

SEGMENTAL MOTION IN PSEUDOMONIC ACID A:  
A CARBON-13 SPIN-LATTICE  
RELAXATION TIME STUDY AT TWO FIELD STRENGTHS

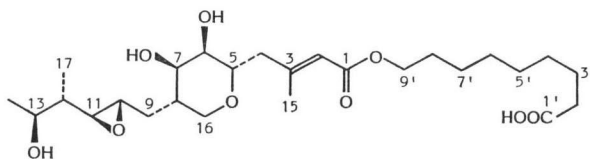
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(Received for publication November 28, 1983)

The mobility of pseudomonic acid A in dimethyl sulfoxide- $d_6$  solution has been determined from  $^{13}\text{C}$  spin-lattice relaxation time measurements at two field strengths. The central tetrahydropyran ring reorientates isotropically with a rotational correlation time of  $\sim 280$  picoseconds/radian. Both side-chains exhibit marked segmental motion with correlation times decreasing to only  $\sim 50$  picoseconds/radian at the end of the nonanoic acid side-chain.

Pseudomonic acid A is a naturally occurring antibiotic produced by fermentation of a strain of *Pseudomonas fluorescens*.<sup>1)</sup> The gross structure was elucidated on the basis of spectroscopic and degradative data.<sup>2)</sup> This structure was confirmed and the absolute stereochemistry shown to be **I** by further spectroscopic and X-ray work.<sup>3)</sup>



**I**

We have now measured the  $^{13}\text{C}$  spin-lattice relaxation times ( $T_1$ 's) of all the carbon atoms in **I** in order to gain information about the mobility of **I** in solution. This information is potentially useful in the understanding of both the biological activity of **I** and also the interaction of **I** with other molecules and ions in solution. The  $T_1$  data has also served as a check on the  $^{13}\text{C}$  spectral assignments.

### Results

Table 1 gives the 20 MHz  $^{13}\text{C}$   $T_1$  and nuclear overhauser effect (NOE) results, the 63 MHz  $^{13}\text{C}$   $T_1$  and NOE results and the effective  $^{13}\text{C}$  correlation times for a dimethyl sulfoxide- $d_6$  solution of **I**. Fig. 1 shows three partially relaxed 63 MHz  $^{13}\text{C}$  spectra of **I** in dimethyl sulfoxide- $d_6$ .

### Experimental

All experiments were conducted at ambient temperature on  $\sim 0.6$  M solutions of pseudomonic acid A in DMSO- $d_6$ . The 20 MHz  $^{13}\text{C}$  NMR data was acquired on a Brüker WP 80 using 10 mm tubes. The sweep width was 3787.9 Hz over 8 K data points. The  $90^\circ$  pulse for  $^{13}\text{C}$  was  $\sim 17$   $\mu$ seconds. The free induction decays (FIDs) were zero-filled to 16 K points and a line broadening of 1 to 1.5 Hz was applied prior to Fourier transformation.

The 63 MHz  $^{13}\text{C}$  data was acquired on a Brüker WM250 using 5 mm tubes in a  $^{13}\text{C}/^1\text{H}$  dual probe. The sweep width was 11627.9 Hz over 16 K data points. The  $90^\circ$  pulse for  $^{13}\text{C}$  was  $\sim 10$   $\mu$ seconds.

Table 1.

Carbon	ppm <sup>a</sup>	M <sup>b</sup>	NT <sub>1</sub> (20 MHz) <sup>c</sup> (seconds)	NT <sub>1</sub> (63 MHz) (seconds)	NOE (63 MHz)	$\bar{\tau}$ (picoseconds)
1	165.8	s	4.7	3.6 <sup>h</sup>	0.5	—
2	116.8	d	0.26	0.32	1.5	~190
3	157.9	s	2.1	1.6 <sup>h</sup>	0.8	—
4	42.6	t	<sup>d</sup>	0.27	1.5	<sup>d</sup>
5	74.6	d	0.18	0.23	1.4	~280
6	68.4	d	0.19	0.23	1.4	~280
7	69.6	d	0.15	0.21	1.2	~280
8	40.1	d	<sup>d</sup>	0.21	<sup>d</sup>	<sup>d</sup>
9	31.6	t	0.29	0.32	1.6	~170
10	54.8	d	0.33	0.38	1.6	~150
11	59.2	d	0.36	0.39	1.6	~140
12	41.9	d	<sup>d</sup>	0.45	1.8	<sup>d</sup>
13	68.0	d	0.43	0.47	1.8	~115
14	20.2	q	2.00	1.87	2.2	—
15	18.7	q	2.70	3.51	1.4	—
16	64.8	t	0.19	0.25	1.3	~280
17	11.7	q	2.26	2.16	2.1	—
1'	174.5	s	3.3	5.8 <sup>h</sup>	1.1	—
2'	33.7	t	0.93	0.99	1.8	~55
3'	24.6	t	0.95	1.00	1.8	~50
4'	28.58 <sup>f</sup>	t	} 0.78 <sup>e</sup>	0.91	1.7	} ~65 <sup>e</sup>
5'	28.62 <sup>g</sup>	t		0.81	1.7	
6'	28.7 <sup>g</sup>	t		0.81	1.7	
7'	25.5	t	0.67	0.74	1.8	~75
8'	28.3	t	0.53	0.56	1.6	~95
9'	63.1	t	0.35	0.45	1.6	~140

<sup>a</sup> Relative to DMSO-*d*<sub>6</sub>=39.6 ppm (at 63 MHz).

<sup>b</sup> Multiplicity in off-resonance spectrum.

<sup>c</sup> NOE (20 MHz)=2.0±0.1 (standard deviation) for all protonated carbons. N=number of attached protons.

<sup>d</sup> Signals obscured by solvent resonances.

<sup>e</sup> Signals unresolved at 20 MHz.

<sup>f</sup> Assigned on basis of longer NT<sub>1</sub>.

<sup>g</sup> Assignment uncertain.

<sup>h</sup> Values from linear regression.

The FIDs were zero-filled to 32 K points and line broadening of 1 to 2 Hz was applied prior to Fourier transformation.

The <sup>13</sup>C T<sub>1</sub>'s were measured by the inversion-recovery method using 10 to 12 values of the variable delay (VD). A relaxation delay (RD) of 10 to 20 seconds was used depending upon whether the non-protonated carbons were being studied or not. The T<sub>1</sub>'s were determined by fitting the experimental signal intensity data to equation [1] . . .

$$I = I_0 - A \cdot e^{-VD/T_1} \quad [1]$$

. . . where I=signal intensity for variable delay (VD).

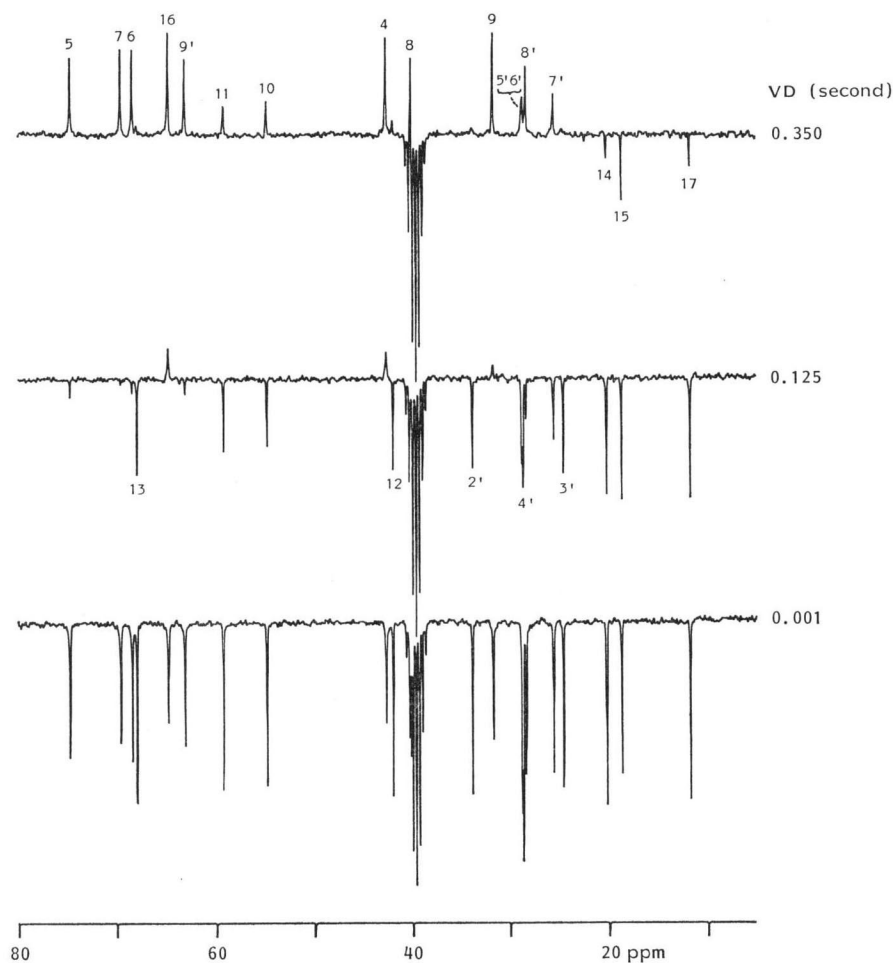
I<sub>0</sub>=normalised signal intensity with complete relaxation.

A=the <sup>13</sup>C 180° flip angle in units of  $\pi/2$ .

The fitting was done using the Brüker DISNMR program.

The <sup>13</sup>C {<sup>1</sup>H} NOE values were measured by the acquisition of decoupled with NOE and decoupled without NOE FIDs. A relaxation delay of 8 seconds was used.

Fig. 1. The high field regions of three partially relaxed 63 MHz  $^{13}\text{C}$  NMR spectra of I in  $\text{DMSO-}d_6$ . The pulse sequence for acquisition was RD-180 $^\circ$ -VD-90 $^\circ$ -Acquire.



### Discussion

The assignment of the  $^{13}\text{C}$  NMR spectrum of the methyl ester of I has been published by FELINE *et al.*<sup>4)</sup> The assignments here follow those, except that C-13 is assigned to high field of C-6 and C-7 on the basis of its long  $\text{NT}_1$  value.

At 20 MHz all the protonated carbons of I showed an NOE of  $2.0 \pm 0.1$  (Table 1, footnote c). This is in good agreement with the theoretical value of 1.988<sup>5)</sup> for a protonated carbon undergoing purely  $^{13}\text{C}$ - $^1\text{H}$  dipolar relaxation. Thus the relaxation of all the protonated carbons is purely dipolar at 20 MHz.

At 20 and 63 MHz the tetrahydropyran (THP) ring carbon  $\text{NT}_1$  values are all equal within an estimated experimental error of 10~15%, indicating that the THP ring is undergoing isotropic reorientation.<sup>6)</sup> However, as one goes away from the central THP ring the  $\text{NT}_1$  values of the carbon atoms on each side chain undergo a monotonic increase. The molecule can thus be considered to be reorienting isotropically overall but to possess in addition, multiple internal motion. The dipolar relaxation of this class of molecule has been treated theoretically by LEVINE *et al.*<sup>7)</sup> The theory is very complex but simplifies considerable under certain conditions.

The simplified equation<sup>7)</sup> for  $T_1$  is [2] . . .

$$\frac{1}{T_1} = \frac{1}{20} \cdot |F_0|^2 \cdot [J(\omega_H - \omega_C) + 3J(\omega_C) + 6J(\omega_H + \omega_C)] \quad [2]$$

where  $J(\omega_j)$ , the spectral density function, is given<sup>5)</sup> by [3] . . .

$$J(\omega_j) = \frac{2\bar{\tau}}{1 + \omega_j^2 \cdot \bar{\tau}^2} \quad [3]$$

and

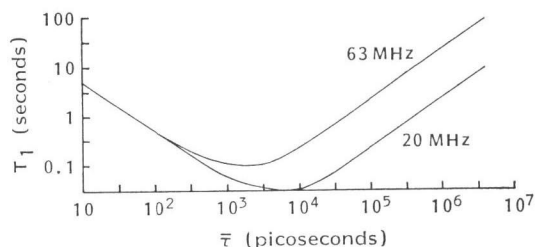
$$F_0 = \frac{\gamma_H \cdot \gamma_C \cdot \hbar}{r_{CH}^3} \quad [4]$$

. . . where  $r_{CH}$  is the carbon, hydrogen bond length (1.1 Å here<sup>5)</sup>) and  $\gamma_H$ ,  $\omega_H$  and  $\gamma_C$ ,  $\omega_C$  are the magnetogyric ratios and Larmor frequencies (radians/second) for hydrogen and carbon respectively. The effective correlation time ( $\bar{\tau}$ ) is the weighted average of all the separate correlation times that contribute to the overall motion of a particular carbon.<sup>6)</sup> At 20 MHz but not at 63 MHz for  $^{13}\text{C}$  the simplification does hold for I.

Fig. 2 shows a log-log plot of  $T_1$  vs.  $\bar{\tau}$  calculated using equation [2] for the two carbon frequencies 63 and 20 MHz. As can be seen, any given value of  $T_1$  could be due to either of two  $\bar{\tau}$  values. The correct solutions were chosen by reference to the 20 and 63 MHz NOE data and the 63 MHz  $T_1$  data.

Table 1 shows that the methine and methylene carbons  $NT_1$  values fall between 0.15 and 0.95 seconds at 20 MHz. The full ( $2.0 \pm 0.1$  average) NOE at 20 MHz (calculated 2.0) and the NOE values of between 1.2 and 1.8 at 63 MHz (calculated 1.5 to 2.0) for these carbons indicate that the solution of Fig. 2 lies on the short  $\bar{\tau}$  side of the graph. This is further corroborated by the 63 MHz  $^{13}\text{C}$   $T_1$  values of these carbons which are all just slightly larger than the corresponding 20 MHz values. If the solution of  $\bar{\tau}$  for the 20 MHz  $T_1$  values lay on the long  $\bar{\tau}$  side of the graph the expected<sup>6)</sup> NOE's at both field strengths would be  $\sim 0.15$  and the 63 MHz  $T_1$ 's would be approximately ten times the 20 MHz  $T_1$  values. This is clearly not the case. Table 1 gives the  $\bar{\tau}$  values calculated from the 20 MHz  $^{13}\text{C}$   $T_1$  data using [2].

Fig. 2. A log-log plot of  $T_1$  vs.  $\bar{\tau}$  for two  $^{13}\text{C}$  observation frequencies, using points calculated by equation [2].



Two approaches have been taken in analysing the results of this study. The 20 MHz derived  $\bar{\tau}$  values have been used to indicate quantitatively the mobility of the molecule in solution. In addition, the 63 MHz  $NT_1$  values have been used to provide a qualitative picture of the molecular motion.

Within experimental error, the 63 and 20 MHz  $NT_1$  values of the carbons of the THP ring are equal, thus indicating that the ring is reorienting isotropically. The 20 MHz  $NT_1$  values were averaged to derive a correlation time of  $\sim 280$  picoseconds for the THP ring. The THP ring is the slowest moving part of the molecule and acts as an effective "anchor" to the ends of the two side chains.

The  $\bar{\tau}$  values of the epoxide-containing side chain show a monotonic decrease from 170 picoseconds to 115 picoseconds as one goes from the anchoring THP ring out to C-13. This increasing mobility along the side chain is reflected in the 63 MHz  $NT_1$  values. These latter values also show that there is a negligible increase in mobility from C-10 to C-11, as is to be expected from two carbon atoms locked in an epoxide ring. They also show that there is little increase in mobility on going from C-12 to C-13, indicating that the C-13 hydroxyl group is involved in hydrogen bonding which restricts rotation around the C-12, C-13 bond.

The  $\bar{\tau}$  values of the nonanoic acid side-chain indicate segmental motion from C-9' to C-3'. However, the magnitude of the bond-to-bond increase in mobility becomes smaller as one goes to the end of the chain. In fact, the  $\bar{\tau}$  and  $NT_1$  values indicate a decrease in mobility from C-3' to C-2' which must be due to the carboxylic acid head group being involved in motion-restricting hydrogen bonding. The 63 MHz  $NT_1$  values also indicate a monotonic increase in mobility from C-5 to C-9' *i.e.* from the THP ring out to the nonanoic acid side-chain. It will be noted, however, that the increase in mobility from C-5 to C-4 is small in relation to that from C-8 to C-9. This is in accord with the observation

of a vicinal coupling of  $\sim 9.5$  Hz between H-5 and one H-4 proton in the 250 MHz  $^1\text{H}$  NMR spectrum of **I** in  $\text{CD}_3\text{OD}$ . The maximal value of this coupling indicates little averaging due to rotation of the C-4, C-5 bond.

The derivation of accurate  $\bar{\tau}$  values for the methyl groups is not simple and has not been attempted. However, the  $\text{NT}_1$  values shed some light on the behavior of these groups. The  $\text{NT}_1$  values of the three methyl groups are the largest of all the protonated carbon  $\text{NT}_1$  values. This is expected since there is little restriction of motion about the C- $\text{CH}_3$  bonds. At 20 MHz the  $\text{NT}_1$  of C-15 is  $\sim 10$  times the  $\text{NT}_1$  of C-2. This shows that there is essentially free rotation around the C-3, C-15 bond (C-2 is rigidly linked to C-3). The theoretical ratio of  $\text{NT}_1$  values in this case<sup>9)</sup> is 9. By contrast the 20 MHz  $\text{NT}_1$  of C-14 is only  $\sim 5$  times that of C-13, indicating some restriction to the rotation of the 14-methyl group. The hydrogen-bonding of the C-13 hydroxyl group to solvent molecules and/or other molecules of **I** is probably responsible for this hindrance to rotation of C-14, as it was likewise for the restriction of segmental motion between C-12 and C-13. The motion of C-17 is also restricted but to a lesser extent than for C-14.

Studies on the interaction of **I** with other molecules are in progress and will be reported in the future.

#### Acknowledgments

We thank Dr. K. COLEMAN for assistance with computer programming. Thanks also to Dr. J. M. BAILEY for allowing us access to the WP 80 spectrometer and to Dr. O. HOWARTH for helpful discussions.

#### References

- 1) FULLER, A. T.; G. MELLOWS, M. WOOLFORD, G. T. BANKS, K. D. BARROW & E. B. CHAIN: Pseudomonic acid: an antibiotic produced by *Pseudomonas fluorescens*. *Nature* 234: 416~417, 1971
- 2) CHAIN, Sir E. B. & G. MELLOWS: Pseudomonic acid. 1. The structure of pseudomonic acid A, a novel antibiotic produced by *Pseudomonas fluorescens*. *J. Chem. Soc., Perkin I* 1977: 294~309, 1977
- 3) ALEXANDER, R. G.; J. P. CLAYTON, K. LUK, N. H. ROGERS & T. J. KING: The chemistry of pseudomonic acid. 1. The absolute configuration of pseudomonic acid A. *J. Chem. Soc., Perkin I* 1978: 561~565, 1978
- 4) FELINE, T. C.; R. B. JONES, G. MELLOWS & L. PHILLIPS: Pseudomonic acid. 2. Biosynthesis of pseudomonic acid A. *J. Chem. Soc., Perkin I* 1977: 309~318, 1977
- 5) DODDRELL, D.; V. GLUSHKO & A. ALLERHAND: Theory of nuclear overhauser enhancement and  $^{13}\text{C}$ - $^1\text{H}$  dipolar relaxation in proton-decoupled carbon-13 NMR spectra of macromolecules. *J. Chem. Phys.* 56: 3683~3689, 1972
- 6) ALLERHAND, A.; D. DODDRELL & R. KOMOROSKI: Natural abundance carbon-13 partially relaxed Fourier transform nuclear magnetic resonance spectra of complex molecules. *J. Chem. Phys.* 55: 189~198, 1971
- 7) LEVINE, Y. K.; P. PARTINGTON & G. C. K. ROBERTS: Calculation of dipolar nuclear magnetic relaxation times in molecules with multiple internal rotations. I. Isotropic overall motion of the molecule. *Mol. Phys.* 25: 497~514, 1973
- 8) DILL, K. & A. ALLERHAND: Small errors in C-H bond lengths may cause large errors in rotational correlation times determined from carbon-13 spin-lattice relaxation measurements. *J. Am. Chem. Soc.* 101: 4376~4378, 1979
- 9) LYERLA, J. R., Jr.; H. M. MCINTYRE & D. A. TORCHIA: A  $^{13}\text{C}$  nuclear magnetic resonance study of alkane motion. *Macromolecules* 7: 11~14, 1974