TABLE IV

IDENTIFICATION OF OLEFINIC PRODUCTS

Olefin	Aldehyde	B.p. °C.	Mm.	$n^{25}D$	2,4-Dinitrophenylhydrazone, m.p., °
1-Nonene	Formaldehyde[a]				165–166[b]
	Octanal	100–101	700	1.4278	106–107[c]
1-Phenyl-1-nonene	Benzaldehyde	100–102	700		233[d]
	Octanal[e]				106–107
1,3-Diphenylpropene	Benzaldehyde	110	751		234
	Phenylacetaldehyde	138–140	752		120–121[f]
1,4-Diphenyl-1-butene	Benzaldehyde[g]	M.p. 45–46			236–237
	Hydrocinnamaldehyde[g]				144–145[h]
3-Phenyl-1-hendecene	Formaldehyde[a]				162–163
	2-Phenylcapraldehyde	101–102	733	1.4111	122–123[i]
4-Ethyl-2-octene	Acetaldehyde[a]				145–146[j]
	2-Ethylcaproaldehyde[e]				119–120[k]
4-Phenyl-2-octene	Acetaldehyde[a]				138–139[l]
	2-Phenylcaproaldehyde[m]	120–121	742	1.3979	107–108[n]
2,2,5-Trimethyl-3-tridecene	Pivalaldehyde[o]	81–82	744	1.3709	103–104[p]
	2-Methyldecanal[q]	119–120	744	1.4205	63–64[r]
5,5-Dimethyl-4-phenyl-2-hexene	Acetaldehyde[a]				146–147[s]
	2-Phenyl-2,2-dimethylbutanal	115–118	744		70–71[t]
1,1-Diphenyl-2-dodecene	Phenylacetaldehyde	179–181	748		121–122[u]
	2-Phenylcapraldehyde[e]				85–86[v]

[a] Compound not isolated but allowed to pass into 2,4-dinitrophenylhydrazine reagent trap during ozonolysis. [b] Reported m.p. 166° by S. M. McElvain, "The Characterization of Organic Compounds," The Macmillan Co., New York, N. Y., 1953, p. 207. [c] Reported m.p. 107°, footnote *b*. [d] Literature m.p. 237° by R. L. Shriner and R. C. Fuson, "Identification of Organic Compounds," John Wiley and Sons, Inc., New York, N. Y., 1948, p. 229. [e] Found in aqueous portion of ozonolysis mixture. [f] Reported m.p. 121°, footnote *d*; mixed m.p. with an authentic specimen of phenylacetaldehyde hydrazone was not lowered. [g] Reported m.p. 47° by "Handbook of Chemistry and Physics," Chemical Rubber Co., Cleveland, Ohio, 1952. [h] Reported m.p. 149°, footnote *d*. [i] *Anal.* Calcd. for $C_{22}H_{28}O_4N_4$: N, 13.60. Found: N, 13.77. [j] M.p. reported 147°, footnote *d*. [k] Literature m.p. 114–115° by L. R. Drake and C. S. Marvel, *J. Org. Chem.*, **2**, 387 (1937) and 121°, footnote *d*. *Anal.* Calcd. for $C_{14}H_{20}O_4N_4$: N, 18.18. Found: N, 18.17. [l] *Anal.* Calcd. for $C_8H_8O_4N_4$: N, 25.00. Found: N, 25.25. [m] Observed $n^{20}D$ 1.3979, d^{20}_4 0.879. [n] *Anal.* Calcd. for $C_{18}H_{20}O_4N_4$: N, 15.73. Found: N, 15.80. [o] Reported b.p. 75°, d^{17} 0.793, footnote *g*; observed $n^{20}D$ 1.3709, d^{23} 0.817. [p] *Anal.* Calcd. for $C_{11}H_{14}O_4N_4$: N, 21.13. Found: N, 21.87. [q] Observed d^{23}_4 0.8948, $n^{20}D$ 1.4205. [r] *Anal.* Calcd. for $C_{17}H_{26}O_4N_4$: N, 16.00. Found: N, 16.44. [s] *Anal.* Calcd. for $C_8H_8O_4N_4$: N, 25.00. Found: N, 25.16. [t] *Anal.* Calcd. for $C_{18}H_{20}O_4N_4$: N, 15.73. Found: N, 15.80. [u] *Anal.* Calcd. for $C_{14}H_{12}O_4N_4$: N, 18.66. Found: N, 18.80. [v] *Anal.* Calcd. for $C_{22}H_{28}O_4N_4$: N, 13.59. Found: N, 13.58.

ozone. The procedure followed for decomposition of the ozonides and isolation of the ozonolysis products was similar to that reported in the third paper of this series.[3]

Physical constants of the ozonolysis products and their derivatives are described in Table IV.

NASHVILLE 8, TENNESSEE

[CONTRIBUTION FROM THE DEPARTMENT OF BIOCHEMISTRY, THE CHICAGO MEDICAL SCHOOL]

Ultraviolet Absorption Spectra of Peptides. III. N,N-Dialkylamides Including Polyvinylpyrrolidone[1]

BY LEO J. SAIDEL

RECEIVED JANUARY 10, 1955

The ultraviolet absorption spectra of aqueous solutions of the N,N-dialkylamides: N,N-dimethylacetamide, N,N-diethylacetamide, acetyl-L-proline and glycyl-L-proline in the various ionic forms, 1-ethyl-2-pyrrolidone, and polyvinylpyrrolidone (PVP) in the region from 200 to 240 mμ all suggest an absorption maximum slightly below 200 mμ. The exact location and intensity of this band depends to some extent upon the nature of the substituents on the amide group. Contrary to the findings of others, the spectrum of PVP does not exhibit maxima above 200 mμ and does not vary appreciably with concentration or *p*H (6.2–12.2). Below 224 mμ, three preparations of PVP of markedly different molecular weight (25,000–251,000) exhibited practically identical spectra, which were lower than that of 1-ethyl-2-pyrrolidone.

Introduction

If the so-called end absorption of proteins is to yield information about protein structure, it is essential to collect data on the spectra of the various kinds of amide links, which occur in proteins, because the amide links contribute a major component of the end absorption. Upon spectroscopic examination in the 200 to 240 mμ region of a large number of compounds containing a single monoalkyl substituted amide link,[2] and of glycine peptides containing more than one substituted amide link,[3] such factors as the presence and ionic state of the carboxyl and amino groups on either side of the peptide link, and the presence of other peptide links within

(1) Presented at the 126th Meeting of the American Chemical Society, New York, N. Y., September 12, 1954.

(2) L. J. Saidel, *Arch. Biochem. Biophys.*, **54**, 184 (1955).

(3) L. J. Saidel, *ibid.*, **45**, in press (1955).

the peptide chain were found to have an appreciable effect upon the spectrum of an individual peptide link. These studies are extended here to peptides and similar compounds containing the dialkylamide link. Because of its superficial resemblance to proteins and because it has been reported[4] to exhibit absorption maxima around 215 mμ, polyvinylpyrrolidone (PVP) has been included in this study.

Experimental

All absorption data were obtained with a Beckman DU spectrophotometer. The procedures, which have been found to give the best results in this region of the spectrum, have been described.[2,5,6] In order to obtain satisfactory absorption data with 0.02% solutions of PVP and with any solutions at *p*H 12, it was necessary to use a quartz cell of approximately 0.02 cm. path length.[5,7] All spectra were obtained in aqueous solution at various *p*H values.

The author is grateful to Dr. O. K. Behrens of Eli Lilly and Company for a sample of L-prolylglycine·H_2O and to General Aniline and Film Corporation for a sample of 1-ethyl-2-pyrrolidone (b.p. 104° at 20 mm.) and three samples of polyvinylpyrrolidone (K-30, K-60 and K-90) of different molecular weights. Acetyl-L-proline·H_2O was synthesized by the method of du Vigneaud and Meyer[8] and gave a satisfactory melting point. The other compounds were obtained commercially. The N,N-dimethylacetamide and 1-ethyl-2-pyrrolidone were distilled before use. All samples were analyzed by titration in water with NaOH, by electrometric titration in glacial acetic acid with $HClO_4$ or by semimicro-Kjeldahl N determination. All gave analyses of 100% ± 1% of the theoretical values except N,N-diethylacetamide (96.4% of theory), and 1-ethyl-2-pyrrolidone (95.6% of theory), which at the time of spectroscopic examination, gave the indicated analyses. The absorption data for these compounds were corrected in each instance on the assumption that the deviation from the theoretical analysis was caused by imperfect drying. The nitrogen analyses of the PVP samples were as follows: K-30, 12.03% N; K-60, 11.70% N; and K-90, 11.85% N. From the formula of Miller and Hamm relating molecular weight as determined by the sedimentation velocity-diffusion method to the intrinsic viscosity[9] and from the manufacturer's estimate of the intrinsic viscosity of these preparations as indicated by the K value,[10] the approximate average molecular weights of these preparations were found to be 25,000, 107,000 and 251,000, respectively. However, the manufacturer states that the K-30 preparation has an average molecular weight, determined osmometrically, of 40,000.[10]

Results and Discussion

Recent absorption data of Hunt and Simpson[11] with formamide vapor indicate that unsubstituted amides exhibit a strong band with maximum absorption around 171.7 mμ. Monoalkyl substitution on the amide N shifts this maximum to about 185 mμ.[12] Dialkyl substitution produces a greater red shift of this band so that the maximum falls very close to 200 mμ. This is best illustrated in Fig. 1 by the spectrogram of diethylacetamide (curve B) with a molar absorptivity (ϵ)[13] at 200 mμ of 9100 (*cf.* spectrogram of methylacetamide, curve L). Unfortunately, the wave length of maximum absorption of these spectra cannot quite be reached with the Beckman spectrophotometer, but that it is really close to 200 mμ is indicated by the work of Hunt and Simpson,[11] who found that dimethylformamide vapor exhibits an intense broad band with maximum absorption at 197.4 mμ and with an ϵ value of 8700. These authors also found weak bands in the 215–225 mμ region, which is the approximate location of a weak amide band exhibited by aqueous solutions of acetamide[14] and glycylglycine at *p*H 1[2] (*cf.* also curve E). However,

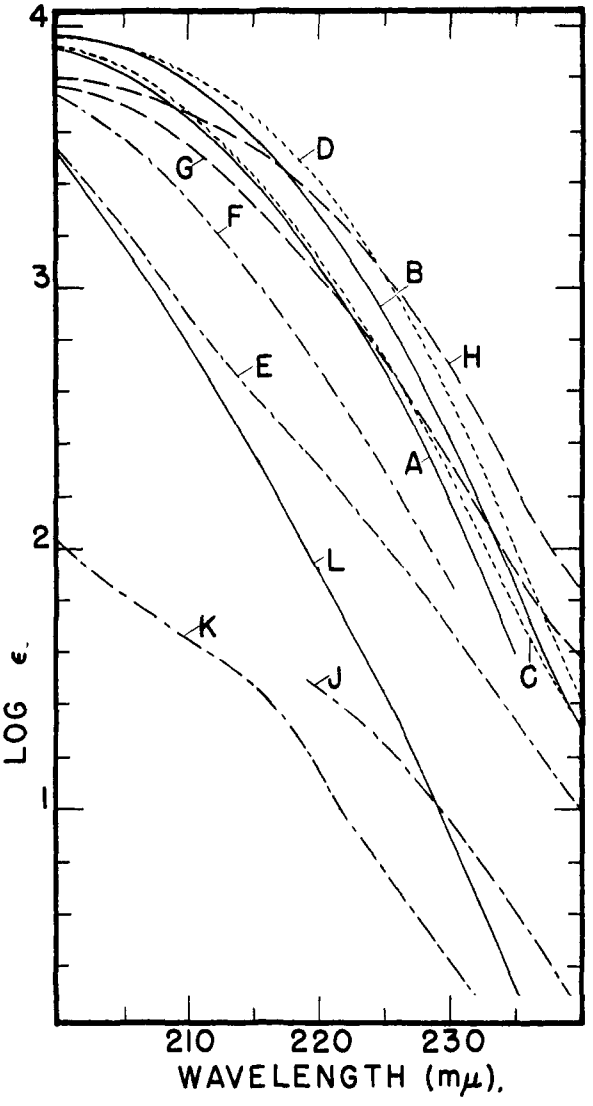

Fig. 1.—Spectrograms of various dialkylamides compared with those of related compounds: A, N,N-diethylacetamide at *p*H 6.1–6.2 in water; B, N,N-diethylacetamide at *p*H 5.4–5.7 in water; C and D, acetyl-L-proline at *p*H 1.0 and *p*H 7.0, respectively; E and F, L-prolylglycine at *p*H 1.0 and in water, respectively; G and H, glycyl-L-proline at *p*H 1.0 and *p*H 6.4–6.5 in water, respectively; J, proline·HCl in water (Ley and Arends)[14]; K, L-proline in water (Ley and Arends)[14]; L, N-methylacetamide at *p*H 4.3–5.6 in water.[2]

(4) G. Oster and E. H. Immergut, THIS JOURNAL, **76**, 1393 (1954).

(5) L. J. Saidel, A. R. Goldfarb and W. B. Kalt, *Science*, **113**, 683 (1951).

(6) L. J. Saidel, A. R. Goldfarb and S. Waldman, *J. Biol. Chem.*, **197**, 285 (1952).

(7) *Cf.* J. M. Vandenbelt, C. Henrich and S. L. Bash, *Science*, **114**, 576 (1951).

(8) V. du Vigneaud and C. E. Meyer, *J. Biol. Chem.*, **98**, 295 (1932).

(9) L. E. Miller and F. A. Hamm, *J. Phys. Chem.*, **57**, 110 (1953).

(10) "Plasdone" Polyvinylpyrrolidone, compiled and published by General Aniline and Film Corporation, New York, 1951.

(11) H. D. Hunt and W. T. Simpson, THIS JOURNAL, **75**, 4540 (1953).

(12) J. S. Ham and J. R. Platt, *J. Chem. Phys.*, **20**, 335 (1952).

(13) The nomenclature suggested in Report No. 6 of the Joint Committee on Nomenclature in Applied Spectroscopy is used. H. K. Hughes, *Anal. Chem.*, **24**, 1349 (1952).

(14) H. Ley and B. Arends, *Z. physik. Chem.*, **B17**, 177 (1932).

the spectrograms of aqueous solutions of none of the dialkylamides presented here show any evidence of such bands.

Proline derivatives in which the amino group of proline is acylated may be considered dialkyl substituted amides. Accordingly, the spectra of these compounds exhibit the expected red shift with resulting high absorption in the region of interest (curves C, D, G and H). That the pyrrolidine ring *per se* does not produce this type of spectrum is indicated in Fig. 1 by the spectrograms of proline[14,6] (curves J and K) and L-prolylglycine (curves E and F), neither of which compound has its pyrrolidine N acylated. Thus, at *p*H 1 the ϵ value for glycyl-L-proline is over 5 times greater than that for L-prolylglycine from about 212 mμ to 225 mμ.[15]

The spectra of these N-acylated proline compounds may be of considerable interest in the study of protein spectra. Dialkylamide groups probably cannot produce a detectable band; but they may make a quantitatively important contribution to certain protein spectra and this may reflect structural elements.

As with all other peptides except those involving tryptophan, the spectra of these compounds undergo a hyperchromic change in this region when a proton is removed from the carboxyl group alpha to the peptide link.[2] Moreover, these spectrograms (*cf.* curve C with D, and G with H) appear to indicate that this hyperchromic change may be attributable to a bathochromic shift of the peptide band. Somewhat different from most dipeptides (including L-prolylglycine), which show some hypochromic change in spectrum when a proton is removed from the ammonium group alpha to the peptide link,[2] glycyl-L-proline shows a very slight hyperchromic change when similarly treated.

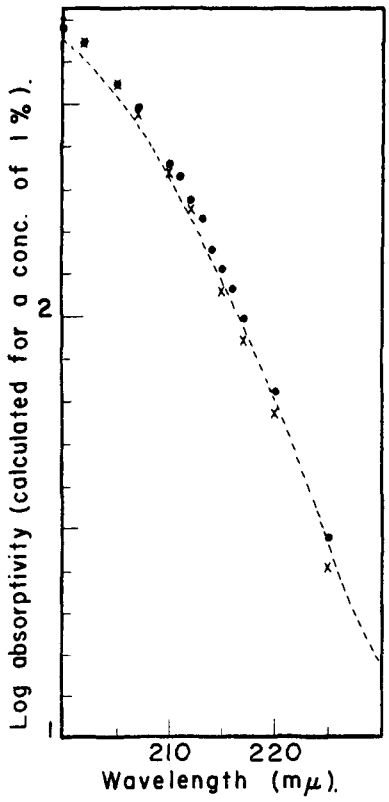

Fig. 2.—Spectrograms of PVP (K-30) at various concentrations (*c*), *p*H values and cell depths (*d*): (-----), c = 0.00103–0.0111% in water, *p*H 6.2–6.3, d = 1 cm.; (. . . . .), c = 0.0206% in water, *p*H 6.2, d = 0.0191 cm.; (xxxx), c = 0.0204% in 0.02 *N* NaOH, *p*H 12.2, d = 0.0191 cm.

(15) The change in spectrum produced on reversing the sequence of residues here is much greater than that observed with other kinds of dipeptides.[2] *Cf.* also M. A. Magill, R. E. Steiger and A. J. Allen, *Biochem. J.*, **31**, 188 (1937).

PVP and 1-ethyl-2-pyrrolidone contain the dialkylamide group (in a ring structure) and may be expected to have spectral properties similar to the other dialkylamides. Thus the recent report of Oster and Immergut[4] that the spectra of solutions of PVP and 1-ethyl-2-pyrrolidone are very sensitive to *p*H extremes is of considerable interest, for, if the dialkylamide links in the proteins behave similarly, they should be considered as one of the causes of the increased absorption at low wave lengths observed on alkalinization of proteins. These authors reported about a tenfold increase in absorption of PVP solutions at 213 mμ on increasing the *p*H from 5 to 12. They also reported that the spectra of these solutions were concentration dependent, exhibiting no maximum above 210 mμ at a concentration of 0.001%, but exhibiting a series of five narrow bands in the region from 212 to 217 mμ at a concentration of 0.02%.

When the absorption of the samples used (both solvent and solution) was kept within safe limits by decreasing the cell depth,[7] no appreciable alteration of the PVP spectrum was observed in our laboratory with change of *p*H (6.2–12.2) and concentration (Fig. 2).[16] It is very likely that the discrepancies between the results obtained in the two laboratories are in large part attributable to a higher percentage of unreacted vinyl groups in the sample of PVP used by Oster and Immergut. However, it should be appreciated that absorbance readings as high as those reported by these authors are subject to considerable error because of the large amount of stray radiation usually present at low wave lengths.[5]

In the analysis of protein spectra in terms of constituent peptide spectra, one of the key problems concerns the change, if any, of the spectrum of an individual peptide link when it is incorporated in a protein. The solution to this problem is not likely to be a simple one, but some light may come from comparing the spectrum of the polyamide, PVP, with that of the corresponding monoamide, 1-ethyl-2-pyrrolidone as is done in Fig. 3.

The plateau in the spectrograms of the polymers around 235 mμ is of questionable significance because as little as 0.7 to 1.1% unreacted vinyl groups could cause the observed differences in absorption at 235 mμ, the wave length of maximum absorption for vinylpyrrolidone. The manufacturer states that the unreacted monomer content of different preparations of PVP varies from about 0.2 to 0.9%.[10]

Below 224 mμ the three preparations of PVP,

(16) The PVP examined in both laboratories came from the same manufacturer and was designated by the manufacturer to be of the same average molecular weight.

though of markedly different average molecular weight, have practically identical spectrograms, which are appreciably lower than that of 1-ethyl-2-pyrrolidone.

It is clear then that building up a long chain polymer from this particular dialkylamide does result in diminished average absorption per amide link on the long wave length shoulder of the amide band. It also appears that beyond a certain point there is no further decrease in low wave length absorption with increase in molecular size.[17] Unfortunately, the spectra of the simpler intermediate polymers are not available.

However, extension of the neutral glycine peptide chain (up to hexapeptide) results in a similar change in the average absorption per peptide link.[3] And because all of the intermediates are available, it was possible there to offer a reasonable explanation of such results on the basis of an unequal distribution of absorption among the different peptide links in a given chain. The unequal distribution was attributed to the different structural environment surrounding each peptide link. The two systems are not entirely analogous, but it is possible that a similar explanation could be offered here.

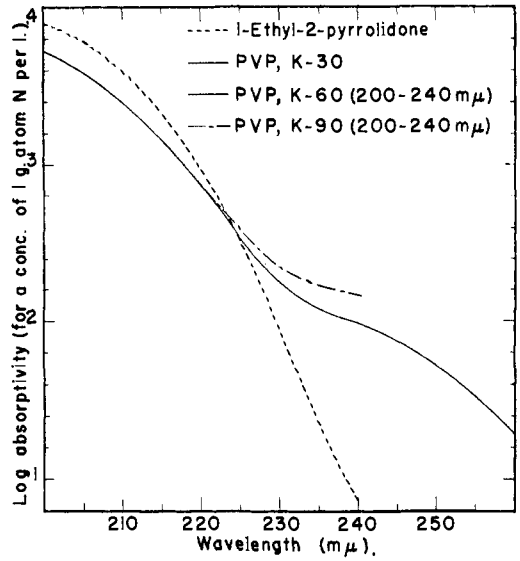

Fig. 3.—Spectrograms of PVP compared with that of 1-ethyl-2-pyrrolidone.

Acknowledgment.—The author is indebted to Miss Mildred Mosby for the N determinations.

CHICAGO 12, ILLINOIS

(17) It is possible that there is actually a progressive decrease in chromophoric absorption; but beyond a certain molecular size, this decrease is exactly compensated by an increase in Tyndall scattering. On the other hand, it may be more reasonable to conclude that Tyndall scattering by molecules of this size does not add significantly to the intense chromophoric absorption found at low wave lengths.

[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, IOWA STATE COLLEGE]

Effect of Temperature on the Intrinsic Viscosity and Optical Rotation of Bovine Plasma Albumin in Acid Media[1]

BY JOSEPH F. FOSTER[2a] AND JEN TSI YANG[2b]

RECEIVED OCTOBER 11, 1954

It has been shown previously that bovine serum albumin undergoes a rapid reversible structural alteration, at room temperature, upon reducing the *p*H of the aqueous solution below approximately 4.0. This alteration, which has been tentatively interpreted by the authors as an all-or-none transition from a compact to an expanded form, is accompanied by a marked increase in intrinsic viscosity and in levorotation. In an attempt to evaluate the thermodynamic parameters studies have now been carried out over the temperature range 2 to 80°. The expansion equilibrium is favored by elevated temperature but the effect is not marked and interpretation of the results is complicated by two general features. In the first place the temperature dependence, which is fairly normal above 20°, disappears below that temperature. Secondly, above about 40° a slow, irreversible alteration takes place. This denaturation is characterized by a marked *decrease* in intrinsic viscosity from that of the reversibly swollen form, but the optical rotation remains substantially constant. That this change is not due primarily to hydrolysis or to aggregation is indicated by preliminary light scattering studies. The kinetics of the process are not simple first-order. It is suggested that the irreversible alteration proceeds primarily through the compact form, so that the low *p*H expanded form may be regarded as a reversibly "protected" structure.

There is now considerable evidence to indicate that bovine plasma albumin (BPA) is subject to drastic reversible alterations in configuration upon change of *p*H, particularly in acid solution. The authors have shown[3] that there is marked increase in intrinsic viscosity and a parallel increase in optical rotation upon lowering the *p*H from the isoelectric point to approximately 2.0. The failure of such solutions to yield flow birefringence was taken as evidence that the alteration is essentially an isotropic expansion and the magnitude of the viscosity increase is such as to indicate an increase of some twenty-fold in effective hydrodynamic volume of the protein molecule.

The present study was undertaken in the hope of clarifying further the mechanism of this reversible alteration. In particular it was desired to ascertain the temperature dependence and attempt to evaluate the thermodynamic parameters characterizing the equilibrium which was postulated to be an all-or-none transition from one to another discrete form of the protein. Experimental measurements have been made of the effect of temperature, at various *p*H values acid to the isoelectric point, on the

(1) Journal Paper Number J-2595 of the Iowa Agricultural Experiment Station, Ames, Iowa. Proj. 1223. This research was carried out under contract Nonr-803 (00) between the Office of Naval Research and Iowa State College. Presented before the Division of Biological Chemistry of the American Chemical Society, September, 1954.

(2) (a) Department of Chemistry, Purdue University, Lafayette, Indiana; (b) Department of Chemistry, Harvard University, Cambridge, Massachusetts.

(3) J. T. Yang and J. F. Foster, THIS JOURNAL, **76**, 1588 (1954).