Tritium Nuclear Magnetic Resonance Spectroscopy. Part II.[1] Chemical Shifts, Referencing, and an Application

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With the aid of compounds partially monotritiated by base-catalysed exchange with labelled water, corresponding triton and proton chemical shifts measured from internal tritiated water under the same conditions are shown to be the same. Applying this result and using the accurate resonance frequencies of the two nuclei measured at constant field on many of the compounds in organic solvents, a mean value, 1·06663975 ($\pm 3 \times 10^{-8}$), has been obtained for the ratio of the Larmor frequencies, ω_T/ω_H. This value enables an accurate ghost reference for any ^{3}H n.m.r. spectrum to be derived from the observed ^{1}H n.m.r. frequency of the normal internal reference. The convenience of ^{3}H n.m.r. spectroscopy for solving mechanistic problems is illustrated in respect of the Reimer–Tiemann reaction.

TRITIUM has found extensive application as a tracer isotope of hydrogen in studies of reaction pathways and mechanisms, both chemical and biochemical.[2-4] Whilst the radioactivity makes it easy to measure the rate of participation of a tritiated compound in a reaction and may aid the isolation of products, the exact location of the label therein is frequently difficult to determine. Usually a tedious sequence of specific degradations has to be performed,[5] or perhaps derivatisation coupled with radio g.c.[6] Equally troublesome can be the unambiguous demonstration of the position of the label in a starting material. These difficulties are readily overcome by the use of ^{3}H n.m.r spectroscopy which we have shown [1] can be performed safely and in a routine manner, and is of course non-destructive. Applications of tritium as a tracer are thereby greatly facilitated.

Preliminary work with relatively simple n.m.r. equipment indicated [1] that triton chemical shifts parallelled proton shifts. Now we establish with the aid of a pulse spectrometer that ^{3}H and ^{1}H chemical shifts are identical (within experimental error) when measured under the same conditions from corresponding references. This fact solves the problem of internal referencing of ^{3}H n.m.r. spectra in that it enables a correct ghost reference to be derived from a normal ^{1}H internal reference.

Thus ^{3}H n.m.r. spectroscopy becomes immediately useful. There is not the trouble of establishing correlations between chemical shifts and structure as there still is with ^{13}C n.m.r. spectroscopy because the enormous wealth of information and the reasoning in ^{1}H n.m.r. spectroscopy is at once applicable. In illustration of the convenience of ^{3}H n.m.r. for reaction mechanism studies, an experiment concerning the Reimer–Tiemann reaction is described.

For the present studies, a range of *C*-tritiated compounds was prepared by chemical exchange using inexpensive tritiated water as the source of the label. It is pertinent to mention that the development of highly basic media [7] makes possible the synthesis of a very wide variety of specifically tritiated compounds by this method, and compounds so labelled are stable at near neutral pH and in normal solvents and do not back-exchange with the environment. Hence they may be used as biochemical tracers [8] without trivial loss of label.

EXPERIMENTAL

Partially Monotritiated Compounds (*see Tables* 1 *and* 2).—(*a*) Compounds (1) and (2) (100 mCi each) (Radiochemical Centre) were each dissolved in tritiated water (50 Ci ml^{-1}) to 30 μl. (*b*) Compounds (3)—(5), (7)—(9), (13), and (14) (8—34 mg), each separately in 0·1N-sodium hydroxide in tritiated water (6—25 μl), were kept for 18 h at 20°. (*c*) Similarly, compound (19) was treated at 75° for 30 h and compounds (20) and (22) at 75° for 20 h (in sealed ampoules) and then dimethyl sulphoxide (10—25 μl) was added to the last two. (*d*) Compounds (6), (10), and (11) (9—20 mg) in dioxan (10 μl) or dimethyl sulphoxide (5 μl) were treated as in (*b*). Compound (12), in dioxan and the tritiated water (10 μl), was kept with solid sodium carbonate and then the solution was decanted. (*e*) Compounds (21) and (24)—(26) (25 mg), each in dimethyl sulphoxide (25—33 μl), were kept with 0·2N-sodium hydroxide in tritiated water (12 μl) for 1 h at 20°. (*f*) Compounds (15), (18), and (23) (25 mg) were each kept in tritiated water (35 μl) for 18 h at 25°. Compounds (16) and (17) were exchanged similarly but using tritiated water (15 μl) and dimethyl sulphoxide (20 μl). (*g*) [2′-^{3}H]Acetophenone (Table 2) was best obtained by keeping a mixture of acetophenone (1 ml), dioxan (1 ml), tritiated water (10 μl), and solid sodium hydroxide (0·5 pellet) at 20° for 18 h. The liquid was decanted into saturated sodium chloride (10 ml) and the organic phase was separated and clarified (Na_2SO_4), and a portion (50 μl) dissolved in perdeuteriated dimethyl sulphoxide (50 μl). (*h*) Labelled water (Table 2) was prepared by dilution of 50% tritiated water with deuterium oxide to 1 Ci ml^{-1}.

N.m.r. Measurements.—The foregoing samples were

[1] Part I, J. Bloxsidge, J. A. Elvidge, J. R. Jones, and E. A. Evans, *Org. Magnetic Resonance*, 1971, **3**, 127.
[2] E. A. Evans, 'Tritium and its Compounds,' Butterworths, London, 2nd edn., 1974.
[3] J. K. Lee and F. Schmidt-Bleek, *Adv. Analyt. Chem. Instrumentation*, 1968, **7**, 67.
[4] Proceedings of a Symposium on the Detection and Use of Tritium in the Physical and Biological Sciences, International Atomic Energy Agency, Vienna, 1962.
[5] J. R. Catch, 'Patterns of Labelling,' review no. 11, Radiochemical Centre, Amersham, 1971; and *e.g.* G. W. Kirby, S. W. Sha, and E. J. Herbert, *J. Chem. Soc.* (*C*), 1969, 1916.
[6] *E.g.* H. J. Ache, A. Thiemann, and W. Herr, *Z. analyt. Chem.*, 1961, **181**, 551.
[7] J. R. Jones, 'The Ionisation of Carbon Acids,' Academic Press, New York, 1973, ch. 11.
[8] J. M. A. Al-Rawi, J. A. Elvidge, D. K. Jaiswal, J. R. Jones, and R. Thomas, *J.C.S. Chem. Comm.*, 1974, 220.

sealed either (i) in microbulbs [1] (30 μl), or (ii) after neutralisation, together with an equal volume of perdeuteriated dimethyl sulphoxide in cylindrical microcells (100 μl) (Wilmad), and these were inserted into standard n.m.r. tubes. Triton and proton spectra were recorded on samples (i) with a Perkin-Elmer R10 instrument operating at 64 and 60 MHz respectively, and on samples (ii) with a Bruker WH90 pulse (Fourier transform) spectrometer at 96 and 90 MHz, the internal deuteriated compound providing the field-locking signal. The chemical shifts, δ_T and δ_H (Table 1) were measured from the internal tritiated water.[1]

N.m.r. Detection Sensitivity.—Progressive dilution of the tritiated water sample (*g*) with deuterium oxide showed that even at 0·5 mCi total activity in 30 μl an adequate signal-to-noise ratio of 3 : 1 was obtained with the Bruker spectrometer after only $1·9 \times 10^4$ pulses (of 2 μs at 1·7 s intervals).

Reimer–Tiemann Reaction.—Phenol (150 mg) in tritiated water (50 μl; 50 Ci ml^{-1}) was added to a stirred solution of sodium hydroxide (245 mg) in tritiated water (250 μl) at 60°. Then at 67°, chloroform (230 μl) was added during 5 min. After 1·5 h, the solution was cooled in ice, acidified (pH <2) with 3N-sulphuric acid, and extracted with ether (5 × 5 ml). The extract was dried (Na_2SO_4) and evaporated, and the residue distilled (high vacuum line) to give a liquid (80 mg), which in a parallel experiment with ordinary water comprised (g.l.c.) phenol and salicylaldehyde (5 : 3). The ^3H and ^1H n.m.r. spectra of the tritiated product (30 μl) showed singlets at δ 4·15 and 4·20 respectively. The intensity of the former signal, calibrated from ^3H n.m.r. signals from known tritiated water, corresponded to 50 mCi of tritium.

RESULTS AND DISCUSSION

Triton and Proton Chemical Shifts.—By definition, the chemical shift δ_x of any nucleus X in a sample, and the frequency of the resonance line ν_x^s and of the relevant internal reference ν_x^r, are related as in equation (1).

$$\delta_x = (\nu_x^s - \nu_x^r)/\nu_x^r = (\nu_x^s/\nu_x^r) - 1 \qquad (1)$$

The condition for resonance of any nucleus X at constant field B_0 is given by equation (2). Combination of

$$\nu_x = \gamma_x B_0(1 - \sigma_x) \qquad (2)$$

appropriate equations (1) and (2) for the triton and proton gives equation (3). If the nuclear screening

$$\delta_T/\delta_H = \frac{[(1 - \sigma_T^s)/(1 - \sigma_T^r)] - 1}{[(1 - \sigma_H^s)/(1 - \sigma_H^r)] - 1} \qquad (3)$$

constants σ_T and σ_H are mainly functions of the local molecular environment, which for a single isotopic replacement is virtually unchanged, then $\sigma_T^s \approx \sigma_H^s$ and $\sigma_T^r \approx \sigma_H^r$ so that equation (3) reduces to the approximation (4).

$$\delta_T/\delta_H \approx 1 \qquad (4)$$

The experimental results (Table 1) show that triton and proton chemical shifts, measured under the same conditions, are indeed virtually identical, the mean value for the ratio δ_T/δ_H being 1·00: a plot of δ_T against δ_H gives a straight line of slope unity and zero intercept. Hence the assumption is justified that a single isotopic replacement of ^3H for ^1H in a molecule will not appreciably alter the shielding at that position. Similar observations and conclusions have been reported concerning the isotopic pairs ^1H and ^2H,[9] ^{14}N and ^{15}N,[10] and ^{117}Sn and ^{119}Sn,[11] although for the tin isotopes the primary isotope effect on shielding 'sometimes lies beyond the experimental error.' The importance of the direct correspondence between triton and proton chemical shifts is of course that the vast compilation of proton chemical shift data in the literature applies equally to the prediction and assignment of triton n.m.r. spectra, so that there is little or no interpretive hindrance to applications of ^3H n.m.r. spectroscopy. The very close shift approximation (4) holds in spite of the relatively enormous isotopic mass difference, evidently because the effects of the different electronegativities, zero point energies, bond lengths, and van der Waals radii upon the screening constants are very small.

TABLE 1

Triton and proton chemical shifts (p.p.m.) [a] from internal tritiated water

No.	Compound (position tritiated)	δ_T	δ_H	δ_T/δ_H
(1)	Sodium acetate (2)	−2·91	−2·87	1·01
(2)	Acetic acid (2)	−2·77	−2·72	1·02
(3)	Acetone (1)	−2·66	−2·64	1·01
		−2·59	−2·59	1·00
(4)	2-Picoline (1′)	−2·58	−2·59	1·00
(5)	Acetonitrile (2)	−2·25	−2·22	1·01
		−1·16	−1·14 [b]	1·02
(6)	Propionitrile (2)	−2·21	−2·22	0·99(5)
(7)	Dimethyl sulphoxide (1)	−2·08	−2·09	0·99(5)
		−1·99	−2·04	0·97(5)
(8)	Prop-2-yn-1-ol (3)	−1·68	−1·67	1·00(5)
(9)	Sodium malonate (2)	−1·60	−1·63	0·98
(10)	Nitromethane (1)	−1·17	−1·18 [b]	0·99
		−0·42	−0·41	1·02
(11)	Malononitrile (2)	−0·75	−0·76	0·99
(12)	Diethyl malonate (2)	−0·50	−0·47	1·06
(13)	[2,3-^{2}H]Prop-2-en-1-ol (3)	0·44	0·43	1·02
(14)	2-Methylresorcinol (4)	1·47	1·50	0·98
(15)	Imidazole (2)	2·63	2·62	1·00
(16)	Benzimidazole (2)	2·94	2·91	1·01
		3·26	3·24 [b]	1·01
(17)	1-Methylbenzimidazole (2)	3·02	3·03	1·00
(18)	Purine (8)	3·09	3·07	1·01
(19)	Pyridine 1-oxide (2)	3·42	3·42	1·00
(20)	Quinoline 1-oxide (2)	3·45	3·44	1·00
(21)	Benzoxazole (2)	3·63	3·63	1·00
(22)	Isoquinoline 1-oxide (1)	3·95	3·99	0·99
	(3)	3·33	3·29	1·01
(23)	1,3-Dimethylbenzimidazolium bromide (2)	4·33	4·36	0·99
(24)	Benzothiazole (2)	4·40	4·37	1·01
		5·69	5·65 [b]	1·01
(25)	Chloroform (1)	5·07	5·16	0·98
(26)	Benzoselenazole (2)	5·28	5·34	0·99

[a] See ref. 1. [b] Samples prepared by method (g).

Ratio of the Larmor Frequencies.—It follows from the experimentally verified close approximation (4) that the ratio of the Larmor frequencies ω_T/ω_H for any site, at constant applied field, should be virtually constant.

[9] P. Diehl and T. Leipert, *Helv. Chim. Acta*, 1964, **47**, 545.
[10] R. Price, Ph.D. Thesis, London, 1969; E. W. Randall and D. G. Gilles, *Progr. N.M.R. Spectroscopy*, 1971, **6**, 135.
[11] A. Tupciauskas, N. M. Sergeyev, and Yu. A. Ustynyuk, *Mol. Phys.*, 1970, **21**, 179.

Measurements of n.m.r. line frequencies made on a selection of the partially monotritiated compounds (Table 2) show that this is so and provide a mean value

TABLE 2

Measurement of the ratio of ^3H and ^1H Larmor frequencies

Compound (see Table 1)	Observed relative line frequencies (Hz) ν_T then ν_H	Digitisation error *	ω_T/ω_H
(1)	96 022 445·9809	*a*	1·066639716
	90 023 317·6693		
(3)	96 022 475·1430	*a*	1·066639744
	90 023 342·5816		
(5)	96 022 676·4118	*b*	1·066639734
	90 023 532·2121	*a*	
(7)	96 022 521·3040	*a*	1·066639758
	90 023 384·6558		
(10)	96 022 904·7448	*b*	1·066639748
	90 023 744·9926	*a*	
(16)	96 023 277·0216	*a*	1·066639779
	90 024 091·3890		
(21)	96 023 398·7388	*a*	1·066639731
	90 024 209·6180		
(24)	96 023 384·7883	*a*	1·066639781
	90 024 192·2620		
(25)	96 022 723·7976	*a*	1·066639718
	90 023 577·6204	*b*	
Acetophenone	96 022 727·5934	*b*	1·066639747
	90 023 579·1041		
Water	96 022 724·7000	*c*	1·066639765
	90 023 574·7900		

* The error is ± 1 channel which is precisely $a = \pm 0{\cdot}1465$, $b = \pm 2a$, $c = \pm 0{\cdot}5a$ Hz.

of 1·06663975 ($\pm 3 \times 10^{-8}$) for the ratio. Of previous measurements of this Larmor frequency ratio,[12-14] the most accurate are those of Duffy.[13] Our result agrees with his. In our experiments, the frequencies in the Bruker spectrometer are locked together to a master 5 MHz oscillator, and to the field *via* a deuteron signal derived from the sample, and all are accurately known. The limits to the precision of our frequency measurements are the computer horizontal and vertical digitisation errors and the uncertainty in spectral line position as a result of the natural line width. The first two errors respectively decrease and increase the uncertainty of location of a line as the spectral frequency range is reduced. Frequency ranges to give display spectral widths of 600 or 300 Hz were found to be optimal, and at these ranges the horizontal digitisation error includes the other errors because of the quantisation, inherent in the whole spectrometer system. The errors quoted in Table 2 are quantised errors, the quotients of the spectral width and the number of channels in which the digitised spectrum was held after Fourier transformation, the free induction decay having been acquired into twice as many channels.

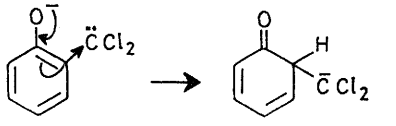
Referencing.—We have previously discussed the particular problem of referencing ^3H n.m.r. spectra,[1] and others have discussed the general problem of referencing for any magnetic nucleus.[15] We mentioned the impracticability (for radiochemical and n.m.r. reasons) of tritiated organic references analogous to those used for proton work, and so employed tritiated water, initially. In spite of the inherent disadvantages of water as an n.m.r. reference, tritiated water at least provided an equivalent reference for both ^3H and ^1H measurements made in parallel on the same sample. This equivalence derives from fast site exchange, and is confirmed by the very small fractionation factors for neutral water (which indicates absence of any appreciable differential hydrogen bonding). However, having obtained a sufficiently precise value for the ratio of the Larmor frequencies of the two nuclei from a set of representative observations, it is now possible to reference any ^3H n.m.r. spectrum accurately and conveniently without the need for an actual tritium reference at all. Tetramethylsilane (TMS), sodium 4,4-dimethyl-4-silapentane-1-sulphonate (DSS), or tetramethylammonium chloride,[16] is added to the sample in the normal way. This provides the generally accepted internal proton reference and of course facilitates the taking of the ^1H n.m.r. spectrum, if required. The accurate resonance frequency of the internal proton reference signal, available from the Bruker spectrometer output, is then multiplied by the Larmor frequency ratio 1·06663975 to provide the reference frequency for the ^3H n.m.r. spectrum. This corresponds accurately to the origin of the ^3H n.m.r. signal which would arise if monotritiated TMS (or DSS, *etc.*) were present in the sample. The appropriate commands to the computer then place this ghost internal reference signal upon the right hand ordinate of the chart paper. The chemical shifts of the ^3H n.m.r. signals from the sample are then obtained from the printout or from the chart in the usual way.

Isotopic Shifts.—For monotritiated compounds, the ^3H and ^1H chemical shifts, so measured, will for all practical purposes be the same, the primary isotopic shift being negligible. The upfield shift in ^1H or ^3H resonance resulting from the $+I$ effect of another tritium, the secondary isotope effect, is also very small and comparable with the normal experimental errors in measuring samples at different times. Nevertheless the secondary isotopic shift does show, for example, in the ^1H n.m.r. spectrum of a tritiated compound having at least a few percent abundance of the isotope at one site, and in the ^3H n.m.r. spectrum of a compound having more than one triton per labelled site. Previously we obtained [1] from the ^1H n.m.r. spectrum of tritiated sodium acetate (in water) a rough value of 0·05 p.p.m. to high field for the isotopic shift $\sigma_H{}^s(CH_2TCO_2Na) - \sigma_H{}^s(CH_3CO_2Na)$: accurate measurements (in D_2O) now provide the value 0·018 $\pm$ 0·002

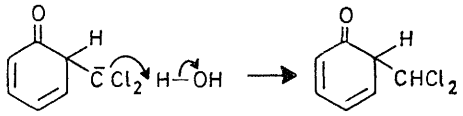
[12] F. Bloch, A. C. Graves, M. Packard, and R. W. Spence, *Phys. Rev.*, 1947, **71**, 551.
[13] W. Duffy, *Phys. Rev.*, 1959, **115**, 1012.
[14] R. W. Huggins and J. H. Sanders, *Proc. Phys. Soc.*, 1965, **86**, 53.
[15] W. McFarlane, *J. Chem. Soc.* (*A*), 1968, 2280; *Ann. Rev. N.M.R. Spectroscopy*, 1968, **1**, 135; F. H. A. Rummens, *Org. Magnetic Resonance*, 1970, **2**, 209; P. G. Harrison, S. E. Ulrich, and J. J. Zuckerman, *J. Amer. Chem. Soc.*, 1971, **93**, 5398; E. D. Becker, *J. Magnetic Resonance*, 1971, **4**, 142; G. C. Levy and J. D. Cargioli, *ibid.*, 1972, **7**, 143.
[16] D. H. Live and S. I. Chan, *Org. Magnetic Resonance*, 1973, **5**, 275.

p.p.m. For the isotopic shift $\sigma_T^s(CHT_2CO_2Na) - \sigma_T^s(CH_2T{\cdot}CO_2Na)$ measured on the same sample we obtain 0·021 ± 0·003 p.p.m. to high field.

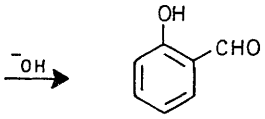
Example of an Application of ^{3}H *N.m.r. Spectroscopy.*— Some years ago Hine [17] showed that dichlorocarbene, formed from chloroform by alkali, was the essential electrophilic reagent in the Reimer–Tiemann reaction, effecting substitution of the phenol as in reaction (5).



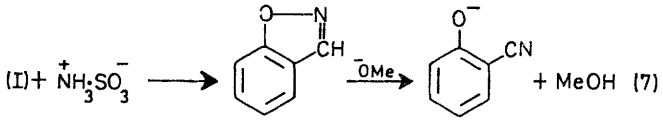
(5)



(6)



(I)



(7)

It remained uncertain whether this stage was followed by an internal proton transfer or whether there was protonation of the side-chain carbon atom by the solvent water, as in reaction (6). In 1971 Kemp [18] used tritiated water and showed that the eventual product, salicylaldehyde (I), was radioactive. Although this suggested mechanism (6), a proof of the mechanism necessitated location of the label in the product, because phenols exchange hydrogen at the *ortho*- and *para*-positions with water under alkaline conditions. Kemp therefore treated the isolated salicylaldehyde with hydroxylamine-*O*-sulphonic acid to give an isoxazole and then caused the latter to react with methoxide in methanol to give *o*-cyanophenol, as in reaction (7). That the methanol became radioactive proved that the aldehyde side-chain in the salicylaldehyde was tritium-labelled and so verified the mechanistic step (6).

To show how much more simple and direct it is to employ ^{3}H n.m.r. spectroscopy for locating the label, we have repeated this Reimer–Tiemann reaction on phenol in tritiated water. The ^{1}H n.m.r. spectrum of the product showed the aldehyde proton singlet at δ 4·2 (from HTO). The ^{3}H n.m.r. spectrum showed a similar singlet at δ 4·15, this then being from an aldehyde triton. The intensity of the ^{3}H n.m.r. signal was such as to show that mechanism (6) occurred to the exclusion of alternatives.

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[17] J. Hine and J. M. van der Veen, *J. Amer. Chem. Soc.*, 1959, **81**, 6446.
[18] D. S. Kemp, *J. Org. Chem.*, 1971, **36**, 202.