



COSY SPECTRUM OF PIPERINE

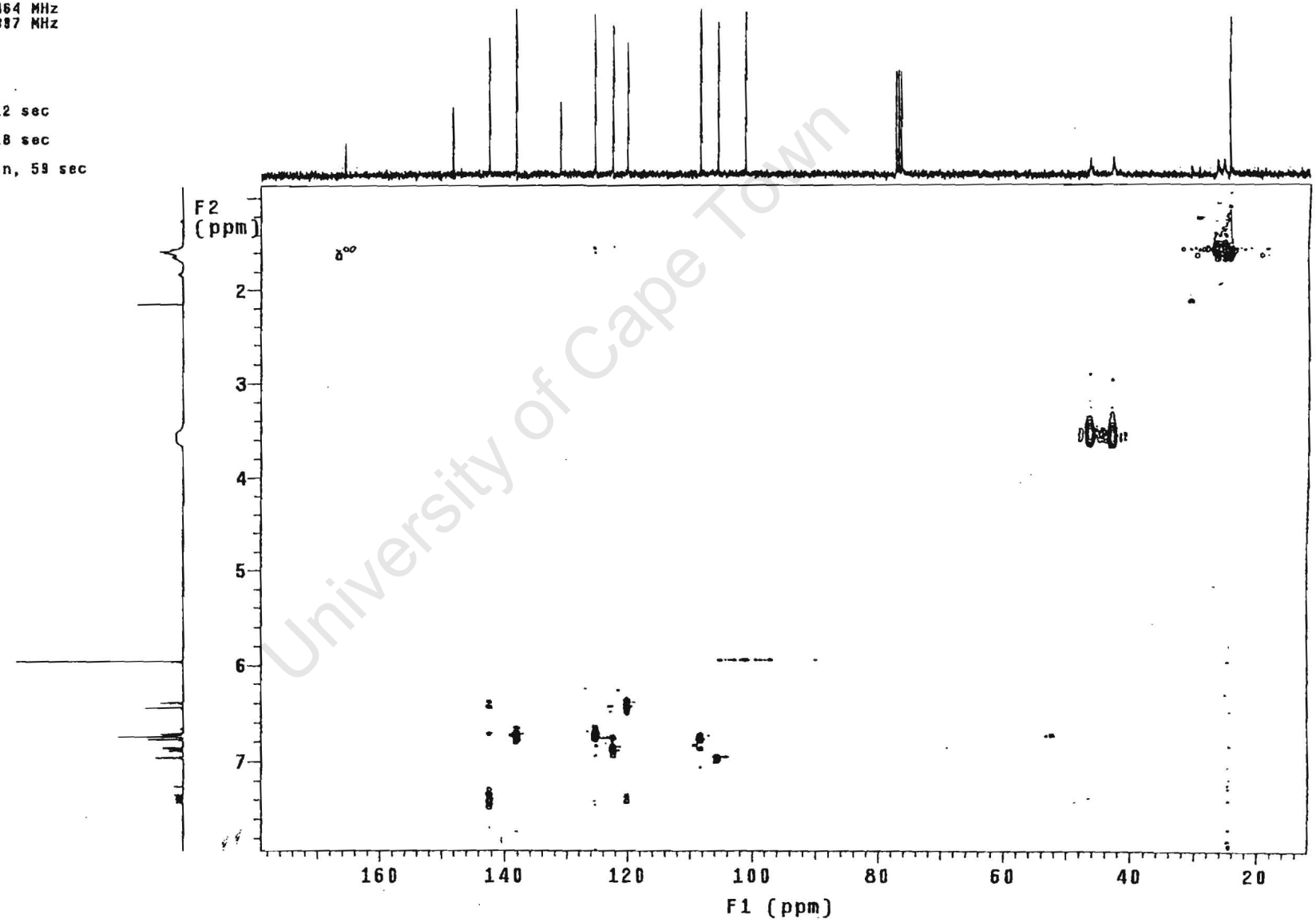
HSQC SPECTRUM OF PIPERINE

Zolani-54_1h

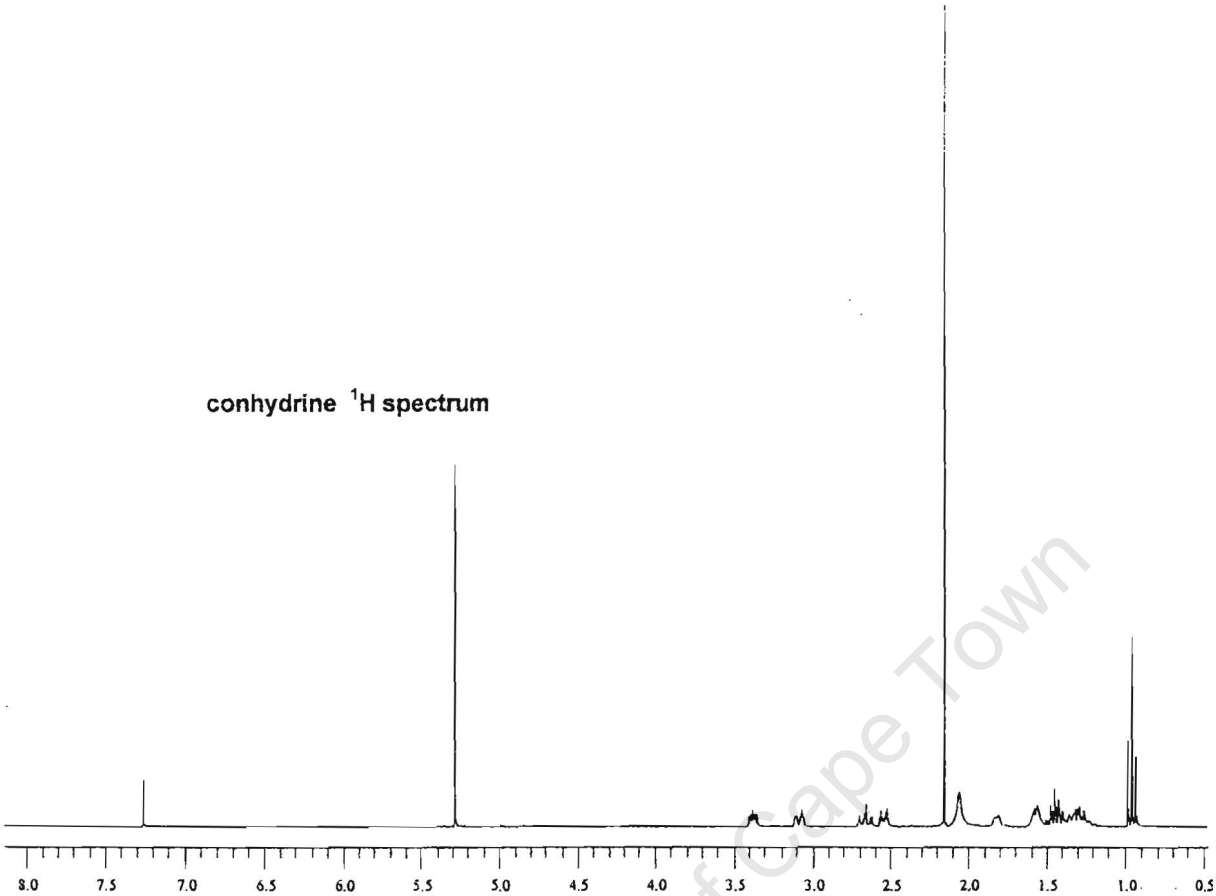
Pulse Sequence: gHSQC

Solvent: CDCl3
Temp. 30.0 C / 303.1 K
File: Zolani-54_gHSQC
INOVA-500 "eland"

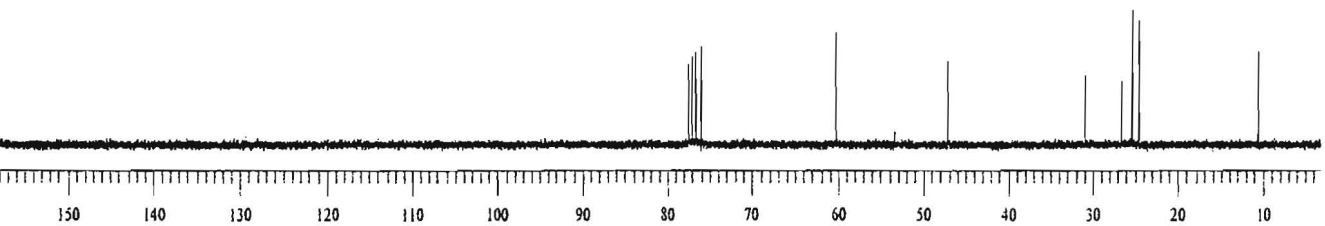
PULSE SEQUENCE: gHSQC
Relax. delay 1.000 sec
Acq. time 0.242 sec
Width 2115.1 Hz
2D Width 12594.5 Hz
64 repetitions
2 x 256 increments
OBSERVE H1, 900.0742464 MHz
DECOUPLE C13, 75.4609397 MHz
Power 41 dB
on during acquisition
off during delay
GARP-1 modulated
DATA PROCESSING
Gauss apodization 0.112 sec
F1 DATA PROCESSING
Gauss apodization 0.018 sec
FT size 1024 x 2048
Total time 12 hr, 22 min, 59 sec



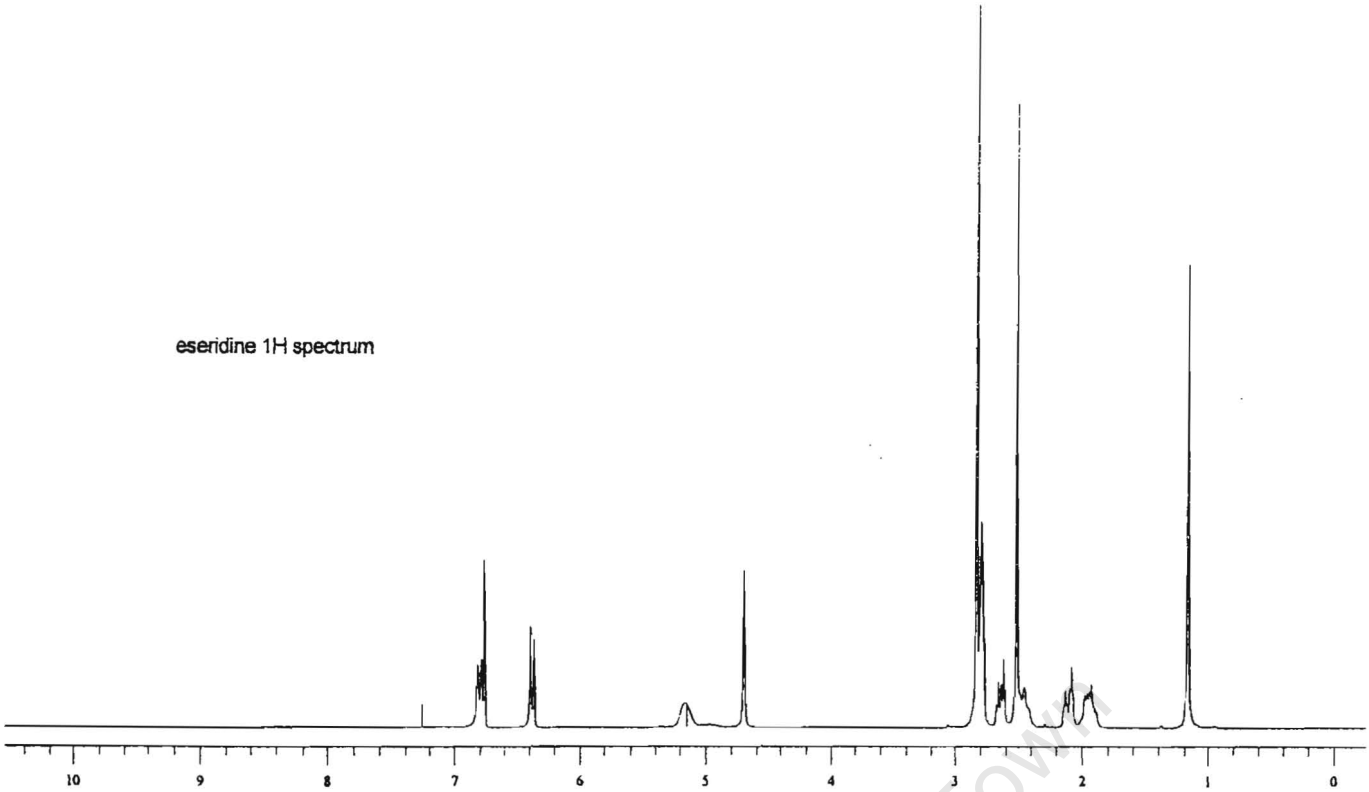
conhydrine ^{1}H spectrum



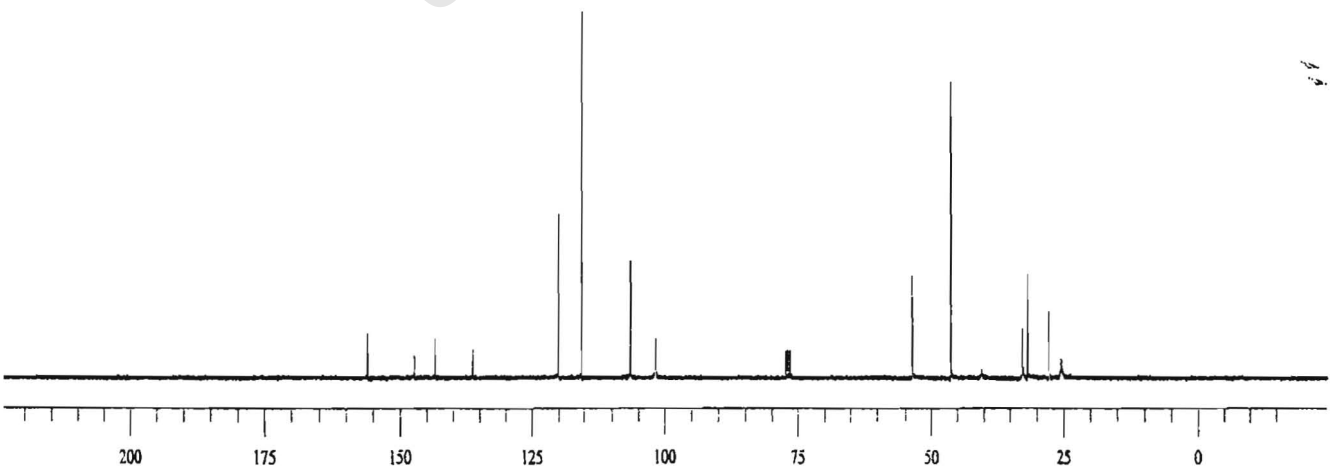
conhydrine C13 spectrum



eseridine 1H spectrum



eseridine C13 spectrum



HMBC SPECTRUM OF ESERIDINE

solani-36_gHMBC

Pulse Sequence: gHMBC

Solvent: CDCl3
Temp. 30.0 C / 303.1 K
File: Zolani-36__gHMBC
INOVA-500 "eland"

PULSE SEQUENCE: gHMBC
Relax. delay 1.000 sec
Acq. time 0.228 sec
Width 2242.2 Hz
20 Width 12886.6 Hz
8 repetitions
400 increments
OBSERVE H1, 300.0742464 MHz
DATA PROCESSING
Sine bell 0.114 sec
F1 DATA PROCESSING
Sine bell 0.011 sec
FT size 1024 x 2048
Total time 1 hr, 14 min, 43 sec