and ρ_{K_a} is the dissociation constant for the nitroalkane. An increase in α would then correspond to an increase in ρ_{k_1}.

The theory has been tested by determining ρ_{k_1} values for the reactions of 1-arylnitroethanes (m-CH_3, H, m-CH_3O, m-Cl, and m-NO_2) with hydroxide ion, piperidine, diethylamine, piperazine, and morpholine in water (Table I); ρ_{k_1} was also determined for the reactions of arylnitromethanes with hydroxide ion, morpholine, and 2,4-lutidine (Table II).

Table I. Hammett ρ Values for Reactions of 1-Arylnitroethanes with Bases in Water at 25°

Base	ΔpK^a	ρ_{k_1}	r	$\sigma_{p\text{-}NO_2}$
Hydroxide	−8.6	1.18 ± 0.02	0.9990	0.823
Piperidine[b]	−4.1	1.02 ± 0.03	0.9985	0.852
Diethylamine[b]	−4.0	0.99 ± 0.01	0.9997	0.993
Piperazine[b]	−2.5	0.93 ± 0.03	0.9984	0.956
Morpholine[b]	−1.6	0.96 ± 0.02	0.9994	0.978

[a] For $PhCHMeNO_2$; ρ_{K_a} = 1.03; statistically corrected. [b] Ionic strength, μ = 0.10.

Table II. Hammett ρ Values for Reactions of Arylnitromethanes with Bases in Water at 25°

Base	ΔpK^a	ρ_{k_1}	r	$\sigma_{p\text{-}NO_2}$
Hydroxide	−8.9	1.28 ± 0.02	0.9998	0.916
Morpholine[b]	−1.8	1.07 ± 0.05	0.9985	1.15
2,4-Lutidine[b]	+0.55	1.08 ± 0.04	0.9986	1.15

[a] For $PhCH_2NO_2$; ρ_{K_a} = 0.83; statistically corrected. [b] Ionic strength, μ = 0.10.

Interestingly enough the ρ_{k_1} (or α) values do not increase as ΔpK approaches zero. If anything the trend is in the opposite direction. The ρ values for amine bases are appreciably smaller in each system than that of hydroxide ion. These data, together with the earlier observation that Brønsted α's for deprotonation reactions can be larger than one and less than zero, negate the use of α as a guide to transition-state structures.[7] The decrease in α (or ρ) as the base type changes from hydroxide to amine bases indicates that, in the transition state, the negative charge on the benzylic carbon atom *decreases* as ΔpK becomes more positive. At the same time there is a slight increase in (calculated) σ_{p-NO_2} as we approach $\Delta pK = 0$ (Tables I and II). This suggests that the decreased charge is accompanied by a slight increase in delocalization of the negative charge from the benzylic carbon atom to the benzene ring. The changes in ρ and $\sigma_{p\text{-}NO_2}$ for a ΔpK change of *ca.* 7 units and a rate change of *ca.* 300-fold are surprisingly small, and the indication is that neither the charge nor geometry of the transition state has changed much over this range.

Brønsted β values calculated for the amines, piperidine, piperazine, and morpholine, reacting with $PhCHMeNO_2$ or its derivatives (data in Table I) are given in Table III, along with other values from the literature.

Table III. Brønsted β Values for Deprotonations of Carbon Acids

Carbon acid	ΔpK range	β	Ref
1-Arylnitroethanes	−4.7 to −2.2	0.52 to 0.56	Table I
Phenylnitromethane[a]	−6 to +2	0.65	*b*
Nitroethane	−2.5 to +4.5	0.50 to 0.55	*c*
3-Nitropropene	−0.8 to 0.0	0.59	*d*
Nitroethane	+2.0 to +3.4	0.65	*e*
Menthone	−1.0 to +5.3	0.48	*f*
Acetone	+13.4 to +14.8	0.64 to 0.72	*g*

[a] A variety of base types (H_2O, RCO_2^-, HPO_4^{2-}, etc.) was used. [b] V. M. Belikov, Ts. B. Korchemnaya, and N. G. Faleev, *Bull. Acad. Sci. USSR, Div. Chem. Soc.*, 1383 (1969). [c] M. J. Gregory and T. C. Bruice, *J. Amer. Chem. Soc.*, **89**, 2327 (1967); J. E. Dixon and T. C. Bruice, *ibid.*, **92**, 905 (1970). [d] J. A. Hautala, unpublished results. [e] R. G. Pearson and F. V. Williams, *J. Amer. Chem. Soc.*, **76**, 258 (1954). [f] Reference 6d. [g] J. A. Feather and V. Gold, *J. Chem. Soc.*, 1752 (1965).

Examination of Table III shows that over a ΔpK range of almost 20 units β for deprotonation of nitroalkanes and ketones shows little variation, and no consistent trend. One must conclude that either β is a very poor guide to the extent of proton transfer in the transition state or that transition-state structures for these reactions vary but little over wide ΔpK ranges. It is conceivable that both parts of this statement are true.

Acknowledgment. This work was supported by the National Science Foundation (GP 11384).

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(7) F. G. Bordwell, W. J. Boyle, Jr., J. A. Hautala, and K. C. Yee, *J. Amer. Chem. Soc.*, **91**, 4002 (1969). See also ref 5.

(8) National Institutes of Health Predoctoral Fellow, 1967–1970.

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Kinetic Isotope Effects as Guides to Transition-State Structures in Deprotonation Reactions

Sir:

Westheimer has presented theoretical arguments to show that for a series of related proton transfers the isotope effect, k^H/k^D, will be a maximum when the transition state is symmetrical.[1] Although Westheimer's treatment ignores bending frequencies and makes other simplifying assumptions,[1] its overall conclusion has been generally accepted,[2] and the magnitude of the primary isotope effect has been frequently used as a guide to the structure of the transition state in reactions where proton transfer is rate limiting.[3]

Bell and Goodall suggested for a simple proton transfer, $B^- + HA \rightarrow BH + A^-$, that the most symmetrical transition state should occur when B^- and A^- are of approximately equal basicity, *i.e.*, when

$$\Delta pK = pK_{HA} - pK_{HB} = 0$$

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(1) F. H. Westheimer, *Chem. Rev.*, **61**, 265 (1961).

(2) See (a) R. A. More O'Ferrall and J. Kouba, *J. Chem. Soc. B*, 985 (1967); (b) L. C. Gruen and F. A. Long, *J. Amer. Chem. Soc.*, **89**, 1287 (1967); (c) W. P. Jencks, "Catalysis in Chemistry and Enzymology," McGraw-Hill, New York, N. Y., 1969, Chapter 4, for discussions and literature references.

(3) (a) C. G. Swain and E. R. Thornton, *J. Amer. Chem. Soc.*, **84**, 817 (1962); (b) E. R. Thorton, *J. Org. Chem.*, 27, 1943 (1962); (c) K. B. Wiberg, "Physical Organic Chemistry," Wiley, New York, N. Y., 1964, p 361; (d) K. C. Brown and W. H. Saunders, Jr., *J. Amer. Chem. Soc.*, **92**, 4292 (1970), and earlier papers in this series; (e) R. A. More O'Ferrall, *J. Chem. Soc. B*, 785 (1970), writes: "For reactant or product-like transition states small isotope effects are normally observed, and for symmetrical transition states quite large ones."

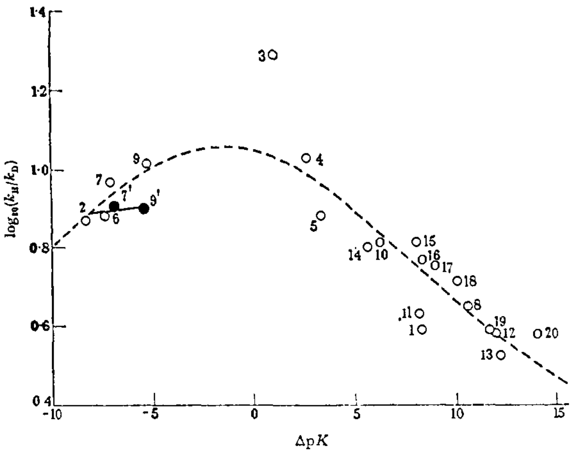

Figure 1. Relation between kinetic isotope effect and pK difference of the reacting system. (Reproduced from ref 4. Points 7′ and 9′ correspond to points 7 and 9 corrected for secondary isotope effects; these and the solid line have been added—see text.)

They presented a plot of log (k^H/k^D) *vs.* ΔpK for a number of different carbon acids with different bases (20 points in all), and demonstrated what appeared to be a general trend of increasing log (k^H/k^D) with decreasing absolute ΔpK (Figure 1).[4]

Other investigators have presented data in support of an apparent maximum near ΔpK = 0,[5] but the data cover a much smaller range of ΔpK and are more uncertain and less convincing.

Unfortunately, the Bell and Goodall plot is nearly devoid of points between ΔpK = −5 to +5 (Figure 1). Dixon and Bruice[5c] have recently reported data in this region (−2.5 to +3) using nitroethane and ten amine bases. Their values were widely scattered and no experimental support for a maximum was obtained. However, they found values of k^H/k^D to change by *ca.* 3 units for a range of 5.5 ΔpK units, and suggested that perhaps k^H/k^D "is a very sensitive probe for transition state symmetry, particularly near ΔpK = 0." Bell and Cox[5d] have found an apparent maximum for the racemization of (−)-methone catalyzed by hydroxide ion in water to which DMSO was added to change the H_- function used as a measure of pK of the base. Although the ratio does rise and fall around pK = 0, total variation in k^H/k^D is small (<15%) over the 6.3-unit range of ΔpK studied.

The apparent approach to a maximum from the left side of the Bell–Goodall curve is based on four points, and rests heavily on the points for HCH_2NO_2, $MeCH_2NO_2$, and Me_2CHNO_2 with hydroxide ion (points no. 9, 7, and 2). This is disturbing because both steric effects and secondary isotope effects could be important here. Steric effects can clearly play an important role as shown by the very large value obtained for the reaction of 2-nitropropane with 2,6-lutidine (no. 3 in Figure 1). In the present work calculation of the secondary isotope effects from rates of neutralization of $PhCH_2NO_2$, $PhCHDNO_2$, and $PhCD_2NO_2$ in water at 25° gave 1.14 and 1.18 for hydroxide ion (two methods) and 1.12 for morpholine. These agree well with the value of 1.18 determined by Bell and Goodall for nitroethane and hydroxide ion,[4] and with the value of 1.15 for the reaction of toluene with cesium cyclohexylamide in cyclohexylamine.[6] When the k^H/k^D values for HCH_2NO_2, $MeCH_2NO_2$, and Me_2CHNO_2 are corrected for secondary isotope effects (using 1.15 as an average value) they change from 10.2, 9.3, and 7.4, to 7.8, 8.1, and 7.4. The latter values show no significant trend, and make the left side of the Bell–Goodall curve almost flat (solid line in Figure 1—points 2, 6, 7′, and 9′).

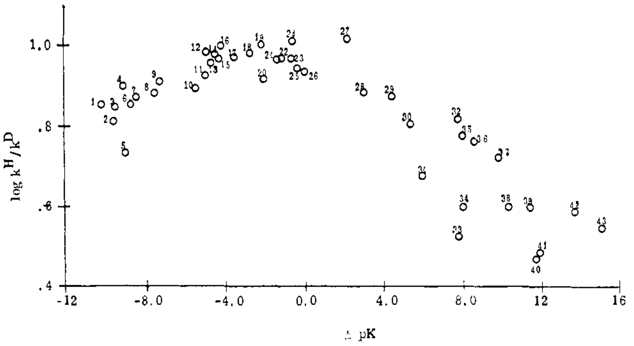

Figure 2. Variation of the primary isotope effect with ΔpK.

Twenty-one additional points have been obtained for the Bell–Goodall curve in the range ΔpK −10.1 to +0.3 by measuring rates of deprotonation and dedeuteration of the nitroalkanes $ArCHMeNO_2$, $ArCH_2NO_2$, and $CH_2{=}CHCH_2NO_2$[7] with bases ranging in strength from hydroxide ion to pyridine. When these points are included one obtains the pattern shown in Figure 2.

Points 1–7 are for $ArCHMeNO_2$ or $ArCH_2NO_2$ with HO^-, points 11–20 are for $ArCHMeNO_2$ or $ArCH_2NO_2$ with amine bases, and points 21–26 are for $CH_2{=}CHCH_2NO_2$ with pyridines.[7] Points no. 27–43 are mainly data with ketones, β-diketones, and β-keto esters taken from the Bell–Goodall curve (corrected for secondary isotope effects, where necessary).

Examination of Figure 2 reveals an apparent, but poorly defined maximum in the neighborhood of ΔpK = −1 to −2. The distinguishing feature of the graph, however, is the gentle slope with which values decrease on either side of the apparent maximum. Thus, at ΔpK = −10, k^H/k^D has decreased by only 30% from the maximum, while for positive values of ΔpK, where the scatter is more severe, a typical point, no. 39, shows that k^H/k^D has decreased by about 60% at ΔpK = +12.

The scatter of the points in Figure 2 is not surprising when one considers: (a) the inherent inaccuracy of most of the data,[8] (b) the likelihood that steric and/or tunneling effects may alter k^H/k^D, and (c) the possibility that low k^H/k^D ratios observed for positive ΔpK values may result from a prerate-limiting equilibrium.[9] In

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[4] R. P. Bell and D. M. Goodall, *Proc. Roy. Soc., Ser. A*, **294**, 273 (1966).

[5] (a) J. L. Longridge and F. A. Long, *J. Amer. Chem. Soc.*, **89**, 1292 (1967); (b) A. J. Kresge, D. S. Sagatys, and H. L. Chen, *ibid.*, **90**, 4174 (1968); (c) J. E. Dixon and T. C. Bruice, *ibid.*, **92**, 905 (1970); (d) R. P. Bell and B. J. Cox, *J. Chem. Soc. B*, 194 (1970).

[6] A. Streitwieser, Jr., and D. E. Van Sickle, *J. Amer Chem. Soc.*, **84**, 254 (1962).

[7] Data of J. A. Hautala.

[8] Errors of ±5% in the rate data for deprotonation and dedeuteration will lead to an error of about ±10% in the k^H/k^D ratio.

[9] D. J. Cram, D. A. Scott, and W. D. Nielsen (*J. Amer. Chem. Soc.*, **83**, 3696 (1961)) have attributed the low k^H/k^D ratio observed or the reaction of $HexCHMeSO_2Ph$ with HO^- (Δp$K \cong +15$) to this mechanism [see also W. T. Ford, E. W. Graham, and D. J. Cram, *ibid.*, **89**, 4661 (1967)].

view of these uncertainties and the gentleness of the slopes in Figure 2 one might question the reality of the apparent maximum. The maximum is still apparent, however, if one selects only points with RCH_2NO_2 (R = H, Me, Ar, $CH_2{=}CH$) in order to eliminate changes in type of substrate and to minimize steric effects (points 5, 9, 10, 20, 21–26, 31, 33, and 40).

Even though the maximum appears real, it seems clear that the shape of the curve is relatively flat, and that either k^H/k^D is relatively insensitive to the symmetry of the transition state or that the symmetry does not change over wide ranges of ΔpK. The same conclusion is reached from an examination of the relationship of solvent isotope effects and transition state structures in deprotonation reactions,[10] and with respect to the relationship of Brønsted β coefficients to the symmetry of transition states for deprotonation reactions.[11] Expanding on Hammett's statement concerning solvent isotope effects,[12] we conclude that *the hope, which at one time seemed bright, for a simple general correlation of Brønsted coefficients, kinetic isotope effects, and solvent isotope effects with the extent of proton transfer in the transition state has proved vain.*

Acknowledgment. This work was supported by the National Science Foundation (GP 11384).

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(11) F. G. Bordwell and W. J. Boyle, Jr., *J. Amer. Chem. Soc.*, **93**, 511 (1971).

(12) L. P. Hammett, "Physical Organic Chemistry," 2nd ed, McGraw-Hill, New York, N. Y., 1970, p 131.

(13) National Institutes of Health Predoctoral Fellow, 1967–1970.

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Hydration of Macromolecules. III. Hydration of Polypeptides

Sir:

We have described a method for determination of the hydration of proteins.[1] We now report results for water-soluble polypeptides. In this communication we focus on the question: can the hydration of a globular protein be described in terms of the hydration of its constituent amino acids?

Our experiment measures the amount of water which does not freeze when an aqueous macromolecular solution is rapidly frozen and then equilibrated at −20 to −60°. The high-resolution proton nmr spectrum of such a solution shows a single, broad (0.2–2 kHz), lorenztian signal whose area is a direct indication of the amount of unfrozen water. Experimental details and a discussion of the rationale of ascribing this water to "water of hydration" are available.[1,2]

Polypeptides were obtained from commercial sources and used without further purification.[3] All solutions contained 0.01 *M* KCl as well as sufficient KOH or HCl to set the pH as desired. Macromolecular concentrations were 5–10% by weight where possible; otherwise saturated solutions were used. We measured the concentrations of the polypeptides by evaporating a known quantity of solution to dryness (60° for 36 hr, followed by 12 hr at 100°). Absolute hydrations of the polypeptides were obtained by comparing the area of the nmr signal each produced with that found with a 10% bovine albumin sample. The hydration of the albumin had been determined earlier using a D_2O–H_2O–LiCl solution.[1,2]

Our results are given in Table I, with hydration expressed as moles of water per mole of amino acid. The indicated errors include the uncertainties in area and concentration determinations. We find that all the amino acids with ionized side chains are heavily hydrated. In addition, polyproline and the uncharged forms of the basic amino acids hold considerable water. Nonpolar groups hold much less. The nmr water signal increased linearly with polymer concentration (1–10% range), so that the hydration per mole is independent of polymer concentration. No molecular weight dependence was observed. The data indicate a marked temperature dependence for the amount of water associated with the charged residues; a very strong pH effect is seen for the negatively charged glutamic, aspartic, and tyrosyl side chains. We note in passing that the globular proteins, nucleic acids, and nonpolar polypeptides do not show pronounced temperature effects in this temperature range.[1,2]

Four sources of systematic error should be considered. First, we have made no correction for the possible presence of "exchangeable" hydrogens contributing to the water signal. Although many of the polypeptide protons are "readily" exchangeable at room temperature when assayed by conventional methods,[4] it is very unlikely that they are mobile enough to exchange rapidly (by nmr standards) at −30°.[5] Second, we are reasonably confident that there are no contributions to the nmr signal from the CH_2 and CH_3 groups of the side chains. At relatively high temperatures (−10°) the water line is sharp enough so that the CH regions of the spectra are exposed. Under these conditions, only quite small amounts of side-chain resonances are detected, and on lowering the temperature, these quickly broaden and lose intensity as the side chains become immobilized in the ice. Polyvaline is the only exception so far noted. An unusually sharp water peak and a clearly defined CH_3 peak were detectable down to −30°.[6] We cannot report at this time ionic strength and counterion effects. Protein work[1,7] suggests that such effects will

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(1) I. D. Kuntz, T. S. Brassfield, G. Law, and G. Purcell, *Science*, **163**, 1329 (1969).

(2) I. D. Kuntz and T. S. Brassfield, *Arch. Biochem. Biophys.*, in press.

(3) The source, molecular weight as supplied by the manufacturer, and concentration used in the nmr experiments (wt %) are given for each polypeptide: DL-Ala, S, 1600, 4%; L-ArgHCl, S, 50,000, 5%; L-Asn, P, mol wt unknown, 5–9%; Gly, S, 10,000, 0.35%; L-LysHBr, M, 12,000 and 44,000, 2–9%; DL-LysHBr, M, 60,000, 7%; L-OrnHBr, P, 105,000, 6–10%; L-Pro, S, 7500 and 200,000, 2 and 3.5%, respectively; L-Tyr, P, 100,000, 3%; L-Val, M, mol wt unknown, 0.4%; $Lys^{40}Glu^{60}$, from W. Kauzmann who received it from E. Blout, mol wt unknown, 5–9%; $Lys^{50}Phe^{50}$, M, 180,000, 5%; $Lys^{33}Phe$,^{67} M, mol wt unknown, 1.5%. Abbreviations used are: S, Sigma (St. Louis, Mo.); P, Pilot Division of New England Nuclear (Boston, Mass.); M, Miles (Elkhart, Ind.); Sch, Schwartz Biochem. (Orangeburg, N. Y.).

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(5) A. Hvidt and S. O. Nielsen, *Advan. Protein Chem.*, **21**, 287 (1966); A. Wishnia and M. Saunders, *J. Amer. Chem. Soc.*, **84**, 4235 (1962).

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