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Conformations of Model Compounds of Proteins II. Infrared Spectra of N-Acetyl-amino Acid Methylamides

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Synopsis

N-Acetyl-amino acid methylamides $CH_3CONHCHRCONHCH_3$ were prepared from L- and DL-alanine, L- and DL-α-amino-n-butyric acid, L- and DL-norvaline, DL-norleucine, L- and DL-methionine, L- and DL-leucine, L-aspartic acid and DL-phenylalanine. The deuterium homologs of the type $CH_3CONDCHRCONDCH_3$, $CD_3CONHCHRCONHCH_3$ and $CH_3CONHCHRCONHCD_3$ were also prepared. The infrared spectra of these compounds were measured down to 300 cm^{-1} in the crystalline state. The infrared spectra of N-isopropylacetamide $CH_3CONHCH(CH_3)_2$, N-methylisobutyramide $(CH_3)_2CH$-$CONHCH_3$ and their deuterium homologs were also measured. The C=O in-plane and out-of-plane bending vibration bands of the CH_3CONHC^{α} group (amide IVa and VIa) and those of the $-C^{\alpha}CONHCH_3$ group (amide IVb and VIb) were assigned from these data. Two crystalline modifications, form I and form II, were found for the compounds prepared from L-alanine, DL-leucine, L-aspartic acid and DL-phenylalanine. The two forms show quite different skeletal vibrations, which suggest rotational isomerism. Two distinct patterns were found as to the positions of the amide IVa and VIa bands for the above compounds. The two amide bands were found near 630 and 600 cm^{-1} in form I, whereas they were found near 600 cm^{-1} in form II. The crystals of the remaining compounds were also classified into form I or form II on the basis of the arrangement of the amide bands. The X-ray structure analyses suggest that these two forms have different hydrogen-bond structures.

INTRODUCTION

In a previous paper[1] we reported the infrared spectra of crystalline N-acetylglycine methylamide $CH_3CONHCH_2CONHCH_3$, a model compound of the polypeptide chain with the glycine residue, with the emphasis on their relationship to the stable conformations. In the present paper we deal with N-acetyl-amino acid methylamides $CH_3CONHCHRCONHCH_3$ having various side chains R.

N-Acetyl-amino acid methylamides prepared from alanine, valine, leucine, norleucine and proline were studied by Mizushima et al.[2–6] They measured the near-infrared spectra of these compounds in carbon tetrachloride and chloroform solutions and found two rotational isomers, the extended form with free N—H bonds and the bent form with an intramolecular hydrogen bond.

We measured the infrared spectra down to 300 cm^{-1} for crystalline N-acetyl-amino acid methylamides $CH_3CONHCHRCONHCH_3$ prepared from L- and DL-alanine (R = CH_3), L- and DL-α-amino-n-butyric acid (R = CH_2CH_3), L- and DL-norvaline (R = $CH_2CH_2CH_3$), DL-norleucine (R = $CH_2CH_2CH_2CH_3$), L- and DL-methionine (R = $CH_2CH_2SCH_3$), L- and DL-leucine (R = $CH_2CH(CH_3)_2$), L-aspartic acid (R = CH_2COOH) and DL-phenylalanine (R = $CH_2C_6H_5$). We tried to correlate the C=O in-plane bending (amide IV), C=O out-of-plane bending (amide VI) and skeletal deformation bands with the hydrogen-bond structure and the conformation of the peptide backbone.

EXPERIMENTAL

The following four methods were adopted for the preparation of N-acetyl-amino acid methylamides.

Method A: Acetyl-amino acid (10 mM) was esterified by the use of etherial diazomethane as described by Neuberger.[7] Oily methyl ester was dissolved in methanol (20 ml) and the solution was saturated with gaseous methylamine and then allowed to stand at room temperature for two or three days. The solvent was evaporated in vacuo and the residue was recrystallized.

Method B: A mixed anhydride prepared in tetrahydrofuran (40 ml) from acetyl-amino acid (10 mM), triethylamine (11 mM) and isobutyl chloroformate (11 mM) was added to the solution of methylamine (20 mM) in tetrahydrofuran (20 ml). The mixture was stirred in an ice-salt bath for two hours and kept at room temperature overnight. The reaction mixture was evaporated to dryness *in vacuo*. The residue was dissolved in 50% ethanol (50 ml) and the solution was treated with Amberlite IR-120 (H^+ form) and IR-400 (OH^- form) by a batch system. Finally, the solvent was evaporated *in vacuo* and the residue was recrystallized.

Method C: Acetyl-amino acid hydrazide was prepared from acetyl-amino acid methyl ester and hydrazine hydrate in methanol. A solution of sodium nitrite (5.5 mM) in cold water (3 ml) was added dropwise to the solution of acetyl-amino acid hydrazide (5.0 mM) in 1 N hydrochloric acid (10 ml) and tetrahydrofuran (15 ml) cooled with ice. After 10 minutes the azide solution was adjusted to pH 7.5 with triethylamine and added to the solution of methylamine hydrochloride (5.0 mM) and triethylamine (5.0 mM) in cold water (5.0 ml). The mixture was stirred for two days at 4°C and then the solvents were evaporated *in vacuo*. The residue was treated in the same manner as described in Method B.

Method D: A mixed anhydride prepared in tetrahydrofuran (25 ml) from benzyloxycarbonyl-amino acid (10 mM), triethylamine (10 mM) and isobutylchloro formate (10 mM) was added to the solution of methylamine (10 mM) in tetrahydrofuran (10 ml). The mixture was stirred in an ice-salt bath for two hours and then stored in a refrigerator overnight. The solvent was evaporated *in vacuo* and the residual solid was dissolved in

ethyl acetate (50 ml) and water (20 ml). The ethyl acetate layer was separated, washed with water, 5% sodium hydrogen carbonate aqueous solution, 1 N hydrochloric acid and water and then dried over anhydrous sodium sulfate. The solvent was evaporated *in vacuo* and the residue was recrystallized from ethyl acetate-n-hexane. Benzyloxycarbonyl-amino acid methylamide thus obtained (10 mM) was dissolved in 0.4 N hydrochloric acid-methanol (28 ml, 11 mM) and methanol (15 ml) and was hydrogenated with palladium black (0.30 g). The catalyst was removed by filtration and the filtrate was dried *in vacuo*. The residual solid was dissolved in water (20 ml) and ethyl acetate (10 ml) and the aqueous layer was separated and dried *in vacuo*. The obtained amino acid methylamide hydrochloride (10 mM) was acetylated with acetic anhydride (10 mM) in acetic acid (10 ml) at room temperature (Method Da) or with acetyl chloride (12 mM) and triethylamine (12 mM) in chloroform (25 ml) in an ice-salt bath (Method Db). The solvent was evaporated *in vacuo* and the residue was treated in the same manner as described in Method B.

Method of preparation, yield and melting point are listed in Table I. Optical Activity and the result of elemental analyses are also listed for the new compounds.

N-Isopropylacetamide was prepared from acetylchloride and isopropylamine and N-methylisobutyramide was prepared from isobutyrylchloride and methylamine.

All the acetyl- and N-methyl- deuterated homologs were prepared by the use of acetic acid-d_4 and methyl-d_3-amine hydrochloride, respectively. The N-deuterated homologs were prepared by the exchange reaction in deuterium oxide solution.

Infrared spectra were measured with a Japan Spectroscopic Co. 402G grating spectrometer (4000–700 cm^{-1}) and a Hitachi EPI-L grating spectrometer (700–200 cm^{-1}). The KBr disc method was used unless otherwise mentioned.

Infrared Spectra and Two Crystalline Modifications

In the course of the study on the infrared spectra of these compounds, two crystalline modifications were found for some of them. N-Acetyl-L-alanine methylamide, N-acetyl-DL-leucine methylamide and N-acetyl-L-aspartic acid methylamide give well-grown crystals at room temperature. The absorption curves of the above compounds in the region of 700–500 cm^{-1} are similar to each other as shown in Figure 1*a*. They have two peaks near 630 and 600 cm^{-1}. These crystals are called *form I*. If on the other hand, the crystals are grown from melts between two KBr or silicone plates, these crystals give quite different spectra, which indicate the difference in the modifications. These modifications, called *form II*, again give the spectra similar to each other in the region of 700-500 cm^{-1} as shown in Figure 1*b*. They have a single peak or almost overlapped two peaks near 600 cm^{-1}. When these samples are kept at room temperature for a few days, they give the spectra of form I indicating that form II is a metastable

TABLE I. Preparation of N-Acetyl-amino Acid Methylamides

Residue		Method	Yield (%)	m.p.	$[\alpha]_D^{25}$ (g/100 ml)	Formula	Elemental Analysis, Found (Calc. (%)) C	H(+D)	N	S
ALA	L[12]	A		182°						
	DL[3]	B		162°						
ABU	L	A	82	205–6°	−63.8° (1.080 EtOH)	$C_7H_{14}O_2N_2$	53.19	8.64	17.63	
	DL	B	53	161°			53.21	8.77	17.52	
							(53.15)	(8.92)	(17.70)	
NVAL	L	A	53	197°	−44.6° (1.190 EtOH)	$C_8H_{16}O_2N_2$	55.65	9.09	16.09	
	DL	B	60	160°			55.73	9.14	16.05	
							(55.79)	(9.37)	(16.26)	
NLEU	DL[5]	B	66	173–4°						
MET	L[12]	A	46	181°	−17.8° (1.170 EtOH)					
	DL	B	71	133°		$C_8H_{16}O_2N_2S$	47.03	7.72	13.65	15.93
							(47.04)	(7.89)	(13.71)	(15.70)
LEU	L[12]	A	81	165–6°	−39.7° (1.007 MeOH)					
	DL[2]	B	82	152–3°						
ASP	L	Da	77	190°	−51.0° (0.243 EtOH)	$C_7H_{12}O_4N_2$	44.25	6.07	14.81	
							(44.68)	(6.28)	(14.89)	
PHE	DL	B	78	182°		$C_{12}H_{16}O_2N_2$	65.63	7.23	12.45	
							(65.46)	(7.33)	(12.72)	
					(Deuterated Compounds)[a]					
L-ALA	CD_3—	Db	36	184°	−56.5° (1.027 EtOH)	$C_6H_9D_3O_2N_2$	49.32	9.58	18.99	
	—CD_3	C		181°	−51.3° (1.053 EtOH)		49.05	9.94	19.13	
							(48.97)	(10.27)	(19.03)	
DL-ABU	CD_3—	Db	73	162–3°		$C_7H_{11}D_3O_2N_2$	52.20	10.27	17.32	
L-ABU	—CD_3	C	57	199–200°	−47.8° (1.028 EtOH)		52.19	10.12	17.32	
							(52.15)	(10.69)	(17.38)	
DL-LEU	CD_3—	Db	36	153–4°		$C_9H_{15}D_3O_2N_2$	57.14	10.84	14.61	
	—CD_3	B	62	151°			57.11	10.97	14.49	
							(57.11)	(11.18)	(14.80)	

[a] CD_3—: $CD_3CONHCHRCONHCH_3$, —CD_3: $CH_3CONHCHRCONHCD_3$

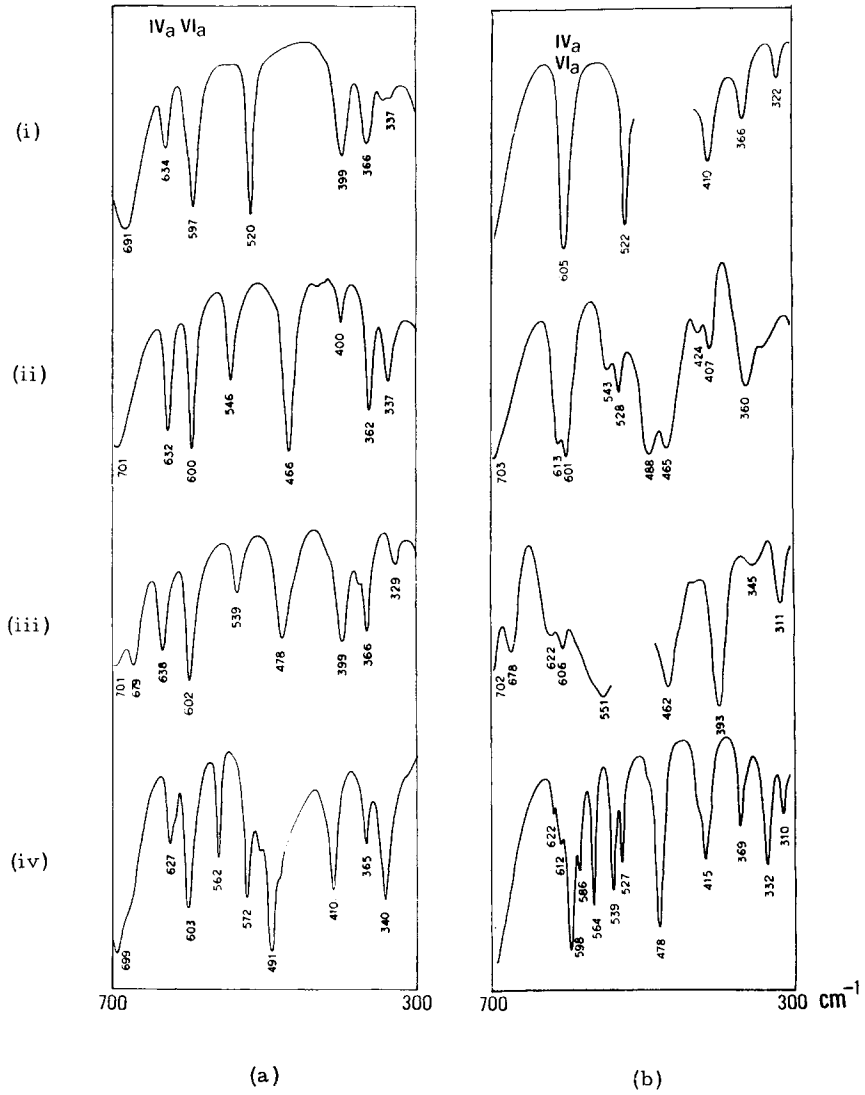

Fig. 1. Infrared spectra of two crystalline modifications, (a) form I and (b) form II, in the region 700–300 cm^{-1}. (i) N-Acetyl-L-alanine methylamide, (ii) N-acetyl-DL-leucine methylamide, (iii) N-acetyl-L-aspartic acid methylamide and (iv) N-acetyl-DL-phenylalanine methylamide.

state at room temperature. When the crystals of form I are mixed with polyethylene and rolled at high temperature, they undergo the phase transformation and the rolled film gives the spectrum of form II. In this case, the change is irreversible even if it is kept at room temperature.

N-Acetyl-DL-phenylalanine methylamide has also two crystalline modifications (Fig. 1). However, the behavior is different. When the sample is recrystallized from water, we obtain plate crystals showing the spectrum

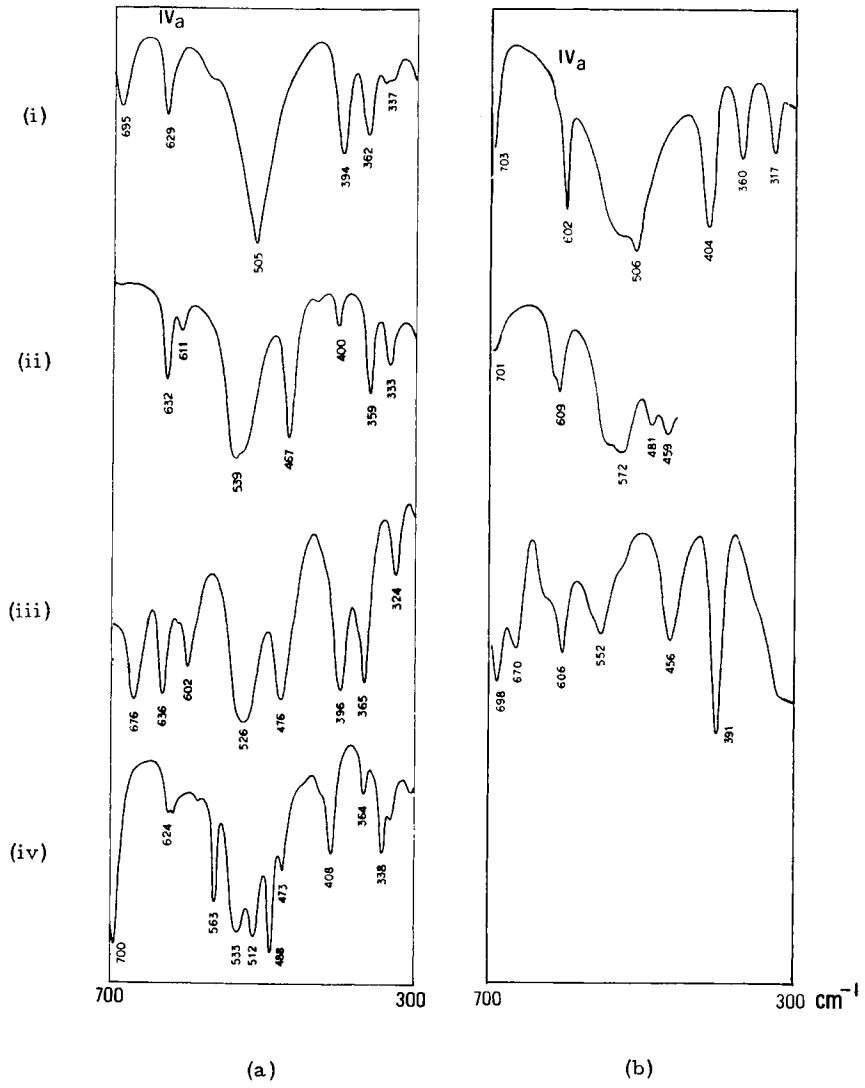

Fig. 2. Infrared spectra of two crystalline modifications, (a) form I and (b) form II, in the region 700–300 cm^{-1} for N-deuterated species. (i) N-Acetyl-L-alanine methylamide, (ii) *N*-acetyl-DL-leucine methylamide, (iii) N-acetyl-L-aspartic acid methylamide and (iv) N-acetyl-DL-phenylalanine methylamide.

of form I. When it is recrystallized from acetone, we obtain fibrous solid which gives the spectrum of form II. The transition to form I occurs when the form II crystal is melted and cooled between two KBr plates.

N-Acetyl-DL-alanine methylamide gives plate crystals showing the spectrum of form I and no transformation is observed. N-Acetyl-L(and DL)-α-amino-n-butyric acid methylamide, N-acetyl-L(and DL)-norvaline methylamide, N-acetyl-DL-norleucine methylamide, N-acetyl-L(and DL)-

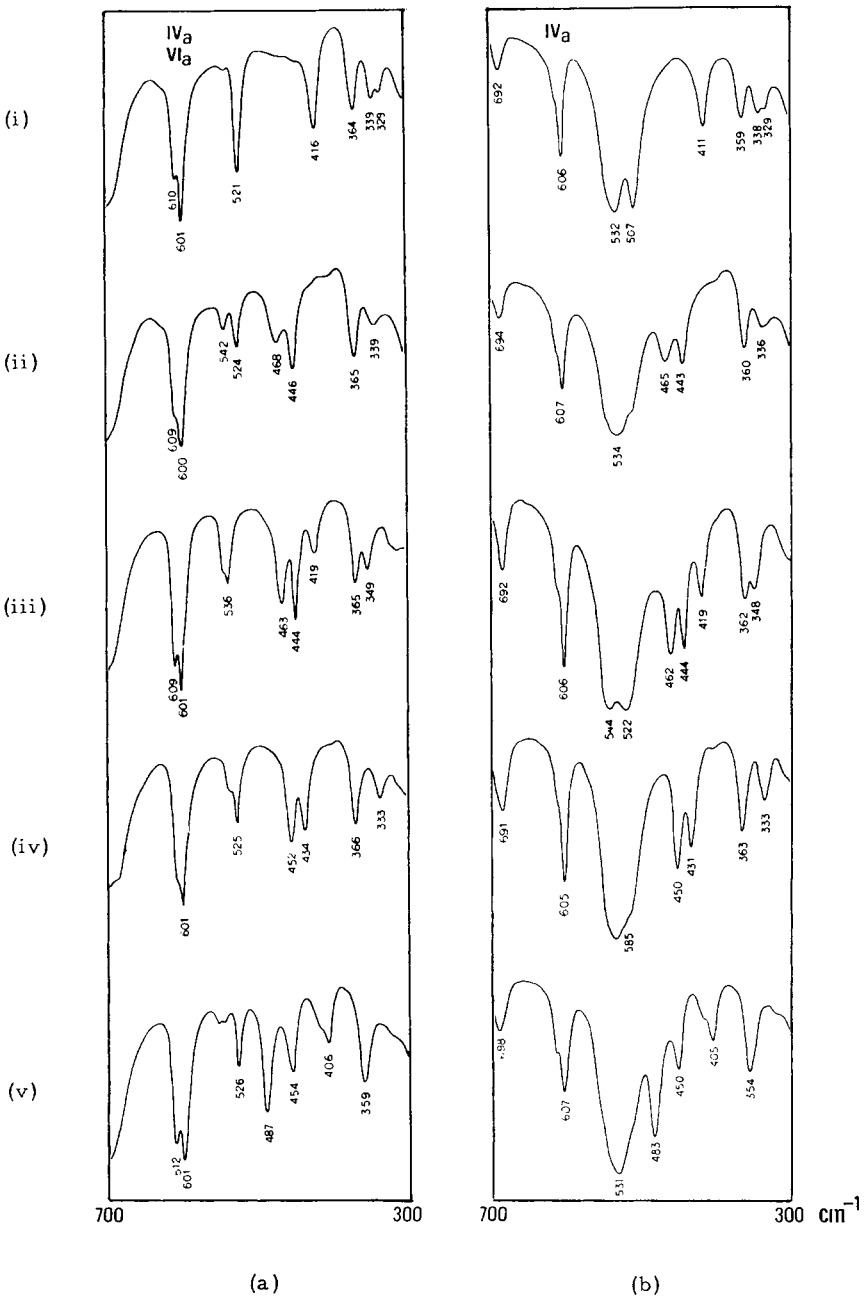

Fig. 3. Infrared spectra of (a) undeuterated and (b) N-deuterated species for (i) N-acetyl-α-amino-n-butyric acid methylamide, (ii) N-acetyl-norvaline methylamide, (iii) N-acetyl-DL-norleucine methylamide, (iv) N-acetyl-methionine methylamide and (v) N-acetyl-L-leucine methylamide in the region 700–300 cm^{-1}. No significant spectral difference was observed between the L- and DL- crystals for the compounds (i), (ii), and (iv).

TABLE II
Two Forms of N-Acetyl-amino Acid Methylamides

Residue			Crystallizing condition[a,b] Form I	Form II
ALA	—CH_3	L	(AcOEt)	from melt
		DL	(AcOEt)	—
ABU	—CH_2CH_3	L	—	(AcOEt)
		DL	—	(AcOEt)
NVAL	—$CH_2CH_2CH_3$	L	—	($EtOH$-Et_2O)
		DL	—	(AcOEt)
NLEU	—$CH_2CH_2CH_2CH_3$	DL	—	(AcOEt)
MET	—$CH_2CH_2SCH_3$	L	—	(AcOEt)
		DL	—	(AcOEt)
LEU	—$CH_2CH(CH_3)_2$	L	—	(AcOEt)
		DL	(AcOEt)	from melt
ASP	—CH_2COOH	L	($MeOH$-Et_2O)	from melt
PHE	—$CH_2C_6H_5$	DL	(H_2O), from melt	(acetone)

[a] ( ): Solvent used for recrystallization.
[b] ——: Metastable at room temperature.

methionine methylamide and N-acetyl-L-leucine methylamide show the spectra similar to those of form II of the above compounds as shown in Figure 3*a* and are classified into form II. Other modifications were not observed. In general form I is hard and crystalline and form II is soft and fibrous. The conditions for the two forms are summarized in Table II.

Assignments of Amide IV and VI Bands

These model compounds have two peptide groups and two amide IV and two amide VI bands. The bands due to the CH_3CONHC^{α}— group are called amide IVa and VIa, and those due to the —$C^{\alpha}CONHCH_3$ group are called amide IVb and VIb.

In order to establish the assignments of these bands, the infrared spectra were analyzed for N-isopropylacetamide $CH_3CONHCH(CH_3)_2$, N-methylisobutyramide $(CH_3)_2CHCONHCH_3$, N-acetyl-L-alanine methylamide $CH_3CONHCH(CH_3)CONHCH_3$, N-acetyl-α-amino-n-butyric acid methylamide $CH_3CONHCH(CH_2CH_3)CONHCH_3$, N-acetyl-DL-leucine methylamide $CH_3CONHCH(CH_2CH(CH_3)_2)CONHCH_3$ and their deuterium homologs.

N-Isopropylacetamide and N-Methylisobutyramide

The infrared spectra of these molecules are shown in Figure 4. In the region of 700–500 cm^{-1} N-isopropylacetamide has only a broad band at 606 cm^{-1}. This band is assigned to amide IV overlapped by amide VI, since it splits into two bands at 577 and 544 cm^{-1} on C-methyl deuteration.

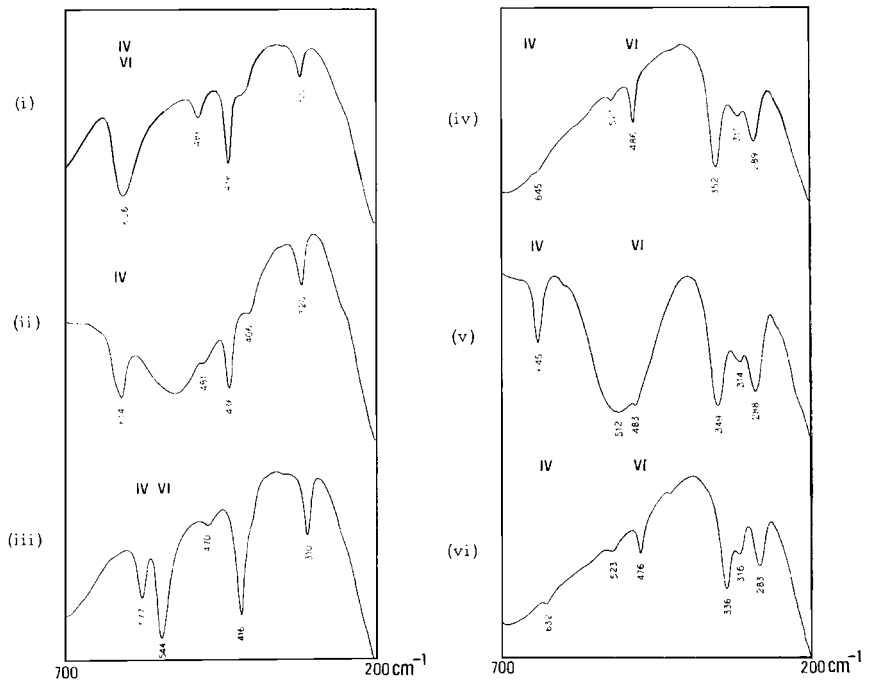

Fig. 4. Infrared spectra of N-isopropylacetamide, N-methylisobutyramide and their deuterium homologs in the region 700–300 cm^{-1}. (i) $CH_3CONHCH(CH_3)_2$, (ii) $CH_3CONDCH(CH_3)_2$, (iii) $CD_3CONHCH(CH_3)_2$, (iv) $(CH_3)_2CHCONHCH_3$, (v) $(CH_3)_2CHCONDCH_3$, and (vi) $(CH_3)_2CHCONHCD_3$.

A characteristic behavior of the amide VI band is that it disappears or becomes very weak on N-deuteration unless it is strongly coupled with other vibrations. This fact is shown for N-methylacetamide[8], N-ethylacetamide and N-acetylglycine methylamide.[1] As for the case of N-isopropylacetamide the situation is the same. On N-deuteration the band at 606 cm^{-1} gets narrower and its peak shifts to 614 cm^{-1}, which can be assigned to amide IV.

The two bands at 645 and 486 cm^{-1} of N-methylisobutyramide are assigned to the amide IV and VI bands, respectively. The band at 645 cm^{-1} is unaffected by N-deuteration and shifts to 632 cm^{-1} on N-methyl deuteration. The band at 486 cm^{-1} also shifts to 476 cm^{-1} on N-methyl deuteration. In this case, the amide VI band remains in the N-deuterated species and its frequency is far lower than that of N-isopropylacetamide. This fact is ascribed to the coupling between amide VI and the skeletal deformation vibration of $(CH_3)_2CHCO$— group. A weak band at 521 cm^{-1} which is unaffected by N-methyl deuteration cannot be assigned to any of characteristic amide bands.

The C=O bending vibrations of CH_3CONH—$CH(CH_3)$—C— and —N— $CH(CH_3)$—$CONHCH_3$ may be regarded as similar to those of CH_3CONH—$CH(CH_3)$—CH_3 and CH_3—$CH(CH_3)$—$CONHCH_3$, respectively.

Accordingly, the above assignments suggest that the four amide bands of N-acetylalanine methylamide $CH_3CONH—CH(CH_3)—CONHCH_3$ are in the following order: amide IVb, (IVa, VIa) and VIb.

N-Acetyl-L-alanine Methylamide

The assignments of the amide IVb, IVa, VIa and VIb bands are given on the basis of their behavior on C-methyl and N-methyl deuteration.

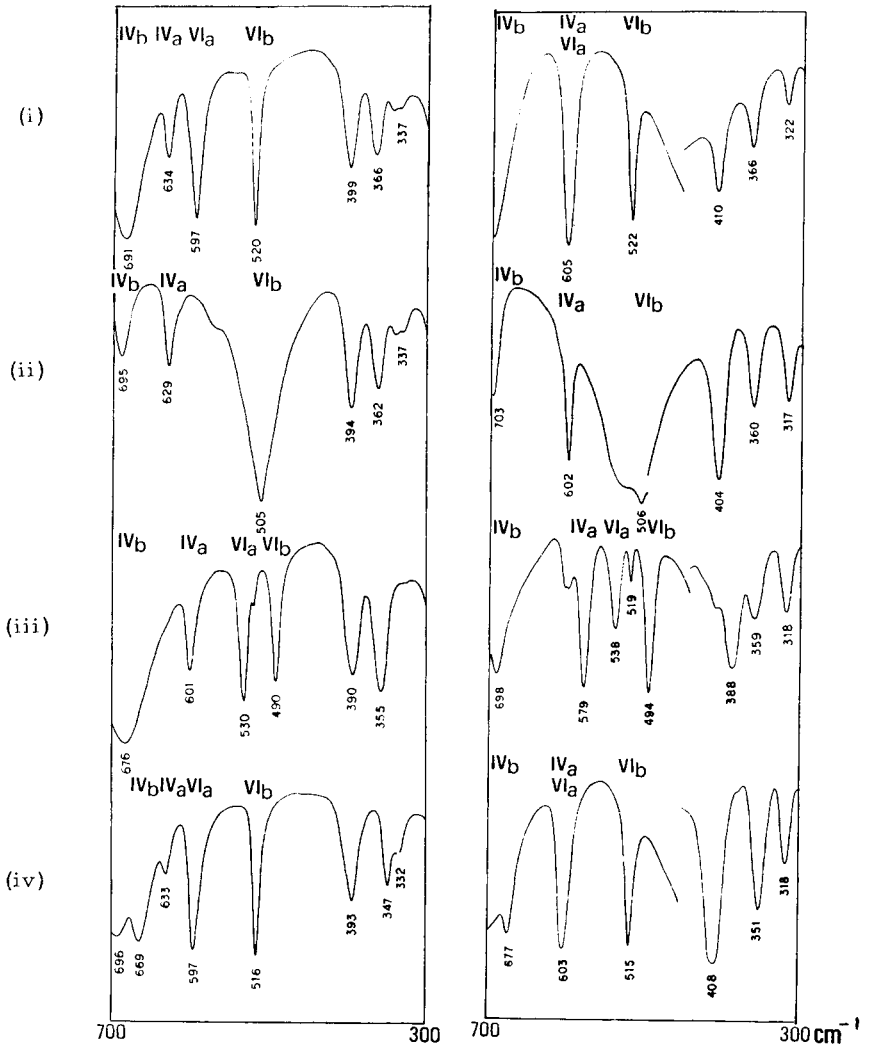

Fig. 5. Infrared spectra of two crystalline modifications, (a) form I and (b) form II, of *N*-acetyl-L-alanine methylamide and its deuterium homologs in the region 700–300 cm^{-1}. (i) $CH_3CONHCH(CH_3)CONHCH_3$, (ii) $CH_3CONDCH(CH_3)CONDCH_3$, (iii) $CD_3CONHCH(CH_3)CONHCH_3$ and (iv) $CH_3CONHCH(CH_3)CONHCD_3$.

The deuteration on one part of the peptide plane must have a stronger effect upon the vibrations mainly associated with the same peptide plane. Therefore, the vibrations which are influenced by N-methyl deuteration are related to the modes in the —$C^{\alpha}CONHCH_3$ group. The disappearance of the amide VIa band on N-deuteration was also used for the assignment. The assignments for this compound are given in Figure 5.

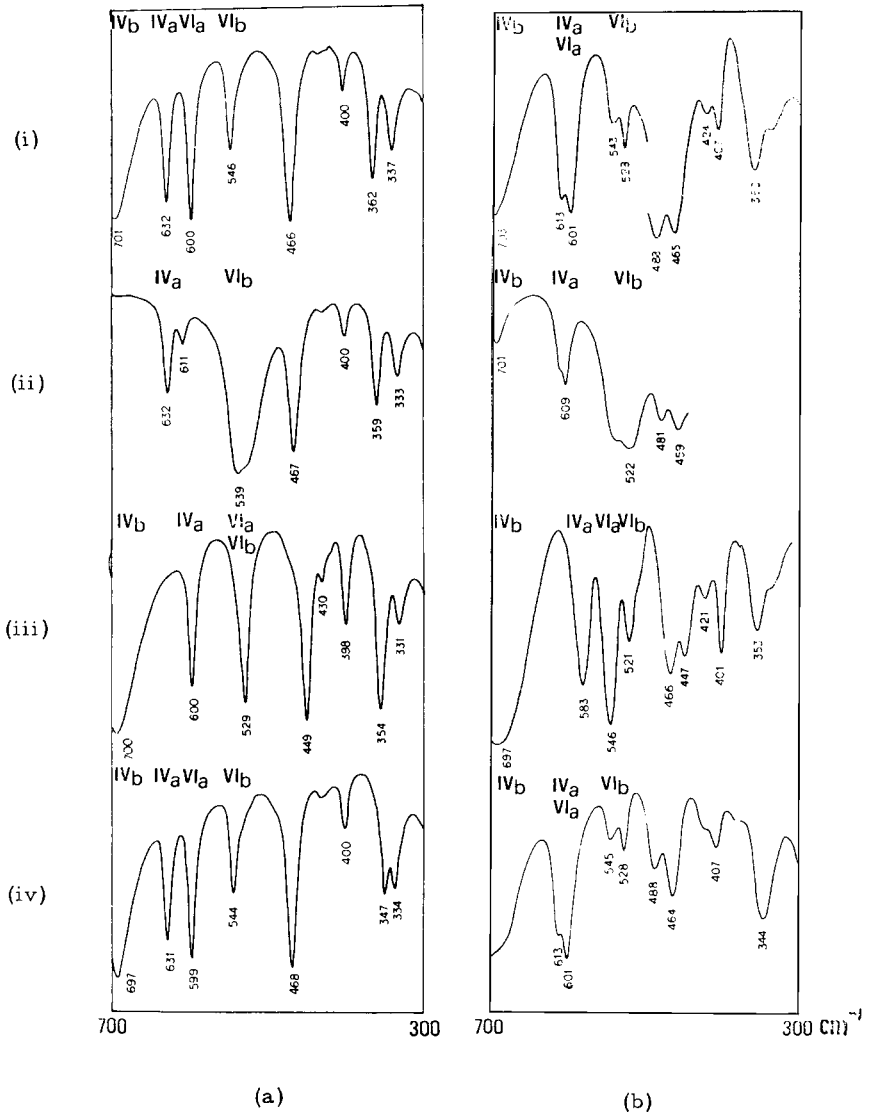

Fig. 6. Infrared spectra of two crystalline modifications, (a) form I and (b) form II, of N-acetyl-DL-leucine methylamide and its deuterium homologs in the region 700–300 cm^{-1}. (i) $CH_3CONHCHRCONHCH_3$, (ii) $CH_3CONDCHRCONDCH_3$, (iii) CD_3-$CONHCHRCONHCH_3$ and (iv) $CH_3CONHCHRCONHCD_3$, where R is $CH_2CH(CH_3)_2$.

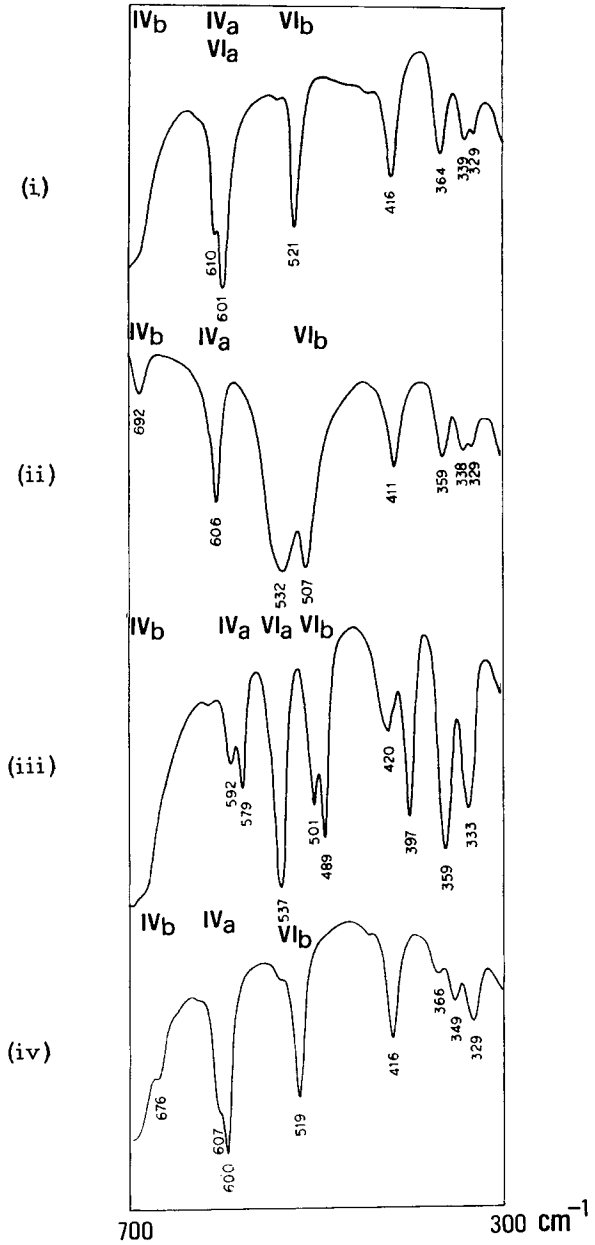

Fig. 7. Infrared spectra of N-acetyl-α-amino-n-butyric acid methylamide and its deuterium homologs in the region 700–300 cm^{-1}. N osignificant spectral difference was observed between the L- and DL- crystals. (i) $CH_3CONHCHRCONHCH_3$, (ii) $CH_3CONDCHRCONDCH_3$, (iii) $CD_3CONHCHRCONHCH_3$ and (iv) $CH_3CONHCHRCONHCD_3$, where R is CH_2CH_3. VIa overlaps IVa in (iv).

For form I the amide IVa and VIa bands are separated and appear at 634 and 597 cm^{-1}. For form II they are overlapped with each other and appear at 605 cm^{-1}. On N-deuteration the amide VIa band at 597 cm^{-1} of form I disappears and the band at 605 cm^{-1} of form II becomes narrower. On C-methyl deuteration these bands move, respectively, to 601 and 530 cm^{-1} for form I and to 579 and 538 cm^{-1} for form II. On N-methyl deuteration these bands are unaffected.

The amide VIb band, which corresponds to the band at 486 cm^{-1} of N-methylisobutyramide, appears at 520 cm^{-1} for form I and 522 cm^{-1} for form II. On N-deuteration it is overlapped with the broad amide V band and appears at 505 and 506 cm^{-1} for forms I and II, respectively. It remains on N-deuteration as in the case of N-methylisobutyramide. On N-methyl deuteration it shifts to 516 and 515 cm^{-1} for forms I and II, respectively. On C-methyl deuteration it shifts to 490 and 494 cm^{-1} for forms I and II, showing that this vibration is coupled with amide IVa and VIa.

The amide IVb band is regarded as overlapped with the amide V band. The sharp bands remaining in the N-deuterated compound at 695 and 703 cm^{-1} for forms I and II are assigned to amide IVb. On N-methyl deuteration these bands are separated from amide V bands and appear at 669 and 677 cm^{-1}, supporting the above assignments. There are a few weak bands, whose assignments are not certain, in the region of 700–500 cm^{-1} for C-methyl deuterated compounds. These bands are not found in the DL-crystal.

*N-Acetyl-*DL*-leucine Methylamide and N-Acetyl-α-amino-n-butyric Acid Methylamide*

The assignments of the four amide bands for these compounds are obtained in the same way as described above, the results being shown in Figures 6 and 7. As for the amide IV and VI bands, the spectra and their behavior on deuteration for forms I and II of N-acetyl-DL-leucine methylamide correspond exactly to those for forms I and II of N-acetyl-L-alanine methylamide, respectively. The crystal of N-acetyl-L(and DL)-α-amino-n-butyric acid methylamide, whose spectra and spectral change on deuteration closely resemble to those of form II of N-acetyl-L-alanine methylamide, is classified into form II. The amide IVa and VIa bands for form II are observed separately for these compounds.

For the remaining compounds, the amide IVa and VIa bands are assigned on the basis of their positions and their behavior on N-deuteration and are shown in Figures 1–3.

Conformation of Peptide Backbone

In a previous paper[1] we reported the rotational isomerism of N-acetylglycine methylamide $CH_3CONH—CH_2—CONHCH_3$ in the crystalline state, which causes a phase transformation. The skeletal stretching and deformation vibration bands as well as the amide IV and VI bands in-

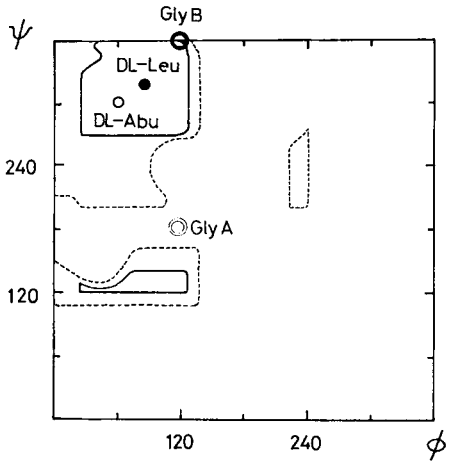

Fig. 8. Conformation map for N-acetyl-amino acid methylamides. ◎ Gly A, ○ Gly B: N-acetylglycine methylamide[1]; ○ DL-Abu: N-acetyl-DL-α-amino-n-butyric acid methylamide[10]; ● DL-Leu: N-acetyl-DL-leucine methylamide[9]. The regions shown with hard and broken lines are those presented by Ramachandran et al.[11]

dicated that the rotational angles (φ, ψ) in one modification are near (120°, 180°) and that those in another are near (120°, 0°). Since this rotational isomerism is caused by the internal rotation potential around the CH_2—CONH axis, the same type of rotational isomerism is expected for the present molecules.

N-Acetyl-L-alanine methylamide has also two crystalline modifications. The marked difference observed in their spectra in the region of 500–300 cm^{-1}, where the skeletal deformation vibration bands appear, suggests that the phase transformation involves the rotational isomerism. The steric interaction between the peptide backbone and the side chain methyl group should affect the stable conformations. The rotational angles (φ, ψ) of the two rotational isomers may be somewhat different from those of N-acetylglycine methylamide and may roughly correspond to those in the two sterically allowed regions shown in Figure 8.

Two crystalline modifications found in N-acetyl-DL-leucine methylamide, N-acetyl-L-aspartic acid methylamide and N-acetyl-DL-phenylalanine methylamide also give marked spectral changes in the region of 500–300 cm^{-1}. However, they cannot be ascribed only to the rotational isomerism about the CHR—CONH axis, because the rotational isomerism in the side chain is also possible for these compounds.

The crystal structure of N-acetyl-DL-leucine methylamide was determined by Ichikawa and Iitaka.[9] The space group is $P2_1/a$ and four molecules are in a unit cell with the dimensions, $a = 8.46$, $b = 17.33$, $c = 8.24$ Å and $\beta = 113.5°$. The molecules in the crystal are connected by hydrogen-bonds, forming a two-dimensional network parallel to the ab-plane. The peptide backbone takes a β-like conformation ($\varphi = 86°$, $\psi = 319°$ for L-molecules).

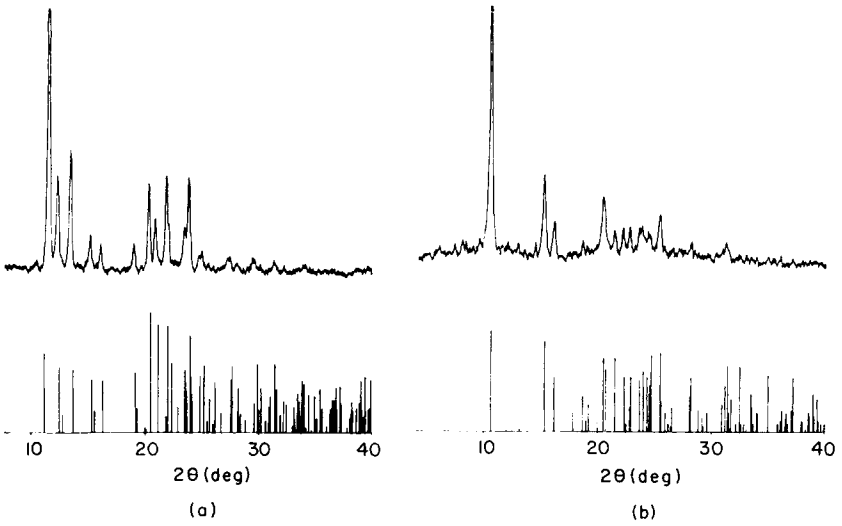

Fig. 9. Observed and calculated X-ray powder patterns for (a) N-acetyl-DL-leucine methylamide (form I) and (b) N-acetyl-DL-α-amino-n-butyric acid methylamide (form II).

The crystal structure of N-acetyl-DL-α-amino-n-butyric acid methylamide has recently been determined by Koyama, Shimanouchi and Iitaka.[10] The space group is $P2_1/n$ and four molecules are in a unit cell with the dimensions, $a = 16.52$, $b = 11.58$, $c = 4.84$ Å and $\beta = 90.9°$. In the crystal the molecules are connected by the hydrogen bonds, which form a one-dimensional linear chain parallel to the c-axis. The peptide backbone takes also a β-like conformation (φ is near 60° and ψ is near 300° for L-molecules).

In order to correlate the infrared spectrum with the conformation of the peptide backbone for these compounds, we measured the X-ray powder pattern of the sample used for the infrared measurement. The X-ray-powder patterns of N-acetyl-DL-leucine methylamide (form I) and N-acetyl-DL-α-amino-n-butyric acid methylamide (form II) are shown in Figure 9. They are explained in terms of the cell dimensions and the structure factors used in the X-ray analyses.

As described above, two patterns were found as to the positions of the amide IVa and VIa bands. The two amide bands were found near 630 and 600 cm^{-1} in form I, whereas they were found near 600 cm^{-1} in form II. These two forms were also distinguished by their physical appearance. For N-acetyl-DL-leucine methylamide the crystals of form I are plates and give the amide IVa and VIa bands at 632 and 600 cm^{-1}. For N-acetyl-DL-α-amino-n-butyric acid methylamide the crystals (form II) are fine needles and give the amide bands at 610 and 601 cm^{-1}. The X-ray structure analyses revealed that these two patterns do not directly reflect the conformation of the peptide backbone and suggest that they reflect the difference in

the way of hydrogen bondings. It is reasonable that the two-dimensional hydrogen bondings give plate crystals and that the one-dimensional hydrogen bondings give fine needles.

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