FORM NRC-7
(5-79)
10 CFR 110

U.S. NUCLEAR REGULATORY COMMISSION

APPROVED BY GAO
B-180225(R0362)

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

PDR

1. APPLICANT'S USE →	a. DATE OF APPLICATION	b. APPLICANT'S REFERENCE	2. NRC USE →	a. DOCKET NO.	b. LICENSE NO.
	Oct. 12, 1982	D-100		11002994	XMAT0264

3. APPLICANT'S NAME AND ADDRESS	RIS	4. SUPPLIER'S NAME AND ADDRESS *(Complete if applicant is not supplier of material)*	RIS
a. NAME: Sigma Chemical Company		a. NAME	
b. STREET ADDRESS: P.O. Box 14508		b. STREET ADDRESS	
c. CITY: St. Louis; STATE: MO; ZIP CODE: 63178		c. CITY; STATE; ZIP CODE	
d. TELEPHONE NUMBER *(Area Code – Number – Extension)*: (314) 771-5765 ext. 202			

5. FIRST SHIPMENT SCHEDULED	6. FINAL SHIPMENT SCHEDULED	7. APPLICANT'S CONTRACTUAL DELIVERY DATE	8. PROPOSED LICENSE EXPIRATION DATE	9. U.S. DEPARTMENT OF ENERGY CONTRACT NO. *(If Known)*
Est. - as soon as license issued	Est. - 2 yrs from license	Not established issue	2 yrs. from issue	N/A

10. ULTIMATE CONSIGNEE	RIS	11. ULTIMATE END USE *(Include plant or facility name)*	–
a. NAME: K. RAJ & CO.		NMR Application in Analytical Chemistry (see attached)	
b. STREET ADDRESS: 5/58 Jaldarshan, 51 Nepean Sea Rd.			
c. CITY – STATE – COUNTRY: Bombay 400 036 India		11a. EST. DATE OF FIRST USE	

12. INTERMEDIATE CONSIGNEE	RIS	13. INTERMEDIATE END USE	
a. NAME			
b. STREET ADDRESS			
c. CITY – STATE – COUNTRY		13a. EST. DATE OF FIRST USE	

14. INTERMEDIATE CONSIGNEE	RIS	15. INTERMEDIATE END USE	
a. NAME			
b. STREET ADDRESS			
c. CITY – STATE – COUNTRY		15a. EST. DATE OF FIRST USE	

16. NRC USE	17. DESCRIPTION *(Include chemical and physical form of nuclear material; give dollar value of nuclear equipment and components)*	18. MAX. ELEMENT WEIGHT	19. MAX. WT. %	20. MAX ISOTOPE WT.	21. UNIT
	Compounds containing Deuterium; application is for shipment of equivalent of 5 kg of D_2O liquid over a 2-year period.				

22. COUNTRY OF ORIGIN.– SOURCE MATERIAL	23. COUNTRY OF ORIGIN–SNM 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	a. SIGNATURE	b. TITLE
	Willard Klein	Vice President

SIGMA
CHEMICAL COMPANY
P. O. BOX 14508, ST. LOUIS, MO. 63178 U.S.A.

XMAT026Y

APPLICANT'S REFERENCE D-100

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 D_2O liquid.

Example: If 1000 gm of Deuterated Acetic Acid (CD_3COOD) 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 D_2O.

Thus, 625 gm D_2O would be used for determining amount shipped under this license. Since this shipment contains more than the equivalent of 90 gm D_2O, 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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National Research Council of Canada*
Ottawa, Ontario, Canada K1A 0R6

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 ^2H at natural abundance;[2] 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.[2] 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 molecules 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 2.3%, 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.[3,4]

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 reactions[5-7] 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.[6,7] As an isotopic tracer in metabolic or biosynthetic studies ^2H is an inexpensive substitute for ^{13}C.[8]

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.[9] 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.[1,2] The relatively short T_1 values of ^2H[9,10] allow rapid data accumulation in the pulsed Fourier transform mode of acquisition.

In highly ordered systems such as lyotropic liquid crystals[11] or biological membranes[12] 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-

*Issued as NRCC Publication Number 16204.

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 δ= 2.50 and 2.80 ppm in agreement with the cycloalkyne pathway.

More recently Stothers and coworkers have investigated the mechanism of homoenolization in bicyclic ketones.[6,7] In both studies the shift reagent Pr(fod)$_3$ was used to obtain better dispersion of the ^2H resonances. A ^2H nmr spectrum of fenchone-$d_{1.94}$ 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 1. 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.

Scheme I

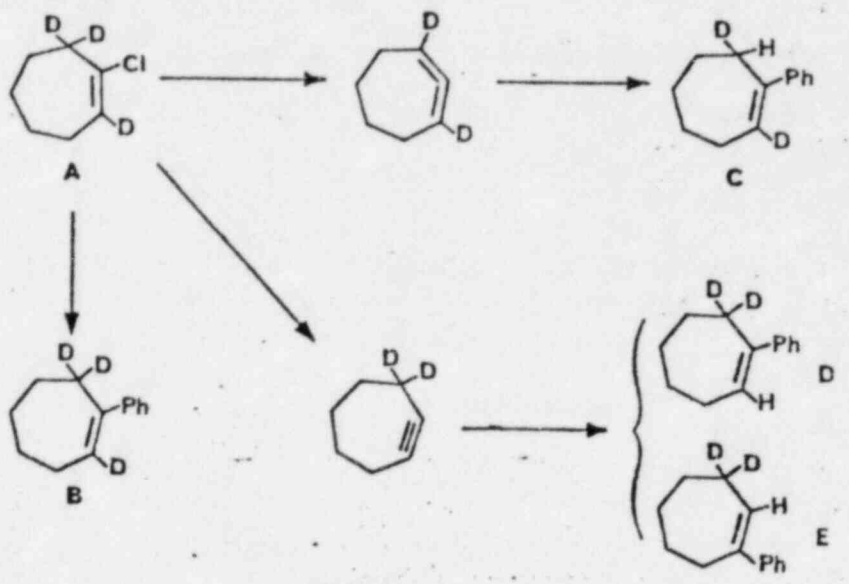


TABLE 1
Quantitation by ^2H-NMR of ^2H Incorporation in Fenchone as a Function of Reaction Time.[7]

	C-6		CH_3			
Time (h)	*Exo*	*Endo*	Total	*Exo*	*Endo*	Bridge
10	0.26	0.08	0.01_8			
20	0.34	0.12	0.02_5			
40	0.70	0.38	0.10	0.09		0.01
100	0.75	0.58	0.33	0.26	0.05	0.01_8
200	0.68	0.68	0.39			
300	0.65	0.64	0.65	0.42	0.16	0.06

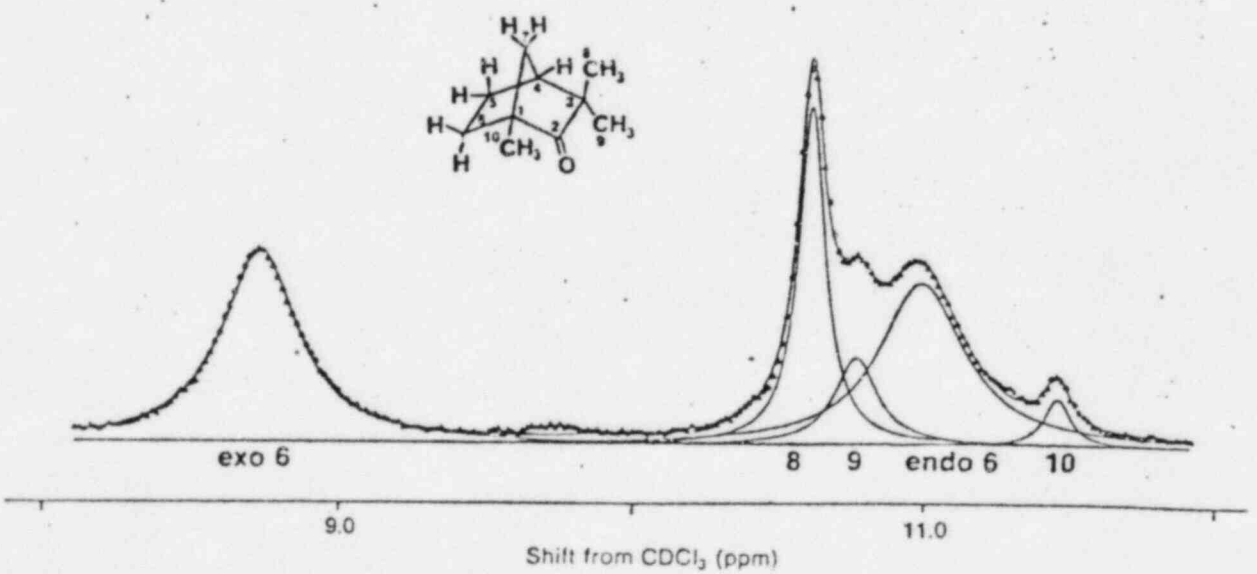


Figure 1 Experimental (Δ) and computer-fitted (—) ^2H nmr spectra (15.4 MHz) of fenchone-$d_{1.94}$ containing 0.26 equivalent of Pr(fod)$_3$. The spectrum was obtained with noise-modulated ^1H-decoupling in a solvent system of $CHCl_3$:C_6F_6 (1:4 v/v). Individual Lorentzian curves for each absorption are shown beneath the composite envelope.[7]

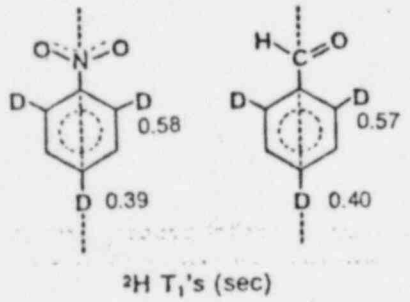
The mechanism of formation of nortricyclanone during deamination of 2-*exo*-aminonorbornan-3-one was elucidated by Edwards *et al.*[15] Figure 2 shows the ^2H nmr spectra of the product with and without ^1H-decoupling. The resonance at 1.3 ppm is clearly coupled to a geminal proton (^2J = 1.5 Hz) whereas 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 coworkers[16] presented evidence for an asymmetrical, nonrotating, corner-protonated cyclopropane intermediate in the electrophilic ring opening of substituted cyclopropanes by $^2H^+$.

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, $1/T_1(^2H) = (3e^2qQ/8h)^2\tau_c$, where e^2qQ/h is the quadrupole coupling constant in radian sec^{-1} and τ_c is the correlation time for rotation of the C-^2H bond. As a rule of thumb one can say the longer is T_1, the more mobile is the C-^2H bond. For more complex or slower motions the relationships become more complicated.[2]

Mantsch *et al.* measured the T_1 values for a wide range of deuterated compounds.[9,10] 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 C-^2H bond and the external magnetic field. The T_1 value at this 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.

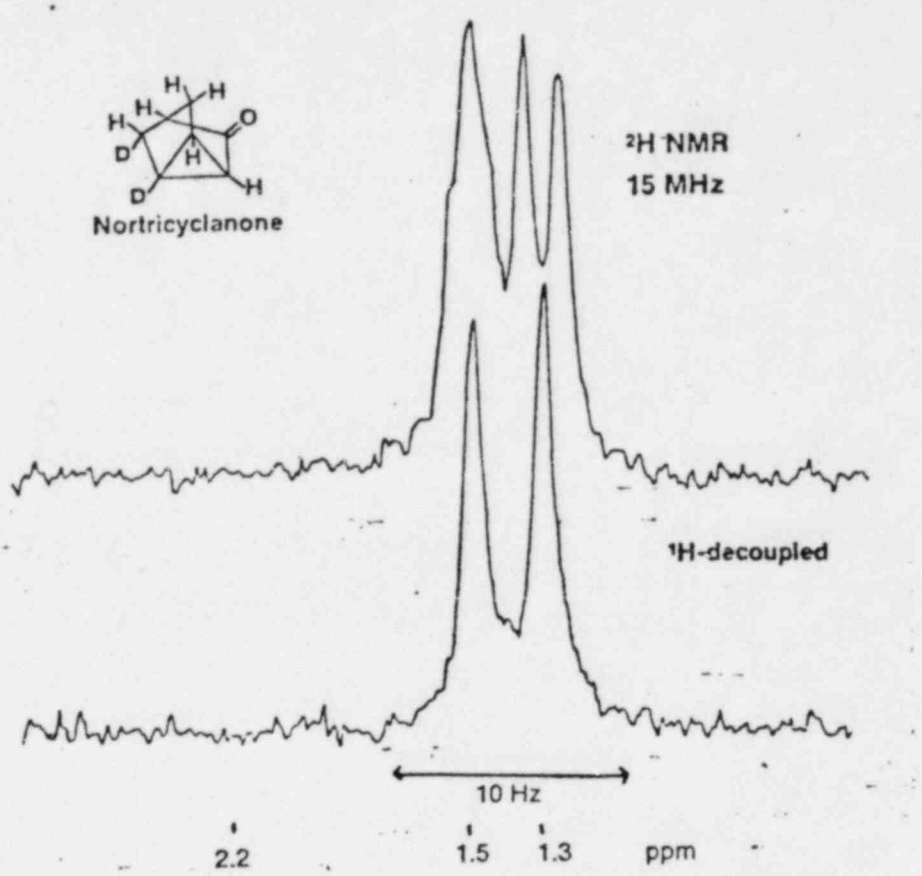
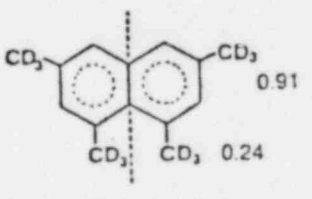


Figure 2 ^2H nmr spectra (15.4 MHz) of nortricyclanone with and without broad band ^1H-decoupling.[15]

The relative T_1 values of the α- and β-deuteromethyl groups of naphthalene-$\alpha,\alpha',\beta,\beta'$-$(C^2H_3)_4$ 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[17] 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.[18] 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, 0.40 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.[8] An example of the resolution of the technique is the spectrum of griseofulvin shown in Figure 3.[19] 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

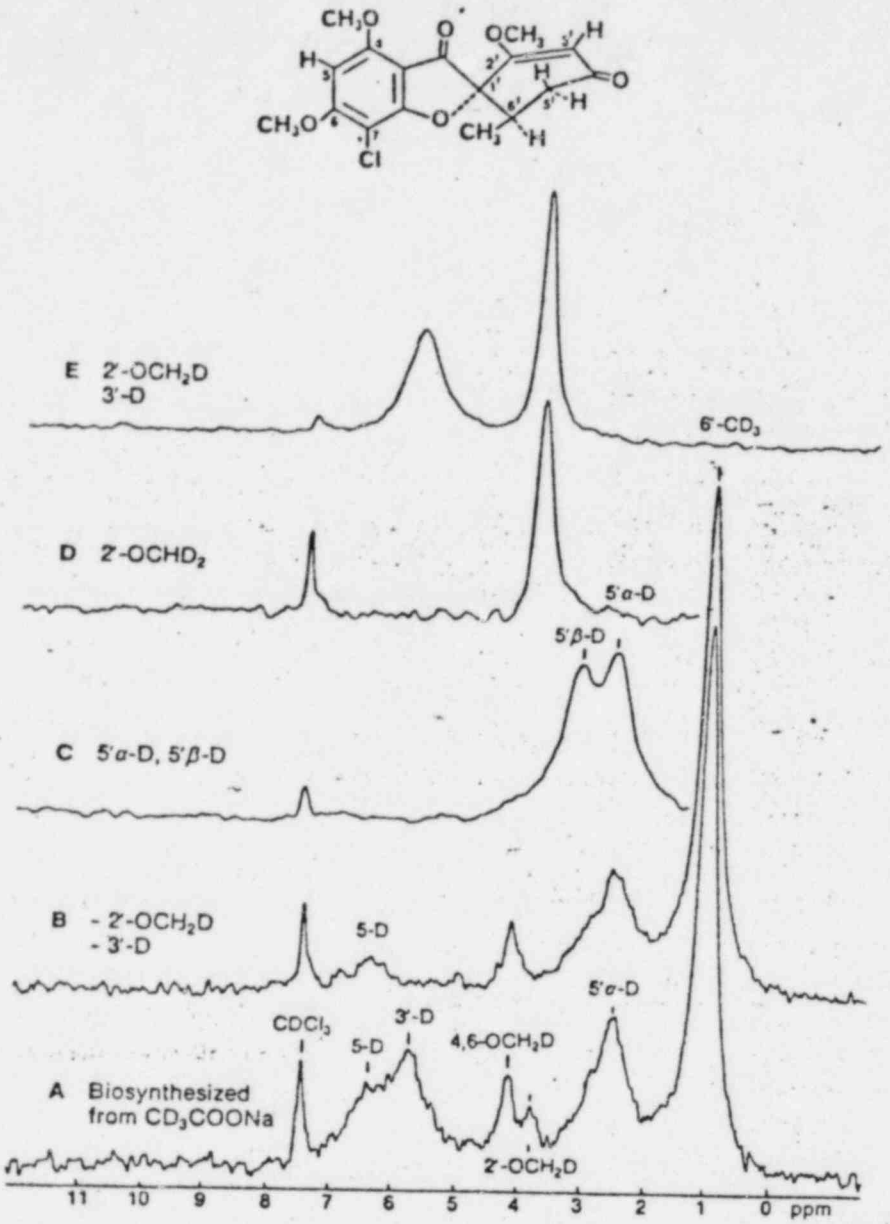


Figure 3 ^2H nmr spectra (15.3 MHz, ^1H-decoupled) of $CHCl_3$ solutions of griseofulvin (A) deuterated by growth of *P. urticae* on sodium acetate-2-d_3 and of a series of specifically deuterated derivatives: B, without ^2H at 2' and 3'; C, 5'α,5'β-D_2; D, 2'-$OCHD_2$; E, 2'-OCH_2D, 3'-D. Comparison 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 at physiological temperatures in a state resembling liquid crystals, *i.e.*, the chains are fixed at the carboxyl end due to attachment to a pseudoionic 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 deuteration. 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*.[23,24] A review of this technique has appeared recently.[12]

The type of spectrum obtained from a biological membrane is shown in Figure 4. The membrane phospholipids were enriched 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. Using 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 dipalmitoyl[21] and egg lecithin[22] 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.[24]

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 ^2H 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.[2] 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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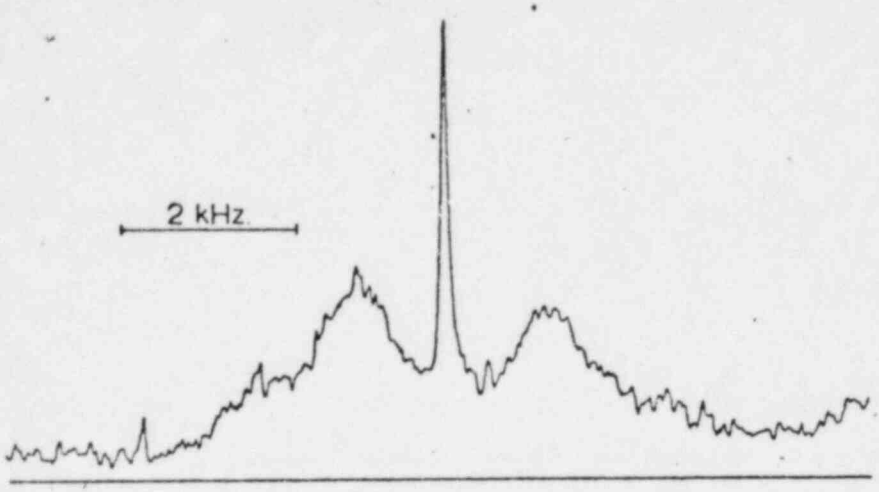

Figure 4 ^2H nmr spectrum (15.4 MHz) of the cytoplasmic membranes of *A. laidlawii* enriched in the phospholipids with palmitic acid-16,16,16-d_3. The spectrum was taken in H_2O at 43° and required the accumulation of 2×10^5 free-induction decays before Fourier transformation.[12,23,24]

Chem. Soc., in press.

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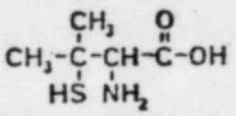
ABOUT THE AUTHOR

Dr. Ian C.P. Smith received his B.Sc. (1961) and M.Sc. (1962) 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.

L-(+)-Penicillamine



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. Riaute, *16th. Proc. Int. Conf. Coord. Chem.*, 1.7b (1974); *Chem. Abstr.*, 85, 63322g (1976).
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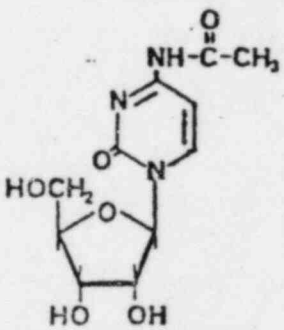
19,631-2 L-(+)-Penicillamine
1g $6.50; 5g $21.00

Interesting Nucleoside

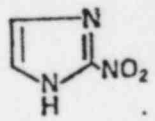


N^4-Acetylcytidine is a minor component of *E. coli* methionine *t*RNA.

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

86,082-4 N^4-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 protozoacides.

1) F. Hoffmann-La-Roche and Co., A.-G., Fr. Patent 2,260,996 (1975); *Chem. Abstr.*, 84, 150631e (1976).
2) A.G. Beaman and W.P. Tautz, U.S. Patent 3,865,823 (1975); *Chem. Abstr.*, 82, 170944w (1975).

19,565-0 2-Nitroimidazole (azomycin)
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51 NEPEAN SEA RD
BOMBAY 400 036 INDIA

"AVOID BILLING ERRORS"
PUT YOUR CUSTOMER NUMBER ON EVERY ORDER"

PHONE ORDER FROM - TOLANI, MR

SIGMA® chemical company
P.O. BOX 14508
ST. LOUIS, MO. 63178 U.S.A.

REAGENTS FOR MEDICAL RESEARCH

INVOICE # 82-281-0864

K.RAJ & CO
5/58 JALDARSHAN
51 NEPEAN SEA RD
BOMBAY 400 036 INDIA

GENERAL EXPORT
LIC G DEST

-PHONE-
-ORDER-

INT. CODE 67

YOUR ORDER NUMBER	SHIP VIA
PHN 10-8-82	AIR FREIGHT CR

82-281-0864

CUSTOMER ORDER NUMBER	SHIPPED VIA	YOUR CUSTOMER NUMBER	FILE	PACKING SLIP NO.	DATE	82-281-0864 01036
PHN 10-8-82	AIR FREIGHT CR	4-450-73270	8 01034	82-281-0864	10/08/82	

QUANTITY SHIPPED	QUANTITY NOT SHIPPED	PACKAGE SIZE	CATALOG OR STOCK NUMBER	PRODUCT DESCRIPTION	LOT NUMBER	WAREHOUSE LOCATION	VALUE	
2		25 G	S-8251	SUCCINYLCHOLINE CHLORIDE DIHYDRATE	038C0444	2008	7.02	P
1		100 G	M-2256	N-METHYL-N-NITROSO-P-TOLUENE SULFONAMIDE CRYSTALLINE	012F0423	880	12.82	P
6		5 G	P-2250	D-PANTOTHENIC ACID HEMI-CALCIUM	012F0343	CC-210-10	10.80	P
1		100 ML	D-4501	DEUTERIUM OXIDE 99.8 ATOM % D	031F0429	1837	36.85	PP/S TO A.J.---DEUTERATED COMPOUND ---DMF---
1		1 MG	N-3008	NUCLEOSIDE PHOSPHORYLASE FROM BOVINE SPLEEN	091F0004	CC-270-2	10.35	A
1		25 ML	D-4011	N,N-DIMETHYL-1-NAPHTHYLAMINE	139C0343	1343	11.92	PORN-A 11330 N16938 PASS NO LIMIT CARGO NO LIMIT
1		25 ML	M-1754	METHYLBENZETHONIUM HYDROXIDE	012F0065	CC-300-4	9.31	PFLAMMABLE LIQUID 11113 UI230 PASS 1L CARGO 40L F054

SPECIAL ORDER AND OR CUSTOMER DATA

MUST HAVE CREDIT APPROVAL
2 ORDERS ONE AIR FRT
ALL ORDERS MUST BE REGISTERED OR INSURED

OK 10/8/82

CREDIT EXCEEDED, NEEDS CREDIT CK.

DG
pls ck
acct is inactive

STOCK PULLED BY	CHECKED BY
SHIPPED BY	DATE
INSURANCE NO.	
REGISTRATION NO.	
C.O.D. NO.	

VALUE		POST EDIT	ACK'D	CRISPER NO.
GROSS	8105.65			
DISC	810.58			
NET	895.07			

NANO 10/08/82 11:20:46

SEE REVERSE SIDE FOR TERMS AND CONDITIONS OF SALE.

Art
per our telecon
B 10/11/82