

SECOND MOMENT ANALYSIS OF THE WIDE-LINE NMR SPECTRA OF THREONINE AND ITS ISOMERS

S. GANAPATHY, K. RAMANI AND R. SRINIVASAN

Department of Crystallography and Biophysics, University of Madras, Guindy Campus, Madras 600 025, India

ABSTRACT

The three crystalline forms of the isomers of threonine arising from the presence of two asymmetric carbon atoms have been analysed by wide-line NMR. A second moment analysis shows interesting configurational variations.

1. INTRODUCTION

RECENTLY we have been interested in the wide-line PMR studies of the amino acids and related compounds. Such a study would be an important adjunct to the X-ray^{1,2} and neutron diffraction data³ and would enable one to correlate and/or complement the structural and conformational information from the different sources. Not much of wide-line NMR work has been done on such compounds, although organic and inorganic substances have been studied as shown by a recent survey.

This paper deals with the results of the second moment analysis of the amino acid, threonine and its isomers. Threonine is an amino acid containing two asymmetric carbon atoms and therefore has a number of isomers. In fact, a number of different crystalline forms involving threonine and allothreonine have been X-ray analysed in this laboratory⁵⁻⁸ and compared^{9,10} with structural and conformational information available for other threonine derivatives in the literature.

The close isomorphism of L-threonine and L-allothreonine as determined by X-ray method, led to the possibility of growing a mixed crystal of the two isomers and the X-ray analysis has been reported on a 1:1 mixed crystal system⁸. Evidence of equal proportion of the two isomers from the X-ray data is reasonably convincing, however, it was thought worthwhile to examine these systems by PMR technique since such a study on organic molecules does not appear to have been reported in the literature.

2. EXPERIMENTAL

L-threonine and L-allothreonine were obtained from Produits Chimiques—Department of Pharmaceutique, Paris and Nutritional Biochemicals Corporation, Cleveland, Ohio, U.S.A., respectively. The mixed crystal was prepared by the method described elsewhere⁸.

The proton magnetic resonance spectra of the polycrystalline samples were recorded at room temperature with a wide-line NMR spectrometer constructed in this laboratory, employing 215 Hz magnetic field

modulation and phase sensitive detection. The radio frequency was 11.69 MHz. The audio frequency modulation was kept at about 0.3 G, and the radio frequency amplitude was kept below the saturation level. The line-widths were measured as separation in gauss of points of maximum and minimum slope of the derivative spectra. The experimental second moments for each compound were obtained as a mean from a dozen derivative tracings by numerical integration and were corrected for modulation broadening by standard procedures.¹¹ All the computations were performed on an IBM-1130 digital computer. A typical derivative tracing of the PMR signal in one of these compounds is shown in Fig. 1.

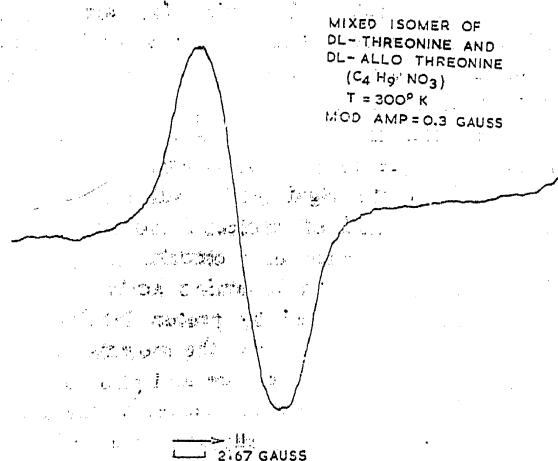


Fig. 1. A typical derivative tracing of PMR Signal in polycrystalline mixed crystal of L-Threonine and L-Allothreonine taken at room temperature.

3. DISCUSSION

Table I shows the second moments deduced from the observed spectra for the three cases. The rigid lattice values of second moment were calculated using Van Vleck's formula for a powdered sample.¹²

$$M_2 = (3/5) \gamma_1^2 \hbar^2 I(I+1) N^{-1} \sum_{j>k} r_{jk}^{-6} \quad (1)$$

where the symbols have their usual meaning and these are also given in Table I. The contribution to the second moment due to magnetic nuclei other than

TABLE I
Second Moment Data for Threonine and its Isomers

Compound	Room temperature second moment (G ²)	Theoretical second moment for a rigid lattice (G ²)		Total
		Intra	Inter	
L-Threonine	13.28 (0.77)	22.40	11.64	34.04
L-Allothreonine	8.54 (0.26)	24.25	12.31	36.56
Mixed crystal of L-Threonine and L-Allothreonine	11.35 (0.23)	24.24	11.57	35.81

Note: Values in parentheses denote standard deviation in experimental values.

protons has been found to be negligible. The sum in (1) is taken over all proton-proton distances less than 10.0 Å. The contribution for $r > 10.0$ Å was estimated to be 0.09 G² by integration over a continuous distribution of protons. For L-threonine proton coordinates from neutron diffraction study has been used¹³, while geometrically fixed positions of hydrogens have been used for L-allothreonine and the mixed crystal, since no neutron diffraction data are available for these. The contribution to the second moment from within a molecule (intra) and neighbouring molecules (inter) is listed separately in Table I. It is seen from Table I that the mean square width (second moment) for the three compounds is very much reduced as compared to the rigid lattice value. This must be due to some kind of molecular motion in these structures. The presence of molecular motions due to NH₂ and CH₃ groups in amino acids have been identified and characterised by proton NMR relaxation¹⁴ and C₁₃¹⁵ studies. Since the molecular motions at room temperature for the pure and allo forms are different, as evinced by different values of the second moments, it is reasonable to expect that a 1:1 mixed crystal would give a value of second moment which is in between those of the pure and allo forms. Our experimental value of the second moment for the mixed crystal (11.35 G²) lies between those of the pure (13.28 G²) and allo (8.54 G²) forms. This is in

accordance with the earlier X-ray work done in our laboratory.⁸ Since these structures have very close cell dimensions, space group and packing, in particular same hydrogen bonding scheme,¹⁰ the intermolecular contribution to the second moment which takes into account the molecular environment is practically the same. Thus the intramolecular contribution reflects the change in configuration of protons at C^β atom. A detailed study of these compounds and other amino acids at low temperatures is in progress and the results will be reported in due course.

ACKNOWLEDGEMENT

One of the authors (S.G.) thanks the University Grants Commission for financial help in the form of a Junior Research Fellowships

1. Ramachandran, G. N. and Lakshminarayanan, A. V., *Biopolymers*, 1966, **4** 495.
2. Lakshminarayanan, A. V., Sasisekharan, V. and Ramachandran, G. N., In *Conformation of Biopolymers*, Ramachandran, G. N. (Ed.), Academic Press, London, 1967, **1**, 61.
3. Ramanadham, M. and Chidambaram, R., *Acta Crystallogr.*, 1975, **A 31** (supplement), S 179.
4. Ganapathy, S. and Srinivasan, R., *J. Sci. Ind. Res.*, 1975, **34**, 134.
5. Swaminathan, P., *Acta Crystallogr.*, 1975, **B31**, 1608.
6. — and Srinivasan, R., *Ibid.* 1975, **B 31**, 217.
7. — and —, *J. Cryst. Mol. Struct.*, 1975, (In Press).
8. — and —, *Ibid.*, 1975 (In Press).
9. Srinivasan, R., Varughese, K. I. and Swaminathan, P., *Acta Crystallogr.*, 1974, **B 30**, 2270.
10. Swaminathan, P. and Srinivasan, R., *Int. J. Peptide and Protein Res.*, 1975 (In Press).
11. Andrew, E. R., *Phys. Rev.*, 1953, **91**, 425.
12. Van Vleck, J. H., *Ibid.*, 1948, **74**, 1168.
13. Ramanadham, M., Sikka, S. K. and Chidambaram, R., *Pramāna*, 1973, **1**, 247.
14. Andrew, E. R., Hinshaw, W. S., Hutchins, M. G. and Canepa, P. C., *chem. Phys. Letters*, 1974, **26**, 50.
15. Ratkovic, S., *Ibid.*, 1972, **17**, 623.