

INFLUENCE OF THE HYDROGEN BOND ON THE N-H STRETCHING FREQUENCIES IN AMINO-ACIDS

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1. INTRODUCTION

It is well known that glycine, the simplest member of amino-acids, exists as zwitter ion ($\text{NH}_3^+\text{CH}_2\text{COO}^-$) in the crystalline state. It easily forms addition compounds in inorganic acids and acid salts. These addition compounds contain "glycinium ion" or the glycine cation ($\text{NH}_3^+\text{CH}_2\text{COOH}$). In the higher homologues of amino-acid, zwitter ions of similar type exist. It has been established from structure analysis that in the solid state of these substances, the NH_3^+ group forms hydrogen bonds with the oxygen atoms of the COOH or COO^- groups of the neighbouring ions or with other electro-negative atoms. These hydrogen bonds form a three-dimensional network and are the determining factor in their crystal structure. They are also responsible for the ferroelectric behaviour exhibited by some of these crystals. Hence a study of the spectroscopy of the hydrogen bond in these substances is of very great interest. Systematic investigations on the Raman effect and infra-red absorption of glycine, its addition compounds and the higher amino-acids were therefore started in our laboratory nearly six years ago. The Raman spectra of the following substances in the single crystal form have so far been investigated: α -glycine (1958), triglycine sulphate (1958), γ -glycine (1962), diglycine hydrochloride, diglycine hydrobromide and diglycine nitrate (1961), *L*-asparagine monohydrate (1962), *c*-deuterated γ -glycine (1963), triglycine selenate (1963) and diglycine barium chloride (1964). The infra-red spectra of some of these substances have also been investigated (Narayanan and Khanna, 1959; 1964). The spectra of these substances both in Raman effect and infra-red absorption exhibited a series of broad and intense lines falling in the region of frequency shifts from 3250 cm.^{-1} to 2500 cm.^{-1} , the intensity decreasing with decreasing frequency shift (see Table I). Although the mean frequency shifts of these lines varied from substance to substance, the general pattern was similar for the different

substances. These Raman lines were found to persist in the spectra at lower temperatures (1963). Similar observations have been made in infra-red absorption by Bellamy (1954). In the case of deuterated compounds these Raman lines appear with correspondingly lower frequencies (Dodd, 1959).

TABLE I
Frequency shifts in cm.^{-1} of Raman lines appearing in the region
2500–3250 cm.^{-1} (values are in cm.^{-1})

α -gly- cine	γ -gly- cine	Trigly- cine sul- phate	Trigly- cine sele- nate	Digly- cine hydro- chloride	Digly- cine hydro- bromide	Digly- cine ni- trate	Digly- cine barium chloride mono- hydrate	<i>l</i> -aspa- gine mono- hydrate
2530	2546	2528	2610	2459	2592	2630	2596	2480
2630	2612	2651	2731	2553	2715	2707	2700	2588
2750	2665	2763	2857	2613	2785	2751	2745	2614
2830	2733	2874	2932	2652	2874	2806	2785	2693
2895	2788	2930	3150	2713	2910	2882	2865	2750
3145	2876	3150	3230	2790	3029	3039	2900	2838
..	3120	3230	..	2853	3067	3143	2920	2883
..	..	3270?	..	3040	3122	3252?	3033	3120?
..	3066	3221	3339??	3054	3240??
..	3126	3141	..
..	3229	3220	..

The Raman lines appearing in the region from 3250 cm.^{-1} to 2500 cm.^{-1} in the spectra of the amino-acids and glycine addition compounds have been assigned to the N-H vibrations, the frequencies of which have been lowered due to the hydrogen bonding. The characteristic C-H vibration frequencies appearing in this region have been excluded. In some of these compounds the hydrogen bond lengths (N...O) distances are known from X-ray studies

In such cases, we attempted to calculate the N-H...O vibration frequencies from the known bond lengths using the correlation curve of N-H...O stretching frequencies *versus* the H bond lengths given by Nakamoto *et al.* (1955) and Pimental and Sederholm (1956) (see curve *a* of Fig. 1). It was found that the calculated frequencies were restricted to the region from 3300 cm^{-1} to

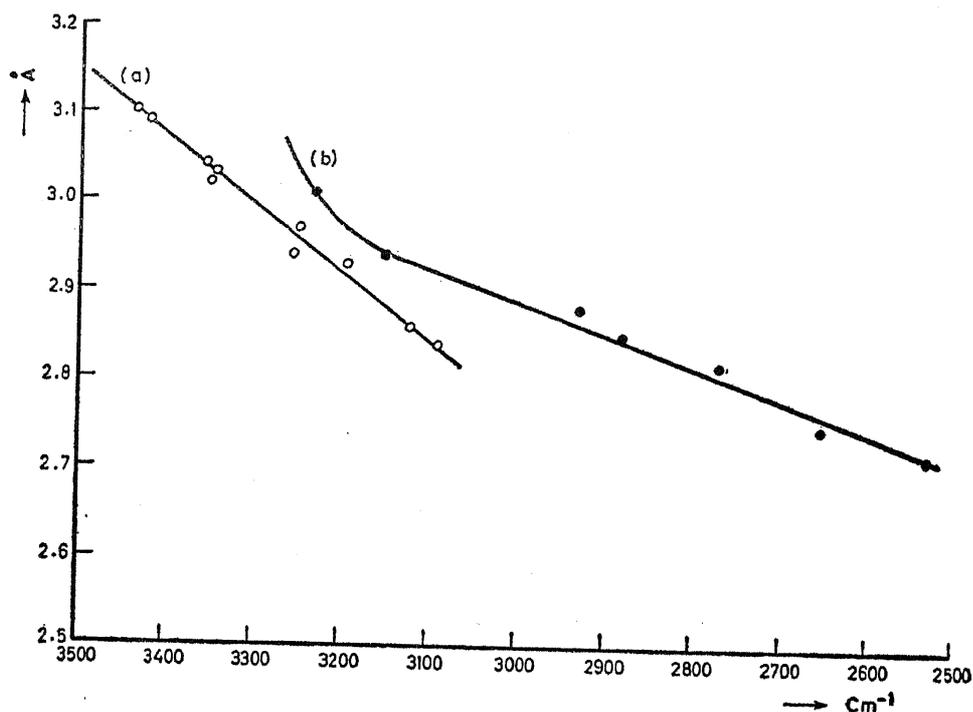


FIG. 1. Stretching frequency as a function of hydrogen bond length for
 (a) N-H...O bonds
 (b) N⁺-H...O bonds

2900 cm^{-1} , whereas the frequency shifts of the observed Raman lines due to N-H vibration extended as low as 2500 cm^{-1} . The Raman lines of lower frequency shifts could not be explained on the basis of Nakamoto-Pimental correlation curve for N-H...O vibration. The object of the present investigation was therefore to examine the problem in relation to the nature of the N-H bond.

2. NATURE OF THE N-H...O BONDS IN AMINO-ACIDS

It has been established from other studies, that in the amines and similar compounds, the N atom of NH_2 groups has the covalency of three and is termed tricovalent. In amino-acids, on the other hand, the N atom of the NH_3^+ groups has the covalency of four and is termed quadricovalent. These two types of N-H bonds should exhibit different behaviour when hydrogen-bonded. The structural notations used for these two types of hydrogen

bonds are as follows: (1) N-H...O which occur in amines, and (2) N⁺-H...O which occur in amino-acids. In this connection it is interesting to note that N⁺-H...O bonds are usually shorter than N-H...O bonds (Fuller, 1959). Raman lines due to N-H...O stretching vibrations are usually sharper than the N⁺-H...O stretching vibrations (Edsall, 1940). The Nakamoto-Pimental correlation curve is for the bonds of the first type, namely, N-H...O and therefore it is not surprising to find that it does not explain the N⁺-H...O vibrational frequencies observed in the Raman spectra of amino-acids. When Nakamoto *et al.* (1955) and Pimental (1956) realised this point, they could not draw any correlation curve for the N⁺-H...O vibration due to the lack of experimental data. For explaining the observed Raman spectra of amino-acids, it is necessary to know the correlation between the N⁺-H bond lengths and the corresponding N⁺-H stretching frequencies. This has been worked out by us here.

3. CORRELATION BETWEEN THE BOND LENGTHS AND THE STRETCHING FREQUENCIES FOR N⁺-H...O BONDS

Table I gives the different hydrogen bonds and their lengths in α -glycine (Marsh, 1957), γ -glycine (Iitaka, 1961), triglycine sulphate (Hoshino, 1959), diglycine hydrochloride (Hahn and Buerger, 1957), diglycine dibromide (Hahn *et al.*, 1956) *l*-asparagine monohydrate (1961) and *dl*-alanine (1950) obtained from structure data. It is seen from Table II that triglycine sulphate has the maximum number of N⁺-H...O bonds and its Raman spectrum has been very thoroughly investigated (Krishnan and Balasubramanian, 1958). The nine hydrogen bonds of the type N⁺-H...O observed in the crystal structure of triglycine sulphate have lengths varying from 3.009 Å to 2.644 Å. From one is able to identify the Raman lines and assign them to the corresponding N⁺-H...O bonds for triglycine sulphate, one can draw the correlation curve.

A perusal of the data collected by Fuller (1959) on the bond lengths and bond angles of hydrogen bonds shows that except for bifurcated bonds the lengths of the N⁺-H...O bonds between the NH₃⁺ groups and the COO⁻ groups are less than 3 Å. It can, therefore, be assumed that the longest N⁺-H bond for the amino-acids and for the glycine addition compounds is about 3 Å. Lacher *et al.* (1957) have shown that the free N⁺-H stretching frequency of the NH₃⁺ group in *dl*-alanine and in other amino-acids lies between 3250 cm.⁻¹ and 3180 cm.⁻¹. In glycine and its addition compounds one can assume that the free N⁺-H stretching frequency is about 3250 cm.⁻¹. A re-examination of the Raman spectrum of triglycine sulphate reproduced by Krishnan and Balasubramanian (1958) indicates that the highest N⁺-H

stretching frequency shift is 3230 cm.^{-1} which has been assigned to the vibration of the $\text{N}^+-\text{H}\dots\text{O}$ bond of length 3.009 \AA . The one at 3270 cm.^{-1} reported by these authors is not a genuine Raman line, but is only the end of a continuum. The prominent Raman lines with frequency shifts 3150, 2930, 2880, 2770, 2650 and 2530 cm.^{-1} of triglycine sulphate have been assigned to vibrations of the $\text{N}^+-\text{H}\dots\text{O}$ bonds of lengths 2.941, 2.879, 2.853, 2.822, 2.745 and 2.717 \AA respectively (see Table II). Using these values the frequency *versus* bond length curve has been drawn for the $\text{N}^+-\text{H}\dots\text{O}$ bonds, and is reproduced in Fig. 1 (b). It is satisfying to note that all the points lie on a smooth curve. As is to be expected the correlation curves for the $\text{N}-\text{H}\dots\text{O}$ and for the $\text{N}^+-\text{H}\dots\text{O}$ bonds are quite different. The general shape of the correlation curve for the $\text{N}^+-\text{H}\dots\text{O}$ bond is similar to that for other hydrogen bond systems, e.g., $\text{O}-\text{H}\dots\text{O}$ (Nakamoto *et al.*, 1955). It will be seen from Table II that triglycine sulphate has two short $\text{O}-\text{H}\dots\text{O}$ bonds of lengths 2.438 and 2.540 \AA . The Raman lines corresponding to these bonds should appear well below 2500 cm.^{-1} . All the lines appearing in the region from 3230 cm.^{-1} to 2530 cm.^{-1} are due to the $\text{N}^+-\text{H}\dots\text{O}$ bonds only. The straight line portion of the curve 1 (b) can be represented by the relation, $\nu = 2.714 (R - 1.784) \times 10^3$ where R is the H bond length in \AA .

4. $\text{N}^+-\text{H}\dots\text{O}$ STRETCHING VIBRATIONS IN GLYCINE AND OTHER COMPOUNDS

Using the correlation curve (b) (Fig. 1) the frequencies of the $\text{N}^+-\text{H}\dots\text{O}$ vibrations of glycine and other compounds whose hydrogen bond lengths are known have been estimated. The calculated values are entered in the fourth column of Table II. The observed frequency shifts of the prominent Raman

TABLE II
Hydrogen bond lengths and $\text{N}^+-\text{H}\dots\text{O}$ frequencies in amino-acids and glycine compounds

Compound	H-bond type	Length in \AA	Frequencies in cm.^{-1}	
			Predicted	Observed
α -glycine $\text{N}^+-\text{H}\dots\text{O}$	2.768	2670	2630
		2.850	2890	2895
		2.949*	3165	3145
		3.074*	3270	..
γ -glycine $\text{N}^+-\text{H}\dots\text{O}$	2.801	2750	2733
		2.817	2820	2876
		2.970	.. 3190	3120

TABLE II (Contd.)

Compound	H-bond type	Length in Å	Frequencies in cm. ⁻¹	
			Predicted	Observed
Triglycine sulphate	.. O-H...O	2.438
		2.540
	N ⁺ -H...O	2.644
		2.717	..	2530
		2.745	..	2650
		2.822	..	2770
		2.853	..	2880
		2.879	..	2930
		2.910
		2.941	..	3150
3.009	..	3230		
Diglycine hydrochloride	.. O-H...O	2.570
		N ⁺ -H...O	2.90	3035
	N ⁺ -H...Cl	2.93	3115	3126
		2.98*	3200}	3229
		3.04*	3250}	
		3.13
		3.22
		2.23*
		3.32*
Diglycine hydrobromide	.. O-H...O	2.46	..	{3029
		N ⁺ -H...O	2.90	3035
	N ⁺ -H...Br	2.94	3150	3122
		3.04*	3250}	3221
		3.10*	3280}	
		3.37
		3.33
		<i>l</i> -asparagine monohydrate	.. O-H...O	2.80
2.84
N-H...O	2.92	
	3.02	
N ⁺ -H...O	2.80		2750	2750
	2.81		2780	2838
	2.85		2890	2883
<i>dl</i> -alanine N ⁺ -H...O	2.80	2750	2716
		2.84	2870	2839
		2.88	2975	3042

* Bifurcated H bonds.

lines in this region for the different substances are also entered in Table II. In the case of *dl*-alanine, the observed frequencies given in the table are those obtained by Leifer and Lippincott (1957) in infra-red absorption. There is reasonably good agreement between the calculated and observed values of the frequencies for the $N^+-H...O$ vibrations.

In α -glycine, γ -glycine, diglycine hydrochloride and diglycine hydrobromide there are a few comparatively weaker Raman lines in the region above 2500 cm^{-1} . It is well known that the H bond stretching mode due to $N^+...O$ vibration which has a frequency of about 150 to 200 cm^{-1} often combines with the mode corresponding to $N^+-H...O$ stretching vibration and gives rise to combinations (summationals and differentials) in the Raman spectrum. All the additional weaker lines in these substances could be assigned to such combinations. Often the summationals fall on the intense C-H Raman lines, whereas the differentials fall on the lower frequency shift side and are easily identified. In the case of diglycine hydrochloride, the lines at 2459 and 2553 cm^{-1} are due to the $O-H...O$ vibrations and the lines at 2790 and 2854 cm^{-1} are due to the $N^+-H...Cl$ vibrations. In the case of diglycine hydrobromide the Raman lines at 2785 , 2874 and $2907-2917\text{ cm}^{-1}$ are due to the $N^+-H...Br$ vibrations. The present study could not be extended to other amino-acids for want of reliable experimental data in Raman and infra-red spectra.

In this connection, it is necessary to mention a word about the characteristic infra-red band near 2100 cm^{-1} observed in the case of all the amino-acids (Koegel *et al.*, 1957). This has often been assigned to the NH_3^+ stretching vibrations. From the considerations outlined in the earlier part of this paper, it is obvious that the absorption at this frequency cannot arise from NH_3^+ stretching vibration. Leifer and Lippincott (1957) have suggested that this band might be due to the bending vibration of the NH_3^+ group. Actually, this band is similar to the 2095 cm^{-1} band observed in water, which has been assigned as the combination of bending and librational modes of water (1962). Similarly, the 2100 cm^{-1} band in amino-acids may be the combination band due to the NH_3^+ deformation (around $1500-1600\text{ cm}^{-1}$) and NH_3^+ torsion (around 500 cm^{-1}).

5. SUMMARY

The spectra of glycine, its addition compounds and other amino-acids exhibit Raman lines in the region from 3250 cm^{-1} to 2500 cm^{-1} . It has been shown that these lines cannot be assigned to $N-H...O$ stretching vibrations, where the N atom has the covalency of three, but to $N^+-H...O$ stretching

vibration where the N atom has the covalency of four. Using the data obtained with triglycine sulphate which has the largest number of N-H...O bonds and whose H bond lengths are known, the correlation curve showing the relation between the N-H...O stretching frequencies and the corresponding H bond lengths has been drawn. Using this correlation curve, the N-H...O stretching frequencies appearing in α -glycine, γ -glycine, α -hydrochloride, diglycine hydrobromide, *l*-asparagine monohydrate and *dl*-alanine have been satisfactorily accounted for on the basis of the hydrogen bond lengths in these substances.

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7. REFERENCES

1. Balasubramanian, K. — *Ph.D. Thesis*, Indian Institute of Science, Bangalore.
2. ————— .. *Proc. Ind. Acad. Sci.*, 1961, **53 A**, 105.
3. ——— and Krishnan, R. S. *Ibid.*, 1963, **58 A**, 209.
4. ———, ——— and Itaka, Y. .. *Bull. Chem. Soc. Japan*, 1962, **35**, 1303.
5. Bellamy, L. J. .. *The Infra-red Spectra of Complex Molecules*, Wiley and Co., London, 1954, p. 200.
6. Dodd, D. M. .. *Spectrochim. Acta*, 1959, **12**, 1072.
7. Donohue, J. .. *J. Am. Chem. Soc.*, 1950, **72**, 949.
8. Edsall, J. T. and Scheinberg, H. .. *J. Chem. Phys.*, 1940, **8**, 520.
9. Fuller, W. .. *J. Phys. Chem.*, 1959, **63**, 1705.
10. Hahn, T. and Buerger, M. J. .. *Z. Krist.*, 1957, **108**, 419.
11. ———, Barney, E. and Buerger, M. J. .. *Ibid.*, 1956, **108**, 130.
12. Hoshino, S., Okaya, Y. and Pepinsky, R. .. *Phys. Rev.*, 1959, **115**, 323.
13. Itaka, Y. .. *Acta Cryst.*, 1961, **14**, 1.
14. Kartha, G. and de Vries, A. .. *Nature*, 1961, **192**, 862.
15. Koegel, R. J., McCallun, R. A., Greenstein, J. P., Winitz, M. and Birnbaum, S. .. *Ann. N.Y. Acad. Sci.*, 1957, **69**, 94.
16. Krishnan, R. S. and Balasubramanian, K. .. *Proc. Ind. Acad. Sci.*, 1958, **48 A**, 55.

17. Krishnan, R. S. and Balasubramanian, K. .. *Proc. Ind. Acad. Sci.*, 1958, 48 A, 138.
18. ————— .. *Ibid.*, 1964, 59 A, 14.
19. Krishnan, K. and Krishnan, R. S. *Ibid.*, 1962, 55 A, 153.
20. Lacher, J. R., Croy, V. D., Kianpour, A. and Park, J. D. *J. Phys. Chem.*, 1954, 58, 206.
21. Leifer, A. and Lippincott, E. R. *J. Am. Chem. Soc.*, 1957, 79, 5807.
22. Marsh, R. E. .. *Acta Cryst.*, 1958, 11, 654.
23. Nakamoto, K., Margoshes, M. and Rundle, R. E. *J. Am. Chem. Soc.*, 1955, 77, 6480.
24. Narayanan, P. S. and Khanna, R. K. *Proc. Symp. on Raman and Infra-red Spectroscopy, Nainital*, 1959, pp. 52-58.
25. ————— .. 1964 (To be published).
26. Neelakantan, P., Krishnan, R. S. and Iitaka, Y. *Proc. Ind. Acad. Sci.*, 1963, 58 A, 275.
27. Pimental, G. C. and Sederhohn, C. H. *J. Chem. Phys.*, 1956, 24, 639.
28. Walrafen, G. E. — *Ibid.*, 1962, 36, 1035.