state were calculated from data given by Rossini[14] and were found to be −1397.6 and −1685.6 kcal./mole, respectively.

With the above values, the calculated heats of formation from the atoms were obtained as

$$\Delta H_f(298°\text{K.})\ \text{cyclopentane(g)} - \text{B(C–H)} + \text{B(C–N)} + 2\text{B(N=N)} \quad (7)$$
$$= \Delta H_f \text{ of structure I for cyclopentyl azide}$$

$$\Delta H_f(298°\text{K.})\ \text{cyclohexane(g)} - \text{B(C–H)} + \text{B(C–N)} + 2\text{B(N=N)} \quad (8)$$
$$= \Delta H_f \text{ of structure I for cyclohexyl azide}$$

The structure II was not used as a reference for the stabilization energy calculation; as shown by Sidgwick,[15] there is no nitrogen–nitrogen triple bond value that can be used without ambiguity.

On the basis of equations 7 and 8 the calculated heats of formation were −1571.1 and −1859.1 kcal., respectively. Comparison of these values with those observed leads to a resonance energy of 41.0 kcal. for cyclopentyl azide and 44.8 kcal. for cyclohexyl azide.

Acknowledgment.—One of us (TFF) wishes to acknowledge his indebtedness to the Research Corporation for a grant which made this and continuing investigations possible.

(14) F. D. Rossini, "Selected Values of Properties of Hydrocarbons," National Bureau of Standards, Circular c461, Washington, District of Columbia, 1947.

(15) N. V. Sidgwick, *Trans. Faraday Soc.*, **30**, 801 (1934).

NEW ORLEANS, LOUISIANA

[CONTRIBUTION FROM THE CHEMICAL LABORATORY, FACULTY OF SCIENCE, TOKYO UNIVERSITY]

Near Infrared Spectra of Compounds with Two Peptide Bonds and the Configuration of a Polypeptide Chain V

BY SAN-ICHIRO MIZUSHIMA, MASAMICHI TSUBOI, TAKEHIKO SHIMANOUCHI AND MASATOMO ASAI

RECEIVED JUNE 9, 1954

The near infrared spectra of acetylpiperidine α-carboxylic acid N-methylamide have been measured in carbon tetrachloride and chloroform solutions. A considerable difference in the behavior of the NH bands has been found between this substance and acetylproline N-methylamide. This has been explained as arising from the different steric effects of the five-membered and six-membered rings on the internal rotation of these two substances. The piperidine ring affects the internal rotation in such a way that the internal hydrogen bond N–H . . . O of acetylpiperidine α-carboxylic acid N-methylamide becomes much weaker than that of acetylproline N-methylamide. *Intermolecular* hydrogen bonding in these two substances has also been discussed.

In this series of researches the near infrared spectra of various acetylaminoacid N-methylamides, $CH_3CONHCHRCONHCH_3$, have been measured in carbon tetrachloride and chloroform solutions.[1-5] From the experimental results it was concluded that there is in general an equilibrium of three molecular configurations: extended, folded and associated configurations shown in Fig. 1, A, B and C, respectively. These configurations correspond to the stable positions of internal rotation about the four single bonds I, II, III and IV shown in Fig. 1A.[6] In the case of acetylproline N-methylamide in carbon tetrachloride the molecules exist only in the folded configuration shown in Fig. 1D,[3] which is partly converted to another form such as shown in Fig. 1E in chloroform in which the internal hydrogen bonding is reduced.[5] The interesting behavior of this substance different from that of other acetylaminoacid N-methylamide arises from the facts that the molecule has only one N–H bond and that owing to the ring formation, the C–N (I) and C–C (III) bonds cannot form the *trans* configuration. It would, therefore, be interesting to make a similar research on acetylpiperidine α-carboxylic acid N-methylamide which has six-membered rings instead of five (Fig. 1F), as the two rings affect the internal rotations quite differently. Such a study will make a further contribution to the determination of the configurations of aminoacid residues building up a polypeptide chain.

(1) S. Mizushima, T. Shimanouchi, M. Tsuboi, T. Sugita, E. Kato and E. Kondo, THIS JOURNAL, **73**, 1330 (1951).

(2) S. Mizushima, T. Shimanouchi, M. Tsuboi and R. Souda, *ibid.*, **74**, 270 (1952).

(3) S. Mizushima, T. Shimanouchi, M. Tsuboi, T. Sugita, K. Kurosaki, N. Mataga and R. Souda, *ibid.*, **74**, 4639 (1952).

(4) S. Mizushima, T. Shimanouchi, M. Tsuboi, K. Kuratani, T. Sugita, N. Mataga and R. Souda, *ibid.*, **75**, 1863 (1953).

(5) S. Mizushima, M. Tsuboi, T. Shimanouchi, T. Sugita and T. Yoshimoto, *ibid.*, **76**, 2479 (1954).

(6) As to the summary of the work on internal rotation, see, for example: S. Mizushima, Reilly Lectures V, University Press of Notre Dame, 1951; "Structure of Molecules and Internal Rotation," Academic Press, Inc., New York, N. Y., 1954.

Experimental

Fourteen grams of acetylpiperidine α-carboxylic acid ethyl ester was dissolved in 35 cc. of methanol cooled at −50° by a mixture of Dry Ice and ethanol. To this solution was added 10 g. of methylamine cooled by the same cryogen and the reaction product was allowed to stand for two weeks. After removal of remaining methylamine and solvent, colorless crystal of acetylpiperidine α-carboxylic acid N-methylamide was obtained. This was recrystallized from chloroform and was used in the measurement, m.p. 107–108°.

Anal. Calcd. for $C_9H_{16}O_2N_2$: C, 58.67; H, 8.71; N, 15.21. Found: C, 58.36; H, 8.59; N, 15.05.

The infrared absorption measurement was made, using the LiF prism in the reflection monochromator constructed in our laboratory.

Results and Discussions

A. Intramolecular and Intermolecular Hydrogen Bonds of Acetylpiperidine α-Carboxylic Acid N-Methylamide.—The results of the absorption measurements in the 3 μ region are shown in Figs. 2 and 3, each of which consists of two parts, one referring to the molar absorption coefficient *versus* wave length and the other to the dependence of the absorption coefficient on the concentration of the solution.

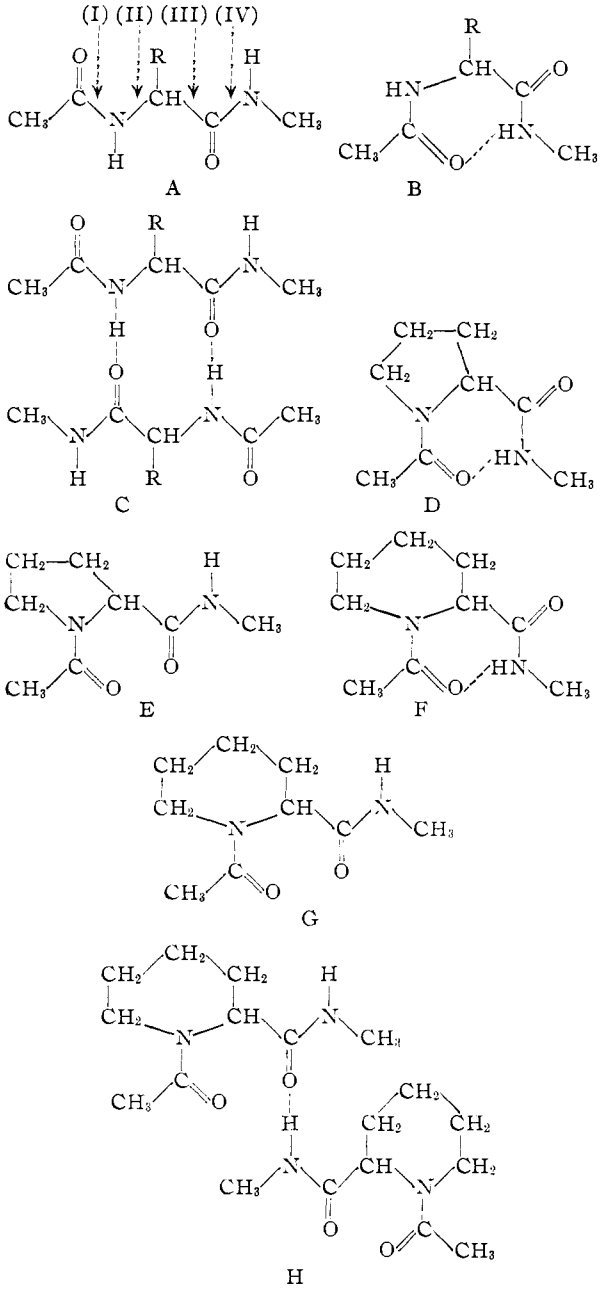


Fig. 1.—The molecular configurations of acetylamino acid N-methylamides.

As shown in Fig. 2, acetylpiperidine α-carboxylic acid N-methylamide exhibits two NH bands at 2.89 and 2.95 μ in dilute carbon tetrachloride solutions. The molar absorption coefficient of these two bands is independent of concentration, if this is less than 0.0005 mole/l. Therefore, both of these two bands arise from NH vibrations of free molecules and the one at 2.89 μ can be assigned to the free NH vibration and the other at 2.95 μ to that involved in internal hydrogen bond. In other words we have an equilibrium between a configuration with internal hydrogen bond (Fig. 1F) and another or others without this bond (such as shown in Fig. 1G).

The intensity ratio of the 2.89 μ band to the 2.95 bμ and was measured at 30° and at 60° and was

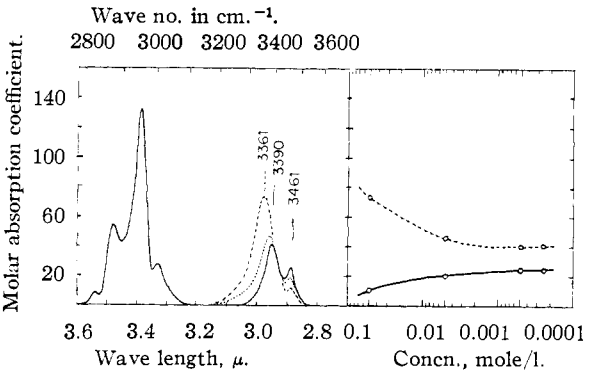


Fig. 2.—3 μ region infrared absorption spectra of acetylpiperidine α-carboxylic acid N-methylamine in carbon tetrachloride solutions at 30°.

found to increase by only 3 ~ 4% at the higher temperature. This shows that in carbon tetrachloride solutions the configuration with internal hydrogen bond is more stable than another without this bond by only 200 ~ 300 cal./mole. In other words the internal hydrogen bond in this case is much weaker than that in the case of acetylglycine N-methylamide for which we have found a considerable temperature dependence of the intensity ratio of the free NH band to the bonded band.

As the concentration of the carbon tetrachloride solution is raised (0.0005 mole/l.), the intensity of the free NH band at 2.89 μ becomes weaker and the bonded NH band at 2.95 μ is overlapped by the band at 2.97 μ arising from the *intermolecular* hydrogen bond and having the same wave length as that of the corresponding NH band of N-methylacetamide.[7] Furthermore, the *intermolecular* hydrogen bond begins to be appreciable at a concentration of 0.001 mole/l., just as in the case of N-methylamide. Therefore, the associated molecule can be considered to have a form as shown in Fig. 1H. The fact that the 2.97 μ band is not shifted toward longer wave lengths at higher concentrations or even in crystalline state indicates that the associated molecules are not long chain polymers.[7-9] This is probably due to the steric hindrance of the piperidine ring.

In chloroform solutions the wave lengths and the molecular absorption coefficients of the free and bonded NH bands at 2.89 and 2.94 μ become independent of concentration, when this is lower than 0.01 mole/l. (see Fig. 3). This shows that in this range of concentration the molecules of acetylpiperidine α-carboxylic acid N-methylamide are in the free state and are in both the hydrogen-bonded and non-bonded configurations. From the intensity ratio of the bonded and non-bonded NH bands shown in Figs. 2 and 3 we see that in chloroform solutions the relative amount of the configuration with internal hydrogen bond is far less than that in carbon tetrachloride solutions. This is due to the reduction of hydrogen bonding in chloroform solutions as reported in a previous paper.[5] The tem-

(7) S. Mizushima, T. Shimanouchi, S. Nagakura, K. Kuratani, M. Tsuboi, H. Baba and O. Fujioka, THIS JOURNAL, **72**, 3490 (1950); M. Tsuboi, *Bull. Chem. Soc., Japan*, **22**, 215 (1949).

(8) M. Tsuboi, *ibid.*, **22**, 255 (1949).

(9) M. Tsuboi, *ibid.*, **25**, 385 (1952).

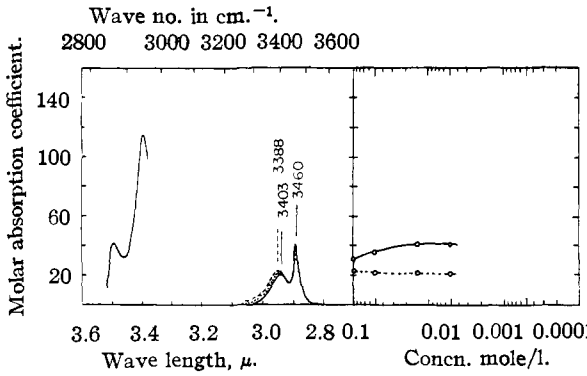


Fig. 3.—3 μ region infrared absorption spectra of acetylpiperidine α-carboxylic acid N-methylamine in chloroform solutions at 30°.

perature dependence of the intensity ratio of the 2.89 μ band to the 2.94 μ band was found to be very small in this case also.

However, if the concentration becomes higher than 0.01 mole/l., the relative intensity of the free NH band at 2.89 μ is reduced and the bonded NH band at 2.94 μ is overlapped by the 2.97 μ band arising from the intermolecular hydrogen bond.

Therefore, at higher concentrations the association takes place in chloroform solutions also, although this is much weaker than that in carbon tetrachloride.

B. Difference in Hydrogen Bonding between Acetylproline N-Methylamide and Acetylpiperidine α-Carboxylic Acid N-Methylamide. Configuration of Proline Residue in Polypeptide Chain.—As described above, the internal hydrogen bond of acetylpiperidine α-carboxylic acid N-methylamide behaves differently from that of acetylproline N-methylamide.[3,5] In the first place the ratio of the number of molecules with internal hydrogen bond to that without this bond in acetylproline N-methylamide is much larger than that in acetylpiperidine α-carboxylic acid N-methylamide as can be seen from the intensity ratio of the two NH bands shown in Table I.

TABLE I

THE INTENSITY RATIO OF THE FREE NH BAND TO THE BONDED NH BAND

	In CCl_4	In $CHCl_3$
Acetylproline N-methylamide	0:10	4:6
Acetylpiperidine α-carboxylic acid N-methylamide	4:6	6:4

In the second place the shift in the NH frequency due to the formation of the internal hydrogen bond of acetylproline N-methylamide is considerably greater than that of acetylpiperidine α-carboxylic acid N-methylamide as shown in Table II.

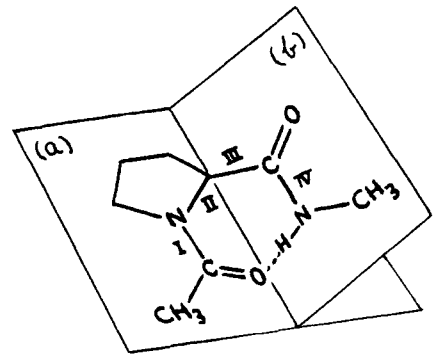
TABLE II

SHIFT IN NH FREQUENCY DUE TO THE FORMATION OF INTERNAL HYDROGEN BOND

	In CCl_4	In $CHCl_3$
Acetylproline N-methylamide	~100	115
Acetylpiperidine α-carboxylic acid N-methylamide	71	57

These results show that the formation of the internal hydrogen bond in acetylproline N-methylamide is much stronger than that in acetylpiperidine α-carboxylic acid N-methylamide. This can be explained from the difference in molecular configuration, especially in reference to the internal rotation between these two substances.

The molecule of each of these two substances has two peptide bonds which have been shown to be in the planar *trans* configuration in a previous paper.[7] These planes of the two peptide bonds are designated by (a) and (b) in Figs. 4 and 5. In acetylproline N-methylamide shown in Fig. 4 the five-numbered ring can be considered to have almost a planar configuration[10] and, therefore, the C–C bond (III) is in the gauche position with respect to the N–C bond (I). This gives rise to a favorable configuration for the formation of internal hydrogen bond, as can easily be seen from Fig. 4.



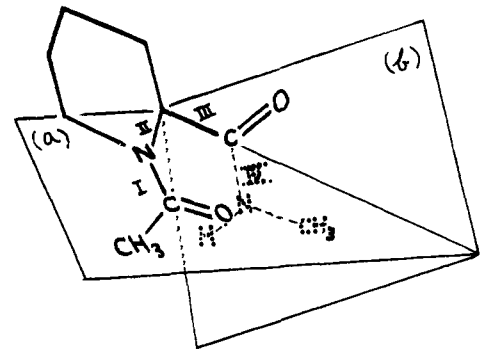


Fig. 4.—Top figure, molecular configuration of acetylproline N-methylamide.

Fig. 5.—Bottom figure, molecular configuration of acetylpiperidine α-carboxylic acid N-methylamide.

On the other hand, in acetylpiperidine α-carboxylic acid N-methylamide the six-membered ring is considered to have a chair form as in the case of cyclohexane.[11] This results in the *cis* relation between the C–C (III) and the N–C bond (I), where the C–C (III) bond is an equatorial bond of the piperidine ring. Therefore, although the N–C bond (IV) can be in the gauche position with respect to the N–C bond (II) in the ring, the N–H bond be-

(10) C. W. Beckett, K. S. Pitzer and R. Spitzer, THIS JOURNAL, **69**, 2483 (1947).

(11) C. Finbak and O. Hassel, *Arch. Math. Naturvidenskab*, **45**, No. 3, 8 (1941); C. W. Beckett, K. S. Pitzer and R. Spitzer, THIS JOURNAL, **69**, 2488 (1947).

longing to (IV) does not point to the O-atom of the C=O group (see Fig. 5). This configuration does not result in a strong hydrogen bond. If the N–C bond (IV) is in the *trans* position with respect to the N–C bond (II), the configuration cannot at all form an internal hydrogen bond. However, if the C–C bond (III) is a polar bond of the piperidine ring, we may again expect the formation of the N–H . . . O internal hydrogen bond which can be concluded to be very weak from the inspection of the relative position of N- and O-atoms. In short we cannot consider the formation of a strong internal hydrogen bond in all the conceivable configurations of acetylpiperidine α-carboxylic acid N-methylamide in which the N–C bond (I) cannot be in the gauche position with respect to the C–C bond (III).

Besides internal hydrogen bond acetylpiperidine α-carboxylic acid N-methylamide differs from acetylproline N-methylamide in *intermolecular* hydrogen bond. As referred to above, the former substance begins to show association at 0.005 mole/l. in carbon tetrachloride solution and at 0.05 mole/l. in chloroform solution, while the latter substance shows no association even at 0.05 mole/l. in these two solutions. This is due to the fact that in the latter substance the N–H group is involved in strong internal hydrogen bonding and almost no N–H group (in carbon tetrachloride solutions) or few N–H groups (in chloroform solutions) are left free to form *intermolecular* hydrogen bond.

Summarizing the results obtained with regard to the hydrogen bonding, all the acetylaminoacid N-methylamides of the type of $CH_3CONHCHRCONHCH_3$ (where R = H, CH_3, $CH(CH_3)_2$, $CH_2CH(CH_3)_2$ and $CH_2CH_2CH_2CH_3$) so far studied in this series of researches[1-5] showed the NH bands involved in internal hydrogen bonding in carbon tetrachloride and chloroform solutions, when these molecules are in the folded configurations. The shift in frequency from the free NH band was found to amount to about 100 cm.$^{-1}$ just as in the case of the bonded NH band of acetylproline N-methylamide. The formation of this fairly strong hydrogen bond is due to the gauche relation between the N–C bond (I) and the C–C bond (III). For the corresponding amino acid residues contained in the folded polypeptide chain (*i.e.*, glycine, alanine, valine, leucine and norleucine residues), we can consider configurations similar to this. However, as we have seen from the different behavior between acetylpiperidine α-carboxylic acid N-methylamide and acetylproline N-methylamide, a change in the internal rotation state will affect considerably the *intramolecular* and *intermolecular* hydrogen bonds of a polypeptide chain and hence also the configuration of the chain.

Acknowledgment.—The authors thank Prof. J. Noguchi of Kanazawa University for the preparation of piperidine α-carboxylic acid. Their thanks are also due to Prof. K. Kozima for the loan of the LiF prism and to Mr. Ishino and Miss Mitsui, Faculty of Agriculture, for the performance of the microanalysis here recorded.

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[CONTRIBUTION FROM THE CHEMISTRY DEPARTMENT, BROOKHAVEN NATIONAL LABORATORY]

A Chlorophyll Substance Possessing a Spectrum Very Similar to that of Chlorophyll-b[1]

BY SIMON FREED, KENNETH M. SANCIER AND ALFRED H. SPORER

RECEIVED APRIL 28, 1954

A chlorophyll substance is obtained along with chlorophyll-*b'* when chlorophyll-*b* has been heated in solvents and also without solvent, in the absence of oxygen and water. Its spectrum, and its fluorescence and solvation properties are almost identical with those of chlorophyll-*b*. Despite the fact that it does not undergo the Molisch phase test, reasons are advanced that the three substances, chlorophyll-*b*, chlorophyll-*b'* and the new substance, may constitute the three, long-discussed possible tautomers of chlorophyll-*b* whose structures may be written for the chlorophyll molecule by bonding magnesium atom in turn to three different pairs of pyrrol nitrogen atoms. There are indications that a corresponding substance exists in the chlorophyll-*a* series also and it is tentatively proposed that these substances be known as chlorophyll-*b''* and chlorophyll-*a''*.

In the preparation[2] of chlorophyll-*b'* from chlorophyll-*b* it was observed during the chromatographic analyses that in addition to the two zones of chlorophylls-*b* and -*b'*, a distinct third zone appeared. The order of the zones, from the top of the column consisted of the substance X under consideration, chlorophyll-*b* and finally chlorophyll-*b'*, roughly in the proportions 1:4:2, respectively.

X has a spectrum very similar to those of chlorophyll-*b* and -*b'*, just distinguishable from them by the slight displacements of the corresponding maxima. From the table and the figures it may be noted that at room temperature X in solution has the principal features of its spectrum closer to those of chlorophyll-*b* than does chlorophyll-*b'*

TABLE I

WAVE LENGTHS OF ABSORPTION MAXIMA OF SOLUTIONS OF CHLOROPHYLL SUBSTANCES

Wave lengths were reproducible to ±3 Å. At 300°K. the solvent was 10% *n*-propyl ether in methylcyclohexane and at 75°K. it was 10% *n*-propyl ether in 1:1 propane, propene.

Substance	75°K.	300°K.	75°K.	300°K.
Chlorophyll-*b*	4765	4512	6436	6411
Chlorophyll-*b'*	4756	4535	6447	6427
X	4736	4506	6443	6411
Allomerized chlorophyll-*b*		4530		6600
Ethyl chlorophyllide-*b*		4523		6425
Pheophytin-*b*		3950		. .

(1) Research performed under the auspices of the U. S. Atomic Energy Commission.

(2) H. H. Strain and W. M. Manning, *J. Biol. Chem.*, **146**, 275 (1942).