IMPROVED PROCEDURE FOR SETTING UP, RUNNING, AND INTERPRETING 2D-NMR SPECTRUM USED FOR STRUCTURAL ELUCIDATION

by

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A thesis submitted in conformity with the requirements for the degree of Master of Science in the Department of Chemistry University of Toronto

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Abstract

Improved Procedure for Setting Up, Running, and Interpreting 2D-NMR Spectrum Used For Structural Elucidation

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The heteronuclear single quantum coherence (HSQC) experiment has several advantages over the more widely used heteronuclear multiple quantum coherence (HMQC) experiment. The ability of the HSQC sequence to eliminate $^1$H-$^1$H homonuclear coupling in the $f_1$ domain is the most important one. Three HSQC based sequences were examined in detail. The comparison between HSQC and HMQC sequences shows that HSQC has better resolution and sensitivity in both $f_1$ and $f_2$ domains. The two-dimensional HSQC-TOCSY sequence added important bond connectivity information to the heteronuclear shift correlation spectrum. A coupled HSQC sequence presented a new way of obtaining clear proton multiplet structures, as well as displaying an interesting virtual coupling effect. Together these three HSQC based sequences were used to aid the full assignment of a plant steroid, clionasterol.

A computer program written in Varian's magnetic instrumental control and analysis language II (MAGICAL II), called ZUNITY, constitutes the later part of this thesis. The ZUNITY program is an user-friendly program which allows one to run series of routine NMR experiments on the Varian UNITY spectrometers.
To My Mentor,

Professor Nancy G. Dengler

And

To My Families,

My Parents, Seika Tay, Taiko Tay, Ching-Chi Tai, and Jue-Chu Tseng

My Sisters, Lily and Leanne Tai

With Love And Gratitude
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Chapter 1 Basic NMR Principles

1.1 Introduction

Nuclear Magnetic Resonance (NMR) spectroscopy is one of the most popular and powerful techniques for the study of complex molecular structures. It started out as a physics experiment at some 50 years ago, but has since revolutionized chemistry.

The technique was initially designed as a potentially accurate method for measuring nuclear magnetogyric ratios. This original intent turned out to be far afield from its ultimate practical application. Results of this experiment revealed that the radio-frequency (rf) magnetic susceptibility measured turned out to be a rather complicated function exhibiting many sharp, close-lying resonances. The realization that these complex resonance patterns were due to the fine characteristics of the electronic and magnetic environment of the nuclei embedded in the molecule began the development of nuclear magnetic resonance as a high resolution spectroscopic technique in the study of complex molecular structure. Today, the applications arising from the NMR technique span all basic fields of sciences from Physics to Chemistry and more recently, to medicine (in the form of NMR imaging, or MRI). However, the most profound impact remains in the field of Chemistry.
The physical foundation of NMR lies in the magnetic properties of atomic nuclei and their interaction with external magnetic fields. Like electrons, atomic nuclei also exhibit intrinsic angular momentum. When placed in an external magnetic field, these nuclei split into discrete energy levels (which are known as eigenstates) due to the quantization of the magnetic energy of the nucleus. Transitions between different energy levels can be induced by an external electromagnetic radiation of proper frequency in the form of a radio-frequency (rf) pulse. The transition between these discrete energy states (eigenstates) results in a net absorption in energy which can be detected, amplified and recorded as spectral lines. It is also the basis of a 1D NMR spectrum.

Experimental-wise, the NMR phenomenon was first demonstrated in 1945 by two group of physicists working independently -- Bloch, Hansen and Packard at Stanford University and Purcell, Torey and Pound at Harvard University. However, chemical application became possible only after the chemical shift effect was discovered in 1949. Initially, spectra were acquired using the Continuous Wave (CW) technique. Due to its poor sensitivity arising from the excitation of only one part of spectrum at a time, this technique was later replaced by pulsed Fourier transform NMR.
Pulsed Fourier transform method was first introduced by Ernst and Anderson\textsuperscript{4,5}. It has one important advantage over the CW method in that a normal high-resolution NMR spectrum can be excited by a single short radio frequency pulse. The data is collected as time signals and stored in a digital computer. A mathematical operation, Fourier transform, is then applied to convert signals from the time domain into the frequency domain. Since the FT method is able to excite the entire spectrum in a single short pulse, it takes a very short time to complete one single scan. However, the same experiment is usually performed multiple times and stored in the same space in the computer in an additive mode as a way to improve the signal-to-noise ratio (S/N). True signals will always show up in the fixed frequency whereas the random noise generated by the electronics shows up randomly. Over time, the intensity of the true signals grows much faster than the noise and hence improves the S/N. Of course, such operation can be applied to the CW method, but CW method requires a much longer time frame to build up similar S/N, since it excites only part of the spectrum each time, and each part of the spectrum must be scanned numerous times to build up the desired S/N ratio. The difference in measuring time was the main factor which led to the complete replacement of CW spectrometers by pulse Fourier transform spectrometers.
Aside from the advances in the hardware aspect of NMR, the NMR pulse sequences also grew in complexity, especially in the past twenty years owing to a spectacular break through in the development of two-dimensional NMR experiments. The concept was first introduced by Jeener in 1971 but, unfortunately, it was never published. It was Ernst who published the first 2D NMR experiment five years later. The 2D NMR concept has made a profound impact in NMR. It later leads to the development of 3D and more recently 4D NMR spectra for the study of complex biological molecules.

A 2D NMR spectrum is basically a collection of a series of one-dimensional NMR spectra. In each 1D spectrum, the magnetization will undergo an evolution period $t_1$ prior to the detection period $t_2$. The 2D spectrum consists of a series of 1D spectra with a systematic increment $t_1$ by a factor of $\Delta t_1$. For each $t_1$ increment, a separate free induction decay (FID) signal is detected in $t_2$. Hence a signal is dependent on two separate time variables, $t_1$ and $t_2$. After Fourier transforming each of the 1D spectra from the $t_2$ time domain into the $f_2$ frequency domain, it was found that each of the FID in the $f_2$ domain are different in intensity and/or phase. This is because the signals measured in each of the 1D spectra retain a "memory" of what had happened during the previous evolution period, and hence show a modulation in the amplitude and/or phase. A second Fourier transform over the $t_1$ time domain into the $f_1$ frequency domain (which is orthogonal to $f_2$) produces the desired 2D spectrum.
In other words, 2D NMR spectroscopy is essentially a way to record the coherence transfer. The intensity of the signal is determined by the transfer efficiency during the mixing time which is the period of time that separates the evolution and detection period. The transferability of coherence depends on two factors. One of them is the properties of the spin system, for example, the molecular structure, dynamics of the molecule and other molecular properties. The other dependence is on the sequence of the rf pulses to which the spins are subjected. This series of rf pulses designed to manipulate the spins during an NMR experiment is called a "pulse sequence".

The two-dimensional NMR concept provided a fertile research ground for spectroscopists. Two-dimensional NMR pulse sequences have grown tremendously, both in number and complexity, in the past twenty years. Much of the work in this thesis has gone into the study of 2D $^1$H-$^{13}$C heteronuclear correlation sequences, which will be discussed later in Chapter 2.
1.2 Basic Principles of Nuclear Magnetic Resonance

Similar to electrons, an atomic nucleus also exhibits an intrinsic angular momentum associated with its spin. A nucleus with a non-zero spin $I$ has an angular momentum $P = I \hbar$, where $\hbar = h/2\pi$ and $h$ is Planck's constant. This angular momentum is related to the magnetic moment $\mu$ through magnetogyric ratio $\gamma$ by the following relation.

$$\mu = \gamma P$$

According to quantum mechanics, the nuclear spin $I$ is quantized, hence, both angular momentum and nuclear magnetic moment can only take on certain sets of values. The observed magnetic moment at the atomic level is the projection of magnetic moment $\mu$ upon the external static magnetic field direction which is arbitrarily chosen in the $z$-direction in the Cartesian coordinate system. Therefore, the magnitude of the magnetic moment in the $z$ direction is given by

$$\mu_z = \gamma \hbar M_I$$

where $M_I$ is the magnetic quantum number whose values are restricted to $I$, $(I-1), (I-2), (I-3), ..., -I$. 
Due to the restriction on \( M_l \), there are a total of \((2I+1)\) eigenstates in which a nucleus can exist. When a nucleus with a z-component magnetic moment \( \mu_z \) is placed in an external magnetic field \( B_0 \) (in the z-direction), the magnetic moment experiences an magnetic energy \( E \) which is given by the following relation.

\[
E = -\mu \cdot B_0 = -\mu_z B_0
= -\gamma \hbar M_l B_0
\]

Again, one can conclude that magnetic energy \( E \) is also quantized since \( M_l \) can only takes on certain sets of values. Obviously, in the absence of a magnetic field, the energy of an ensemble of nuclei is degenerate. However, the presence of the magnetic field will split the ensemble into \((2I+1)\) Zeeman energy levels due to the interaction of the magnetic moment \( \mu \) with external magnetic field \( B_0 \). Each of these energy states corresponds to an allowed eigenstate with a characteristic energy value known as eigenvalue. Each allowed energy level is populated in thermal equilibrium according to Boltzmann’s distribution.

For spin \( \frac{1}{2} \) particles (e.g. \(^1\)H, \(^{13}\)C, and \(^{15}\)N), Zeeman splitting produces only two eigenstates corresponding to the two allowed \( M_l \) values, namely, \( M_l = \pm \frac{1}{2} \). They are commonly denoted as \( \alpha \) and \( \beta \) spin states. The \( \alpha \) spin state is the lower spin state and it corresponds to \( M_l = +\frac{1}{2} \). The Zeeman splitting pattern of a spin \( \frac{1}{2} \) nucleus under the influence of a static magnetic field \( B_0 \) is shown in fig 1.1.
Referring to fig. 1.1, the energy separation between the two levels can be expressed as
\[ \Delta E = \gamma \hbar B_0. \]
and the transition between the two states can be stimulated by applying an electromagnetic field in the form of a radio-frequency pulse with energy equal to the separation energy between the two Zeeman levels. In other words, a rf pulse with energy \( E = \hbar \nu = \gamma \hbar B_0 \) or more precisely, with an angular velocity \( \omega = \gamma B_0 \) (where \( \omega \) is the angular velocity and is equal to \( 2\pi \nu \)) will be able to promote such a transition.

In a classical approach, it is known that a magnetic dipole moment, \( \mu \), in a magnetic field \( B_0 \) will experience a time rate change of angular momentum (\( d\mu/dt \)) or a torque. Since the direction of the torque is perpendicular to both the magnetic moment \( \mu \)
and the static magnetic field $B_0$, the resultant motion causes the magnetic moment $\mu$ to precess about the $B_0$ field (in $z$-direction). The angular velocity of this precessional motion is known as the Larmor angular velocity and is given by $\omega_0 = \gamma B_0$. Fig. 1.2 shows the Larmor precession of a spin $\frac{1}{2}$ particle.

![Diagram of Larmor precession](image)

**Fig 1.2** The Larmor precession of a spin-$\frac{1}{2}$ nucleus

The precessing moment traces out two cones which make an angle $\theta$ to $B_0$. Refer to fig. 1.2. The angle $\theta$ relates to $M_z$ and $I$ through the following expression.

$$\cos \theta = \frac{M_z}{\sqrt{I(I+1)}}$$
In a general NMR experiment, another weak magnetic field $B_1$ is applied perpendicular to the $B_0$ field. If $B_1$ carries an exact amount of energy equal to the energy separation between the two spin states, then it has an angular frequency equal to the Larmor frequency. This $B_1$ pulse will be able to cause the nuclei to "jump" from a lower energy $\alpha$ spin state to the higher energy $\beta$ spin state and hence generate the "resonance" condition. The absorption in $B_1$ energy is then detected, amplified and recorded as NMR signals.

If the frequency of NMR transitions were entirely dependent on the frequency $\nu$

$$\nu = \left( \frac{\omega}{2\pi} \right) = \left( \frac{\gamma}{2\pi} \right)B_0,$$

the technique would have little chemical application. Nuclei of different elements would give resonances in different parts of the spectrum due to the variation of the magnetogyric ratio $\gamma$. It would be very difficult to compare signals at very different frequencies. However, the absorption frequency of a nucleus depends on its chemical environment. This difference in resonance condition is known as the chemical shift. The existence of the chemical shift allows an NMR spectroscopist to distinguish between different chemical environments and makes NMR spectroscopy a powerful technique for structure elucidation.

The actual magnetic field experienced by a nucleus in a molecule differs very slightly from the applied field $B_0$, due to the magnetic field produced by the molecular electrons. The field produced by these molecular electrons usually opposes the applied
field $B_0$ and is proportional to $B_0$. This induced contribution to the magnetic field can be expressed as $(-\sigma_i B_0)$, where $\sigma_i$ is known as the shielding constant for nucleus $i$. Therefore, the overall magnetic field experienced by the nucleus $i$ is expressed as $B_i = B_0 (1 - \sigma_i)$.

Substituting this expression into the frequency $\nu$, expression above, the NMR frequencies of a molecule is expressed as $\nu_i = \left( \frac{\gamma}{2\pi} \right) B_0 (1 - \sigma_i)$. Thus if one observes two different nuclei of the same kind, e.g. $^1\text{H}$, the frequency difference is expressed as

$$\nu_1 - \nu_2 = \frac{\gamma}{2\pi} B_0 (1 - \sigma_1) - \frac{\gamma}{2\pi} B_0 (1 - \sigma_2) = \frac{\gamma}{2\pi} B_0 (\sigma_2 - \sigma_1)$$

Obviously, different kinds of nuclei have very different $\gamma$ values. Therefore, their NMR peaks will occur at very different frequencies, while nuclei of the same kind will give signals in a much narrower frequency range. These frequencies are usually measured relative to the frequency of a reference compound. The compound (CH$_3$)$_4$Si. tetramethylsilane (TMS), is usually used as an internal reference for $^1\text{H}$ and $^{13}\text{C}$. To express the chemical shift of a particular nucleus $i$, $\delta_i$ is defined as follows.

$$\delta_i = (\sigma_{\text{ref}} - \sigma_i) \times 10^6$$

where $\sigma_{\text{ref}}$ is the shielding constant for the $^1\text{H}$ and $^{13}\text{C}$ of TMS. Note that defined in this way, $\delta_i$ is independent of the magnetic field strength of the spectrometer, allowing easy comparison of chemical shifts measured at different field strength.
Proton spectra are usually more complex due to the existence of the spin-spin coupling. A nucleus with non-zero spin exhibits a nuclear magnetic moment. The magnetic field due to this moment also affects the magnetic field experienced by a neighboring nucleus, thereby slightly changing the absorption frequency of the neighboring nucleus. However, rapid molecular motions in liquid average out the direct nuclear spin-spin interactions, but not indirect interactions. Indirect interaction are transmitted through bonding electrons and is responsible for the splitting patterns of proton peaks in solution.

Although the coupling constant, like the chemical shift, depends on the magnetic environment of a molecule, it does not depend on the field strength and operating frequency of the spectrometer, since it arises from interactions between pairs of nuclear magnetic dipoles and will be present even in the absence of an external field $B_0$. For this reason, coupling constants are expressed in frequency units (Hz) rather than field dependent units. When there are more than two magnetically different nuclei present in a molecule, coupling may occur between each pair of nuclei. The coupling constant between each pair of nuclei will usually be different unless some nuclei are related by symmetry.
Each coupling constant, $J_{ik}$, causes equal splittings of the resonances of nuclei $i$ and $k$. For a system with $n$ non-equivalent spin-$\frac{1}{2}$ nuclei, each nucleus will in principle give rise to $2^{n-1}$ lines. However, it is possible that some of these lines may overlap or that many of the coupling constants may be too small to observe. In more general terms, $n$ equivalent protons split the absorption peaks of its neighboring coupled protons into $(n+1)$ lines. The intensity of the splitting peaks follows that of a Pascal’s triangle. For example, a doublet splitting pattern has the peak intensity ratio of 1:1 and a triplet peak intensity ratio is 1:2:1.

The splitting patterns due to spin-spin coupling provides NMR spectroscopists with detailed information of the molecular environment and the magnitude of the coupling constants reveal conformational information. Together with chemical shift, this information make NMR a powerful tool for structural studies.
1.3 One-Dimensional NMR

Since a two-dimensional NMR spectrum can be viewed as a collection of one-dimensional spectra in the $f_2$ domain, it is important to discuss the underlying principles of one-dimensional NMR spectroscopy.

As mentioned in the previous section, a spin 1/2 particle in the presence of a static magnetic field $B_0$ will split into two spin states, $\alpha$ and $\beta$. Both spin states are filled at thermal equilibrium according to a Boltzmann distribution and the population ratio of the two spin states is given by the Boltzmann equation.

$$\frac{N_\alpha}{N_\beta} = \exp\left[-\frac{\Delta E}{KT}\right]$$

Where $N_\alpha$ and $N_\beta$ are the number of spins in the $\alpha$ and $\beta$ spin states. $\Delta E$ is the energy difference between $\alpha$ and $\beta$ spin states. $K$ is Boltzmann's constant and $T$ is the absolute temperature.

Since the $\alpha$ spin state is lower in energy, it has a slight excess of spin population. The excess population determines the bulk magnetization of the nuclear spin ensemble. Furthermore, all of the individual spins in this ensemble have the same magnetic quantum number $M_1$, and hence are all precessing at an angle $\theta$ to $B_0$ at an angular frequency equal to the Larmor frequency. However, each individual spin precesses with a random phase.
leading to the cancellation of the magnetic moments in the xy-plane. This gives rise to a net magnetization \( M_z \) that is parallel to \( B_0 \). This situation is depicted in Fig. 1.3 below.

![Diagram](image)

**Fig. 1.3** (a) An ensemble of nuclei precessing at Larmor frequency
(b) The components of magnetic moments projected on the xy-plane. All components in the xy-plane cancel each other leading to a zero net magnetic moment in the xy-plane.

The overall cancellation in the xy-plane results in a net magnetization \( \vec{M} \) in the z direction, parallel to the \( B_0 \) field, indicated in (a).

At this point, the ensemble of spins is irradiated at the Larmor frequency by a radio-frequency field \( B_1 \) which is perpendicular to \( B_0 \). This electromagnetic radiation interacts with the spin system through its magnetic dipole component, causing the net magnetization to precess around the \( B_1 \) field as well as the static \( B_0 \) field. The two precessional motions superimposed upon each other lead to a rather complicated net motion. To simplify and eliminate confusion, the transmitter frequency (\( B_1 \) frequency) is used as a reference for the overall motion rather than the Larmor frequency. This is
accomplished by transforming the laboratory (or the static) reference frame into a rotating reference frame, which has a rotational frequency equal to the transmitter frequency. Hence, in the rotating frame, $B_1$ appears to be static.

In such a reference frame, it is possible to represent the motion of an ensemble of spins pictorially by the "vector diagram". Such representation is a simple and convenient way to describe the motion of the bulk magnetization during an NMR experiment. However, such a pictorial representation becomes ambiguous when treating a phenomenon depending on quantum mechanical properties. Under this situation, a formalism called the product operator formalism is often employed to describe the motion of the spin ensemble under various interactions. The fundamental basis of the product operator formalism will be discussed in section 1.4.

The linearly polarized $B_1$ field is actually a superposition of two circularly polarized fields, which can be viewed as two counter-rotating vectors. If the $B_1$ frequency is chosen to match the Larmor frequency (as it is required for the resonance condition to occur), one component of the $B_1$ field rotates at the Larmor frequency and the other at twice the Larmor frequency in the opposite direction. The second component can be neglected since it does not meet the resonance condition. This leaves the first component of $B_1$ field static in the rotational frame. Hence, a sample with a bulk magnetization $M_z$ experiencing a static $B_1$ field will have its net magnetization flipped from the $z$-direction into the $xy$-plane, where the net magnetization precesses with phase
coherence. The tipping angle $\alpha$ is given by $\alpha = \gamma B_1 t_p$, where $t_p$ is the pulse duration. For example, a $90_\circ$ $B_1$ pulse will tip the net magnetization into $-y$ axis, and a $180_\circ$ pulse flips the magnetization down to the $-z$ axis, as described in the vector diagrams in fig. 1.4.

![Vector representation of the net magnetization under the influence of a 90$^\circ$ and 180$^\circ$ B$_1$ pulse.](image)

90$^\circ$ pulse flips net magnetization from $z$-direction to $-y$. 180$^\circ$ B$_1$ pulse flips $M_z$ from $+z$ to $-z$.

The radio frequency pulse $B_1$ is usually very short lived. Upon the removal of the $B_1$ field, nuclei continue to feel the effect of the static $B_0$ field which causes the magnetization to precesses about $B_0$. The $z$-component of the magnetization will gradually increase since the system tries to restore the normal Boltzmann distribution by relaxing back to equilibrium state. Part of the energy absorbed from the $B_1$ field is transferred to the environment (or the lattice) around the spins, hence the name spin-
lattice relaxation is used to describe such a process. It is also known as longitudinal relaxation. The new equilibrium magnetization $M_z$ is a function of longitudinal relaxation time $T_1$ and the strength of the $B_1$ field.

In addition to spin-lattice relaxation, there is one other factor which decreases the transverse magnetization in the $xy$ plane. It is known as spin-spin relaxation or transverse relaxation. As the name suggests, it is based on the energy transfer within the spin system. This effect is caused by the loss of the phase coherence of the spins in the $xy$ plane, which is largely due to the inhomogeneities of the $B_0$ field. $B_0$ inhomogeneities causes different parts of the sample to experience slightly different magnetic fields and therefore to lose phase coherence. Obviously, as the spins lose their phase “memory” while precessing about $B_0$, net magnetization splits into many different components, all precessing in the $xy$-plane with different phase. Fig. 1.5. shows the “fanning out” effect of the spins during the $T_2$ relaxation period. Overall, this will leads to the diminishing of the net magnetization in the $xy$ plane because different components of transverse magnetization begin to cancel each other out. However, this spin-spin relaxation has no effect on the new equilibrium magnetization $M_z$. In general, the $T_2$ relaxation time is usually shorter than $T_1$ and together these two mechanisms bring the spin system back to the equilibrium state to achieve the Boltzmann distribution once again.
Fig. 1.5 Fanning out the transverse magnetization during $T_2$ relaxation.

(a) Transverse magnetization precessing with phase coherence.
(b) As the transverse magnetization loses phase coherence, a "fanning out" effect is as shown.

Only the transverse magnetization is recorded to produce NMR signals. It is done in the following way. As the transverse magnetization precesses in the $xy$ plane, a receiver coil is placed in the same plane to detect the rotating magnetization. As the transverse magnetization decreases, the oscillating voltage from the coil also decays till it reaches zero when all of the transverse magnetization diminishes. The decaying receiver voltage is recorded as a function of time and this signal is called a "free induction decay" (FID). The signal induced in the coil is a free precession signal and, owing to its decay, is called a free induction decay. The magnetization is allowed to precess freely during $T_2$ relaxation. The oscillating voltage observed by the receiver is generated through an induction process. Lastly, the signal detected is a decaying signal. Therefore, the process is called "free
induction decay”. This time dependent FID signal can be re-expressed as a function of frequency via the operation of Fourier transform: which converts time domain data into frequency domain.

During the signal detection, the detectable transverse magnetization is represented by a vector precessing in the xy plane in the rotating frame of reference. A single detector aligned along the x or y axis can detect the rotational frequency but is unable to distinguish the direction of the rotation. In other words, two vectors rotating in the opposite direction with same frequency will show up as the same signal. This problem is solved by employing the quadrature detection technique. Unlike single detection which observes the oscillatory motion from one axis, quadrature detection simultaneously detects two signals which are perpendicular to each other, e.g., one along the x-axis and the other along the y-axis. By using two phase shifted detectors, one can obtain both sine and cosine components of the magnetization. The two components are digitized separately to give rise to the imaginary (from the sine component) and real part (from the cosine component) of a spectrum. When two sets of data are combined and Fourier transformed, a spectral line with the correct frequency is produced. This process is illustrated in fig. 1.6.
Fig. 1.6 The principle of quadrature detection:
(a) The net magnetization \( M \) rotates clockwise, a positive sine function is detected on the \( x \)-axis and positive cosine observed on the \( y \)-axis. (b) Net magnetization \( M \) rotates counter-clockwise, a negative sine is observed on the \( x \)-axis and a positive cosine on the \( y \)-axis. The result of the Fourier transformation of the signals and addition of frequency spectra is shown in (c).

Sometimes, instrument imperfection can lead to the imperfect cancellation of the unwanted peaks (see figure 1.6) and hence produce the so-called "quad-images" and other artifacts. These artifacts can sometimes be recognized by their different phase property.
Fortunately these images can be eliminated or greatly attenuated by the CYCLOPS phase cycling.

When NMR signals are quadrature detected and Fourier transformed, each point in the frequency domain has two coefficients associated with it, one real and one imaginary. The real set of coefficients generate signals in the absorptive mode and imaginary data generates dispersive mode signals. Sometimes, an absolute value mode is use by spectroscopists as well. An absolute value coefficient is calculated as the square root of the sum of the squares of imaginary and real coefficients. This mode is used extensively in processing 2D NMR spectra.

In a general 1D NMR experiment, a pulse sequence is repeated many times to build up a good signal-to-noise ratio, (S/N). The number of transient (nt) represents the number of times a pulse sequence is repeated. Also, a pre-saturation delay period, d1. is applied prior to the pulsing of each sequence to avoid saturation. The Nyquist theorem demands that the sampling rate be at least twice the maximum frequency acquired. This leads to the following relationship between spectral width (sw), acquisition time (at) and total number of sampling point (np).

\[ np = at \times 2 (sw) \]

The FT NMR method provides several post acquisition techniques to be used in improving experimental results to either achieve a better spectral resolution or to obtain a
better signal-to-noise ratio. A better S/N can be achieved by multiplying each of the recorded data point by an exponential function, \( \exp\left(\frac{-jT_c}{N}\right) \) where \( T_c \) is an empirical time constant, \( j \) is the number of the particular data point and \( N \) is the total number of data points. This exponential function is known as weighting function. It is routinely applied to enhance the S/N of the FT NMR spectra. It also plays an important role in the 2D NMR spectra.

Resolution enhancement can be achieved via a process called “zero-filling”. It is done by adding blocks of zeros to the FID data. To do so, the spectrometer system must have enough disk memory to take in the additional “zero” data. This provides a large number of data points in the frequency domain and allows a better production of the spectrum. Most importantly, this additional data is obtained without the expense of additional spectrometer time.
1.4 Product Operator Formalism

It is possible to describe certain aspects of the spin motion under the influence of the radio-frequency pulses in the rotating frame by vector diagrams. Such pictorial presentation plays an important role in understanding the basic NMR phenomena. However, the vector diagram approach which is built upon the framework of classical physics is only valid for isolated nuclei without spin-spin interactions. When the spin-spin interaction becomes important, vector diagrams can no longer provide an accurate picture. One needs to adopt the quantum mechanical approach in order to take the scalar coupling effect into account.

The calculation of the effect of a pulse sequence on the AX type spin system (or those of higher order) is based on the time-dependent Schrodinger equation. The theoretical tool to perform such a calculation is the density matrix formalism. In the density matrix formalism, the state of the spin ensemble is completely specified by a Hermitian density matrix of dimension \((2I+1) \times (2I+1)\). Therefore, instead of dealing with each spin state individually, the average value of the overall coherence between the quantum states of the individual spin systems is used to describe the net magnetization of the ensemble. In the case of a spin-\(\frac{1}{2}\) nucleus, the average spin state of the spin ensemble is represented by a \(2\times2\) density matrix. In many one- and two-dimensional NMR experiments, there are often two spin systems involved. Therefore, a \(4\times4\) density matrix
is required to represent such a system of two spin-$\frac{1}{2}$ ensembles. The effect of each pulse and free precession period is calculated by a unitary transformation of the density matrix, and each transformation requires the multiplication of several $4 \times 4$ matrices. For a simple pulse sequence, this is not a big problem. However, the process becomes tedious and cumbersome when a complicated pulse sequence is involved or when applied to a larger spin system, where an even higher order Hermitian matrix is required.

A simplified procedure introduced by Ernst and co-workers\textsuperscript{11,12} called the "product operator" formalism, which can greatly reduce the calculation process, has become increasingly popular in describing the effect of pulse sequences on spin ensembles. The product operator formalism is limited in its application to weakly coupled spin systems and it neglects all relaxational effects. However, in spite of this shortcoming, it is used extensively in the literature.

The product operator formalism employs physically meaningful vectors (in the form of operators) to represent the bulk magnetization of the spin ensemble. Simple transformation rules allow one to track the motions of the spins through multiple-pulse experiments. Similar to the density matrix formalism, the state of an ensemble system is described by the linear combination of the basis states. As mentioned earlier, for a two spin-$\frac{1}{2}$ ensemble, a $4 \times 4$ matrix is required to describe the ensemble. In other words, there are 16 linearly independent basis states involved in describing a two spin-$\frac{1}{2}$ ensemble.
Similarly, 16 product operators are required to represent the same system. These 16 linearly independent product operators are as follows:

\[
\frac{1}{\sqrt{16}}
\]

\[I_x, I_y, I_z, S_x, S_y, S_z\]

\[2I_xS_x, 2I_yS_y, 2I_zS_z, 2I_xS_x, 2I_yS_y, 2I_zS_z, 2I_xS_x, 2I_yS_y, 2I_zS_z\]

Where

1 is the unity operator.

\[I_x, I_y, I_z\] is the x-, y-, and z-magnetization of spin I.

\[S_x, S_y, S_z\] is the x-, y- and z-magnetization of spin S.

\[2I_xS_x\] is the anti-phase x-magnetization of spin I. (refer to fig. 1.7)

\[2I_yS_y\] is the anti-phase y-magnetization of spin I. (refer to fig. 1.7)

\[2I_zS_z\] is the anti-phase x-magnetization of spin S.

\[2I_xS_y\] is the anti-phase y-magnetization of spin S.

\[2I_xS_z\] is the anti-phase z-magnetization of spin I or spin S. (refer to fig. 1.7)

\[2I_xS_x, 2I_yS_y, 2I_zS_z\] are the two-spin coherence of spin I and S.
During a pulse sequence, product operators are transformed. Three factors cause the transformations to take place: first, the effect of radio-frequency pulses; second, the effect of Larmor precession (also known as chemical shift); and third, the effect of scalar coupling. These effects on a spin ensemble is described by the following transformation:

\[ \exp(-i\phi U_m) U_n \exp(i\phi U_m) \]

where \( \phi U_m \) corresponds to the Hamiltonian \( H_m \). For each of the three effects, the relevant Hamiltonian is given as follows:
First, $\theta I_k$ is the Hamiltonian function for radio-frequency pulses with flip angle $\theta$ and phase $k$. Second, $\omega I_z$ is the Hamiltonian for chemical shift. Third, $2\pi J_1 I_z S_z$ is the Hamiltonian for the evolution under weak scalar coupling.

With the Hamiltonian as defined above, the transformation rules of the product operators are summarized as follows:

**Transformation due to the effect of radio-frequency pulses**

\[
I_{i,y} \xrightarrow{\theta_{i,y}} I_{i,y} \\
I_z \xrightarrow{\theta_{i,y}} I_z \cos \theta \pm I_{i,y} \sin \theta \\
I_{i,y} \xrightarrow{\theta_{i,y}} I_{i,y} \cos \theta \mp I_z \sin \theta
\]

**Transformation due to the chemical shift evolution**

\[
I_{i,y} \xrightarrow{\omega_{i,y}} I_{i,y} \\
I_z \xrightarrow{\omega_{i,y}} I_z \cos \omega_0 t - I_{i,y} \sin \omega_0 t \\
I_i \xrightarrow{\omega_{i,y}} I_i \cos \omega_0 t + I_{i,y} \sin \omega_0 t
\]

**Transformation due to scalar coupling**

\[
I_z \xrightarrow{\pi J_1 I_z S_z} I_z \\
I_{i,y} \xrightarrow{\pi J_1 I_z S_z} I_{i,y} \cos \pi J t - 2 I_z S_z \sin \pi J t
\]
Product operator formalism is used to analyze some of the pulse sequences mentioned in this thesis.
1.5 Polarization Transfer

Although nuclear magnetic resonance spectroscopy is an ideal choice in many chemical applications, its poor sensitivity sometimes hampers its potential. When measuring the NMR signals of certain nuclei such as $^{13}\text{C}$, $^{15}\text{N}$, and $^{29}\text{Si}$, the poor sensitivity becomes apparent due to the nuclei's low magnetogyric ratio ($\gamma$). In addition, the low-natural abundance of $^{13}\text{C}$ and $^{15}\text{N}$ also contributes to the sensitivity problem.

An ensemble of spin-$\frac{1}{2}$ particles placed under a static magnetic field $B_0$ will split into two spin states $\alpha$ and $\beta$ with an energy separation of $\Delta E$, where $\Delta E = \gamma h B_0$. The population ratio of the two states is given by Boltzmann's distribution equation.

\[
\frac{N_\beta}{N_\alpha} = \exp\left(\frac{-\Delta E}{KT}\right)
\]

where $N_\beta$ and $N_\alpha$ are the population of $\beta$ and $\alpha$ spin state, respectively.

In simpler terms, the population difference between the two states is proportional to $\Delta E$ in an exponential way. Therefore, the smaller the difference in $\Delta E$, the smaller the excess population is in the $\alpha$ spin state and hence a smaller signal intensity results. Since $\Delta E = \gamma h B_0$, a stronger magnetic field $B_0$ will certainly widen the energy difference and hence improve the population difference. This factor prompted the hardware development to incorporate the superconducting magnet in order to achieve higher $B_0$. 


field strength. However, $\Delta E$ also depends on $\gamma$ (magnetogyric ratio), which explains why the low-$\gamma$ nuclei suffers from poor sensitivity problem under fixed $B_0$ field. For a measurement at any given $B_0$, if the polarization of a high-$\gamma$ and high abundant nucleus (e.g. $^1H$), could be transferred to the less abundant, low-$\gamma$ nucleus (e.g. $^{13}C$), this can dramatically improve the signal sensitivity of the later nuclei. This is done by polarization transfer through Selective Population Inversion (SPI) in the coupled spin systems.

In a weakly coupled heteronuclear AX spin system with spin-$\frac{1}{2}$ particles (such as a C-H system), the population ratio between the two spin states in both nuclei is proportional to their magnetogyric ratios. Since $\gamma_H$ is four times greater than $\gamma_C$, the population transition between proton are four times greater than that of carbon transition. The relative population difference is depicted in figure 1.8.
Referring to fig. 1.8 above, the number circled indicates the population difference. (It also corresponds to the difference in the number of arrows between two energy level.) The number of arrows in each state indicates the relative populations in that energy level. The population difference for the transitions from energy level $1 \to 2$ (or $|\alpha\alpha\rangle \to |\alpha\beta\rangle$) and $3 \to 4$ (or $|\beta\alpha\rangle \to |\beta\beta\rangle$) are proportional to $\gamma_C$, indicating the transition between the two $^{13}\text{C}$ spin states. Similarly, transitions of $2 \to 4$ (or $|\alpha\beta\rangle \to |\beta\beta\rangle$) and $1 \to 3$ (or $|\alpha\alpha\rangle \to |\beta\alpha\rangle$) correspond to the transition of the two spin states of $^1\text{H}$, and therefore are proportional to $\gamma_H$. As fig. 1.8 indicates, the ratio of $\frac{\gamma_H}{\gamma_C}$ is indeed 4.
Now, suppose one of the proton transitions is selectively inverted. As shown in fig. 1.8 (b), transition \( |\alpha\alpha\rangle \rightarrow |\beta\alpha\rangle \) (or \( 1 \rightarrow 3 \)) is selectively inverted. The inversion cause the population differences in the three other transitions to change. As a result, the new population ratio between the two carbon transitions \( 1 \rightarrow 2 \) and \( 3 \rightarrow 4 \) changes from 1:1 to -3:5. This implies that an initial peak intensity of 1:1 carbon doublet becomes a -3:5 doublet. In other words, the overall intensities of the carbon peaks has been enhanced through Selective Population Inversion.

The SPI experiments leads to anti-phase multiplets which are asymmetric in intensity. The overall enhancement is given by the ratio of the \( \frac{\gamma_{\text{inverted}}}{\gamma_{\text{observed}}} \) spin. In the case of \(^{13}\text{C}-^{1}\text{H} \) spin system, the signal intensity is enhanced by an overall ratio of 4. Experimentally, a proton transition is inverted by a \( 180^0 \) proton pulse. This is followed by a pair of \( 90^0 \) pulses on both nuclei in order to observe their signals without decoupling. Fig. 1.9 shows the \(^{13}\text{C} \) line intensities of the a \(^{13}\text{C}-^{1}\text{H} \) spin system before and after population transfer.
Fig. 1.9 The $^{12}$C line intensities.
(a) Before Selective Population Inversion
(b) After Selection Population Inversion
The number indicates relative intensity.
1.6 A Brief view of INEPT pulse sequence

Insensitive Nuclei Enhanced by Polarization Transfer (INEPT)\textsuperscript{13} is a polarization transfer pulse sequence often used for $^{13}$C assignment. Fig. 1.10 gives the five-pulse INEPT sequence.

![INEPT sequence diagram](image)

Fig. 1.10 INEPT sequence.

INEPT is an extension of the two-particle polarization transfer experiment described in section 1.5. It is different from the two spin $\frac{1}{2}$ nuclei system in that INEPT includes the
excitation of all the $^{13}\text{C} - ^1\text{H}_n$ groups (where n=1, 2, 3) simultaneously through the use of non-selective pulses and proper delays.

As fig. 1.10 shows, INEPT is basically a polarization transfer sequence with a pair of $180^0$ pulses inserted in the middle of two different sets of $90^0$ pulses. This pair of $\pi$ pulses serve to re-focus the chemical shifts of the proton spins, while retaining their coupling to $^{13}\text{C}$ spins. Such re-focusing pulses are used frequently in many other NMR pulse sequences, including the HSQC sequence described in the next chapter.

To trace out the evolution of the spins, a product operator based calculation is carried out as follows for each step of the INEPT sequence. (Again, referring back to fig. 1.10.)

First of all, let $I$ denotes the more sensitive $^1\text{H}$ spins and $S$ denotes the less sensitive $^{13}\text{C}$ spins. At thermal equilibrium, the initial net magnetizations for both nuclei are in the z-direction as the product operator formalism shown below. The index $i$ of overall magnetization $M_i$ corresponds to the numbers in fig. 1.10.

$$M_0 = I_z$$

After the first proton $90^0$ pulse in x-direction, proton magnetization is flipped by $90^0$ about the x-axis, from the z direction to -the y direction.

$$M_1 = -I_x$$
During the first delay period of \((4J)^{-1}\), proton spins evolve according to both the chemical shift and the scalar coupling Hamiltonians described in chapter 1.4. The resultant magnetization at stage 2 (refer to fig. 1.10) is as follows.

\[
M_2 = \frac{1}{\sqrt{2}} \cos \Delta \tau(-I_x + 2I_zS_z) + \frac{1}{\sqrt{2}} \sin \Delta \tau(I_x + 2I_zS_z)
\]

At this point, a pair of \(180^\circ\) pulses are applied and the magnetization becomes:

\[
M_3 = \frac{1}{\sqrt{2}} \cos \Delta \tau(-I_x + 2I_zS_z) + \frac{1}{\sqrt{2}} \sin \Delta \tau(-I_x - 2I_zS_z)
\]

Again, magnetization \(M_3\) is allowed to evolve through a period of \((4J)^{-1}\) under both chemical shift and scalar coupling Hamiltonians. At the end of this second evolution period, the magnetization \(M_4\) results.

\[
M_4 = I_xS_z
\]

Lastly, a pair of \(90^\circ\) pulses produce the final magnetization state \(M_5\) which is detected and Fourier transformed into NMR spectrum.

\[
M_5 = I_zS_y
\]

As the last set of magnetization \(M_5\) indicates, the detectable magnetization will be \(^{13}\text{C}\) magnetization, which lies in the y direction. The vector diagram analysis of this very same sequence is shown in fig. 1.11. Final magnetization \(M_5\) consists of the term \(I_zS_y\) that is a pair of anti-phase \(^{13}\text{C}\) magnetizations on the y-axis. This \(I_zS_y\) magnetization is observable if the multiplet structure is resolved. However, if the decoupler is turned on during acquisition, this coupled signal will be destroyed.
So far, the calculation has been limited to the CH system, however, it can be extended to account for CH$_2$, and CH$_3$ systems. The resultant intensity for the CH$_2$ peaks is 9:2:7 as opposed to the usual triplet splitting pattern of 1:2:1; and the group CH$_3$ splits into 13:15:-9:-11 rather than 1:3:3:1 quartet intensity peaks$^{14}$. Therefore, taking into account of the absolute intensities, signal enhancement factor for CH, CH$_2$, and CH$_3$ are 1, 1, and 1.5 units of $\frac{\gamma_H}{\gamma_C}$. These intensity ratios are produced based on the extension of selective population inversion experiments.$^{15}$
One of the disadvantages of INEPT sequence is that $^{13}$C residual magnetization can produce artifacts within the spectra. Fortunately, it can be eliminated by phase cycling to the first $90^0$ proton pulse. Although INEPT offers an elegant way to distinguish the CH$_n$ groups in the molecule, the anomalies present in the multiplets tends to distort peaks. In practical applications of polarization transfer experiments for resonance assignments, the DEPT$^{16}$ (Distortionless Enhancement by Polarization Transfer) sequence is usually preferred.
Chapter One References:

7. R. R. Ernst, Chimia, 29, 179, 1975

Chapter 2  Heteronuclear Correlation Experiments

2.1 Comparison of the HMQC and HSQC Experiments

Both the INEPT and DEPT sequences described earlier allow the assignment of $^{13}\text{C}$ signals with respect to their multiplicity and yield information about the number of attached protons. However, more experiments are required in order to determine the precise assignment of each signal in the chemical structure. This is often done with the aid of two-dimensional homonuclear and heteronuclear shift correlation experiments.

Homonuclear shift correlation experiments, such as COSY and many of its derivative sequences, provide important bond connectivity information through proton-proton shift correlation. However, the resolution of these homonuclear correlation experiments is limited by the degree of crowding and overlapping of the proton resonances. On the other hand, heteronuclear shift correlation experiment exhibits less spectral crowding by correlating the proton (or the I nucleus) signals with S nucleus that has a much larger shift dispersion than proton, such as $^{13}\text{C}$ or $^{15}\text{N}$. In the heteronuclear shift correlation experiments, resonance frequencies of the scalar coupled nuclei (e.g. $^{13}\text{C}$-$^1\text{H}$) are correlated and presented as cross peaks in a two dimensional NMR spectrum. The correlation is based on the heteronuclear scalar coupling constant over one bond. Therefore, the nuclei which shows cross peaks are the direct neighbors in the particular molecule. This allows the assignment of a proton with a particular resonance frequency to
the directly bonded carbon as directed by the correlated cross peak, and vice versa.

Furthermore, an extension of the short range correlation experiments is the heteronuclear long range correlation experiments. The long range correlation sequences based on the heteronuclear long-range coupling can be used to yield bond connectivity information as in the homonuclear shift correlation experiments, and may provide further aid towards the final solution of a structural elucidation problem. HSQC-TOCSY is one pulse sequence designed for this purpose. It will be discussed later in this chapter.

There are many different pulse sequences available for the two-dimensional heteronuclear shift correlation between a sensitive I nucleus and insensitive S nucleus. One of them, which has been in use for more than fifteen years, is based on the polarization transfer experiments. This is the HETCOR\(^1\) (HETeronuclear CORrelation) experiment. Figure 2.1 below shows the HETCOR pulse sequence.
Another one involves heteronuclear multiple quantum phenomena and is known as heteronuclear multiple quantum correlation (HMQC) experiment. Yet another experiment which utilizes the polarization transfer technique and the sensitivity advantage of the inverse detection feature was introduced by Bodenhausen and Ruben in 1980. It is more recently referred to as the heteronuclear single quantum correlation (HSQC) experiment.

In the HETCOR experiment, magnetization is transferred from the sensitive nucleus (e.g. $^1$H) to the insensitive nucleus (e.g. $^{13}$C) and the $^{13}$C signal is detected. In the case of HMQC, multiple quantum magnetization is generated and allowed to evolve. The

![Fig. 2.1 HETCOR (heteronuclear shift correlation) pulse sequence]

$$\Delta_1 = \frac{1}{2J_{XZ}} \text{ and } \Delta_2 \text{ is responsible for refocusing of the anti-phase X magnetization.}$$
magnetization is then transferred into detectable single quantum magnetization and the more sensitive nuclei ($^1$H) is used for signal detection. Since the signals are detected on the more sensitive nuclei, HMQC has the additional spectral sensitivity advantage over the conventional HETCOR experiment. This applies to the HSQC experiment as well. HMQC and HSQC experiments are, therefore, known as the inverse shift correlation or inverse detection experiments. In this section, the study will be focused on these two inverse detection experiments.

2.1.1 Concept of Coherence

Before carrying out further analysis of HMQC and HSQC pulse sequences, it is important to examine the concept of coherence, in an attempt to gain a general understanding of the multiple and single quantum coherence phenomenon. In theory, a coherence between two spin states corresponds to a transition in the NMR energy diagram. In general NMR terms, coherence describes all the possible mechanisms for the exchange of spin population between two different states, even though not all transitions end up as observable NMR signals. In fact, only the coherences which obey the quantum mechanical selection rules can be directly detected. In addition, only transitions between states of different symmetry can give rise to coherence phenomenon. Coherence between symmetric eigenstates is forbidden.
A system consists of two spin-1/2 nuclei (e.g. IS system) usually has its basis eigenstates expressed as product functions in the Dirac notation, $|IS\rangle$, where the first entry in each eigenbasis indicates the state of the first nucleus and the second entry that of second nucleus. Therefore, the eigenbasis for the spin-1/2 (IS) system is given by $(|\alpha\alpha\rangle, |\alpha\beta\rangle, |\beta\alpha\rangle, |\beta\beta\rangle)$, which can be labeled as states 1, 2, 3, and 4, respectively. This convention is also used in describing the selective polarization transfer in chapter 1.5. Figure 1.8 shows the energy-level diagram of these four eigenstates. In general, the states of the ensemble can be described completely by a $4 \times 4$ density matrix, $\sigma_{RS}$. By definition, the diagonal elements, $\sigma_{kk}$ ($k = 1, 2, 3, 4$), of the density matrix represent the relative populations of the particular state $k$. Coherence is represented by the non-zero off-diagonal elements $\sigma_{RS}$ (where $R \neq S$) between the eigenstate $|R\rangle$ and $|S\rangle$. The order of coherence, $q$, is given by the difference of the total magnetic quantum number $M$ of states $|R\rangle$ and $|S\rangle$. If the total magnetic quantum number $M$ of R and S states differ by $q$ unit, $\sigma_{RS}$ then represents the $q^{th}$ order quantum coherence. Clearly, each transition between the RS states has two coherences $\sigma_{RS}$ and $\sigma_{SR}$ associated with it, and the coherence order are determined by $(M_R - M_S)$ and $(M_S - M_R)$, respectively. Therefore, in the system of two spin-1/2 ensemble, first order coherence, or more generally known as single quantum coherence is represented by the matrix elements of $\sigma_{12}$, $\sigma_{13}$, $\sigma_{24}$, $\sigma_{34}$ and their Hermitian conjugates $\sigma_{21}$, $\sigma_{31}$, $\sigma_{42}$, $\sigma_{43}$. Second order coherence, or double quantum coherence is represented by $\sigma_{14}$ and $\sigma_{41}$. Similarly, $\sigma_{23}$ and $\sigma_{32}$ represent zero-quantum coherence.
Within the framework of product operator formalism, 16 operators are required to describe a two spin-$\frac{1}{2}$ ensemble. In addition to these 16 operators described in chapter 1.4, two additional operators are required to characterized the non-observable coherences. They are the $\hat{I}^+$ and the $\hat{I}^-$ or the raising and lowering operators, respectively. In quantum mechanical terms, these two operators are defined as follows.

\[ \hat{I}^+ = \hat{I}_x + i\hat{I}_y \]
\[ \hat{I}^- = \hat{I}_x - i\hat{I}_y \]

Only single quantum coherence which resulted from $\Delta M=\pm 1$ transition can give rise to observable transverse magnetizations. Therefore, the operators $I_x, I_y, I_x, S_x,$ and $S_y$ which represent the in-phase transverse magnetizations, as well as, $I_xS_x, I_yS_y, I_xS_y, I_yS_x,$ for the anti-phase transverse magnetizations, all corresponds to single quantum coherences. These operators, therefore, represent the observable magnetizations.

The non-observable double quantum coherences are characterized by the products of $\hat{I}^+ \hat{S}^+$ or $\hat{I}^- \hat{S}^-$ and the zero quantum coherences are given by $\hat{I}^+ \hat{S}^-$ or $\hat{I}^- \hat{S}^+$. From the definition of the double and zero quantum coherences, the operator products with two transverse components $I_xS_x, I_yS_y, I_xS_y$ and $I_yS_x$ all contains double and
zero quantum contributions. However, the pure double and pure zero quantum transitions can be obtained through the linear combination of these four product operators.

From the product operator description, it is apparent that both zero and double quantum coherences lead to non-observable magnetization components, whereas single quantum transitions produce detectable magnetization. In fact, single quantum coherence corresponds to transitions between two states with $\Delta M = \pm 1$, which indeed satisfy the selection rules for magnetic dipole. Since a single quantum transition is the only detectable coherence, signals from the multiple quantum experiments must be converted back to single quantum coherence prior to detection, as in the HMQC experiments.

### 2.1.2 Product Operator analysis of HMQC and HSQC

With a general understanding of coherence concepts, the detailed analysis of HMQC and HSQC sequences can then proceed. Figure 2.2 depicts the standard HMQC pulse sequence. Product operator formalism can be used to trace out the detailed spin motion in the course of this sequence, as follows. During the calculation, $\sigma_I$ (where $I = 0, 1, ...$) is used to represent the net magnetization at a specific stage of the pulse sequence and the index $I$ corresponds to the numbering in fig. 2.2.
Initially, bulk magnetization aligns with the external magnetic field $B_0$, as $z$-magnetization and is represented by $\sigma_0$, where

$$\sigma_0 = I_z.$$ 

After the first $90^\circ$ proton pulse in the $x$-direction, net magnetization becomes

$$\sigma_1 = -I_x.$$ 

The transverse magnetization evolves through time period $\Delta$ (or $\frac{1}{2J_{CH}}$) and results in $\sigma_3$. 

$$\sigma_3 = (\cos \omega \Delta)(2I_xS_z) + (\sin \omega \Delta)(2I_yS_x)$$

Next, a $90^\circ_c$ carbon pulse is applied to give $\sigma_4$.

$$\sigma_4 = (\cos \omega \Delta)(-2I_xS_y) + (\sin \omega \Delta)(-2I_yS_x)$$
At this stage, it is clear that the magnetization has evolved into terms which are the linear combination of both zero and double quantum coherences. Unlike previous $\sigma_3$ magnetization, $\sigma_4$ cannot evolve to produce any observable magnetization. Therefore, these multiple quantum coherence terms need to be transformed back to single quantum coherence to give observable NMR signals.

Next, a 180° proton pulse sandwiched in the $t_1$ evolution period interchanges the zero- and double-quantum terms leading to the refocusing of the proton chemical shift and heteronuclear scalar coupling, but not the proton-proton scalar coupling as indicated by $\sigma_5$.

$$\sigma_5 = 2(I_x \cos \omega s t_1 - I_y \sin \omega s t_1)(S_x \cos \omega s t_1 - S_y \sin \omega s t_1)(\cos \pi J_{HH} t_1)$$

A 90° carbon pulse is then applied next to give $\sigma_6$.

$$\sigma_6 = -2S_z (\cos \omega_s t_1)(\cos \pi J_{HH} t_1)(I_x \cos \omega s - I_y \sin \omega s)$$

Lastly, magnetization evolves through the $\Delta$ period again and gives the final observable magnetization as described in $\sigma_7$.

$$\sigma_7 = I_z (\cos \omega_s t_1)(\cos \pi J_{HH} t_1)$$

Other terms which do not give rise to observable magnetization are omitted. Under the experimental conditions, the phase cycle indicated in figure 2.3 is applied to remove undesired artifacts.
Similarly, the product operator calculation for HSQC sequence is outlined below.

Figure 2.3 describes the HSQC sequence and the numbering in the diagram, again, corresponds to the subscript of $\sigma_i$ magnetization in the calculation following figure 2.3.

![Diagram of HSQC sequence]

Fig. 2.3 Heteronuclear single quantum correlation (HSQC) sequence, with $v1 = x \cdot x \cdot x \cdot x$; and $v2 = x \cdot x \cdot x$. $\Delta = 1/(2J_{CH})$

The number corresponds to the magnetization in the HSQC product operator calculation.

Initially, net magnetization $\sigma_0$ is given as follows.

$$\sigma_0 = I_2$$

A 90$^\circ$ pulse on proton gives raise to $\sigma_1$.

$$\sigma_1 = -I_1$$

$\sigma_1$ evolves through $2\Delta$ period ($\Delta = \frac{1}{2J_{CH}}$) with a pair of 180$^\circ$ pulses sandwiched in between, to give $\sigma_2$. 
\[ \sigma_2 = -2I_z S_z \]

A pair of 90° pulses is then applied, (90, on \(^1\)H and 90, on \(^{13}\)C), to produce \( \sigma_3 \).

\[ \sigma_3 = +2I_z S_z \]

The magnetization then evolves through \( t_1 \). The 180, pulse on protons during the \( t_1 \) evolution period refocuses the heteronuclear \( J_{CH} \) coupling, leading to the single-quantum \(^{13}\)C-spin coherence described in \( \sigma_4 \).

\[ \sigma_4 = 2I_z S_z \cos \omega_s t_1 - 2I_z S_z \sin \omega_s t_1 \]

Immediately following \( t_1 \), a pair of 90° pulses are applied, with 90, on proton and 90, on carbon, which results in \( \sigma_5 \). The second term in \( \sigma_5 \) will not evolve into any observable magnetization and is omitted from the calculation.

\[ \sigma_5 = 2I_z S_z \cos \omega_s t_1 - 2I_z S_z \sin \omega_s t_1 \]

Finally, magnetization evolves through 2\( \Delta \) period, again, with the last pair of 180, pulses sandwiched in between. The final observable magnetization is given by \( \sigma_6 \) as follows.

\[ \sigma_6 = -I_z (\cos \omega_s t_1) \]

In essence, the HSQC sequence consists of two INEPT sequences with an evolution period \( t_1 \) inserted in the middle. The first INEPT transfers \(^1\)H magnetization to \(^{13}\)C magnetization, which then evolves through \( t_1 \). The \(^{13}\)C magnetization is then transferred back to \(^1\)H magnetization by the second inverse INEPT and \(^1\)H signals are detected.
The HSQC sequence has several advantages over HMQC. the most important one lies in its ability to eliminate the proton-proton homonuclear coupling in the \( F_1 \) domain. This can be explained with the aid of the product operator calculation above. During the \( t_1 \) evolution period of the HMQC sequence, net magnetization contains the terms \( 2I_xS_y \) and \( 2I_yS_y \) which is a linear combination of the zero and double quantum coherence. This magnetization evolves not only according to the chemical shifts, but also according to the homonuclear J-coupling Hamiltonian of the proton spins. Consider the effect of a second proton \( K \) which is coupled to the \( I \) or \( S \) spin. During the \( t_1 \) evolution period, dephasing caused by the \( J_{SK} \) heteronuclear coupling is refocused by the \( 180^0 \) proton pulse. However, dephasing caused by the homonuclear scalar coupling, \( J_{IK} \), is not refocused since both spins \( I \) and \( K \) experienced the non-selective \( 180^0 \) pulse. Consequently, the scalar coupling term, \( \cos(\pi J_{HH}t_1) \) remains in the final observable magnetization \( \sigma_f \) of the HMQC product operator calculation. This leads to the \( ^1\text{H}-^1\text{H} \) scalar coupling in the carbon domain of the HMQC spectrum. Such induced homonuclear scalar coupling is redundant and leads to the loss of sensitivity in the spectrum. In practice, this is usually observed as the line broadening effect, which affects the overall spectral sensitivity and resolution. The HSQC sequence proposed by Bodenhausen and Ruben is able to overcome this line broadening problem present in the HMQC spectrum.

Unlike the HMQC sequence, there are no multiple quantum terms during the \( t_1 \) evolution period as indicated by \( \sigma_4 \) (\( \sigma_4 = 2I_xS_y \cos\omega_st_1 - 2I_yS_y \sin\omega_st_1 \)) of the HSQC product operator calculation. Therefore, no homonuclear proton-proton scalar coupling
will contribute to the evolution. Hence, the $t_1$ domain of HSQC spectrum is free of the line broadening effect caused by the homonuclear $J$-coupling of the protons. This leads to a much better spectral sensitivity and ultimately leads to a better resolution in the HSQC spectrum.

The sensitivity improvement in the HSQC sequence over HMQC has been known to NMR spectroscopists and this property was demonstrated through the study of protein and other macromolecules via $^1$H-$^{15}$N correlation experiments$^5$. Recently, the introduction of the gradient version of HSQC sequence$^6$ which is capable of more effective solvent suppression further improves the preference towards the single quantum correlation experiments. However, the advantage of HSQC is less clear for the $^1$H-$^{13}$C correlated experiments on the macromolecules due to unfavorable $^{13}$C transverse relaxation$^5$.

The line width in the $F_1$ domain depends on the $t_2$ transverse relaxation. In the case of a $^1$H-$^{15}$N or $^1$H-$^{13}$C (IS) spin system, transverse relaxation is considered to be purely dipolar and the S-nucleus ($^{13}$C or $^{15}$N) is relaxed solely by interaction with its bonded protons, $H_N$, while $H_N$ relaxes both with $^{15}$N or $^{13}$C and m other protons. Therefore, the transverse relaxation rate depends on the $t_2$ relaxation rates of spin I, spin S, and multiple quantum coherence. For an $^{15}$N-$^1$H system, the homonuclear dipolar relaxation is stronger than the effect of the heteronuclear coupling on transverse relaxation. For a $^{13}$C-$^1$H system, the heteronuclear dipolar coupling to a directly attached $^1$H is approximately a factor of 2 larger than for $^{15}$N and therefore it usually dominates

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transverse relaxation of $^{13}$C magnetization and the proton attached to it\(^5\). However, the loss of sensitivity due to the unfavorable $^{13}$C transverse relaxation in the macromolecule should have a substantially lower effect on smaller natural product compounds. The advantage of HSQC over HMQC sequence is significant in the $^{13}$C-$^1$H correlation experiments for the smaller natural product compounds. The following experimental results confirm this.

2.1.3 Experimental Verifications

To demonstrate the advantage of the HSQC sequence, standard HMQC and HSQC sequences from Varian software library were employed to obtain the heteronuclear shift correlation spectra.

Figure 2.5a and 2.5b shows the contour plot of the HMQC and HSQC spectra of the clionasterol, respectively. It is readily noticeable that HSQC signals in 2.5b shows much better resolution particularly in the carbon (or F\(_1\)) domain. The narrowing effect in the F\(_1\) domain of the HSQC spectra is largely due to the elimination of the homonuclear proton-proton J coupling during the t\(_1\) evolution period. Figure 2.6 zooms into the region between $\delta_c=30$ - 32 PPM and $\delta_h=1.3$-2.0 PPM region of the HMQC (2.6a) and HSQC (2.6b) spectra. The resolution and sensitivity improvement in HSQC spectrum is apparent. The assignment of clionasterol is presented in the paper by Prof. Reynolds et. al.\(^7\). The HMQC spectrum in Figure 2.6a shows a cluster of un-resolved peaks at $\delta_c 31.8$ /
\( \delta_H 1.5 \). The same signal is partially resolved by the HSQC sequence shown in figure 2.6b. From fig.2.6b, one can also observe the striking improvements in sensitivity of the HSQC spectrum in the carbon domain. Figure 2.6c and 2.6d shows the trace of the proton cross section at \( \delta_H 1.5 \), where the spectrum (2.6d) gathered via HSQC sequence shows a better sensitivity and resolution of the carbon doublets as compared to the similar cross section of HMQC spectra in 2.6c. This demonstrates the improvements of the HSQC experiment in the carbon domain. In addition, the traces through carbon domain at \( \delta_C 31.8 \) PPM are shown in fig. 2.7a and 2.7b for HMQC and HSQC, respectively. Again, cross-section through HMQC in 2.7a shows two proton peaks with somewhat ambiguous multiplet patterns, whereas 2.7b depicts more sensitive and better resolved proton peaks via an HSQC experiment. This further demonstrated that the spectral improvement of the HSQC experiment is not limited to the \( F_1 \) domain, the proton (or \( F_2 \)) domain seems to improve in sensitivity and resolution as well. Another cross-section through the carbon domain at \( \delta_C 26.4 \) is shown in fig. 2.8 for both experiments. Again, this shows similar general improvement as noted in the \( \delta_C 31.8 \) PPM cross section.

In summary, it is shown that HSQC has significant advantages over its HMQC counterpart in the case of natural product molecules. The improvement in \( F_1 \) and \( F_2 \) domain resolution of the HSQC spectrum produced by eliminating the \(^1H-^1H\) scalar coupling during \( t_1 \) evolution period is significant in natural product molecules, therefore, it is preferred over the multiple quantum experiment.
Though HSQC has several advantages over HMQC, it is not without faults. A general disadvantage of HSQC sequence lies in its greater number of pulses which give rise to more opportunity for generation of artifacts and loss of signal intensity due to pulse imperfection. All these factors may decrease its sensitivity. However, such factors are small compared to the overall gain by employing the HSQC sequence.
Figure 2.5 (a)

HMOC contour plot of Clionasterol

Figure 2.5(b)

HSQC contour plot. It shows improvement in both sensitivity and resolution, especially in Carbon domain.
Figure 2.6 (a)

HMQC spectrum of Clionasterol shows unresolved Carbon peak.

Figure 2.6(b)

HSQC plot of similar region shows two partially resolved carbon peaks.
Figure 2.6 (c)

Proton cross section of HMQC spectrum at $\delta_H 1.5$ showing the C-2 peaks.

Figure 2.6 (d)

Similar cross section of the HSQC spectrum shows sensitivity and resolution improvement in carbon domain.
Figure 2.7 (a)

$^{13}$C cross section of HMQC spectrum at $\delta_c$ 31.8. It shows H-7 $\alpha$ and $\beta$ proton peaks.

![Graph of Figure 2.7 (a)](image)

Figure 2.7 (b)

Similar cross section of the HSQC spectrum shows much better sensitivity and resolution even in the proton domain.

![Graph of Figure 2.7 (b)](image)
Figure 2.8 (a)

$\delta_c = 26.4$

HMQC cross section

Figure 2.8 (b)

$\delta_c = 26.4$

HSQC cross section

Figure 2.8 (c)

$\delta_c = 28.2$

HMQC cross section

Figure 2.8 (d)

$\delta_c = 28.2$

HSQC cross section
2.2 Heteronuclear Shift Relay experiment

Both HMQC and HSQC sequences are one-bond heteronuclear shift correlation experiments. To further utilize the advantages of less resonance congestion in the heteronuclear shift correlation experiment and at the same time obtain important bond connectivity information, a heteronuclear correlated relay experiment is constructed to carry out this work. Since the HSQC sequence has the added advantage of superior spectral sensitivity over HMQC, consequently, HSQC relay experiments, also known as the HSQC-TOCSY experiment, are preferred for multiple bond connectivity studies over the HMQC based relay experiment. HSQC-TOCSY combines the advantage of increased resolution provided by single quantum coherence with a simple relay (TOCSY or HOHAHA) sequence to yield a more complete and fully resolved $^1$H-$^1$C spectrum. This adds valuable information to the structure elucidation problem.

Since a detailed analysis of HSQC sequence is covered in the previous section, it is suffice to examine the second building block - TOCSY component of the HSQC-TOCSY sequence. TOCSY, also known as total correlation spectroscopy, is another cross-polarization experiment, which is also known as the Homonuclear Hartman Hahn (HOHAHA) experiment. It is based on the idea proposed by Hartmann and Hahn for solid state NMR but which has been applied to liquid NMR. Fig. 2.9 below shows the two-dimensional homonuclear TOCSY experiment.
TOCSY sequence with a MLEV-17 spin lock sequence.

Similar to the polarization transfer experiments discussed in chapter one, the objective of TOCSY is to improve the detection of the insensitive S nucleus of low natural abundance by magnetization transfer from a sensitive I nucleus of high abundance and sensitivity. The first 90° pulse flips magnetization from z-direction to the y-direction in the rotating frame of reference. The MLEV pulse acts as spin-lock that locks the proton magnetization in the y-direction of the rotating frame of reference. Under this condition, the transfer of polarization between the protons took place. In a solid state TOCSY experiment, magnetization transfer is based on the dipolar coupling between spins. In liquid, fast molecular motion averages out the dipolar interactions; however, magnetization transfer is still possible through the scalar spin-spin coupling effect.
Magnetization transfer in TOCSY takes place during the mixing time \( (t_m) \) period. The transfer of magnetization proceeds beyond the directly coupled nuclei, to the remote nuclei and finally to throughout the entire network of nuclei that are scalar coupled, hence the name, total correlation spectroscopy. In a TOCSY experiment, magnetization transfer is governed by the length of the mixing time. A short mixing time produces cross peaks of the strongly coupled protons, while a longer mixing time allows transfer of magnetization to remote protons further away in the spin system. This applies to the HSQC-TOCSY sequence as well. With a longer mixing time, magnetization transfer is allowed to proceed further to the weakly coupled protons. Through the long range propagation of magnetization in protons, TOCSY spectrum reveals bond-connectivity information.

The HSQC-TOCSY sequence is basically the HSQC sequence with a TOCSY spin-lock (in the form of MLEV-17 pulses) attached at the end. The TOCSY component of HSQC-TOCSY allows the magnetization transfer of the proton spins. Figure 2.10 shows the HSQC-TOCSY pulse sequence.
Product operator analysis of HSQC-TOCSY sequence is essentially the same as for the HSQC sequence in section 2.1 with a slight difference due to the effect of the spin lock sequence at the end. During the MLEV-17 spin lock sequence, $^1$H coherences are mixed and the magnetization is "locked" along the chosen spin-lock axis to allow the magnetization transfer between the $^1$H spins. A trim pulse inserted prior to the MLEV-17 pulses act as a filter to remove the magnetization which does not lie along the spin-lock axis. Finally, after the mixing period, $^1$H magnetization is detected during $t_2$ period. The experiment is repeated many times with incrementing $t_1$ to generate the final 2D heteronuclear relay spectrum.
As stated earlier, the biggest advantage in selecting a heteronuclear relay experiment over its homonuclear counterpart lies in the better spectral resolution in the spectrum produced by the former experiment. In addition, heteronuclear relay experiments retain the relay peaks as in the homonuclear relay experiments. The two-dimensional heteronuclear relay sequence, HSQC-TOCSY, is, therefore, particularly suited for structural assignment problems of the compounds with strong overlap in 1H resonances. The compound clionasterol ((24S)-24-ethylcholest-5-en-3β-ol) is selected to test the HSQC-TOCSY sequence.

Clionasterol is the C24 epimer of the common plant sterol, β-Sitosterol. The two epimers are distinguished by small differences in the $^{13}$C and $^1$H chemical shift of the side chain methyl groups. $^8$ β-Clionasterol presents an ideal challenge for the HSQC-TOCSY heteronuclear relay experiment since there is extreme $^1$H resonance crowding. Figure 2.11 shows the structure of the β-clionasterol. As a comparison, a two-dimensional TOCSY relay experiment was run. In the paper by Reynolds$^7$ et. al., HSQC-TOCSY experiment along with series of other 1D and 2D NMR experiments were used to provide a full $^1$H assignment of the β-clionasterol compound.
Some of the HSQC-TOCSY spectra are presented here as an attempt to illustrate several characteristics of this sequence. First of all, figure 2.12 shows a part of the TOCSY spectrum, where one can see the obvious crowding and overlapping in many of the proton resonances. Part (b) of figure 2.12 is the HSQC-TOCSY spectrum obtained with mixing time of 0.018s. One can immediately notice that the HSQC-TOCSY spectrum retains the sensitivity enhancement in the $^{13}$C domain from the HSQC sequence; it also shows substantially less crowding of the proton resonances. Most importantly, HSQC-TOCSY provides better resolved relay peaks from the TOCSY component of the HSQC-TOCSY pulse sequence.

As discussed earlier, the longer mixing time allows further magnetization transfer to the weakly coupled protons. This effect is very apparent in the $^1$H cross section through C-14 of Clionasterol with the mixing time ranging from 0.006s to 0.018s. Figure 2.13 shows the polarization transfer of the protons neighboring to the C-14 nucleus. As pointed out in the paper by Reynolds et. al., in the C-14 ($\delta_c=56.77$) cross section, H-14$_a$ proton showed an initial large transfer to H-15$_3$ in figure 2.13, consistent with the large anti-phase coupling between these protons. As the length of the mixing time increases, the spectrum showed further polarization transfer to proton at $\delta 1.84$ (mixing time 0.05s) as predicted. It is difficult to distinguish the protons which are directly coupled to the carbon nuclei in the relay spectrum. However, this problem can be resolved by cross checking the relay spectrum with the HSQC or HMQC one bond correlation experiments. Both HMQC and HSQC provides information on the protons directly coupled to $^{13}$C.
nuclei. Another route to solve this problem was proposed by Domke in 1991. Domke inserted a delay of $2\Delta$ after the spin lock sequence to achieve a phase difference between directly bonded and remote correlation signals. This provides an area for further study.

Lastly, it should be noted that although the HMBC (heteronuclear multiple bond correlation) experiment essentially presents similar spectral information as HSQC-TOCSY, it has a similar limitation in $^{13}\text{C}$ resolution as HMQC does. That is, proton-proton homonuclear coupling effect shows up in the carbon dimension and hence decreases the sensitivity and resolution in the $t_1$ domain.

Therefore, in cases where there will be noticeable carbon resonance crowding, this effect could become a major problem. However, if one has sufficient sample, a $^{13}\text{C}$-detected sequence such as FLOCK is capable of providing excellent $^{13}\text{C}$ and $^1\text{H}$ resolution to overcome the HMBC resolution problem in the $t_1$ domain. Otherwise, HSQC-TOCSY remains a better choice in heteronuclear relay experiments.
Figure 2.11

Structure of β-clionasterol. Assignment of Proton and carbon chemical shift is listed in table 2.1 and discussed briefly in chapter 2.3.
Figure 2.12 (a)

TOCSY contour plot of clionasterol. It shows of $^1$H resonance overlapping.
Figure 2.12 (b)

HSQC-TOCSY spectrum correlates the $^1$H and $^{13}$C cross peaks to reduce spectral congestion as in TOCSY.
Figure 2.13 (a)

HSQC-TOCSY cross section of Clionasterol through $\delta_c = 56.88$ (C-14).

Mixing time = 0.006 sec.

Figure 2.13 (b)

Same cross section with mixing time = 0.012 sec.

More relay peaks showed up during the slightly longer mixing time. As the mixing time increase, magnetization transfer propagates further causing more relay peaks to show up.

Figure 2.13(c)

Mixing time = 0.03 sec.
Figure 2.13(d)
Mixing time = 0.05

Figure 2.13(e)
Mixing time = 0.075
2.3 Coupled HSQC Sequence

It has been proposed that coupled HMQC spectra can be used to determine individual $^1$H multiplet patterns. Unfortunately, presence of $^1$H multiplet structure along both axes sometimes leads to distorted cross-sections. Nevertheless, similar effects can be observed in a slightly modified HSQC sequence without the spectral distortion problem. To distinguish from the original HSQC sequence, the modified HSQC is referred to as CHSQC sequence; short for coupled HSQC sequence. Figure 2.15 below depicts the CHSQC sequence.

![Diagram of CHSQC sequence]

Fig. 2.15 Coupled heteronuclear single quantum correlation (CHSQC) sequence with $\nu_1 = x_1 x_2 x_3 x_4$ and $\nu_2 = x_1 x_2$. $\Delta = 1/(2J_{CH})$

It is essentially HSQC sequence with the last delay removed and the decoupler on carbon not turned on during acquisition.
Compare it with the original HSQC sequence in figure 2.3. CHSQC lacks the last two $\Delta \left( \frac{1}{2J_{CH}} \right)$ delay period between the final polarization transfer and the acquisition period. Again, the product operator analysis will be identical to the HSQC sequence up to the last pair of $90^0$ pulses. According to the HSQC product operator calculation, the CHSQC magnetization prior to acquisition should correspond to $\sigma_3$ of the HSQC sequence in chapter 2.1, with

$$\sigma_3 = 2I_zS_z\cos\omega_1t_1 - 2I_zS_z\sin\omega_1t_1$$

The second term consists of non-observable zero and double quantum mixing terms and can be neglected, whereas the first term consist of $I_zS_z$ indicates a pair of observable anti-phase x magnetization.

CHSQC differs from the HSQC sequence in that the additional $2\Delta$ period in HSQC refocuses the two anti-phase magnetizations and eventually produces observable in-phase magnetization. A small phase distortion is observed in the original HSQC sequence due to the evolution of $^1H-^1H$ coupling under the fixed delay. This problem is eliminated in the CHSQC sequence.
Two CHSQC experiments were run on the standard testing sample, kauradienoic acid and clionasterol. Figure 2.16 shows an excellent proton resolution which allows one to estimate the coupling constants and assign individual protons as axial or equatorial based on the number of large vicinal coupling. This study is carried out in the paper by Reynolds et. al.\(^7\)

Figure 2.17 shows the structure of kauradienoic acid. In the case of kauradienoic acid, most of the carbon cross-sections of the CHSQC spectra show symmetric multiplet structures of positive and negative peaks resulted from the anti-phased proton magnetization. Figure 2.18 and 2.19 shows the symmetry of these anti-phase proton multiplets of both kauradienoic acid and clionasterol, respectively. Also figure 2.20 shows the C-6 and C-7 cross section of the kauradienoic acid. The asymmetry between the anti-phase doublets of proton magnetization is due largely to the virtual coupling effect previous observed in the \(^{13}\)C-detected HSQC sequence as pointed out by Reynolds’ paper.

The simplest spin system to show virtual coupling between \(^1\)H and \(^{13}\)C is an ABX system where there are resolvable \(J_{AX}\) and \(J_{AB}\) coupling. As the name indicates, the system has two nuclei, A and B, with very similar chemical shifts and are coupled to the third nucleus X with resonance frequency \(v_X\) which is very different from \(v_A\) and \(v_B\). Suppose there are three protons are labeled as A, B, and C, and two carbons with the
olefinic methylene carbon labeled as X and methine carbon as Y. J_{AB} coupling are usually much weaker than J_{AX} coupling. Under this condition, the heteronuclear shift correlation spectrum shows a simple doublet with splitting J_{AX} and a doublet at v_A in the F_1 domain. However, if the difference between v_A and v_B is equal to (1/2)J_{AX}, one of the ^13C satellite proton spectrum will split the multiplet of A into two halves: one distant from B and the other on top of B. Similarly, C proton will do the same to proton B and carbon Y will produce the same effect on proton A and C. This results in a virtual coupling effect in the coupled spectrum.¹¹

In kauradienoic acid, the virtual coupling condition is fulfilled for H-7_a (δ=1.98) and H-6_b (δ=1.85) since v_{ab} is close to that of (1/2)J_{CH} as shown in 2.20. However, some of the asymmetrical splitting patterns of clionasterol are slightly more complicated. Therefore, the problem of asymmetric proton anti-phase magnetization still awaits further investigation.

The CHSQC sequence has proven to be very useful in the spectral assignment of \(^1H\) chemical shift of clionasterol, especially in assigning the α or β methylene protons. This allows the correction of several α and β proton assignments reported for cholesterol which only differs from clionasterol in the absence of the C-24 ethyl group.³
To utilize the advantages provided by the HSQC based sequences introduced in this chapter, a rigorous assignment of clionasterol was carried out. As mentioned before, clionasterol is a suitable compound for this purpose due to its extreme proton resonance crowding. In addition, its $^{13}$C spectrum indicated three protonated carbon peaks crowded in the region of 31.7 to 31.9 PPM. The superior carbon resolution in the HSQC based sequences provided valuable information in assigning these three carbon peaks.

To arrive at the overall assignments, the following experiments were run: normal 1H, 13C, high resolution DEPT (focusing on the aliphatic region), COSY, HMQC, HMBC, NOESY, HSQC, CHSQC and 2D HSQC-TOCSY.

In figure 2.6, both HSQC and HMQC shows the one-bond $^{13}$C-$^1$H correlation peaks which corresponds to the three closely overlapped $^{13}$C peaks. The HMQC spectrum (figure 2.6a) spectrum showed the unresolved peak centered at $\delta_C$ 31.92 and $\delta_H$ 1.5. which corresponds to cross-peaks C-2, C-7 and C-8. On the other hand, the HSQC spectrum in figure 2.6b shows a partially resolved cross peak at $\delta_C$ 31.68 (with $\delta_H$ at 1.5 and 1.84) which corresponds to C-2. However, the other two carbon peaks at $\delta$ 31.92 and $\delta$ 31.93 were unresolved due to the small 13C chemical shift difference and the two protons have relatively similar chemical shifts centered at $\delta_H$ 1.49. Both HSQC-TOCSY and COSY spectra suggested that olefinic hydrogen were coupled at $\delta_H$ 1.98 and $\delta_H$ 1.53, and were assigned as the C-7 methylene protons. The chemical shifts of H-8, H-11$_a$ and H-11$_b$ are very similar and are all vicinally coupled to H-9 making the assignment
difficult. However, the CHSQC spectrum showed distinct multiplet peaks at $\delta_H 1.98, 1.53$ and 1.45 which allow the assignment of $\delta_H 1.45$ to H-8. Lastly, the high resolution DEPT spectrum allowed the assignment of C-7 at 31.93 and C-8 at 31.92 based on their different number of attached protons. The HSQC based experiments combined with the other routine 1D and 2D experiments listed earlier allowed the full assignment of the basic $^1H$ and $^{13}C$. The result is first presented in the paper by Reynolds et al.\textsuperscript{7} Table 2.1 on page 82 shows the complete assignment of $^{13}C$ and $^1H$ chemical shift for clionasterol.

Throughout this chapter, a series of HSQC and HSQC based sequences have been investigated in detail, showing its superiority over the HMQC based experiments. Overall, the elimination of $^1H$-$^1H$ coupling in the $F_1$ domain which leads to an enhancement of both resolution and sensitivity remains the most important reason in developing these single quantum based sequences. In addition, the coupled HSQC (CHSQC) sequence, presents an new way of obtaining clear proton multiplet structure. The CHSQC sequence also brings up an interesting virtual coupling effect which produces asymmetric proton coupling in CHSQC spectrum. However, as the paper by Reynolds et. al points out,\textsuperscript{7} it is not clear that the asymmetry was the result of the redistribution of magnetization through strongly coupled spin system and/or from the pulse imperfections. This factor also awaits further study.
In addition to the assignment of clionasterol discussed earlier, the full assignment of a new imide compound isolated from the leaves and twigs of the plant, *Piper Verrucosum*, was also carried out with more routine NMR experiment.

Analysis of $^1$H, $^{13}$C, COSY, HMQC and HMBC spectra enabled the complete structural assignments of this new imide compound, leading to its formulation as 3,4-epoxy-2-oxopiperdinyl. The complete assignment and detail analysis of this newly isolated compound is presented in the paper by Jacobs et al.\textsuperscript{12} and is appended as appendix C of this thesis.
Table 2.1: $^1$H and $^{13}$C Chemical Shift For Clionasterol$^a$

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</tbody>
</table>

a. $^{13}$C and $^1$H chemical shifts in CDCl$_3$, relative to internal (CH$_3$)$_4$Si

b. The olefinic hydrogen cannot be designated as either $\alpha$ or $\beta$

c. Side-chain methylene protons are listed in order of increasing chemical shift without designation as $\alpha$ or $\beta$

d. C-26 and C-27 are assigned as previously since the spectra provided no means of distinguishing these two methyl groups
Figure 2.17

Structure of Kauradienoic acid.

Figure 2.16

CHSQC cross section through $\delta_c = 37.38$ shows symmetric peaks of clionasterol.
Figure 2.19

CHSQC cross section of Clionasterol at $\delta_c = 39.84$.

Figure 2.18

CHSQC cross section of Kauradienoic acid.
Figure 2.20

C-6 and C-7 cross section of kauradienoic acid. The asymmetric $^1$H peaks are due to the virtual coupling effect.
Chapter Two Reference:

All the NMR experiments, including HMQC, HSQC, CHSQC, HSQC-TOCSY, and HMBC were carried out on a Varian UNITY-500 NMR spectrometer equipped with a 5 mm inverse detection probe. The 90° proton pulse width was set at 9.5 μs and the 13C decoupler pulse width at 9.4 μs. Data were acquired at a temperature of 25°C. All of the clionasterol spectra were obtained with 8 mg of clionasterol dissolved in 1 ml of CDCl₃ in a 5 mm tubes. The solution contains 0.1% of TMSO, (CH₃)₄Si, as an internal reference.

All HMQC, HMBC and TOCSY spectra were obtained with the standard pulse sequences provided in the Varian software library. The HSQC and the CHSQC sequences were extensively modified from an earlier version of HSQC sequence in the user’s library. The CHSQC sequence was constructed by removing the last two delay periods as well as the last pair of 180° pulses from the HSQC sequence. Similarly, the HSQC-TOCSY sequence was written by modifying the HMQC-TOCSY sequence to convert it to a HSQC-TOCSY sequence. This process was somewhat tricky since the phase cycles of the two sequences are very different. Extra care must be taken to ensure the phase cycles were programmed correctly in the HSQC-TOCSY sequence. All three programs were written in the C programming language. However, the executable files generated by the C source codes cannot be processed directly by the NMR software that runs the actual experiment. Macros must be constructed to direct the NMR software to the correct source code and parameter sets. Therefore, related macros and standard parameter sets were also
programmed. All three pulse sequences, namely, HSQC, HSQC-TOCSY and CHSQC, as well as the related macros are included in appendix A.

Decoupled HSQC and HMQC spectra were acquired in a phase sensitive mode with 256 data points, zero filled to 512 and both with 256 times $t_1$ increments. The relaxation delay ($d_1$) was set to 1.6s. 16 spectra were collected per $t_1$ increment and $^{13}$C Garp decoupling was applied during $t_2$ acquisition. A Gaussian weighting function was applied prior to Fourier transformation. The Gaussian time constant for the $f_1$ domain was set to 0.176s for linear prediction up to 2048 points.

Coupled HSQC spectra were obtained using 2048 data points, zero filled to 4096, with $f_1$ linear prediction up to 1024 points and zero filled to 2048. There are 32 transients per $t_1$ increment and the $d_1$ delay used in this experiment was 0.6s.

HSQC-TOCSY spectra were obtained for a series of mixing times ranging from 0.006s to 0.05s.
Chapter 4:

Developing ZUNITY program:

A user friendly software to perform 1D and 2D NMR experiments

4.1 Introduction

Among the many spectroscopic methods available to chemists for the use in structure elucidation, nuclear magnetic resonance is the most popular spectroscopic technique today. NMR has made tremendous amount of progress in an extraordinary short period of time. In 1945, the successful observation of NMR phenomenon in liquid and solid were made by two group of physicists working independently, Bloch, Hansen and Packard at Standford University\textsuperscript{1} and Purcell, Torrey, and Pound at Harvard University\textsuperscript{2}.

Over the past 50 years, NMR spectroscopy quickly developed to completely change the way of studying of complex molecular structures. From the earlier continuous-wave spectrometers to the new generation of pulse Fourier transformed spectrometer, each generation strives to achieve higher and higher magnetic field strength (and hence improve the spectral resolution as well as sensitivity). One other profound change in the new generation of spectrometers is the adaptation of more sophisticated computers and computer interfacing technology, which has made the recent generation of
spectrometers more powerful than ever. In the case of Varian’s high field spectrometers, (e.g. UNITY models), the popular UNIX operating system is employed to interface with SUN-based workstations as well as the VNMR software which eventually runs the spectrometer. The advanced computer technology provides users more flexibility, yet it also adds tremendous complexity to programming routine NMR experiments.

The advances of NMR spectroscopy are not limited to the hardware aspect because the developments of many popular one-dimensional and two-dimensional pulse sequences are the soul of this spectroscopic technique. With more and more complex molecular structures being investigated, many of these two-dimensional experiments are used on a routine basis. Needless to say, the set-up of these 2D experiments is much more complicated than the general 1D experiments, mainly because of the large number of parameters involved in the 2D experiments. This coupled with the complexity of the interfacing software are two serious obstacles for general users. One needs to be familiar with the UNIX operating environment as well as having significant amount of experience in running NMR experiments in order to set up the large number of parameters efficiently and correctly. The non-NMR spectroscopists are often overwhelmed by the large number of parameters which are required in setting up a 2D experiment. More importantly, without extensive experience in running these experiments, many will not know what values that some of these parameters should be set to.
To aid the general users in setting up and running some of the more routine experiments, a user friendly program - ZUNITY was developed. ZUNITY guides the users to set-up parameters, acquiring as well as processing data in several popular 1D and 2D NMR experiments. The program greatly simplifies the set-up procedure and reduces the amount of time, knowledge and effort required from users.

4.2 ZUNITY - A Modified Version of UNITYNMR Program

ZUNITY programs were adapted and modified from the UNITYNMR program written by J.P. Yang. Yang’s UNITYNMR program provided users choices of six 1D and 2D experiments, namely, Proton, Carbon, Attached Proton Test (APT)\(^3\), Proton-Proton Correlation Spectroscopy (COSY)\(^4\), Proton-Carbon Correlation Spectroscopy (HETCOR)\(^5\), Proton-Carbon Correlation Spectroscopy via long range coupling (FLOCK)\(^6\). However, it fails to include two important and routinely used 2D experiments- Heteronuclear Multiple Quantum Correlation (HMQC)\(^7\) and Heteronuclear Multiple Bond Correlation (HMBC)\(^8\). The newly developed ZUNITY program incorporated these two experiments along with the other six available from UNITYNMR program into one coherent and user friendly software.
Although HETCOR and HMQC both are capable in producing 2D heteronuclear shift correlation between a sensitive A nucleus (e.g. $^1$H, $^{19}$F, $^{31}$P) and an insensitive X nucleus (e.g. $^{13}$C, $^{15}$N), the principles used to obtain these results are intrinsically different between the two sequences. HETCOR utilizes the polarization transfer from the sensitive A nucleus to the insensitive X nucleus and the signals of the insensitive X nucleus (often $^{13}$C or $^{15}$N) are detected, whereas in HMQC, multiple quantum magnetization is produced and then transferred into detectable magnetization. In the case of HMQC, the more sensitive A nucleus is used for signal detection. Therefore, HMQC is capable of generating a more sensitive spectrum compared to HETCOR. Hence, it is important to provide users the option of this sequence in addition to HETCOR. In addition to HMQC, HMBC provides another heteronuclear shift correlation experiment via multiple bond connectivity. Combination of these two experiments along with $^1$H and $^{13}$C experiments have been used extensively for structure elucidations. ZUNITY program provides an easy and quick route in setting up these experiments.
4.3 ZUNITY Features

ZUNITY program is written in Varian’s MAGnetic Instrument Control and Analysis Language II. (MAGICAL II). MAGICAL II is a high-level VNMR software language, and is designed to run on the Varian SUN-based NMR spectrometers (e.g. the UNITY systems). The program exists as a coherent package of individual macros. Each macro is basically a collection of VNMR commands and can be used independently to carry out a specific task outside of the main ZUNITY program. For example, one can invoke the “setbc” macro to setup the HMBC experiment without entering the main program. This allows the user the freedom to change any of the parameters set by the program before running the experiment.

One other noticeable feature of the ZUNITY program is its user friendliness. Similar to UNITYNMR program, ZUNITY will automatically select an experimental working area, set and transfer necessary parameters, acquire data as well as processing them when the acquisition is completed. In addition, numerous on-line comments have been implemented to inform the user of the current status of the spectrometer and to guide users through several user-input questions which are necessary in setting up the experimental parameters.
The greatest advantage of ZUNITY lies in its versatility. The program provides users a total of eight routinely used 1D and 2D experiments including \(^1\)H, \(^{13}\)C, APT, COSY, HETCOR, FLOCK, HMQC and HMBC. In each experiment, user have a choice of ten solvents, namely, CDCL3, C6D6, CD2CL2, Acetone, DMSO, D2O, C6D5CD3, C6D12, CD3COOD. Since VNMR software provides the queuing and multitasking feature, ZUNITY employs these features to efficiently utilize the spectrometer time. ZUNITY can queue up multiple experiments and when one experiment is done, it proceeds to process the data while the other experiment is in progress. This will help to save valuable spectrometer time.

4.4 Programming Strategy for HMQC and HMBC Options

The working strategy of the first six experiments in ZUNITY are similar to Yang's UNITYNMR program and were described in detail in chapter 5 of Yang's Ph.D. thesis. Here, the programming strategy of both HMQC and HMBC will be discussed.

As ZUNITY program is invoked, it will prompt users for the solvent used in the sample. As soon as the solvent is entered, ZUNITY sets up the \(^1\)H and \(^{13}\)C parameters according to the solvent chosen. It then prompts for which of the eight experiments one wishes to run. Option 7 and 8 will bring user into the HMQC and HMBC experiments respectively.
**HMJC options**

Once the HMJC option is chosen, an on-line comments will guide the user in selecting an appropriate sensitivity level. ZUNITY will set up the number of transients (nt) according to the sensitivity choice selected. ZUNITY then proceed to set up necessary parameters including the four plotting parameters, sp1, rfi1, rfp1, and wp1, transferred from the $^{13}\text{C}$ parameter set. Also, a sub-macro "setqc" is invoked to select the HMJC pulse sequence, pre-set the weighting function and the linear prediction variables. Then acquisition will begin in the background and one is free to set up more experiments or perform other tasks on the VNMR software.

**HMBC option**

The HMBC option has a very similar working strategy as the HMJC option with the following exceptions. In addition to sensitivity, users will be prompted to enter the approximate molecular weight of the sample. This information is used to select an appropriate pre-acquisition delay time (d1 length). If HMJC has been set or run previously, ZUNITY simply moves HMJC parameters into the current experimental working space and sets up HMBC experiments with several minor alterations. The "setbc" sub-macro is used to load the pulse sequence and re-adjust some of the
parameters. Of course, this sub-macro (as well as "setq") can be used independently if the user wishes to customize some of the acquisition parameters.

Like HMQC, weighting function and linear prediction variables are pre-set and the processing of the data begins immediately following the completion of acquisition. When all the acquisition and processing are completed, the experimental data (both raw data and the Fourier Transformed data) is stored in the corresponding working area, so that users can access these data sets for further processing at a later time.

4.5 Experimental Results

Since the first six experiments (namely, $^1$H, $^{13}$C, APT, COSY, HETCOR, and FLOCK) have been tested by Yang and proven to work successfully, it is suffice to test the HMQC and HMBC options of ZUNITY program.

A testing sample of 6 mg of kauradienoic acid dissolved in 1 mg of deuterated chloroform (CDCl$_3$) containing 0.1% of tetramethylsilane (TMS) as internal reference was ran on a Varian UNITY-500 spectrometer equipped with a 5 mm inverse detection $^1$H multinuclear probe and a Sun IPX computer. The HMQC and HMBC spectra produced by ZUNITY program are shown in fig. 4.1 and fig 4.2, respectively. The
spectrometer operates at a probe temperature 25 °C. The relaxation delay time (d1) for HMQC is set at 20 times of the acquisition time (at) and a GARP decoupling pulse sequence is applied during acquisition. The results show that ZUNITY works successfully in generating both HMQC and HMBC spectra.

4.6 Further Improvement

ZUNITY can be further improved by incorporating more of the routinely used 2D experiments such as, NOESY, TOCSY (HOHAHA) and ROESY. All of these sequences are valuable tools and they all require significant amounts of user input to set-up and process these experiments. This further modification of ZUNITY program can provide a short cut for the experienced users as well as making these sequences accessible for the general users.

Full version of the ZUNITY program is presented in the appendix B of this thesis.
Figure 4.1

HMOC spectrum of Kauradienoic acid obtained by ZUNITY program
Figure 4.2

HMBC spectrum of Kauradienoic acid obtained by ZUNITY program
Chapter Four references:

APPENDIX
Appendix A

A.1 HSQC Program

/*/ 
This HSQC program is stored under the name of "delenn.c" in 
/unity500/data/krish/vnmrsys/psplib directory of the UNITY500 host. This is to 
distinguish it from the original HSQC source code. Executable is in the seqlib of the Krish 
account. Executable is generated by the seqgen command in the UNIX window.

Last modification: Nov. 15, 1996 by Li-Lin Tay 
This sequence is modified from the overbdn1.c sequence in the user's library.

overbdn1.c - heteronuclear Overbodenhausen experiment using REVINEPT

Parameters:

sspull = 'y': selects for sstrim(x)-sstrim(y) sequence at the start 
of the pulse sequence;
'n': normal experiment
fadd = 'y': TPPI axial-peak displacement;
'n': standard phasecycle
f1180 = 'y': the first t1 point is sampled at half the t1 dwell time;
'n': the first t1 point is sampled at t1 = 0
satmode = 'yn': presaturation during relaxation period (satdly) with xmtr
'nn': no presaturation during relaxation period (satdly)
'ny': presaturation during only the null period
satfreq = presaturation frequency
satdly = saturation time during the relaxation period
satpwr = saturation power for all periods of presaturation with xmtr
hs = 'yn': homospoil pulse (hst) during the d1 relaxation delay
null = delay associated with the BIRD nulling
tpwr = power level for 1H transmitter pulses
pw = 90 degree xmtr pulse length for protons (the observed nucleus)
pxxql = power level for X decoupler pulses
pxx = 90 degree decoupler pulse length for X
xrfdev = RF device for the X heteronucleus (2 for 1st dec., 3 for 2nd dec.)
jh = one-bond heteronuclear coupling constant to X (in Hz)
deltaxh = 1/(4*jxh) if jxh = 0.0; otherwise, the entered value is used; the delay
used in the REVINEPT subsequences
dm(dm2) = 'nnnn': no broadband decoupling of X during acquisition
'nnny': broadband heteronuclear decoupling of X during acquisition
phase = 1.2: hypercomplex experiment with F1 quadrature (complex F1-FT)

#include <standard.h>
#include <math.h>

#define MIN_J 0.1 /* Hz */
#define MIN_DELAY 0.2e-6 /* sec */
#define MIN_NULL 0.0001 /* sec */
#define MAX_SSTRIM 0.1 /* sec */
/*#define POWER_DELAY 4.2e-6 */ /* sec */

static int phs1[4] = {1,1,3,3}.
phs2[2] = {0,2}.
phs3[8] = {0,0,0,0,0,0,2,2.2}.
phs4[16] = {0,0,0,0,0,0,0,2,2,2,2,2,2,2}.
phs5[16] = {0.2,2,0.2,0.2,0.2,0.2,0.2,0.2,0.2,0.2,0.2,0.2,0.2,0.2};

static double d2_init = 0.0;

/*------------------------------------------
 |          | 1
 | 1  pulsesquence()/0  | 1
 | 1
 +-----------------------------------------*/

103
pulsesquence()
{
    /* VARIABLE DECLARATION */
    char /* satmode[MAXSTR], */
        sspul[MAXSTR],
        fad[MAXSTR],
        f1180[MAXSTR];
    int phase,
        satmove,
        t1_counter;
    double ss,
        sstrim,
        t1evol,
        xdecpwr = 0.0, /* safety precaution */
    /* sw1, pwxlvl, */
    pwx, /*
        jxh,
        deltahxh,
        bird,
        /* satfrq. */
    satdly, satpwr */
    null:

    /* Load variables */
    /*
        satfrq = getval("satfrq");
        satdly = getval("satdly");
        satpwr = getval("satpwr");
        pwxlvl = getval("pwxlvl");
        pwx = getval("pwx"); /*
        jxh = getval("jxh");
        deltahxh = getval("deltahx");
        ss = getval("ss");
        /* sw1 = getval("sw1"); */
        sstrim = getval("sstrim");
        null = getval("null");
        phase = (int) (getval("phase") + 0.5);
    */
getstr("sspul", sspul);
getstr("f1180", f1180);
getstr("fad", fad);
getstr("satmode", satmode);

/* Load phase tables */
settable(t1, 4, phs1);
settable(t2, 2, phs2);
settable(t3, 8, phs3);
settable(t4, 16, phs4);
settable(t5, 16, phs5);

/* Check X RF device */
/*
  if ( (xrfdev != DODEV) & (xrfdev != DO2DEV) )
  {
    text_error("invalid X RF device
    abort(1);
  }
  */

/* Adjust delays */
if (jxh > MIN_J)
{
  bird = 1/(2*jxh);
  deltah = 0.4*bird;
}
else
{
  bird = 0.0;
}

/* Check for 1H frequency change */
satmove = (fabs(tof - satfrq) >= 0.1);
/* Check for correct 'dm/dm2' and 'dpwr/dpwr2' settings */
if ( (dm[A] == 'y') || (dm[B] == 'y') || (dm[C] == 'y') ||
    (dm[D] == 'y') )
{
    text_error("DM must be set to either 'nnnnn' or 'nnnny\n");
    abort(1);
}

xdecpwr = dpwr;

if (sstrim > MAX_SSTRIM)
{
    text_error("'sstrim' is > maximum value\n");
    abort(1);
}

/* Determine steady-state mode */
if (ss < 0)
{
    ss *= (-1);
    initval(ss, ssval);
    initval(ss, ssctr);
}

/* Phase incrementation for hypercomplex 2D data */
if (phase == 2)
    tsadd(t2, 1, 4);
/* FAD phase incrementation */
if (fad[A] == 'y')
{
    if (ix == 1)
        d2_init = d2;
    t1_counter = (int) ( (d2 - d2_init)*sw1 + 0.5 );
    if (t1_counter % 2)
    {
        tsadd(t2, 2, 4);
        tsadd(t5, 2, 4); /* receiver phase cycle */
    }
}

/* BEGIN ACTUAL PULSE SEQUENCE CODE */

status(A);
   rlpower(tpwr, TODEV);
   rlpower(pwx1l.DODEV);

if (sspul[A] == 'y')
{
    rgpulse(sstrim, zero, rof1, 1.0e-6);
    rgpulse(sstrim, one, rof1, rof2);
    hsdelay(d1);
}
else
{
    hsdelay(d1);
}

 /* selective saturation period */
if (satmode[A] == 'y')
{
    if (satmove)
        offset(satfrq, TODEV);

    rlpower(satpwr, TODEV);
    rgpulse(satdly, zero, 4.0e-5, 0.2e-6);
    if (satmove)
        offset(tof, TODEV);
rlpower(tpwr, TODEV);
delay(1.0e-5);

}

status(B);
/* Bird pulse and nulling period for both C13 and N15 */
if (null > MIN_NULL)
{
    rgpulse(pw, zero, rof1, 0.0);
delay(bird - rof1 - 1.0e-6 - 2*pwx - 0.5*pw);
rcvroff();
decrgpulse(pwx, one, rof1, 0.0);
simpulse(2*pw, 2*pwx, zero, zero, 1.0e-6, 0.0);
decrgpulse(pwx, one, 1.0e-6, 0.0);
rcvron();
delay(bird - rof1 - 1.0e-6 - 2*pwx - 0.5*pw);
rgpulse(pw, two, rof1, 1.0e-6);

if (satmode[B] == 'y')
{
    if (satmove)
        offset(satfrq, TODEV);

    rlpower(satpwr, TODEV);
    rgpulse(null, zero, 1.0e-5, 0.2e-6);
if (satmove)
        offset(tof, TODEV);

    rlpower(tpwr, TODEV);
delay(1.0e-5);
}
else
{
    delay(null);
}
status(C);
rcvroff();
rgpulse(pw. zero. rofI. 0.0);

decphase(zero);
txphase(zero);
delay(deltaxh - pw x);
simpulse(2*pw. 2*pwx. zero. zero. 0.0. 0.0);
txphase(t1);
decphase(t2);
delay(deltaxh - pw x);
simpulse(pw. pwx. t1. t2. 0.0. 0.0);

txphase(zero);
decphase(t4);

/* Calculate t1 delay */
tlevol = d2;
if (fl180[A] == 'y')
   tlevol += 0.5/sw1;

if (tlevol > MIN_DELAY)
{
   tlevol -= 2*pw + (4*pwx/M_PI);
   if (tlevol < MIN_DELAY)
      tlevol = 0.0;
}
delay(tlevol/2);
rgpulse(2*pw. zero. 0.0. 0.0);
txphase(t3);
delay(tlevol/2);
status(D);
simpulse(pw, pwx, t3, t4, 0.0, 0.0);
txphase(zero);
decphase(zero);
delay( deltah - pw - (2*pwx/M_PI) );
simpulse(2*pw, 2*pwx, zero, zero, 0.0, 0.0);

rlpower(xdecpwr, DODEV);  /* X decoupling power level */
delay(rof2);
rcvron();
delay(deltah - pw - POWER_DELAY - rof2);

status(E);
setreceiver(t5);
}
A.2 HSQC-TOCSY Program

/* HSQC-TOCSY sequence

This is an HSQC sequence with clean-toesy(MLEV16) proton relay.

The program will support hypercomplex and TPPI implementation.
Also hypercomplex FAD is supported as well -- highly recommended.
The trimx, trimy, ssulse pulse also supported.
All the "xrfdev" variable were replaced with "DODEV". */

/* Written by Li-Lin Tay, Oct. 12, 96 */

/ *
Modified from the HSQC (overbdn.l.c) program, TOCSY (toesy.c) and
HMQCTOCYSIS program (hmqctoecy.c) All three program can be find in
the krish user's pulse sequence library. The path on the UNITY500 host is
/unity500/data/krish/vnmrsys/psglib/hsqctoecy.c. The executable is in the seqlib under
vnmrsys directory and macro for this program is in the maclib directory of the vnmrsys
directory.

PARAMETERS:

sspull='y': selects for sstrip(x)-sstim(y) sequence at the start of of pulse sequence
'n': normal experiment
fad='y': TPPI axial-peak displacement
'n': standard phasescycle
f1180='y': the first tl point is sampled at half the tl dwell time
'n': the first tl point is sampled at t1=0
satmode='yn': presaturation during relaxation period (satdly) with xmtr
'nn': no presaturation during relaxation period (satdly)
'ny': presaturation during only the null period
sstrimx= gives a (1ms) trim ssulse before d1.
satfrq= presaturation frequency
satdly= presaturation time during the relaxation period
satpwr= saturation power for all periods of presaturation with xmtr
hs='yn': homospoil pulse (hst) during the d1 relaxation delay
  'yy': homospoil pulse during both d1 and the 'null' period
  'nn': recommended if sstrim is used.
null= delay associated with the BIRD nulling or the nulling time
  for the C12-H1 pair
tpwr= power level for H1 transmitter pulses
pw= 90 degree decoupler pulse length for X
pxxvl= power level for X decoupler pulses (for decoupler nuclei)
pxx= 90 degree decoupler pulse length for X
xrfdev= DODEV, RF device for the x heteronucleus
  (this parameter has been replaced by DODEV in this program.)

ejh= one-bond heteronuclear coupling const to X (in Hz)
deltah= 1/(4ejh) if jh = 0.0; otherwise, the entered value is used;
  the delay used in the REVINEPT subsequences
dm/dm2= /nnnn/: no broadband decoupling of x during acquisition.
  /nnny/: broadband heteronuclear decoupling of X during acquisition.

window= clean-tocys window (in us)
mix= mixing time for isotropic mixing
trim= length of trim pulse preceding the MLEV-16 spin lock

phase= 1.2: hypercomplex experiment with F1 quadrature (complex F1-FT)
  =3: TPPI phase-sensitive experiment with F1 quadrature

*/

#include<stdio.h>

#define MIN_J 0.1 /* Hz */
#define MIN_DELAY 0.2e-6 /* sec */
#define MIN_NULL 0.0001 /* sec */
#define MAX_SSTRIM 0.1 /* sec */
#define MIX_MAX 0.3 /* sec */
/* define the MLEVa pulses -- 90x 180y 90x  */
/* and the MLEVb pulses -- 90-x 180-y 90-x */

mleva()
{
  rgpulse(p1, zero, 0.0, 0.0);
  rgpulse(2.0*p1, one, 0.0, 0.0);
  rgpulse(p1, zero, 0.0, 0.0);
  delay(getval("window"));
}

mlevb()
{
  rgpulse(p1, two, 0.0, 0.0);
  rgpulse(2.0*p1, three, 0.0, 0.0);
  rgpulse(p1, two, 0.0, 0.0);
  delay(getval("window"));
}

/* Define the phase cycles (similar to HSQC sequence) */

static int phs1[4] = { 1,1,3,3 };
static int phs2[2] = { 0.2 };
static int phs3[8] = { 0,0,0,0,2,2,2,2 };
static int phs4[16] = { 0,0,0,0,0,0,0,2,2,2,2,2,2,2,2,2 };
static int phs5[16] = { 0,2,2,0,2,0,2,2,0,0,2,0,2,2,0,2 };

static double d2_init = 0.0;

/*----------------------------------------*/
| |
pulsesquence() /o |
| |
+-------------------*/
pulsequence()
{
    /* Variable declaration */

    char satmode[MAXSTR]. /* presaturation mode */
    sspul[MAXSTR], /* selecting sstrim before pulse seq. */
    fad[MAXSTR]. /* TPPI axial-peak displacement selection */
    f1180[MAXSTR]: /* Determine when to sample first t1 */

    int phase. /* track phase for hypercomplex or TPPI phase */
    satmove,
    t1_counter: /* for the phase incrementation */

    double
    ss,
    sstrim,
    swl,
    tlevol, /* t1 evolution */
    xdecpwr = 0.0, /* safety precaution */
    pwxlvl. /* pwr level for X decoupler pulse */
    pwx. /* 90 degree decoupler pulse for X nucleus */
    jxh. /* 1 bond heteronuclear coupling constant */
    deltaxh, /* deltaXH= 1/4Jxh */
    bird. /* delay in the bird pulse */
    satfreq. /* presaturation frequency */
    satdly. /* saturation time during relaxation period */
    satpwr. /* saturation pwr for all period of saturation */
    null. /* delay associated with bird pulse */

    window, /* delay window for the MLEV pulse */
    mix, /* mixing time */
    trim, /* trim pulse time */
    cycles, /* for cycles calculation in TOCSY */
    p1lvl: /* pwr level for MLEV-16 and trim pulses */
/* Load variable */
/* whatever is declared as double, use getval to load variable 
& getstr similarly is for char variables 
& gettable used to load the table elements, here the phase angles. 
All getval, getstr, and gettable do are looking up the parameter 
values from current experiment parameter list and import the 
values into the sequence. If a parameter is not found, its 
value is set to zero and pulse sequence produces a warning msg. */

ss = getval("ss");
sstrim = getval("sstrim");
sw1 = getval("sw1");
pwxlvl = getval("pwxlvl");
pwx = getval("pwx");
jxh = getval("jxh");
deltaxh = getval("deltaxh");
satfrq = getval("satfrq");
satdly = getval("satdly");
satpwr = getval("satpwr");
null = getval("null");

/* tlevol used to calculate t1 evolution time. no need to load 
it in as a variable. So as "bird" variable. */

window = getval("window");
mix = getval("mix");
trim = getval("trim");
pll lvl = getval("pll lvl");

phase = (int)(getval("phase") + 0.5);

getstr("sspul", sspul);
getstr("f1180", f1180);
getstr("fad", fad);
getstr("satmode", satmode);
/* Phase cycle tables */

settable(t1, 4, phsi);
settable(t2, 2, phs2);
settable(t3, 8, phs3);
settable(t4, 16, phs4);
settable(t5, 16, phs5);

/* Safety checks */
/* Adjust delays; calculate delay for bird pulse */
if (jxh > MIN_J)
{
bird = 1/(2*jxh);
deltaxh = 0.4 * bird;
}
else
{
  /* if jxh < MIN_J then set bird delay=0 */
bird = 0.0; /* therefore skip bird nulling. Use this if */
}
/* sample is labelled */

/* check for H1 frequency change */
satmove = (fabs(tof-satfrq) >= 0.1);

/* check for correct dm and dpwr settings */
if ((dm[A] == 'y') || (dm[B] == 'y') || (dm[C] == 'y') || (dm[D] == 'y'))
{
  text_error("dm must be set to either 'nnnnn' or 'nnnny' \n");
  abort(1);
}

xdecpwr = dpwr;
if (sstrim > MAX_SSTRIM)
{
    text_error("sstrim > maximum value \n");
    abort(1);
}

/* Determine steady-state mode */
if (ss < 0)
{
    ss *= (-1);
    initval(ss, ssval);
    initval(ss, ssctr);
}

/* Check for mixing time */
if (mix > MIX_MAX)
{
    text_error(" mixing time > MIX_MAX allowed value \n");
    abort(1);
}

/* phase incrementation for hypercomplex 2D data */
if (phase == 2)
    tsadd(t2, 1, 4); /* add 1 to t2 then take mod4, result stored in t2 */

/* FAD phase incrementation */
if (fad[A] == 'y')
{
    if (ix == 1)
        d2_init = d2;
    t1_counter = (int) ( (d2 - d2_init)*sw1 + 0.5);
    if (t1_counter % 2)
    {
        tsadd(t2, 2, 4);
        tsadd(t5, 2, 4); /* receiver phase cycle */
    }
}

/** BEGIN ACTUAL PULSE SEQUENCE CODE */

status(A); /* presaturation period */
rlpower(tpwr, TODEV); /* set transmitter power */
rlpower(pwxlvl, DODEV); /* set decoupler power */
if (sspol[A] == 'y') /* this selects for ssstrim */
    { /* beginning of pulse sequence */
        rgpulse(sstnm, zero, rof1, 1.0e-6);
        rgpulse(sstnm, one, rof1, rof2);
        hsdelay(d1);
    }
else
    {
        hsdelay(d1);
    }

/* Selective saturation period */
if (satmode[A] == 'y')
    {
        if (satmove)
            offset(satfrq, TODEV);

        rlpower(satpwr, TODEV);
        rgpulse(satdly, zero, 4.0e-5, 0.2e-6);

        if (satmove)
            offset(tof, TODEV);
        rlpower(tpwr, TODEV);
        delay(1.0e-5);
    }
status(B); /* BIRD pulse and nulling for C12-H1 pair */

if (null > MIN_Null)
{
  rgpulse(pw, zero, rof1, 0.0);
  delay(bird - rof1 - 1.0e-6 - 2*pwx - 0.5*pw);
  rcvoff();
  decrgpulse(pwx, one, rof1, 0.0);
  simpulse(2*pw, 2*pwx, zero, zero, 1.0e-6, 0.0);
  decrgpulse(pwx, one, 1.0e-6, 0.0);
  rcv(on);
  delay(bird - rof1 - 1.0e-6 - 2*pwx - 0.5*pw);
  rgpulse(pw, two, rof1, 1.0e-6);
}

if (satmode[B] == 'y') /* nulling time for H1-C12 pair */
{
  if (satmove)
    offset(satfrq, TODEV);

  rlpower(satpwr, TODEV);
  rgpulse(null, zero, 1.0e-5, 0.2e-6);
  if (satmove)
    offset(tof, TODEV);

  rlpower(tpwr, TODEV);
  delay(1.0e-5);
}
else
{
  delay(null);
}
}
status(C);       /* actual HSQC sequence begin here */

rcvoff();
rgpulse(pw, zero, rof1, 0.0);       /* H1 90x pulse */

decphase(zero);       /* decoupler phase=x */
txphase(zero);       /* transminter phase=x */
delay(deltah - pwx);
simpulse(2*pw, 2*pwx, zero, zero, 0.0, 0.0);
       /* H1 180x + C13 180x pulse */
txphase(t1);       /* transminter phase=t1 */
decphase(t2);       /* decoupler phase=t2 */
delay(deltah - pwx);
simpulse(pw, pwx, t1, t2, 0.0, 0.0);
       /* H1 90t1 + C13 90t2 pulse */

txphase(zero);
decphase(t4);

/* calculate t1 delay */
tlevol = d2;
if (f1 180[A] \text{==}'y')       /* first t1 sampled at t1/2 time */
        tlevol += 0.5/sw1;

if (tlevol > MIN_DELAY)
{
    tlevol -= 2*pw + (4*pwx/M_PI);
    if (tlevol < MIN_DELAY)
        tlevol = 0.0;
}

delay(tlevol/2);       /* if f1 180[A]=n, sample 1st t1 at t1=0 */
rgpulse(2*pw, zero, 0.0, 0.0);       /* H1 180x pulse */
txphase(t3);
delay(tlevol/2);
/*---------------------------------------------------------------*/

status(D);  /* 2nd INEPT + MLEV pulses */

simpulse(pw, pwx, t3, t4, 0.0, 0.0);
txphase(zero);
decphase(zero);
delay(deltaTxh - pwx - (2*pwx/M_PI));
simpulse(2*pw, 2*pwx, zero, zero, 0.0, 0.0);

rlpower(xdecpwr, DODEV);  /* set decoupler power */
delay(rof2);

/* Begin here, TOCSY H1 relay */

delay(deltaTxh - pwx - POWER_DELAY - rof2);
rlpower(p1lvl, TODEV);

if (mix >0)
{
  cycles = (mix-trim)/(64.66 *p1 + 32 * window);
cycles = 2.0 * (double) (int) (cycles/2.0);
initval(cycles, v1);

if (cycles > 1.0)
{

  rgpulse(trim, zero, rof1, 0.0);  /* trimx pulse */

  starthardloop(v1);
mleva(); mlevb(); mlevb(); mleva();
mlevb(); mlevb(); mleva(); mleva();
mlevb(); mleva(); mleva(): mlevb();
mleva(); mleva(); mleva(); mlevb();
rgpulse(0.66*p1, zero, 0.0, 0.0);
endhardloop();
}
}
status(E);
delay(rof2);
txphase(zero);
rcvron();
setreceiver(t5);
}
A.3 CHSQC Program

/* CHSQC sequence also known as the Coupled HSQC sequence

CHSQC500.C
Program is under the name of "chsqc500.c". It is stored in the Krish
account of the UNITY500 host. Source code is under
/krish/vnmrsys/psglib/chsqc500.c, the executable is under seqlib directory
and the macro is under maclib directory. This sequence is very similar to the
HSQC sequence except the last two delay of the original HSQC sequence and
the last pair of 180 x pulses were removed. In other words,
acquisition begins immediately after the second pair of 90deg pulse.

written by Li-Lin Tay, Nov. 15, 1996

Parameters:

sspul = 'y': selects for sstrim(x)-sstrim(y) sequence at the
    start of the pulse sequence
'n': normal experiment
fad = 'y': TPPI axial-peak displacement
    'n': standard phasecycle
f1180 = 'y': the first t1 point is sampled at half the t1 dwell time
    'n': the first t1 point is sampled at t1 = 0
satmode = 'yn': presaturation during relaxation period (satdly) with xmtr
    'nn': no presaturation during relaxation period (satdly)
    'ny': presaturation during only the null period
satfrq = presaturation frequency
satdly = saturation time during the relaxation period
satpwr = saturation power for all periods of presaturation with xmtr
hs = 'yn': homospoil pulse (hst) during the d1 relaxation delay
null = delay associated with the BIRD nulling
tpwr = power level for 1H transmitter pulses
pw = 90 degree xmtr pulse length for protons (the observed nucleus)
pwxlvl = power level for X decoupler pulses
pwx = 90 degree decoupler pulse length for X
xrfdev = RF device for the X heteronucleus(2 for 1st dec., 3 for 2nd dec.)
jh = one-bond heteronuclear coupling constant to X (in Hz)
deltaxh = 1/(4*jh) if jh = 0.0; otherwise, the entered value is used;
the delay used in the REVINEPT subsequences
dm(dm2) = 'nnnnn': no broadband decoupling of X during acquisition
'nnnny': broadband heteronuclear decoupling of X during acquisition
phase = 1.2: hypercomplex experiment with F1 quadrature (complex F1-FT)

#include <standard.h>
#include <math.h>

#define MIN_J 0.1 /* Hz */
#define MIN_DELAY 0.2e-6 /* sec */
#define MIN_NULL 0.001 /* sec */
#define MAX_SSTRIM 0.1 /* sec */
/* #define POWER_DELAY 4.2e-6 */ /* sec */

static int phs1[4] = {1,1,3,3},
        phs2[2] = {0,2},
        phs3[8] = {0,0,0,0,2,2,2,2},
        phs4[16] = {0,0,0,0,0,0,0,2,2,2,2,2,2,2,2,2},
        phs5[16] = {0,2,2,0,2,0,2,2,0,0,2,0,2,0,2,0};

static double d2_init = 0.0;

/*---------------------------------------------------------------------------
 |                        |  
 |    pulsesquence()/0    |  
 |                        |  
 +------------------------*/
pulsesquence()

{
/* VARIABLE DECLARATION */

char    /* satmode[MAXSTR], */
        sspul[MAXSTR],
        fad[MAXSTR],
        f1180[MAXSTR];

int     phase,
        satmove,

double   ss,
         sstrim,
         tlevol,
         xdecpwr = 0.0,         /* safety precaution */

        swl,
        pwxlvl,
        pwx,              /*

        jxh,
        deltah,
        bird,

        satfrq,
        satdly,
        satpwr,     */
        null;

/* safety precaution */
/* Load variables */
/*
satfrq = getval("satfrq");
satdly = getval("satdly");
satpwr = getval("satpwr");
pwxlvl = getval("pwxlvl");
pwx = getval("pwx"); */

jxh = getval("jxh");
deltaxh = getval("deltaxh");
ss = getval("ss"); */

swl = getval("swl"); */
sstrim = getval("sstrim");
null = getval("null");
phase = (int) (getval("phase") + 0.5);

getstr("sspul", sspul);
getstr("f1180", f1180);
getstr("fad", fad);
getstr("satmode", satmode);

/* Load phase tables */
settable(t1, 4, phs1);
settable(t2, 2, phs2);
settable(t3, 8, phs3);
settable(t4, 16, phs4);
settable(t5, 16, phs5);

/* Check X RF device */
/*
if ( (xrfdev != DODEV) && (xrfdev != DO2DEV) )
{ text_error("invalid X RF device
"); abort(1);
}
*/
/* Adjust delays */
if (jxh > MIN_J)
{
    bird = 1/(2*jxh);
    deltaxh = 0.4*bird;
}
else
{
    bird = 0.0;
}

/* Check for 1H frequency change */
satmove = ( fabs(tof - satfrq) >= 0.1 );

/* Check for correct 'dm/dm2' and 'dpwr/dpwr2' settings */
if ( (dm[A] == 'y') ll (dm[B] == 'y') ll (dm[C] == 'y') ll
     (dm[D] == 'y') )
{
    text_error("DM must be set to either 'nnnnn' or 'nnnny\n");
    abort(1);
}

xdecpwr = dpwr;

if (sstrim > MAX_SSTRIM)
{
    text_error("'sstrim' is > maximum value\n");
    abort(1);
}

/* Determine steady-state mode */
if (ss < 0)
{
    ss *= -1;
    initval(ss, ssval);
    initval(ss, ssctr);
}
/* Phase incrementation for hypercomplex 2D data */
if (phase == 2)
  tsadd(t2, 1, 4);

/***********/
/* FAD phase incrementation */
if (fad[A] == 'y')
{
  if (ix == 1)
    d2_init = d2;
  t1_counter = (int) ((d2 - d2_init)*sw1 + 0.5);
  if (t1_counter % 2)
  {
    tsadd(t2, 2, 4);
    tsadd(t5, 2, 4); /* receiver phase cycle */
  }
}

/*********************************************/
/* BEGIN ACTUAL PULSE SEQUENCE CODE */

/***************************************************/
/* status(A); */
rlpower(tpwr, TODEV);
rlpower(pwx1vl, DODEV);

if (sspul[A] == 'y')
{
  rgpulse(sstrim, zero, rof1, 1.0e-6);
  rgpulse(sstrim, one, rof1, rof2);
  hsdelay(d1);
}
else
{
  hsdelay(d1);
}
/* selective saturation period */
   if (satmode[A] == 'y')
   {
      if (satmove)
         offset(satfreq, TODEV);

         rlpower(satpwr, TODEV);
         rgpulse(satdly, zero, 4.0e-5, 0.2e-6);
      if (satmove)
         offset(tof, TODEV);

         rlpower(tpwr, TODEV);
         delay(1.0e-5);
   }

/*---------------------------------------------------------------*/
status(B);

/* Bird pulse and nulling period for both C13 and N15 */
   if (null > MIN_NULL)
   {
      rgpulse(pw, zero, rofl, 0.0);
      delay(bird - rofl - 1.0e-6 - 2*pwx - 0.5*pw);

      rcvoff();
      decrgpulse(pwx, one, rofl, 0.0);
      simpulse(2*pw, 2*pwx, zero, zero, 1.0e-6, 0.0);
      decrgpulse(pwx, one, 1.0e-6, 0.0);
      rcvron();

      delay(bird - rofl - 1.0e-6 - 2*pwx - 0.5*pw);
      rgpulse(pw, two, rofl, 1.0e-6);
if (satmode[B] == 'y')
{
    if (satmove)
        offset(satfrq, TODEV);
    rlpower(satpwr, TODEV);
    rgpulse(null, zero, 1.0e-5, 0.2e-6);
    if (satmove)
        offset(tof, TODEV);

    rlpower(tpwr, TODEV);
    delay(1.0e-5);
}
else
{
    delay(null);
}
}

status(C):

rcvroff();
rgpulse(pw, zero, rof1, 0.0);

decphase(zero);
txphase(zero);
delay(deltaxh - pwx);
simpulse(2*pw, 2*pwx, zero, zero, 0.0, 0.0);
txphase(t1);
decphase(t2);
delay(deltaxh - pwx);
simpulse(pw, pwx, t1, t2, 0.0, 0.0);

txphase(zero);
decphase(t4);
/* Calculate t1 delay */

tlevol = d2;
if (f1180[A] == 'y')
    tlevol += 0.5/sw1;

if (tlevol > MIN_DELAY)
{
    tlevol -= 2*pw + (4*pwx/M_PI);
    if (tlevol < MIN_DELAY)
        tlevol = 0.0;
}

delay(tlevol/2);
rgpulse(2*pw, zero.0.0.0);
txphase(t3);
delay(tlevol/2);

/**************************** remove delay and pair of 180 pulses *****************/
delay( deltah - pwx - (2*pwx/M_PI) );
simpulse(2*pw, 2*pwx, zero.0.0.0);

delay(rof2);

rlpower(xdecpwr, DODEV); /* X decoupling power level */
rcvron();

/**************************** remove last delay ******************************/
delay(deltaxh - pwx - POWER_DELAY - rof2);

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/*-----------------------------------------------*/
status(E);

setreceiver(t5);
}
Appendix A.4 HSQC Macro Program

"hsqc-macro to convert a presat parameter set to hsqc"
"Program is stored under the name of ‘delenn’ in the macro library"
"of the Krish account. The path is /unity500/data/krish/vnmrsys/maclib/delenn"

“Written by Li-Lin Tay”
“Last modification: Nov, 15, 1996”

clear(2)
banner(‘wait! setting up parameters now’,‘cyan’)
psgset(‘delenn’,’sspal’,’sstrim’,’satmode’,’satdly’,’satpwr’,’satfrq’,’fad’)
psgset(‘delenn’,’f1180’,’swl’,’ni’,’phase’,’jxh’,’null’,’pwx’,’pxlvl’)
psgset(‘delenn’,’dg’,’dg1’,’ap’,’dm’,’dpw’)
psgset(‘delenn’,’df’,’dseq’,’dres’,’dn’)
psgset(‘delenn’,’dof’,’array’,’deltahx’,’dmm’)

ni=256 phase=1 if (at>.08) then at=.06 endif

dm=’nnnny’  jxh=140  axis=’pd’
"sw1=140d  dof=-30d"
pwx=9.4  dmm=’ccccp’  np=512  pw=9.5
dl=1.6  ss=16
satmode=’nnn’
satdly=0  satpwr=’n’  fad=’n’  dpwr=41
ni=256  swl=16991.6
dof=-4000  il=’n’
nul=0.4  gain=’y’

atext(’13C HSQC EXPERIMENT’)
dg
dps(‘delenn’)

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Appendix A.5 HSQC-TOCSY Macro Program

“This is the macro to run the hsqctocsy sequence. It is stored in the “Krish account under the name of ‘hsqctocsy’ in the macro library.”

“************************************************************************
hsqctocsy - sets up parameters in accordance with the hsqctocsy pulse sequence.
************************************************************************

“written by Li-Lin Tay, Oct. 12, 1996”

banner('WAIT! Now setting HSQC-TOCSY parameters','cyan')
psgset('hsqctocsy','p1lavl','p1','dn','mix','trim','window','dg','ap')
psgset('hsqctocsy','dm','dmm','dmf','dpwr','j','jxh','deltaxh','null')
psgset('hsqctocsy','pwxvl','pxv','sstrim','homo','satmode','satfrq','ss')
psgset('hsqctocsy','phase','array','satdly','satpwr','ni','swl','hs','dof')
psgset('hsqctocsy','fad','f1180','sspul','sstrim')

at=.2 fn=np nt=16
bs=8 il='n' dl=1.2
pw(90) axis='pd'
clear(2)
banner('Parameter setting completed','cyan')
dps('hsqctocsy')
dg
Appendix A.6 Coupled HSQC (CHSOC) Macro Program

"chsqc500 - a macro to run the chsqc pulse sequence. The macro"
"runs the chsqc500 executable from the seqlib of the krish account"
"in the UNITY500 host"

"Chsqc500 is basically hsqc with last two delay and last "
"pair of 180 deg pulse taken off, ie, acquisition followed"
"immediately after the second 90 deg polarization transfer pulses"

"Written by Li-Lin Tay"
"Last modification: Nov. 15, 1996"

clear(2)
banner('wait! setting up parameters now','cyan')

psgset('chsqc500'.'sspu1.'sstrim'.'satmode'.'satdly'.'satpwr'.'satfrq'.'fad')
psgset('chsqc500'.'fl180.'swl.'ni.'phase'.'jxh'.'null'.'pwx'.'pwxvlv')
psgset('chsqc500'.'dg'.'dg1.'ap.'dm.'dpwr')
psgset('chsqc500'.'dmf.'dseq.'dres.'dn')
psgset('chsqc500'.'dof.'array.'deltaxh.'dmm')
ni=256 phase=1 if (at>.08) then at=.06 endif

    dm='nnnnn'    jxh=140    axis='pd'
"swl=140d    dof=-30d"
pwx=9.4    dmm='ccccp'    np=512
pw=9.5    dl=1.6    ss=16
satmode='nnn'    null=0.3
satdly=0    satpwr='n'
      fad='n'
dpwr=41    ni=256
swl=16991.6    dof=-4000
phase=1.2

atext('13C HSQC EXPERIMENT')
dg
dps('chsqc500')
Appendix B  ZUNITY Programs

"zunity - the program is the modification of the unitynmr program"
"This modification will add the HMBC and HMQC procedures into the"
"macro allowing the user to run the automated HMBC, and HMQC"
"spectrum. The program is stored under the name 'zunitybc2' in the macro"
"library of the Krish account"

"Written by Li-Lin Tay"
"Last update: July 29, 96"

"zunitybc - macros to do 1D or/and 2D nmr experiments"

banner('Welcome to UNITY system','yellow')
write('graphics'.55.160,'would you like to run H1 exp? (y/n)')
write('graphics'.55.140,'If no, the H1 parameters is moved from the')
write('graphics'.55.120,'exp1 will be used to setup experiments')
input:$41
if $41='y' then
  jexp1 solset:$1
  setup('H1',$1)
endif
solset:$1
jexp2 setup('C13',$1)
"jexp1"
zexpset:$11,$12,$13,$14,$15,$16,$17,$18,$19,$20,$21,$22

if $11=1 then
  runh($1,'nolock') nt=16 np=2*sw/$19 fn=2*np wexp='h1p'
  if $15=5 or $16=6 then
    wexp='seth'
  endif
  au
endif
if $S_{12} = 2$ then
  \text{jexp2 runc('no1ock')} \quad d_1 = 20 \quad sn = 25 \quad bs = 32 \quad nt = 20000
  \quad dp = 'y' \quad dpwr = 43 \quad wbs = 'testsn' \quad werr = 'react' \quad wexp = 'c13pro'
  \quad au
endif

if $S_{13} = 3$ then
  \text{unlock(3) mp(2,3) apt('1')} \quad d_1 = 20 \quad dp = 'y' \quad d_2 = 7e-3
  \quad sn = 25 \quad bs = 32 \quad nt = 20000 \quad wbs = 'testsn' \quad werr = 'react'
  \quad wexp = 'papi1' \quad dpwr = 43
  \quad au
endif

if $S_{14} = 4$ then
  \text{jexp4 runh('no1ock')} \quad nt = 16 \quad np = 2*sw/$s_{19} \quad fn = 2*np
  \quad wexp = 'rcosy'
  \quad au
endif

if $S_{15} = 5$ then
  if $S_{11} = 0$ and $S_{14} = 0$ then
    \text{jexp1 runh('no1ock')} \quad nt = 16 \quad fn = 2*np \quad wexp = 'seth'
    \quad au
  endif
  unlock(5) mp(2,5) dept('1') \quad dp = 'y' \quad r_2 = 20 \quad r_3 = 21 \quad r_4 = 22
  \quad d_1 = r_2 \quad mult = 0.5 \quad sn = 25 \quad bs = 32 \quad nt = 10000 \quad wbs = 'testsn'
  \quad wexp = 'setch' \quad dpwr = 43
  \quad au
endif

if $S_{16} = 6$ then
  if $S_{11} = 0$ and $S_{15} = 0$ and $S_{14} = 0$ then
    \text{jexp1 runh('no1ock')} \quad fn = 2*np \quad nt = 16 \quad wexp = 'seth'
    \quad au
  endif
  unlock(6) mp(2,6) apt('1') \quad dp = 'y' \quad r_2 = 20 \quad r_3 = 21 \quad r_4 = 22
  \quad d_1 = r_2 \quad d_2 = 7e-3 \quad bs = 32 \quad sn = 25 \quad nt = 10000 \quad wbs = 'testsn'
  \quad werr = 'react' \quad wexp = 'setcf' \quad dpwr = 43
  \quad au
endif

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if $17=7$ then
   echo('now entering exp #7')
   $40=''
   clear echo('Would you like to run H1 exp? (y/n) ')
   clear(2)
   write('graphics',55,160,'would you like to run H1 exp? (y/n) ')
   write('graphics',55,140,'If you already ran it and H1 ')
   write('graphics',55,120,'parameters are stored in exp1. ')
   write('graphics',55,100,'Please enter "n" ')
   write('graphics',55,080,'The program will then move the H1 ')
   write('graphics',55,060,'parameters from exp1 to exp7 and ')
   write('graphics',55,040,'set up HMQC parameters for you')
   write('graphics',55,020,'Otherwise, enter "y" to run H1 exp')
   input:$40$
   if $40='n' then $11=1$ endif

"*******************************************************************"
" * Run H1 exp. if haven't already done.    *
"*******************************************************************"

"* $11=1 *
   if $11=0$ and $15=0$ and $14=0$ and $16=0$ then
      jexp1 runh($1,'nolock')
      clear(2) banner('run H1 now','white')
      fn=2*np nt=16 wexp='seth'
      au
   endif

"* Move H1 parameters into HMQC exp7 space  *
"* and set up some HMQC parameters.         *

unlock(7) mp(1,7)
np=512 dp='y' r3=$21
r4=$22 nt=r4 dpwr=41
tpwr=61 pw=9.5
temp=25 spin='n'
clear(2) banner('move C13 parameters to exp7')
"* get C13 parameters "
jexp2
$sp=sp
$rfl=rfl $rfp=rfp $wp=wp

"* bring them back to HMQC "
jexp7
sp1=$sp
rfl1=$rfl rfp1=$rfp wp1=$wp

wbs = 'testsn'
werp = 'react'
wexp = 'setqc'
au
endif

if S18=8 then
  if S17=7 then
    mp(7,8) "move HMQC parameters into HMBC"
    np=1024 $dl=r2
    wbs='testsn'
    werp='react'
    wexp='setqc'
    au
  endif
endif
clear(2) banner('run H1 exp?? (y/n) ')
input:$$41\,$$
if $$41='n'\,$$ then $$11=1\,$$ endif

if $$11=0\,$$ and $$14=0\,$$ and $$15=0\,$$ and $$16=0\,$$ and $$17=0\,$$ then
  jexp1 runh($$l\,'\text{nolock}'\,$$
  fn=2*np
  nt=8
  wexp='\text{seth}'
  au
endif

unlock(8) mp(1.8)
np=1024 dp='y'
d1=0.6 r3=$$21\,$$ r4=$$22\,$$
dpwr=41 tpsr=61 pw=9.5
temp=25 spin='n'

"* get C13 parameters  
  jexp2
  $$sp=sp\,$$
  rfrf=rfp $wfp=wp$

"* bring them back to HMBC  *
  jexp8
  sp=$$sp\,$$
  rfrf=rfp $wfp=wp$

  wbs='\text{testsn}'
  werr='react'
  wexp='\text{setbc}'
  au
endif

clear(2)
banner('Experiments progress now.','.\,'yellow') dg
zexpset - select nmr expseriment
"this one is used to set up the parameters prior to"
"the experiment"

clear

note
"$17 for Resol. $18 for d1, $19 for ni, $20 for nt"
clear expts $17=0 $18=0 $19=0 $20=0
$21=0 $22=0 $23=0 $24=0 $25=0 $26=0 $27=0 $28=0
$3=0 $5=0 $7=0 $9=0 $11=0 $13=0 $15=0
input('Enter a number for desired experiment and return:'):S1
if $1<1$ or $1>8$ then repeat
  write('error'.The number entered is out of the range.')
  input('Please enter again and return:'):S1
  until $1>=1$ and $1<=8
endif
if $1=1$ then
  reset:$17 $21=1$ else
  if $1=4$ then reset:$17 $24=4
endif
endif
if $1=2$ then
  d1set:$18 $22=2$ else
  if $1=3$ then d1set:$18 $23=3
endif
endif
if $1=5$ then $26=6$
  d1set:$18 niset:$19 senset:$20 $25=5$ else
  if $1=6$ then d1set:$18 niset:$19 senset:$20
endif
endif

"* setting HMQC exp parameters  *
"* although d1 value is set, but this value is not used  *
"* d1 of HMQC is set to be 15*at in the qcpparamset proc  *

if $1=7$ then $27=7$
  if $18=0$ then d1set:$18$ endif "value not used though"
  if $20=0$ then qcsenset:$20$ endif
endif

if $1=8$ then $28=8
if $18=0$ then $d\text{1set}\!:18$ endif
if $20=0$ then $qcsen\text{set}\!:20$ endif
endif

$2='$

echo(' ') echo(' ')
input('Do another experiment? (y/n):'):$2
if $2='y'$ then
expts
input('Enter the experiment and return:'):$3
if $3<1$ or $3>8$ then repeat
write('error', 'The number entered is out of the range. ')
input('Please enter again and return:'):$3
until $3>=1$ and $3<=8$
endif
if $3=1$ then $21=1$
if $17=0$ then reset:$17$ endif
endif
if $3=4$ then $24=4$
if $17=0$ then reset:$17$ endif
endif
if $3=2$ then $22=2$
if $18=0$ then $d\text{1set}\!:18$ endif
endif
if $3=3$ then $23=3$
if $18=0$ then $d\text{1set}\!:18$ endif
endif
if $3=5$ then $25=5$
if $18=0$ then $d\text{1set}\!:18$ endif
if $19=0$ then $n\text{iset}\!:19$ endif
if $20=0$ then $s\text{enset}\!:20$ endif
endif
if $3=6$ then $26=6$
if $18=0$ then $d\text{1set}\!:18$ endif
if $19=0$ then $n\text{iset}\!:19$ endif
if $20=0$ then $s\text{enset}\!:20$ endif
endif

if $3=7$ then $27=7$
if $18=0$ then $d\text{1set}\!:18$ endif
if $20=0$ then $q\text{csen}\text{set}\!:20$ endif
endif
if $S3=8$ then $S28=8$
    if $S18=0$ then d1set:$S18$ endif
    if $S20=0$ then qcsenset:$S20$ endif
endif
else
clear(2)
return($S21,S22,S23,S24,S25,S26,S27,S28,S17,S18,S19,S20$)
endif

$S4='$
input('Wish to do another experiment? (y/n):'):$S4$
if $S4='y'$ then
    expts
    input('Enter a number for desired experiment and return:'):$S5$
    if $S5<1$ or $S5>8$ then repeat
        write('error'.The number entered is out of the range. ')
        input('Please enter again and return:'):$S5$
        until $S5>=1$ and $S5<=8$
endif
if $S5=1$ then $S21=1$
    if $S17=0$ then reset:$S17$ endif
endif
if $S5=4$ then $S24=4$
    if $S17=0$ then reset:$S17$ endif
endif
if $S5=2$ then $S22=2$
    if $S18=0$ then d1set:$S18$ endif
endif
if $S5=3$ then $S23=3$
    if $S18=0$ then d1set:$S18$ endif
endif
if $S5=5$ then $S25=5$
    if $S18=0$ then d1set:$S18$ endif
    if $S19=0$ then niset:$S19$ endif
    if $S20=0$ then senset:$S20$ endif
endif
if $S5=6$ then $S26=6$
    if $S18=0$ then d1set:$S18$ endif
    if $S19=0$ then niset:$S19$ endif
    if $S20=0$ then senset:$S20$ endif
endif
if S5=7 then $27=7$
  if $18=0$ then d1set:$18$ endif
  if $20=0$ then qcsenset:$20$ endif
endif
if S5=8 then $28=8$
  if $18=0$ then d1set:$18$ endif
  if $20=0$ then qcsenset:$20$ endif
else
  clear(2)
  return($21,22,23,24,25,26,27,28,17,18,19,20)$
endif

$6='$
input('Do another experiment? (y/n):'):$6$
if $6='y'$ then
  expts
  input('Enter the experiment and return:'): $7$
  if $7<1$ or $7>8$ then repeat
    write('error'. 'The number entered is out of the range.')
    input('Please enter again and return:'): $7$
    until $7>=1$ and $7<=8$
  endif
  if $7=1$ then $21=1$
    if $17=0$ then reset:$17$ endif
  endif
  if $7=4$ then $24=4$
    if $17=0$ then reset:$17$ endif
  endif
  if $7=2$ then $22=2$
    if $18=0$ then d1set:$18$ endif
  endif
  if $7=3$ then $23=3$
    if $18=0$ then d1set:$18$ endif
  endif
  if $7=5$ then $25=5$
    if $18=0$ then d1set:$18$ endif
    if $19=0$ then niset:$19$ endif
    if $20=0$ then senset:$20$ endif
  endif
  if $7=6$ then $26=6$
    if $18=0$ then d1set:$18$ endif

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if \( S_{19} = 0 \) then \( \text{iset} : S_{19} \) endif
if \( S_{20} = 0 \) then \( \text{senset} : S_{20} \) endif
if \( S_{7} = 7 \) then \( S_{27} = 7 \)
if \( S_{18} = 0 \) then \( \text{d1set} : S_{18} \) endif
if \( S_{20} = 0 \) then \( \text{qcsenset} : S_{20} \) endif
if \( S_{7} = 8 \) then \( S_{28} = 8 \)
if \( S_{18} = 0 \) then \( \text{d1set} : S_{18} \) endif
if \( S_{20} = 0 \) then \( \text{qcsenset} : S_{20} \) endif
else
\( \text{clear} (2) \)
return(\( S_{21}, S_{22}, S_{23}, S_{24}, S_{25}, S_{26}, S_{27}, S_{28}, S_{17}, S_{18}, S_{19}, S_{20} \))
endif

\( S_{8} = '>' \)
input('Do another experiment? (y/n):') \( S_{8} \)
if \( S_{8} = 'y' \) then
\( \text{expts} \)
input('Enter the desired experiment and return:') \( S_{9} \)
if \( S_{9} < 1 \) or \( S_{9} > 8 \) then repeat
write('error','The number entered is out of the range.')
input('Please enter again and return:') \( S_{9} \)
until \( S_{9} \geq 1 \) and \( S_{9} \leq 8 \)
endif
if \( S_{9} = 1 \) then \( S_{21} = 1 \)
if \( S_{17} = 0 \) then \( \text{reset} : S_{17} \) endif
endif
if \( S_{9} = 4 \) then \( S_{24} = 4 \)
if \( S_{17} = 0 \) then \( \text{reset} : S_{17} \) endif
endif
if \( S_{9} = 2 \) then \( S_{22} = 2 \)
if \( S_{18} = 0 \) then \( \text{d1set} : S_{18} \) endif
endif
if \( S_{9} = 3 \) then \( S_{23} = 3 \)
if \( S_{18} = 0 \) then \( \text{d1set} : S_{18} \) endif
endif
if \( S_{9} = 5 \) then \( S_{25} = 5 \)
if \( S_{18} = 0 \) then \( \text{d1set} : S_{18} \) endif
if \( S_{19} = 0 \) then \( \text{niset} : S_{19} \) endif

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if $S20=0$ then senset:$20$ endif
endif
if $S9=6$ then $S26=6$
    if $S18=0$ then d1set:$18$ endif
    if $S19=0$ then niset:$19$ endif
    if $S20=0$ then senset:$20$ endif
endif
if $S9=7$ then $S27=7$
    if $S18=0$ then d1set:$18$ endif
    if $S20=0$ then qcsenset:$20$ endif
endif
if $S9=8$ then $S28=8$ endif
else
clear(2)
return($S21,S22,S23,S24,S25,S26,S27,S28,S17,S18,S19,S20)$
endif

$S10='$
input('Do another experiment? (y/n):'):$S10$
if $S10='y'$ then
    expts
    input('Enter the desired experiment and return:'):S$11$
    if $S11<1$ or $S11>8$ then repeat
        write('error'.The number entered is out of the range.')</n        input('Please enter again and return:'):S$11$
        until $S11>=1$ and $S11<=8$
    endif
    if $S11=1$ then $S21=1$
        if $S17=0$ then reset:$17$ $S21=1$ else $S21=1$ endif
    endif

    if $S11=4$ then $S24=4$
        if $S17=0$ then reset:$17$ endif
    endif
    if $S11=2$ then $S22=2$
        if $S18=0$ then d1set:$18$ endif
    endif
    if $S11=3$ then $S23=3$
        if $S18=0$ then d1set:$18$ endif
    endif
    if $S11=5$ then $S25=5$
        if $S18=0$ then d1set:$18$ endif
endif
if $5=0$ then niset:5 endif
if $5=0$ then senset:5 endif
endif
if $11=6$ then $26=6$
  if $18=0$ then dierset:18 endif
  if $19=0$ then niset:19 endif
  if $20=0$ then senset:20 endif
endif
if $11=7$ then $27=7$
  if $18=0$ then dierset:18 endif
  if $20=0$ then qcsenset:20 endif
endif
if $11=8$ then $28=8$
  if $18=0$ then dierset:18 endif
  if $20=0$ then qcsenset:20 endif
endif
else
  clear(2)
  return($21,$22,$23,$24,$25,$26,$27,$28,$s_17,$s_18,$s_19,$s_20)
endif

$s_12='$
input('Do another experiment? (y/n):'):$s_12
if $s_12='y'$ then
  expts
  input('Enter the desired experiment and return:'):$s_13
  if $s_13<1$ or $s_13>8$ then repeat
    write('error', 'The number entered is out of the range.')
    input('Please enter again and return:'):$s_13
    until $s_13>=1$ and $s_13<=8$
  endif
  if $s_13=1$ then $21=1$
    if $17=0$ then reset:$17$ $21=1$ else $21=1$ endif
  endif
  if $s_13=4$ then $24=4$
    if $17=0$ then reset:$17$ endif
  endif
  if $s_13=2$ then $22=2$
    if $18=0$ then dierset:$18$ endif
endif
if $S_{13} = 3$ then $S_{23} = 3$
  if $S_{18} = 0$ then d1set:$S_{18}$
endif
endif
if $S_{13} = 5$ then $S_{25} = 5$
  if $S_{18} = 0$ then d1set:$S_{18}$
  if $S_{19} = 0$ then niset:$S_{19}$
  if $S_{20} = 0$ then senset:$S_{20}$
endif
if $S_{13} = 6$ then $S_{26} = 6$
  if $S_{18} = 0$ then d1set:$S_{18}$
  if $S_{19} = 0$ then niset:$S_{19}$
  if $S_{20} = 0$ then senset:$S_{20}$
endif
if $S_{13} = 7$ then $S_{27} = 7$
  if $S_{18} = 0$ then d1set:$S_{18}$
  if $S_{20} = 0$ then qcsenset:$S_{20}$
endif
if $S_{13} = 8$ then $S_{28} = 8$
  if $S_{18} = 0$ then d1set:$S_{18}$
  if $S_{20} = 0$ then qcsenset:$S_{20}$
endif
else
  clear(2)
  return($S_{21}, S_{22}, S_{23}, S_{24}, S_{25}, S_{26}, S_{27}, S_{28}, S_{17}, S_{18}, S_{19}, S_{20}$)
endif

$S_{14} = '$
input('Do another experiment? (y/n):')$S_{14}$
if $S_{14} = y$ then
  expts
  input('Enter the desired experiment and return:')$S_{15}$
  if $S_{15} < 1$ or $S_{15} > 8$ then repeat
    write('error', 'The number entered is out of range.')</n    input('Please enter again and return:')$S_{11}$
    until $S_{15} >= 1$ and $S_{15} <= 8$
  endif
  if $S_{15} = 1$ then $S_{21} = 1$
    if $S_{17} = 0$ then reset:$S_{17}$ $S_{21} = 1$ else $S_{21} = 1$
  endif
  if $S_{15} = 4$ then $S_{24} = 4$
    if $S_{17} = 0$ then reset:$S_{17}$
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endif
if $15=2$ then $22=2$
  if $18=0$ then d1set:$18$ endif
endif
if $15=3$ then $23=3$
  if $18=0$ then d1set:$18$ endif
endif
if $15=5$ then $25=5$
  if $18=0$ then d1set:$18$ endif
  if $19=0$ then niset:$19$ endif
  if $20=0$ then senset:$20$ endif
endif
if $15=6$ then $26=6$
  if $18=0$ then d1set:$18$ endif
  if $19=0$ then niset:$19$ endif
  if $20=0$ then senset:$20$ endif
endif
if $15=7$ then $27=7$
  if $18=0$ then d1set:$18$ endif
  if $20=0$ then qcsenset:$20$ endif
endif
if $15=8$ then $28=8$
  if $18=0$ then d1set:$18$ endif
  if $20=0$ then qcsenset:$20$ endif
endif

clear(2)
return($21,22,23,24,25,26,27,28,17,18,19,20)$ else
clear(2)
return($21,22,23,24,25,26,27,28,17,18,19,20)$ endif
"qcsenset - set up nt value"

clear(2)
write('graphics','yellow',1,160,'program is trying to set the spectral')
write('graphics','yellow',1,140,'sensitivity. If the sample conc. is very')
write('graphics','yellow',1,120,'low, try to choose the high sensitivity')
write('graphics','yellow',1,110,'option. This way "nt" will be set at a higher')
write('graphics','yellow',1,100,'number and give a better 2D spectrum.')

clear echo('') echo('')
echo(' 1. Give a low sensitivity 2D spectrum.')
echo(' 2. Give a medium sensitivity 2D spectrum.')
echo(' 3. Give a high sensitivity 2D spectrum.')
input('Enter choice of sensitivity and return:'): $1

if $1<1 or $1>3 then repeat
  write('error','The number you entered is out of the range.')
  input('Please enter again and return:'): $1
  until $1>=1 and $1<=3
endif

if $1=1 then $2=4 else
  if $1=2 then $2=8 else
    if $1=3 then $2=16 endif
  endif
endif

clear(2)
return($2)
"qcparamset -- this sets the parameters for HMQC and HMBC"

"* parameters include weighting parameters, d1, ni. and nt  *"
"* Flock and Hetcor experiments both use sw1/16 or sw1/8 to set ni value  *"
"* this is not appropriate since the sw1 in HMQC is transfered from C13  *"
"* the value of sw1 is HUGE compare to the ones transfered from H1  *"

\[ \text{d1} = \text{at}^*20 \]
\[ \text{nt} = \text{r}4 \]

"* setting number of increment (ni) value  *"

if \( \text{sw1} \geq 15000 \) then ni=256 else
if \( \text{sw1} < 15000 \) then ni=128 endif
endif

"* setting Fourier transform number (fn) value  *"

if ni \leq 64 then fn1=128 else
if ni > 64 and ni \leq 128 then fn1=256 else
if ni >128 and ni \leq 256 then fn1=512 else
if ni >256 and ni \leq 512 then fn1=1024 else
if ni >512 and ni \leq1024 then fn1=2048
endif endif endif endif endif endif

"* the above procedure should have the same effect with the one below  *"
"* try the first one first, if successful it'll be used for paramset  *"
"* procedure as well.  *"

"* if ni = 128 then fn1=256 else  *"
"* if ni = 256 then fn1=522 endif  *"
"* endif  *"
"dlset - set up dl value"

clear echo(' ') echo(' ')  
echo(' Your sample molecular weights')  
echo(' ----------------------------------')  
echo(' 1. More than 10 and less than 200')  
echo(' 2. More than 200 and less than 300')  
echo(' 3. More than 300 and less than 400')  
input('Please enter 1, 2 or 3 for your sample MW:'):$1  
if $1<1 or $1>3 then repeat  
  write('error. The number you entered is out of the range.')  
  input('Please enter again and return:'):$1  
  until $1>=1 and $1<=3  
endif  
if $1=1 then $2=1 else  
  if $1=2 then $2=0.8 else  
    if $1=3 then $2=0.65  
    endif  
  endif  
endif  
return($2)

"setqc - Sets parameters and invoke HMQC sequence"

"this procedure is different from setch (hetcore processing) and"  
"setcf (flock processing) because there is no need to reset nt"

banner('now in setqc, but entering hmqc1','white')  
hmqc1  
qcparamset "* this macro sets up the parameters. *"  
dp='y'  
wexp='proqc' dp='y'  
dg  
au
"hmqc1 -- set parameters for the hmqc experiment."
"modified from the hmqc proc from /home/wfr/vnmrsys/maclib/hmqc"
"The modified procedure supports C13 (X nucleus) only, however, the
"original program is capable of supporting both C13 and N15."
"Modified by Li-Lin Tay"

psgset('hmqc13','satdly','satfrq','satpwr','satflg','array','null','taumb')
psgset('hmqc13','dmf','dn','j','pwxlvl','pwx','dpwr','dof','swl','ni','mbond')
psgset('hmqc13','phase','dg','ap','ss','dm','dmm','dl','at','dgs','hs')
psgset('hmqc13','dseq','pw')
seqfil='hmqc'
clear(2)
banner('Calling up HMQC sequence’, ‘cyan’)

if (nt < 16) then
  nt=16
else
  nt=nt/16
  nt=nt*16
endif

ph bs='n' homo='n' wshim='n'
ss=32 pw=9.5
dmf=15873
hs='nn'
swl=19991.6
rlf1=566.7
spin=0
np=512

qcwtpset

lpset " this macro sets linear prediction"
dglp
echo(' ') echo('')
echo('LINEAR PREDICTION PARAMETERS sets to the above values')
"bcparamset -- this sets the parameters for HMBC"

"* parameters include weighting parameters, d1, ni, and nt  *

d1 = 0.6    nt = r4

"* setting number of increment (ni) value  *

if sw1 >= 15000 then ni=256 else
if sw1 < 15000 then ni=128 endif
endif

clear(2)
banner('bcparamset, setting ni and fn1 value','yellow')

if ni <= 64 then fn1=128 else
if ni > 64 and ni <= 128 then fn1=256 else
if ni >128 and ni <= 256 then fn1=512 else
if ni >256 and ni <= 512 then fn1=1024 else
if ni >512 and ni <=1024 then fn1=2048 endif endif endif endif endif

"setbc - Sets parameters and invoke HMBC sequence"

banner('now in setbc, but entering hmbc l','white')

hmbc l
bcparamset dp='y'
wexp=proqc' dp='y'
dg
au
"HMBC -- set parameters for the HMBC experiment."
"modified from the hmqc proc from /home/wfr/vnmrsys/maclib/hmqc1"

"hmbc - sets up parameters in accordance with the hmbc pulse sequence."

psgset('hmqc13','satdly','satfrq','satpwr','satflg','array','null','taumb')
psgset('hmqc13','dmf','dn','j','pwxlvl','pwx','dpwr','dof','swl','ni','mbond')
psgset('hmqc13','phase','dg','ap','ss','dm','dmm','dl','at','dgs','hs')
psgset('hmqc13','dseq')
seqfil='hmqc1'

clear(2)
banner('Calling up HMBC sequence.' 'cyan')

ph bs='n' homo='n' wshim='n'
pmode='full'

if (nt < 16) then
  nt=16
else
  nt=nt/16
  nt=nt*16
endif

ss=32 pw=9.5 dmm='ccc' dseq='garp1'
dmf=15873 pwx=9.4 pwxlvl=63 hs='nn'
dm='nnn' gain='y' temp=25 spin='n'
swl=27991.6 dof=1500 av
ph l null=0 sp l=-566.7 rfl=566.7
rfpl=0 axis='pd' spin=0 mbond='y'
np=1024 taumb=0.063

clear
echo('setting weighting parameters')
bcwtpset

lpset " this macro sets linear prediction "
dglp
echo('LINEAR PREDICTION PARAMETERS sets to the above values')
"wtpset - set weighting parameters along both F1 and F2 axes for processing"
if pslabell='relayh' or pslabel='cosy' then
  if $#>0 then $a=$1 else $a=0.0625 endif
  if $#>1 then $b=$2 else $b=0.25 endif
  if $#>2 then $c=$3 else $c=0.0625 endif
  if $#>3 then $d=$4 else $d=0.25 endif
  lb = - 0.318 / ($a*at) gf = $b*at gfs = 'n' sb = 'n' sbs = 'n' awc = 'n'
  exists('ni','parameter'):Se
  if ($e>0) then
    if (ni>1) then
      Sat1 = ni/sw1 $1=fn1/ni
      if $1=2 then lb1 = -0.318 / ($c*Sat1) gfl = $d*Sat1
      else
        $2=$1/2 lb1 = -0.318 / ($2*$c*Sat1) gfl = $2*$d*Sat1
      endif
      gfl1 = 'n' sb1 = 'n' sbs1 = 'n' awc1 = 'n'
      endif
    endif
  endif
endif

"* pulse label for both HMQC and HMBC are the same, both    *
"* labeled as HMQC sequences. Therefore, similar weighting    *
"* will be applied to both sequences.      *

if pslabell='hetcor' or pslabel='flock' or pslabel='dodo'
or pslabel='hmqc' then
  gf=0.625*at lb='n' sb=gf sbs=(-1/3)*gf $1=fn1/ni
  if $1=2 then
    gfl=0.643*(ni/sw1) lb1='n' sb1=gfl sbs1=(-1/3)*gfl
  else
    $2=$1/2 gfl=$2*0.643*(ni/sw1) sb1=gfl lb1='n' sbs1=(-1/3)*gfl
  endif
endif
"LPSET - set up parameters for linear prediction along f1 axis"
parlp(1) lpop1=f lpfilt1=8 $1=ni lnpnupts1=$1
strtlp1=$1 strtext1=$1+1 lpext1=(4*$1-$1) $2=2*(lpext1+strtext1)
if $2>=1 and $2<=8 then fn1=8 else
if $2>8 and $2<=16 then fn1=16 else
if $2>16 and $2<=32 then fn1=32 else
if $2>32 and $2<=64 then fn1=64 else
if $2>64 and $2<=128 then fn1=128 else
if $2>128 and $2<=256 then fn1=256 else
if $2>256 and $2<=512 then fn1=512 else
if $2>512 and $2<=1024 then fn1=1024 else
if $2>1024 and $2<=2048 then fn1=2048 else
if $2>2048 and $2<=4096 then fn1=4096 else
if $2>4096 and $2<=8192 then fn1=8192
endif endif endif endif endif endif endif endif endif
proc l1='lp'

"proqc - process HMQC experiments"

"Weighting function and linear prediction parameters are set"
"in the proc hmqc1 already. No need to reset. Simply FT the"
"data to give the desired spectrum"
wft2da nm2d dconi
ppa(35,140) pcon
page
dconi
"solset - set up solvet"

clear echo(' ') echo(' ')
echo(' NMR solvents used')
echo(' --------------------------')
echo(' 1. Chloroform')
echo(' 2. Benzene')
echo(' 3. Acetone')
echo(' 4. Deuterium Oxide')
echo(' 5. Cyclohexane')
echo(' 6. DMSO')
echo(' 7. Toluene')
echo(' 8. Acetic Acid')
echo(' 9. Methyl Alcohol')
echo(' 10. Methylene Chloride')
input('Enter a number for the solvent you used and return:'):$1
if $1<1 or $1>10 then repeat
  write('error','The number you entered is out of the range.')
  input('Please enter again and return:'):$1
until $1>=1 and $1<=10
endif
if $1=1 then $2='CDCL3' else
if $1=2 then $2='C6D6' else
if $1=3 then $2='CD3COCD3' else
if $1=4 then $2='D20' else
if $1=5 then $2='C6D12' else
if $1=6 then $2='DMSO' else
if $1=7 then $2='C6D5CH3' else
if $1=8 then $2='CD3COOD' else
if $1=9 then $2='CD3OD' else
if $1=10 then $2='CD2CL2' else
endif endif endif endif endif endif endif endif endif
return($2)
"expts - choose nmr experiments with desired parameters"
clear echo(' ') echo(' ')

1. Proton (1H) 
2. Carbon (13C) 
3. Attached Proton Test (APT) 
4. Proton-Proton Correlation (COSY) 
5. Carbon-Proton Correlation (HETCOR) 
6. Long range Carbon-Proton Correlation (FLOCK) 
7. H1-detected Carbon-Proton Correlation (HMQC) 
8. H1-detected Long-range Carbon-Proton Correlation (HMBC) 

"PAPTS - plot APT spectrum"
wft aph f vsadj noislm vp=70 setrefC pl pscale apa page

"pro2d - process 2D experiments"
wft2d nm2d dcon ppa(35.140) pcon page
"runc - setup parameters and run for carbon"

if ($# >= 1) then
    $solv = $1
    "use passed argument as solvent"
else
    $solv = 'cdcl3'
    "change this assignment if a"
endif
setup('C13',$solv)
    "set up parameters"
fixup
    "fix frequency positioning"
tof = tof - 500
    "allow some baseline upfield"
rfl = rfl + 500
macro = 'c13'
if ($# >= 2) then
    $lockmode = $2
    "check locking option"
else
    $lockmode = 'lock'
endif
if ($lockmode = 'lock') then
    setlk(solvent)
    "set lock power and gain"
endif
aLock='s'
if (macro = 'c13') then
    wbs = 'testsnp
exists('c13par','maclib'):Se
    if ($e > 0.5) then
        "user macro to set acquisition parameters"
        c13par
    endif
    wexp='c13p'
    "select appropriate processing at the end"
    werr = 'react'
else
endif

160
"runh - set up parameters and run proton experiment"

if ($# >= 1) then
  $solv = $1
  "use passed argument as solvent"
else
  $solv = 'cdcl3'
  "change this assignment if a"
endif
setup('H1', $solv)
"set up parameters"
fixup
"fix frequency positioning"
tof = tof - 500
"allow some baseline upfield"
rf1 = rf1 + 500
macro = 'h1'
if ($# >= 2) then
  $lockmode = $2
else
  $lockmode = 'lock'
endif
if (Slockmode = 'lock') then
  setIk(solvent)
  "set lock power and gain"
endif
if (auto = 'n') then
  if (Slockmode = 'lock') then
    readlk:Slevel
    "check if locked yet"
    if (Slevel > 100) then
      lockgain = lockgain - 10
    endif
    if (Slevel < 10) then
      "do lock optimization"
      alock = 's'
    endif
  endif
endif
if (macro = 'h1') then
  exists('h1par', 'maclib'):Se
  if ($e > 0.5) then
    "user macro to set acquisition parameters"
    h1par
  endif
  werr = 'react'
endif
Appendix C
Structural assignments of 3,4-Epoxy-8,9-dihydrioplatinum
Structural Assignments of 3,4-Epoxide-8,9-dihydropiplartine

As part of the ongoing work on the phytochemistry of a selected species of the endemic Jamaican flora, we have fractionated extracts of the leaves and twigs of Piper verrucosum Sw. (Piperaceae), a tree of relatively infrequent occurrence in central Jamaica.\(^1\)

The ground leaves and twigs were extracted with hexanes. The concentrated extract, upon repeated column chromatography and crystallization yielded only 3,4-epoxide-8,9-dihydropiplartine. (1) (0.0003%). Fractionation and crystallization of the Me\(_2\)Co extract obtained from the marc afforded 3,4,5-trimethoxybenzenepropanoic acid (0.0015%), identified from spectral data and comparison of the mp with the literature value.\(^2\)

![Chemical structure of compound 1](image)

Compound 1. C\(_{17}\)H\(_{21}\)NO\(_6\), mp 85-87 \(^\circ\)C, [\(\alpha\)]\(_D\) -121.6\(^\circ\), exhibited bands in its IR spectrum that indicated an aromatic ring (1596, 1548, 1473 cm\(^{-1}\)) and two carbonyl groups (1726, 1698 cm\(^{-1}\)). The \(^1\)H-NMR spectrum displayed peaks characteristic of a trimethoxybenzenepropanoyl moiety.\(^3\) These consisted of coupled triplets for the C-8 and C-9 methylenes (\(\delta\) 3.23 and \(\delta\) 2.91, each 2H, J=7.4 Hz); signals for three methoxyl groups, two of which are equivalent (\(\delta\) 3.85, 6H, and \(\delta\) 3.82, 3H), and a shielded aromatic singlet integrating for two protons (\(\delta\) 6.46). The integration of the latter three signals demonstrated that the aromatic ring is symmetrically substituted as shown. The corresponding carbon signals (see Table 1) were assigned with the aid of HMQC spectra. Cross peaks in the HMBC spectra between both pairs of methylene protons (\(\delta\) 3.23 and \(\delta\) 2.91) and a carbon at \(\delta\) 174.6 established this as the carbonyl of the substituted benzenepropanoyl group. Fragment ions in the EIMS at m/z 222, 194, 181, and 179 were consistent with the presence of this group.\(^4\)

\(^1\)H and \(^13\)C signals for the C\(_5\)H\(_6\)NO\(_2\) residue indicated an amidic carbonyl, a disubstituted epoxide, and two methylene groups. HMBC cross peaks between the Carbonyl carbon (\(\delta\) 169.6) and the epoxymethine proton at \(\delta\) 3.56 demonstrated that the epoxy and carbonyl functionalities are vicinal. Analysis of HMQC, COSY, and HMBC data enabled the complete assignment of the signals for this residue (see table 1) leading to its formulation as 3,4-epoxy-2-oxopiperdinylo.
<table>
<thead>
<tr>
<th>position</th>
<th>δC</th>
<th>δH a</th>
<th>HMBC b</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>169.6</td>
<td>3.56 (1H, d, J= 4.1 Hz)</td>
<td>3.56</td>
</tr>
<tr>
<td>3</td>
<td>52.3</td>
<td>3.70 (1H, dd, J= 4.1, 5.8 Hz)</td>
<td>3.70, 2.42</td>
</tr>
<tr>
<td>4</td>
<td>53.4</td>
<td>2.42 (1H, m) 2.00 (1H, ddd, J=15.0, 5.8, 13.2 Hz)</td>
<td>4.34, 2.42</td>
</tr>
<tr>
<td>5</td>
<td>23.8</td>
<td>4.34 (1H, dddd, J= 13.5, 5.8, 1.5, 1.5 Hz) 3.20 (1H, m)</td>
<td>4.34, 3.70</td>
</tr>
<tr>
<td>6</td>
<td>35.6</td>
<td>2.91 (2H, t, J=7.4 Hz)</td>
<td>2.00</td>
</tr>
<tr>
<td>7</td>
<td>174.6</td>
<td>3.23 (2H, t, J=7.4 Hz)</td>
<td>3.23, 2.91</td>
</tr>
<tr>
<td>8</td>
<td>41.2</td>
<td>6.46 (2H, s)</td>
<td>2.91</td>
</tr>
<tr>
<td>9</td>
<td>31.2</td>
<td>6.46, 3.23</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>136.6</td>
<td>6.46, 3.23, 2.91</td>
<td></td>
</tr>
<tr>
<td>11, 15</td>
<td>105.4 (doubled)</td>
<td>6.46 (2H, s)</td>
<td>6.46, 2.91</td>
</tr>
<tr>
<td>12, 14</td>
<td>153.1 (doubled)</td>
<td>6.46, 3.85</td>
<td></td>
</tr>
<tr>
<td>13</td>
<td>136.3</td>
<td>3.82</td>
<td></td>
</tr>
<tr>
<td>16,18</td>
<td>56.0</td>
<td>3.85 (6H, s)</td>
<td></td>
</tr>
<tr>
<td>17</td>
<td>60.8</td>
<td>3.82 (3H, s)</td>
<td></td>
</tr>
</tbody>
</table>

a. Values were established by HMQC.
b. Protons that correlate with carbon resonance.
The relative stereochemistry was determined from the magnitude of the vicinal coupling between H-3 and H-4 and the results of a NOESY experiment. The J value and the results of a NOESY experiment. The J value ($J_{3,4} = 4.1$ Hz) was exactly as expected for a cis coupling with a near-zero dihedral angle in a 1,2-epoxycyclohexane.5 A trans coupling in the same system should have a coupling of ~0 Hz. The NOESY spectrum showed a strong cross peak between H-3 and H-4, again consistent with a cis stereochemistry. H-4 showed typical gauche coupling along with NOESY cross peaks to both C-5 protons, suggesting that it is in a pseudoequatorial orientation in the six-membered ring. This ring will obviously be distorted from a chair form by the carbonyl and epoxide functionalities.

Compound 1 is a new amide alkaloid and may be regarded as the 3,4-epoxy-8,9-dihydro derivative of a known compound pipartine, which occurs in the roots of the Indian plant *Piper longum* L. 6 A similar compound in which the heterocyclic residue is 3,4-didehydro-2-oxopyrrolidinyl has been isolated from *Piper demeraranum* (Miq.) C. DC. of Trinidad.3

Epoxides are not common in the *Piper* genus and have been reported only in the Old World species *P. polysyphonum* C. DC. of China and *P. hookeri* Hook, *P. cubeba* (Miq.), and *P. brachystachium* Wall of India. These plants produce the chorismate-derived cyclohexane epoxides, crotepoxide, and related compounds.7-9

**Experimental section**

**General Experimental Procedures.** Melting points were determined on a Thomas-Hoover capillary melting point apparatus. The optical rotation was measured on a Perkin-Elmer 24MC polarimeter. IR spectra were taken on a Perkin-Elmer 735B spectrophotometer. MS were obtained on a VG 70-250S mass spectrometer. NMR spectra were determined using a Bruker ACE 200 and a Varian UNITY-500 spectrometer with TMS as internal standard. Column chromatography utilized SiO2, Mallink-rodt, 230-400 mesh.

**Plant Material.** The leaves and twigs of *P. verrucosum* Sw. (Piperaceae) were collected near Quickstep, Trelawny, Jamaica, in March 1995. Avoucher specimen (no. 33621) is lodged in the Herbarium at the University of West Indies, Mona, Jamaica.

**Extraction and Isolation.** Dried leaves and twigs (1.64kg) were grounded and extracted by cold percolation with hexanes. Evaporation yielded a gum (37g), a portion (12g) of which was chromatographed (gradient elution with Me2CO-hexanes). The residue (1.5g) from the 20% Me2CO fractions was chromatographed in 5% Me2CO-hexanes) to yield pure 1 (157mg).
Extraction with cold Me₂CO of the marc from the hexane extract and evaporation of the solvent gave a gum (57g), a portion (12g) of which was chromatographed (Me₂CO-hexanes gradient). The fractions eluted with 50% Me₂CO were rechromatographed, again using a Me₂CO-hexanes gradient. 3,4,5-Trimethoxybenzenepropanoic acid (500mg) was obtained from fractions eluted with 25% Me₂CO-hexanes.

3,4-Epoxy-8,9-dihydropiplartine (1): Recrystallized from Me₂CO-ligroin, mp 85-87 °C; [α]D -121.6° (c 0.020, CHCL₃); IR ν max 1726, 1698, 1596, 1548, 1473 cm⁻¹; UV λ max (Ethol) (log ε) 260 (2.92) nm; EI MS m/z [M]+ 335 (95), 222 (100), 194 (66), 181 (69), 179 (66); HRMS m/z found 335.1384, calcd 335.1369 for C₁₇H₁₁O₆; ¹H and ¹³C-NMR data, see Table 1.

References:


