Tritium Nuclear Magnetic Resonance Spectroscopy. Part III.[1] Coupling Constants and Isotope Effects, and Calculation of $^2J_{HH}$ Coupling Constants

By **Jasim M. A. Al-Rawi, John A. Elvidge,*** and **John R. Jones,** Chemistry Department, University of Surrey, Guildford GU2 5XH
E. Anthony Evans, The Radiochemical Centre Ltd., Amersham, Buckingham HP7 9LL

J_{HT} Couplings are closely predictable from J_{HH} couplings and *vice versa*: a new set of $^2J_{HH}$ coupling constants has thus been derived, of higher precision than any previously calculated from $^2J_{HD}$ couplings. Primary isotope effects become noticeable for the larger $^1J_{CT}$ couplings. Various secondary isotope effects on chemical shifts were noted.

We have demonstrated the practicability of ^3H n.m.r. spectroscopy [2] and have begun to explore its utility.[1-3] Having solved the referencing problem and shown that the primary isotope effect on ^3H chemical shifts is normally negligible so that proton chemical shift information is directly applicable to the interpretation of ^3H n.m.r. spectra,[1] we have now examined some triton coupling

---

[1] Part II, J. M. A. Al-Rawi, J. P. Bloxsidge, C. O'Brien, D. E. Caddy, J. A. Elvidge, J. R. Jones, and E. A. Evans, *J.C.S. Perkin II*, 1974, 1635.

[2] J. P. Bloxsidge, J. A. Elvidge, J. R. Jones, and E. A. Evans, *Org. Magnetic Resonance*, 1971, **3**, 127.

[3] J. M. A. Al-Rawi, J. A. Elvidge, D. K. Jaiswal, J. R. Jones, and R. Thomas, *J.C.S. Chem. Comm.*, 1974, 220.

constants. For smaller couplings, *e.g.* $^{3}J_{HT}$ and $^{4}J_{HT}$, the values agree with those predicted from the corresponding proton–proton couplings when measured on the same samples. Larger triton couplings however, *e.g.* $^{1}J_{CT}$ couplings, show significant differences from the calculated values, but these apparent primary isotope effects are not large enough to upset normal use of the coupling constants for ascertaining the position and stereochemistry of labelling in tritiated compounds.

EXPERIMENTAL

Tritiated water (50 Ci ml^{-1}) and the uridines were from The Radiochemical Centre, deuteriated solvents and reagents from Ryvan, and ^{13}C-labelled compounds (90% enriched) from Prochem.

Isotopic Labelling.—(a) Acetone (35 μl) was kept with tritiated water (10 μl) and 0·1N-sodium hydroxide (5 μl) for 18 h at 20°. The liquid was neutralised (HCl) and diluted (to 100 μl) with perdeuteriated acetone.

(b) Acetonitrile (0·25 ml) and [2-^{13}C]acetonitrile (0·25 ml) were each treated with tritiated water (15 μl) and solid sodium hydroxide (1 pellet). After 18 h, the liquids were decanted and clarified (Na_2SO_4) and a portion of each (50 μl) was diluted with perdeuteriated acetonitrile (50 μl).

(c) Acetophenone and [2′-^{13}C]acetophenone were each tritiated as before.[1] For deuteriation, acetophenone (2 ml) in [*O*-^{2}H]methanol (3 ml) was kept with deuterium oxide (1 ml) and 10% sodium deuterioxide in deuterium oxide (10 μl) for 18 h at 20°. The methanol was evaporated under reduced pressure. Saturated aqueous sodium chloride (10 ml) was added, and the organic phase was decanted, clarified (Na_2SO_4), and distilled at 45° and 16 mmHg. The whole procedure was repeated: the acetophenone was then added to an equal volume of perdeuteriated dimethyl sulphoxide. The ^{1}H n.m.r. spectrum indicated that the acetophenone comprised 80% of the [2′-$^{2}H_3$]compound.

(d) [^{13}C]Chloroform (0·15 ml) in dimethyl sulphoxide (1 ml) was kept with tritiated water (30 μl) and 10% sodium hydroxide (2·5 μl) for 18 h at 20°. Deuteriochloroform (1 ml) and water (10 ml) were added (shaking), and the organic phase was separated and dried (Na_2SO_4).

(e) Diethyl malonate (0·25 ml) and diethyl [2-^{13}C]malonate (0·25 ml) were each kept in dioxan (0·5 ml) with tritiated water (10 μl) and anhydrous sodium carbonate (10 mg) for 48 h at 20°. The liquids were decanted into saturated aqueous sodium chloride (10 ml portions), the organic phases were separated and dried (Na_2SO_4), and a portion of each (70 μl) was diluted with perdeuteriated dimethyl sulphoxide (30 μl).

Deuteriation of diethyl malonate, as for acetophenone but without distillation, afforded diethyl [2-^{2}H]malonate (70% labelled; ^{1}H n.m.r. spectrum); no [$^{2}H_2$]compound was obtained.

(f) Dimethyl sulphoxide (2 g), tritiated water (0·1 ml), and 10% sodium hydroxide (60 μl) were kept together at 90° for 18 h. After neutralisation (HCl), water was distilled off at 40° and 16 mmHg, and then the tritiated dimethyl sulphoxide at 110° and 16 mmHg. A portion (50 μl) was diluted with perdeuteriated dimethyl sulphoxide (50 μl).

(g) Nitromethane (0·5 ml) was kept with tritiated water (15 μl) and solid sodium hydroxide (1 pellet) at 20° for 18 h. The liquid was decanted and dried (Na_2SO_4) and a portion (50 μl) diluted with perdeuteriated dimethyl sulphoxide (50 μl).

(h) Sodium [2-^{13}C]acetate (0·1 g), sodium hydroxide (1 pellet), water (100 μl), and tritiated water (60 μl) were heated together in a sealed tube at 90° for 1 week. The solution was neutralised (to phenolphthalein) with perdeuteriated acetic acid, and freeze-dried. The residue was then dissolved in deuterium oxide to give a saturated solution.

(i) [5-^{3}H]Uridine (50 mCi, 25 Ci mmol^{-1}), [6-^{3}H]uridine (30 mCi, 20 Ci mmol^{-1}), and [5,6-^{3}H]uridine (44 mCi, 29 Ci mmol^{-1}) were each examined in perdeuteriated dimethyl sulphoxide and then the first two solutions in admixture.

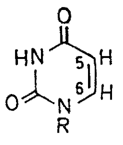
N.m.r. Measurements.—^{1}H, ^{3}H, and ^{13}C Spectra were obtained at 25 ± 1° with a Bruker WH90 pulse (Fourier transform) spectrometer operating at 90, 96, and 22·6 MHz (nominal), respectively, and constant field locked to the deuteron signal from the deuteriated compound or solvent in the sample. For ^{1}H and ^{3}H n.m.r. measurements, solutions were sealed in cylindrical microcells (100 μl; Wilmad) which were then inserted into standard n.m.r. tubes (5 mm). For the ^{13}C measurements made on the natural abundance in deuteriated samples, perdeuteriated acetonitrile and deuteriochloroform were placed direct in 10 mm diam. n.m.r. tubes, the deuteriated acetophenone and the deuteriated diethyl malonate were examined as solutions (1 : 1 and 7 : 3, respectively) in perdeuteriated dimethyl sulphoxide, whilst the sodium acetate solution was sealed in a 1 ml cylindrical cell (Wilmad) placed in a 10 mm n.m.r. tube.

The free-induction decay signals were mostly acquired into 8K channels and the frequency range was usually chosen to give a display spectral width (after Fourier transformation) of 300 Hz. Errors in the results, taken from the printout, were expressed accordingly.[1] Sets of results (mostly 6—12) were averaged (see Tables).

For calculating values of J (Tables 1—4), the ratios γ_T/γ_H and γ_H/γ_D were taken as 1·06664 [1,4] and 6·5144 [5] respectively. These values are strictly the Larmor frequency ratios but suffice because the ratios of the isotopic nuclear screenings must be virtually unity.[4]

RESULTS AND DISCUSSION

Primary Isotope Effects on Proton Coupling Constants.—Earlier observations [2] made with simple equipment indicated that a J_{HT} coupling was only roughly predictable from the J_{HH} coupling constant. More accurate measurements with the fully stabilised pulse spectrometer show that the smaller J_{HT} couplings in organic compounds are closely predictable, as illustrated in Table 1 which lists the three-bond couplings in isotopically labelled uridine (I). A significant difference between the J(5-H,6-T) and J(5-T,6-H) was not detected, as had been considered possible.[2] Also, J_{HT} and J_{TT} coupling constants calculated from the (most precisely measured) J_{HH} coupling were not significantly different from those



(I) R = β-D-ribofuranose

---

[4] W. Duffy, *Phys. Rev.*, 1959, **115**, 1012.

[5] T. F. Wimett, *Phys. Rev.*, 1953, **91**, 499.

observed, except for J(5-T,6-H) where there is a discrepancy of 0·1 ± 0·06 Hz which may represent a ^{3}H primary isotope effect on this ^{3}J coupling in uridine.

Table 1

Vicinal hydrogen–hydrogen nuclear spin couplings in uridine and tritiated uridines from ^{1}H and ^{3}H n.m.r. spectra

^{3}J/Hz	Obs. value (Hz)		Calc. value from $^{3}J_{\rm HH}$/Hz
5-H, 6-H	8·06 ± 0·04		
5-H, 6-T	8·55 ± 0·07	mean of set 8·52 ± 0·04	8·60 ± 0·04
5-T, 6-H	8·50 ± 0·05		
5-T, 6-T	9·01 ± 0·24 *		9·17 ± 0·05

* Precision spoiled by line broadening resulting from radiolysis.

Table 2 shows differences between observed $J_{\rm CT}$ coupling constants and calculated values [derived from the relation $J_{\rm CT}$(calc.) = $J_{\rm CH}$ × 1·06664] as primary isotope effects. These last are about ±0·22 Hz for direct CH couplings of *ca.* 130 Hz. The larger effect found for chloroform, −1·52 ± 0·06 Hz, was substantiated by separate sets of measurements on different occasions. Proportionality between a primary isotope effect and the numerical value of the coupling would have led to a predicted effect of |0·35| Hz for tritiochloroform. The observed discrepancy is 4·3 times larger: reasons are being sought.

Table 2

Direct carbon–proton and –triton couplings measured from the ^{1}H and ^{3}H n.m.r. spectra, and primary isotope effects

Compound (position tritiated)	$^{1}J_{\rm CH}$/Hz	$^{1}J_{\rm CT}$/Hz	$J_{\rm CT}$(calc.)/Hz	Primary isotope effect/Hz
Acetonitrile (2)	136·20 ± 0·02 [a,c]	145·02 ± 0·08	145·26 ± 0·02	−0·24 ± 0·08
Acetophenone (2′)	127·34 ± 0·02	136·09 ± 0·07	135·81 ± 0·02	+0·28 ± 0·07
Chloroform	209·12 ± 0·05 [b,d]	221·51 ± 0·02	223·03 ± 0·05	−1·52 ± 0·06
Diethyl malonate (2)	132·28 ± 0·04	140·90 ± 0·08	141·08 ± 0·04	−0·18 ± 0·09
Sodium acetate (2)	127·12 ± 0·05	135·78 ± 0·06	135·58 ± 0·05	+0·20 ± 0·08

N. Muller and D. E. Pritchard, *J. Chem. Phys.*, 1959, **31**, 1471, give [a] 136 and [b] 209. E. Breitmaier, G. Jung, W. Voelter, and L. Pohl, *Tetrahedron*, 1973, **29**, 2485, give [c] 107·4 and [d] 209·9.

Quantum mechanical analysis incorporating Fermi contact theory indicates that the coupling $J_{\rm AX}$ between adjacent nuclear spins is proportional to the product of the magnetogyric ratios $\gamma_{\rm A}\gamma_{\rm X}$.[6] 'The Fermi-type coupling is also likely to give the principal contribution to the coupling between protons in other molecules where there is no direct bond.'[7] Fraser *et al.*[8] point out that the theory neglects changes in electron distribution resulting from an isotopic substitution so that discrepancies may be found between predicted and measured couplings. Early attempts to observe such primary isotope effects were negative,[9] possibly because the precision was insufficient for the magnitudes of the couplings investigated. With the very large electron–proton spin couplings in radicals, appreciable deuteron isotope effects are apparent.[10] With $^{2}J_{\rm HF}$ and $^{1}J_{\rm HP}$ couplings, comparison with the corresponding deuteron couplings revealed just significant primary isotope effects.[11] For $^{1}J_{\rm CH}$ and $^{1}J_{\rm CD}$ in the methyl group of toluene, results have been ambiguous.[12] Recently, Fraser *et al.*[8] claimed a significant primary isotope effect on $|^{2}J_{\rm HH}|$ (12·97 Hz) for the methylene group of benzyl methyl sulphoxide, monodeuterium substitution lowering the value (calc.) by 0·46 ± 0·2 Hz. The possibility that this was caused by the ^{2}H quadrupole relaxation was apparently eliminated. The results of Gold *et al.*[13] on deuterium isotope effects on $^{1}J_{\rm CD}$ couplings are unfortunately not quite significant except for chloroform (1 ± 0·9 Hz) because the precision of measurement was too poor (for inherent reasons). Gold's conclusion [13] that observed $J_{\rm CH}$ coupling constants seem on average to be greater than those calculated from observed $J_{\rm CD}$ couplings agree fully with our observations, differently expressed in Table 3. We find that observed $J_{\rm CT}$ coupling constants (Table 2) are also larger than those calculated from the observed $J_{\rm CD}$ couplings.

Table 3

Direct carbon–deuteron coupling constants measured from ^{13}C n.m.r. spectra, and derived values from the corresponding proton and triton couplings

Compound	$^{1}J_{\rm CD}$/Hz (obs.)	Calc. from $J_{\rm CH}$/Hz [a]	Calc. from $J_{\rm CT}$/Hz [b]
Acetonitrile	20·78 ± 0·13	20·91 ± 0·01	20·87 ± 0·03
Acetophenone	19·25 ± 0·32	19·55 ± 0·01	19·59 ± 0·03
Chloroform	31·98 ± 0·14	32·10 ± 0·02	31·88 ± 0·01
Diethyl malonate	20·15 ± 0·32	20·31 ± 0·02	20·28 ± 0·03
Sodium acetate	19·51 ± 0·12	19·51 ± 0·02	19·54 ± 0·02

[a] From the ^{1}H n.m.r. spectrum (see Table 2). [b] From the ^{3}H n.m.r. spectrum (see Table 2).

---

[6] J. A. Pople, W. G. Schneider, and H. J. Bernstein, 'High-resolution Nuclear Magnetic Resonance,' McGraw-Hill, New York, 1959, pp. 184—188.

[7] Ref. 6, p. 189.

[8] R. R. Fraser, M. A. Petit, and M. Miskow, *J. Amer. Chem. Soc.*, 1972, **94**, 3253.

[9] H. S. Gutowsky, V. D. Mochel, and B. G. Somers, *J. Chem. Phys.*, 1962, **36**, 1153; P. Diehl and T. Leipert, *Helv. Chim. Acta*, 1964, **47**, 545.

[10] R. G. Lawler, J. R. Bolton, M. Karplus, and G. K. Frankel, *J. Chem. Phys.*, 1967, **47**, 2149.

[11] C. N. Banwell, J. N. Murrell, and M. A. Turpin, *Chem. Comm.*, 1968, 1466; A. A. Borisenko, N. M. Sergeyev, and Yu. A. Ustynyuk, *Mol. Phys.*, 1971, **22**, 715.

[12] M. Murray, *J. Magnetic Resonance*, 1973, **9**, 326.

[13] H. N. Colli, V. Gold, and J. E. Pearson, *J.C.S. Chem. Comm.*, 1973, 408.

This may imply, as does the greater line width of deuteron induced splittings, that the J_{CD} couplings are really slightly reduced couplings as a result of the deuteron relaxation. It seems that apparent ^2H primary isotope effects on couplings need to be assessed with caution.

TABLE 4

Geminal triton–proton couplings measured from ^3H n.m.r. spectra and derived proton–proton values, with deuteron–proton and derived proton–proton values for comparison

Compound (position tritiated)	$\lvert ^2J_{HT} \rvert$/Hz (obs.)	$\lvert ^2J_{HH} \rvert$/Hz (calc. from J_{HT})	$\lvert ^2J_{HH} \rvert$/Hz (calc. from J_{HD})	$\lvert ^2J_{HD} \rvert$/Hz (obs.)	$\lvert ^2J_{HD} \rvert$/Hz (calc. from J_{HT})
Acetone (1)	15·27 ± 0·03	14·32 ± 0·03	14·9 ± 0·3 [a] 14·33 ± 0·07 [c]	2·27 ± 0·05 [b] 2·20 ± 0·01 [c]	2·20 ± 0·01
Acetonitrile (2)	17·15 ± 0·06	16·08 ± 0·06	16·9 ± 0·3 [a]	2·58 ± 0·04 [a]	2·47 ± 0·02
Acetophenone (2′)	15·38 ± 0·05	14·42 ± 0·05	14·40 ± 0·26 [d]	2·20 ± 0·04 [e]	2·21 ± 0·02
Dimethyl sulphoxide	13·24 ± 0·04	12·41 ± 0·04	12·12 ± 0·26 [c]	1·86 ± 0·04 [e]	1·91 ± 0·02
Diethyl malonate (2)	16·84 ± 0·04	15·79 ± 0·04			2·42 ± 0·01
Nitromethane	13·69 ± 0·10	12·84 ± 0·10	13·2 ± 0·2 [a]	2·02 ± 0·03 [a]	1·97 ± 0·04
Sodium acetate (2)	14·95 ± 0·03	14·02 ± 0·03	14·2 ± 0·3 [f]	2·16 ± 0·05 [f]	2·15 ± 0·01

[a] M. Barfield and D. M. Grant, *J. Amer. Chem. Soc.*, 1961, **83**, 4726. [b] H. S. Gutowsky, M. Karplus, and D. M. Grant, *J. Chem. Phys.*, 1959, **31**, 1278. [c] This work. [d] Calc. from J_{HD} given in ref. *e*. [e] C. G. Macdonald, J. S. Shannon, and S. Sternhell, *Austral. J. Chem.*, 1964, **17**, 38. [f] M. Barfield and D. M. Grant, *J. Amer. Chem. Soc.*, 1963, **85**, 1899.

Just as the $^1J_{CD}$ couplings given in Table 3 have inherently poor precision, so $^2J_{HD}$ couplings measured from the ^1H n.m.r. spectra of deuteriated compounds have poor precision because of their small magnitudes and the inferior line widths. Moreover, they too may be slightly reduced couplings.

Geminal Proton–Proton Couplings.—Table 4 records $^2J_{HT}$ couplings measured from ^3H spectra, together with $^2J_{HH}$ coupling constants calculated from them. Thus a new set of geminal proton–proton couplings is obtained (where the two nuclei are isochronous) of higher precision than any calculated hitherto.

Table 4 includes for comparison the rather imprecise $^2J_{HH}$ coupling constants in the literature, which have been calculated from observed $^2J_{HD}$ couplings. These latter are also listed, together with $^2J_{HD}$ couplings calculated from the $^2J_{HT}$ coupling constants.

Carbon Secondary Isotope Effects on $^2J_{HT}$ *Couplings.*—The effect of replacing ^{12}C by ^{13}C on the $^2J_{HT}$ coupling constant for a tritiated methyl or methylene group, using the compounds available, was undetectable.

Secondary Isotope Effects on Chemical Shifts.—Incidentally, several secondary isotope effects on ^{13}C, ^1H, and ^3H chemical shifts were observed. Table 5 shows that the effects of hydrogen isotope substitution on ^{13}C chemical shifts are of the order of 1—10 Hz at 22·6 MHz but the effects of carbon isotope substitution on ^1H or ^3H chemical shifts are only a few tenths Hz at 90 and 96 MHz, reflecting of course the much wider range of chemical shifts for ^{13}C as opposed to hydrogen nuclei. No simple correlation between different compounds for an induced shift and the number of isotopic substitutions emerges. Thus the change from a $^{13}CH_3$ group to a $^{13}CD_3$ group produces an upfield ^{13}C chemical shift of 0·46—0·17 p.p.m. amongst the compounds examined. In all the present examples of secondary isotope effects on chemical shifts, the heavier isotope produces an upfield shift.

TABLE 5

Secondary isotope effects on some chemical shifts

Compound (group)	Isotopic shift/Hz	P.p.m. upfield
Acetonitrile (2)	$\sigma_C(^{13}CD_3) - \sigma_C(^{13}CH_3) = 10·33 \pm 0·17$	0·46
Acetophenone (2′)	$\sigma_C(^{13}CD_3) - \sigma_C(^{13}CH_3) = 5·48 \pm 0·03$	0·24
	$\sigma_H(^{13}CH_3) - \sigma_H(^{12}CH_3) = 0·11 \pm 0·03$	0·001
Chloroform	$\sigma_C(^{13}CD) - \sigma_C(^{13}CH) = 4·77 \pm 0·18$	0·21
	$\sigma_H(^{13}CH) - \sigma_H(^{12}CH) = 0·37 \pm 0·05$	0·004
	$\sigma_T(^{13}CT) - \sigma_T(^{12}CT) = 0·33 \pm 0·02$	0·004
Diethyl malonate (2)	$\sigma_C(^{13}CHD) - \sigma_C(^{13}CH_2) = 5·85 \pm 0·35$	0·26
	$\sigma_H(^{13}CH_2) - \sigma_H(^{12}CH_2) = 0·16 \pm 0·03$	0·002
Sodium acetate (2)	$\sigma_C(^{13}CD_3) - \sigma_C(^{13}CH_3) = 3·79 \pm 0·29$	0·17

We thank the S.R.C. and The Radiochemical Centre for support and the Director of the latter, Dr. W. P. Grove, for permission to publish. We also thank the University of Basrah, Iraq, for study leave to Dr. Al-Rawi.

[4/1618 *Received, 2nd August,* 1974]