

Application of Carbon-13 NMR Spectroscopy to the Chemistry of Natural Products

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Carbon-13 NMR spectroscopy (abbreviated CMR) is an extremely powerful strategy for the study of natural organic molecules. The information derivable from CMR is often complementary to that obtained from proton NMR spectroscopy (PMR). Because of low natural abundance of ^{13}C nucleus (1.1%) coupled with low inherent sensitivity relative to ^1H (about 1/64), CMR experiment is approximately 6000 times less sensitive than PMR. Despite of this, now it is possible to measure CMR of small amount of compound by the development of three significant

ingenious techniques, i.e. a) computer time-averaging, b) wide-band (or noise modulated) proton decoupling, and c) pulsed Fourier transform (FT) NMR: For general recognition of CMR, its fundamental aspects of CMR are briefly reviewed.

SAMPLING FOR THE MEASUREMENT OF CMR

Solvent: Choice of appropriate NMR solvent extremely important though it is considered not to be (Fig. 1).

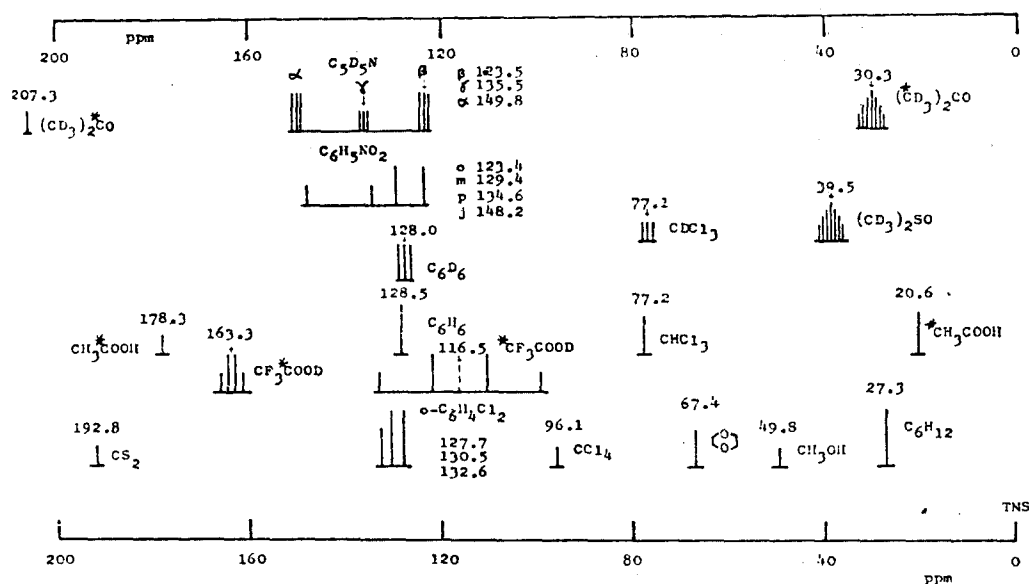


Fig. 1: Chemical shifts for common standards and solvents [Courtesy of JEOL Company]

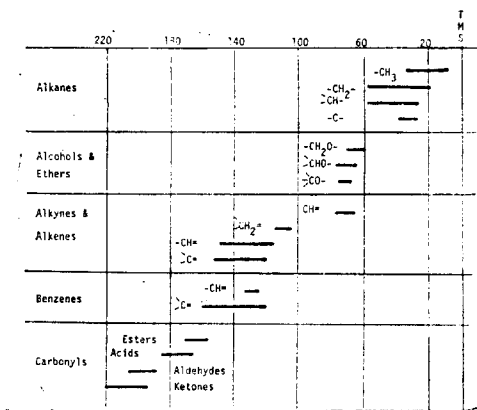


Fig. 2: ^{13}C Chemical shifts (δ)

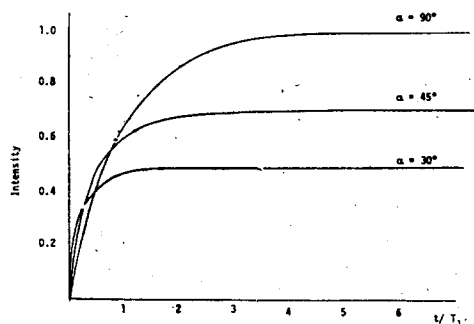


Fig. 3: Intensity of resonance as function of pulse flipping angle (α), repetition time (t), and spin lattice relaxation time (T_1)

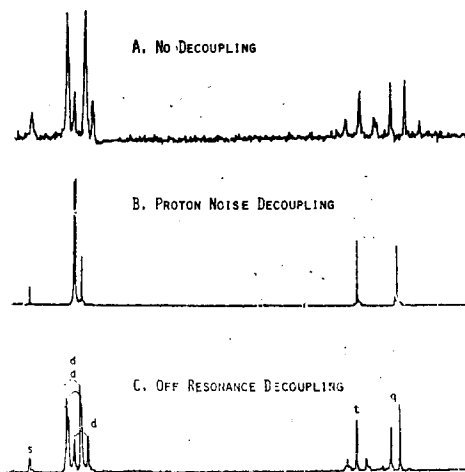


Fig. 4: CMR of 90 % ethyl benzene^o

Reference (Standard): TMS, CS,
Dioxane... (Fig. 1)

Sample Tube: Mainly decided by instrument.

MEASUREMENT OF CMR

Resolution (Accuracy of horizontal axis):
 $R = \pm 2F/NF$ (ppm) F: Frequency range,
 N: Data points, F: Observed Frequency
Frequency Range is decided by the (anticipated) structure of the sample. Appropriate CMR chemical shifts (from TMS) of various groups are shown in Fig. 2.

Pulse Flipping Angle (α) and Repetition Time (t)

$$M = \frac{\left(1 - e^{-\frac{t}{T_1}}\right) \sin \alpha}{1 - e^{-\frac{t}{T_1}} \cos \alpha}$$

M: Magnetization (Peak intensity) T_1 : spin-Lattice Relaxation Time (see Fig. 3)

Number of Scans (n): Signal/Noise = \sqrt{n}

INFORMATION AND MEASURING MODE

Most important parameter derivable from CMR is chemical shift (δ in ppm scale from standard, usually TMS). Other two main parameters are spin-spin coupling constants (J), and spin lattice relaxation times (T_1). The latter two are not always measured, but at least qualitative estimation of the two gives favourable information for structure elucidation.

Proton Noise Decoupling (Ordinary Mode):
 All signals appear as singlets (Fig. 4-B).
 --- Chemical Shift (δ)

Off Resonance (Single Frequency) Decoupling: Residual ^1H - ^{13}C coupling patterns (multiplicities of peak) give the number of proton attached to carbon (Fig. 4-C).

No Proton Decoupling (Proton Coupled): Proton- ^{13}C coupling constants can be obtained ($J_{\text{C-H}}$, Fig. 4-A). However, lacking in NOE enhancement causes poor s/n ratio (sensitivity).

Gated Decoupling, With NOE Mode: Measurement of $J_{\text{C-H}}$ with NOE.

Gated Decoupling, Without NOE Mode: For quantitative study.

Proton Selective Decoupling: Correlation of PMR to CMR, etc.

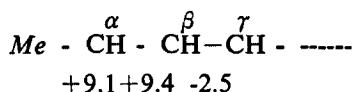
CORRELATION OF CHEMICAL SHIFTS AND CHEMICAL STRUCTURES

The main contribution to the CMR chemical shift arises from local paramagnetic term. Although much progress has been made in theoretical calculations of CMR chemical

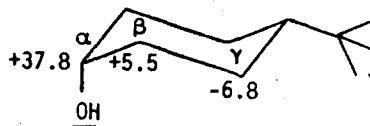
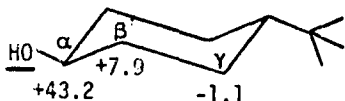
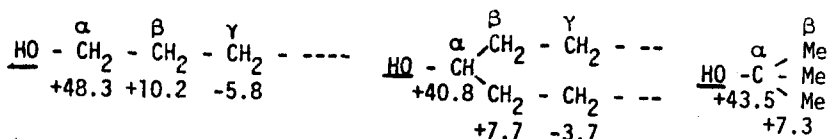
shift, practical spectrum analysis depends almost entirely on empirical relationships determined in simple model compounds. Experimentally, it has been found that the effects of substituents on CMR chemical shifts are often more or less additive. Hybridization (s-character) and electronic charge have large effects on chemical shifts (Fig.2). Calculation has been made for acyclic hydrocarbons, olefins, substituted benzenes, introduction of OH groups.¹⁾ Recently unique explanation for γ -shift and calculation method of cyclic (six membered ring) was reported.²⁾ Esterification shift³⁾, shift caused by O-alkylation⁴⁾, and glycosylation shift are extremely useful for structure elucidation. There are some examples.

Substitution Effect

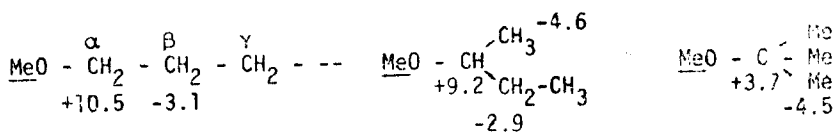
1. C-Methylation $\Delta\delta = \delta(\text{CH}_3\text{-R}) - \delta(\text{H-R})$

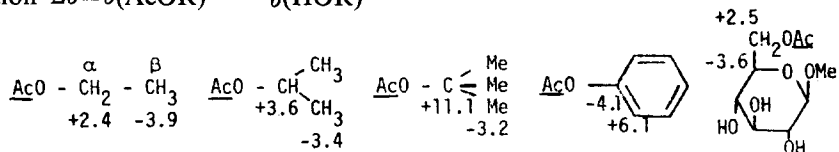
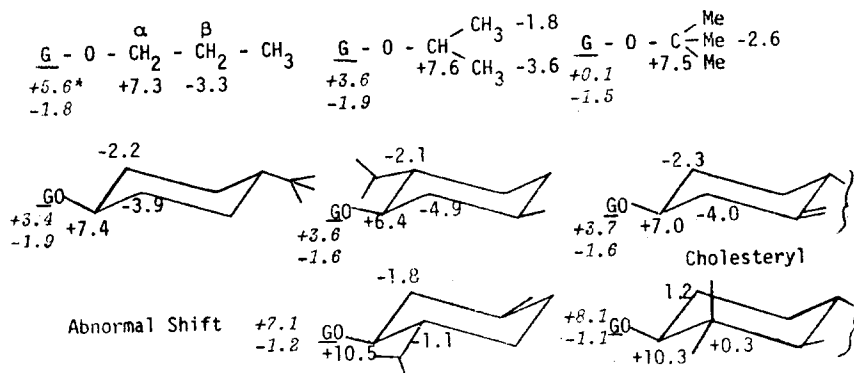


2. Hydroxylation $\Delta\delta = \delta(\text{HO-R}) - \delta(\text{H-R})$



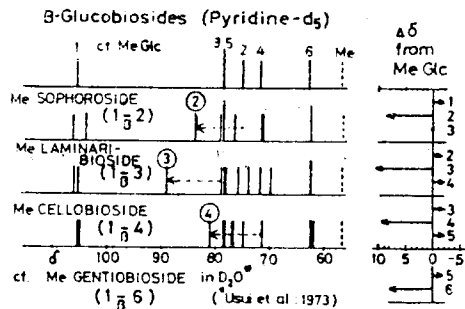
3. O-Methylation (MeO-R)



4. Acetylation $\Delta\delta = \delta(\text{AcOR}) - \delta(\text{HOR})$ 5. Glycosylation $\Delta\delta = \delta(\text{GO-R}) - \delta(\text{HO-R})^*$ G = β -D-Glucosyl

**Italic Figure* : Glucosyl Carbon (C-1 and C-2) Shift from β -D-Glucose

5' Glycosylation to Another Glucose



coupling constants in simple molecules are listed in Table I, from which it is apparent that the coupling increases markedly with increasing s character of the carbon hybridization.

This parameter can be used for structure analysis although practically it is sometimes difficult to measure J_{C-H} due to overlapping of CMR peaks resulted from appearance of many long range $^{13}C-^1H$ couplings (usually 1 - 6 Hz, but 26.7 Hz is observed in CH_3-CHO).

SPIN-SPIN COUPLING CONSTANT (J)

Some representative data for $^{13}C-^1H$

Table I: $^{13}C-^1H$ Couplings in Simple Molecules

Compound	(Hybridization)	J_{C-H} (Hz)	Compound(Hybridization)	J
CH_3-CH_3	sp^3	+125.0	$CH_2=CH_2$	sp^2 +156.2
Cyclohexane	sp^3	+125.0	Benzene	sp^2 157.5
$CH_3-(OCH_3)$	sp^3	139.6	$H_2C=C_2=CH_2$	sp^2 168
$CH_3-(OCH_3)$	sp^3	161.8	$HC\equiv CH$	sp +248.7

Table II: $^{13}\text{C}_1-^1\text{H}_1$ Constant (J) and Chemical Shift (δ) of $\text{C}_1^{6)}$

	axial- H_1		equatorial- H_1	
	β	δ J	α	δ J
D-glucopyranose	β	96.3 160Hz	α	92.4 169.5Hz
D-galactopyranose	β	97.0 159.5	α	92.8 169.5
D-mannopyranose	β	94.6 160	α	95.2 170
D-xylopyranose	β	97.3 160	α	92.8 170

bearing equatorial proton has always larger $^1\text{J}_{\text{C-H}}$ value (by ca. 10 Hz) than the axial case. This method is practically very useful because the anomeric carbon signals in CMR spectra are usually isolated from "crowded" other sugar carbon resonances.

SPIN-LATTICE RELAXATION TIME (T_1)

The third parameter, T_1 also significantly reflects the molecular structure.

Measurement: Inversion recovery method is most commonly used, although by no means the only one available. In this method the first pulse (180° pulse) inverts the nuclear magnetization. The nuclei recover their normal magnetization with a time constant

equal to T_1 . After a specific waiting time (t), the magnetization of various nuclei can be sampled by the application of a second pulse (90° pulse), thus resulted partially relaxed Fourier transform (PRFT) spectrum. The T_1 's for all the carbon can be calculated from the changes in line intensities with time. (see equation and Fig. 5)

Pulse sequence : $(180^\circ - t - 90^\circ - T_1)_n$

Peak intensity : S_t ($S_0 = S_\infty$)

$$M = \frac{S_\infty - S_t}{2S_\infty} = \exp(-t/T_1), \text{ or } \ln M = -t/T_1$$

T_1 and Structure: Relaxation is dominated by various mechanisms, however, $^{13}\text{C}-^1\text{H}$ dipole-dipole (DD) relaxation is generally contribute much more than the others in "ordinary" natural products.

$$1/T_1 = R_1 = R_1^{\text{DD}} + R_1^{\text{others}}$$

$$R_1^{\text{DD}} = 1/T_1^{\text{DD}} = \gamma_{\text{H}}^2 \gamma_{\text{C}}^2 h^2 \sum_i r_{\text{CH}_i}^{-6} \tau_c$$

R: Relaxation rate

r_{CH} : Distance of C-H

τ_c : Reorientational Correlation Time (Important!)

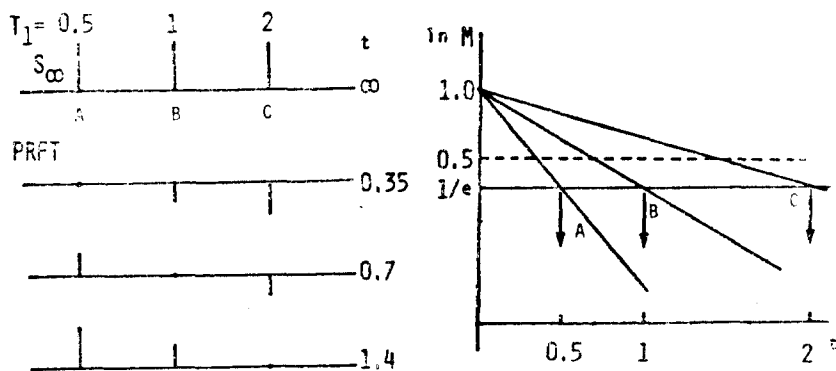


Fig. 5: Simulated PRFT spectra and calculation of T_1

for proton bearing carbon :

$$NT_1 = k \frac{r_{CH}^6}{\tau_c} = \frac{K}{\tau_c}$$

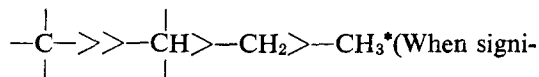
N : The number of directly attached proton

k, K : constant

From the above equations, the relative order of T_1 can be estimated as shown below.

Comparison of T_1 values in various factors

1. Molecular Weight: Small > Large
2. Concentration: Diluted > Concentrated
3. Temperature: Higher > Lower
4. Numbers of directly attached proton (in a same molecule):



5. Segment molecular movement (in a same molecule and same number of attached protons): Fast > Slow [Important for structure analysis !] (Fig. 6)

APPLICATION OF CMR TO STRUCTURE ANALYSIS OF PLANT GLYCOSIDES

From the chemotaxonomical view, it sometimes occurs that a plant or closely

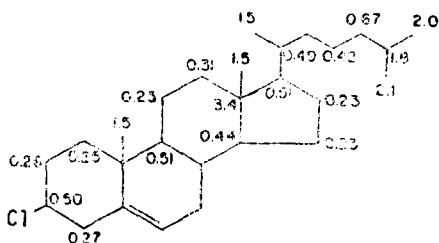


Fig. 6: T_1 , in seconds, for cholesteryl chloride, 1 M in CCl_4 at 42° .

related plants contains several glycosides with the same or similar aglycones. Therefore, when CMR of several anticipated aglycones

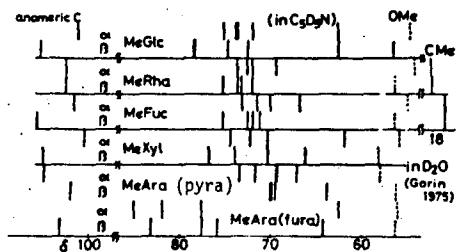


Fig. 7: CMR of several methyl glycosides

Note that each compound has unique CMR pattern between α and β anomers and also that it is between pyranose and furanose types.

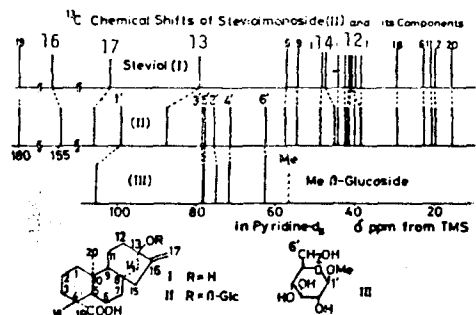


Fig. 8: Comparison of CMR signals of glycoside and its component

Note that the CMR signals of the glycoside are combination of its components. Significant shifts are observed only in the carbons around the combined position.

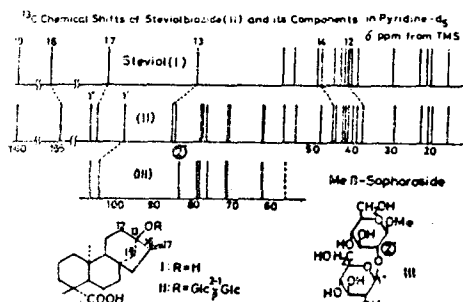
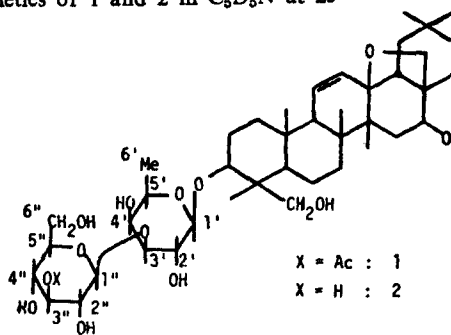


Fig. 9: Examples of glucobioside (cf. 4-5)

Table III ^{13}C Chemical Shifts (δ) of Sugar Moieties of 1 and 2 in $\text{C}_5\text{D}_5\text{N}$ at 25°

	1	2		
fucosyl	C-1'	105.9	106.5	
	2'	70.8	70.9	
	3'	84.8	84.9	
	4'	72.0	72.0	
	5'	71.5	71.7	
	6'	17.2	17.2	$\Delta\delta$
glucosyl	1"	105.9	105.8	+0.1
	2"	73.4	75.6	-2.2
	3"	79.1	78.3	+0.8
	4"	69.1	71.5	-2.4
	5"	78.3	78.6	-0.3
	6"	62.0	62.7	-0.7
Ac	21.1			
	170.7			



Acylated position of a saponin obtained from *Bupleurum falcatum* was decided by

the comparison of the CMR spectra of 1 and 2.

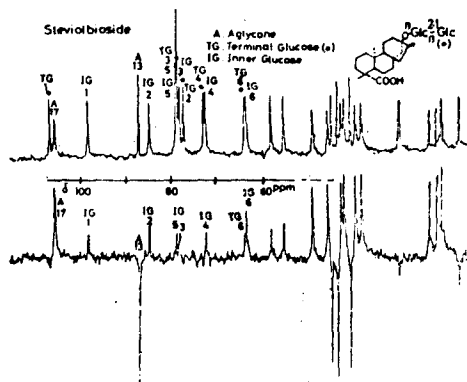


Fig. 10: Normal(upper) and PRFT spectra of the glucobioside

By the difference of peak intensities in PRFT spectrum, signals of inner glucose unit and terminal one are distinguished clearly. (cf. 6-4, and 6-5)

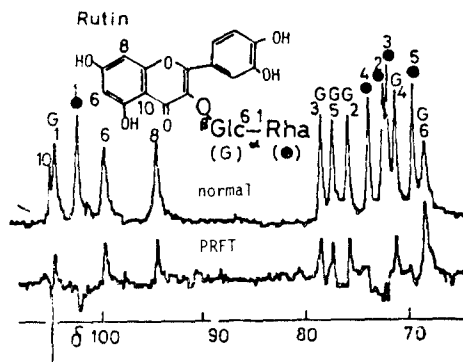


Fig. 11: Heteroglycoside signals associated to terminal sugar are distinguished by PRFT spectrum.

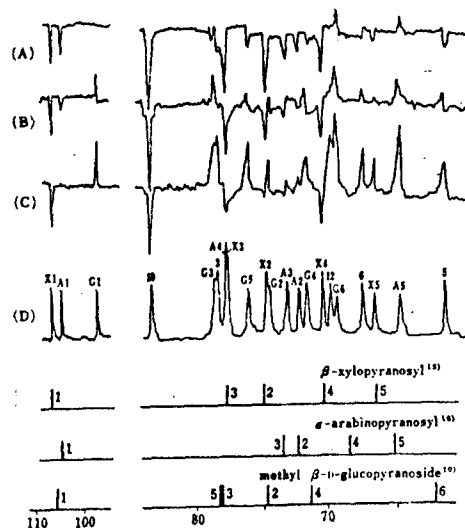
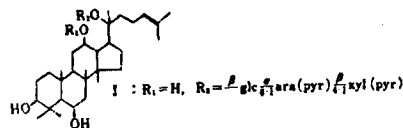


Fig. 12: PRFT Spectra of chikusetsusaponin-L₅(I) at 60° Sugar sequence of I, isolated from *Panax japonicus* was decided mainly by CMR using PRFT measurement.¹⁰⁾

(A-C) PRFT spectra: The intervals (sec) are; (A): 0.09 (B): 0.12, (C): 0.14, with a recycle time of 3.6sec. (D) Normal Fourier transform spectrum using a recycle time of 3.6sec. δ ppm from internal TMS in $\text{C}_5\text{D}_5\text{N}$.

For assignment of the aglycone moiety, in addition to ordinal method (see 3) shift reagent, shift derivative, and specific isotope (deuterium) labelling method were effectively used.¹¹⁾

and sugars are available, comparisons of these spectra to that of unknown glycoside can readily lead to determine the structure without consumption of any sample. Glucosylation (or generally glycosylation) shift plays important role at this time (See 4-5). Even if the aglycone is not identical to any compound available, the structure can be anticipated by the regular consideration of CMR spectrum including substitution effect.

Recent work in our laboratory are shown in the following figures.

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