Temperature-Dependent Infrared Study on L- and DL-α-Alanine

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(Received November 21, 1974)

Infrared spectra from 4000 to 30 cm^{-1} of L- and DL-α-alanine and their *N*-deuterated analogues were measured at temperatures ranging from 295 to 100 K. Highly temperature-dependent bands were observed at 972 cm^{-1} (at 100 K) for L-α-alanine and 975 cm^{-1} for DL-α-alanine. The temperature dependence of the half-widths of these bands indicates that above 200 K the vibrational modes are considerably modulated by some motions, possibly arising from breaking of hydrogen bonds. These bands are thus assigned to the first overtone of the NH_3^+ torsional mode.

Temperature studies of vibrational spectra of amino acids would provide valuable information on the nature of hydrogen bonding as well as molecular motions in these biologically important substances. Although numerous studies have been published on amino acids, temperature studies of the infrared and Raman spectra are very scarce.[1–5)]

It is the purpose of this paper to report on the changes due to the temperature variation observed in the infrared spectra of L- and DL-α-alanine and their *N*-deuterated analogues.

Experimental

L-Alanine and DL-α-alanine were recrystallized several times from aqueous solution. *N*-Deuterated analogues were prepared by successive exchanges with D_2O.

Infrared spectra were recorded on a Hitachi EPI-G3 spectrophotometer for the 4000 to 400 cm^{-1} range and a Hitachi FIS-3 spectrophotometer for the 400 to 30 cm^{-1} range. The spectra were obtained from Nujol mulls between KRS-5 or polyethylene plates (depending on the frequency range). A Dewar-type low temperature cell with a copper-constantan thermocouple was used. The frequencies are estimated to be accurate within ±2 cm^{-1}.

Results

L-α-Alanine. Infrared spectra from 1800 to 30 cm^{-1} of L-α-alanine at both room and liquid nitrogen temperatures are given in Fig. 1. A band appears at 972 cm^{-1} which does not seem to have been reported

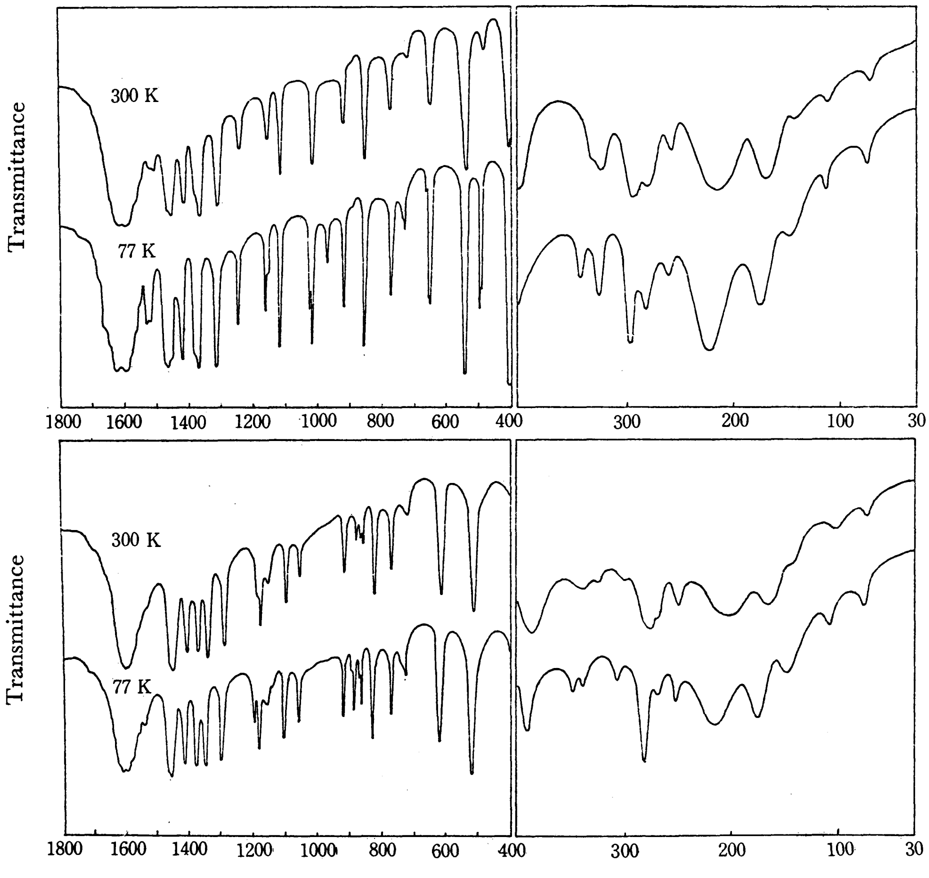

Fig. 1. Infrared spectra of L-α-alanine (upper) and its *N*-deuterated powder (bottom) suspended in Nujol, observed at room temperature and liquid nitrogen temperature.

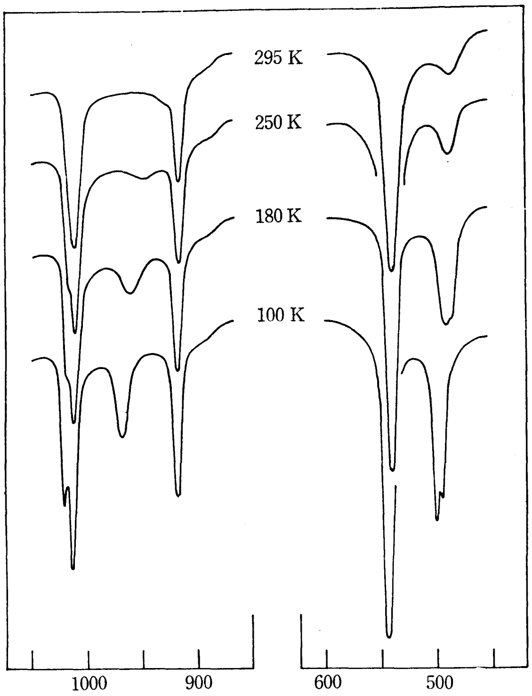

Fig. 2. Temperature dependence of the bands at 972 and 487 cm^{-1} for L-α-alanine.

before—this is the most interesting feature of the liquid nitrogen temperature spectrum. This band disappears by deuteration of the NH_3^+ group as shown in Fig. 1.

The temperature variation of the band is shown in Fig. 2 together with that of the band at 487 cm^{-1}. At room temperature the band is recognizable as a very weak and rather broad shoulder on the 920 cm^{-1}. With a fall in temperature the shoulder becomes sharper, the intensity increasing and shifting towards higher frequency. A similar behavior is observed for the band at 487 cm^{-1}, except that it exhibits splitting as well. This band has been assigned to the NH_3^+ torsional vibration.[2)]

DL-α-*alanine*. The liquid nitrogen temperature spectrum from 1800 to 30 cm^{-1} of DL-α-alanine is shown in Fig. 3 along with the room temperature spectrum. The very weak and broad band at 975 cm^{-1} and the band at 497 cm^{-1} show a similar temperature effect to that of the bands at 972 cm^{-1} (at 100 K) and 487 cm^{-1} of L-α-alanine. See Fig. 4 for its detailed feature. The 497 cm^{-1} band has been assigned to the NH_3^+ torsional vibration.[6)] The 975 cm^{-1} band, not previously reported, is absent from the spectrum of the *N*-deuterated analogue as shown in Fig. 3.

Infrared spectra of α- and γ-glycine, L- and DL-valine and DL-leucine were also measured at various low temperatures. Although NH_3^+ torsional frequencies were

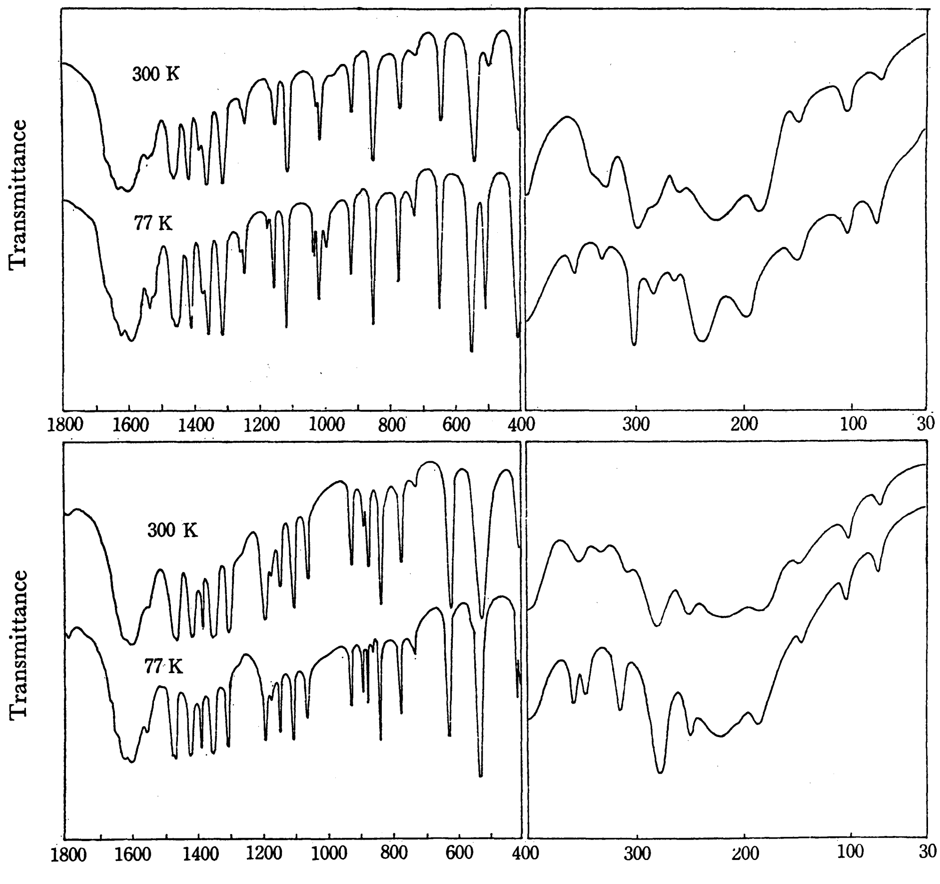

Fig. 3. Infrared spectra of DL-α-alanine (upper) and its *N*-deuterated powder (bottom) suspended in Nujol, observed at room temperature and liquid nitrogen temperature.

observed respectively at 525, 560, 575, 565, and 580 cm^{-1} for α-, γ-glycine, L-, DL-valine, and DL-leucine, no temperature-dependent band was detected between 1200 and 900 cm^{-1}.

Discussion

Since the bands at 972 cm^{-1} (at 100 K) for L-α-alanine and at 975 cm^{-1} for DL-α-alanine are both far (over 40 cm^{-1}) from adjacent bands, it is unlikely that they arise from crystal-field splitting.

We find that the temperature effect of the bands at 972 and 975 cm^{-1} is very similar to that of the NH_3^+ torsional vibrations at 487 and 497 cm^{-1} and that the frequencies are about twice as high as the corresponding torsional frequencies. We may infer that the temperature dependence of overtone vibrations is analogous to that of the fundamental vibrations, and, hence, the newly observed bands at 972 cm^{-1} for L-α-alanine and at 975 cm^{-1} for DL-α-alanine should be assigned to the first overtone of the NH_3^+ torsional vibration.

The above assignment can be substantiated by the spectra of *N*-deuterated analogues. Disappearance of the bands at 972 and 975 cm^{-1} by deuteration of the NH_3^+ group indicates that they originate from the motion of the NH_3^+ group. However, the location of the first overtone of the ND_3^+ torsional vibration is not clear. We see from Figs. 1 and 3 that the ND_3^+ torsional fundamental is observed at 346 cm^{-1} for L-α-alanine and at 354 cm^{-1} for DL-α-alanine at room temperature. The first overtone is thus expected to appear around 700 cm^{-1} at liquid nitrogen temperature. However, no new band is detected around this frequency. It is possible that the overtone bands are hidden under the Nujol band at 720 cm^{-1} or the intensities are too low to be observed.

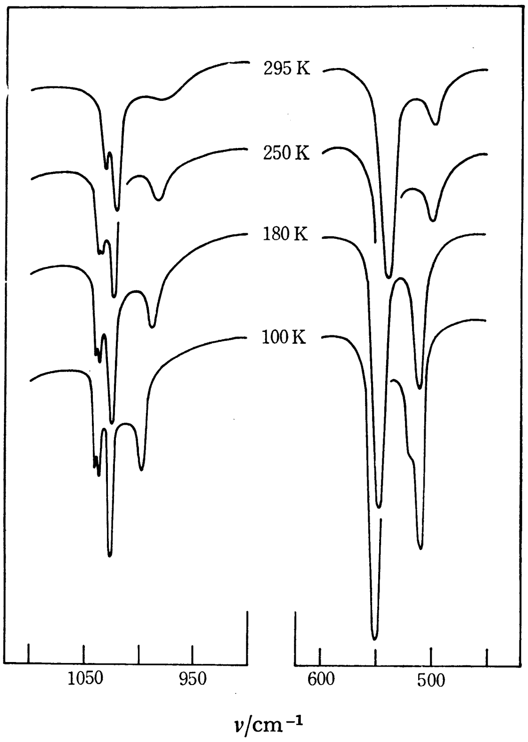

Fig. 4. Temperature dependence of the bands at 975 and 497 cm^{-1} for DL-α-alanine.

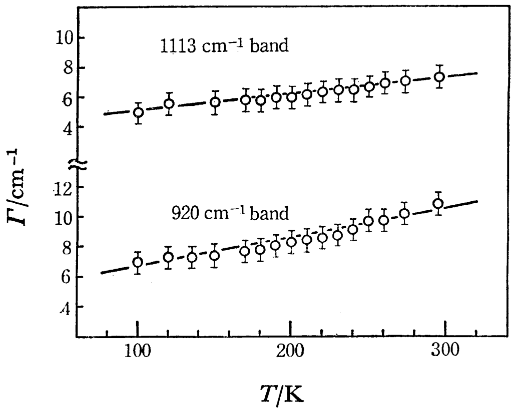

Fig. 5. The half-width of the band at 1113 cm^{-1} (CH_3 rock[4]) and that of the band at 920 cm^{-1} (CCN sym. stretch[4]) for L-α-alanine *versus* absolute temperature. The spectral slit-width is 3 cm^{-1}.

The appearance of the first overtone of the NH_3^+ torsional vibrations in the infrared spectra can be explained if we assume that the NH_3^+ torsions in L-α-alanine and DL-α-alanine are very anharmonic, as one would expect from any vibration associated with hydrogen bonds.

The theory given by Maradudin and Fein[7] predicts that the phonon width due to anharmonicity increases linearly with increasing temperature when the temperature is sufficiently high and is proportional to the square of the cubic force constant. The half-width of the band at 1113 cm^{-1} (CH_3 rock[4]) and that of the

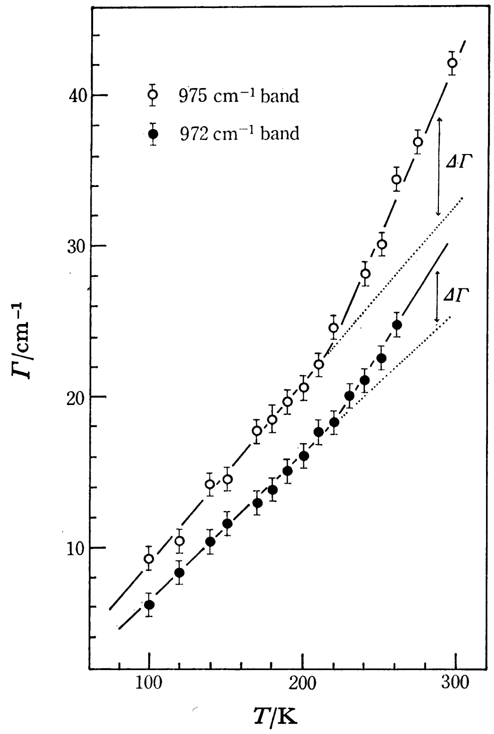

Fig. 6. The half-width of the band at 972 cm^{-1} for L-α-alanine and that of the band at 975 cm^{-1} for DL-α-alanine *versus* absolute temperature. The spectral slit-width is 3 cm^{-1}.

band at 920 cm^{-1} (CCN sym. stretch[4]) of L-α-alanine are plotted against temperature as shown in Fig. 5. They show a gradual temperature dependence, varying linearly with temperature, which indicates that the anharmonicity is small. In Fig. 6, the half-width of the 972 cm^{-1} band for L-α-alanine as well as that of the 975 cm^{-1} band for DL-α-alanine are plotted as a function of temperature. In the temperature range from 100 to 200 K, the half-widths of both bands also increase linearly with increasing temperature. However, the slopes are much steeper in this case, indicating considerable anharmonicity. An interesting feature in this figure is that the deviation from the linearity starts at about 200 K.

Wang and Storms[2,3] reported that a similar deviation was observed above 220 K for the half-width of the NH_3^+ torsional Raman band at 484 cm^{-1} for L-α-alanine. They explained the deviation as due to the modification of the NH_3^+ torsional mode by breaking of a –NH···O– hydrogen bond above 220 K.

If the extra broadening of the 972 and 975 cm^{-1} bands is also caused by relaxation induced by the rupture of a –NH···O– hydrogen bond, it is reasonable to assume that the extra broadening $\Delta\Gamma$ can be expressed as

$$\Delta\Gamma = \Delta\Gamma_0 \exp(-\Delta E/RT)$$

where ΔE is the activation energy of the hydrogen breaking process. In fact, a straight line was obtained when the observed values of $\ln(\Delta\Gamma)$ were plotted against $1/T$. From the slope of this straight line, ΔE was calculated to be 3.6 ± 0.6 kcal/mol for L-α-alanine and 3.7 ± 0.7 kcal/mol for DL-α-alanine.

These values are quite reasonable for the energy of a –NH···O– hydrogen bonding, being in good agreement with the hydrogen bonding energy of 3.5 ± 0.6 kcal/mol calculated by Wang and Storms from the half-width of the torsional Raman band at 484 cm^{-1} for L-α-alanine This may be regarded as additional evidence that the newly observed infrared bands at 972 cm^{-1} for L-α-alanine and at 975 cm^{-1} for DL-α-alanine are due to the first overtone of the NH_3^+ torsional mode.

The half-widths of the NH_3^+ torsional infrared bands at 487 cm^{-1} for L-α-alanine and at 497 cm^{-1} for DL-α-alanine were also measured at various temperatures. However, the splitting of these bands at low temperatures (Figs. 2 and 4) made it almost impossible to evaluate half-width accurately and ΔE was not calculated for these bands.

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