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Conformation of α-Aminobutyric Acid in Aqueous Solution

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Raman spectrum of α-aminobutyric acid has been observed in its 15% aqueous solution (neutral). By comparing it with the Raman and infrared spectra of crystal A (in which 40% of the total molecules are the T-form molecules, 36% G_a-, and 24% G_b-form molecules), crystal B (which consists only of T-form molecules), and a complex crystal of this amino acid with calcium chloride (in which 85% are the G_a-form molecules), it has been shown that the G_a-form molecules are predominant in the aqueous solution. Here, T-, G_a-, and G_b-forms have respectively the amino group, the hydrogen atom, and the carboxy group in the position trans to the methyl group in the internal rotation around the C_α-C_β bond. Proton magnetic resonance spectrum was observed of a C_γ-deuterated product ($CD_3CH_2CH\ NH_3{}^+COO^-$) in its aqueous solution at several temperatures in the −1.5 to 91°C range. It has been indicated that the C_α-form is more stable by about 300 cal/mole than the other two forms.

For DL-α-aminobutyric acid, two crystalline modifications, A and B, were previously found.[1] An analysis of X-ray diffraction[2,3] indicated that in crystal A the methyl group on the β-carbon does not take a fixed position. It was found to be distributed among the three sites in the internal rotation around the C_α–C_β axis. One site is nearly trans to the amino group (designated as T-form), another is nearly trans to the hydrogen atom (G_a-form), and the other nearly trans to the carboxy group (G_b-form) (see Fig. 1). The occupancy factors of these three positions were found to be 40, 36, and 24%, respectively, at room temperature. In crystal B, only T-form was found. [2,3] Recently, Akimoto and Iitaka[4] found that, in a complex crystal of DL-α-amino-*n*-butyric acid with calcium chloride, 85% of the amino acid molecules are in G_a-form. Thus, it is probable that T-, G_a-, and G_b-forms are three stable rotational isomers. The purpose of the present work is to examine which of

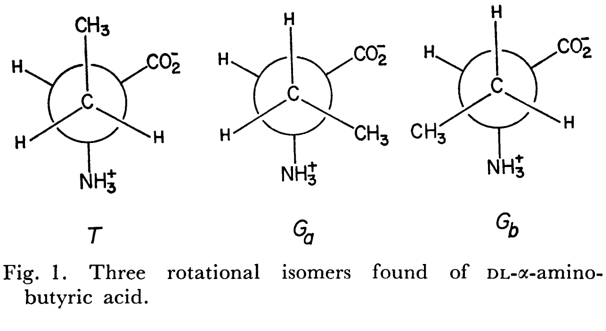

Fig. 1. Three rotational isomers found of DL-α-amino-butyric acid.

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[1] M. Tsuboi, Y. Iitaka, S. Suzuki, and S. Mizushima, This Bulletin, **32**, 529 (1959).

[2] T. Ichikawa, Y. Iitaka, and M. Tsuboi, *ibid.*, **41**, 1027 (1968).

[3] T. Ichikawa and Y. Iitaka, *Acta Cryst.*, **B24**, 1488 (1968).

[4] T. Akimoto and Y. Iitaka, to be published. See also T. Akimoto, Doctor Thesis, University of Tokyo, 1970.

these three forms are involved in neutral aqueous solution of this amino acid.

Experimental

DL-*α-Aminobutyric Acid and Its Crystals.* The sample of DL-α-aminobutyric acid used in this investigation was obtained from a commercial source and was purified by recrystallization with water and ethanol.

Crystal A was obtained by dissolving the amino acid in water and by adding ethanol. When a small amount of precipitate came out, this was dissolved again by adding a small amount of water. The solution was then placed in a refrigerator at 5—10°C, and, a few days after, crystals were obtained which were found to be all in A-form.

From aqueous solutions with 1 M NaCl, always crystal B was obtained. Crystal B could be also obtained, however, by adding a greater amount of ethanol into an aqueous solution of this amino acid. When a good amount of precipitate came out, this mixture was placed in a refrigerator. The crystals thus obtained were found to be in B form.

The crystal of the complex with $CaCl_2$ was obtained by adding $CaCl_2$ into an aqueous solution of the amino acid so that the mole ratio of amino acid/$CaCl_2$ became about 2/1.5, and then by gradual evaporation of the solvent. What was obtained was a hygroscopic, monoclinic crystal elongated along the c axis. Elementary analysis was made of a sample completely dried by means of an Abderhalden's dryer.

Found: C, 27.4; H, 6.35; N, 8.00%. Calcd for $C_8H_{20}N_2O_6CaCl_2$: C, 27.35; H, 5.70; N. 8.00%.

A crystallographic analysis[4)] of this crystal indicated that its chemical unit is $CaCl_2$ - 2(DL-α-amino-*n*-butyric acid) - $2H_2O$.

Raman Spectrum Raman spectrum of DL-α-aminobutyric acid was observed in its neutral aqueous solution by the use of a Perkin-Elmer LR-1 Raman Spectrometer with a 10 milliwatt He–Ne laser. The concentration was about 15% and the solution was placed in a 2.5-m*l* multi-reflection cell.

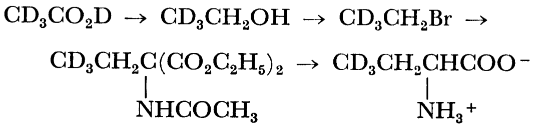
The Raman spectrum of this aqueous solution was observed also by the use of 5145 Å radiation of an argon ion laser. The results of the measurements with two different light sources agreed well, so that the Raman lines of this amino acid in its neutral aqueous solution could be fixed with a high degree of certainty.

The Raman spectra of the crystals were observed in the form of powder by the use of a JRS-01B spectrophotometer of Japan Electron Optics Laboratory Co., Ltd. with the 4880 Å and/or 5145 Å line of an argon ion laser.

Infrared Spectra. Infrared spectrum of an aqueous solution with the concentration 15% was observed with a CaF_2 cell by the use of a Perkin-Elmer 621 spectrometer.

Infrared spectra of crystals were observed in the forms of the Nujol and hexachlorobutadiene mulls. The spectral region of 4000—300 cm^{-1} was covered by the use of a Perkin-Elmer 621 Spectrometer. Each spectrum was observed not only at a room temperature but also at lower temperatures by the use of dry ice+acetone and of liquid nitrogen as cryogens. At lower temperatures, most absorption bands were observed at slightly higher frequencies, were narrower in their widths, and were observed with a higher resolution than those observed at the room temperature.

Preparation of DL-*α-aminobutyric-4-*d_3 *Acid.* The C-deuterated product $CD_3CH_2CH(NH_3^+)COO^-$ was prepared from acetic acid-d_4 containing over 99% deuterium (purchased from E. Merck and Co.) *via* the following steps:



$$CD_3CO_2D \rightarrow CD_3CH_2OH \rightarrow CD_3CH_2Br \rightarrow \underset{\displaystyle NHCOCH_3}{CD_3CH_2C(CO_2C_2H_5)_2} \rightarrow \underset{\displaystyle NH_3^+}{CD_3CH_2CHCOO^-}$$

Ethanol-2-d_3: A solution of 11.0 g (0.17 M) of acetic acid-d_4 in 50 m*l* of diethyl carbitol was added to a slurry of 10 g of lithium aluminum hydride in 100 m*l* of diethyl carbitol at room temperature. The reaction mixture was kept stirring for 8 hours at 60—70°C. After the reaction was completed, the mixture was cooled, and 260 m*l* of monobutyl carbitol was added below 20°C with stirring. Stirring was continued for additional 4 hr. The resulting ethanol-2-d_3 was distilled under 10^{-3}—10^{-4} mmHg at the bath temperature of 60—70°C, and collected in a trap chilled with liquid nitrogen. The yield was 7.3 g (87%).

Ethyl-2-d_3 *Bromide*: To 7.3 g of ethanol-2-d_3, prepared as described above, was added 27 g of phosphorus tribromide at −10°C. The reaction mixture was subjected to a fractional distillation, and the product was dried over potassium carbonate. Yield 10.3 g (62%).

Ethyl Acetamido (Ethyl-2-d_3*) Malonate*: In a solution of 16.3 g of ethyl acetamidomalonate plus 30 m*l* of absolute ethanol was dissolved 1.73 g of sodium. To the solution was added the deuterated ethyl bromide dissolved in 20 m*l* of ethanol. The mixture was refluxed for 13 hr, and then poured into ice-cold water. The resulting crystals were collected by filtration, washed with cold water, and recrystallized from ethanol-water. Yield 15 g (86%).

α-Aminobutyric-4-d_3 *Acid*: The deuterated ethyl acetamido (ethyl)malonate was hydrolyzed and decarboxylated by refluxing with 100 m*l* of concentrated hydrochloric acid for 7 hr. The mixture was concentrated to dryness. The residue was dissolved in a small amount of aqueous ethanol and 15 m*l* of pyridine was added dropwise to the solution. By filtration of the precipitate, 3.8 g (57%) of the crude crystals was obtained. It was recrystallized from hot 96% ethanol.

Proton Magnetic Resonance. Proton magnetic resonance of DL-α-aminobutyric-4-d_3 acid, $CD_3CH_2CH(NH_3^+)COO^-$, was observed in D_2O solution by the use of a Varian HA-100 spectrometer. The concentration of the sample was again about 15%. The temperature of the sample solution was controlled with a Varian V6040 Controller.

Results and Interpretations

Raman spectrum of DL-α-aminobutyric acid in its aqueous solution (15%) is shown at the top of Fig. 2 and also of Fig. 3. Raman spectra of the same amino acid in its crystalline states (crystal B, complex with $CaCl_2$, and crystal A) are given in the lower part of Fig. 2. Infrared absorption spectra of the aqueous solution and crystals are given (with absorption upward) in Fig. 3. The infrared spectra of the three crystals given here are all what were observed at about −180°C (with liquid nitrogen). By comparing the Raman spectra of the three crystals with one another, many of the Raman lines can be assigned to the T, G_a, or G_b form molecule. By a similar comparison of the infrared spectra, the assignments of the absorption bands are also made. In addition, by comparing the Raman and infrared spectra, it is shown that each of the broad Raman peaks of crystal A at 650, 770, 875, 1050, 1120, 1170, and 1270 cm^{-1} must consist

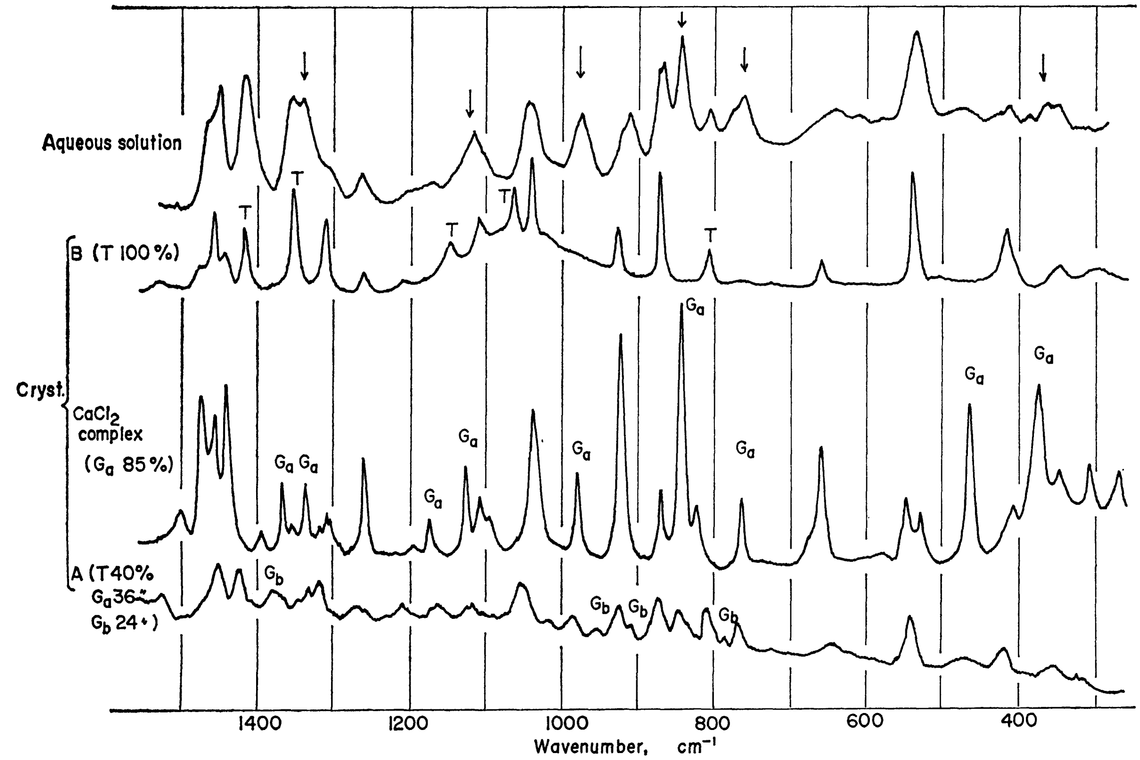


Fig. 2. Raman spectra of DL-α-aminobutyric acid in aqueous solution (top) and of three crystals: B-form, $CaCl_2$-complex, and A-form.

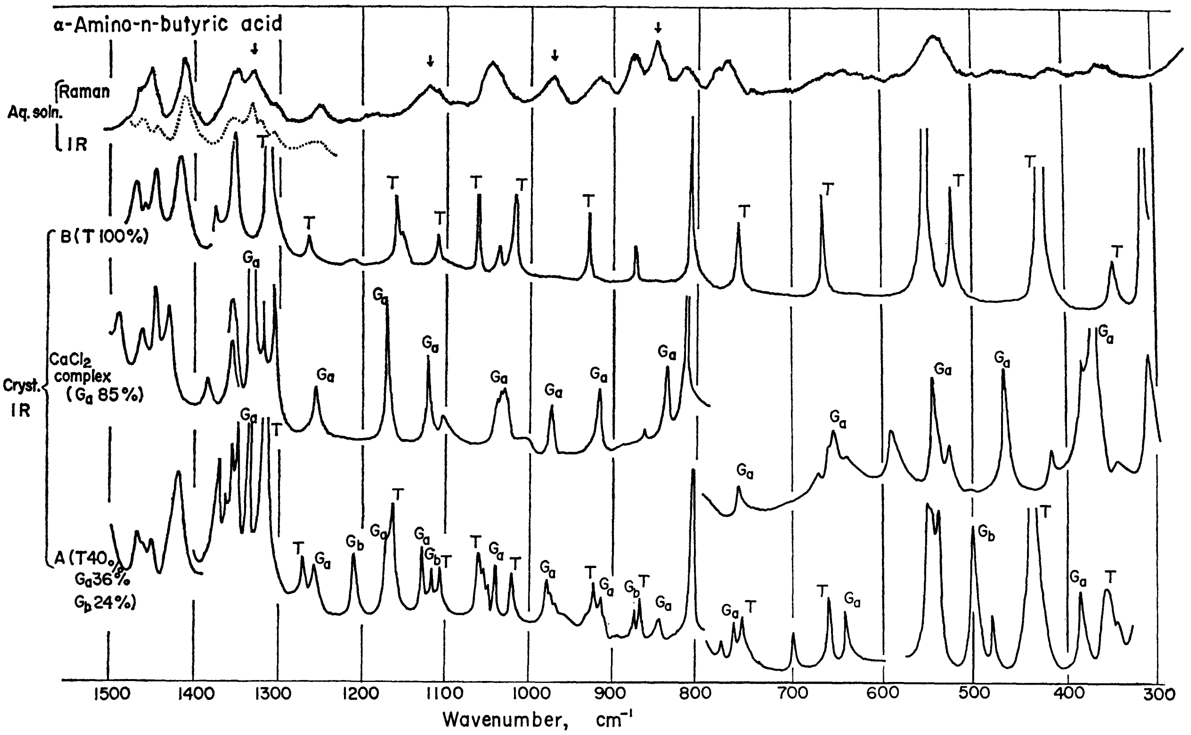


Fig. 3. Raman and infrared spectra of DL-α-aminobutyric acid in aqueous solutions (top) and infrared spectra of three crystals: B-form, $CaCl_2$-complex, and A-form.

of T, G_a, and/or G_b lines. The assignments of the Raman lines and infrared bands to the T, G_a, and G_b forms are given in Figs. 2 and 3. The appearance of strong Raman lines at 765, 843, 970, 1120, and 1340 cm^{-1} (indicated by arrows in Fig. 2) suggests that the G_a form is predominant in the aqueous solution at room temperature. A strong infrared band at 1340 cm^{-1} and a weak Raman line at 375 cm^{-1} give a support to this idea.

Proton magnetic resonance spectrum (Fig. 4) of deuterated α-aminobutyric acid ($CD_3CH_2CH(ND_3^+)COO^-$) in D_2O solution, gives its methylene (AB)

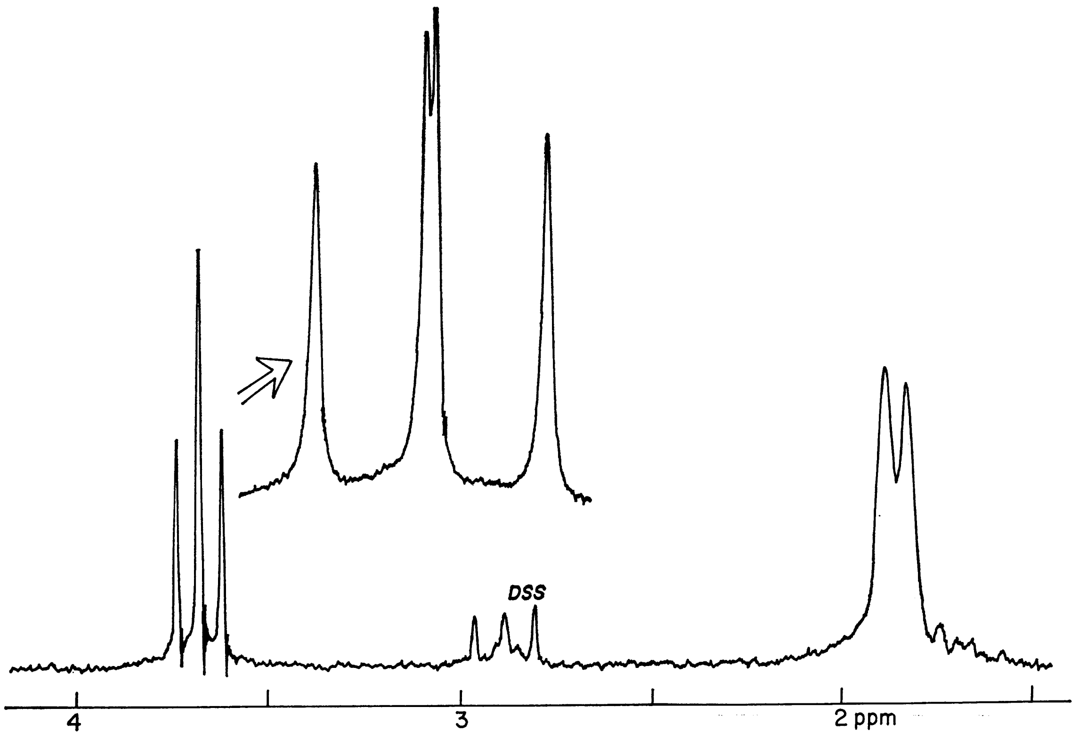


Fig. 4. Magnetic resonance signal of the proton on the α-carbon in α-aminobutyric acid-4-d_3 in D_2O solution.

signal (doublet) at 1.85 ppm (from internal DSS[5]) and the signal of the proton on C_α at 3.68 ppm (X). The latter signal consists of four components (see Fig. 4) which are caused by coupling with the methylene protons. Each value of the coupling constants J_{AX} and J_{BX} cannot easily be determined from the spectrum, but the value of $|J_{AX}+J_{BX}|$ can be determined by measuring the separation between the outermost peaks. As is shown in Table 1 and in Fig. 5, this value was found to change slightly but appreciably with the temperature of the solution.

Table 1. Vicinal coupling constants in α-aminobutyric acid-4-d_3 and the population of G_a form

Temperature °C	$J_{AX}+J_{BX}$ Hz	$\alpha(G_a)$	K	ΔF kcal/mol
−1.5	11.70	0.407	1.37_2	−0.165
8.5	11.82	0.396	1.31_1	−0.152
34	11.92	0.387	1.26_3	−0.142
46.5	11.99	0.381	1.23_1	−0.132
69	12.00	0.380	1.22_5	−0.136
91	12.04	0.376	1.20_5	−0.135

The temperature effect of the $|J_{AX}+J_{BX}|$ value is attributable to a conformational change of the amino acid in the aqueous solution. The fact that the $|J_{AX}+J_{BX}|$ value *increases* with the temperature indicates that G_a form is the most stable form among the three postulated rotational isomers. Thus, in form G_a both of the methylene C-H bonds are at gauche positions with respect to the C_α–H bond around the C_α–C_β axis, whereas in forms T and G_b one at trans and the other at gauche positions (see Fig. 1). As is now well known, the vicinal coupling constant (J_g) is much smaller when the H–C–C–H system is in a gauche form than that (J_t) when it is in a trans form. Therefore, the $|J_{AX}+J_{BX}|$ value should be smaller for G_a form than that for T or G_b form, and the fact that this value increases with the temperature should mean that the population of form G_a decreases and those of form T and/or form G_b increase with the temperature.

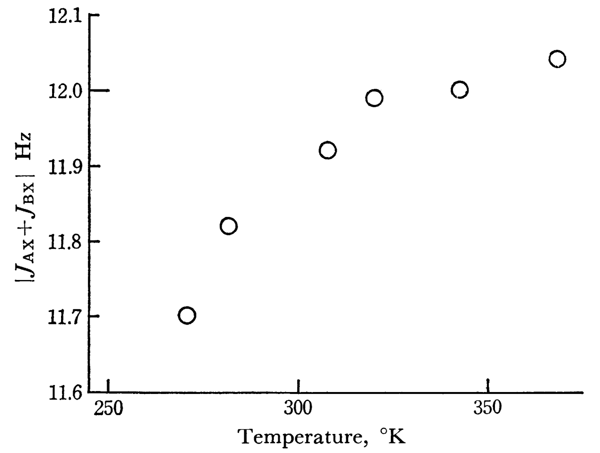


Fig. 5. Temperature dependence of the *vicinal* coupling constants in α-aminobutyric acid-d_3.

If, according to Pachler,[6] the J_g and J_t values are assumed to be 2.60 and 13.56 Hz, respectively, then the mole fraction $\alpha(G_a)$ of G_a form molecules can be calculated by an equation,

$$\alpha(G_a) = [(J_t+J_g) - |J_{AX}+J_{BX}|]/(J_t-J_g)\,.$$
$$= (16.16 - |J_{AX}+J_{BX}|)/10.96 \qquad (1)$$

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[5] DSS=Sodium-2,2-dimethyl-2-silapentane-5-sulfonate

The equilibrium constant K between G_a form and the other two forms is given as

$$K = \alpha(G_a) \Big/ \frac{[1-\alpha(G_a)]}{2}. \tag{2}$$

Here, it is tentatively assumed that T and G_b forms have always equal populations to each other. The $\alpha(G_a)$, K and ΔF (free energy difference) values calculated from the $|J_{AX}+J_{BX}|$ value at each temperature are given in the last three columns of Table 1. The energy difference ΔE between the G_a form and the other two forms is to be estimated as usual from the slope of the ln$K$ *versus* $1/T$ curve. As may be seen in Fig. 6, the ln$K$-$1/T$ plot does not result in a straight line. This fact may be interpreted as indicating that the ΔE value depends on the temperature. It is also possible, however, that this dependence is merely an apparent one which is caused by neglecting the free energy difference of the T and G_b forms. The apparent ΔE value is obtained as $\Delta E = -0.4$ kcal/mol at 20°C and $\Delta E = -0.2$ kcal/mol at 50°C.

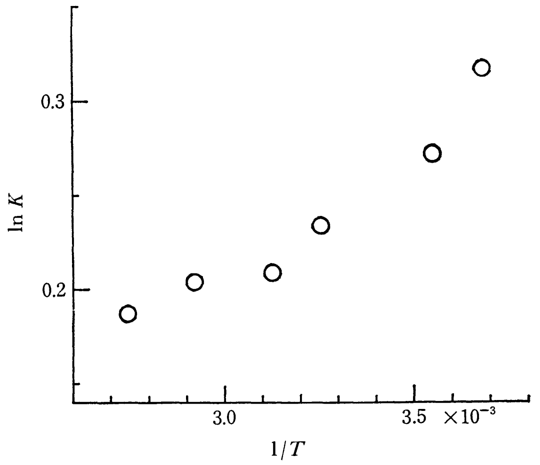

Fig. 6. Natural logarithm of the equilibrium constant K (see Eq. 2) plotted against $1/T$.

Discussion

Rotational isomerism of α-aminobutyric acid in aqueous solution was previously studied by Pachler.[6,7] He drew a conclusion that all of the three isomers have almost equal residence times to one another at about 30°C. This conclusion, however, was based upon the results of his NMR examination of an alkaline and an acidic solutions, while our present study was made of a neutral solution. In addition, he obtained only an approximate (±0.5 Hz) vicinal coupling constant of the CH_2–CH system. A more precise NMR study of undeuterated α-aminobutyric acid should involve a rather elaborate A_3MNX treatment for the CH_3CH_2CH system. Even if a decoupling experiment were done, this would not provide coupling constant values with an accuracy of ±0.02 Hz.

To overcome the difficulties, we have deuterated the methyl group of this amino acid in our present study. The NMR of the CH_2CH group can now be treated as an ABX problem. A precise value of $|J_{AX}+J_{BX}|$ can now be obtained, and a detailed discussion of the temperature effect on this value is now significant. We have also obtained in our present study a quite independent set of data—*i.e.* the vibrational spectrum.

As has been described above, our present NMR study clearly indicated that G_a form is the most stable among the three rotational isomers. Both of our NMR and Raman data showed that, in a neutral aqueous solution at room temperature, G_a form molecules are most abundant. From the NMR data, the mole fraction of G_a form has been estimated to be 0.387 which is only slightly greater than 1/3. It should be pointed out here, however, that this figure is based upon an assumption that every proton in the CH_2–CH system in every rotational isomer is located at an exact trans or an exact gauche position with respect to another proton, so that the proton-proton coupling constant should be 13.56 or 2.60 Hz. It is not improbable that the actual rotational isomers take conformations somewhat deviated from such "standard" conformations. For such deviated conformations, the effective J_t value would be slightly smaller than 13.56 Hz and the effective J_g value would be slightly greater than 2.60 Hz. If so, $\alpha(G_a)$ should be estimated (Eq. (1)) to be somewhat greater than 0.387.

One might be puzzled by our present conclusion that G_a form is the most stable among the three possible rotational isomers, because this form should undergo the strongest intramolecular steric repulsion. The steric repulsion between the methyl group and the carboxyl and/or amino group looks certainly a factor of making G_a form less stable. Thus, when the methyl group is substituted by the OH group (which is smaller than CH_3) the population of G_a form becomes greater (0.61 at room temperature[8]). When the methyl group is substituted by the carboxyl or SH group (which is greater than CH_3), on the other hand, the population of G_a form becomes smaller (0.22 for L-aspartic acid and 0.38 for L-cystein[6,7]). For all of these amino acids, however, it is necessary to postulate a factor which makes G_a form more stable. It is not likely that this factor is related with the electric dipole moment of the molecule or with an electrostatic interaction within the molecule, because the position of the methyl group should not cause great change in the dipole moment or in the electrostatic interaction. It is probable that the factor is related with the extent to which the water structure is disturbed by bringing the amino acid molecule into water. Perhaps it is worthwhile to point out here that G_a form is the most globular among the three rotational isomers. A preliminary examination of a space-filling model has indicated that the largest and smallest radii of the molecule are respectively 6.8 and 5.2 Å in G_a form whereas they are 7.2 and 4.4 Å in both T and G_b forms.

In the present study we could estimate only the

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[6] K. G. R. Pachler, *Spectrochim. Acta*, **19**, 2085 (1963).
[7] K. G. R. Pachler, *ibid.*, **20**, 581 (1964).
[8] H. Ogura, S. Fujiwara, and Y. Arata, *J. Mol. Spectry.*, **23**, 76 (1967).

population $\alpha(G_a)$ of G_a form but could not estimate those of T and G_b forms. The latter would be possible if we can deuterate selectively one of the two hydrogens on the β-carbon. An NMR spectrum of such a deuterated product $CD_3CHDCH(NH_3^+)COO^-$ should give J_{AX} or J_{BX} value.