

APPLICATION FOR LICENSE TO EXPORT NUCLEAR
MATERIAL AND EQUIPMENT (See Instructions on Reverse)

1. APPLICANT'S USE		2. NRC USE		3. DOCKET NO.		4. LICENSE NO.	
3-27-81		D-73		1100 2428		XMAT0175	
5. APPLICANT'S NAME AND ADDRESS				6. SUPPLIER'S NAME AND ADDRESS			
a. NAME Sigma Chemical Company				b. LICENSE NO. RIS			
c. STREET ADDRESS 3500 DeKalb Street				d. NAME			
e. CITY St. Louis		f. STATE MO		g. ZIP CODE 63118		h. STREET ADDRESS	
i. TELEPHONE NUMBER (Area Code - Number - Extension) (314) 771-5765 Ext. 202				j. CITY			
k. STATE				l. ZIP CODE			
7. FIRST SHIPMENT SCHEDULED		8. FINAL SHIPMENT SCHEDULED		9. APPLICANT'S CONTRACTUAL DELIVERY DATE		10. PROPOSED LICENSE EXPIRATION DATE	
Est. as soon as license issued		Est. 2 yrs from license		Not established issue		2 yrs. from issue	
11. U.S. DEPARTMENT OF ENERGY CONTRACT NO. (If Known)				12. N/A			
13. ULTIMATE CONSIGNEE				14. ULTIMATE END USE			
a. NAME Flobio				(For use plant or facility name)			
b. STREET ADDRESS 15 Rue Ormand-Silvestre				NMR Applications in Analytical Chemistry (see attached)			
c. CITY - STATE - COUNTRY 92400 Courbevoie France				15. EST. DATE OF FIRST USE			
16. INTERMEDIATE CONSIGNEE				17. INTERMEDIATE END USE			
a. NAME				18. EST. DATE OF FIRST USE			
b. STREET ADDRESS				19. INTERMEDIATE END USE			
c. CITY - STATE - COUNTRY				20. EST. DATE OF FIRST USE			
21. INTERMEDIATE CONSIGNEE				22. INTERMEDIATE END USE			
a. NAME				23. EST. DATE OF FIRST USE			
b. STREET ADDRESS				24. INTERMEDIATE END USE			
c. CITY - STATE - COUNTRY				25. EST. DATE OF FIRST USE			
26. NRC USE		27. DESCRIPTION		28. MAX. ELEMENT WEIGHT		29. MAX. WT. %	
(Include chemical and physical form of nuclear material; give dollar value of nuclear equipment and components)		Compounds containing Deuterium; application is for shipment of equivalent of 5 kg of D ₂ O liquid over a 2-year period. See attached.		13. MAX. ELEMENT WEIGHT		19. MAX. WT. %	
						20. MAX. ISOTOPE WT.	
						21. UNIT	
22. COUNTRY OF ORIGIN - SOURCE MATERIAL		23. COUNTRY OF ORIGIN - ENM WHERE ENRICHED OR PRODUCED		24. COUNTRIES WHICH ATTACH SAFEGUARDS (If Known)			
U.S.A.				Unknown			
25. ADDITIONAL INFORMATION (Use separate sheet if necessary)							
26. The applicant certifies that this application is prepared in conformity with Title 10, Code of Federal Regulations, and that all information in this application is correct to the best of his/her knowledge.							
27. AUTHORIZED OFFICIAL		28. SIGNATURE		29. DATE		30. TITLE	
		[Signature]		3/27/81		Walter C. Stern V.P.	



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APPLICANT'S REFERENCE D-73

Item No. 17 continued

For purposes of accounting for material shipped under this license, the Deuterium content of each compound will be expressed as weight D₂O liquid.

Example: If 1000 gm of Deuterated Acetic Acid (CD₃COOD) is shipped this would contain $\left(\frac{8}{64}\right)$ (1000 gm) or 125 gm of D or the equivalent of $\left(\frac{20}{4}\right)$ (125) = 625 gm D₂O.

Thus, 625 gm D₂O would be used for determining amount shipped under this license. Since this shipment contains more than the equivalent of 90 gm D₂O, it would not be permitted under the general license authorized by 10 CFR 110.25 and would need to be covered by this specific license.

ASM/ev
10/20/80

Deuterium NMR - A Useful Technique in Chemistry, Physics, and Biology

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INTRODUCTION

Deuterium nmr has been much neglected as a means to study chemical structures and processes. Its enormous potential was demonstrated in a thorough report by Diehl and Leipert in 1964,¹ but due to the unavailability of spectrometers and to an ingrained prejudice in nmr textbooks it has only come to the fore in the last five years. A recent comprehensive review cites 350 references.²

The nuclear moment of ^2H is considerably lower than that of ^1H ; it resonates at 15 MHz in a magnetic field of 23 kG. Its detection sensitivity relative to ^1H is 0.00965. This, combined with a natural abundance of 0.015%, makes detection in unenriched compounds relatively difficult.³ Nonetheless, ordinary tap water yields a signal-to-noise ratio of 2/1 for a single scan of ^1H at natural abundance;⁴ as we shall see later this can cause difficulties in some experiments where H_2O is used as a solvent. With the availability of sensitive

FT spectrometers detection of deuterium has become routine. Enrichment in ^2H is reasonably inexpensive, and becomes more so as the use of deuterated compounds increases.

Deuterium has a quadrupole moment, albeit relatively small; the quadrupole coupling constant varies from 130 to 210 kHz depending upon the chemical nature of the compound.⁵ The quadrupole moment was presumed to be a source of prohibitively broad resonances as it will dominate the relaxation behavior of ^2H . However, for small molecular line widths of 0.5-2 Hz are common. Some of the broad resonances observed previously were due to the presence of unresolved ^1H - ^2H couplings which are easily removed by ^1H -decoupling.

The low magnetogyric ratio of ^2H leads to spin-spin couplings much lower than those observed for ^1H ; ^2H - ^1H and ^2H - ^2H couplings will be only 15% and 23%, respectively, of their ^1H - ^1H analogs. This greatly simplifies ^2H spectra. Although ^2H chemical shifts are essentially identical in ppm to those of the corresponding ^1H , the scale in Hz is only 15% of that of ^1H . The greatly reduced or unobservable spin-spin couplings compensate partly for this apparent disadvantage. In the analysis of complex ^1H spectra the chemical shifts of ^2H , which are easily determined, yield a valuable starting point.^{1,4}

Deuterium chemical shifts are as sensitive as those of ^1H to conformation or configuration, although small differences may be masked by the greater line widths of large molecules.² ^2H nmr has been used to follow the paths of chemical reactions⁶⁻⁷

and is particularly valuable when exchange of hydrogen is incomplete. It is an excellent method to distinguish simply the degree of exchange of two very similar hydrogens.⁸ As an isotopic tracer in metabolic or biosynthetic studies ^2H is an inexpensive substitute for ^{14}C .⁹

The spin-spin and spin-lattice relaxation times (T_1 and T_2 , respectively) of ^2H are useful in studies of molecular dynamics as they are completely dominated by a quadrupolar exchange mechanism.¹⁰ They are insensitive to the presence of oxygen and less sensitive than those of ^1H and ^{13}C to the presence of paramagnetic metal ions.¹² The relatively short T_1 values of ^2H ¹⁰ allow rapid data accumulation in the pulsed Fourier transform mode of acquisition.

In highly ordered systems such as lyotropic liquid crystals¹¹ or biological membranes¹² the partially averaged quadrupole splittings of ^2H yield valuable estimates of the degree of molecular order. For highly symmetric ions (such as ND_4^+) interacting with colloids or ionic surfaces, similar quadrupolar splittings are indicative of distortions from tetrahedral or cubic symmetry.¹³

SOME CHEMICAL APPLICATIONS

A classic example of the usefulness of ^2H nmr was reported in pre-Fourier transform days by Montgomery *et al.*¹⁴ They explored the mechanism of substitution of cyclic olefinic halides with strongly basic nucleophiles. Three mechanisms are possible: (1) direct substitution, (2) formation of a cycloalkyne intermediate or (3) formation of a cycloallene intermediate. Direct substitution of Cl in 1-chlorocycloheptene-

2,7,7- d_3 (A) (See Scheme I) by the phenyl moiety of phenyllithium would yield B whose ^2H nmr spectrum would comprise two groups of resonances of relative intensity 2:1, the former in the allylic and the latter in the olefinic region. Allenic elimination of chlorine and subsequent addition to the center of the allenic system would lead to C with resonances in similar positions but of relative intensities 1:1. Cycloalkyne formation at carbon-1 would lead to two possible products D and E depending upon how phenyllithium added to the triple bond. The ^2H nmr spectrum of the product revealed two resonances of equal intensity at $\delta = 2.50$ and 2.80 ppm in agreement with the cycloalkyne pathway.

More recently Strothers and coworkers have investigated the mechanism of homoenolization in bicyclic ketones.^{6,7} In both studies the shift reagent $\text{Pr}(\text{fod})_3$ was used to obtain better dispersion of the ^2H resonances. A ^2H nmr spectrum of fenchone- d_{15} is shown in Figure 1. Even with the aid of the shift reagent some overlap of resonances is evident. The spectra were computer-simulated using the input resonances shown below the experimental and calculated composite spectra. The degree of substitution as a function of time was thus measured, and the results are summarized in Table I. Note that for shorter reaction times substitution of the *endo* and *exo* protons at position 6 showed a preference for the *exo* proton, whereas after three hundred hours the two were equally exchanged. A strong preference for the *exo* methyl group was maintained. The authors claimed an accuracy of 1-2% for this method.

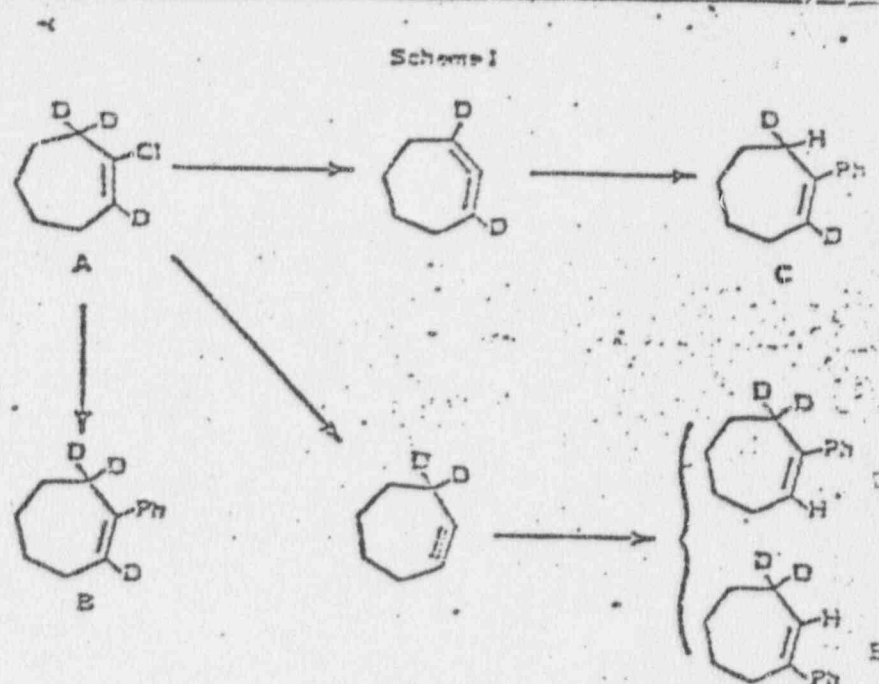


TABLE I
Quantitation by ^2H -NMR of ^2H Incorporation in Fenchone
as a Function of Reaction Time.⁷

Time (h)	C-6		Total	CH ₃		E
	Exo	Endo		Exo	Endo	
10	0.26	0.03	0.01			
20	0.34	0.12	0.02			
40	0.70	0.33	0.10	0.09		
100	0.75	0.58	0.33	0.26	0.05	
200	0.63	0.63	0.39			
300	0.65	0.64	0.65	0.42	0.16	

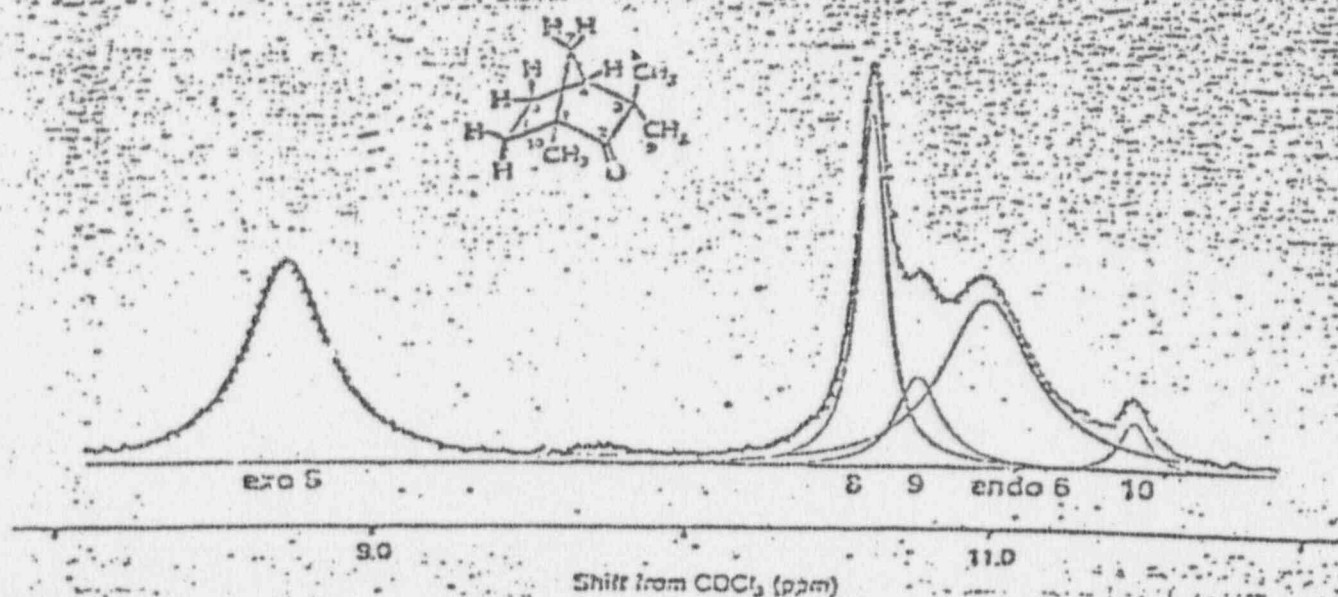


Figure 1 Experimental (A) and computer-fitted (—) ^2H nmr spectrum (15.6 MHz) of fenchone- d_{15} , containing 0.25 equivalent of $\text{Pr}(\text{fod})_3$. The spectrum was obtained with noise-modulated ^1H -decoupling in a solvent system of CHCl_3 - C_6H_6 .

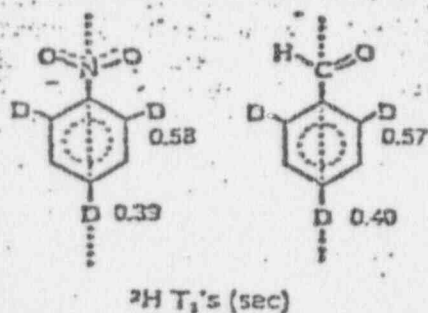
The mechanism of formation of nortricyclanone during deamination of 2-*exo*-aminonorboman-3-one was elucidated by Edwards *et al.*¹¹ Figure 2 shows the ^2H nmr spectra of the product with and without ^1H -decoupling. The resonance at 1.3 ppm is clearly coupled to a geminal proton ($^2J = 1.5 \text{ Hz}$) whereas the resonance at 1.5 ppm is broadened due to three unequal and small vicinal couplings. This demonstrates that the product has the structure shown in Figure 2. The positions of deuteration were confirmed by ^{13}C nmr which, however, could not distinguish whether the *endo* or *exo* proton at position 6 had been substituted.

Using ^2H nmr DePuy and coworkers¹² presented evidence for an asymmetrical, nonrotating, corner-protonated cyclopropane intermediate in the electrophilic ring opening of substituted cyclopropanes by $^2\text{H}^+$.

MOLECULAR DYNAMICS

The relaxation behavior of ^2H is totally dominated by a quadrupolar mechanism and therefore is indicative of molecular dynamics (internal and/or overall motion) at the position of substitution. This makes interpretation of relaxation data much simpler for ^2H than for ^1H or ^{13}C . For the simplest case of rapid isotropic motion, $1/T_1(^2\text{H}) = (3e^2qQ/8h)^2\tau_c$, where e^2qQ/h is the quadrupole coupling constant in radian sec^{-1} and τ_c is the correlation time for rotation of the $\text{C}-^2\text{H}$ bond. As a rule of thumb one can say the longer is T_1 , the more mobile is the $\text{C}-^2\text{H}$ bond. For more complex or slower motions the relationships become more complicated.²

Mantich *et al.* measured the T_1 values for a wide range of deuterated compounds.^{13,14} The data for benzaldehyde and nitrobenzene are shown below. The inequality of the T_1 values for the *ortho* and *para* deuterons is, in each case due to



anisotropic motion of the molecules, with the rate about an axis through the *para* and substituted carbons being considerably more rapid than those about the two other orthogonal axes. Note that rotation about this particular axis does not change the angle between the *para* $\text{C}-\text{H}$ bond and the external magnetic field. The T_1 value at this

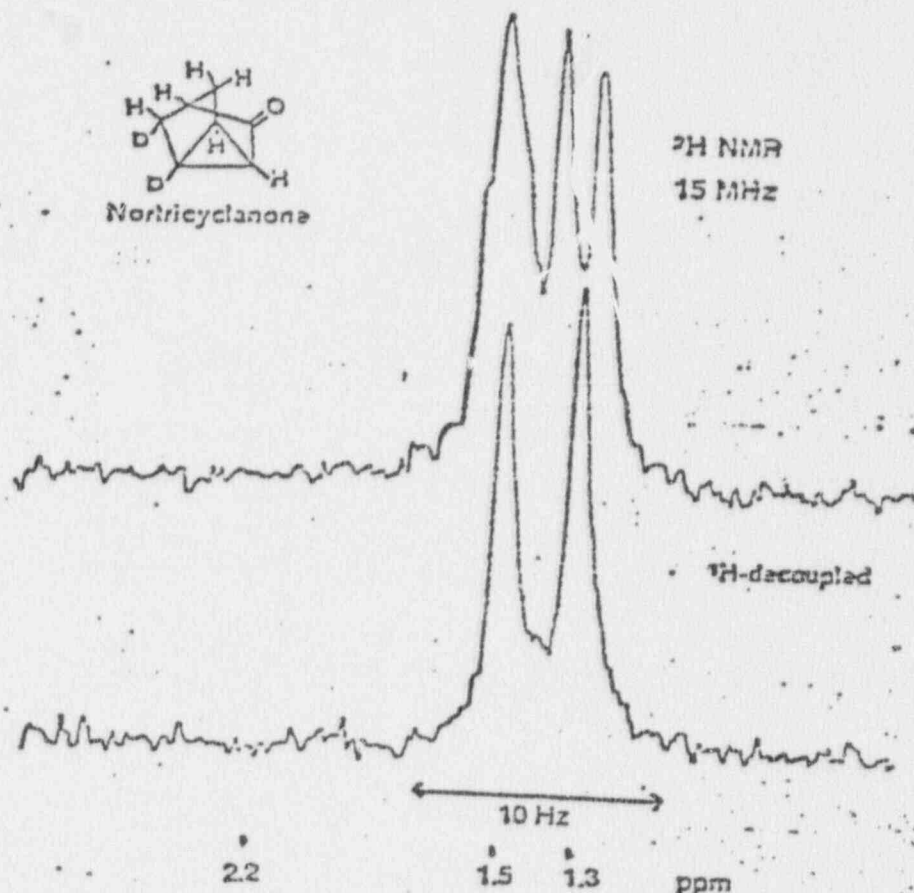
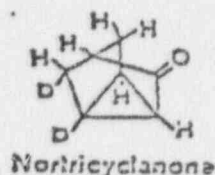
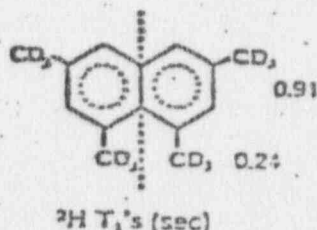


Figure 2 ^2H nmr spectra (15.4 MHz) of nortricyclanone with and without broad band ^1H -decoupling.¹¹

position is therefore determined only by the slower motions and is shorter than that for the *ortho* deuterons which are affected by the rapid motion.

The relative T_1 values of the α - and β -deuteromethyl groups of naphthalene- $\alpha,\alpha',\beta,\beta'$ -(C^2H_5)₂ are a dramatic example of the influence of steric hindrance on the rate of methyl-group rotation. In contrast to ^{13}C , where the relative importance of dipole-dipole and spin-rotation mechanisms must be determined, the ^2H data may be simply interpreted in terms of the modulation of the quadrupole interaction by rotation of the methyl groups relative to the naphthalene framework. Clearly the proximal methyl groups (α,α') interfere with each other's motion, whereas the β -methyl groups approximate free rotors.



The conclusion from ^{13}C nmr that a high degree of rapid intracyclic motion (pseudorotation) occurs in the amino acid proline was confirmed by ^2H nmr T_1

measurements on a series of deuterated prolines.¹⁵ The ^2H T_1 value of the α -carbon (0.27 sec) was considerably less than those of the β , γ , and δ -deuterons (0.42, 0.44, 0.49 sec, respectively) which had been thought to take part in rapid ring-puckering.

BIOSYNTHETIC MECHANISMS

Just as with organic reactions, ^2H nmr can be very valuable in establishing the pathways by which large molecules are synthesized biologically.² An example of the resolution of the technique is the spectrum of griseofulvin shown in Figure 3.¹⁶ The compound was produced by growth of *Penicillium urticae* on a medium containing sodium acetate-2- d_3 . Synthesis of a series of derivatives with different positions of deuteration confirmed the assignments shown in Figure 3.

The metabolic product of the urinary antibiotic nalidixic acid administered orally to a monkey was elucidated using ^2H nmr.²⁰ Freeze-dried urine was dissolved in trifluoroacetic acid and the spectra run without further purification. In separate experiments with nalidixic acid deuterated at either the methyl or ethyl group it was shown that the principal metabolic product involves hydroxylation of the methyl group. The advantages of the ^2H nmr method are that only resonances derived

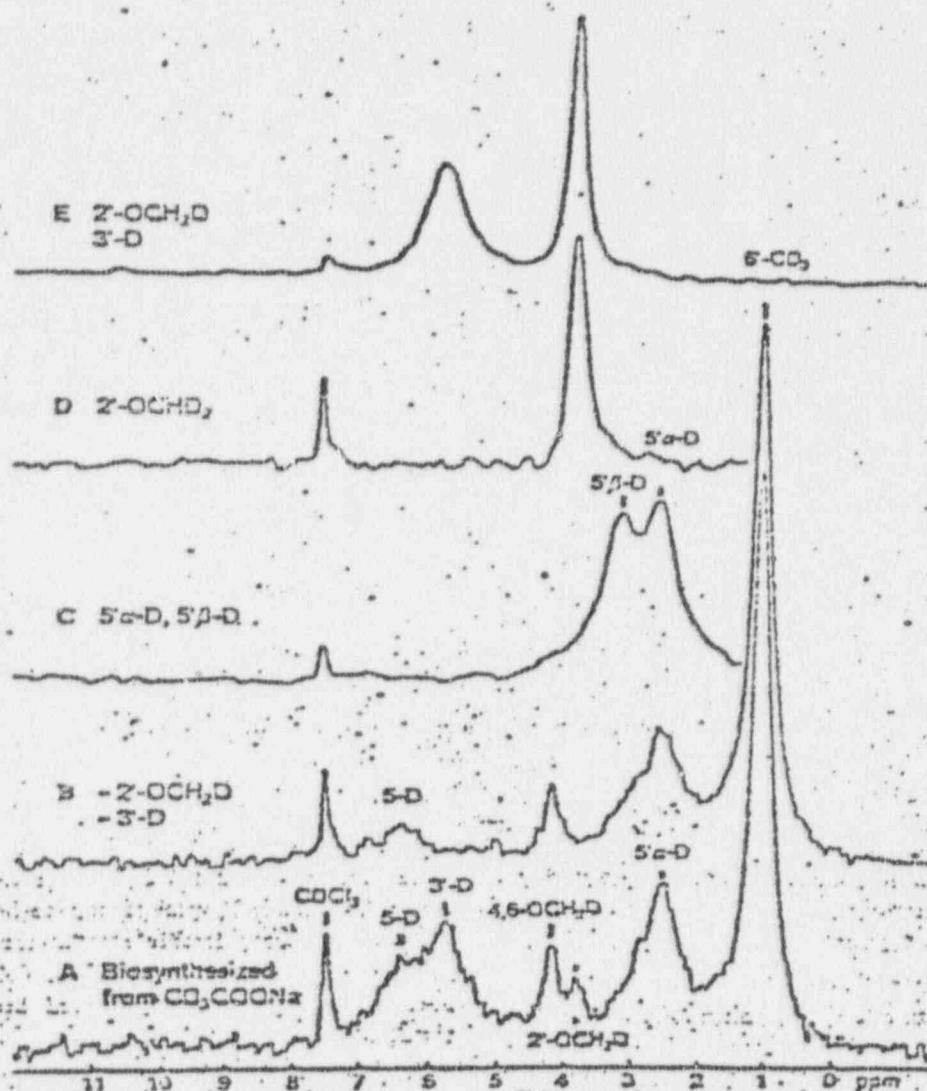
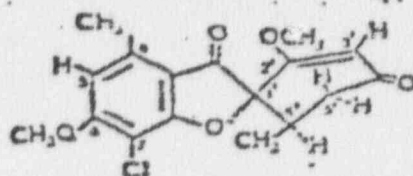


Figure 3. ^1H nmr spectra (113 MHz, ^1H -decoupled) of CHCl_3 solutions of griseofulvin (A) deuterated by growth of *P. utiformis* on sodium acetate-2- D_2 and of a series of specifically deuterated derivatives: B, without ^1H at 2 and 3; C, 5a, 5b- D_2 ; D, 2-O CH_2D_2 ; E, 2-O CH_2D_2 , 3-D. Comparison of ^1H -coupled and ^1H -decoupled spectra demonstrated that the two peaks centered around 4 ppm in A were coupled to ^1H .¹⁹

from the original compound are observed, the measured chemical shift is indicative of the chemical nature of the compound, and no separation of the product must be made to avoid interference with other compounds present in the physiological fluid.

It is expected that ^2H nmr will be applied to a wide variety of biosynthetic problems in the near future.

BIOLOGICAL MEMBRANES

By far the most fruitful biological application of ^2H nmr has been to the problem of the degree of molecular organization in membranes. The fatty acyl chains of the phospholipids in membranes are known to exist in a disordered

state resembling liquid crystals, i.e., the chains are fixed at the carboxyl end due to attachment to a pseudo-ionic lattice, but are relatively free at the terminal methyl groups. Rapid motion occurs about the C-C bonds of the fatty acyl chain; its rate and amplitude are expected to vary considerably from one end of the chain to the other. Incomplete averaging of the ^2H quadrupole interaction takes place in the case of such anisotropic motion, and partially averaged quadrupole splittings are observed. These splittings are directly related to the degree of molecular order (packing, relative numbers of *gauche* and *trans* C-C bonds) at the position of deuterium. A variety of model systems

has been studied^{21,22} and the method has been applied successfully to the plasma membrane of the microorganism *Acholeplasma laidlawii*.^{13,23} A review of the technique has appeared recently.¹²

The type of spectrum obtained from biological membranes is shown in Figure 4. The membrane phospholipids were deuterated in palmitic acid-16,16,16- d_3 by growth on a medium supplemented with this compound. The relatively small quadrupole doublet observed is due to a low degree of molecular packing at this position. Unsaturated lipids specifically deuterated at different positions along the chain it was found that the degree of order for the first ten carbon positions of the sixteen-carbon chain was relatively high and constant, and that it decreased rapidly with position thereafter to a minimum value at the terminal methyl group. This is similar to the behavior seen in the model systems of dipalmitoyl²⁴ and egg lecithin²⁵ and serves to justify the use of these model systems as well as providing the first detailed insight into the structure of biological membranes at the molecular level.²⁶

A technical problem encountered with ^2H nmr of membrane systems is the strong resonance observed in the center of the spectrum. This is due to ^2H at natural abundance in water (0.015%). As the quadrupole splittings become larger, difficulties are encountered with this peak due to dynamic range limitations of the spectrometer. By the use of ^2H -depleted water (ca. 0.00015%) this problem is minimized. The ^2H -depleted water is also useful in ^1H nmr studies of small molecules in H_2O where the chemical shifts of the positions of interest lie close to that of water.

PROGNOSIS

^2H nmr has finally come of age. Its usefulness in chemistry, physics, and biology has been well documented. With the greater availability of multinuclear spectrometers we are now in a position to take advantage of its versatility and advantages, and I expect that we shall witness a literature explosion in this subject in the near future.

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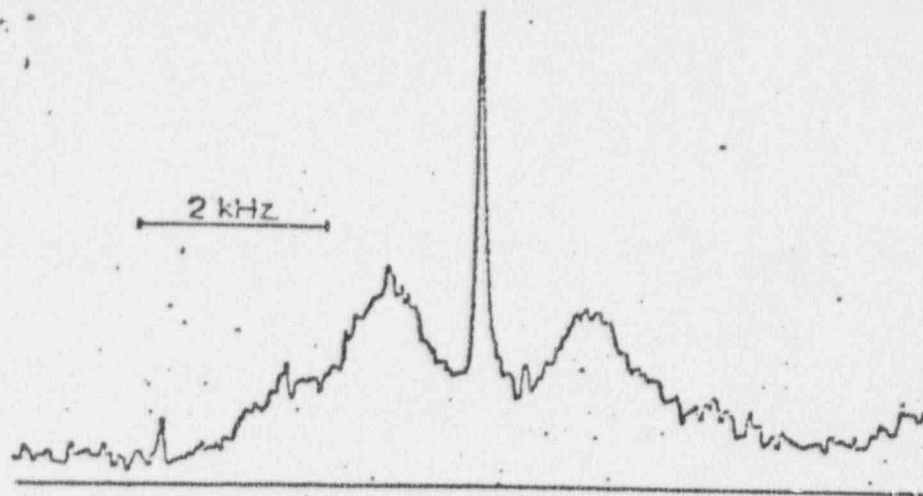


Figure 4 ^2H nmr spectrum (15.4 MHz) of the cytoplasmic membranes of *A. laidlawii* enriched in the phospholipids with palmitic acid-15,16,16- d_3 . The spectrum was taken in H_2O at 43° and required the accumulation of 2×10^3 free-induction decays before Fourier transformation.^{12,13,24}

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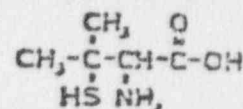
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ABOUT THE AUTHOR

Dr. Ian C.P. Smith received his B.Sc. (1961) and M.Sc. (1963) degrees from the University of Manitoba, Canada, and his Ph.D. (1965) from Cambridge University, England. He was elected a Fellow of the Chemical Institute of Canada in 1973 and of the Royal Society of Canada in 1977. His principal interest is application of magnetic resonance spectroscopy to biological problems, with an emphasis on membranes.

Currently he is a Senior Research Officer at the National Research Council of Canada, Division of Biological Sciences, Ottawa, Canada as well as an Adjunct Professor of Chemistry at Carleton and Ottawa Universities, Ottawa, and Adjunct Professor of Physiology and Biophysics at the University of Illinois, Chicago, U.S.A.

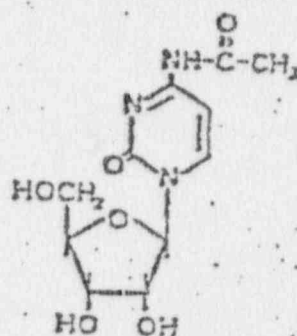
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Deuterium-depleted Water
19,529-4 25g \$25.00



L-(+)-Penicillamine is a metal chelating agent^{1,2} and a useful agent for the induction of experimental convulsions in the rat.³

- 1) R.P. Martin and J. Riande, 16th *Proc. Int. Conf. Coord. Chem.*, 1.75 (1974); *Chem. Abstr.*, 83, 63322g (1976).
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- 19,631-2 L-(+)-Penicillamine
1g \$6.50; 5g \$21.00

Interesting Nucleoside

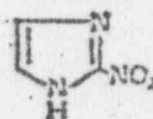


N⁴-Acetylcytidine is a minor component of *E. coli* methionine tRNA.

- 1) Z. Ohashi, K. Murao, T. Yahagi, D.L. von Minden, J.A. McCloskey and S. Nishimura, *Biochim. Biophys. Acta*, 262, 209 (1972).

86,082-4 N⁴-Acetylcytidine
100mg \$36.00

Antimicrobial Agent



2-Nitroimidazole (azomycin) possesses antimicrobial activity and is an important intermediate in the preparation of 2-nitroimidazolyl carbamates,^{1,2} potential protozoocides.

- 1) F. Hoffmann-La-Roche and Co., A.G., Fr. Patent 2,260,995 (1975); *Chem. Abstr.*, 84, 150631c (1976).
- 2) A.G. Benman and W.P. Tautz, U.S. Patent 3,865,523 (1975); *Chem. Abstr.*, 82, 17094w (1975).

19,565-0 2-Nitroimidazole (azomycin)
250mg \$8.00; 1g \$21.00