

INFRARED SPECTRA OF N-ACETYL-L- $\alpha$ -AMINO-ACID N'-METHYL-AMIDES WITH ALIPHATIC SIDE CHAINS IN NON-POLAR SOLVENTS\*

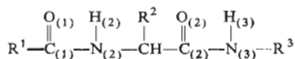
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Infrared spectra of the L-alanine (*I*), L-leucine (*II*), L-valine (*III*) and L-tert-leucine (*IV*) N-acetyl N'-methylamides were measured. Amides *I*–*IV* are not self associated in tetrachloromethane in the concentration  $2 \cdot 10^{-5} \text{ mol l}^{-1}$  at room temperature and in tetrachloroethylene in the concentration  $1.5 \cdot 10^{-4} \text{ mol l}^{-1}$  at temperatures above  $65^\circ\text{C}$ . True conformational changes are observable only with the least flexible amide *IV* which exists at room temperature in a  $C_5$  conformation. This conformational type is also highly populated in the valine derivative *III*, but is less important in the alanine and leucine derivatives *I* and *II* in which the intramolecularly bonded  $C_7$  and the distorted hydrogen-nonbonded conformations contribute seriously.

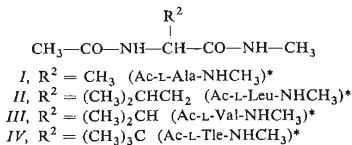
A sequence of homoconjugated amide (peptide) groups is the most characteristic section of all peptide and protein molecules. The smallest segment of the polypeptide backbone still retaining this structural feature is the dipeptide unit represented on the molecular level by N'-alkylamides of N-acyl- $\alpha$ -amino acids.



These diamides are very simplified models of the actual situation in polypeptides because they can imitate the mutual interaction only of neighbouring peptide groups. Nevertheless, great effort was devoted to their investigation<sup>1</sup>, particularly to conformational studies on N-acetyl- $\alpha$ -amino-acid N'-methylamides ( $\text{R}^1 = \text{R}^3 = \text{CH}_3$ ). As early as in 1959 Tsuboi and coworkers<sup>2</sup> analysed the infrared spectra (N—H stretching vibrations) of such amides derived from glycine, DL-alanine, L-valine, DL-norleucine, and L-proline. When studying the temperature and concentration dependences of four bands present in  $3300\text{--}3650 \text{ cm}^{-1}$  region they discovered that the absorption band in the range  $3300\text{--}3360 \text{ cm}^{-1}$  did not disappear totally neither by dilution nor by temperature increase (*i.e.* under conditions under which

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intermolecular hydrogen bonds are usually disrupted) and that the relation of the intensity of this band to intensities of other bands remains constant in diluted solutions at elevated temperatures. This band was assigned to the stretching vibration of the  $N_{(3)}-H_{(3)}$  group bonded intramolecularly to the  $C_{(1)}=O_{(1)}$  group. The band at  $3420\text{ cm}^{-1}$  was assigned to the stretching vibration of the  $N_{(2)}-H_{(2)}$  group which is free in the same conformer. Two remaining bands located at highest wavenumbers were assigned to  $\nu(N-H)$  vibrations in extended conformers not participating in hydrogen bonds. This assignment and also the conformational interpretation was changed by Marraud and coworkers<sup>3-6</sup>. The assignment made by Tsuboi and coworkers<sup>2</sup> was retained for the band with the lowest wavenumber. In addition, two conformers have been distinguished differing in location of  $R^2$  on the seven-membered ring ( $C_7^{eq}$  and  $C_7^x$  folded conformation). The band at  $3415-3420\text{ cm}^{-1}$  was assigned to the stretching vibration of the  $N_{(2)}-H_{(2)}$  group hydrogen-bonded to  $C_{(2)}=O_{(2)}$  under formation of a five-membered ring in an extended conformation ( $C_5$ , extended, or  $\beta$ ). Two bands with highest wavenumbers were considered to be caused by stretching vibrations of remaining  $N-H$  groups which are not involved in hydrogen bonds in mentioned conformations. The conformations  $C_5$  and  $C_7$  have been regarded as the decisive conformational types in all following stereochemical analyses based not only on infrared spectroscopy but also on other physico-chemical measurements<sup>7</sup> and quantum chemical calculations<sup>1,8,9</sup>. In these studies model compounds have been used in which hydrogen-bonding is limited due to alkylation on nitrogen atoms and/or in which the side chains  $R^1$ ,  $R^3$  restrict the conformational mobility of the molecule due to steric or chemical factors (*e.g.* restricted  $\Phi$  angle range in N-acetyl-L-proline amides<sup>2,6,10,11</sup> or fixation of the folded conformation due to the side-chain interaction in N-acetyl-L-serine N'-methylamide<sup>10</sup>). Newly, we suggested to use the tert-leucine derivative *IV* (ref.<sup>12</sup>) in which the extremely bulky side chain  $R^2$  ought to restrict seriously the conformational mobility without changing the substitution patterns on nitrogen atoms, *cf.*<sup>13</sup>. The location of the tert-butyl substituent in the central part of the molecule should mimic sensibly the conformational situation valid generally for the dipeptide unit. The key compound *IV* was examined together with already known derivatives of L-alanine (*I*), L-leucine (*II*)



\* Formulae written in symbols common in peptide chemistry<sup>15</sup>. Abbreviation Tle is used for tert-leucine<sup>16</sup>.

and L-valine (III). In the present paper we describe the infrared spectra of this series of compounds measured in tetrachloromethane and tetrachloroethylene. Very recently, Scheraga and coworkers published a paper<sup>14</sup> in which similar studies on diamides I and II are included.

## EXPERIMENTAL

Compounds I–IV were the same preparations as in the following paper<sup>12</sup>. Spectra were measured on an infrared spectrophotometer, Perkin–Elmer model 580, operated in the scale expansion mode. The concentration dependence was studied in 10 cm Hellma cells, the temperature dependence in Hellma cells, length 2 or 4 cm, respectively. Temperature was measured directly in the sample using a thermocouple. The reversibility was checked in all temperature dependence measurements. Because some of bands (mainly in the range 3300 to 3400  $\text{cm}^{-1}$ ) exhibited significant sensibility to the humidity all solvents were dried immediately before use, tetrachloroethylene by filtration through a column of active silica gel, tetrachloromethane by azeotropic distillation.

## RESULTS AND DISCUSSION

*Association changes.* Compounds I–IV are not associated in tetrachloromethane at room temperature in concentrations  $2 \cdot 10^{-5} \text{ mol l}^{-1}$  or lower (Table I) and in tetrachloroethylene in concentration  $1.5 \cdot 10^{-4} \text{ mol l}^{-1}$  at temperatures 65°C or higher (Table II). Spectra of compounds I and II measured in our laboratory under mentioned conditions were equal to those measured by Tsuboi and coworkers<sup>2</sup> in tetrachloromethane at 60°C and concentration  $1 \cdot 10^{-4} \text{ mol l}^{-1}$ . The transformation of intermolecularly associated entities into non-associated entities exhibited the same spectral variation regardless of way in which the dissociation was reached (concentration lowering or temperature increase). The most significant spectral changes were observed on the band located between 3300 and 3400  $\text{cm}^{-1}$  which has been until now assigned to the stretching vibration of the  $\text{N}_{(3)}\text{—H}_{(3)}$  group intramolecularly hydrogen-bonded to the atom  $\text{O}_{(1)}$  in conformation  $C_7$ . Decrease in intensity and shift to higher wavenumbers of this band was observed in the spectra of I, III and IV with decreasing concentration and increasing temperature. In the spectra of II, two bands have been found at room temperature in concentrations equal or higher than  $1 \cdot 10^{-4} \text{ mol l}^{-1}$  in tetrachloromethane and  $1.5 \cdot 10^{-4} \text{ mol l}^{-1}$  in tetrachloroethylene. The lower wavenumber band in this couple disappeared without changing the wavenumber when the concentration was further lowered or the temperature increased. The maximum of the other band in the couple was shifted under these conditions to higher wavenumbers. Bands in the discussed spectral region are shifted from the position given for  $C_7^{\text{eq}}$  conformation towards the position proposed by Cung and coworkers<sup>6</sup> for the  $C_7^{\text{ax}}$  conformation. A shift to higher wavenumbers was observed also for the band at 3420  $\text{cm}^{-1}$  which has

been assigned<sup>6</sup> to the  $N_{(2)}-H_{(2)}$  group intramolecularly hydrogen-bonded onto the  $O_{(2)}$  in a  $C_5$  conformation. However, this shift is rather small and is accompanied by a simultaneous decrease in intensity of the band<sup>17</sup>. The decrease of the intensity of the band at  $3440\text{ cm}^{-1}$ , assigned to the stretching vibration of the free  $N_{(2)}-H_{(2)}$  group<sup>2-7</sup>, is well observable with decreasing association in temperature dependence studies. A change in overlapping with the band at  $3420\text{ cm}^{-1}$  which is sensitive to the degree of association can explain this behaviour. In addition, the universal decrease in  $\epsilon_{\text{max}}^a$  value with increasing temperature should accentuate the observed variation of the band at  $3440\text{ cm}^{-1}$ .

An important increase in band intensity with decreasing association was observed (using temperature dependence measurements) only with the tert-leucine derivative on the band at  $3460\text{ cm}^{-1}$  which has been assigned to the stretching vibrations of the free  $N_{(3)}-H_{(3)}$  bond (Fig. 1). With other three derivatives *I-III* the increase of intensity of this band is smaller and is again concealed by the decrease of the  $\epsilon_{\text{max}}^a$  value with increasing temperature.

The above depicted changes in the spectra of *I, III* and *IV*, especially the gradual shifts of the bands in  $3300-3400\text{ cm}^{-1}$  region, can be rationalized as resulting

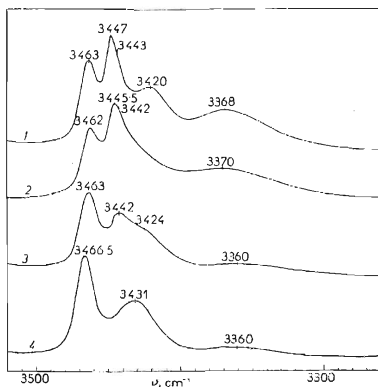


FIG. 1

Infrared Spectra of Compounds *I-IV* Measured in Tetrachloromethane

1 Ac-L-Ala-NH-CH<sub>3</sub> (*I*), 2 Ac-L-Leu-NH-CH<sub>3</sub> (*II*), 3 Ac-L-Val-NH-CH<sub>3</sub> (*III*), 4 Ac-L-Tle-NH-CH<sub>3</sub> (*IV*). Concentration  $2 \cdot 10^{-5}\text{ mol l}^{-1}$ , cell length 10 cm, the ordinate scale ten times expanded.

from stepwise degradation of higher associates rather than from direct dissociation of cyclic dimers. The latter possibility was considered by Marraud and coworkers<sup>17,18</sup> for explanation of spectral changes observed with a different set of model diamides ( $R^1 = \text{CH}_3, (\text{CH}_3)_2\text{CH}, (\text{CH}_3)_3\text{C}$ ;  $R^2 = \text{CH}_3, \text{CH}_3\text{CH}_2, (\text{CH}_3)_2\text{CH}$ ). The difference of association properties of these two sets of models is probably caused by different side-chain distribution. In our set, the end-groups substituents ( $R^1$  and/or  $R^3$ ) are substantially smaller (methyls) when compared with bulky substituents  $R^1$  and/or  $R^3$  in Marraud's models<sup>18</sup>. Association in cyclic dimers, which should be characterized<sup>17,18</sup> by the band at  $3326 \text{ cm}^{-1}$ , was observed in our set only with the leucine derivative *II* at room temperature in concentrations  $2-1 \cdot 10^{-4} \text{ mol l}^{-1}$ . However, even in this case, higher associates are present which generate after dissociation the  $C_7$  conformers.

### Conformational Changes of Non-Associated Molecules

The thermal degradation of associates in tetrachloroethylene is accomplished at  $65^\circ\text{C}$  (only for *I* a little bit higher temperature is necessary). The spectral changes registered at temperatures above this limit must be caused by changes in the population of single

TABLE I

Wavenumbers ( $\text{cm}^{-1}$ ) of Bands in the Region of the N—H Stretching Vibrations: Concentration Dependence in Tetrachloromethane

Com- pound	Concentra- tion	$\nu(\text{N—H})$ free				$\nu(\text{N—H})$ bonded <sup>b</sup>	
		$\text{N}_{(3)}\text{—H}_{(3)}$		$\text{N}_{(2)}\text{—H}_{(2)}$			
<i>I</i>	0.0002	3 463	3 447	3 443 sh	3 418	3 350	—
<i>I</i>	0.0001	3 463	3 447	3 443 sh	3 419	3 356	—
<i>I</i>	0.00002 <sup>a</sup>	3 463	3 447	3 443 sh	3 420	3 368 <sup>c</sup>	—
<i>II</i>	0.0002	3 461.5	3 445.5	3 442.5	3 428	3 360	3 326
<i>II</i>	0.0001	3 462	3 445.5	3 443	3 428	3 360	3 324
<i>II</i>	0.00002 <sup>a</sup>	3 462	3 445.5	3 442	3 426	3 370 <sup>c</sup>	—
<i>III</i>	0.0002	3 463	3 439	—	3 424	3 334	—
<i>III</i>	0.0001	3 463	3 439	—	3 424	3 340	—
<i>III</i>	0.00002	3 463	3 441	—	3 424	3 360 <sup>c</sup>	—
<i>IV</i>	0.0002	3 466.5	—	—	3 428.5	3 335	—
<i>IV</i>	0.0001	3 466.5	3 452	—	3 429	3 338	—
<i>IV</i>	0.00002	3 466.5	<sup>d</sup>	—	3 431	3 360 <sup>c</sup>	—

<sup>a</sup> In concentration  $1 \cdot 10^{-5} \text{ mol l}^{-1}$  the same values; <sup>b</sup> intermolecular hydrogen bonds unless stated otherwise; <sup>c</sup> intramolecular hydrogen bond; <sup>d</sup> exact wavenumber not estimated.

TABLE II

Temperature Dependence of the Infrared Spectra of Compounds I-IV in Tetrachloroethylene in the Range 3 500-3 250 cm<sup>-1</sup> (wavenumbers in cm<sup>-1</sup>)

Temperature °C	$\nu(\text{N}_{(3)}-\text{H}_{(3)})^a$ free	$\nu(\text{N}_{(2)}-\text{H}_{(2)})^b$ free	$\nu(\text{N}_{(2)}-\text{H}_{(2)}\dots^c$ $\dots\text{O}_{(2)}=\text{C}_{(2)})$ intramolecular	$\nu(\text{N}_{(3)}-\text{H}_{(3)}\dots^d$ $\dots\text{O}_{(1)}=\text{C}_{(1)})$ intramolecular	$\nu(\text{N}_{(3)}-\text{H}^e$ intermolecular associated
Compound I					
27	3 463	3 446	3 443 sh	3 413 <sup>f</sup>	3 346
64	3 463	3 446	3 443 sh	3 416	3 362
96	3 463	3 446	3 443 sh	3 416	—
110	3 463	3 446	3 443 sh	3 416	—
Compound II					
27	3 461.5	3 444.5	3 440 sh	(3 425) <sup>g</sup>	3 360
64	3 461.5	3 444.5	3 440 sh	(3 425) <sup>g</sup>	—
97	3 461.5	3 444.5	3 440 sh	(3 425) <sup>g</sup>	—
Compound III					
27	3 464	3 439 <sup>h</sup>	—	3 418 <sup>i</sup>	3 360
64	3 464	3 439 <sup>h</sup>	—	3 418 <sup>i</sup>	—
97	3 464	3 439 <sup>h</sup>	—	3 370	—
111	3 464	3 439 <sup>h</sup>	—	3 370	—
Compound IV					
28	3 466	—	—	3 425 <sup>f</sup>	3 340
64	3 466	3 452	—	3 428	—
97	3 466	3 452	—	3 429	—
110	3 466	3 452	—	3 429	—

<sup>a</sup> Conformers C<sub>5</sub> and hydrogen-nonbonded conformers; <sup>b</sup> conformer C<sub>7</sub> and hydrogen-nonbonded conformers; <sup>c</sup> conformers C<sub>5</sub>; <sup>d</sup> conformer C<sub>7</sub>; <sup>e</sup> intermolecular association; <sup>f</sup> intramolecular hydrogen bond in associates; <sup>g</sup> the exact wave number value cannot be estimated; <sup>h</sup> for assignment see Results and Discussion; <sup>i</sup> the degradation of associates is accompanied by an intensity decrease.

conformers of the non-associated species. Generally, spectral variations due to these conformational transitions seem to be by one order of magnitude smaller than those arising from association processes. For compounds *I–III* the spectral variations caused by conformational factors in the temperature range 65–110°C are masked by the universal decrease in  $\epsilon_{\max}^a$  values with increasing temperature. The overall effect of increasing temperature on the spectra of *I–III* is, in greater or lesser extent, the decreased intensity of all absorption bands under study. Clearly, the differences in conformers population due to temperature increase are not substantial with diamides *I–III*. The only one model compound for which the spectral changes arising from conformational transitions are experimentally evidenced is the tert-leucine derivative *IV*. Here, the increase in temperature results in an increase of intensity of an inflex at 3452  $\text{cm}^{-1}$  which should be assigned to the stretching vibration of the non-associated  $\text{N}_{(2)}\text{—H}_{(2)}$  bond.

**Conformational considerations.** In the spectra of non-associated models *I* and *II* the absorption bands assigned to stretching vibrations of free N—H bond are the most prominent. The band at 3460  $\text{cm}^{-1}$  is related to the  $\text{N}_{(3)}\text{—H}_{(3)}$  vibrational mode, the band at 3440  $\text{cm}^{-1}$  to that of  $\text{N}_{(2)}\text{—H}_{(2)}$  bond regardless of the overall molecular conformation — either intramolecularly hydrogen-bonded  $C_7$  or a hydrogen-nonbonded more extended alternative. The intensity of the band at 3440  $\text{cm}^{-1}$  does not correspond to the population of the  $C_7$  conformer (36%) found earlier<sup>10</sup> at 90°C in tetrachloroethylene using band resolution technique. Taking into account results published by Marraud and Neel<sup>19</sup> and our results on solvation of amides<sup>20</sup> we have to conclude that the participation of the hydrogen-nonbonded conformers is not negligible<sup>21</sup>. In addition, the  $C_5$ -conformational type must be considered. Similar situation was also met with the leucine derivative *II*. From visual inspection of spectra we may guess that the populations of both conformations,  $C_7$  and hydrogen-nonbonded, are in *II* the same or smaller when compared with values for *I*. A quantitative evaluation is disqualified in this case by extensive overlapping of the  $\nu(\text{N}_{(2)}\text{—H}_{(2)})$  free band with the sum of bands related to the conformation  $C_5$ . The conformational type denoted usually as  $C_5$  should be regarded as a set

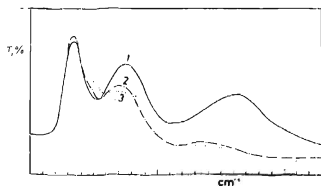


FIG. 2  
The Temperature Dependence of the Infrared Spectra of the N-Acetyl-L-tert-leucine N'-Methylamide (*IV*) in Tetrachloroethylene  
1 At 33°C; 2 at 74°C; 3 at 111°C. Concentration  $2 \cdot 10^{-4} \text{ mol l}^{-1}$ , cell length 4 cm.

of congenerous variants differing in the extent in which the  $H_{(2)}$  proton is influenced either by direct hydrogen-bond formation or by dipole-dipole interaction. This situation is clearly reflected in the IR spectra: On the lower wavenumber side of the free  $N_{(2)}-H_{(2)}$  band (at  $3440\text{ cm}^{-1}$ ) a broad, slowly decreasing slope is shown.

Different situation was met with the valine (*III*) and tert-leucine (*IV*) derivatives in which the  $C_5$ -conformational type prevails. The population of the  $C_7$  conformer is in both cases negligible if any. In the spectrum of *III* the  $\nu(N_{(2)}-H_{(2)})_{free}$  absorption band is broad and clearly composed of two or more components. However, an inflex similar to that in the spectra of *I* and *II* is not manifested (Fig. 2). Also the wave number of the maximum is slightly smaller. There are several possibilities for interpretation of these observations, however at present, they are only of hypothetical value, cf.<sup>22</sup> With *IV* the increase in temperature extends the population of the hydrogen-nonbonded conformers (the absorption intensity of the inflex at  $3452\text{ cm}^{-1}$  is significantly augmented). The energetical minima corresponding to single stable conformers seems to be better separated in the diamide *IV* with an extremely bulky side-chain than in diamides *I-III* with less bulky substituent  $R^2$ . This is the reason why we were able to detect unambiguously the changes in population of conformers of non associated molecules with increasing temperature only with diamide *IV*.

From our study of concentration and temperature dependence of IR spectra within the series of models *I-IV* we may conclude the following points: 1) The folded conformation  $C_7$  (equatorial or axial) is not the generally prevailing conformational type. Its participation is considerable, even in non-polar media, only in specific structural situations: In the series of N-acetyl- $\alpha$ -amino-acid N'-methylamides only in derivatives with non-bulky side-chains. 2) Extended conformers similar to  $C_5$  are the most common constituents of the conformational equilibrium mixture. In these conformers the wavenumber of the  $\nu(N_{(2)}-H_{(2)})$  band is modified by the varying proximity of the  $C_{(2)}-O_{(2)}$  group. The character of this interaction is not clear. With the exception of the tert-butyl derivative *IV* we should accept that a family of closely similar conformers, differing only slightly in  $\phi$  and  $\psi$  angle values, is present. The results of quantum chemical calculations support this concept. Very broad minima are depicted on conformational maps in the region  $\phi = -155^\circ$  to  $-55^\circ$  and  $\psi = 160^\circ$  to  $120^\circ$ . 3) Even at room temperatures we must allow the participation of hydrogen-nonbonded conformers mainly in compounds with small side-chains<sup>8,19</sup>. In compounds with bulky substituents conformers of this kind participate at higher temperatures.

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