J.E. Celis, J.D. Smith and S. Brenner, Nature New Biol. *241*, 130–132 (1973).
D. Cummings and R.W. Bolin, Bact. Rev. *40*, 314–359 (1975).
A.H. Doermann, F.A. Eiserling and L. Boehner, J. Virol. *12*, 374–385 (1973).
R.H. Epstein, A. Bolle, C.M. Steinberg, E. Kellenberger, E. Boy de la Tour, R. Chevalley, R.S. Edgar, M. Susman, G.H. Denhardt and A. Lielausis, Cold Spring Harb. Symp. quant. Biol. *28*, 375–394 (1963).
T. Ishii and M. Yanagida, J. molec. Biol. *97*, 655–660 (1975).
T. Ishii, Y. Yamaguchi and M. Yanagida, J. molec. Biol. *120*, 533–544 (1978).
F. Jacob and E.L. Wollman, Annls Inst. Pasteur *90*, 282–302 (1956).
E. Kellenberger, in: Principles of Biomolecular Organizations. A Ciba-Foundation Symposium, pp. 192–228. Ed. G.E.W. Wolstenholme and M. O'Connor. J. & A. Churchill, London 1966.
E. Kellenberger, in: Polymerization in Biological Systems. A Ciba-Foundation Symposium, pp. 189–206. Ed. G.E.W. Wolstenholme and M. O'Connor. Ass. Scient. Publ., Amsterdam 1972.
E. Kellenberger, Trends biochem. Sci. *3*, N135–137 (1978).
J. Kistler, U. Aebi, L. Onorato, B. ten Heggeler and M.K. Showe, J. molec. Biol. *126*, 571–589 (1978).
U.K. Laemmli, L.A. Amos and A. Klug, Cell *7*, 191–203 (1976).
A. Lwoff and L. Siminovitch, C.r. Acad. Sci. *233*, 1397–1399 (1951).
D.W.A. Mount, A.W. Harris, C.R. Fuerst and L. Siminovitch, Virology *35*, 134–149 (1968).
C. Schaerli and E. Kellenberger, J. Virol., in press (1980).
M.K. Showe, E. Isobe and L. Onorato, J. molec. Biol. *107*, 35–69 (1976).
A.C. Steven, U. Aebi and M.K. Showe, J. molec. Biol. *102*, 373–407 (1976a).
A.C. Steven, E. Couture, U. Aebi and M.K. Showe, J. molec. Biol. *106*, 187–221 (1976b).
A.C. Steven and J.L. Carrascosa, J. supramolec. Struct. *10*, 1–11 (1979).
L. Onorato, B. Stirmer and M.K. Showe, J. Virol. *27*, 409–426 (1978).
W.B. Wood, R.S. Edgar, J. King, I. Lielausis and M. Henninger, Fed. Proc. *27*, 1160–1166 (1968).
M. Wurtz, J. Kistler and T. Hohn, J. molec. Biol. *101*, 39–56 (1976).

SPECIALIA

The editors do not hold themselves responsible for the opinions expressed in the authors' brief reports. – Les auteurs sont seuls responsables des opinions exprimées dans ces brèves communications. – Für die Kurzmitteilungen ist ausschliesslich der Autor verantwortlich. – Per le brevi comunicazioni è responsabile solo l'autore. – Ответственность за короткие сообщения несёт исключительно автор. – Solo los autores son responsables de las opiniones expresadas en estas comunicationes breves.

^{13}C NMR-study of flexibilide, an anti-inflammatory agent from a soft cora[1]

R.S. Norton[2] and R. Kazlauskas

Roche Research Institute of Marine Pharmacology, Dee Why (N.S.W. 2099, Australia), 26 April 1979

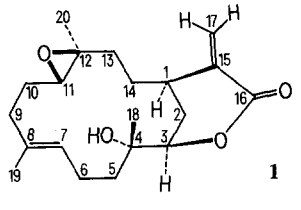
Summary. The use of ^{13}C spin-lattice relaxation measurements as a probe of structure in solution is illustrated for flexibilide. Analysis of quaternary carbon relaxation behaviour indicates that the structure in solution differs from the crystal structure. The effects of lanthanide shift and relaxation reagents on ^{13}C spectral parameters are also reported.

Flexibilide (**1**), a cembranoid lactone isolated from the soft coral *Sinularia flexibilis*[3,4], exhibits anti-inflammatory and anti-arthritic activity in the rat[5]. We have examined the structure of **1** in solution with the use of ^{13}C NMR spin-lattice relaxation measurements, placing particular emphasis on the analysis of quaternary carbon relaxation behaviour as a structural probe.

Materials and methods. Flexibilide was isolated from *S. flexibilis* as described previously[3]. Yb(fod)$_3^6$ and Gd(fod)$_3$ were obtained from Norell Chemical Co., Landisville, NJ, and Eu(fod)$_3$ and Pr(fod)$_3$ from Pierce Chemical Co., Rockford, Ill. The solvents $CDCl_3$, $(CD_3)_2SO$ and D_2O were all spectroscopic grade (> 99% deuterated). All materials were used as received.

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 OD spinning sample tubes. Probe temperature was 32 ± 1 °C. 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, and the NMR-tubes were capped and sealed with Parafilm. Spin-lattice relaxation times (T_1) were measured by the inversion-recovery method[7]. The 90° pulse width was 17 μsec.

Results and discussion. The figure shows the ^{13}C-spectrum of **1** in $CDCl_3$. The table summarizes ^{13}C chemical shifts, spin-lattice relaxation times (T_1) and integrated intensities for the 20 carbons of **1** in $CDCl_3$ and DMSO-d_6. Resonance assignments are based on chemical shifts[8], multiplicities in single-frequency off-resonance proton-decoupled spectra, and T_1-values. In both solvents all carbons (protonated and quaternary) have nuclear Overhauser enhancements (NOE) of 3.0 ± 0.3, indicating that their spin-lattice relaxation is dominated by ^{13}C-^{1}H dipolar interactions[9,10]. Furthermore, in each solvent the T_1-values of the methine carbons are identical within experimental error, and are twice as long as the values for the methylene carbons. This indicates[9,10] that **1** undergoes essentially isotropic reorientation in $CDCl_3$ and DMSO-d_6, and that any internal motions in the molecule are slow relative to the rate of overall molecular tumbling. The exceptions are the methyl groups, which would have T_1-values of 0.33 and 0.13 sec in $CDCl_3$ and DMSO-d_6, respectively, in the absence of internal motion. The data in the table indicate that all 3 methyl groups



1

undergo internal rotation, with C-19 and C-20 undergoing essentially free rotation[9,10].

With the use of equations given elsewhere[9-12], and assuming a C–H bond length of 1.09 Å, the rotational correlation times for **1** in $CDCl_3$ and DMSO-d_6 are calculated to be 0.05 and 0.11 nsec, respectively. As the quaternary carbon resonances have the full NOE (table), we may calculate their T_1-values by assuming that the relaxation of these carbons is dominated by dipolar interactions with hydrogens 2 bonds removed[10-12], C–H distances being obtained from the X-ray structure[3]. These calculated T_1-values are given in the table.

The measured T_1-values for C-4, C-8 and C-12 agree well with their calculated values (table). In contrast, the measured T_1-values for C-15 and C-16 are significantly shorter than those calculated using distances from the X-ray crystal structure[13] (table). Taking into account all hydrogens closer than 3 Å we calculate[9-12] T_1-values for C-15 and C-16, respectively, of 17 and 95 sec in $CDCl_3$, and 7.2 and 39 sec in DMSO-d_6. This indicates that in solution there are hydrogens closer to the β-methylene lactone moiety than in the crystal. These additional contributions could be made up as follows[14]: in $CDCl_3$, for C-15, 1 hydrogen 2.0 Å away, 2 at 2.2_5 Å or 3 at 2.4 Å, and for C-16, 2.2 (1), 2.4 (2) or 2.6 (3). Corresponding values for DMSO-d_6 are C-15, 2.2_5 (1) 2.5_5 (2) or 2.7 (3); C-16, 2.4 (1), 2.7 (2) or 2.9 (3). In order to determine if the hydroxyl proton is responsible for enhanced relaxation of C-15 and C-16, we measured their T_1-values in $CDCl_3$ following deuteration of the hydroxyl group[11,12]. The T_1 of C-15 is unaffected within experimental error, while that of C-16 increases from 37 to 47 sec (following correction for the slight decrease in protonated carbon T_1-values caused by the presence of D_2O). The NOE of the C-16 resonance is not measurably affected, so we can estimate the distance between the hydroxyl proton and C-16 directly from the difference in T_1-values. We obtain a value of about 2.6 Å. The corresponding distance in the crystal[3] is 3.65 Å. However, the hydroxyl proton is not close enough to C-15 and C-16 to fully account for the observed T_1-values. It is likely that in solution the conformation of the methylene lactone ring is altered in a way that brings (C-3)H and/or one of (C-2)H closer to C-15 and C-16 than in the crystal. Model building studies confirm that such conformational changes are possible in solution.

In an attempt to obtain further information on the solution conformation we examined the effects on the ^{13}C-spectrum of the relaxation agent $Gd(fod)_3$ and lanthanide shift reagents (LSR) $Yb(fod)_3$, $Eu(fod)_3$ and $Pr(fod)_3$. T_1-values were measured at a number of $Gd(fod)_3$ concentrations, the paramagnetic contributions ($T_{1,p}$) were obtained by subtracting the diamagnetic values (table), and the $T_{1,p}$ values were expressed as a ratio relative to that of peak 1 (C-16). The inverse ratios are given in the last column of the table.

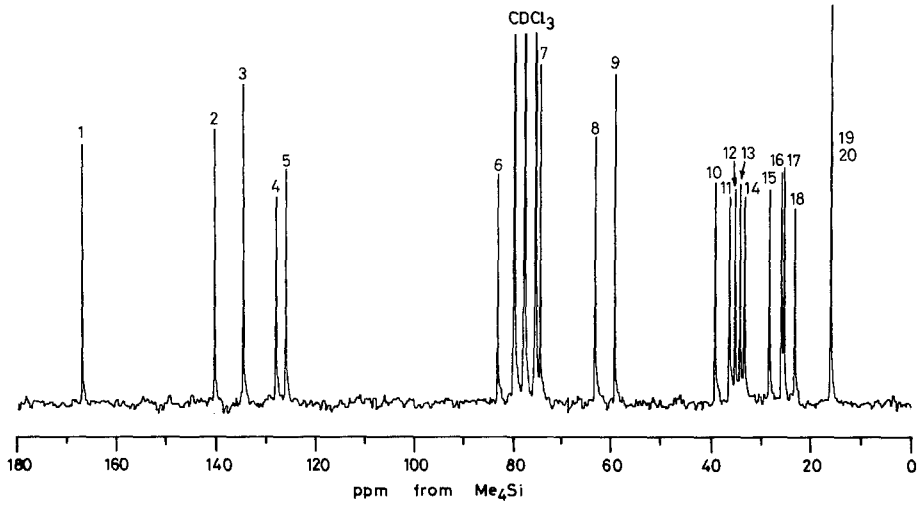
LSR effects were measured by adding **1** to a low (and constant) amount of LSR[16]. The LSR data were analyzed as described by Armitage et al.[16] to yield values of Δ_B, the lanthanide-induced shift (LIS) in the fully formed complex, and K_B, the LSR-substrate association constant. The ratios of Δ_B for each resonance to Δ_B for peak 1 were then calculated for each LSR[17,18].

Relaxation rate enhancements induced by $Gd(fod)_3$ are proportional to r^{-6} (where r = Gd-C distance)[17]. Thus the data in the table indicate that the carbonyl and epoxide groups are closest to the metal, with the hydroxyl slightly further away. Analysis of the LIS data[16] indicates that the effects are due to a site[19] with $K_B \approx 200\ M^{-1}$. Thus, **1** appears to have a single $Ln(fod)_3$ binding site involving the

Chemical shifts and spin-lattice relaxation times of the carbons of flexibilide[a]

Peak[b]	Multiplicity[c]	Assignment[d]	Chemical shift[e]		T_1 (integrated)[f]		Calculated T_1^h		$1/T_{1,p}^i$
			$CDCl_3$	DMSO-d_6	$CDCl_3$	DMSO-d_6	$CDCl_3$	DMSO-d_6	Ratios
1	s	C-16	167.0	166.3	37 (2.7)	21 (2.9)	95	39	1.00
2	s	C-15	140.0	140.5	12 (3.0)	5.9 (3.0)	17	7.2	0.12
3	s	C-8	134.2	133.1	9 (3.0)	4.4 (3.0)	10	4.1	0.02
4	t	C-17	127.6	126.5	0.45 (3.2)	0.20 (2.8)			0.12
5	d	C-7	125.6	125.7	0.98 (3.1)	0.40 (3.1)			0.02
6	d	C-3	82.6	81.8	0.97 (2.8)	0.37 (2.9)			0.07
7	s	C-4	73.9	72.5	7 (3.3)	3.3 (2.9)	8	3.6	0.13
8	d	C-11	62.8	61.7	0.99 (3.1)	0.37 (2.9)			0.30
9	s	C-12	58.9	58.3	9 (3.0)	4.4 (3.1)	10	4.1	0.27
10	t		38.7	40.1	0.50 (2.7)	0.22 (–)[g]			0.06
11	t		35.8	35.1	0.50 (3.0)	0.22 (–)[g]			< 0.04[j]
12	t		34.7	34.4	0.50 (3.1)	0.21 (3.1)			0.13
13	d	C-1	33.7	32.9	0.97 (3.1)	0.38 (3.1)			0.05
14	t		32.8	32.0	0.49 (3.2)	0.19 (3.2)			0.03
15	t		27.7	27.3	0.50 (2.8)	0.20 (2.8)			0.05
16	t		25.3	24.9	0.51	0.23 (2.8)			0.14
17	q	C-18	24.7	23.9	0.84 (6.1)	0.53 (3.3)			0.08
18	t		22.6	22.2	0.49 (2.8)	0.24 (2.8)			< 0.01[k]
19	q	C-19	15.4	15.1	2.8 (5.9)	1.3 (6.3)			0.02
20	q	C-20	15.4	15.0	2.8	1.4			

[a] 0.4 m in degassed solvent at 32 °C. [b] Numbered as in the figure. [c] Multiplicity of ^{13}C resonance in single frequency off-resonance proton-decoupled ^{13}C-spectrum (s, singlet; d, doublet; t, triplet; q, quartet). [d] See text. Numbering system is shown in **1**. [e] In ppm from internal Me_4Si. [f] T_1-values in sec and integrated intensities (in parentheses). T_1-values were obtained from 3 sets of inversion-recovery spectra in each solvent (1 for protonated carbons, 1 for the carbonyl, and 1 for the remaining four quaternary carbons). The quoted T_1-values for the protonated carbons and 4 quaternary carbons of flexibilide in $CDCl_3$ are the average of values obtained for 2 different solutions of the same concentration. Estimated experimental errors are ± 10% for protonated carbon resonances and ± 15% for quaternary carbon resonances. Integrated intensitites are the average of values obtained from 2 spectra in each solvent (the figure shows a typical spectrum used for measuring integrated intensities). For $CDCl_3$ the arithmetic average of the intensities of all protonated carbon resonances was set equal to 2.99, whilst for DMSO-d_6 the intensities of peaks 10–13 were omitted from the average because of interference from solvent resonances. [g] Overlaps with solvent resonance. [h] Predicted T_1, calculated as described in text. [i] Average of ratios obtained at $Gd(fod)_3$: flexibilide molar ratios of 0.0005_7, 0.0009_2 and 0.0015_2. [j] Change in $T_{1,obs}$ within experimental error at 1 [$Gd(fod)_3$]. [k] Change in $T_{1,obs}$ within experimental error at 3 [$Gd(fod)_3$].



Natural-abundance ^{13}C NMR-spectrum of a degassed solution of **1** (0.4 M) in $CDCl_3$ at 32 °C. 391 scans were accumulated with a recycle time of 150 sec.

carbonyl and epoxide oxygens, and possibly also the hydroxyl oxygen. The alternative explanation, that there are 2 or 3 separate binding sites with very similar K_B-values, is possible but less likely. The LIS ratios are not constant from one LSR to the other, indicating that the complexes are not isostructural, that they are not axially symmetric[17,20], and/or that scalar interactions[17,20] contribute significantly to LIS. Present methods for the separation of dipolar, scalar, and diamagnetic contributions to LIS[20] are not sufficiently accurate to justify an attempt to use the LIS data for structural analysis. Nevertheless, it should be noted that simultaneous binding of the epoxide and carbonyl moieties to the lanthanide reagents is not consistent with the crystal structure[3]. We cannot, however, exclude the possibility of a metal-induced conformational change.

We have shown how ^{13}C spin-lattice relaxation measurements on quaternary carbons can be used as probes of through-space distances and hence of conformation. The distance of any hydrogen from a quaternary carbon can be determined by measuring the effect of replacement of the hydrogen by deuterium on the ^{13}C T_1- and NOE-values, as illustrated for the hydroxyl proton of **1** in the present case[15]. Distances can be obtained fairly accurately by this means because of the 6th power dependence of T_1-values on internuclear distances[9–12,15]. Thus, quaternary carbon T_1-values can be used as conformational probes, as well as for resonance assignments[11,21] and covalent structure determinations[22–24].

In the present case the data indicate that the conformations of **1** in $CDCl_3$ and DMSO-d_6 differ from the crystal structure, at least in the region of the methylene lactone ring. Further studies, will be required to define the solution structure. The effects of $Ln(fod)_3$ reagents on ^{13}C spectral parameters, while not useful for structural analysis, indicate that **1** can form a bi- or, possibly, tri-dentate complex with $Ln(fod)_3$ in solution. This property may have biological significance, as there is evidence to suggest that Cu complexes of anti-inflammatory agents may be the active metabolites[25]. Thus, it may be that a common feature of **1** and the non-steroid anti-inflammatory drugs is their ability to bind metal ions. Furthermore, the high solubility of **1** in nonaqueous media[3] may enable it to act as an ionophore, with 2 molecules of **1** binding 1 metal ion.

1 Acknowledgment. We thank R.J. Wells, RRIMP, for helpful discussions.
2 Queen Elizabeth II Fellow in Marine Science, 1976–1978.
3 R. Kazlauskas, P.T. Murphy, R.J. Wells, P. Schönholzer and J.C. Coll, Aust. J. Chem. *31*, 1817 (1978).
4 A.J. Weinheimer, J.A. Matson, M. Bilayet Hossain and D. van der Helm, Tetrahedron Lett. 2923 (1977).
5 K.M. Taylor and B.A. Baldo, Aust. J. Pharm. Sci. *8*, 25 (1979).
6 fod = 6,6,7,7,8,8,8-heptafluoro-2,2-dimethyl-3,5-octanedionate.
7 G.C. Levy and I.R. Peat, J. magn. Reson. *18*, 500 (1975).
8 J.B. Stothers, Carbon-13 NMR Spectroscopy. Academic Press, New York 1972.
9 J.R. Lyerla and D.M. Grant, in: Magnetic Resonance, Physical Chemistry Series One, vol.4, p.155. Ed. C.A. McDowell. University Park Press, Baltimore 1972.
10 A. Allerhand, D. Doddrell and R. Komoroski, J. chem. Phys. *55*, 189 (1971).
11 E. Oldfield, R.S. Norton and A. Allerhand, J. biol. Chem. *250*, 6368 (1975).
12 R.S. Norton and A. Allerhand, J. Am. chem. Soc. *98*, 1007 (1976).
13 According to the X-ray crystal structure[3] the nearest hydrogens to C-16 are (C-17)H at 2.6_5 Å and (C-3)H at 2.8_5 Å.
14 T_1-values were calculated with the use of $r_{CH} = 1.09$ Å for all protonated carbons. Duplicate calculations with $r_{CH} = 1.10$ Å were carried out following recent reports in the literature which suggest that this value may be more appropriate (V.R. Cross and J.S. Waugh, J. magn. Reson. *25*, 225 (1977); M. Llinás, W. Meier and K. Wüthrich, Biochim. biophys. Acta *492*, 1 (1977/reference 15). These calculations yield nearly identical non-bonded C-H distances.
15 L.M. Jackman and J.C. Trewella, J. Am. chem. Soc. *98*, 5712 (1976).
16 I. Armitage, G. Dunsmore, L.D. Hall and A.G. Marshall, Can. J. Chem. *50*, 2119 (1972).
17 R.A. Dwek, NMR in Biochemistry, Oxford University Press, London 1973.
18 Δ_B values for C-16 in the presence of $Yb(fod)_3$, $Eu(fod)_3$, and $Pr(fod)_3$ are 823, 154, and −346 Hz, respectively.
19 Note that neither solvent nor LSR was dried before use.
20 C.N. Reilley, B.W. Good and R.D. Allendoerfer, Analyt. Chem. *48*, 1446 (1976).
21 F.W. Wehrli, in: Topics in Carbon-13 NMR Spectroscopy, vol.2, p.343. Ed. G.C. Levy. Wiley-Interscience, New York 1976.
22 R.S. Norton, Tetrahedron *33*, 2577 (1977).
23 R.S. Norton, R.G. Warren and R.J. Wells, Tetrahedron Lett. 3905 (1977).
24 R.S. Norton and R.J. Wells, in preparation.
25 J.R.J. Sorenson, J. med. Chem. *19*, 135 (1976).