quency ratio and consequently cannot affect the value of the actual temperature-independent factor.

Johnston and co-workers[6] have studied the temperature dependence of kinetic isotope effects and shown that at very high temperatures (>1000°K. for his hydrogen isotope effects) ln (k/k') becomes linear in $1/T^2$ with an infinite-temperature intercept of ln $(\nu_L^*/\nu_L^{*\prime})$. However, at the intermediate values of $h\nu/\mathbf{k}T$ common in most mechanistic work, plots of ln (k/k') *vs.* $1/T$, although they may appear to be linear over short temperature ranges, have infinite-temperature intercepts which are complex quantities not easily related to theory. Bigeleisen[7] has discussed this phenomenon with respect to equilibrium isotope effects.

Equation 5 may be used generally in combination with an estimate of the temperature-dependent factor in order to calculate an isotope effect at a single temperature. Omission of low bending frequencies for the transition state is then justified because their contribution to τ_* is slightly more than offset by their temperature-dependent contribution, only slightly favoring an inverse effect when they are included. On the other hand, for more rigorous calculations or when bending frequencies are high, the complete contribution τ_* of the transition state to the temperature-independent factor can be calculated by multiplying eq. 5 by eq. 6 for the bending motions ν_3 and ν_4

$$\frac{\nu_3\nu_4}{\nu'_3\nu'_4} = \frac{\mu_{12}r_2^2 + \mu_{23}r_1^2 + 2\mu_2 r_1 r_2}{\mu'_{12}r_2^2 + \mu'_{23}r_1^2 + 2\mu'_2 r_1 r_2} = \frac{\mu_{23} + p\mu_{12} + 2p^{1/2}\mu_2}{\mu'_{23} + p\mu'_{12} + 2p^{1/2}\mu'_2} \qquad (6)$$

where p is now r_2^2/r_1^2. The result is not eq. 2 but is its square times eq. 5. When bending frequencies are included in τ_*, care must be taken that the frequencies assigned in calculating the temperature-dependent factor satisfy eq. 5 and 6. If force constants are assumed and the frequencies are *calculated* by the usual methods, consistent results are obtained.[6,8]

(6) H. S. Johnston and E. Tschuikow-Roux, *J. Chem. Phys.*, **36**, 463 (1962); T. E. Sharp and H. S. Johnston, *ibid.*, **37**, 1541 (1962); G. Chiltz, R. Eckling, P. Goldfinger, G. Huybrechts, H. S. Johnston, L. Meyers and G. Verbeke, *ibid.*, **38**, 1053 (1963).

(7) J. Bigeleisen, *Proc. Sec. U. N. Intern. Conf. Peaceful Uses At. Energy*, **4**, 480 (1958).

(8) J. Bigeleisen and M. Wolfsberg, *J. Chem. Phys.*, **23**, 1535 (1955); J. Bigeleisen, F. S. Klein, R. E. Weston, Jr., and M. Wolfsberg, *ibid.*, **30**, 1340 (1959); H. S. Johnston, *Advan. Chem. Phys.*, **3**, 131 (1961); F. H. Westheimer, *Chem. Rev.*, **61**, 265 (1961).

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Protonation of Amide Groups of Polypeptides by "Helix-Breaking" Solvents

Sir:

It has been observed recently[1] that the formation of an amide hydrogen bond may be followed in a model system, N-methylacetamide in a variety of solvents, by the shift in the overtone of the NH stretching vibration; the absorption maximum in the near-infrared moves from 1.47–1.48 μ when the amide is in the unassociated form to a double-peaked band at 1.53–1.57 μ upon the formation of an amide hydrogen bond. Such shifts would also be expected to occur in connection with the transition of polypeptides from the random coil to the helical state. We have therefore undertaken a study of the spectral properties of several synthetic polyamino acids under conditions where changes in the physical properties of these polymers have been interpreted in terms of a helix-coil transformation.

One procedure for producing these transformations employs solvent mixtures containing varying proportions of apolar and polar components—for example, chloroform and an organic acid. The effectiveness of the acids, dichloroacetic and trifluoroacetic, in producing transitions in optical, rotatory and viscosity parameters of solutions of polypeptides in these mixed solvents[2-4] has been attributed to the ability of the acids to break peptide hydrogen bonds primarily by forming their own competing hydrogen bonds with the peptide C=O and NH groups.[4-6] On this basis, one would expect to observe corresponding changes in the position of the NH absorption peak as it shifts from

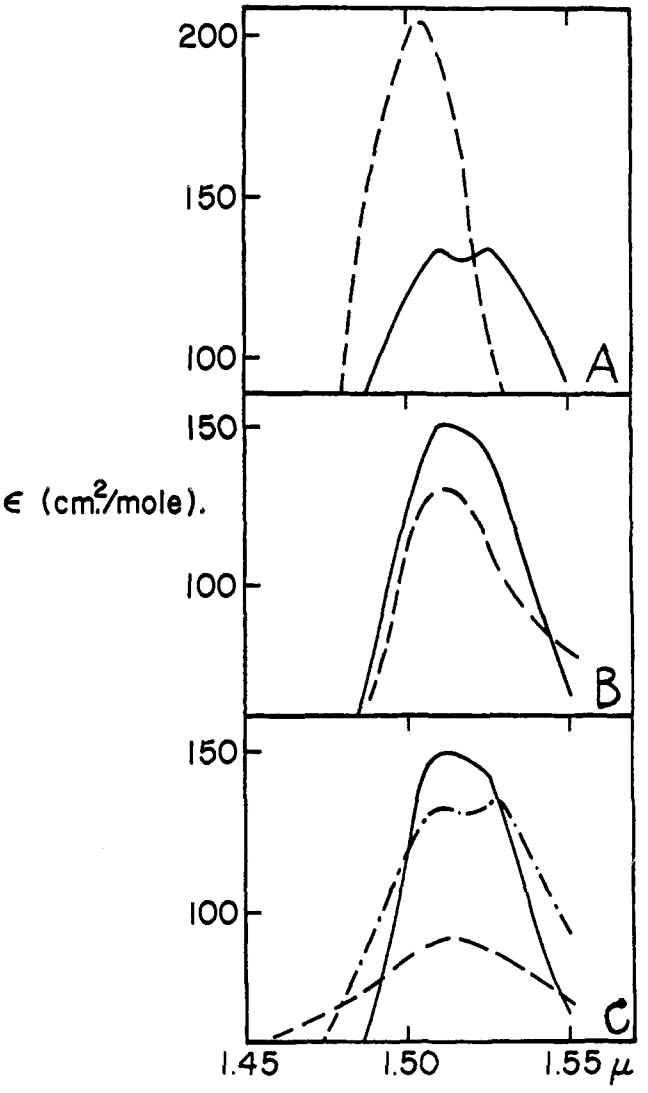


Fig. 1.—Spectra in acidic solvents: A, in CF_3CO_2H: - - - poly-L-alanine (0.442 *M*); ——— N-methylacetamide (0.466 *M*). B, in 8.4 *M* $HClO_4$, 2.5 *M* $C_4H_8O_2$, 19.2 *M* H_2O: - - - poly-L-alanine (0.378 *M*); ——— N-methylacetamide (1.08 *M*). C, N-methylacetamide: - - - in 10.7 *M* HCl, 36.5 *M* H_2O; ——— in 8.4 *M* $HClO_4$, 2.5 *M* $C_4H_8O_2$, 19.2 *M* H_2O; -·-·- in CF_3CO_2H. Spectra are plotted as ϵ, the extinction coefficient in cm.²/mole, on the ordinate *vs.* an abscissa wave length scale in microns.

(1) I. M. Klotz and J. S. Franzen, *J. Am. Chem. Soc.*, **84**, 3461 (1962).

(2) J. T. Yang and P. Doty, *ibid.*, **79**, 761 (1957).

(3) G. E. Perlmann and E. Katchalski, *ibid.*, **84**, 452 (1962).

(4) G. D. Fasman, in "Polyamino Acids, Polypeptides and Proteins," M. A. Stahmann, Ed., University of Wisconsin Press, Madison, Wis., 1962.

(5) C. H. Bamford, W. E. Hanby and F. Happy, *Proc. Roy. Soc.* (London), **A206**, 407 (1951).

(6) S. J. Singer, *Advan. Protein Chem.*, **17**, 1 (1962).

the 1.53–1.57 μ position characteristic of the amide–amide hydrogen bond to another wave length characteristic of the amide–acid interaction.

The spectra of poly-γ-benzyl-L-glutamate in the "helix-forming" solvents, chloroform and pyridine, did indeed reveal double absorption bands at 1.53–1.57 μ with extinction coefficients almost identical with those of the model amide in the amide hydrogen bonded form. Thus, the spectral properties of this interaction are not markedly changed when the C=O and the NH groups are part of a polypeptide structure. Spectra of solutions of poly-γ-benzyl-L-glutamate in dichloroacetic acid, as well as those of solutions of poly-L-alanine in dichloroacetic acid and in trifluoroacetic acid and of poly-L-leucine in trifluoroacetic acid, exhibited only a single peak which, however, appeared in the neighborhood of 1.51 μ, a band position hitherto unobserved in the spectra of the model amide. The spectral characteristics of the polymers in these organic acids are summarized in Table I. A spectrum of poly-L-alanine is shown in Fig. 1-A.

TABLE I

SPECTRAL CHARACTERISTICS OF POLYAMINO ACIDS IN "HELIX-BREAKING" SOLVENTS

Polymer	Solvent	Position of absorption maximum, μ	Extinction coefficient, cm.^{2}/mole amino acid residue
Poly-γ-benzyl-L-glutamate	$CHCl_2CO_2H$	1.510[a]	165[a]
Poly-L-alanine	$CHCl_2CO_2H$	1.510	230
	CF_3CO_2H	1.505	200
Poly-L-leucine	CF_3CO_2H	1.508	172

[a] Spectrum recorded on a Beckman DK-2 spectrophotometer; all others taken on a Cary Model 14 CMR.

To identify the structural state of the peptide corresponding to this 1.51 μ band, we have returned to our model compound, N-methylacetamide. Dissolved in trifluoroacetic acid, this amide (read against a reference solution containing N,N-dimethylacetamide) exhibited a spectrum with a double-humped band between 1.510 and 1.525 μ, as shown in Fig. 1-A. Despite the presence of an extra peak at 1.525 μ in this model amide, the following experimental evidence indicates that both N-methylacetamide and poly-L-alanine are protonated in trifluoroacetic acid.

(1) Protonation of N-methylacetamide in mixed solvents containing perchloric acid, dioxane, and water has been followed by the technique of nuclear magnetic resonance.[7] Under conditions where these data indicate protonation of the amide (predominantly on the oxygen[7]), the near-infrared spectra of N-methylacetamide (read against reference solutions containing N,N-dimethylacetamide) reveal an NH peak at 1.51 μ. This same peak is also exhibited in this perchloric acid solvent when the amide group is incorporated in a polypeptide structure such as poly-L-alanine (see Fig. 1-B). Solutions of N-methylacetamide in other strong acids, sulfuric and hydrochloric, also exhibit maxima at 1.51 μ. The presence of this band in solutions of hydrochloric acid removes any objection that such peaks may reflect specific hydrogen bonding between the amide groups and the oxygens of $HClO_4$, H_2SO_4 or CF_3COOH. Spectra of the amide in these strong acids are shown together in Fig. 1-C.

(2) Measurements of the apparent specific volumes (Table II) of both the low molecular weight amides, N-methylacetamide and N,N-dimethylacetamide, and the polymer, poly-L-alanine, in trifluoroacetic acid indicate a pronounced contraction in the volume of these solutions. This contraction is symptomatic of electrostriction, which would be produced by charged groups created in the protonation reaction.

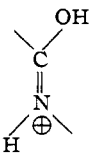
TABLE II

APPARENT SPECIFIC VOLUMES OF MODEL AMIDES AND POLY-L-ALANINE

Solute	Solvent	Concentration, moles/l.	Apparent specific volume, ml./g.
N-Methylacetamide	CF_3CO_2H	2.00	0.700
		.309	.451
		.274	.433
		0	.31 (extrap.)
	CCl_4	0	1.02 (extrap.)
	Pure liquid		1.06
N,N-Dimethylacetamide	CF_3CO_2H	1.00	0.62
		.446	.554
		.270	.498
		0	.35 (extrap.)
	Pure liquid		1.07
Poly-L-alanine	CF_3CO_2H	0.849[a]	0.65
	Amino acid residue		.74[b]

[a] Concentration in moles of amino acid residue/l. [b] Taken from E. J. Cohn and J. T. Edsall, "Proteins, Amino Acids and Peptides as Ions and Dipolar Ions," Reinhold Publishing Co., New York, N. Y., 1943, p. 372.

It may thus be concluded that a peak in the neighborhood of 1.51 μ is diagnostic of the protonated amide form



and that this form characterizes the state of the amide groups of polypeptides in trifluoroacetic and dichloroacetic acids.

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(9) Postdoctorate Fellow of the U. S. Public Health Service.

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(7) A. Berger, A. Loewenstein and S. Meiboom, *J. Am. Chem. Soc.*, **81**, 62 (1959).

In Vitro Alkaloid Biosynthesis in the Amaryllidaceae; Norbelladine O-Methylpherase

Sir:

Radioactive norbelladine (I) is incorporated without degradation into alkaloids such as belladine, galanthamine, crinamine, and lycorine by intact plants of the Amaryllidaceae.[1] However, this approach requires long periods of time and yields are usually low. We have sought to avoid these difficulties by employing partially purified cell-free enzyme systems.

Flowering bulbs of *Nerine bowdenii* were homogenized in a 0.05 *M* Tris buffer (pH 7.4) at 4°, filtered, and centrifuged at 5000*g*. Ammonium sulfate precipitation and dialysis or Sephadex treatment yielded a partially purified preparation which catalyzed the O-methylation of norbelladine (I) and of other catechols. The preferred methyl donor is (−)-S-adenosyl-L-methionine; diastereoisomers of this compound are less active, and such potential methyl donors as S-methylmethionine, betaine, methionine sulfoxide,

(1) Most recent paper: W. C. Wildman, A. R. Battersby and S. W. Breuer, *J. Am. Chem. Soc.*, **84**, 4599 (1962).