Tetrahedron Vol. 33, pp. 2577 to 2581 Pergamon Press 1977. Printed in Great Britain

# IDENTIFICATION OF HALOGEN SUBSTITUENTS IN NATURAL PRODUCTS BY MEASUREMENT OF $^{13}$C SPIN-LATTICE RELAXATION TIMES AND INTEGRATED INTENSITIES

RAYMOND S. NORTON
Roche Research Institute of Marine Pharmacology, P.O. Box 255, Dee Why, 2099, Australia

(*Received in the UK* 19 *April*; *Accepted for publication* 16 *May* 1977)

**Abstract**—A method is proposed for differentiating brominated carbons from chlorinated carbons by means of natural-abundance $^{13}$C NMR spectroscopy. The basis of the method is that the spin-lattice relaxation behaviour of brominated carbons is influenced by carbon–bromine scalar interactions, which can lead to shortened $^{13}$C spin-lattice relaxation times and reduced values of the nuclear Overhauser enhancement. C–Cl scalar interactions make a negligible contribution to the spin-lattice relaxation of chlorinated carbons. These effects are illustrated by measurement of the $^{13}$C spin-lattice relaxation times and integrated intensities of chloro-, bromo and iodobenzene and chloro-, bromo- and iodocyclohexane. The method is then tested on four polyhalogenated marine natural products. The results indicate that $^{13}$C relaxation measurements can be used to distinguish brominated carbons from chlorinated carbons in the case of halogenated quaternary carbons, *sp*$^2$ hydridized methine carbons and some *sp*$^3$ hydridized methine carbons, but not in the case of halogenated methylene carbons or *gem*-dihalo substituted methine carbons.

A number of polyhalogenated metabolites have recently been isolated from marine organisms,[1,2] in particular algae.[3–6] Many of these metabolites contain both bromine and chlorine. In these cases it has often been difficult to locate the halogens in the molecule without recourse to X-ray crystallography, a situation that has arisen partly because natural-abundance $^{13}$C NMR spectroscopy, which is used extensively in structure determination,[7,8] does not permit the differentiation of brominated carbons from chlorinated carbons. The substituent effects of bromine and chlorine on $^{13}$C chemical shifts are similar,[7] and the $^{13}$C–$^{1}$H scalar coupling constants of chlorinated and brominated carbons are comparable.[7]

In this report we show the potential and some limitations of the measurement of $^{13}$C spin-lattice relaxation times and integrated intensities as a means of distinguishing brominated carbons from chlorinated carbons. The basis of this approach is that brominated carbons are influenced by carbon–bromine scalar interactions,[9–12] which can lead to shortened $^{13}$C spin-lattice relaxation times and reduced values of the NOE. On the other hand, C–Cl scalar interactions make a negligible contribution to the spin-lattice relaxation of chlorinated carbons. This behaviour is confirmed in the present communication by measurement of the $^{13}$C spin-lattice relaxation times and integrated intensities of three monohalogenated benzene derivatives, three monohalogenated cyclohexane derivatives, and β-bromostyrene. The general applicability of the method is then examined with the use of a number of polyhalogenated marine natural products.

## THEORETICAL CONSIDERATIONS

A number of interactions can contribute to the relaxation of $^{13}$C nuclei,[8–12] but the relaxation of most protonated carbons of large organic molecules is dominated by $^{13}$C–$^{1}$H dipole–dipole interactions with directly-bonded hydrogens.[8–13] The relaxation of nonprotonated carbons is also dominated by the $^{13}$C–$^{1}$H dipolar mechanism in many cases.[13,14] Contributions from the spin-rotation relaxation mechanism[15,16] can usually be ignored for large organic molecules,[11–14] and chemical shift anisotropy[15,16] makes a negligible contribution to $^{13}$C relaxation at low magnetic field strength.[11,12,17] Dipolar interactions of carbon with nuclei other than protons (e.g. $^{14}$N) must be taken into account, but these contributions can be calculated fairly accurately,[14] provided the appropriate rotational correlation times are known. In the absence of paramagnetic impurities, the only other significant contribution to $^{13}$C relaxation is likely to come from scalar interactions.

If a $^{13}$C nucleus interacts with a nucleus X which is relaxing rapidly, such that its spin-lattice relaxation time $T_1^x$ is short compared with the reciprocal of the $^{13}$C–X spin-spin coupling constant, A (in radians sec$^{-1}$), then the $^{13}$C may undergo relaxation by a mechanism known as "scalar relaxation of the second kind".[15] In the case of a molecule undergoing isotropic rotational reorientation, the contribution of this interaction to the spin-lattice relaxation time ($T_1$) of a $^{13}$C nucleus is given by eqn (1).[15,16]

$$\frac{1}{T_1} = \frac{2A^2}{3} S(S+1) \frac{T_1^x}{1+(\omega_c-\omega_x)^2(T_1^x)^2} \qquad (1)$$

where $S$ is the spin quantum number of nucleus X, $T_1^x$ is the spin-lattice relaxation time of nucleus X, and $\omega_c$ and $\omega_x$ are the resonance frequencies (in radians sec$^{-1}$) of $^{13}$C and X, respectively. Nuclei with $S \geqslant 1$ may undergo efficient relaxation as a result of quadrupolar interactions.[15,16] In this case, $T_1^x$ is given by eqn (2),[15] where

$$\frac{1}{T_1^x} = \frac{3}{40} \frac{2S+3}{S^2(2S-1)} \left(1+\frac{n^2}{3}\right) \left(\frac{e^2Qq}{\hbar}\right)^2 \tau_R \qquad (2)$$

n is the asymmetry parameter[15,18] and ($e^2Qq/\hbar$) the quadrupole coupling constant.[15,18] Note that eqn (2) is valid only in the "extreme narrowing limit."[15,16]

Scalar interactions make a significant contribution to the spin-lattice relaxation of a $^{13}$C nucleus only when $1/T_1^x \approx (\omega_c - \omega_x)$. For those halogen nuclei with $S > 1$, the values of $2\pi(\omega_c - \omega_x) \times 10^{-6}$ at 14.1 kG are:

$^{35}$Cl, 9.21; $^{37}$Cl, 10.20; $^{79}$Br, 0.05; $^{81}$Br, − 1.12; $^{127}$I, 3.08.

Thus, we expect scalar interactions to contribute to the relaxation of brominated carbons and, to a lesser extent, iodinated carbons, but not to that of chlorinated carbons. Indeed, the presence of scalar relaxation for $^{13}$C nuclei directly bonded to bromine has been reported for a number of simple organic molecules.[9–12,19] In bromobenzene, differentiation of $^{13}$C–$^{79}$Br and $^{13}$C–$^{81}$Br relaxation has been reported.[12,19] For carbons bonded to $^{79}$Br scalar relaxation dominates, but for carbons bonded to $^{81}$Br it is likely that $^{13}$C–$^{81}$Br dipolar relaxation and scalar relaxation both contribute significantly to $^{13}$C relaxation.[12,19] These interactions are expressed as a reduction in the $T_1$ (compared with that calculated for purely $^{13}$C–$^{1}$H dipolar relaxation) and the NOE.[7,8,20]

## RESULTS AND DISCUSSION

Table 1 shows the $^{13}$C spin-lattice relaxation times and integrated intensities for some monohalogenated benzene and cyclohexane derivatives. In each case, the $T_1$ values for carbons 2–6 decrease as the size of the halogen substituent increases, and the ratio $T_1$(C-2, C-3, C-5, C-6)$_{av}$/$T_1$(C-4) increases. The latter result probably reflects an increase in the extent of anisotropy of molecular reorientation. The $T_1$ for C-1 of bromobenzene is shorter than that for C-4, whereas in chlorobenzene the $T_1$ for C-1 is considerably longer than that for C-4. As mentioned above, the relaxation of C-1 of bromobenzene has been analysed previously[12,19] in terms of scalar relaxation for $^{13}$C–$^{79}$Br, and a mixture of scalar relaxation and $^{13}$C–$^{81}$Br dipolar relaxation for $^{13}$C–$^{81}$Br. These interactions account for the reduction in NOE of C-1 of bromobenzene (Table 1).

In the case of C-1 of chlorobenzene in $CD_3CN$, the predicted $T_1$ is ~450 sec if only $^{13}$C–$^{1}$H dipolar interactions with the *ortho* protons[14] are considered. However, the results in Table 1 ($T_1 = 96$ sec, intensity = 1.6) indicate that other interactions dominate the relaxation of this carbon. The most likely contributors are spin-rotation[11,12] and $^{13}$C–Cl scalar interactions. We distinguished between these two mechanisms by measuring $T_1$ values and integrated intensities for chlorobenzene in $d_6$-DMSO, where the rate of overall molecular reorientation is decreased (as indicated by the decrease in the $T_1$ values for C-2 to C-6, see Table 1). If scalar interactions dominated the relaxation, a reduction in the $T_1$ of the substituted carbon would be expected, with little or no change in the NOE, whereas, if spin-rotation dominated, increases in both $T_1$ and NOE would be observed.† The results in Table 1 indicate that spin-rotation dominates the

Table 1. Spin-lattice relaxation times and integrated intensities for carbons of monohalogenated benzene and cyclohexane derivatives

| Sample[a] | $T_1$(sec)[b] | | | | Intensity[c] | | | |
|---|---|---|---|---|---|---|---|---|
| | C-1 | C-2 C-6 | C-3 C-5 | C-4 | C-1 | C-2 C-6 | C-3 C-5 | C-4 |
| Chlorobenzene | 96 | 19.5 | 20.0 | 15.7 | 1.6 | 6.2 | 6.5 | 3.0 |
| Chlorobenzene[d] | 118 | 12.9 | 12.2 | 10.5 | 2.1 | 6.4 | 6.4 | 3.0 |
| Bromobenzene | ~7[e] | 16.4 | 15.2 | 11.2 | 1.0 | 6.6 | 6.1 | 3.0 |
| Iodobenzene | 8.9 | 11.8 | 11.6 | 7.1 | 1.1 | 6.0 | 6.1 | 3.0 |
| Chlorocyclohexane | 19.3 | 11.4 | 11.5 | 9.9 | 2.9 | 6.0 | *f* | *f* |
| Bromocyclohexane | ~10[e] | 8.9 | 8.7 | 7.2 | 2.3 | 5.7 | 5.8 | 3.0 |
| Iodocyclohexane | 9.5 | 6.3 | 6.5 | 4.8 | 2.9 | 6.2 | 6.2 | 3.0 |

[a] 70% (v/v) in $CD_3CN$ at 31°, unless otherwise indicated.

[b] Estimated accuracy is ± 10%.

[c] Normalised by setting intensity of C-4 equal to 3.0. Estimated accuracy is ± 10%. In some cases the absolute NOE of carbons 2 to 6 was determined by gated proton decoupling, and found to be equal to 3.0 within experimental error.

[d] 50% (v/v) in $(CD_3)_2SO$ at 31°.

[e] Relaxation not decribed by a single exponential, because relaxation times of carbons bonded to $^{79}$Br and $^{81}$Br differ from one another.[12,19]

[f] C-3/C-5 and C-4 resonances overlap (total intensity = 9.3, when intensity of C-2/C-6 set to 6.0).

---

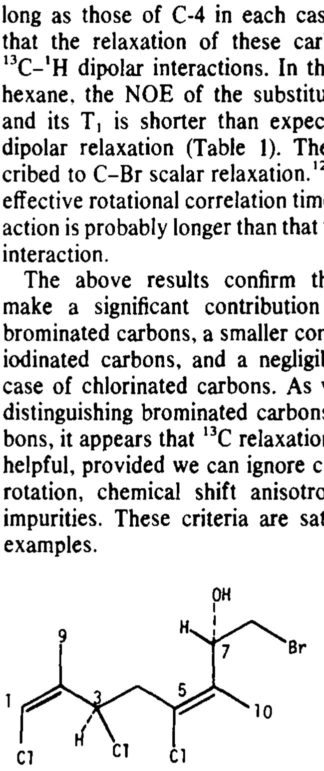
†The rationale for these conclusions is as follows. A decrease in the rate of overall molecular reorientation in DMSO would lead to an increase in the efficiency of the $^{13}$C–$^{1}$H dipolar mechanism ($T_1$ predicted by considering interactions with *ortho* protons decreases to ~300 sec.). The efficiency of scalar relaxation would also increase in DMSO, so we would expect a decrease in the observed $T_1$, with little or no change in NOE (depending on whether or not the *relative* efficiencies of the scalar and $^{13}$C–$^{1}$H dipolar mechanisms altered). On the other hand, the decrease in the rate of overall reorientation would lead to a *decrease* in the efficiency of the spin-rotation relaxation mechanism.[15,16] This decrease, coupled with an increase in efficiency of the $^{13}$C–$^{1}$H dipolar contribution would lead to an increase in NOE. The change in $T_1$ can be predicted by assuming that only the spin-rotation and $^{13}$C–$^{1}$H dipolar mechanisms are important in $CD_3CN$. This yields values of 137 and 320 sec., respectively, for the contributions to $T_1$ of the two mechanisms in $CD_3CN$. From known equations[15,16] we estimate that the corresponding in DMSO would be 209 and 210 sec. This yields a total $T_1$ of 105 sec. and NOE = 2.0. Thus, both $T_1$ and the NOE would increase in DMSO if spin-rotation dominated.

relaxation of C-1 of chlorobenzene in $CD_3CN$ ($T_1$ spin-rotation ≈ 140 sec), consistent with the expectation that $^{13}C$–Cl scalar interactions would be negligible.[11,12]

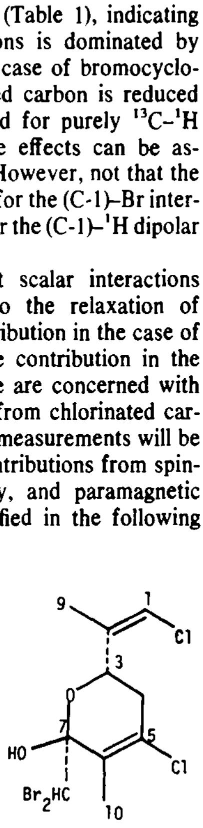
The substituted carbon of iodobenzene has a short $T_1$ and small NOE (Table 1), indicating that interactions other than $^{13}C$–$^1H$ dipolar dominate its relaxation. The $T_1$ values of the *protonated* carbons of iodobenzene are *shorter* than those of chlorobenzene, so that the contribution of spin-rotation to $T_1$ of C-1 is likely to be significantly longer than 140 sec. Thus, the major contribution to the $T_1$ of this carbon probably comes from $^{13}C$–$^{127}I$ scalar interactions ($^{13}C$–$^{127}I$ dipolar interactions make a negligible contribution).† Contributions from $^{13}C$–$^{127}I$ scalar interactions to the relaxation of carbons in substituted methanes have been observed previously,[9,11] but the effects have been small.

In chloro- and iodocyclohexane the substituted carbons have the full NOE, and their $T_1$ values are twice as long as those of C-4 in each case (Table 1), indicating that the relaxation of these carbons is dominated by $^{13}C$–$^1H$ dipolar interactions. In the case of bromocyclohexane, the NOE of the substituted carbon is reduced and its $T_1$ is shorter than expected for purely $^{13}C$–$^1H$ dipolar relaxation (Table 1). These effects can be ascribed to C–Br scalar relaxation.[12] However, not that the effective rotational correlation time for the (C-1)–Br interaction is probably longer than that for the (C-1)–$^1H$ dipolar interaction.

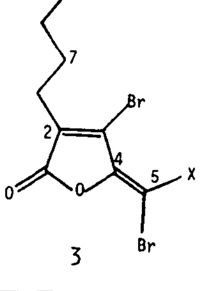
The above results confirm that scalar interactions make a significant contribution to the relaxation of brominated carbons, a smaller contribution in the case of iodinated carbons, and a negligible contribution in the case of chlorinated carbons. As we are concerned with distinguishing brominated carbons from chlorinated carbons, it appears that $^{13}C$ relaxation measurements will be helpful, provided we can ignore contributions from spin-rotation, chemical shift anisotropy, and paramagnetic impurities. These criteria are satisfied in the following examples.



1



2



3

a: X = Br
b: X = H

Costatol (**1**) and costatone (**2**) are polyhalogenated monoterpenes from the red alga *Plocamium costatum*.[4,5] The $T_1$ values and integrated intensities for the carbons of costatol are given in Table 2. These results allow the positions of the halogens to be assigned given the basic skeleton and the information that costatol contains 3 chlorine atoms and 1 bromine atom. Thus, for the non-protonated carbon C-5, the absence of a significant reduction in the $T_1$ and NOE values is not consistent with bromine substitution. A similar argument applies to the halogenated methine carbons C-1 and C-3, which have $T_1$ and NOE values identical with those of C-7. Thus, we must place the bromine on C-8. The relaxation of this carbon is dominated by $^{13}C$–$^1H$ dipolar interactions because C–Br scalar interactions cannot compete effectively when a carbon is bonded to two hydrogens. Note that costatol behaves as an isotropic rigid rotor,[15,16] i.e. the $NT_1$ values (N = number of directly-bonded hydrogens) for the CH and $CH_2$ carbons are the same, although both Me groups undergo internal rotation.

Costatone (**2**) contains 2 chlorine atoms and 2 bromine atoms. The $^{13}C$ $T_1$ values and integrated intensities are given in Table 2. As in the case of costatol, we can establish that C-5 is not brominated. However, in distributing the remaining chlorine and 2 bromines between C-1 and C-8 we *cannot* make use of relaxation measurements. The relaxation behaviour of C-1 is consistent with chlorine substitution, but the contribution of the 2 bromines to C-8 relaxation is much less that expected on the basis of the bromocyclohexane data (Table 1). This effect may be due to a change in the quadrupole coupling constant[18] as a result of a change in the electric field gradient[18] at the bromine, or to a change in the C–Br scalar coupling constant. In any case, it appears that the method may not be useful for *gem*-dihalo substituted protonated carbons (see below).

In order to determine the effect of bromine substituents on the relaxation of olefinic $sp^2$ hydridized carbons, we examined the $^{13}C$ relaxation behaviour of the fimbrolides **3a** and **3b** isolated from the red alga *Delisea fimbriata* (Bonnemaisoniaceae).[6] Measurements were carried out on a mixture of **3a** and **3b** (ratio 1.7 : 1; total solute 1.3M). The results are given in Table 2. In each case the brominated carbon C-3 is readily identified. In **3a** the dibrominated olefinic carbon C-5 has a $T_1$ value approximately half that of the monobrominated carbon C-3, which suggests that the effects of the two bromine substituents on the spin-lattice relaxation rate of C-5 are independent of one another, in contrast to the case of C-8 of **2** (see above).

The relaxation behaviour of C-4 in **3a** emphasizes an important point, viz. that the measurement of integrated intensities alone cannot be used as evidence for bromine substitution in the case of quaternary carbons. The long $T_1$ of this carbon indicates that it is not relaxed by C–Br scalar interactions, and the low intensity simply reflects the fact that $^{13}C$–$^1H$ dipolar interactions make a negligible contribution to its relaxation because the nearest hydrogens are four bonds removed. This carbon is probably relaxed by a mixture of C–Br dipolar interactions and chemical shift anisotropy, weak though these two contributions are. In **3b** the C-5 hydrogen makes a significant contribution to the relaxation of C-4.

In the case of C-5 of **3b** the directly-bonded hydrogen and bromine both contribute significantly to its spin-lattice relaxation. Thus, it appears that relaxation measurements can be used to identify the brominated

---

†The possibility that paramagnetic impurities are responsible for the observed reduction of $T_1$ and NOE was eliminated by a control experiment in which the $T_1$ and NOE of chlorobenzene were measured in the presence of an equal volume of iodobenzene. The $T_1$ and NOE values of the halogenated carbons in *both* species were essentially identical with those given in Table 1.

Table 2. Spin-lattice relaxation times and integrated intensities for carbons of 1, 2, 3a and 3b.[a]

| Carbon[b] | 1 | | 2 | | 3a | | 3b | |
|---|---|---|---|---|---|---|---|---|
| | δ[c] | $T_1$(int.)[d] | δ[c] | $T_1$(int.)[d] | δ[c] | $T_1$(int.)[d] | δ[c] | $T_1$(int.)[d] |
| 1 | 115.6 | $1.8_5$(3.0) | 113.3 | 1.8(2.9) | 164.5 | ~180(2.1) | 165.8 | ~120(1.8) |
| 2 | | *e* | *h* | | 137.9 | 47(2.6) | 133.8 | 52(2.4) |
| 3 | 56.4 | $1.9_5$(2.8) | 67.6 | 1.9(3.0) | 128.2 | 11(1.7[j]) | 129.9 | 8(0.9) |
| 4 | 37.0 | 1.1 (3.1)[f] | 35.0 | 1.0(3.0) | 144.6 | >200(1.2) | 149.9 | ~120(2.0) |
| 5 | | *e* | *h* | | 81.2 | 4.1(0.8) | 90.6 | 2.6(2.1) |
| 6 | | *e* | *h* | | 25.7 | 2.2(2.7)[k] | 25.0 | 2.3(2.9)[k] |
| 7 | 71.2 | 1.9 (3.0) | 96.9 | 27(2.9) | 28.7 | 2.5(3.0) | 28.9 | 3.1[l](3.0) |
| 8 | 41.4 | $1.0_5$(3.1)[f] | 51.6 | 1.5($2.7_5$) | 22.4 | 3.3(3.1) | 22.4 | 3.3(3.1) |
| 9 | 14.4 | 6 (3.0)[g] | 13.8 | 4.9(3.0)[i] | 13.6 | 3.7(3.1) | 13.6 | 3.7(3.1) |
| 10 | 16.2 | 4 (3.0)[g] | 16.0 | 2.6(3.0)[i] | | | | |

*a.* 0.6 - 1.1M solutions in $CDCl_3$ at 32 - 33°.

*b.* Numbering system is shown in structure diagrams.

*c.* Chemical shift (in ppm downfield from TMS).

*d.* Spin-lattice relaxation times in sec and integrated intensities (in parentheses). Integrated intensities were normalised in each case by assigning a value of 3.0 to the average of the intensities of nonhalogenated, protonated carbon resonances, as follows: **1**, C-7, C-9 and C-10; **2**, C-3, C-4, C-9 and C-10; **3a** and **3b**, C-6, C-7, C-8 and C-9. Estimated accuracy of $T_1$ values and intensities is ± 15% for protonated carbon resonances, ± 20% for quaternary carbon resonances.

*e.* Values for the quaternary carbon resonances at 135.9, 134.0 and 129.7 ppm are 27(2.4), 29(2.6) and 28(2.4), respectively.

*f.* Assignments may be reversed.

*g.* Assignments may be reversed.

*h.* Values for the quaternary carbon resonances at 137.1, 131.9 and 125.6 ppm are 26(2.4), 30(2.4) and 41(2.4), respectively.

*i.* Assignments may be reversed.

*j.* A small resonance from an impurity contributes here; true intensity of C-3 resonance < 1.7.

*k.* Assignments of C-6 and C-7 resonances are based on $T_1$ values.

*l.* Estimated accuracy is about ± 25%, due to overlap with larger resonance from C-7 of **3a**.

carbon of a terminal vinyl bromide group. This was verified in the case of β-bromostyrene. The $T_1$ values (in sec) and integrated intensities (in parentheses) for the carbons of *trans* β-bromostyrene in $CDCl_3$ at 32° are: C-1′, 8.3 (3.0); C-2′, 4.3 (2.0); C-1, 69 (2.4); C-2/C-6, 7 (6.0); C-3/C-5, 7 (6.6); C-4, 5 (4.8). The resonances of C-2 to C-6 overlap with resonances from the *cis* isomer (10%), so the $T_1$ and NOE values of these carbons are less accurate than those of C-1, C-1′ and C-2′. However, it is clear that the spin-lattice relaxation of C-2′ is affected significantly by the directly-bonded bromine. The value of the NOE suggests that the bromine and the directly-bonded proton contribute about equally to its spin-lattice relaxation. The $T_1$ values support this conclusion, but deviations from rigid isotropic rotational reorientation prevent a more quantitative interpretation.

Finally, we examined the relaxation behaviour of a marine natural product containing four brominated quaternary carbons, three in Br–C=groups and one in a Br–C–Br group. The rotational correlation time of the molecule in $CDCl_3$ at 32° was about 0.03 nsec. The $T_1$ values (in sec) and integrated intensities for the three brominated allylic carbons were 10(1.0), 16(0.9) and 13(1.0), and for the *gem*-dibromo carbon 10(1.5). Thus, the relaxation of the allylic carbons appears to be dominated by C–Br scalar interactions. However, there is a small contribution to relaxation of the *gem*-dibromo carbon from $^{13}C$–$^{1}H$ dipolar interactions (with the hydrogens on an adjacent $CH_2$ group).

The above examples demonstrate the advantages, and some of the limitations, of the use of $^{13}C$ relaxation measurements for distinguishing brominated carbons from chlorinated carbons. It is essential to measure the $T_1$ *and* NOE of *all* carbons in a molecule, not only to identify the brominated carbons, but also to establish that the molecule behaves as an isotropic rigid rotor. If this condition is satisfied, one can make use of both NOE and $T_1$ values to identify brominated carbons, whereas deviations from this behaviour *may* limit the utility of $T_1$ values in this respect. Finally, it is *essential* to eliminate all possible contributions to $^{13}C$ relaxation other than $^{13}C$–$^{1}H$ dipolar interactions and $^{13}C$-halogen scalar interactions.

It is expected that the approach described in this paper will be useful mainly in the field of marine natural products chemistry (see Introduction), but the recent isolation of some brominated metabolites from terrestrial organisms[21] suggests that applications in this field may be forthcoming. The method is nondestructive and measurements can be carried out on as little as 30–40 mg of material with the use of 10-mm sample tubes. Quantities lower than this would be required if 1-mm sample tubes or micro-cells were employed. In a subsequent paper,[22] we describe the application of this approach to determination of the structure of three polyhalogenated monoterpenes from algae.

## EXPERIMENTAL

*Materials*. Chloro-, bromo- and iodobenzene and chloro-, bromo- and iodocyclohexane were obtained from Fluka (Puriss, or Purum grade) and used as received. β-Bromostyrene from Fluka (Pract. grade, nominally about 20% *cis*) was passed through a column of alumina before use. The sample was found to consist of 90% *trans* isomer and 10% *cis* by $^{13}C$ NMR (see below). The solvents $CDCl_3$, $CD_3CN$ and $(CD_3)_2SO$ were all spectroscopic grade (> 99% deuterated). Samples of **1**, **2**, **3a** and **3b** were kindly provided by Drs. R. Kazlauskas and R. J. Wells of this Institute. The methods of isolation have been described.[4,6]

*Methods*. Natural-abundance $^{13}C$ NMR spectra were obtained at 15.04 MHz on a Jeol FX-60 spectrometer operating in the pulsed Fourier transform mode, with on-resonance noise-modulated proton decoupling and 10 mm o.d. spinning sample tubes. Probe temp. was $32 \pm 1°$. All spectra were accumulated in 8192 time-domain addresses, and processed with 0.7–1.2 Hz exponential broadening. Samples were degassed by bubbling nitrogen through the solutions for at least 1 min.

Spin-lattice relaxation times were determined by the inversion-recovery method,[23] in which a $(180° - \tau\text{-}90° - t)_n$ pulse sequence is employed. The delay time, t, between each 90° radiofrequency pulse and the following 180° radiofrequency pulse was greater than 3.5 times the longest $T_1$ being measured[24] except in the following cases: C-1 of chlorobenzene in $d_6$-DMSO ($3.1 \times T_1$); C-2, C-5 and C-6 of **1** ($3.2 \times T_1$); C-1 and C-4 of **3a** (about $2 \times T_1$). The width of a 90° pulse was 17 μsec. $T_1$ values were determined from a plot of $\ln(M_0 - M_\tau)$ versus $\tau$, where Mo is the thermal equilibrium value of the magnetisation and $M_\tau$ is the initial value of the magnetisation following a 90° pulse at time $\tau$. Only values of $\tau \le T_1$ were employed in calculating $T_1$ values. Estimated accuracies of individual $T_1$ values are given in footnotes to the Tables.

Integrated intensities were obtained digitally from fully-relaxed spectra recorded with 90° radiofrequency pulse excitation. In some cases absolute NOE values were determined by comparing integrated intensities obtained from spectra recorded with complete proton decoupling with those from spectra recorded with gated proton decoupling.[25] Spectra recorded with gated proton decoupling were employed in determining the ratio of **3a** to **3b** (see above). The ratio obtained in this way was in good agreement with that obtained by GC analysis. Chemical shifts were measured digitally, and are reported in parts per million downfield from internal TMS.

Assignments of resonances to specific carbons were obtained from the literature[7,8] where possible. For **1**, **2**, **3a** and **3b**, assignments were made by a combination of chemical shift considerations,[7,8] single frequency off-resonance proton decoupling experiments,[7,8] and spin-lattice relaxation time measurements.

*Acknowledgements*—I wish to thank Drs. R. Kazlauskas and R. J. Wells for supplying the marine natural products used herein and for helpful discussions, and the Queen's Fellowships Committee for the award of a Fellowship in Marine Science.

## REFERENCES

[1] J. T. Baker and V. Murphy. *Handbook of Marine Science. Compounds from Marine Organisms*, Vol. 1. Chemical Rubber Co., Cleveland, Ohio (1976).

[2] F. J. McDonald, D. C. Campbell, D. J. Vanderah, F. J. Schmitz, D. M. Washecheck, J. E. Burks and D. van der Helm, *J. Org. Chem.* **40**, 665 (1975); C. Ireland, M. O. Stallard, D. J. Faulkner, J. Finer and J. Clardy, *Ibid.* **41**, 2461 (1976).

[3] F. X. Woolard, R. E. Moore and P. P. Roller, *Tetrahedron* **32**, 2843 (1976); J. S. Mynderse and D. J. Faulkner, *Ibid.* **31**, 1963 (1975); P. Crews and E. Kho, *J. Org. Chem.* **40**, 2568 (1975); H.-E. Högberg, R. H. Thomson and T. J. King, *J. Chem. Soc.* Perkin I. 1696 (1976); H. H. Sun, S. M. Waraszkiewicz and K. L. Erickson, *Tetrahedron Letters*, 4227 (1976); J. A. Pettus, R. M. Wing and J. J. Sims, *Ibid.* 41 (1977).

[4] R. Kazlauskas, P. T. Murphy, R. J. Quinn, R. J. Well and P. Schönholzer, *Ibid.* 4451 (1976).

[5] D. B. Stierle, R. M. Wing and J. J. Sims, *Ibid.* 4455 (1976).

[6] R. Kazlauskas, P. T. Murphy, R. J. Quinn and R. J. Wells, *Ibid.* 37 (1977).

[7] J. B. Stothers, *Carbon*-13 *NMR Spectroscopy*. Academic Press, New York (1972).

[8] G. C. Levy and G. L. Nelson, *Carbon*-13 *Nuclear Magnetic Resonance for Organic Chemists*. Wiley-Interscience, New York (1972).

[9] J. R. Lyerla, D. M. Grant and R. D. Bertrand, *J. Phys. Chem.* **75**, 3967 (1971).

[10] R. Freeman and H. D. W. Hill, *Molecular Spectroscopy* 1971, Institute of Petroleum, London (1972).

[11] T. C. Farrar, S. J. Druck, R. R. Shoup and E. D. Becker, *J. Am. Chem. Soc.* **94**, 699 (1972).

[12] G. C. Levy, J. D. Cargioli and F. A. L. Anet, *Ibid.* **95**, 1527 (1973).

[13] A. Allerhand, D. Doddrell and R. Komoroski, *J. Chem. Phys.* **55**, 189 (1971).

[14] R. S. Norton and A. Allerhand, *J. Am. Chem. Soc.* **98**, 1007 (1976).

[15] A. Abragam, *The Principles of Nuclear Magnetism*. Oxford University Press, London (1961).

[16] T. C. Farrar and E. D. Becker, *Pulse and Fourier Transform NMR*. Academic Press, New York (1971).

[17] R. S. Norton, A. O. Clouse, R. Addleman and A. Allerhand, *J. Am. Chem. Soc.* **99**, 79 (1977), see Fig. 2.

[18] E. A. C. Lucken, *Nuclear Quadrupole Coupling Constants*, Academic Press, London (1969).

[19] G. C. Levy, *J. Chem. Soc.* Chem. Commun. 352 (1972).

[20] NOE is defined here as the ratio of the integrated intensity of a $^{13}C$ resonance under conditions of proton decoupling to its intensity in the absence of proton decoupling.

[21] A. G. Andrewes, C. L. Jenkins, M. P. Starr, J. Shepherd and H. Hope, *Tetrahedron Letters* 4023 (1976).

[22] R. S. Norton, R. G. Warren and R. J. Wells, *Tetrahedron Letters*, submitted for publication.

[23] R. L. Vold, J. S. Waugh, M. P. Klein and D. E. Phelps, *J. Chem. Phys.* **48**, 3831 (1968); R. Freeman and H. D. W. Hill, *Ibid.* **51**, 3140 (1969).

[24] G. C. Levy and I. R. Peat, *J. Magn. Reson.* **18**, 500 (1975).

[25] R. Freeman, H. D. W. Hill and R. Kaptein, *Ibid.* **7**, 327 (1972).