TABLE I

PHYSICAL PROPERTIES OF R—O—CF_2—CF_2H

R	F.p., °C.	B.p., °C.	Mm.	t, °C.	d^t_4	n^t_D	AR_F	Fluorine, % Calcd.	Found
$-CH_3$	−107	36.5	760	20	1.293	1.3	..	...	..
$-C_2H_5$	Glass	50.7	621.7	25	1.1951	1.2961	1.15	52.0	51.8
$-n$-C_3H_7	Glass	71.7	626.7	25	1.1549	1.3141	1.12	47.5	47.2
$-n$-C_4H_9	Glass	22.0	23.5	25	1.1163	1.3296	1.22	43.7	43.2
$-n$-C_5H_{11}	Glass	31.0	20.3	25	1.0726	1.3480	1.27	C, 44.7	45.2
								H, 6.38	6.7

sured using a Beckman quartz spectrophotometer model DU. Distilled water was used as a reference liquid for liquid samples and the experiments were carried out in a 10-mm. quartz cell. The extinction coefficient, E, defined by $\log I/I_0 = El$ was measured; l is the length of the cell in cm. which is unity in the present case. Readings were made at approximately every 10 Å. in the regions where absorption took place. The results are given in Fig. 1. The inflection or humps in the curves may be attributed to the presence of unresolved vibrational fine structure in the electronically excited state. This resolvable fine structure is shown by some of the ethers made from trifluorochloroethylene.[3]

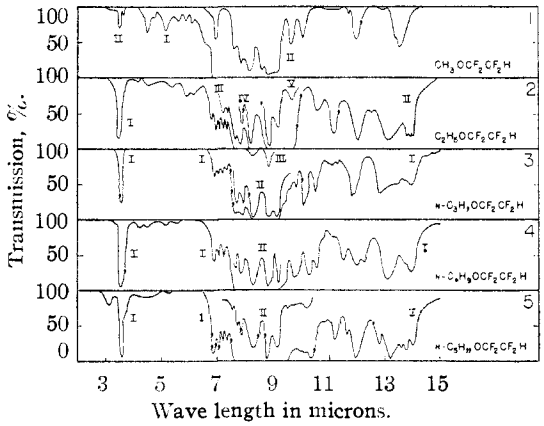

Fig. 1.—The infrared absorption spectrograms at room temperature in a 10 cm. gas cell at ind. pressure: (1) CH_3–O–CF_2–CF_2H; I in a 0.1-mm. liquid cell and II in the gas cell at 20 mm.; (2) C_2H_5–O–CF_2CF_2H; I in a 0.025-mm. liquid cell, II in the gas cell at 50 cm., III at 20 mm., IV at 7 mm., and V at 4 mm.; (3) n-C_3H_7–O–CF_2CF_2H; I in a gas cell at 47 mm., II at 19 mm., and III at 8 mm.; (4) n-C_4H_9–O–CF_2CF_2H; I in a gas cell at 39 mm., and II at 8 mm.; (5) n-C_5H_{11}–O–CF_2CF_2H; I in a 0.025-mm. liquid cell, and II in a gas cell at 19 mm.

The infrared absorption was measured using an automatic recording Perkin–Elmer infrared spectrometer, model 12B with beam chopper attached. The results are given in Fig. 1. Some qualitative assignments of the peaks are possible using the data of Barnes, *et al.*,[9] as previously shown for the infrared absorption spectra for R–O–CF_2–CCl_2H[10] and for R–O–CF_2CFClH.[11]

(9) R. B. Barnes, R. C. Gore, R. W. Stafford and V. Z. Williams, *Anal. Chem.*, **20**, 402 (1948).

(10) J. D. Park, C. M. Snow and J. R. Lacher, *ibid.*, **73**, 861 (1951).

(11) D. C. Smith, J. R. Nielsen, L. H. Berryman, H. H. Claasen and R. L. Hudson, NRL-3567, Final Report on Project NR019-120, Contract N7-onr-398-T.O.1.

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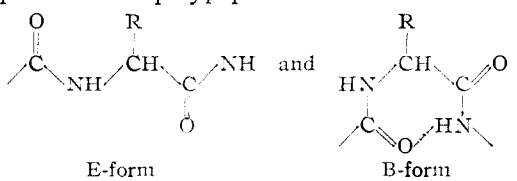
[CONTRIBUTION FROM THE CHEMICAL LABORATORY, FACULTY OF SCIENCE, TOKYO UNIVERSITY]

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

BY SAN-ICHIRO MIZUSHIMA, TAKEHIKO SHIMANOUCHI, MASAMICHI TSUBOI, TADAO SUGITA, EIZO KATO AND EIJI KONDO

Near infrared spectra of compounds containing two peptide bonds have been measured in dilute carbon tetrachloride solutions and for each of them there have been found two absorption peaks at 2.9 and at 3.0 μ arising from the N–H group in the free state and that involved in the *intramolecular* hydrogen bonding, respectively. This affords evidence for the existence of the bent molecular form of these substances and hence also for the existence of the bent configuration of certain amino-acid residues contained in the polypeptide chain.

A few years ago[1,2] two unit structures were proposed of a polypeptide chain based on the energy consideration of the intramolecular rotation about the C–C and C–N bonds as axes. From the combination of these unit structures various configurations of the polypeptide chain can be constructed: for example, EEE.... corresponding to the extended form of the chain proposed by Meyer and Mark in 1928,[3] BBB.... corresponding to a configuration of α-keratin proposed by us in 1947[1] and independently by Ambrose, *et al.*, in 1949,[4]



(1) T. Shimanouchi and S. Mizushima, *Kagaku*, **17**, 24, 52 (1947).

(2) T. Shimanouchi and S. Mizushima, *Bull. Chem. Soc. Japan*, **21**, 1 (1948). See *C. A.*, **43**, 8843 (1949).

(3) K. H. Meyer and H. Mark, *Ber.*, **61**, 1932 (1928).

(4) E. J. Ambrose and W. E. Hanby, *Nature*, **163**, 483 (1949); E. J. Ambrose, A. Elliot and R. S. Temple, *ibid.*, **163**, 859 (1949).

and BBEBBE.... corresponding to another configuration of α-keratin proposed by us in 1948.[2,5] Experimental evidence in support of our view has since been accumulated in our laboratory and in particular evidence for the existence of the bent form (B-form) has been afforded from the investigations of rather simple molecules. This paper presents the experimental results on the infrared absorption of some compounds with two peptide bonds ($CH_3CONHCHRCONHCH_3$ and $CH_3CONHCHRCONHC_6H_5$) which are considered to be capable of taking the bent form.

Materials

Acetylglycine Anilide.—Forty grams of acetylglycine chloride prepared according to the method of Max,[6] was added very slowly to 70 cc. of aniline cooled in water. After the initial vigorous reaction had ceased, the mixture was heated on a water-bath until all the solid mass was dissolved. The resulting reddish solution solidified on cooling. The solid mass was washed with ether, hydrochloric acid and water. This was then recrystallized three times from water and dried over phosphorus pentoxide. It melted at 191° in agreement with the value of Gränacher, *et al.*[7]

Anal. Calcd. for $C_{10}H_{12}O_2N_2$: C, 62.50; H, 6.25; N, 14.25. Found: C, 62.82; H, 6.57; N, 14.3.

Acetylglycine N-Methylamide.—Acetylglycine chloride prepared from 40 g. of acetylglycine was added to 40 cc. of anhydrous methylamine placed in a flask cooled with a mixture of Dry Ice and ethanol. After the initial reaction had ceased, the mixture was placed in a flask kept at room temperature and provided with a reflux condenser cooled with a mixture of Dry Ice and ethanol After removal of the remaining methylamine and drying over sulfuric acid for 3 days acetylglycine N-methylamide was extracted with 300 cc. of boiling ethyl acetate. The material was recrystallized three times from ethyl acetate and was used in the measurement; m.p. 158°.

Anal. Calcd. for $C_5H_{10}O_2N_2$: C, 46.15; H, 7.69; N, 21.5. Found: C, 46.05; H, 7.56; N, 21.3.

Acetyl-DL-leucine Anilide.—Twenty cc. of acetic anhydride was added to 2 g. of acetyl-L-leucine and the mixture was heated on a water-bath for 2 hours and allowed to stand overnight. The excess acetic anhydride and acetic acid was removed at first under reduced pressure (10–15 mm.) at 40–50°. The removal was repeated after adding xylene. To the remaining yellow oily substance (azlactone) was added 1.2 cc. of aniline to obtain the anilide. This was recrystallized from 70% ethanol and was used in the measurement; m.p. 223.5–225° (uncor.).

Anal. Calcd. for $C_{14}H_{20}O_2N_2$: C, 67.31; H, 8.12; N, 11.30. Found: C, 67.47; H, 8.52; N, 11.32.

Acetyl-DL-leucine N-Methylamide.—Two cc. of anhydrous methylamine was added to the previously described azlactone of acetylleucine dissolved in 20 cc. of ether cooled by a mixture of Dry Ice and ethanol. The reaction mixture was kept at room temperature for 2 days and the colorless crystalline precipitate was obtained. After recrystallization from ethyl acetate the sample was used in the measurement; m.p. 153.5–155.5° (uncor.).

Anal. Calcd. for $C_9H_{18}O_2N_2$: C, 58.04; H, 9.68; N, 15.04. Found: C, 57.82; H, 9.95; N, 14.9.

Experimental Results

The infrared absorption measurement on these substances in carbon tetrachloride solutions was carried out in the N–H frequency region from 2.7 to 3.2μ. The optical system of the monochromator consisted of two concave mirrors and a 60° quartz prism. This was used in conjunction with the thermocouple and galvanometer system.[8] The absorption vessel made of quartz and provided with a reflux condenser was about 10 cm. in length, so that we could make the measurement at very low concentrations (0.0001—0.0008 mole/l.). The temperature of the sample was read by a thermometer inserted into the absorption vessel.

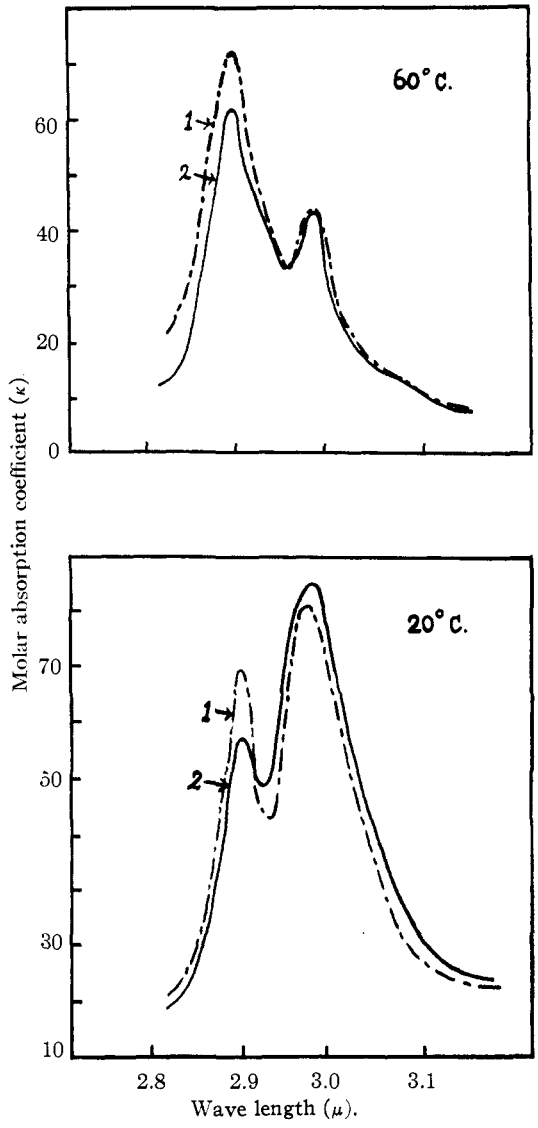


Fig. 1.—Near infrared absorption curves of acetylglycine N-methylamide in dilute carbon tetrachloride solution: (1) 0.000165, (2) 0.000315 mole/l.

Some of the experimental values of the molar absorption coefficient κ are shown in Fig. 1. These were obtained from the observed per cent. transmission I/I_0, concentration of the solution c, and the length of the absorption vessel d through the equation

$$I/I_0 = \exp(-\kappa cd) \qquad (1)$$

All these absorption curves show two peaks at about 2.9 and 3.0μ. This is also the case for all the other results obtained in the present experiment (see Table I).

It is well known that the 2.9μ peak arising from the vibration of free N–H group is very sharp. In the present measurement, however, the 2.9μ

(5) S. Mizushima, T. Shimanouchi, M. Tsuboi, T. Sugita and E. Kato, *Nature*, **164**, 918 (1949).

(6) J. Max, *Ann.* **369**, 286 (1909).

(7) C. Gränacher, V. Schelling and E. Schlatter, *Helv. Chim. Acta*, **8**, 873 (1925).

(8) See also M. Tsuboi, *Bull. Chem. Soc. Japan*, **22**, 215 (1949).

TABLE I

WAVE LENGTH λ OF ABSORPTION PEAK AND MOLAR ABSORPTION COEFFICIENT κ OF THE N–H BANDS OF CH_3-CONHCHRCONHCH$_3$ AND $CH_3CONHCHRCONHC_6H_5$ IN DILUTE CCl_4 SOLUTIONS

Substance	Temp., °C.	Concn., mole/l.	Free N–H λ in μ	Free N–H κ	Free N–H $\kappa_{cor.}$	Hydrogen-bonded N–H λ in μ	Hydrogen-bonded N–H κ
Acetylglycine	60	0.000165	2.90	73	90	2.98	44
N-methyl-	60	315	2.90	62	94	2.98	44
amide	60	525	2.90	55	94	2.98	40
	60	765	2.90	45	89	2.98	45
	20	165	2.90	58	85	2.98	82
	20	315	2.90	71	87	2.98	85
Acetylglycine	60	0.00014	2.90	86	99	3.00	72
anilide	60	285	2.90	67	100	3.00	77
	60	45	2.90	56	95	3.00	68
	20	14	2.90	77	95	3.00	109
	20	285	2.90	64	97	3.00	126
Acetyl-DL-	60	0.000555	2.90	50	82	2.98	44
leucine N-	20	.00032	2.90	53	75	2.98	64
methylamide							
Acetyl-DL-	60	.000155	2.90	59	61	3.00	36
leucine	20	.00016	2.92	42	52	3.02	74
Anilide							

peak was not observed to be much sharper as compared with that at 3.0μ (see Fig. 1). This is due to the fact that the slit width of our monochromator was made in the present case comparable to the width of the 2.9μ absorption band. In such a case the Lambert–Beer law would hold no more and the apparent value of the molar absorption coefficient κ would depend on the value of per cent. transmission I/I_0.

TABLE II

MOLAR ABSORPTION COEFFICIENT OF THE 2.9μ BAND OF ACETANILIDE IN DILUTE CCl_4 SOLUTIONS

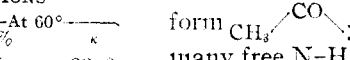
Concn., mole/l.	At 20° I/I_0, %	At 20° κ	At 60° I/I_0, %	At 60° κ
0.00032	84.5	23.0	84.5	23.0
.000615	77	18.6	76	19.4
.001195	65	15.8	65	15.8
.002455	51	12.0	48	12.9

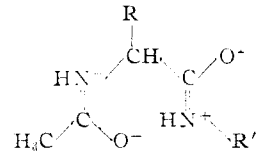
To determine the dependence of κ on I/I_0 we made a measurement on acetanilide which shows only the 2.9μ band of the free N–H group in dilute carbon tetrachloride solutions. The result is shown in Table II. Since this measurement was made at the same slit width as that for the compounds under consideration, we can correct the experimental values obtained for these compounds, using Table II and assuming the molar absorption coefficient of acetanilide as 23. These corrected values for the compounds are shown in the column under κ_{cor} of Table I.

As to the absorption bands at 3.0μ these are much broader than the 2.9μ bands and there is no need to make a correction of the molar absorption coefficient as in the case of the 2.9μ bands.

Discussion

From the experimental results obtained for N-methylacetamide and the other compounds investigated which contain –CO–NH– peptide bond, there can be no doubt that the absorption peak at 2.9μ arises from the N–H group in the free state and that the one at 3.0μ arises from the group involved in the hydrogen bonding.[9] Since these two absorption peaks were exhibited by all the compounds measured in the present experiment (Table I), at least some of their molecules should contain both of the free and the hydrogen-bonded N–H groups. The fact that κ of the 3.0μ band as well as κ_{cor} of the 2.9μ band are practically independent of the concentration (Table I) would indicate that the hydrogen bond is *intramolecular*. This conclusion is confirmed by the fact that N-methylacetamide, acetanilide, etc., which form only the *intermolecular* hydrogen bond show practically no absorption due to the hydrogen-bonded N–H groups in such dilute solutions. Thus our experiment leaves no doubt that the bent or folded form exists for each of the compounds under consideration. This would also afford evidence for the existence of the bent form in certain aminoacid residues contained in the polypeptide chain.

Besides the important conclusion stated above we can derive two others from the experimental result shown in Table I and in Fig. 1. The first one is concerned with the absorption intensity of the free N–H band and the second one with the temperature dependence of the equilibrium ratio of the extended and the bent forms. As stated above we determined the value of κ_{cor} of Table I, assuming κ of acetanilide as 23. Referring to the same standard, we found κ of N-methylacetamide in the free state as 18–20. If, therefore, the free N–H groups of the compounds under consideration have the same bond moment as those of acetanilide and N-methylacetamide, the values of κ for the 2.9μ band should not be much different from 20, in so far as their molecules are in the bent form. And even if all the molecules were in the extended form  with twice as many free N–H groups as in case of the bent form, the value of κ should not much exceed 40. Therefore, the observed values of κ amounting to 70~100 (Table I) indicate that the bond moment of the free N–H group of the compounds with two peptide bonds is greater than those with one peptide bond stated above. This would be explained by considering that the contribution of the resonance structure



for the bent form was made larger by the intramolecular hydrogen bonding.

As to the equilibrium ratio of the extended form to the bent form this can be calculated from the intensity ratio of the 2.9μ band to the 3.0μ band. As shown in Fig. 1 this intensity ratio observed at 60° is considerably different from that observed at 20°. Hence it follows that the equilibrium ratio of these two isomers changes greatly with a temperature change of only 40°.

(9) See *e.g.*, S. Mizushima, T. Shimanouchi, S. Nagakura, K. Kuratani, M. Tsuboi, H. Baba and O. Fujioka, THIS JOURNAL, **72**, 3490 (1950).

We shall next estimate the differences of energy and entropy between these two molecular forms from the temperature dependence of the 3.0μ absorption of a very dilute solution. Let the energy and the entropy of the extended form be larger by ΔE and ΔS, respectively, than those of the bent form and let κ' and κ be the molar absorption coefficient of the 3.0μ band for the pure bent form and for the mixture of the bent and extended forms, respectively. Then we have

$$\kappa = (\kappa')/\left(1 + \exp\left(\frac{\Delta S}{R} - \frac{\Delta E}{RT}\right)\right)$$

since the 3.0μ band arises only from the bent form in a very dilute solution. We cannot uniquely determine the values of ΔE and ΔS from this equation but we can show from the observed value of κ that the value of ΔS cannot be smaller than 15 e.u. If, for example, we take the reasonable values of ΔE from 3.5 to 6.0 kcal./mole, the values of ΔS are calculated as

ΔE(kcal./mole)	3.5	4.0	5.0	6.0
ΔS(e.u.)	16	15	17	19

This large value of the entropy difference would be partly explained by considering that the freedom of the internal rotation of the extended form is much greater than that of the bent form.

We know that proteins are denatured by the elevation of temperature with a large increase in entropy.[10] It is, therefore, quite interesting that the entropy increase of such a magnitude was observed for such compounds as may be considered to be structural units of a polypeptide chain.

It should be realized that the present results were all obtained in carbon tetrachloride solutions. In aqueous solutions of high dielectric constant where the *intramolecular* hydrogen bonding will be enormously suppressed, we may expect quite a different behavior with a tendency of forming more extended forms. There is no inherent inconsistency, therefore, between our results and those in the dielectric increments of aqueous solutions of some peptides.[11]

Acknowledgment.—We wish to express our gratitude to Prof. J. Wyman of Harvard University for his kind discussions during his stay in Tokyo. Our thanks are also due to Dr. H. Oeda for the sample of leucine.

(10) Neurath, Greenstein, Putnam and Erickson, *Chem. Revs.*, **34**, 157 (1944); M. Kunitz, *J. Gen. Physiol.*, **32**, 241 (1948).

(11) J. Wyman and T. L. McMeekin, THIS JOURNAL, **55**, 908 (1933).

BUNKYOKU, TOKYO, JAPAN RECEIVED JULY 31, 1950

[CONTRIBUTION FROM THE SCHOOL OF CHEMICAL ENGINEERING, TULANE UNIVERSITY, AND THE DEPARTMENT OF CHEMISTRY LOYOLA UNIVERSITY]

Thermal Decomposition of Guanidine Chromate and Dichromate

BY CHUK-CHING MA

Pure guanidine chromate and dichromate may be prepared from guanidine carbonate and chromic acid. When these products are heated, they decompose in an orderly manner to yield cyanamide, nitrogen, ammonia, water and Cr_2O_3. A major portion of the cyanamide formed polymerizes to dicyandiamide which in turn hydrolyzes to guanidine, ammonia and carbon dioxide. In addition to these products, ammonium carbonate and traces of melamine are also found. The residual greenish chromic oxide produces an accelerative effect on the process of hydrolysis. In the sealed tube experiments, guanidine dichromate explodes violently when heated. A less powerful effect results when guanidine chromate is heated. These indicate that the chromates of guanidine may be utilized as explosives. From the experimental results, it is concluded that the chromate ion does not oxidize the guanidine molecule as a whole, but that the guanidine rearranges into cyanamide and ammonia followed by the oxidation of ammonia by the chromate ion.

Introduction

When either ammonium chromate or dichromate is heated, or is touched with a flame or spark, a self-sustaining reaction sets in whereby the chromate ion oxidizes the ammonium ion, thereby producing chromic oxide, nitrogen and water. The same reaction does not occur in a boiling aqueous solution. The dry reaction has the unusual feature that the conditions under which it occurs are such that the chromate ion is a peculiarly effective oxidizing agent. It seemed probable that guanidine chromate or dichromate might behave similarly when heated and might yield interesting oxidation products of guanidine. However, during the course of the present investigation, it is found that the guanidine portion of the molecule rearranges to form ammonia and cyanamide, and that the ammonia thus obtained reacts with the chromate ion in the same manner as when it is already combined with it. The purpose of this study is, therefore, to formulate a series of reactions based on the available data obtained from the thermal decomposition of guanidine chromate and dichromate.

Experimental

Preparation of Guanidine Chromate and Dichromate.—The guanidine chromate and dichromate used in all the experiments were prepared from the interaction of guanidine carbonate and a calculated amount of chromic acid. The resulting salts were crystallized from aqueous solutions at 0° since they are extremely soluble in water, especially the dichromate, at room temperature. The products thus prepared were further purified by recrystallization from 50% alcohol solutions at 0°.

Anal. Calcd. for $[NH_2C(NH)NH_2]_2H_2CrO_4$: N, 35.59; Cr, 22.02. Found: N, 35.51; Cr, 21.96. Calcd. for $[NH_2C(NH)NH_2]_2H_2Cr_2O_7$: N, 25.01; Cr, 30.94. Found: N, 24.97; Cr, 30.87.

Decomposition of Guanidine Chromate (a) Qualitative Test.—Five grams of guanidine chromate was placed in an 8" test-tube to which an air-cooled condenser was connected vertically. The test-tube was heated in an oil-bath at 160–270°. As soon as the temperature reached approximately 160°, a strong ammonia odor was noticed. Cyanamide and dicyandiamide were also identified at this stage. When the temperature rose above 190°, water vapor came off and a white crystalline sublimate condensed on the upper part of the test-tube. Under a microscope this sublimate, soluble