CHAPTER II

HISTORICAL

1. ^{13}C Nuclear Magnetic Resonance Spectroscopy

In the early 1970 ^{13}C NMR spectropy has grow from an obscure technique to a well accepted general method of instrumental analysis and used in structure elucidation of natural product .

The first NMR observation of carbon - 13 nuclei have been reported in 1957 and have been developed to enhance the sensitivity and the spectrum resolution. Ernst (6,7) provided the solution of the low sensitivity and overlap problem by using Fourier transform spectroscopy and noise decoupling technique.

Although there are at present a variety of NMR spectrometer using different techniques of determination but the basic theory of NMR is common to all experiment and all nuclei.

1.1 Theory of nuclear magnetic resonance

The fundamental property of the atomic nucleus is the nuclear spin (I). When we place a nucleus ,which has spin quantum number ≠ 0 , into the static magnetic field Ho. Nucleus will spin behave like gyroscope as illustrated in Figure 1. Vo is the frequency of precession known as " Larmor frequency " of the observed nucleus.(8)

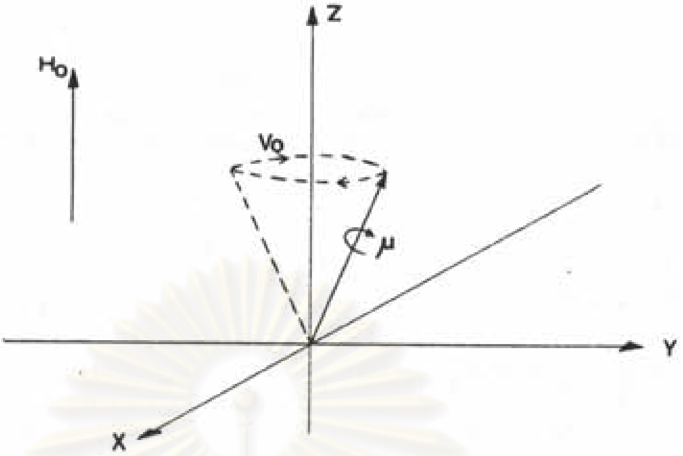


Figure.1 Motion of spinning nucleus in a static magnetic field

The rotational motion of the nucleus can produce the angular momentum when nucleus is placed in the magnetic field (Ho). This angular momentum may have any value depending on the magnitude of the force (Ho) that used to set the body in motion. The law of classical physic which used to explain the atomic particle must be replaced by those of quantum mechanic. According to the quantum mechanic , angular momentum (N) of the nuclues may have only certain value that are always some multiple of Plank's constant, h as in Eq. 1.1

$$N = \frac{I \quad h}{2\pi} \qquad (1.1)$$

where I is the nuclear spin quantum number and can have intregal or half integral value.

The spinning body has its angular momentum dircted along the axis of rotation and these rotating charge can produced a magnetic field , defined as "Magnetic moment". This moment is also directed along the axis of rotation . So, The nuclear angular momentum vector (N) and the nuclear magnetic moment (μ) are therefor collinear. This relation is direct proportion by Eq. 1.2

$$\mu = \gamma N \qquad (1.2)$$

where the proportionality constant, γ is usually refered to magnetogyric ratio and is a very important characteristic of each different nucleus.

From Eq. 1.1 and 1.2 , a nuclear magnetic moment are therefore given as Eq. 1.3

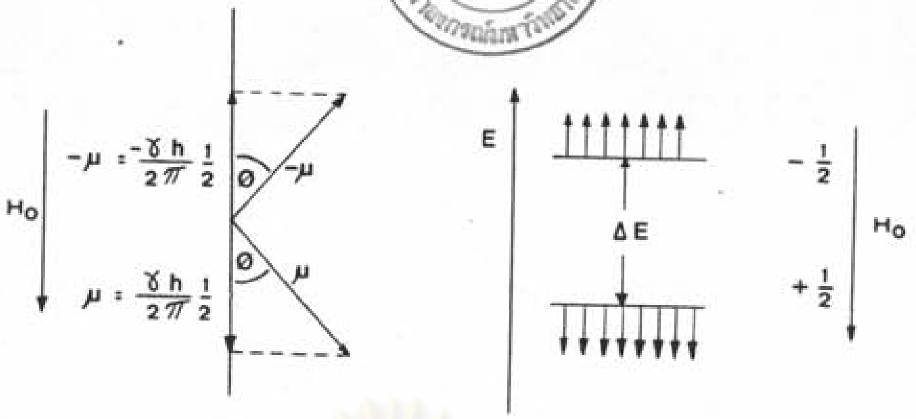
$$\mu = \frac{\gamma I h}{2\pi} \qquad (1.3)$$

The energy (E) of magnetic moment μ in a field Ho is given by the product of Ho and u as eq 1.1

$$E = -\mu Ho = -\frac{\gamma h I Ho}{2\pi} \qquad (1.4)$$

From the quantum mechanic, a nuclues with total spin quantum number I may occupy (2I+1) different energy level when placed in the magnetic field

For ^{1}H, ^{13}C, ^{15}N, ^{19}F, ^{31}P which I= 1/2 two spin aligments relative to Ho are +1/2 and -1/2



(a) (b)

Figure.2 (a) Orientation of the nucleus with I = 1/2
(b) The population of nucleus in the two energy level

The nucleus can precess along and opposite to Ho but in the one along the same direction with Ho have more population because nucleus like to stay in the low energy level. (9)

The energy different (ΔE) between the energies E+1/2 and E-1/2 are derived from Eq. 1.3

$$E_{+1/2} = -\mu H_o = -\frac{\gamma h H_o}{4\pi} \qquad (1.5)$$

$$E_{-1/2} = \mu H_o = \frac{\gamma h H_o}{4\pi} \qquad (1.6)$$

$$\Delta E = E_{-1/2} - E_{+1/2} = 2\,\mu_o H_o = \frac{\gamma h\, H_o}{2\pi} \qquad (1.7)$$

From Eq. (1.7) we can calculate the Larmor frequency Vo of nuclei I= 1/2

$$E = \frac{\gamma h}{2\pi} H_o = hV_o$$

So, $$V_o = \frac{\gamma H_o}{2\pi} \qquad (1.8)$$

In the field strength of 21.3 kilogauss.The Larmor freguency of ^{13}C is in the range of 22 to 23 MHz lower than proton (^{1}H) which has the Larmor freguency at 90 MHz

When we apply the external magnetic field $H_1$with frequency V_1 to irradiate the nucleus which precess in the static field Ho The vector of H_1 must rotate in the plane of precession with the Larmor frequency Vo of the nuclei to be observe

$$V1 = Vo$$

The energy which applied will overcome the energy difference (ΔE) of the two level so that the spin will flip in to the opposite direction againt the Ho. (Figure.3) These phenomenon show that the energy (ΔE) are absorbed from H_1 by the nuclcus. The process is called "Nuclar Magnetic Resonance" (NMR)

In order to observe NMR,a sample containing nuclear spin (eg. ^{1}H, ^{13}C) is placed in a static magnetic field Ho, an alternating field H_1 with radio frequency V_1 is applied perpendicular to Ho, V_1 is increased or decreased slowly and continuously during observation (frequency sweep).When V_1 matches the Larmor frequency of the nuleus to be observed , an absorption signal is recorded in the receiver of the NMR spectrometer.

Continuous irradiation by the radio frequency ^{1}H would soon cause all nuclei to precess againt Ho.

At equilibrium the nuclear magnetic energy level

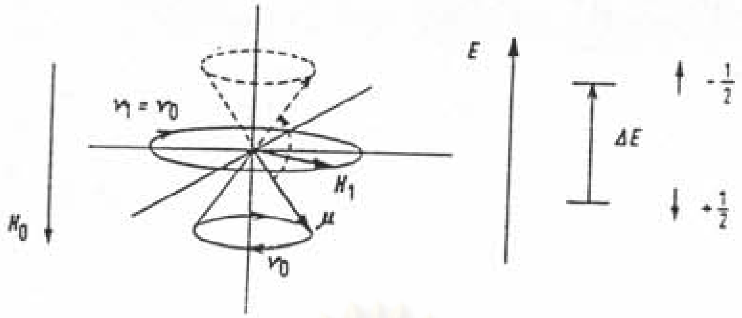


Figure.3 Action of a radio frequency field H1 on a nucleus about Ho direction

are populate according to a Botlzmann distribution which favor the lower state. For the two orientation to Ho of nuclei with I =1/2, The spin population may be expressed by the Boltzmann factor.

$$N+/N- = e^{\frac{\Delta E}{KT}} \approx 1+ \frac{\Delta E}{KT} = 1+ \frac{2\ \mu Ho}{KT} \qquad (1.8)$$

At room temperature, ΔE = 2 μHo is much less than 1 cal, even at the highest field strength. As a result, the term 2 μHo is very small and N+/N- is only slightly qreater than 1 . That mean the population are favor to stay in lower energy.

After we apply H_1 rf field, the equilibrium distribution of the spin in the static field Ho is redistributed. Following the resonance was occure , the nuclear spins will relax to the equilibrium by two processes (10).

1.Spin-spin relaxation (T_2) :this process relax through the exechange of spin between neighboring nuclei

2.Spin-lattice relaxation (T_1) : this process relax through the loss of energy from the nuclei to the surrounding (which is called "Lattice"). The important mechanism of this process is dipole - dipole relaxation.

From the basic of NMR when apply to ^{13}C NMR, we found that the main difficutly problem is the low natural abundance of the ^{13}C and its low gyromagnetic ratio which yeild the low sensitivity of signal detection.

Several methods are available for improving the sensitivity in ^{13}C NMR but the most economical and efficient method of sensitivity enhancement in ^{13}C NMR to organic molecule is the pulse Fourier transform. (11-12)

In the early generation of NMR spcetrometer, the NMR experiment spectra were obtained by sweeping either the excitation frequency or the field through the region of nuclear precission frequencies. The inefficiency of this method is that only line can be observed at a given point in time. In ^{13}C NMR, a total shift range upto 600 ppm. for diamagnetic molecule and typical line width of 1 Hz or less. By this principle it is possible to create spectrum by utilization of several thousand transmitters and an equal number of recievers spaced across the ^{13}C chemical shift range (Figure.4). But this method is impractical in economical scale.(11)

Fortunately there have another method. Instead of sweeping the frequency we can use a high intensity rf pulse to excite all the nuclei which have different Larmor frequencies.

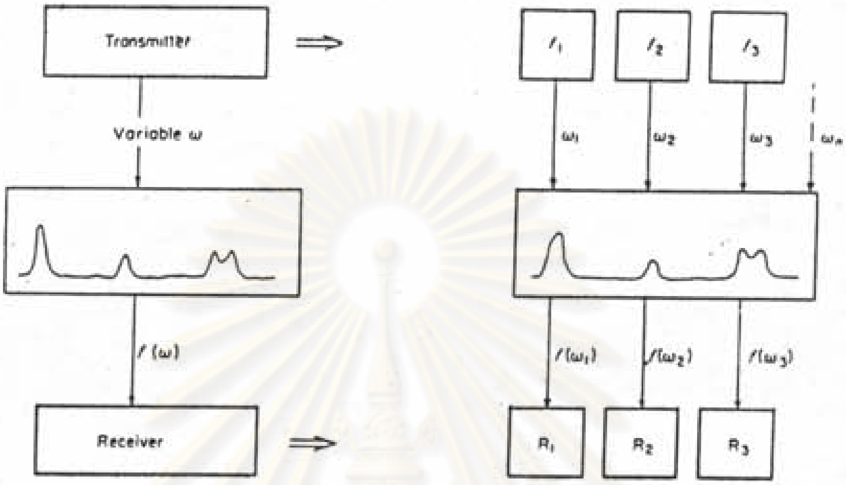


Figure.4 Diagram of a hypothetical multichannel spectrometer

A short rf pulse has the characteristics of rectangular pulse (Figure.5). This type of pulse contain waves of various frequencies which cover a large band of frequencies. It can be shown that the frequencies contain within this pulse are within of the main frequency, where t_p is the duration of the pulse.(8)

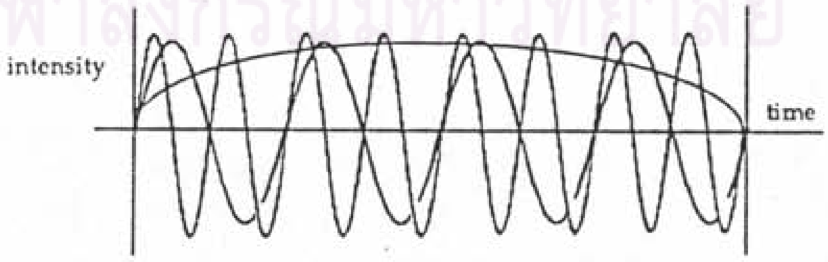


Figure.5 Various frequencies that are added to a rectangular pulse.

In ^{13}C NMR, the range of Larmor frequency in different chemical environment is abont 5,000 Hz at the field of 2T. Therefore a pulse duration (t_p) chosen to produce half the range that is 2,500 Hz and the time is

$$t_p = 1/2500 = 0.4 \text{ millisecond.}$$

At the end of the pulse period, the nuclei will precess freely with their characteristic Larmor frequency reflecting their chemical environment. The resulting macroscopic transverse magnetization induces an a.c. signal in the reciever coil with the amplitude decay in the exponential form. The free precession signal is therefore also called "Free induction decay" (FID)

When two or more absorptions take place the FID become more complex. The way to analyse the spectral information from FID is "Fourier transform" (11)

Fourier Transform analyse all the frequency component out of the complex wave form that present in the FID and give the information in the same form as in continuous wave (cw) experiment (10) (Figure.6)

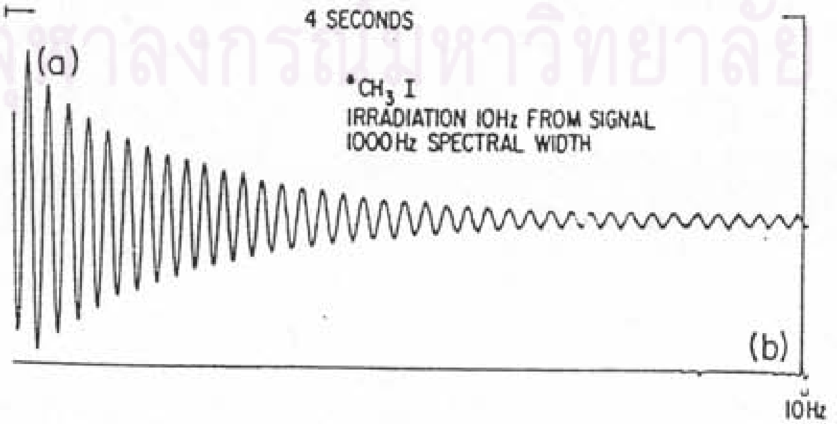


Figure.6 Spectrum of CH_3I (a) Time domain (b) Frequency domain

In combination of fourier transform technique with decoupling method such as proton noise decoupling. Off resonance decoupling can enhance the sensitivity of all multiplet lines in a coupled spectrum. This sensitivity enhancement is called "Nuclear overhauser Effect" (NOE)

The nuclear overhauser effect observed in ^{13}C {^{1}H} experiment arises from an intramolecular dipole-dipole relaxation mechanism. Two such nuclei A and X (e.g. ^{13}C and ^{1}H) in a molecule having intermolecular and intramolecular mobility (translation, Vibration, rotation) give rise to fluctuating magnetic field. Energy transfer between A and X may occure via this fluctuating field.

In an A{X} experiment the transitions of nuclei X are irradiated while the resonance of nuclei A is observed since the irradiating field is very strong. The homonuclcar relaxation process are not adequate to restore the equilibrium population of the nuclei X. The nuclci X transfer their energy to the nuclei A via internuclear dipole-dipole interaction. The nuclei A, recieving these transfered amount of energy, behave as if they have been irradiated themselves and relax. This heteronuclear relaxation process of the nuclei A that induced by double resonance. The population of the lower A level increase. As a result the intensity of a signal is enhanced.

1.2. Assignment technique in ^{13}C spectra

Assignment the chemical shift of carbon in ^{13}C NMR can used several techniques in combination to elucidate structure of organic compound. Some techniques are as following.

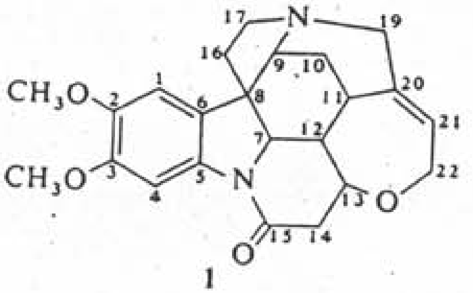
1. Proton noise decoupling (11)

^{13}C NMR spectra always show complex overlapping multiplets that are difficult to interpret. Proton noise decoupling technique provide great spectral simplicity and improve signal to noise because we use wide-band heteronuclear decoupling that result a single sharp peak for each chemically nonequivalent ^{13}C atom

A typical proton noise decoupled ^{13}C NMR spectrum of brucine with data print out, is shown in Figure. 7

2. Off-resonance decoupling (12)

Because the proton noise decoupling simplifies the spectrum and increases peak height but loss of coupling information. Off-resonance decoupling give a simplified spectrum and also retains "residaul" ^{13}C-H coupling information. So, long range coupling is usually lost, from this result we can separate CH_3, CH_2, CH and C by observe the pattern of the coupling, methyl carbon atoms appear as quatets, methylene as triplets, methine as doublets and quaternary carbon as singlet. The Off resonance spectrum of brucine together with



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No.	Int.	ppm	No.	Int.	ppm
1	128	168.63	13	148	59.81
2	91	149.13	14	185	56.44
3	59	146.13	15	205	56.04
4	125	140.28	16	110	52.57
5	84	135.97	17	163	51.82
6	134	127.13	18	106	50.04
7	111	123.46	19	138	48.15
8	125	105.88	20	119	42.34
9	118	101.08	21	134	42.20
10	134	77.62	22	128	31.50
11	110	64.43	23	94	26.72
12	154	60.22	24	82	0.00

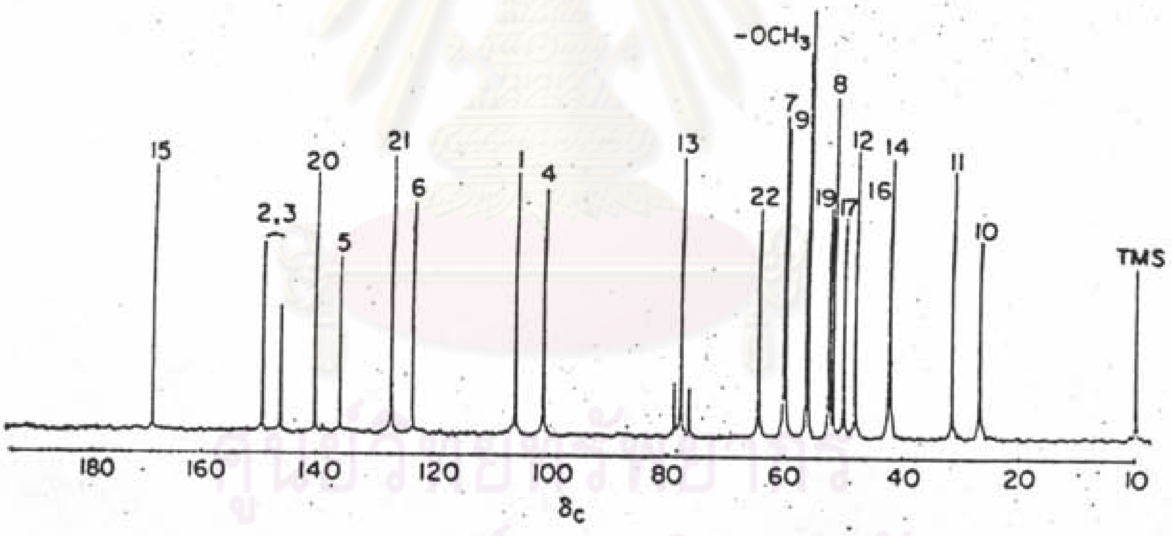


Figure.7 Spectrum of brucine record in duetero chloroform under proton noise decoupling condition

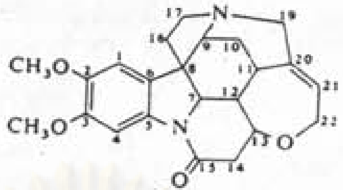
proton noise decoupling spectrum is display in Figure.8 a, and b respectively.

3. Selective proton decoupling (11)

When a specific proton is irradiated at it exact frequency at a lower power level than the one used in Off - resonance decoupling, the absorption of the directly bonded ^{13}C becomes a singlet while the other ^{13}C absorptions show residual coupling. This technique has been used for peak assignment but satisfactory results depend on finding the precise frequency of the proton and the appropriate power level for decoupling . The application of this method is demonstrated on a small molecule , 2 - furfural. Despite its simplicity , the assignment , of ^{13}C NMR, in the distinction between C-3 and C-4 is still obscure. Selective decoupling by irradiation of proton 3 and 4 easily remove this ambiguity. As illustratd in Figure.9. This experiment clearly shows that the highest field line belongs to C-4 .

4. Long-range ^{13}C-H coupling (13)

This technique has been used for complex molecule assignment because the coupling interaction more than 2 bonds give the information that can identify the carbon atom in the different environment of the molecule. The Gated decoupling technique (14,15) can be used to



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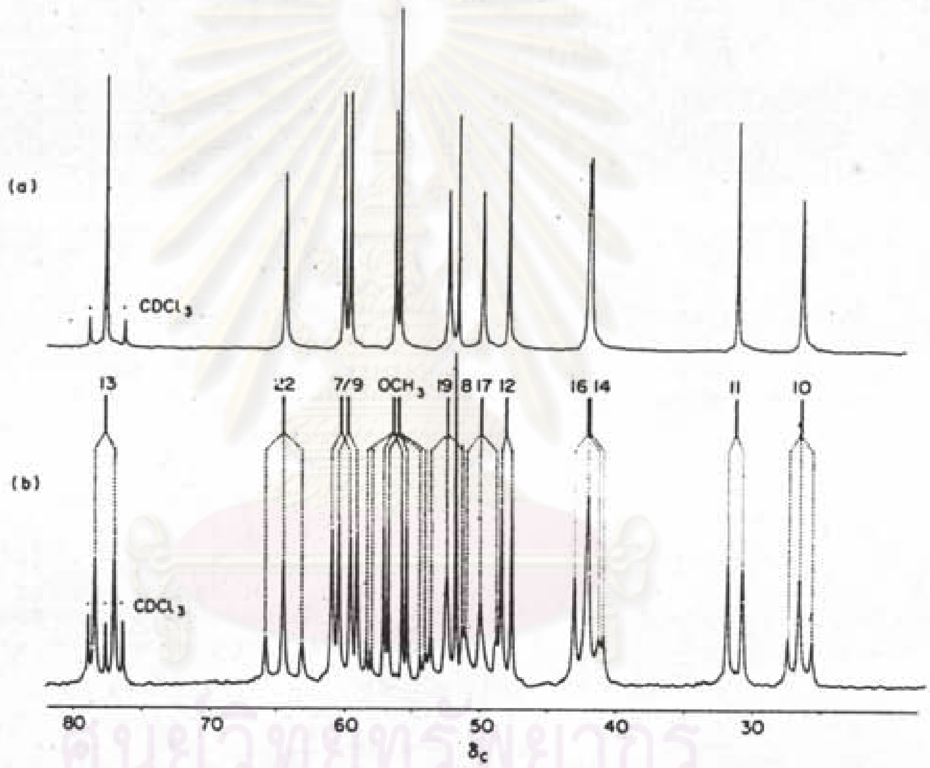


Figure.8 High field region of the spectrum of brucine (a) proton noise decoupling (b) off-resonance decoupled

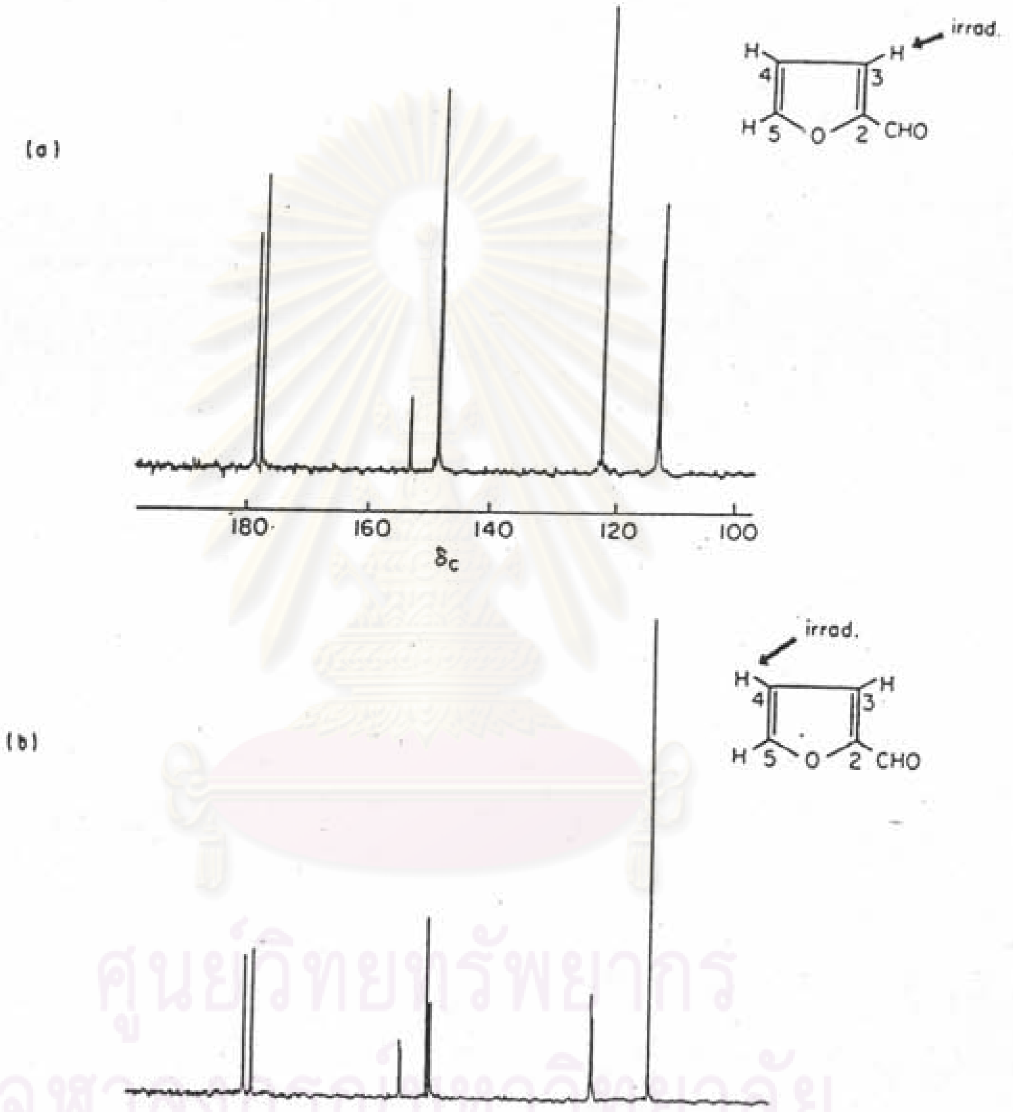
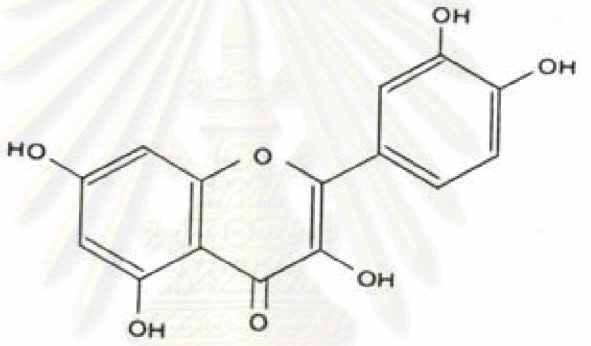


Figure.9 Single frequency selective decopling in 2-furfural
(a) irradiation at the resonance frequency of C3-H
(b) irradiation at the resonance frequency of C4-H

measure the ^{13}C-H long range coupling constant or line splitting. However, this measurement always depends on the experiment resolution and line width of the resonance signal.

Quercetin, which consists of high oxygenated aromatic ring as structure elements , may serve as an example illustrating this method. (16) (Figure.10)



Quercetin

5. Using shift reagent (12)

This technique has found wide application in proton NMR but are relatively less effective in ^{13}C NMR because carbon is shifted by the same amount as the related Proton resonance (about 5 ppm on average).So this shift are not significant for assignment in the complex molecule.

However, the most useful application of shift reagent is to expand the proton spectrum

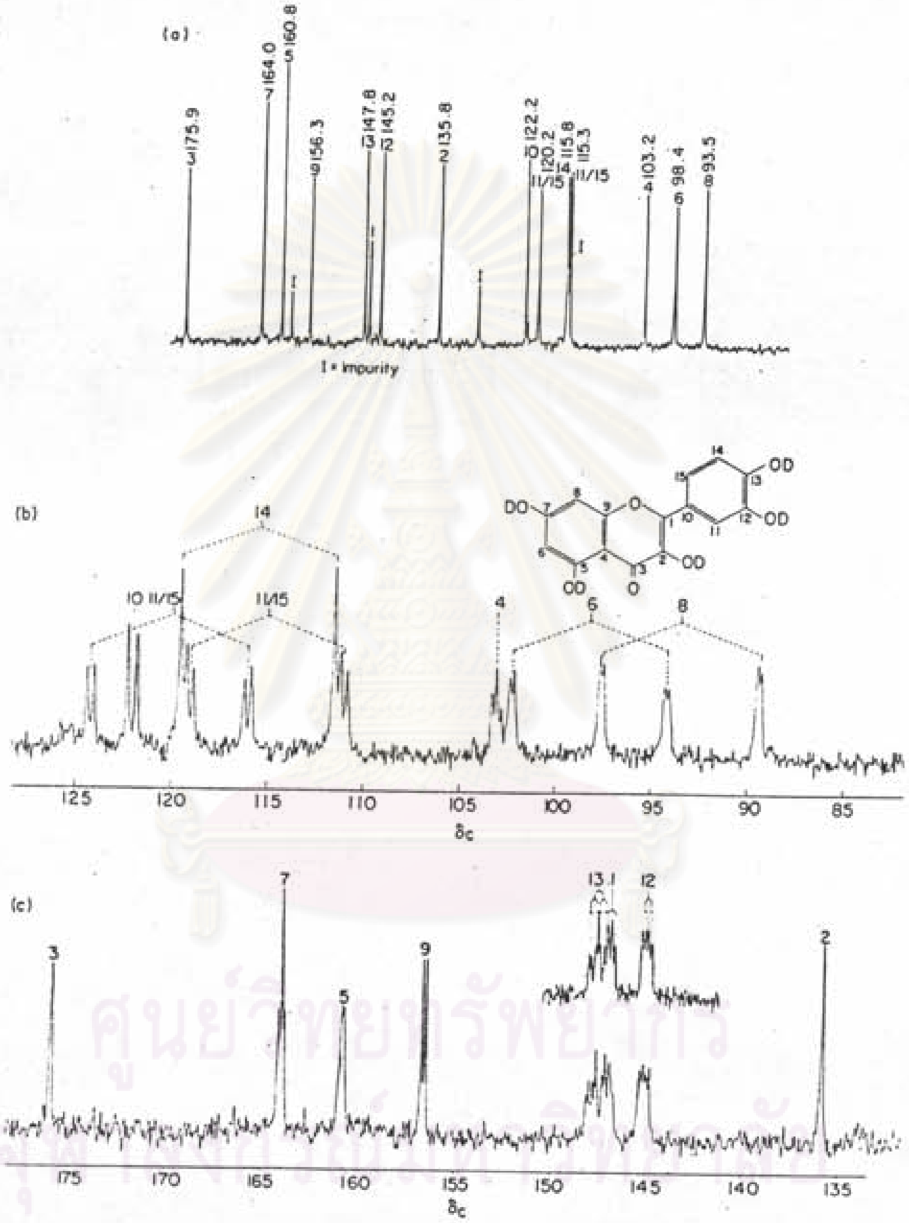


Figure.10 C - 13 NMR spectrum of quercetin (a) proton noise decoupling (b) and (c) Gated decoupling that show the long - range coupling

follow by single frequency decoupling experiment. An example is provide in Figure.11, showing the europium chemical - shift enhanced proton spectrum of geraniol along with partial decoupled ^{13}C NMR spectra obtained by selective low-power irradiation of proton C7-H (Figure.b) and C3-H (Figure.c)

The data prove that the higher field olefinic carbon belongs to C-3, because after irradiation proton C3-H, the carbon peak that bearing those proton will become to singlet.

6. Deuteration (11)

Deuteration of a carbon result in decreasing ^{13}C signal in proton noise decoupling spectrum, because the relaxation time (T_1) of ^{13}C-D is longer than ^{13}C-H due to decreasing dipole - dipole relaxation and the dueteration give no nuclear overhauser effect.

The Isotope effect may also slightly shift the absorption of the carbon chemical up field to 0.25 ppm.

The example of dueteration is ^{13}C studies of steroid by Robert and co-workers (17) that used deuterium labelling in order to sort out carbon resonance in keto steroids, 3,17 - diketoandrostanone and 2,2,4,4,16,16,- hexaduetero derivative. (figure.12)

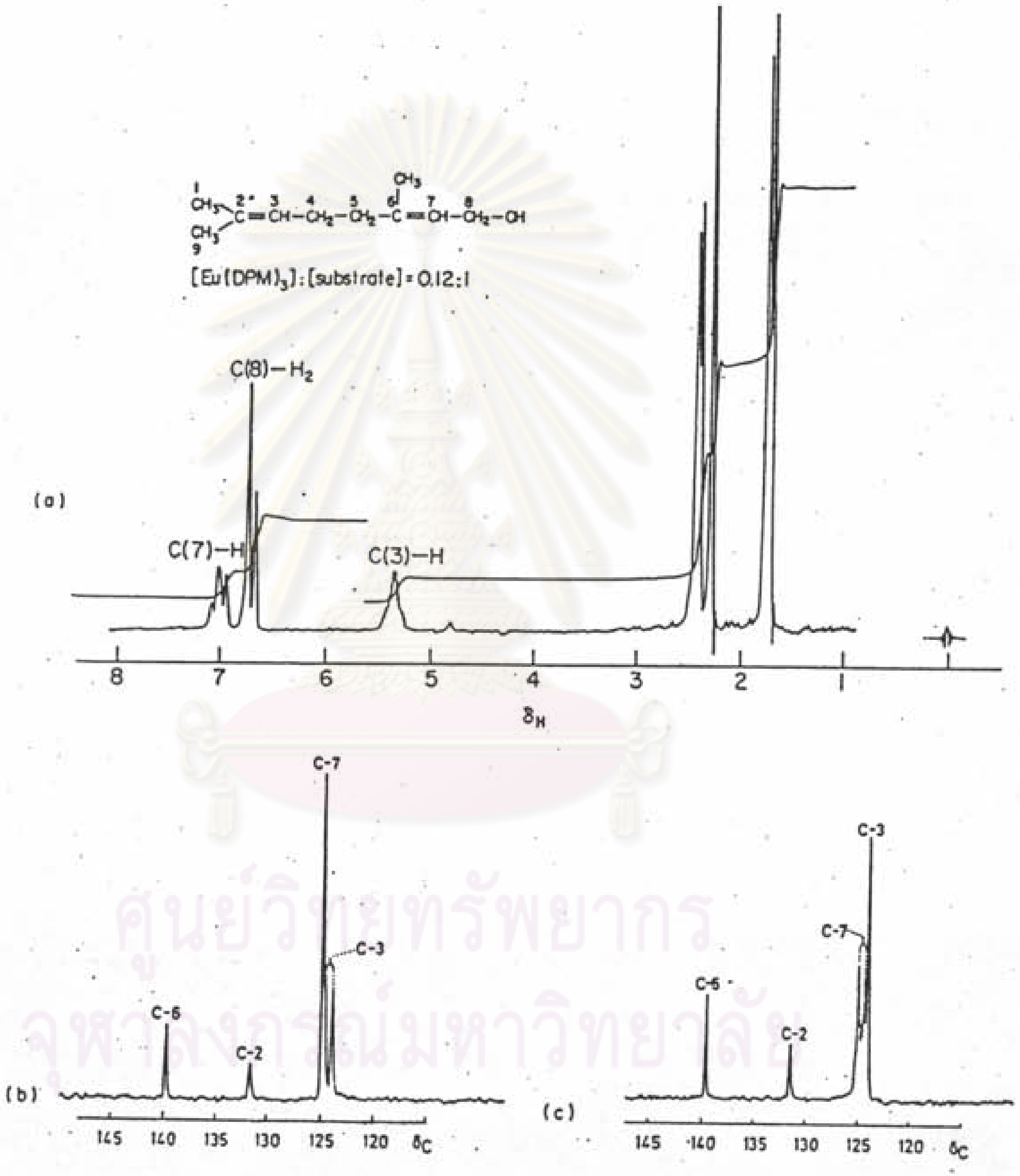
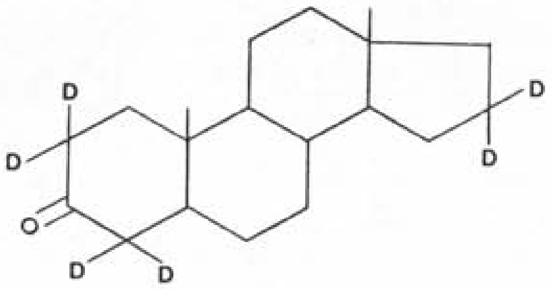


Figure.11 (a) Europium chemical shift enhanced 90 MHz proton spectrum of geraniol (b) olefinic region of the 25.2 MHz ^{13}C NMR spectrum obtained by selective irradiation of proton C7-H (c) same as (b) but with irradiation of C3-H

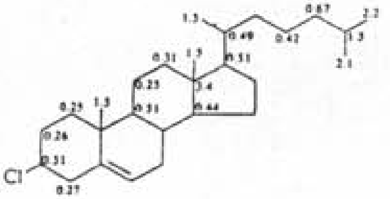


2,2,4,4,16,16,- hexaduetero 3,7 - diketo -
- androstanone

7. Relaxation time (14)

In ^{13}C NMR the quaternary carbon are the most difficult ones to assign especially in the large molecules because of spectral complexcity.

In complex molecule it is possible for individual group to internally reoreint themselve more rapidly than the reoreintation of the molecules as a whole; for example the C_{17} side chain and the methyl group in cholesteryl chloride reoreint more rapidly than overall molecule. Fast oreintation leads to long relaxation times. The intensity of signal is different from another atoms that have slow oreintation.



Cholesteryl chloride

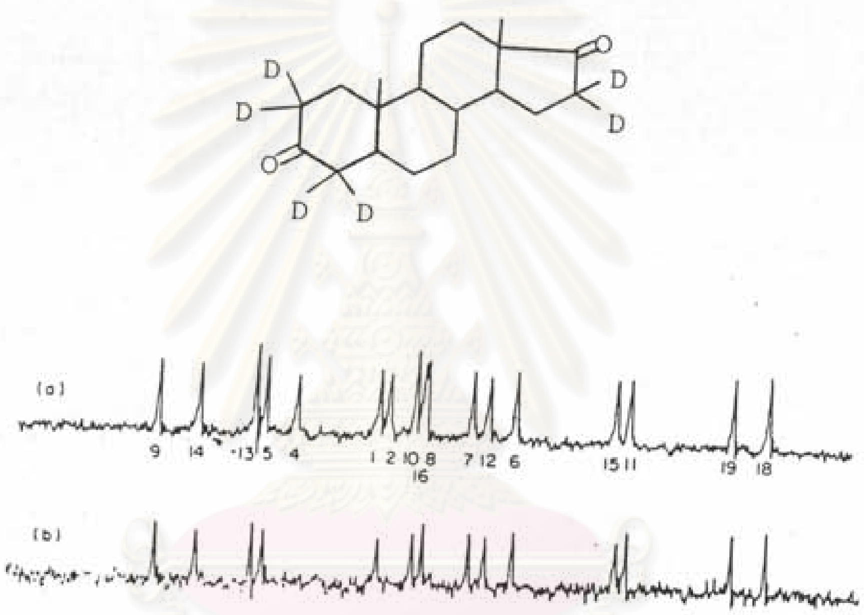


Figure.12 (a) Proton noise decoupling spectrum of androstane-3,17-dione

(b) spectrum of 2,2,4,4,16,16 - hexadeutero androstane -3,17 dione

8. Graphical method (11)

In complex spectra the interpretation of Off - resonance spectra by using residual splitting is difficult because of line overlaping. The graphical method has been proposed to solve this problem. The method use a series of off resonance experiment which the decoupler frequency is increment The carbon peak frequencies is plotted against the proton irradiating frequency.

Example of this method is shown in the Figure.13 The peak frequencies from four off-resonance obtained by varying the decoupler frequency of 1 kHz were plotted as a junction of decoupler frequency. The intersection of the line in the graph are the chemical shift of each carbon:

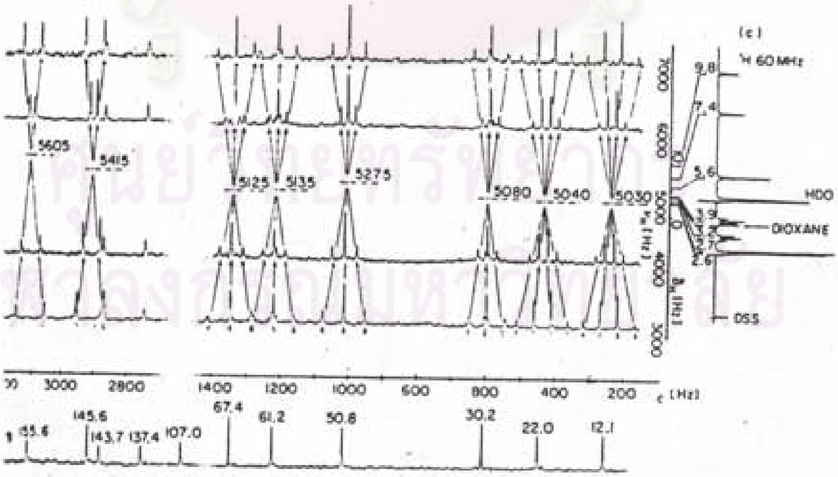


Figure.13 Graphical correlation of Off - resonance decoupled ^{13}C NMR spectra of thiamine hydrochroride and determine of coresponding ^{1}H chemical shift.

1.3 CARBON - 13 NMR OF COUMARINS.

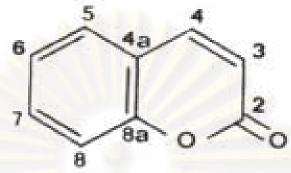
After the development of ^{13}C NMR with pulse Fourier - Transform method and used computer as the sophisticated data acquisition / processing equipment, the Fourier - Transform carbon - 13 NMR has become a sensitive and powerful tool in the structure elucidation of natural product, chemical conformation and other applied field in medical or biological system.

We have found that using ^{13}C NMR as a tool for structure elucidation is increasing very rapidly and become a routine technique. A wide variety of structural and conformation of organic compounds have been studied , most of them have already been studied by other technique eg. proton NMR, infrared spectroscopy, Mass spectroscopy etc.Thus, ^{13}C NMR analysis will reconfirm the structure again. At the present time , the ^{13}C NMR data on coumarin in the literatures is still scarce. However, this investigated results would provide important reference material for the future structure analyses and will be essential in biosynthetic labelling studies .

In general, assignment of carbon's chemical shift in coumarin are base on model studies which compare it with similar structure, Because complex structure cannot be clearly assign by direct comparison, the suitable instrumental techniques are required eg. single freguency decoupling, Long range coupling, deuterium labelling, shift reagent technique.

1.3.1 Spectral assignment

The chemical shift of carbon on coumarin I have been previously assigned (18-20) (Figure.14)



coumarin I

The first position can easily be asssign to C-2; which is a unique quaternary carbon (160.0 ppm.) The assignment is compare to carbonyl carbon of lactone with their characteristic chemical shift, reported in the literature (10,21) .

In proton noise - decoupling experiment, C-2 has low intensity of signal by the loss of Nuclear overhavser enhancement and signal still singlet when the Off resonance experiment is performed (22). The gated decoupling experiment show the absence of a large one-bond C-H conpling but retain the long range coupling information (23). Typically the $^3J(C_2H_4)$ coupling is 11.5 Hz and is much larger than $^2J(C_2H_3)$ interaction (4.5Hz) This relative order of magnitude is similar to that observed for "Long-range" aromatic C-H coupling (24).

Carbon in position C-3 and position C-4 can be assigned by compare with cinnamic acid (25).

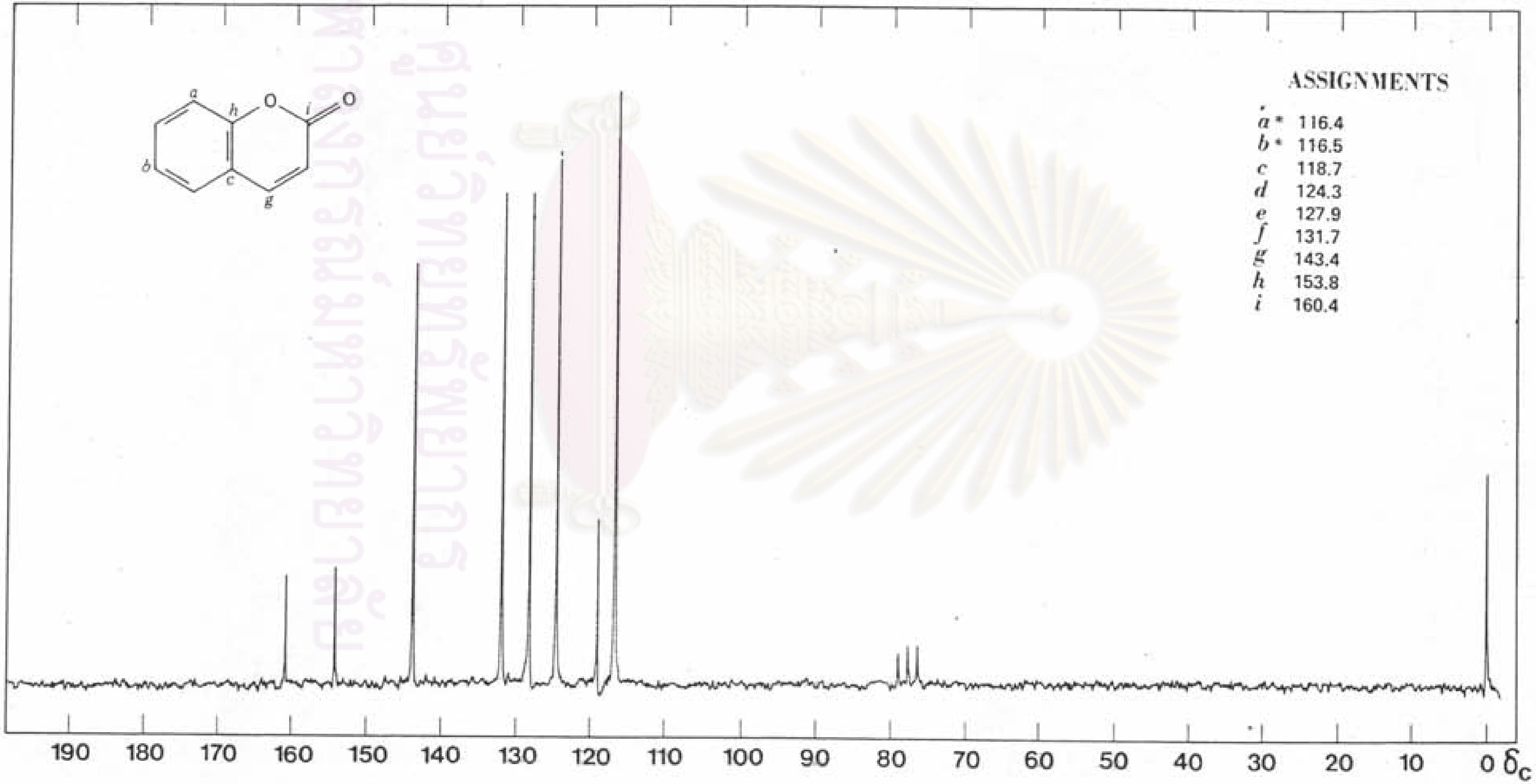
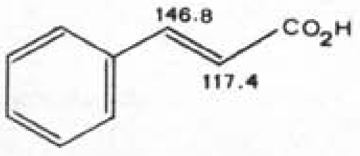
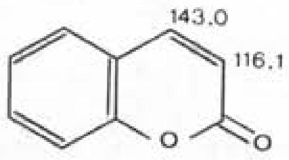


Figure.14 C - 13 NMR spectra of coumarin I



tran-cinnamic acid



Coumarin I

By using the proton coupled experiment (9), $^2J(C_3H_4)$ splitting is observed although the reverve interaction. $^2J(C_4H_3)$ is not present at measurable magnitude. The interring coupling are normally found. Coupling constant of $^3J(C_4H_5)$ is about 5 Hz.Similar effcet also found from C_5 to H_4

The technique that confirm the position of signal of C-3 and C-4 is the single frequency Off - resonance decoupling experiment. By decoupling the proton signal and observing which signal in the ^{13}C NMR spectrum is changed, for example when we decoupling proton signal at H4,the multiplet collapse completely to a singlet at 142.8 ppm. in ^{13}C NMR spectrum. this carbon must then be C_4.

The position of C4a and C8a were first tentatively located with reference to anisole and to o - cresol (26). By using the Off - resonance technique, we can differentiate the quaternary carbons C4a and C8a from the tertiary carbons which we can clearly observe in Figure.12. The signal of C4a and C8a are singlet that means no proton bearing on those

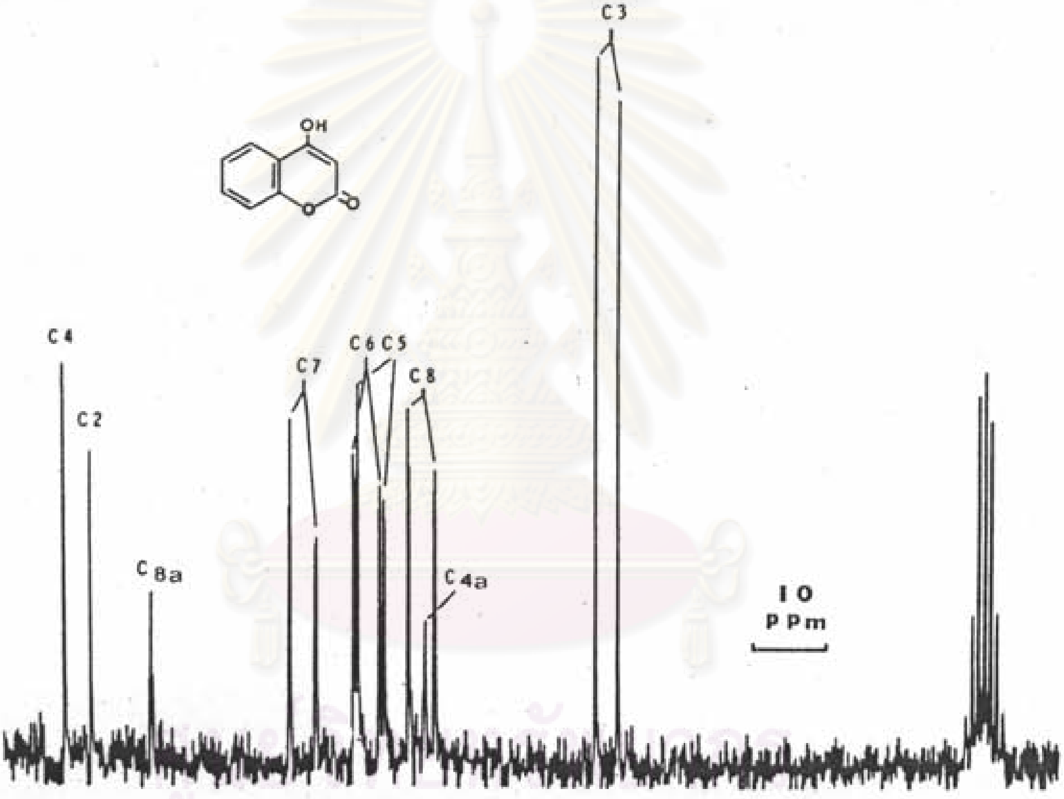
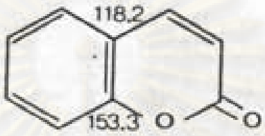


figure.15 Off-resonance spectrum of 4-hydroxycoumarin in DMSO-d

carbon atom and C4a, C8a show a small coupling due to vicinal coupling with proton on the benzenoid ring (27), Since C-8a is attached by oxygen atom, the chemical shift of C-8a will be more downfield than C-4a about 23 ppm. (9) in benzene system and the influence of a vinyl gronp is much smaller. (21)



Coumarin I

We can clearly observe the Long - range coupling through 3 bond in 3,6,8 - tribromocoumarin that show three doublets couplings of C4a with H4, H5 and H7 (the coupling constant are 0 Hz, 9 Hz and 7 Hz respectively) for C8a, $^3J(C_{8a}H_4)$ is about 10 Hz.Cussan and Huckerby (24) explained the usefulness of bromine in this work . The effect of bromine on the chemical shift of aromatic carbon atoms is quite small even at the site of substitution, Thus the effect of such substitution should cause a small perturbation in the carbon resonance position while leaving a signal which may be readily recognised by the loss of the nuclear Overhauser enhancement in the conventional proton noise decoupled spectrum.

The assignment of C-5, C-6, C-7 and C-8 can not be assigned by direct comparison with anisole and o-cresol . The method which Gunther et al.(28)

used to differentiation the chemical shift of four contiguous methine within an aromatic ring can be used to verify the shift assignment of coumarin, This method used a fully proton coupled spectrum that show the signal of four methines carbons C-5, C-6, C-7 and C-8 The group of signals can separated in two set of shapes , the outer methines in the form of broad diffuse doublets and the inner methines as sharply doublet of doublet. This method is called "Fingerprints" method which can distinquish C-6 and C-7 from the other methins.

The example of the fingerprints method is the assignment of napthalene which contained a symmetrically ortho-dissubstituted benzene ring. We used the undecoupled spectra of these compound.

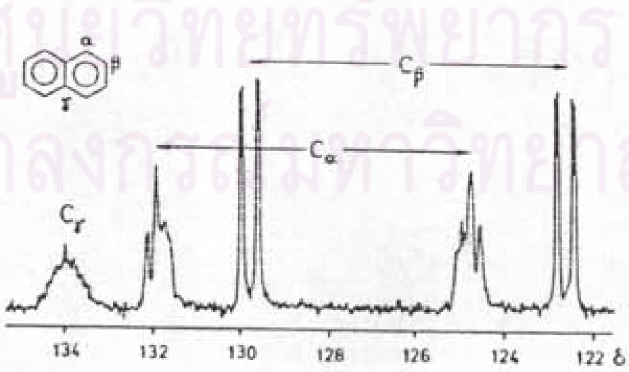
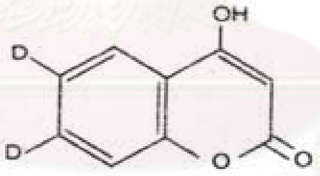


Figure.13 The Fingerprints spectrum of Naphthaleen.

The multiplet structure obtained from the resonance of these two paires of ^{13}C nuclei (α and β carbon) differed characteristically as demonstrate in Figure.16

In coumarin I the four remaining carbon can be assign as α (C-5,C-8), β (C-6,C-7) by Off-resonance decoupling experiment which produced the band shapes of their characteristic substitution position. Thus the signal at 127.4 ppm.and 115.8 ppm.are classified as while the remaining signal at 123.8 ppm. and 131.1 ppm. are assigned to the β

Chan et al. (14) use the dueteration to assignment C-6, C-7 in 4-hydroxy coumarin by partially deuterated to obtain 6,7 - dideuterio - 4 - hydroxycoumarin.



6,7 - diduetero - 4 - hydroxycoumarin

The ^{13}C NMR spectrum of 6,7 - didueterio 4 - hydroxy coumarin can clearly observe the decreasing in intensities of the signal C-6 and C-7 because of dueterium - carbon couplings and the nuclear overhauser effect. C-6 and C-7 are differentiated on the basis of the additive effect of methyl substitution in o-cresol (26)

Cussan and Huckerby (23) used Long-range coupling technique for assignment of the substiuent effect of methyl group on coumarin as show in Table.1

All methyl derivetives show signficant incrcases in chemical shift of carbon to about 10 ppm (+8.6 to +11.8 ppm) show a quartet coupling constant $^{2}J(C-CH_3)$ of 5-6 Hz, a quartet spitting $^{3}J(C-C-CH_3)$ about 5 Hz also being produced at the carbon ortho to the site of substitution.

The effect of the methyl substitution on perturbation of ortho carbon chemical shift in benzenoid ring are - 0.4 to + 1.7 ppm. For meta carbon are - 0.5 to + 0.7 ppm., For para carbon are consistent upfield movement about 2 ppm.

Form Table 1 a comparison is made between the substituent chemical shift value predicted for 6,8-dimethyl coumarin by a combination of data for the 6- and 8- derivatives. No value shows a discrepancy of greather than 0.25 ppm. From this basis we predict value of 5 - Me group by subtract from 5,8 - dimethyl coumarin data.

The effect of hydroxy substituent on coumarin are summarised in Table 2 (29). The effect of an OH group when present in the carbocyclic ring is quite characteristic, the newly formed quaternary carbon move approximately 30 ppm.down field from the value observed in coumarin itself. Carbons that ortho and para to the substituent both move upfield by 11-15 and 7-10 ppm. respectively, while the meta carbons have little affect

	$3\text{-}CH_3$[a]	$4\text{-}CH_3$	$6\text{-}CH_3$	$7\text{-}CH_3$	$8\text{-}CH_3$	Predicted 6,8-di-CH_3 (b)	Observed 6,8-di-CH_3	Observed 5,8-di-CH_3	Deduced $5\text{-}CH_3$
C_2	+1.8	-0.4	+0.1	+1.0	+1.0	+0.2	0.0	-0.1	-0.2
C_3	+9.65	-1.85	-0.35	-0.55	-0.45	-0.8	-0.85	-1.35	-0.9
C_4	-6.0	+8.6	-0.2	+0.3	+0.3	+0.1	0.0	-3.0	-3.2
C_5	-7.0	-3.5	-4.0	0.0	-2.4	-2.8	-2.8	-5.4	+7.8
C_6	+0.15	-0.4	+9.45	+1.6	-0.5	+8.95	+8.8	+0.6	+1.1
C_7	-1.0	-0.3	+0.8	+11.8	+1.3	+2.1	+2.2	+0.9	-0.4
C_8	+0.15	-0.05	-0.35	+0.95	+9.95	+9.0	+8.75	+6.95	-2.4
C_9	+0.3	+0.7	-2.0	-0.7	-1.7	-3.7	-3.7	-1.7	+0.4
C_{10}	+1.1	+0.8	-0.5	-1.9	-0.4	-0.9	-0.8	-1.8	-1.4

(a) Substituent Chemical shifts are shown as changes in chemical shifts, expressed in p.p.m between Coumarin (in $CDCl_3$ solvent) and the methyl derivative, also in $CDCl_3$.

(b) Values derived from the data for the 6- and 8-methyl derivatives.

Table 1. Substituent chemical shift value for the methoxycoumarins

	3-Hydroxy Coumarin	4-Hydroxy Coumarin	6-Hydroxy Coumarin	7-Hydroxy Coumarin	8-Hydroxy Coumarin
C_2	-1.1 [a]	+2.6	+0.5	+1.1	+0.4
C_3	+25.85	-24.5	+0.2	-4.4	+0.2
C_4	-28.7	+22.1	+0.1	+0.6	+08
C_5	-1.8	-4.8	-15.7	+1.45	-9.7
C_6	+0.4	-0.4	+29.6	-10.7	+0.3
C_7	+4.0	+0.9	-11.8	+30.1	-13.1
C_8	-0.3	+0.4	+1.0	-13.4	+28.8
C_9	-4.2	+0.4	-7.65	+2.3	-11.0
C_{10}	+2.2	-2.4	+0.6	-7.0	+1.2

[a] All values are expressed as the difference in chemical shift (in ppm) between the derivative and coumarin, both values being from measurements in DMSO.

Table 2. Substituent chemical shift value for the hydroxycoumarins

but move consistently downfield by 0.3 - 2.3 ppm.

Replacement of H by OH also introduces characteristic changes in coupling patterns within the benzenoid ring. The one bond C-H coupling for a carbon ortho to the substituent drops by 2-3 Hz and meta coupling across an oxygen bonded carbon also drop by this amount, on the contrary, meta couplings involving the substituented carbon increase by 2-3 Hz.

Table 3. summarises the substituent effect for 4 - and 7- methoxycoumarin. The pattern is similar to the hydroxy derivative.

With 4 - methoxycoumarin the largest changes produced by substituting the OH proton is - 1.4 ppm. (at C-3) and all the other shifts are static or move slightly upward except C-6 and C-7.

With 7-methoxycoumarin the only significant difference compared with the hydroxy derivative are at C-7 (slightly down field shift) and the ortho sites C-6 and C-8 (upfield shift of 1-2 ppm). The carbon to which the methoxy group attached is clearly,identify by the presence of a 4 Hz quartet splitting on proton - coupling spectra.

From the data that present above, we can predict the chemical shift of some derivatives (methyl - hydroxy, dihydroxy and methyl - dihydroxy) by suitable combination of data with 7-hydroxy-4- methyl conmarin, The largest error in prediction is 0.6 ppm for the two

	4-Methoxy coumarin		7-Methoxy coumarin		7-Hydroxy-4-methyl coumarin	
					Observed	Predicted
C_2	+2.1 [a]	-0.5 [b]	+0.5 [a]	-0.6 [c]	+0.8 [a]	+0.8 [d]
C_3	-25.9	-1.4	-3.6	+0.8	-5.5	-6.1
C_4	+22.1	0.0	+0.1	+0.5	+9.5	+9.4
C_5	-5.5	-0.7	+1.0	-0.45	-1.9	-1.85
C_6	-0.2	+0.2	-12.1	-1.3	-11.2	-10.8
C_7	+1.0	+0.1	+30.9	+0.8	+29.7	+30.1
C_8	+0.4	0.0	-15.4	-2.0	-13.5	-13.2
C_9	-0.7	-1.1	+1.9	-0.4	+1.6	+1.6
C_{10}	-3.4	-1.0	-6.5	+0.5	-6.3	-6.2

a. Expressed as a chemical shift difference (in ppm) from coumarin in DMSO.

b. Expressed as a chemical shift difference (in ppm) from 4-hydroxy coumarin in DMSO.

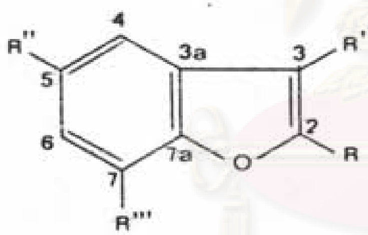
c. Expressed as a chemical shift difference (in ppm) form 7-hydroxy coumarin in DMSO.

d From a combination of SCS parameters for 4-methyl coumarin (in DMSO) and 7-hydroxy coumaris.

Table 3. Substituent chemical shift value for methoxy and methylhydroxy - coumarins

6,7 - dihydroxy derivative the worst discrepancy is 1.0 ppm and for the two 7,8 - dihydroxy compounds the poorest estimate is 1.5 ppm off.

The assignment of furanocoumarin ^{13}C NMR is base on the benzofuran structure (A) (25).The chemical shift analysis depends on comparision with Indole skeleton (26), and many derivative such as benzofuran - 2 - acetic acid (B) benzofuran - 3 - acetic acid (C), 5 - methylbenzofuran (D),and 7 - methylbenzofuran (E) were used as model for interpretation using fully proton - coupled spectrum.



(A) R = R'= R''= R'''= H

(B) R =CH_3COOH,R'= R''= R'''= H

(C) R = R''= R'''= H,
R'= CH_3COOH

(D) R = R'= R'''= H,R''= Me

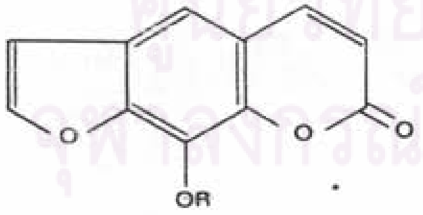
(E) R = R'= R''= H,R'''= Me

Benzofuran (A) carbons in position 3a and 7a are unique, because of lacking the nuclear overhauser enhancement in proton noise decoupled technique. C-7a is attached by oxygen atom , so the signal will shift downfield than C-3a.

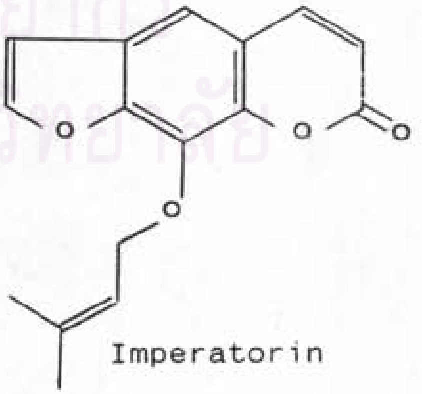
C-2 and C-3 are expectedly the lowest and highest field methines respectively. The finger-printing method (28) differentiates C-4 and C-7 from C-5 and C-6 and the high - field position of C-7 indicates it to be associated with a carbon ortho to an oxy substituted carbon. This point is confirm by C-4 experiencing mild shielding on introduction of γ effect due to side chain in benzofuran - 3 - acetic acid (C).

In 7 - methylbenzofuran (E) structure, methyl substitution on C-7 can differentiate C-5 from C-6 of benzofuran (A) because its change the C-5 coupling pattern by remove the important three bond coupling with C-7.

Form the chemical shift data of coumarin and benzofuran, it can possible to assess the furano coumarins eg. Xanthotoxol Xanthotoxin, and imperatorin. The shift of the tricyclic skeleton are shown in Table 4.



Xanthotoxol R=H
Xanthotoxin R=Me



Imperatorin

The other technique was proposed by Bose et al.(22) which use Titanium tetrachloride and trichloro

	Chemical shift (PPM)		
	Xanthotoxol	Xanthotoxin	Imperatorin
C-2	160.7	160.4	160.1
C-3	114.2	114.5	114.2
C-4	145.8	144.2	144.0
C-4a	116.8	116.3	116.0
C-5	110.5	112.9	112.9
C-6	125.9	126.0	125.6
C-7	145.8	145.0	148.1
C-8a	140.3	139.2	139.2
C-8	130.7	126.0	125.6
C-2'	147.7	146.6	146.2
C-3'	107.5	106.6	106.4

Table 4. C - 13 NMR data of furanocoumarins

(a) Xanthotoxol (b) Xanthotoxin (c) Imperatorin acetylisocyanate (TAI) as shift reagent for ^{13}C NMR studies.

This technique employ running the spectrum in the normal experiment, then adding shift reagent into the sample and running again. The results are the position on carbon chemical shift will move upfield or downfield a little from the normal spectrum.

The small amount of chemical shift change in various carbon are use to compare with other structure of furanocoumarin which the chemical shift have been assigned. This method is easy in assignment because they have the same trend in the different change in chemical shift.

Figure.17 show carbon chemical shift of furanocoumarin.In the spectrum of a conjugated ketone, strong downfield shift are produced by $TiCl_4$ for the carbonyl and B- carbons , while the carbon undergoes a small upfield shift. This could be explained by the dipolar canonical form (I). After add $TiCl_4$, a change in distribution of electron density occure to a great extent at the carbon (31-32).

$$-C=C-C=O \longrightarrow -\overset{+}{C}-C=C-\overset{-}{O}$$

I

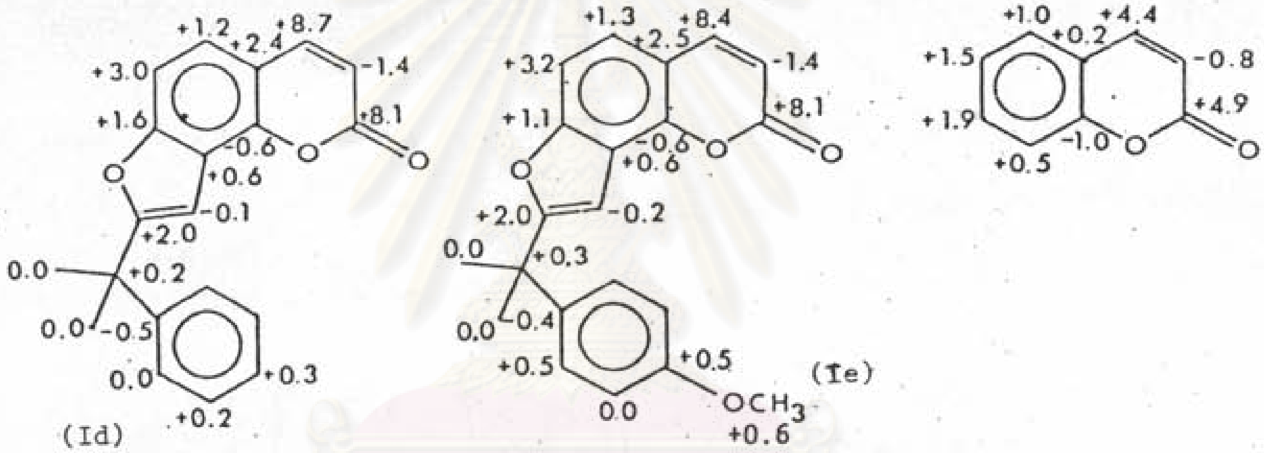


Figure.17 $TiCl_4$ induce shift in the ^{13}C NMR spectra of (Id), (Ie) and coumarin

1.4 Classification of coumarin

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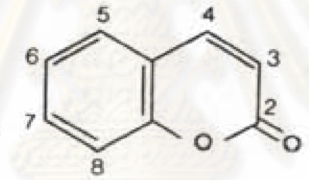
2532 Cu Thesis

Coumarin is a large group of natural occuring compound possessing a 2 H - 1 - benzopyran - 2 - one nucleus.(33) Coumarin was first isolated from seed of Coumarona ordorata Aubl. by Vogel in 1820 . Untill now , about 300 coumarins have been isolated from many source of plant, especially from families Leguminosae , Umbelliferae , Guttiferae , Rutaceae , labiatae , Gramineae, Orchidaceae.(34) They have also been report from micro organisms (35) and animals (36).



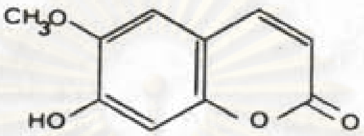
Coumarin

(2H - 1 - benzopyran -2 - one nucleus)

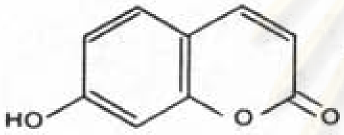
Untill now , no completely satisfactory classification of the coumarins is possible. Steck and Mazureck (37) had devided coumarins into two type. one is "normal" type , Which has an oxygen function at C-7 and hydrogen at C-3 and C-4 (unsaturated pyrone ring).The other is "abnormal type" ,Which either lacks of the C-7 oxygen or possesses pyrone ring substituents. Seshadri and vishwapaul (38) had classified coumarins in to five group as following.

1.Simple coumarin

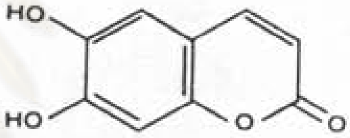
This type of coumarins have hydroxy group and / or methoxy group substituent on benzene ring of the benzopyran nuclues eg. Scopoletin , Umbelliferone , Aesculetin.



Scopoletin



Umbelliferone

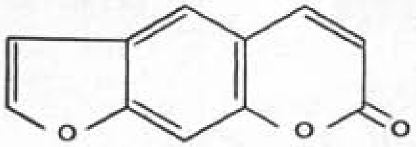


Aesculetin

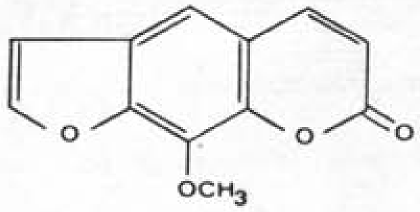
2.Furanocoumarin

This type of coumarins has furan ring fused with the coumarin nucleus at the various position on benzene ring to form linear or angular structure and the furan rings does not carry any substituents . Furanocoumarin had been classified into six subtypes.

2.1 Psorarene type (linear)

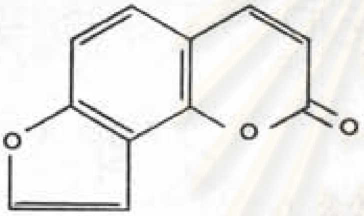


Psolaren

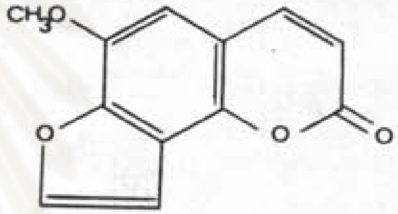


Xanthotoxin

2.2 Angelicin type (angular)

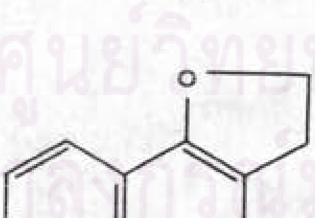


Angelicin

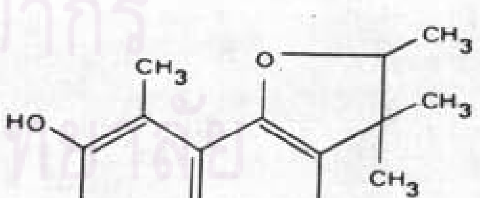


Sphondin

2.3 Dihydrofuranocoumarin (4,3) type

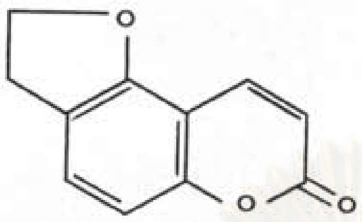


Dihydrofuranocoumarin (4,3) nucleus

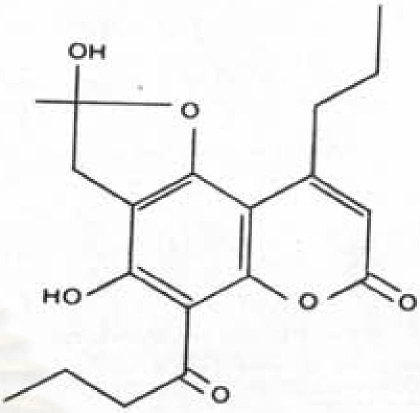


Glaupalol

2.4 Dihydrofuranocoumarin (5,6) type

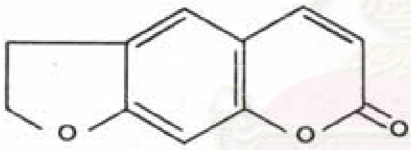


Dihydrofuranocoumarin (5,6) nucleus

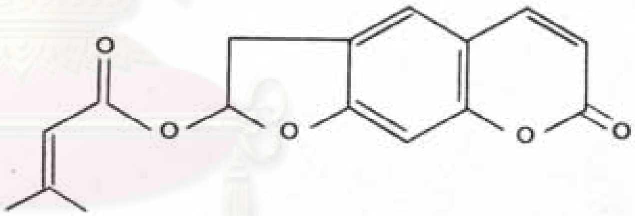


Furomammea A

2.5 Dihydrofuranocoumarin (7,6) type

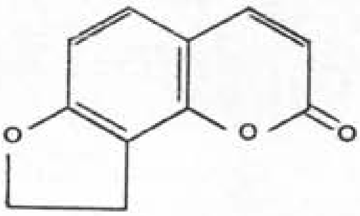


Dihydrofuranocoumarin (7,6) nucleus

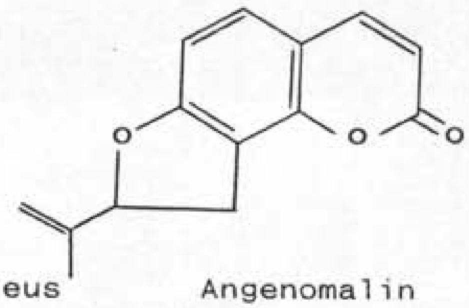


Prantschimgin

2.6 Dihydrofuranocoumarin (7,8) type



Dihydrofuranocoumarin (7,8) nucleus

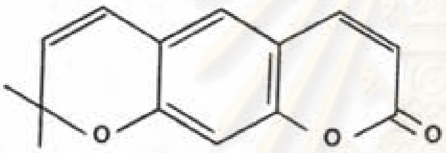


Angenomalin

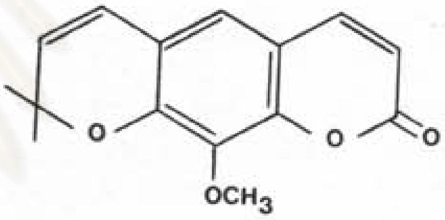
3. Pyranocoumarins

This type of coumarins has pyran ring fused to the benzene ring at various position. These coumarins may be called chromano - coumarins. Consider the fusion of pyran ring , pyranocoumarins may be cliassified into four subtypes. (38)

3.1 Xanthyletin type



Xanthyletin nucleus



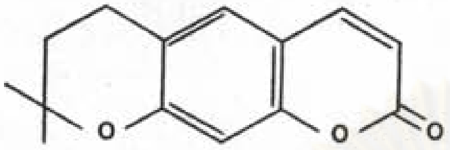
Luvangetin

3.2 Seselin type

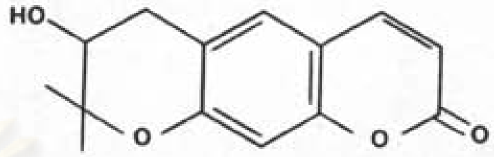
Seselin nucleus

Braylin

3.3 Dihydroxanthyletin type

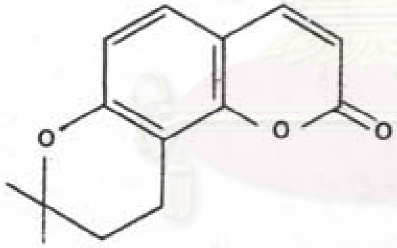


Dihydroxanthyletin nucleus

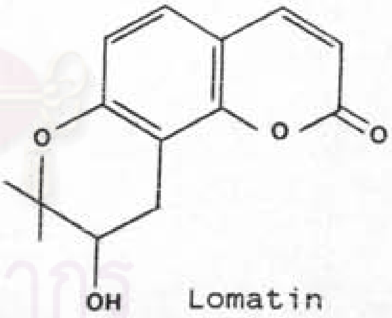


Decursinol

3.4 Dihydroseselin type



Dihydroseslin nucleus

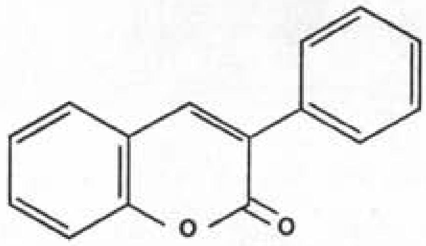


Lomatin

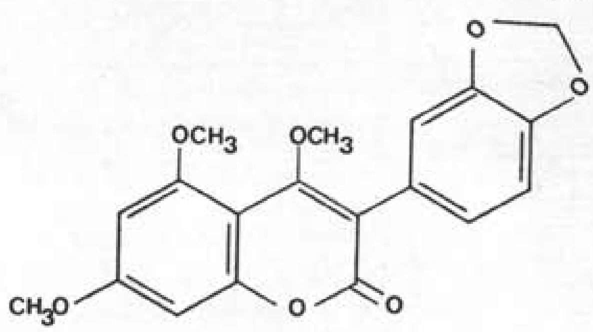
4. Phenylcoumarins

This type coumarin has phenyl ring substitute at C-3 or C-4 of coumarin nucleus. Phenylcoumarins can be classified into seven subtypes

4.1 3 - phenylcoumarin type

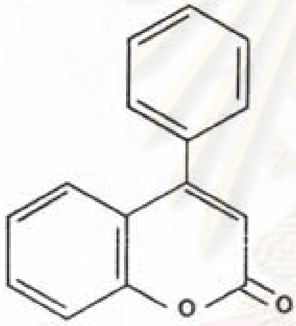


3 - phenylcoumarin nucleus

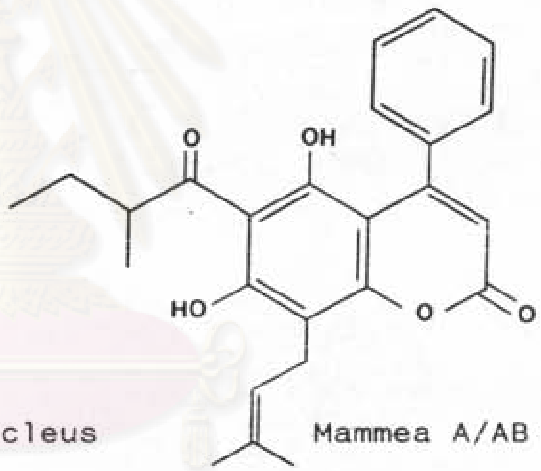


Derrussnin

4.2 4 - phenylcoumarin type

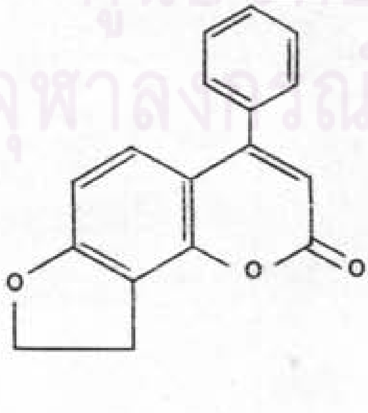


4 - phenylcoumarin nucleus

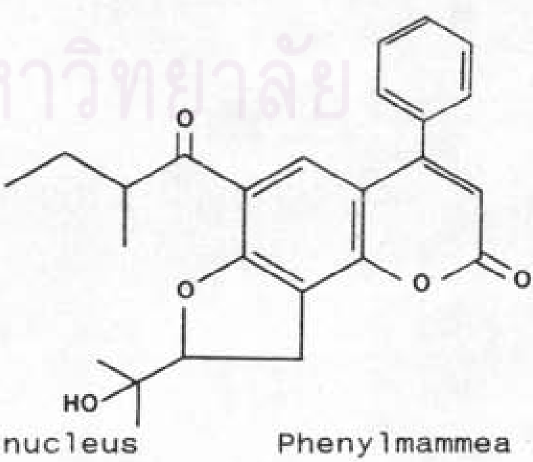


Mammea A/AB

4.3 4 - phenyldihydroangelicin type

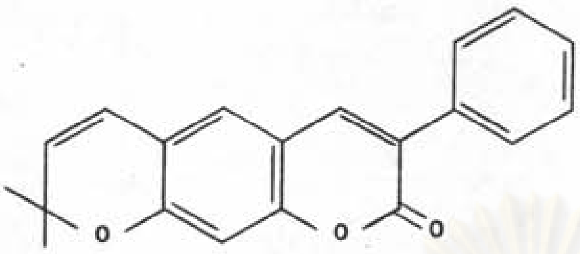


4 - phenyldihydroangelicin nucleus

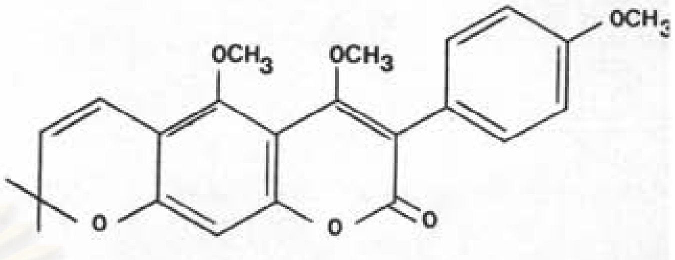


Phenylmammea A

4.4 3 - phenylxanthyletin type

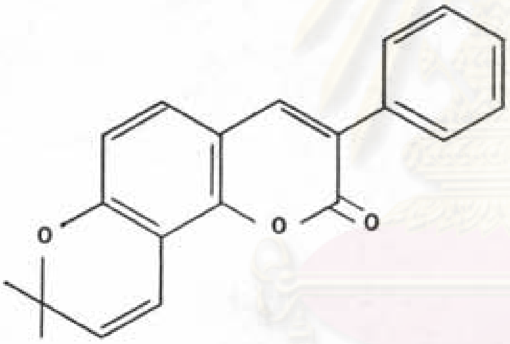


3 - phenylxanthyletin nucleus

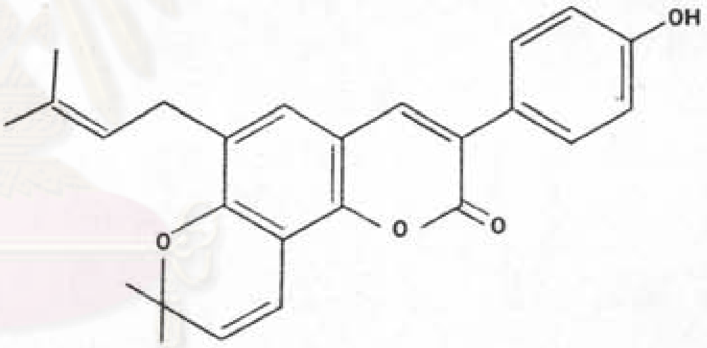


Robustic acid

4.5 3 - phenylseselin type

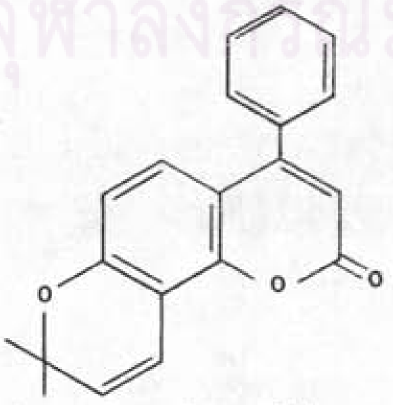


3 - phenylseselin nucleus

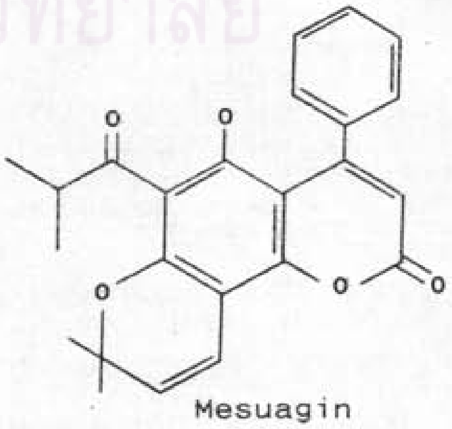


Scandanin

4.6 4 - phenylseselin type

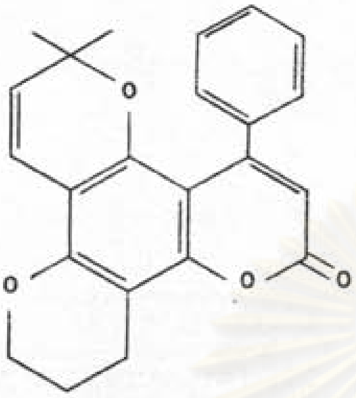


4 - phenylseselin nucleus

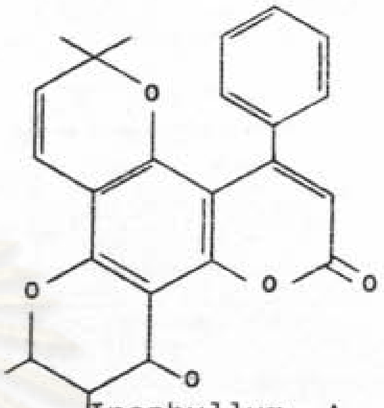


Mesuagin

4.7 Tripyran derivative type



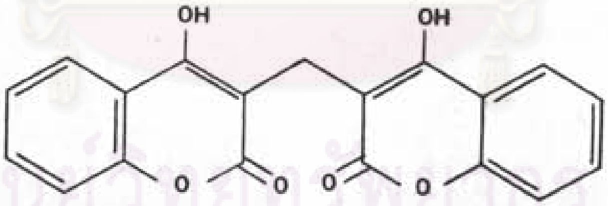
Tripyran nucleus



Inophyllum A

5. Bicoumarins

This type of coumarin consist of two coumarin nucleus bonded with or without methylene group



Dicoumarol