TABLE III

EFFECT OF HEAT ON ABSORPTION SPECTRA OF AZO DYES IN BENZENE

Dye[a]	λ[b]	λ'[c]	Δλ	$A_{\text{hot}}/A_{\text{cold}}$[d]	Dye	λ	λ'	Δλ	$A_{\text{hot}}/A_{\text{cold}}$
I	377	371	6	1.31	VII	410	408	2	1.01
						450	(448)[e]	2	1.10
II	410	405	5	1.62	VIII	427	421	6	1.01
III	422	419	3	2.18	IX	325	323	2	1.00
IV	353	351	2	1.21	X	475	473	1	1.00
V	347	345	2	1.05	XI[f]	407	403	4	1.16
VI	340	339	1	1.03	XII[g]	440	437	3	1.00

[a] For symbols, *cf.* Table I. [b] Wave length of main absorption band of stable form (in mμ) at 25°. [c] Wave length of main absorption band of stable form (in mμ) at 65°. [d] Ratio of intensity of absorption band of hot solution to that of cold solution, both in laboratory illumination. [e] Inflection point. [f] 4-Dimethylamino-4'-hydroxyazobenzene. [g] 1-Benzeneazo-2-naphthylamine.

cis isomers when made up in ordinary laboratory illumination (*cf.* reference 1), undergo considerable changes upon heating. On the other hand, since solutions of weakly phototropic azo dyes contain almost entirely only the *trans* isomers when made up in the laboratory, the heating of these can cause only a slight shift in the equilibrium and hence but little change in the spectra.

The shift of the absorption maxima of these compounds toward shorter wave lengths as a result of raising the temperature by 40° (relative to the position of the absorption band of the stable form at room temperature) is unusual and difficult to explain. This shift may indicate the existence of some resonating complex between dye and solvent molecules which dissociates at higher temperatures; thus lowering the energy difference between the excited state and the ground state when the solution is at room temperature. It has been observed, however, that 4-dimethylaminoazobenzene showed the same hypsochromic shift in 95% ethanol solution, while azobenzene fails to exhibit it in either solvent, indicating that this shift is probably a characteristic of the dye occurring in a variety of solvents of different polarities. It is then perhaps more plausible to suggest that, when light is absorbed by the dye solution at 65°, the molecule is raised from the ground state to one of the higher vibrational levels of the first excited state, resulting in a shift in the absorption maximum to a slightly shorter wave length. Apparently, only azo compounds containing one or more auxochromic substituents exhibit this hypsochromic shift on heating. Further investigation will be necessary, however, in order to establish the scope and limitations of this effect and to arrive at a more complete understanding of this phenomenon.

WASHINGTON 25, D. C.

[CONTRIBUTION FROM THE DIVISION OF MEDICAL PHYSICS, DONNER LABORATORY, AND THE RADIATION LABORATORY OF THE UNIVERSITY OF CALIFORNIA]

Infrared Spectra of Some Long-chain 2-Alkenoic Acids and Their Bearing on the Structure of C_{27}-Phthienoic Acid[1]

BY N. K. FREEMAN

RECEIVED JULY 21, 1952

The infrared spectra of six long-chain 2-alkenoic acids with different types of branching near the double bond are presented and discussed. Several absorption bands are observed which do not appear in the spectra of unconjugated long-chain fatty acids. One of these is the 6.1 μ carbon–carbon double bond stretching vibration band, the intensity of which has evidently been greatly enhanced as a result of conjugation. Other new bands appear in the 10–16 μ region, some of which may prove to be analogous to those in the spectra of alkenes which characterize the arrangement of alkyl groups around the double bond. Such a relationship has not been fully demonstrated with the limited amount of data available. Use of the 7.25 μ band intensity as an index of the number of terminal methyl groups (previously employed in branched alkanoic acids) was found to be valid for this group of alkenoic acids. Comparison of the spectra with that of C_{27}-phthienoic acid provides additional support for previous evidence that this molecule has an α-methyl branch. There is no definite indication regarding the position of a second nearby methyl group, except that the lack of spectral conformity with 2,5-dimethyl-2-heptadecenoic acid rules out this particular structure.

In the course of extensive study of the branched-chain fatty acids of the tubercle bacillus, Cason and collaborators have isolated one of the principal species and shown that it is an α,β-unsaturated acid having twenty-seven carbon atoms and three branching side chains (probably methyl), one of which is on the α-carbon atom.[2,3] This acid has been called C_{27}-phthienoic acid. Infrared spectrometry was employed to aid in establishing these structural features, and certain inferences were based on the results of a broad study of saturated branched-chain fatty acid spectra.[4] In continuing efforts to elucidate the structure of C_{27}-phthienoic acid still further, Cason's group has synthesized several α,β-unsaturated acids with various arrangements of branching methyl groups. The infrared absorption spectra of these acids have been recorded, and are presented here with two purposes in view: (1) to examine the relationships between structure and spectra in this restricted class of compounds; (2) to make direct comparisons with the spectrum of C_{27}-phthienoic acid.

Experimental

Materials.[5]—The first six acids whose spectra are shown in Figs. 2 and 3 are synthetic compounds, whose preparation

---

(1) This work was supported in part by the United States Atomic Energy Commission.

(2) J. Cason and G. Sumrell, *J. Biol. Chem.*, **192**, 405 (1951).

(3) J. Cason, N. K. Freeman and G. Sumrell, *ibid.*, **192**, 415 (1951).

(4) N. K. Freeman, THIS JOURNAL, **74**, 2523 (1952).

(5) The author is greatly indebted to Dr. James Cason of the University of California Chemistry Department for furnishing the compounds which form the basis of this paper. Dr. Cason's series of publications on branched-chain fatty acids will contain descriptions of their synthesis and properties. C_{27}-Phthienoic acid is also the subject of previous publications.[2,3]

and properties will be discussed in other publications. Oleic acid (> 99%) was obtained from the Hormel Institute, Austin, Minnesota. Stearic and elaidic acids were both recrystallized and are regarded as sufficiently pure for illustrative purposes.

Apparatus.—The spectra were recorded on a Baird Associates Model B double beam spectrophotometer equipped with a sodium chloride prism. The region beyond 6.5 μ in the spectra of Figs. 2 and 3 are of liquid films between salt windows. Neither the uncertainties nor the variations in these film thicknesses are regarded as important, since quantitative measurements have only been made on the solution spectra (5.5–6.5 μ).

Discussion

The main points of interest in the interpretation of the spectra (Figs. 2 and 3) are the relationships which involve the double bond, with particular reference to the effects of conjugation and the presence of alkyl branches. The spectral features of long-chain fatty acids in general have been discussed by Shreve, *et al.*,[6] and a more recent publication by Sinclair and collaborators[7] includes the spectra of *cis*- and *trans*-octadecenoic acids. Some effects of chain branching in saturated fatty acids have been reported previously from this Laboratory.[4]

It is well known from the study of alkene spectra[8] that structural differences among simple olefins may be manifested in one or more of three regions of infrared absorption. The first of these is the region of carbon–hydrogen bond stretching vibrations in the range from about 3.2 to 3.5 μ. Olefinic hydrogens can be distinguished in this range, but only with better resolving power than that of the sodium chloride prism in our spectrophotometer.

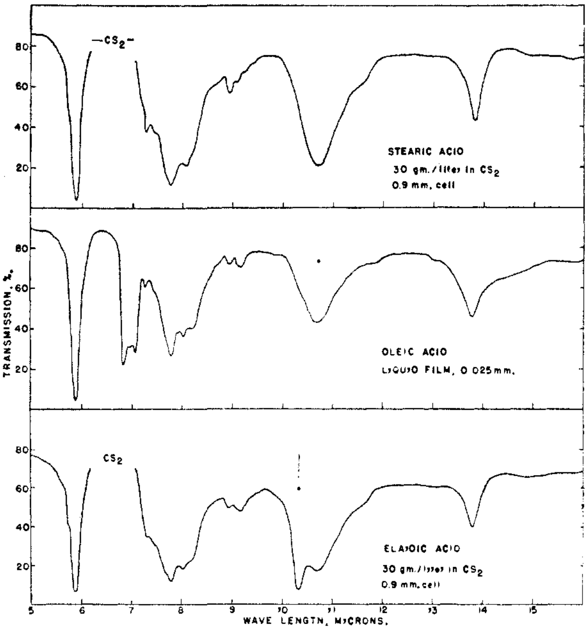


Fig. 1.—Infrared absorption spectra of some non-conjugated fatty acids.

Double Bond Stretching Bands.—The second region is near 6 μ, where the carbon–carbon double bond stretching frequency is located. It is indicated in the correlation of Rasmussen[8] that terminal double bonds have their absorption maxima at slightly longer wave lengths (6.02–6.12 μ) than do internal double bonds (5.98–6.03 μ) and that conjugation with carbonyl groups also tends to displace the peak toward longer wave lengths. The positions of the C=O and C=C stretching bands of the 2-alkenoic acids studied are given in Table I.

TABLE I

2-Alkenoic acid	$\lambda_{C=O}$, μ	$\lambda_{C=C}$, μ	Intensity[a] of C=C band
2-Dodecenoic	5.90	6.05	0.39
5-Methyl-2-hendecenoic	5.87	6.05	.39
2-Methylenedodecanoic	5.89	6.13	.29
3-Methyl-2-nonenoic	5.90	6.10	.63
2-Methyl-2-dodecenoic	5.91	6.08	.33
2,5-Dimethyl-2-heptadecenoic	5.90	6.09	.32

[a] Intensity is expressed as the peak absorbance of the band for a 0.2 *M* solution in chloroform in a 0.1 mm. cell.

The carbonyl bands all lie within a small range near 5.9 μ, whereas in unconjugated long-chain fatty acids (see Fig. 1) the corresponding band is found at about 5.84 μ.[6] The magnitude of the displacement associated with the conjugative effect is about the same as that observed for esters and ketones[8,9] and for the 2-octadecenoic acids.[7]

The C=C stretching bands are all at slightly longer wave lengths than the values quoted above for internal double bonds, and this again is consistent with a conjugative effect. (For oleic and other acids in which the double bond is relatively remote from the carboxyl group, the C=C stretching bands are very weak and not easily resolved from the strong C=O band. They are not discernible in Fig. 1.) A methyl group on either of the unsaturated carbons appears to cause a slightly larger shift. In agreement with the alkene correlation, the terminal double bond (2-methylenedodecanoic acid) has the longest wave length band.

It may be noted in Figs. 2 and 3 that at the same molar concentrations the carbonyl bands of all of these acids have approximately the same intensity (range of peak absorbance is 0.85–0.90). The C=C absorption intensities, on the other hand, vary with different arrangements of groups around the double bond. Peak absorbance values of these bands have been included in Table I. No extension to more refined quantitative intensity measurements has been attempted. Nevertheless in comparison with the two acids which may be regarded as 1-carboxy-2-alkylethylenes, it appears that substitution of an additional methyl group on the β-carbon enhances the intensity, whereas in both cases of α-alkyl substitution the intensity is reduced. If it may be assumed that the vibrational interaction between the conjugated C=C and C=O bonds is small, then the gain in intensity by the C=C band in going from a non-conjugated to a con-

---

[6] O. D. Shreve, M. R. Heether, H. B. Knight and D. Swern, *Anal. Chem.*, **22**, 1498 (1950).

[7] R. G. Sinclair, A. F. McKay, G. S. Myers and R. Norman Jones, THIS JOURNAL, **74**, 2578 (1952).

[8] R. S. Rasmussen in "Progress in the Chemistry of Organic Natural Products," Vol. 5, Vienna, 1948.

[9] E. J. Hartwell, R. E. Richards and H. W. Thompson, *J. Chem. Soc.*, 1436 (1948).

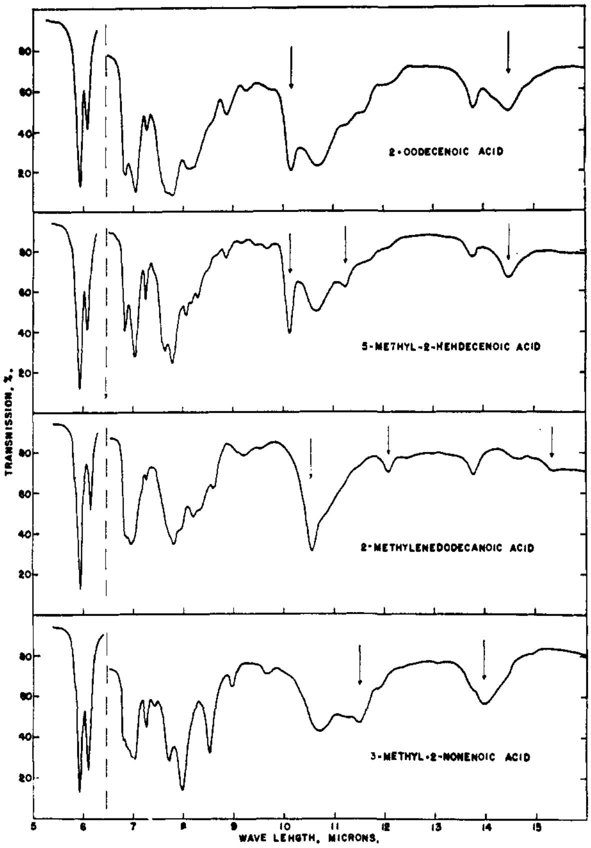


Fig. 2.—Infrared absorption spectra of some α,β-unsaturated fatty acids: 5.5–6.5 μ, 0.2 M in $CHCl_3$, 0.1 mm. cell; 6.5–16 μ, liquid films: upper two curves, 0.025 mm. spacer; lower two curves, no spacer.

jugated position must be attributable mainly to its polarization by the carboxyl group. It may then be inferred that the intensity differences associated with α- *vs.* β-alkyl substitution are a reflection of the relative abilities of a hydrogen atom and an alkyl group to polarize the double bond. The net effect of a hydrogen at one end and an alkyl at the other is in one case competing with polarization by the carboxyl, and in the other case reinforcing it. This description is oversimplified, since the absorption intensity depends on the change of dipole with vibration rather than on the equilibrium dipole directly. Until absolute intensity measurements and a more detailed analysis of the factors influencing the intensity can be made, this hypothesis provides only a tentative and partial explanation of the observations.

Bands in the Range from 10 to 16 μ.—The third region in which double bond configurations may be characterized is beyond 10 μ, where the non-planar vibrations of olefinic hydrogen atoms occur. The well-established correlation of these frequencies with alkylethylene structural types[8,10] is summarized in Table II.

The bands whose maxima have been quoted to the nearest 0.05 μ are usually strong, sharp, and well defined, whereas those for the *cis* and trialkyl types are apt to be broad, less intense, and

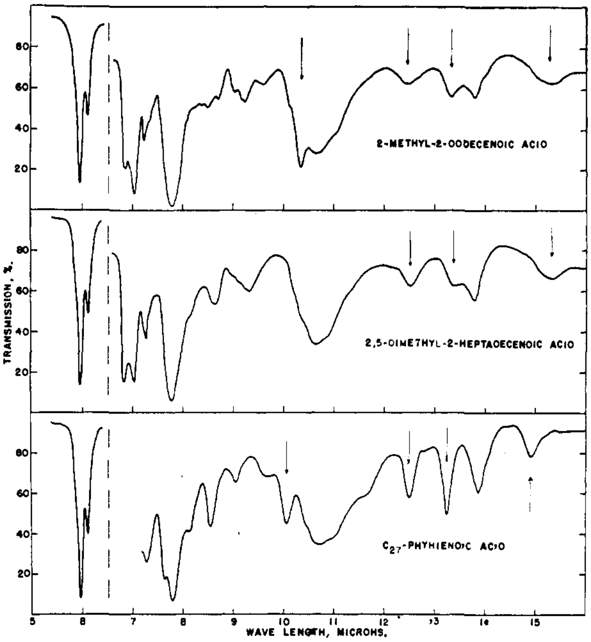


Fig. 3.—Infrared absorption spectra of some α,β-unsaturated fatty acids having α-methyl branches: upper two curves: 5–5.6.5 μ, 0.2 M in $CHCl_3$, 0.1 mm. cell; 6.5–16 μ, liquid films, 0.025 mm. spacer. C_{27}-phthienoic acid: 5.5–6.5 μ, 0.25 M in $CHCl_3$, 0.1 mm. cell; 7–16 μ, 50 g./l. in CS_2, 0.9 mm. cell.

less constant in position. It has been noted by other authors[6,7] that this correlation is valid for non-conjugated fatty acids in which the double bond structure is one of the first three types listed in Table II. (Their work did not include any examples with branching structures. Moreover, as may be seen in Fig. 1, the presence of a 14–15 μ band in the spectrum of oleic acid is difficult to verify.) Rasmussen[8] has shown that these frequencies are altered if one or more of the alkyl groups is replaced by a chlorine atom. Consequently it has not been presumed that the correlation would hold if an alkyl group were replaced by a carboxyl. Since the 2-alkenoic acids can be regarded as carboxyethylenes, it is of interest to examine their spectra with this point in mind. Figure 4 is a chart constructed from the spectra of Figs. 2 and 3, showing the bands between 10 and 16 μ which do not occur in saturated fatty acid spectra. Heights of the solid vertical lines represent roughly the relative band intensities. Dotted lines indicate positions (or ranges) of the characteristic bands for corresponding alkylethylenes, *i.e.*, carboxyl replaced by alkyl.

TABLE II[a]

Alkyl-substituted ethylene	Characteristic wave length, μ
$RCH{=}CH_2$	10.05, 10.95
$RCH{=}CHR'$ (*trans*)	10.35
$RCH{=}CHR'$ (*cis*)	14–15
$RR'C{=}CH_2$	11.25
$RR'C{=}CHR''$	11.5–12.5

[a] These are essentially the values given in ref. 8. Other authors give slightly different limits to the ranges for *cis* and trisubstituted types.

---

(10) N. Sheppard and G. B. B. M. Sutherland, *Proc. Roy. Soc.* (*London*), **A196**, 195 (1949).

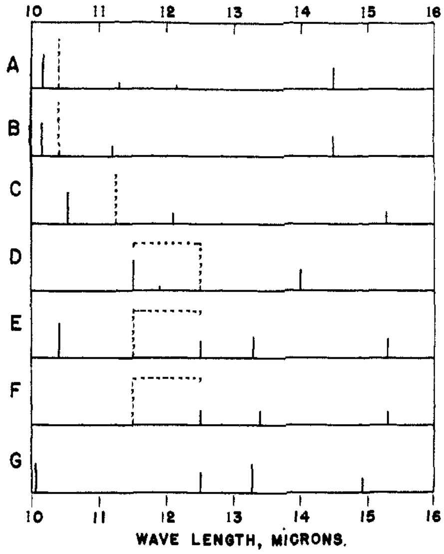


Fig. 4.—Line diagram of the spectra of Figs. 2 and 3 (omitting 10.7 and 13.8 μ bands). Solid lines represent absorption bands. Dotted lines show positions of characteristic absorption by same structure when COOH is replaced by alkyl: A, 2-dodecenoic acid, $HO_2CCH{=}CHR$ (*trans*); B, 5-methyl-2-hendecenoic acid, $HO_2CCH{=}CHR$ (*trans*); C, 2-methylenedodecanoic acid, $HO_2CCR{=}CH_2$; D, 3-methyl-2-nonenoic acid, $HO_2CCH{=}CRR'$; E, 2-methyl-2-dodecenoic acid, $HO_2CCR{=}CHR'$; F, 2,5-dimethyl-2-heptadecenoic acid, $HO_2CCR{=}CHR'$; G, C_{27}-phthienoic acid.

The assignment of a particular band in any of these spectra as the hydrogen non-planar bending vibration is somewhat dubious. The most reliable choices are probably the bands at 10.18 and 10.15 μ in the spectra of 2-dodecenoic and 5-methyl-2-hendecenoic acids, respectively. From the method of synthesis[5] these compounds are presumed to be *trans*, and the suggested band positions are not greatly different from the characteristic one of the *trans*-1,2-dialkylethylene structure (Table II). Sinclair[7] also observed a band at 10.2 μ in the spectrum of *trans*-2-octadecenoic acid which was not exhibited by the *cis* isomer.

For 2-methylenedodecanoic acid the 10.54 μ band is the most reasonable selection, principally on the basis of intensity. This tentative choice is given some support by the appearance of strong bands at 10.6–10.7 μ (rather than 11.25 μ) in the spectra of methyl methacrylate[11] and a number of other methacrylates.[12]

For the compounds which are structurally analogous to trialkylethylenes (only one remaining olefinic hydrogen) the uncertainty regarding *cis–trans* relationships presents an additional obstacle to fuller interpretation. Whereas the non-planar hydrogen bending bands of *cis*- and *trans*-3-methyl-2-pentene appear to be at 12.2 and 12.3 μ, respectively,[11] it must be assumed that the presence of a carboxyl group may have a marked effect on the corresponding vibrations in conjugated acids. It seems evident that until there are available some acids of this type which are unambiguously known to be *cis* or *trans* it will not be possible to disentangle the effects of branching and geometrical isomerism, nor to establish a meaningful correlation of the double bond absorption bands for these compounds.

Number of Branches.—It has been shown in previous work[4] that in a saturated branched long-chain fatty acid the number of methyl groups can be determined by a suitable calibration and intensity measurement of the 7.25 μ absorption band. Measurements were made of this band in the spectra of the 2-alkenoic acids according to the procedure described elsewhere[4] for the saturated branched-chain acids with the results shown in Table III. The values for the 2-alkenoic acids fall within the appropriate ranges, and bearing in mind the slight overlap between the ranges for two and three methyl groups, it appears that the validity of this determination is not impaired by the presence of the double bond.

TABLE III

INTENSITY MEASUREMENTS OF 7.25 μ ABSORPTION BAND

0.1 *M* solutions in CCl_4, 0.5 mm. cell, estimated baselines (see ref. 4)

Number of methyl groups	Absorbance range found in saturated branched-chain acids	2-Alkenoic acid	Absorbance of 7.25 μ band
1	0.03–0.05	2-Dodecenoic	0.05
		2-Methylenedodecanoic	.06
2	.10– .17	3-Methyl-2-nonenoic	.11
		2-Methyl-2-dodecenoic	.10
		5-Methyl-2-hendecenoic	.14
3	.15– .21	2,5-Dimethyl-2-heptadecenoic	.17

Comparison with C_{27}-Phthienoic Acid.—While no satisfactory structural correlation has been achieved for the branched 2-alkenoic acids, it is nevertheless pertinent to make certain comparisons of the available spectra with that of C_{27}-phthienoic acid. Previous chemical and spectral evidence[2,3] has indicated that this acid has an α-methyl branch. Its spectrum has therefore been included in Fig. 3 together with those of the two synthetic acids which have α-methyl branches. All three have absorption bands at about 12.5 and 13.3 μ which do not appear in any of the other spectra (see also Fig. 4). In addition the peak absorbance of the carbon–carbon stretching band of C_{27}-phthienoic acid (when adjusted to 0.2 *M* concentration) was found to be 0.33, in agreement with the values for α-branched acids in Table I. These observations add to the previous evidence for the α-methyl branch, and the correctness of this much of the structure seems well established.

The discrepancies among the spectra of Fig. 3 in the 10–10.5 μ range can only be taken to mean that C_{27}-phthienoic acid does not have the same over-all configuration in the vicinity of the double bond as either of the two synthetic acids. Other evidence has indicated that it has two branches in addition to the α-methyl, one of which is probably on the γ- or δ-carbon. Such a branch near the double bond is also suggested by the

---

(11) Catalog of Infrared Absorption Spectrograms, American Petroleum Institute Research Project No. 44, National Bureau of Standards, Washington, D. C.

(12) "Catalog of Infrared Spectrograms," Samuel P. Sadtler and Son, Inc., Philadelphia, Pa.

more remote branching would not be expected to alter the curve appreciably from that of 2-methyl-2-dodecenoic acid. The absence of a 10.1 μ band in the spectrum of 2,5-dimethyl-2-heptadecenoic acid rules out at least one isomeric form of this structure. The available spectra do not provide any basis for a choice from among the other most likely structures.

NOTE ADDED IN PROOF.—Recently a sample of synthetic 2,4-dimethyl-2-dodecenoic acid has become available from Professor Cason,[5] and its infrared spectrum has been examined. In the region beyond 10 μ, absorption bands are found at the following wave lengths (values for the corresponding bands of C_{27}-phthienoic acid—see Figs. 3 and 4—are given in parentheses for comparison): 10.05 (10.06), 12.3 (12.50), 13.2 (13.26), 14.9 (14.92) μ. Considered together with previous evidence, these data indicate that the second branching methyl group of C_{27}-phthienoic acid is in the γ-position.

Acknowledgment.—The author is grateful to Dr. John W. Gofman and Dr. Hardin B. Jones of these laboratories for their continued interest and encouragement of this work.

BERKELEY 4, CALIFORNIA

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

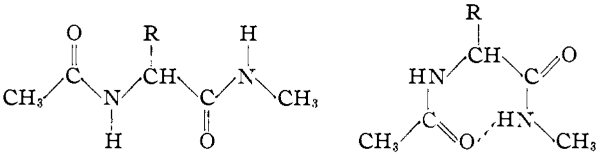
Near Infrared Spectra of Compounds with Two Peptide Bonds and the Configuration of a Polypeptide Chain. III

BY SAN-ICHIRO MIZUSHIMA, TAKEHIKO SHIMANOUCHI, MASAMICHI TSUBOI, KENJI KURATANI, TADAO SUGITA, NOBORU MATAGA AND REISUKE SOUDA

RECEIVED SEPTEMBER 8, 1952

The near infrared spectra of acetylglycine N-methylamide, acetylalanine N-methylamide, acetylleucine N-methylamide, acetylglycine ethyl ester and acetylleucine ethyl ester have been measured in the solid state with polarized incident radiation. For the ethyl esters the absorption measurements in carbon tetrachloride solutions have also been made. From the experimental results configurations of the associated molecules have been suggested.

In a previous work we measured the near infrared spectra of carbon tetrachloride solutions of acetylglycine N-methylamide (AGNA), acetylalanine N-methylamide (AANA), acetylleucine N-methylamide (ALNA) and acetylproline N-methylamide (APNA) and found that the molecules of AGNA, AANA and ALNA exist in the extended and folded forms, while those of APNA exist only in the folded form.[1]



Extended form Folded form

Fig. 1.—The two forms of acetyl aminoacid N-methyl amide.

Our absorption measurements could also show the presence of associated molecules at a concentration as low as 10^{-4} mole/l. for the solutions of AGNA, AANA and ALNA, while there was found no indication of such associated molecules for the solution of APNA even at a concentration as high as 0.05 mole/l.

In order to obtain further information about the structure of the associated molecules of AGNA, AANA and ALNA we have made absorption measurements in the solid state with polarized infrared radiation. In addition, measurements have been made on acetylglycine ethyl ester and acetylleucine ethyl ester in the solid state and in solutions, and the molecular configurations of these ethyl esters have been discussed in relation to those of the N-methylamides.

Acetylleucine N-Methylamide.—As shown in Fig. 2 we observed only two absorption peaks at 3.04 μ and at 3.22 μ in the 3 μ region (from 2.8 to 3.3 μ) and one at 6.19 μ (1616 cm.$^{-1}$) in the 6 μ region for ALNA in the crystalline state. The former two are assigned to the NH stretching vibrations and the latter one to the CO stretching vibration.[2] It is evident from the wave length values of these peaks and from the absence of the 2.9 μ band that the NH and CO groups are involved in hydrogen bonding and are in the *trans* position with respect to each other.[3]

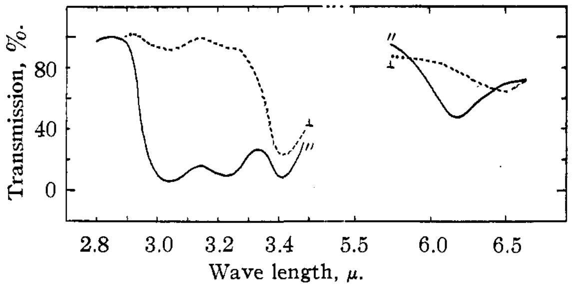


Fig. 2.—Transmission of polarized infrared radiation in the oriented crystal of ALNA.

The solid line of Fig. 2 corresponding to the strong absorption of the NH and CO bands was obtained when the electric vector of the polarized incident radiation was parallel to the long direction of the crystal and the dotted line corresponding to the very weak or almost no absorption was obtained when the electric vector was perpendicular to it. This would mean that the direction of the N—H· · · O hydrogen bond is parallel to the long direction of the crystal and the molecules of ALNA in the lattice are oriented as shown in Fig. 3. Then it would

---

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

(2) S. Mizushima, T. Shimanouchi, M. Tsuboi, T. Sugita, E. Kato and E. Kondo, *ibid.*, **73**, 1330 (1951).

(3) S. Mizushima, T. Shimanouchi, S. Nagakura, K. Kuratani, M. Tsuboi, H. Baba and O. Fujioka, *ibid.*, **72**, 3490 (1950); M. Tsuboi, *Bull. Chem. Soc. Japan*, **22**, 215, 255 (1949).