Internal Rotation and Low Frequency Spectra of Esters, Monosubstituted Amides and Polyglycine

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Monosubstituted amides, polypeptides and proteins have been known to exhibit several prominent bands characteristic to the -CONH- group. The bands at about 3300 cm^{-1} and 3100 cm^{-1} are now considered to be due to the Fermi resonance between the fundamental N-H stretching vibration and the first overtone of the amide II vibration[1]. The amide I (1650 cm^{-1}), the amide II (1550 cm^{-1}), and the amide III (1250 cm^{-1}) vibrations involve the C=O stretching, the C-N stretching and the N-H in-plane bending modes. These vibrations have long been subjects of controversy; however, their nature has finally been elucidated

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[1] R. M. Badger and A. D. E. Pullin, *J. Chem. Phys.*, **22**, 1142 (1954); T. Miyazawa, *J. Mol. Spectroscopy*, **4**, 168 (1960).

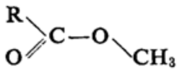
by a normal coordinate treatment of *N*-methylacetamide[2]. The amide IV (650 cm^{-1}), the amide V (750 cm^{-1}), and the amide VI (600 cm^{-1}) bands have been found to be due primarily to the O=C-N bending[3], the N-H out-of-plane bending[4], and the C=O out-of-plane bending modes[3], respectively. The torsional vibration (the amide VII) around the C-N bond is directly related to the potential barrier hindering internal rotation about the C-N bond. For the molecule of *N*-methylformamide the band at 356 cm^{-1} has been assigned to this torsional mode[5].

Internal rotation around the C-N bond of the peptide group is one of the most important factors in determining the conformation or the secondary structure of polypeptides and proteins. Since biological activities of proteins are closely related to their secondary structures an extended spectroscopic study of the hindering potential was undertaken. The torsional vibrations in the low frequency region were assigned and normal coordinate treatments were made of the out-of-plane vibrations for the purpose of determining the potential barrier.

Monosubstituted amides exhibit infrared spectra analogous to those of esters and the analyses of the spectra of esters provide useful information in studying amide spectra. It may be remarked that esters and monosubstituted amides are isoelectronic with each other and, in addition, the mass of NH is not very different from the mass of the oxygen atom. Therefore spectroscopic studies were made of esters as well as of the monosubstituted amides in question.

Internal Rotation around the C-O Bond of Esters

Electron diffration studies of the molecular structures of methyl formate and methyl acetate were made by O'Gorman et al.[6] and the molecule was found to be in the cis form



(R=H or CH_3)

The observed diffraction haloes were in accord with a structure slightly twisted from the planar structure by an internal rotation angle of about 25°. More recently the structure of methyl formate, however, was precisely determined by a microwave absorption measurement and the molecule was established to be in the planar cis form[7]. In the present study, therefore, the infrared spectra of esters will be discussed in terms of the planar cis structure.

Infrared spectra of methyl formate and methyl acetate in the gaseous state and in nonpolar solutions have been measured by Wilmshurst[8] in the region above 300 cm^{-1}. In the present work the measurements were extended out to 190 cm^{-1} so that the torsional frequencies might be observed.

Infrared Absorption Measurements.—For the measurements in the region 400~190 cm^{-1} a Perkin Elmer Model 12C spectrometer equipped with a cesium iodide prism[9] was used. Infrared spectra of methyl formate were measured in the gaseous state, in the solid nitrogen matrix, and in the crystalline state. The vapor spectra were obtained at room temperature with a 10 cm. gas cell and the gas pressure was 30 or 156 mmHg. The observed spectra are shown in Figs. 1 and 2A. A strong band was observed at 325 cm^{-1} with shoulders at about 305 cm^{-1} and at 340 cm^{-1}, in agreement with a previous result[8]. No other strong band, however, was observed below 300 cm^{-1}. The matrix spectra were recorded at 20°K for the molar ratio (N_2: ester) of 32 or 100, where 140 micromoles of the ester sample was dispersed in the matrix over an area of about 2 cm^2. The nitrogen gas used as the matrix material was purified by passing over copper at 600°C and then through a liquid nitrogen bath. The experimental technique of the matrix isolation method was described before[10]. The observed matrix spectra are shown in Fig. 2B. Two prominent bands were observed at

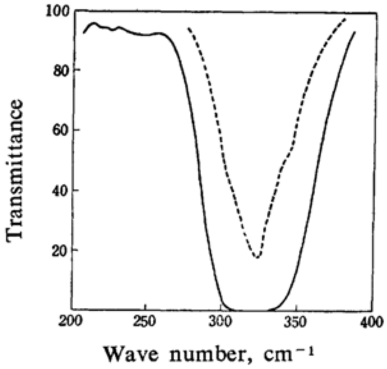


Fig. 1. Infrared spectra of methyl formate vapor, solid line: p=156 mmHg, broken line: p=30 mmHg.

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[2] T. Miyazawa, T. Shimanouchi and S. Mizushima, *J. Chem. Phys.*, **29**, 611 (1958).

[3] T. Miyazawa, T. Shimanouchi and S. Mizushima, ibid., **24**, 408 (1956); T. Miyazawa, *J. Chem. Soc. Japan, Pure Chem. Sec.* (*Nippon Kagaku Zasshi*), **77**, 171, 321, 526 (1956).

[4] H. K. Kessler and G. B. B. M. Sutherland, *J. Chem. Phys.*, **21**, 570 (1953).

[5] D. E. DeGraaf and G. B. B. M. Sutherland, ibid., **26**, 716 (1957).

[6] J. M. O'Gorman, Wm. Shand and V. Schomaker, *J. Am. Chem. Soc.*, **72**, 4222 (1950).

[7] R. F. Curl, *J. Chem. Phys.*, **30**, 1529 (1959).

[8] J. K. Wilmshurst, *J. Mol. Spectroscopy*, **1**, 201 (1957).

[9] T. Miyazawa, *J. Chem. Phys.*, **29**, 421 (1958).

[10] E. D. Becker and G. C. Pimentel, ibid., **25**, 224 (1956).

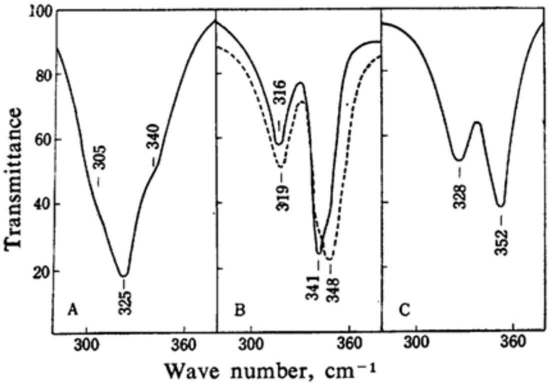

Fig. 2. Infrared spectra of methyl formate. A: vapor, B: matrix (solid line: molar ratio=100, broken line: ratio=32), C: crystal.

316 and 341 cm^{-1} for the molar ratio of 100 and these peaks shifted to 319 and 348 cm^{-1} for a lower ratio of 32. In preparing the crystalline specimen the ester gas was sprayed on a cesium bromide window kept at 140°K and the frozen sample was once slowly warmed up to a temperature slightly below the sublimation point allowing enough time for annealing and then cooled to 150°K for the absorption measurement. The observed crystalline spectrum is shown in Fig. 2C. Two bands were observed at 328 and 352 cm^{-1}, corresponding to the matrix bands at 319 and 348 cm^{-1}, respectively.

Infrared measurements in the vapor phase at room temperature were also made in the region 250~170 cm^{-1} by the use of the grating spectrometer described by Bohn et al.[11] The path length of the vapor cell was 70 cm.; however, no band of methyl formate vapor was observed at a gas pressure of 50~100mmHg. On the other hand a fairly strong band of methyl acetate was observed at 213 cm^{-1} with a gas pressure of 53 mmHg as shown in Fig. 3.

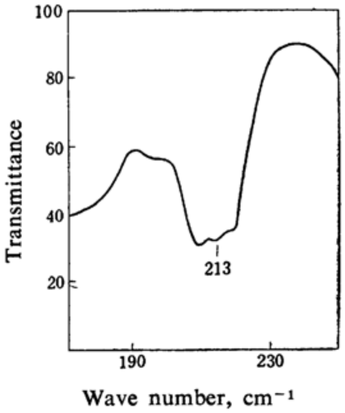

Fig. 3. Infrared spectrum of methyl acetate vapor.

Vibrational Assignment of Methyl Formate. —The vibrational modes of methyl formate expected in the region below 400 cm^{-1} are the C–O–methyl bending mode and the twisting modes around the ester C–O bond and around the O–methyl bond. The strong vapor band at 325 cm^{-1} corresponds to the fairly strong polarized Raman line at 332 cm^{-1} in the liquid state and may be assigned to the C–O–methyl bending mode. The twisting frequencies depend of course upon the potential barrier hindering internal rotation. The three-fold potential barrier of the O–methyl bond has been determined to be 1150±100 cal./mol.[7] and the corresponding twisting frequency will be of the order of 150 cm^{-1}. On the other hand the potential barrier of the ester C–O bond may be of the same order of height as the barrier, 10 kcal./mol., of formic acid monomer[12] and the corresponding twisting frequency may well be in the region 400~200 cm^{-1}. Excepting the 325 cm^{-1} band assigned to the C–O–methyl bending mode, however, no other band with more than 5% absorption was observed with the cell length of 10 cm. and with the gas pressure of 150 mmHg. It may be noted that the 325 cm^{-1} band has a rather peculiar contour, suggesting the possibility of the overlapping of the bands due to the C–O–methyl bending mode and due to the C–O torsional mode.

In the solid matrix the complications arising from rotational fine structures are absent. In the case of formic acid[12] the O=C–O bending mode and the C–O twisting mode exhibit a vibration-rotation band with complicated contour in the vapor phase, whereas in the matrix the two frequencies were resolved. Therefore the matrix isolation technique was used in the present case of methyl formate and the vapor band at 325 cm^{-1} was resolved into two distinct peaks in the nitrogen matrix. In order to establish the fact that these peaks are due to the two vibrational modes, C–O–methyl bending and C–O twisting of a single species, the effect of the molar ratio was observed (see Fig. 2B). On decreasing the molar ratio from 100 to 32, however, the relative intensities of the two peaks did not change appreciably, and these two peaks may now be assigned to the two modes mentioned above. It was not possible to decide which of the two peaks in the matrix was due to the twisting mode. Nevertheless the frequency of 325 cm^{-1} may be taken to be the twisting frequency in the vapor phase.

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[11] C. R. Bohn, N. K. Freeman, W. D. Gwinn, J. L. Hollenberg and K. S. Pitzer, ibid., 21, 719 (1953).

[12] T. Miyazawa and K. S. Pitzer, ibid., 30, 1076 (1959).

Vibrational Assignment of Methyl Acetate.— The molecule of methyl acetate has three skeletal in-plane bending modes and one skeletal out-of-plane bending mode. The Raman lines or the infrared bands at 640, 433, 303 and 610 cm^{-1} have been assigned to these skeletal modes[8,13]. In the region below 400 cm^{-1} three torsional frequencies are expected, one around the ester C–O bond, one around the O–methyl bond, and the last one around the C–methyl bond. As in the case of methyl formate the torsional frequency around the O–methyl bond will be of the order of 150 cm^{-1}. The potential barrier around the C–methyl bond of methyl acetate has not been determined as yet; however, it will not be too different from the value of 483±25 cal./mol. as found for acetic acid monomer[14]. The corresponding torsional frequency will be of the order of 100 cm^{-1}. The observed infrared band of methyl acetate at 213 cm^{-1} is most reasonably assigned to the torsional mode around the ester C–O bond. The reduced moment of inertia for the internal rotation is greater for methyl acetate than for methyl formate and thus a torsional frequency somewhat lower than 325 cm^{-1} of methyl formate may be expected for methyl acetate.

Normal Coordinate Treatment of Out-of-plane Vibrations. — In order to estimate the twofold potential barrier of the ester C–O bond the normal coordinate treatments were made of the out-of-plane vibrations of methyl formate and methyl acetate. The methyl groups of these esters were treated as single dynamic units. The symmetry coordinates and the inverse kinetic energy matrix were derived previously[12]. The bond lengths used in the calculation were $r(\mathrm{C{=}O})=1.22\,\text{Å}$, $r(\mathrm{C{-}O})=1.36\,\text{Å}$, $r(\text{O–methyl})=1.46\,\text{Å}$, $r(\mathrm{C{-}H})=1.07\,\text{Å}$ and $r(\text{C–methyl})=1.52\,\text{Å}$, and all the valence angles were taken to be 120° for simplicity.

The out-of-plane potential function was expressed in terms of the out-of-plane bending constant for the three bonds of the carbonyl carbon atom, H_Ω, the torsional potential constant for the ester C–O bond, H_θ, and the interaction constant, $H_{\Omega\theta}'$. In the case of formic acid monomer[12] the interaction constant $H_{\Omega\theta}'=-0.00_5$ has been found to be negligible as compared to the potential constants, $H_\Omega=0.58_3$ and $H_\theta=0.13_1$. In the present treatments of esters the interaction constant was neglected. In calculating the torsional potential constant the correction for the anharmonicity of the two-fold torsional potential function, $V=V_2\cdot(1-\cos 2\theta)/2$ was made to the observed torsional frequency. The correction $\nu_0-\nu$ is equal to $h/8\pi^2cI_r$ where h is Planck's constant, c the light velocity, and I_r the reduced moment of inertia for intramolecular rotation[12]. The values of the correction were calculated to be 6 and 3 cm^{-1} for the cis form and the trans form of methyl formate and 2 cm^{-1} for both the cis form and trans form of methyl acetate. The CH out-of-plane bending frequency of 1019 cm^{-1} and the corrected torsional frequency of 331 cm^{-1} were used for the calculation of the potential constants of $H_\Omega=0.55_8$ and $H_\theta=0.18_2$ (in unit of 10^{-11} erg). Then the two-fold potential barrier of methyl formate was found to be $13._1$ kcal./mol. The potential constants of methyl acetate were calculated to be $H_\Omega=0.533$ and $H_\theta=0.221$ from the skeletal out-of-plane bending frequency of 607 cm^{-1} and the corrected torsional frequency of 215 cm^{-1}. The two-fold potential barrier of methyl acetate was found to be $15._9$ kcal./mol. It may be noted that the potential barriers of esters are higher than those of formic acid (10 kcal./mol.) and of acetic acid (10 kcal./mol.)[15].

Trans Form of Esters.—The trans isomer of formic acid monomer has been found by the infrared spectra in the vapor phase, and the energy of the trans isomer has been found to be 2 kcal./mol. higher than the more abundant cis isomer[12]. The normal coordinate treatment of the out-of-plane vibrations was also made of the trans form of methyl formate as an aid in assigning the bands due to the trans form. Since the torsional potential constant of the trans isomer has been found to be the same as that of the cis isomer in the case of formic acid, the potential function of the cis form of methyl formate was also used for the treatment of the trans form. The C–H out-of-plane bending frequency and the torsional frequency were calculated to be 1053 and 233 cm^{-1}, respectively. As shown in Fig. 1, however, no band with more than 5% absorption has been observed in the vicinity of 233 cm^{-1}. From the intensity of the 325 cm^{-1} band, the abundance ratio trans/cis was estimated to be less than 1% and the energy difference between the two forms is considered to be greater than 2.7 kcal./mol.

The relaxation absorption of sound in liquid ethyl formate has been ascribed to the rotational isomerism around the ester C–O bond[16]. Actually, however, there is also the rotational isomerism around the $O{-}CH_2$ bond of ethyl formate, and the hindering potential previously estimated from the ultrasonic studies may not be compared with the present results.

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[13] T. Miyazawa, *J. Chem. Soc. Japan, Pure Chem. Sec.* (*Nippon Kagaku Zasshi*), **77**, 619 (1956).

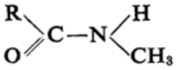
[14] W. J. Tabor, *J. Chem. Phys.*, **27**, 974 (1957).

[15] T. Miyazawa, unpublished.

[16] J. Karpovich, *J. Chem. Phys.*, **22**, 1767 (1954); D. Tabuchi, ibid., **28**, 1014 (1958).

Internal Rotation around the C–N Bond of the Peptide Group

The stable conformation of the peptide group has been established to be the planar trans form by Mizushima et al.[17] The vibrational analysis of *N*-methyl acetamide and *N*-methyl formamide will therefore be made with the planar trans structure,



***N*-Methylacetamide.**—The vibrational assignment as well as the normal coordinate treatment of the in-plane vibrations have been made[2,3,13] and the nature of the skeletal bending vibrations at 436 and 289 cm^{-1} have already been elucidated. The Raman spectrum of liquid *N*-methylacetamide has been carefully measured by Sugita[18] and a new weak line has been observed at 206 cm^{-1}. Since the methyl torsional frequencies around the C–methyl bond and around the N–methyl bond will be lower than 150 cm^{-1} as in the case of methyl acetate, the Raman line at 206 cm^{-1} is most reasonably assigned to the torsional frequency around the C–N bond of the peptide group. It may be noted that this frequency is not very different from the corresponding frequency 213 cm^{-1} of methyl acetate.

In the present study an approximate normal coordinate treatment of the out-of-plane vibrations was made, treating the methyl groups and the NH group as single dynamic units. The bond lengths used were the same as those used in the treatment of the in-plane vibrations[2]. Because of the approximate nature of the present treatment, the correction for the anharmonicity of the torsional frequency was not made. From the observed skeletal out-of-plane bending frequency of 600 cm^{-1} (the amide VI band) and the torsional frequency of 206 cm^{-1} the potential constants were calculated to be $H_\Omega=0.50_8$ and $H_\theta=0.19_5$, and then the two-fold potential barrier was estimated to be 14 kcal./mol. The bond order of the C–N bond of the peptide group may be estimated to be 0.3～0.4 from the torsional potential constant as in the case of formic acid. This value agrees with the bond order 0.3～0.4 derived from the bond distances.

***N*-Methylformamide.** — Infrared and Raman spectra of liquid *N*-methylformamide have been measured by DeGraaf and Sutherland[5]. The polarized Raman line at 302 cm^{-1} has been assigned to the C–N–methyl skeletal bending vibration and the depolarized line at 356 cm^{-1} to the torsional vibration around the peptide C–N bond. These frequencies correspond to the frequency of 325 cm^{-1} observed for methyl formate.

The normal coordinate treatment of the out-of-plane vibrations of *N*-methylformamide was made as in the case of *N*-methylacetamide. The C–H bond length was taken as 1.07 Å and the other lengths were the same as those of *N*-methylacetamide. From the C–H out-of-plane bending frequency[3] of 1015 cm^{-1} and the torsional frequency of 356 cm^{-1} the potential constants were calculated to be $H_\Omega=0.53_2$ and $H_\theta=0.20_2$. This torsional potential constant corresponds to the potential barrier of 14 kcal./mol.

The two-fold potential barrier of the C–N bond of the peptide group has been estimated by Pauling and Corey[19] to be about 30 kcal./mol. As the results of the present spectroscopic studies, however, the values of the potential barrier of *N*-methylacetamide and *N*-methylformamide were both found to be about 14 kcal./mol., and this lower value is to be used in discussing the relative stabilities of the various conformations of a polypeptide chain.

Low Frequency Infrared Spectra of Polyglycine I and II

Infrared absorption spectra have been widely used in studying the conformations of polymer chains. In the case of polypeptide chains the correlations between the chain conformations and the amide I and II frequencies were first noticed by Elliott and Ambrose[20]; that is to say the folded conformation exhibits the amide I and II bands at about 1650 and 1540 cm^{-1} whereas the extended conformation exhibits the bands at 1630 and 1520 cm^{-1}. Recently these correlations were given theoretical interpretations and were refined and extended to cover the random coil conformation, the α helix, the antiparallel-chain extended conformation, and the parallel-chain extended conformation as well as Nylon 66[21]. These refined correlations, although useful for synthetic polypeptides and certain fibrous proteins, may not be too dependable in proteins exhibiting ill-defined amide I and II bands[22]. Therefore it should be worthwhile to find out other characteristic bands whose frequencies change more sensitively than do the amide I and II bands.

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17) S. Mizushima, T. Shimanouchi, S. Nagakura, K. Kuratani, T. Tsuboi, H. Baba and O. Fujioka, *J. Am. Chem. Soc.*, **72**, 3490 (1950).

18) T. Sugita, unpublished.

19) L. Pauling and R. B. Corey, *Proc. Nat. Acad. Sci.*, **37**, 251 (1951).

20) A. Elliott and E. J. Ambrose, *Nature*, **165**, 921 (1950).

21) T. Miyazawa, *J. Chem. Phys.* **32**, 1647 (1960); T. Miyazawa and E. R. Blout, *J. Am. Chem. Soc.*, **83**, 712 (1961).

22) M. Beer, G. B. B. M. Sutherland, K. N. Tanner and D. L. Wood, *Proc. Royal Soc.*, **A249**, 147 (1959).

The N–H stretching vibration is localized in the N–H bond and involves only the nitrogen in the main chain of polypeptides. Its frequency, then, will not change appreciably with the chain conformation. On the other hand the torsional mode around the peptide C–N bond involves the C_α–C–N–C_α atoms in the main chain and its frequency is expected to be most sensitive to the conformational change of the chain. In this connection normal coordinate treatments of the skeletal vibrations of the polymers of the type, $(\text{-CH}_2\text{-})_n$ and $(\text{-CH}_2\text{-O-})_n$, have been made, and in fact the low frequencies primarily due to the torsional modes have been found to change most sensitively with the chain conformation[23].

Polyglycine has been known to exist in two forms[24]; polyglycine I is in the antiparallel-chain extended conformation and polyglycine II is in the helical conformation with three-fold screw axis[21,24,25]. In the present study the infrared spectra of these two forms of polyglycine were measured as Nujol paste in the region 400∼190 cm^{-1}. The samples used were kindly given by Mr. Tsuchiya of the University of Tokyo. As shown in Fig. 4 polyglycine I exhibits a band at 217 cm^{-1}. This frequency appears to correspond to the frequency of 206 cm^{-1} observed for *N*-methylacetamide and is most reasonably assigned to the torsional mode around the C–N bond of the antiparallel-chain extended conformation. On the other hand polyglycine II* exhibits the corresponding band at 365 cm^{-1}. It is remarkable to note that the torsional frequency of polyglycine I is almost doubled by the transformation to polyglycine II. The amide VII band is thus found to be most sensitive to the conformation of polypeptide chains. It would be worth while to find out more extensive correlations between the various conformations and the infrared spectra in the low frequency region.

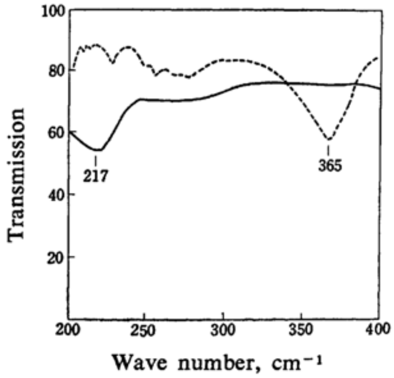

Fig. 4. Infrared spectra of polyglycine I (solid line) and polyglycine II (broken line).

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[25] F. H. C. Crick and A. Rich, ibid., **176**, 780 (1955).

* The weak peaks observed in the region 200∼280 cm^{-1} are presumably due to water vapor.