isolated. There remained a residue of 3.9 g. which might contain polyvalerolactam.

Synthesis of ε-(*p*-Toluenesulfamido)-caproic Acid (XVI).—ε-Aminocaproic acid (131 g., 1.0 mole) was dissolved in 250 ml. of 4 *N* sodium hydroxide and *p*-toluenesulfonyl chloride (190 g., 1.0 mole) dissolved in 500 ml. of acetone was added to the sodium aminocaproate simultaneously with a solution of 250 ml. of 4 *N* sodium hydroxide. The temperature was maintained below 35° during the addition. After the addition was complete the reaction mixture was stirred for three hours at room temperature. Acetone was distilled off and the aqueous solution was acidified with 83 ml. of concentrated hydrochloric acid. After standing overnight in a refrigerator at 0°, 280 g. (98% conversion) of ε-(*p*-toluenesulfonamido)-caproic acid was obtained. Several recrystallizations from 50% aqueous ethanol and subsequently from methyl ethyl ketone gave a material, m.p. 108.8–110.2°, lit.[22] m.p. 104–106°.

Anal. Calcd. for $C_{13}H_{17}O_4NS$: C, 55.15; H, 6.47; N, 4.94. Found: C, 55.46; H, 6.82; N, 4.97.

Synthesis of N-*p*-Toluenesulfonylcaprolactam (XV).—ε-(*p*-Toluenesulfonamido)-caproic acid (5.7 g., 0.02 mole) was heated in a small still under a vacuum of 0.01 mm. at about 125° for two hours. The material then was washed with 5% sodium bicarbonate solution and the residue was extracted with chloroform and treated with charcoal. The chloroform was evaporated under vacuum. The residue was chromatographed on neutral alumina using chloroform as the eluent. Several of the fractions eluted yielded, after recrystallizations from water and methyl ethyl ketone, a compound (55 mg.), m.p. 124–125°. The infrared spectrum of this material was essentially identical with the material obtained by rearrangement of I in chloroform.

Rearrangement of Cyclohexanone Oxime *p*-Toluenesulfonate (I) in Dry Chloroform.—Cyclohexanone oxime *p*-toluenesulfate (89 g., 0.3 mole) dissolved in 250 ml. of dry chloroform, stood for one year at 25° in a sealed flask wrapped in aluminum foil. Thereafter the solution was evaporated to dryness under vacuum leaving 90 g. of a viscous brown material. After numerous attempts at crystallization most of the material solidified to a semi-crystalline mass after being boiled with water and the water removed by freeze-drying. After several recrystallizations from methyl ethyl ketone the material melted at 124.6–125.0°; infrared spectrum (cm.$^{-1}$): 1707 (vs), 1660 (vs), 1448 (s), 1386 (s), 1352 (s) and 1163 (s).

Anal. Calcd. for $C_{13}H_{17}O_3NS$: C, 58.40; H, 6.41; N, 5.24. Found: C, 56.43, 56.65, 57.15, 56.95; H, 7.50, 7.59, 7.55, 7.59; N, 6.62, 6.60.

Acid Hydrolysis of N-*p*-Toluenesulfonylcaprolactam (XV).—N-*p*-Toluenesulfonylcaprolactam, 5.3 g. (0.02 mole), was refluxed with 20 ml. of concentrated hydrochloric acid for five hours. The solution was diluted to about 200 ml. with water and cooled to room temperature. The 3 g. of solid material which precipitated was recrystallized from water several times, m.p. 108–110°. This material did not give a melting point depression with a synthetic sample of ε-(*p*-toluenesulfonamido)-caproic acid, and the infrared spectra of these two samples were identical.

Acknowledgment.—The author wishes to thank Dr. George R. Coraor for many helpful discussions during this investigation.

WILMINGTON, DEL.

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[22] K. Thomas and M. G. H. Goerne, *Hoppe-Seyler's Z. physiol. Chem.*, **104**, 75 (1918); *C.A.*, **13**, 2024 (1919).

[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY AND THE LABORATORY FOR NUCLEAR SCIENCE, MASSACHUSETTS INSTITUTE OF TECHNOLOGY]

Use of Hydrogen Isotope Effects to Identify the Attacking Nucleophile in the Enolization of Ketones Catalyzed by Acetic Acid[1-3]

BY C. GARDNER SWAIN, EDWARD C. STIVERS, JOSEPH F. REUWER, JR., AND LAWRENCE J. SCHAAD

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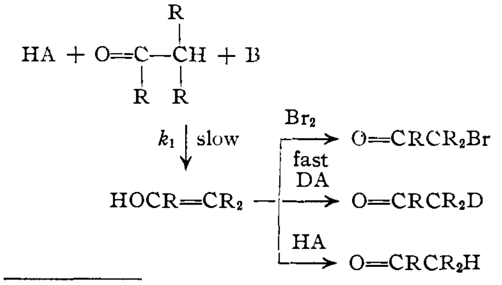
Catalysis of enolization by acetic acid is not due to an acetic acid molecule acting on the ketonic oxygen and a water molecule removing the proton from carbon, but instead to hydrogen ion acting on the ketonic oxygen and acetate ion removing the proton from carbon, in the case of α-phenylisocaprophenone (a ketone with one α-hydrogen) in aqueous solution with 8.4 *M* dioxane (72% dioxane by volume) at 100°. The magnitude of the protium/tritium isotope effect at the carbon is diagnostic of the attacking nucleophile, *increasing* as the basicity and reactivity of the nucleophile increases. For catalysis by acetic acid, it has a value (11.4) which is too high for nucleophilic attack by water, but correct for attack by acetate ion. A relation between tritium (k_H/k_T) and deuterium (k_H/k_D) isotope effects is derived and compared with experimental data.

Enolization is the rate-determining process in the bromination, iodination, deuterium exchange and racemization of ketones.[4]

The first-order rate constant for enolization of acetone in acetic acid buffers in water solution at 25° has the form[5]

$$k_1 = \frac{1}{(\text{ketone})} \times \frac{\mathrm{d(enol)}}{\mathrm{d}t} = k_0 + k_{\mathrm{H^+}}(\mathrm{H^+}) + k_{\mathrm{HO^-}}(\mathrm{HO^-}) + k_{\mathrm{HOAc}}(\mathrm{HOAc}) + k_{\mathrm{AcO^-}}(\mathrm{AcO^-}) + k_{\mathrm{p}}(\mathrm{HOAc})(\mathrm{AcO^-})$$

Table I shows possible combinations of electrophile or acid (HA) and nucleophile or base (B) that may be responsible for each of the terms.[6] The



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[1] This work was supported in part by the research program of the Atomic Energy Commission under Contract No. AT(30-1-)-905.

[2] For Part II on use of hydrogen isotope effects to determine the timing of proton transfer relative to nucleophilic attack, *cf.* C. G. Swain, A. J. Di Milo and J. P. Cordner, THIS JOURNAL, **80**, Nov. 20 (1958).

[3] For complete experimental data, *cf.* E. C. Stivers, Ph.D. Thesis in Organic Chemistry, M.I.T., May, 1956, or (for comparison of deuterium and tritium), J. F. Reuwer, Jr., S.M. Thesis in Organic Chemistry, M.I.T., October, 1956.

[4] A. Lapworth, *J. Chem. Soc.*, **85**, 30 (1904); H. M. Dawson and M. S. Leslie, *ibid.*, **95**, 1860 (1909); P. D. Bartlett, THIS JOURNAL, **56**, 967 (1934); C. K. Ingold and C. L. Wilson, *J. Chem. Soc.*, 773 (1934); C. K. Ingold, S. K. Hsu and C. L. Wilson, *ibid.*, 78 (1938); W. D. Walters and K. F. Bonhoeffer, *Z. physik. Chem.*, **A182**, 265 (1938).

[5] H. M. Dawson and E. Spivey, *J. Chem. Soc.*, 2180 (1930); R. P. Bell and P. Jones, *ibid.*, 88 (1953).

[6] Inclusion of nine terms allows for the operation of either a concerted Lowry mechanism or a stepwise Pedersen mechanism or both at once. The first six terms cannot involve protonation by the acid in a prior equilibrium step, but the last three (VII, VIII and IX) may corre-

TABLE I

ACID-BASE COMBINATIONS CORRESPONDING TO VARIOUS TERMS IN RATE EXPRESSION[6]

HA	B	Rate	Coefficient	Term
H_2O	H_2O	$k_1(H_2O)^2$	$k_1(H_2O)^2 = I$	Uncat.
H_2O	AcO^-	$k_2(H_2O)(AcO^-)$	$k_2(H_2O) = II$	(AcO^-)
H_2O	HO^-	$k_3(H_2O)(HO^-)$	$k_3(H_2O) = III$	(HO^-)
HOAc	H_2O	$k_4(HOAc)(H_2O)$	$k_4(H_2O) = IV$	(HOAc)
HOAc	AcO^-	$k_5(HOAc)(AcO^-)$	$k_5 = V$	$(HOAc)(AcO^-)$
HOAc	HO^-	$k_6(HOAc)(HO^-)$	$k_6K_w/K_{HOAc} = VI$	(AcO^-)
H_3O^+	H_2O	$k_7(H_3O^+)(H_2O)$	$k_7(H_2O) = VII$	(H_3O^+)
H_3O^+	AcO^-	$k_8(H_3O^+)(AcO^-)$	$k_8K_{HOAc} = VIII$	(HOAc)
H_3O^+	HO^-	$k_9(H_3O^+)(HO^-)$	$k_9K_w = IX$	Uncat.

$k_0 = I + IX$ $k_{HO^-} = III$ $k_{AcO^-} = II + VI$

$k_{H^+} = VII$ $k_{HOAc} = IV + VIII$ $k_p = V$

hydronium and hydroxide ion terms are unambiguous. They must involve water and hydroxide ion, respectively, as the base which removes the proton from carbon. However, three terms, *viz.*, the uncatalyzed, acetic acid and acetate ion terms, are ambiguous. For example, catalysis by acetic acid may involve (1) only acetic acid (HA) and water (B), or (2) only hydronium ion (HA) and acetate ion (B) or (3) significant contributions from both of these combinations (IV and VIII).

A kinetic argument based on the relative magnitudes of the experimental catalytic coefficients (k_0, k_{H^+}, . . . , k_p) was presented previously to show that catalysis of enolization by acetic acid is probably due primarily to hydronium ion and acetate ion.[7] The purpose of the work described in the present paper was to check this identification of the participating acid and base by an entirely different approach. In the following paper[2] further experiments are presented which establish the relative timing of the attacks by acid and base, *i.e.*, whether the reaction is concerted with both operating in the same step, or stepwise with the proton transfer from acid to the ketonic oxygen occurring in an equilibrium step preceding the rate-determining step.

For the identification of the base which removes the proton from carbon, the isotope effect for hydrogen at this carbon should be useful, since its magnitude should depend on the particular base involved. Reitz demonstrated this dependence in the bromination of nitromethane (Table II).[8] With nitromethane there is no ambiguity about the identification of the reactants because it shows no acid catalysis, hence any solvation of the oxygens of the nitro group must be done by water molecules only.

Reitz also reported isotope effects for the bromination of acetone (Table III).[9] However, these do not show as large a spread as those for the bromination of nitromethane and do not seem to provide a clear answer to the problem.

Nevertheless it seemed to us that in principle it should be possible to identify the nucleophile in acetic acid catalysis through the magnitude of the isotope effect in the acetic acid term if isotope effects were measured carefully for enough of the other terms in the rate expression.

TABLE II

ISOTOPE EFFECTS IN BROMINATION OF CH_3NO_2 AND CD_3NO_2 IN H_2O AT 25°[8]

Catalyst	pK_B	Catalytic coefficient k_H, M^{-1} sec.$^{-1}$ × 10^6	Isotope effect[a] k_H/k_D
H_2O	15.7[b]	0.0012[e]	3.78
$ClCH_2COO^-$	11.1[c]	4.1	4.28
CH_3COO^-	9.3[d]	76	6.53

[a] Ratio of catalytic coefficients extrapolated to 100% deuterium content. [b] E. J. Roberts, THIS JOURNAL, 52, 3877 (1930). [c] D. D. Wright, *ibid.* 56, 314 (1934). [d] J. F. J. Dippy and F. R. Williams, *J. Chem. Soc.*, 1888 (1934). [e] Second-order rate constant at 25°. Multiply by 55 *M* to obtain first-order constant in sec.$^{-1}$.

TABLE III

ISOTOPE EFFECTS IN BROMINATION OF 0.5 *M* CH_3COCH_3 AND CD_3COCD_3 IN H_2O AT 25°[9]

Catalyst	pK_A	Catalytic coefficient k_H, sec.$^{-1}$ × 10^6	Isotope effect[a] k_H/k_D
H^+	1.7[b]	66	5.00
$ClCH_2COOH$	2.9	3.4	5.15
$HOCH_2COOH$	3.8[c]	0.77	4.43
CH_3COOH	4.7	.21	5.39
CH_3COO^-	..	.58	3.76

[a] Ratio of catalytic coefficient for ordinary acetone and acetone with 92 atom % deuterium. [b] log 55. [c] L. F. Nims, THIS JOURNAL, 58, 987 (1936).

Tritium Isotope Effects in the Enolization of α-Phenylisocaprophenone.—To obtain the isotope effects needed to identify the nucleophile in acetic acid catalysis, we chose to compare the rate of racemization of an optically active ketone (α-phenylisocaprophenone, I) with the rate at which α-tritiated racemic ketone III lost tritium to the solvent, rather than comparing rates of bromination of ordinary *vs.* deuterated ketone. This avoids the problems of autocatalysis by hydrogen bromide produced, of successive replacements and cleavage in basic solution, and of corrections for less than 100% deuteration, and gives larger isotope effects due to the use of the tritiated compound (III) instead of the deuterated compound (II). The main disadvantage is the higher temperature (100°) needed for reaction. In their recent study at 25°, Bell and Jones[5] followed all reactions through less than 1% bromination of the ketone. With race-

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spond to either concerted or stepwise enolizations. We hope to distinguish between concerted *vs.* stepwise enolization and between this interpretation of the product term (V) *vs.* other possibilities in the following paper.[2]

(7) C. G. Swain, THIS JOURNAL, **72**, 4578 (1950).

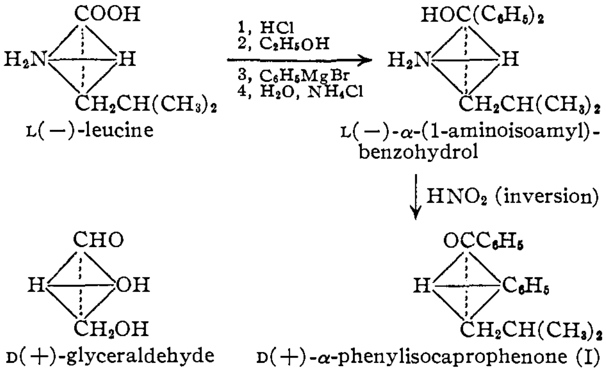
(8) O. Reitz, *Z. physik. Chem.*, **A176**, 363 (1936). The catalytic coefficients listed in Table II and in all of our own work are based on natural logarithms. Reitz reported constants in minutes and common logarithms, larger by a factor of 60/2.3 = 26.

(9) O. Reitz, *ibid.*, **A179**, 119 (1937); O. Reitz and J. Kopp, *ibid.*, **A184**, 429 (1939).

mization as the analytical method, it is desirable to follow the reactions through at least one half-life of the ketone for accurate results, since a property of *unreacted* ketone is being measured rather than bromine or reacted ketone. To follow the reaction this far in a reasonable time (less than a year) requires a higher temperature.

Ionic strength was kept constant at 0.10 M in all reactions by making up any deficiency with sodium chloride. This precaution of constant salt concentration was observed by Bell and Jones[5] but not by Reitz.[9] A solution of 8.4 M dioxane in water (72% dioxane by volume) was used instead of pure water to increase solubility and permit the convenient use of stock solutions of the ketones.

α-Phenylisocaprophenone (I) with a specific rotation $[\alpha]^{25}\text{D}$ of 160.5° in 100% ethanol was prepared in 52% over-all yield from leucine by a stereospecific sequence.[10]



The tritiated ketone, DL-α-phenylisocaprophenone (III), was made by refluxing I with tritiated water, hydrochloric acid and dioxane. In the isotope exchange it was demonstrated that there is no isotope fractionation between ketone and solvent, and no exchange into the methyl groups of the acetic acid used.

Table IV presents the results obtained. It is reasonable to expect that the isotope effect should be larger the stronger the acid involved, provided the attacking base remains constant, because a stronger acid should weaken the bond between carbon and the α-hydrogen resulting in relatively more complete bond breaking at the transition state. The weakening to be considered is not that involved in going from a ketone–acid complex or from protonated ketone to the transition state but rather that from the free ketone to the transition state, since the reactant ketone is predominantly free (not associated with acid) in its ground state in the solution. The weakening is connected with the most difficult shift (only partially complete at the transition state) of an electron pair from a σ-bond from the α-hydrogen to a π-bond to the carbonyl carbon. Certainly this shift should be more complete, relative to σ-bonding of a constant base to the α-hydrogen at the transition state, the stronger the acid associated with the carbonyl oxygen in the transition state.

TABLE IV

ISOTOPE EFFECTS IN ENOLIZATION OF $C_6H_5COCH(C_6H_6)$-$CH_2CH(CH_3)_2$ AND $C_6H_5COCH(C_6H_5)CH_2CH(CH_3)_2$ IN H_2O SOLUTION WITH 8.4 M DIOXANE AND 0.1 M IONIC STRENGTH AT 98°

Catalyst	$k_H{}^a \times 10^6$, M^{-1} sec.$^{-1}$	$k_T{}^a \times 10^6$, M^{-1} sec.$^{-1}$	$k_H/k_T{}^c$	HA	B
H^+	264	27.2	9.7	H_3O^+	H_2O
CH_3COOH	0.0874	0.00768	11.4	H_3O^+	AcO^-
CH_3COO^-	10.7	1.05	10.2	H_2O	AcO^-
HO^-	99200	7680	12.9	H_2O	HO^-

[a] Catalytic coefficient from racemization of I. [b] Catalytic coefficient from exchange of III. [c] This ratio of catalytic coefficients is the isotope effect.

If we make this assumption that the isotope effect should be larger the stronger the acid, then 12.9 (the isotope effect for HO^-) must represent a *minimum* isotope effect for hydroxide ion attacking as a base, since the weakest acid (H_2O) is involved in the hydroxide term. Hence acetate ion catalysis cannot involve hydroxide ion as base and acetic acid as an acid because the acetate isotope effect is even less than 12.9, *viz.*, 10.2. It must therefore involve acetate ion as base and water as acid. If the acetic acid term involves water as the base, its isotope effect should be *less than* 9.7 (the isotope effect for H_3O^+) since it would involve a weaker acid (HOAc) than hydronium ion. If it involves acetate ion and hydronium ion, its isotope effect should be *larger than* 10.2 since it would involve a stronger acid (H_3O^+) than water. The observed value of 11.4 clearly indicates that acetate ion and hydronium ion are the actual reactants. Table IV shows the actual reactants (HA and B) corresponding to each catalyst. No information on isotope effects when molecular acetic acid is the acid (HA) is available since acetic acid catalysis does not involve molecular acetic acid, and the product term, although detected, was too small to be accurately measurable. The uncatalyzed term was not detected.

Our conclusion based on ketone enolization, as well as the conclusion of Reitz[8] based on nitromethane, that the isotope effect *increases* with increasing basicity and reactivity of the attacking base, is contrary to the rule that "in general, in any series of related reactions, the reactions with the lowest activation energy will have the lowest isotope effect."[11] It demonstrates that the proton is *less* tightly bound in the transition state when stronger bases are used. Our rationalization of this interesting conclusion is that the degree of completion of covalent bond formation between an anionic attacking base and the α-hydrogen at the transition state is only very slight. The main role of strong anionic bases, like that of strong acids, is to increase the extent of the electron pair shift from α-hydrogen to the carbonyl carbon (or to nitrogen in the case of nitroalkanes). While the new bond to the α-hydrogen is still long and largely ionic, the

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[10] S. Kanao and T. Yaguchi, *J. Pharm. Soc. Japan*, **48**, 252 (1928); *cf. C. A.*, **21**, 567 (1927); **22**, 2937 (1928); **26**, 5071 (1932); **45**, 5136 (1951); R. P. Bell, O. M. Lidwell and J. Wright, *J. Chem. Soc.*, 1862 (1938). For correlation of configurations and nomenclature, *cf.* W. Klyne, "Progress in Stereochemistry," Vol. 1, Academic Press, Inc., New York, N. Y., 1954, p. 177.

[11] K. B. Wiberg, *Chem. Rev.*, **55**, 713 (1955). In nitromethane ionization in water solution, the Arrhenius activation energy with different catalysis is 22 kcal. with water, 21 kcal. with chloroacetate, 20 with acetate, 15 with ammonia and 12 with hydroxide ion.[12]

[12] National Bureau of Standards Circular 510, "Tables of Chemical Kinetics, Homogeneous Reactions," U. S. Government Printing Office, Washington, D. C., 1951, p. 391.

rupture of the old bond has become very considerable at the transition state and is more complete with hydroxide ion than with acetate ion than with water. Thus hydroxide ion supplies more driving force through coulombic and Pauli repulsions against the electron pair in the old bond than through covalent bond formation with this hydrogen. Since the distances are long in the transition state, most of the directional character of the bonds to the hydrogen and stiffness with respect to lateral movement of the hydrogen are lost. The lateral frequencies are therefore low and the isotope effect very high with strong bases.

Comparison of α-Phenylisocaprophenone and Acetone.—Table V shows the general similarity of the isotope effects observed with α-phenylisocaprophenone to those previously observed with nitromethane[8] and acetone.[9] The relation (equation 7) derived in the next section was used to calculate tritium isotope effects from the observed deuterium isotope effects of all compounds except α-phenylisocaprophenone. This relation is shown to hold for α-phenylisocaprophenone in the section following the next one. Included also are two data measured recently by Emmons and Hawthorne by bromination of phenyl cyclopentyl ketone in approximately 90% acetic acid–10% water at 26.7°.[13]

TABLE V

TRITIUM ISOTOPE EFFECTS (k_H/k_T) IN ENOLIZATION-TYPE REACTIONS OF VARIOUS COMPOUNDS

Catalyst	CH_3NO_2[a]	C_6H_5CO-C_5H_{10}[b]	CH_3CO-CH_3[c]	C_6H_5COCH-$(C_6H_5)CH_2$-$CH(CH_3)_2$[d]
H^+	6.8[e]	7.3	10.2	9.7
$ClCH_2COOH$	8.1	..	10.6	..
CH_3COOH	..	..	11.3	11.4
CH_3COO^-	15.0	13.8	8.3	10.2
HO^-	..	..	..	12.9

[a] Calculated from the data in Table II. [b] Calculated from deuterium isotope effects[13] of 3.96 and 6.17. [c] Calculated from the data in Table III. [d] Observed in current study (Table IV). [e] Uncatalyzed reaction with water.

A Relation between Protium/Tritium and Protium/Deuterium Isotope Effects.[14]—Consider a

$$X\text{-}H^i + A + B + \ldots \xrightarrow{k_i} \text{products} \quad (1)$$

reaction in which an X–H^i– bond is broken where i refers to the particular hydrogen isotope used (1, 2 or 3 for protium, deuterium or tritium), A and B are other reactants, and k_i is the rate constant. In this calculation we assume that all differences except those in zero-point vibrational energy are insignificant, that X is polyatomic and very heavy compared to H, that isotopic substitution affects the vibrations of only the bond which holds the isotope, and this can be treated as a harmonic oscillator; and that all molecules pass over, rather than through, the reaction potential barrier (no "tunneling"). All of these assumptions seem to be reasonable ones for enolization and most other common proton exchange reactions.[15]

Figure 1 is our model for reaction 1 with the protium isotope (i = 1). In the transition state the spring between protium and the rest of the molecule X has broken, and the remaining two springs have altered in strength. When using deuterium or tritium, the force constants f_x, f_y, f_z, $f_y{}^*$ and $f_z{}^*$ for vibrations in the directions indicated in Fig. 1 would still be the same; only the mass of the hydrogen would be different.

The deuterium (k_H/k_D) isotope effect is then

$$\frac{k_1}{k_2} = \prod_j e^{-h(\nu_{1j}{}^* - \nu_{2j}{}^* - \nu_{1j} + \nu_{2j})/2kT} \quad (2)$$

where

$$\nu_{ij} = \frac{1}{2\pi}\sqrt{\frac{f_j}{m_i}} \quad (3)$$

and the asterisk refers to the transition state, h is the Planck constant, k is the Boltzmann constant, T is the absolute temperature (°K.), ν_{ij} is the frequency for hydrogen isotope i and mode j, and m_i is the mass of the hydrogen, which is used instead of the reduced mass $m_x m_i/(m_x + m_i)$ because X is very heavy compared to H^i. Combining equations 1 and 2 gives

$$\frac{k_1}{k_2} = E^{[(1/\sqrt{m_1}) - (1/\sqrt{m_2})]} \quad (4)$$

$$E = e^{[\sqrt{f_x} + \sqrt{f_y} + \sqrt{f_z} - \sqrt{f_y{}^*} - \sqrt{f_z{}^*}]h/4\pi kT} \quad (5)$$

Similarly the tritium isotope effect is

$$\frac{k_1}{k_3} = E^{[(1/\sqrt{m_1}) - (1/\sqrt{m_3})]} \quad (6)$$

Since m_1 = 1.0081, m_2 = 2.0147 and m_3 = 3.0170

$$\left(\frac{k_H}{k_T}\right) = \left(\frac{k_H}{k_D}\right)^{\left[\frac{(1/\sqrt{m_1}) - (1/\sqrt{m_3})}{(1/\sqrt{m_1}) - (1/\sqrt{m_2})}\right]} = \left(\frac{k_H}{k_D}\right)^{1.442} \quad (7)$$

Thus the tritium isotope effect k_H/k_T is the 1.442 power of the deuterium isotope effect. This result would not be changed if we considered also the contribution to E from a new bond to H partly formed at the transition state, because its force constant would likewise be the same for H, D and T; nor would it be changed if we considered the old bond to be incompletely broken at the transition state. The introduction of additional force constants into E cannot affect the validity of equations 4, 6 and 7.

This equation should hold as long as the temperature is sufficiently low that the great majority of the X–H^i bonds are in their lowest vibrational state. This is still true for most bonds to hydrogen at 0–100°C.

Maximum and minimum tritium isotope effects corresponding to any given deuterium isotope effect also can be derived for the model in Fig. 1. Here it is necessary to take account of the distribution of molecules in higher vibrational states, by replacing $e^{-h\nu/2kT}$ by $e^{-h\nu/2kT}(1 - e^{-h\nu/kT})^{-1}$ for each frequency in the transition state or ground state for each isotope.[16] The result is

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[13] W. D. Emmons and M. F. Hawthorne, This Journal, **78**, 5593 (1956).

[14] This relation was derived by L. J. Schaad, Ph.D. Thesis in Organic Chemistry, M.I.T., January, 1957.

[15] However, tunneling has been said to occur in a ketone enolization catalyzed by fluoride ion (though not when catalyzed by water or chloroacetate ion) by R. P. Bell, J. A. Fendley and J. R. Hulett, *Proc. Roy. Soc.* (*London*), **A235**, 453 (1956).

[16] S. Glasstone, K. J. Laidler and H. Eyring, "The Theory of Rate Processes," McGraw-Hill Book Co., Inc., New York, N. Y., 1941, p. 174, eq. 92a, and p. 189, eq. 140; M. Dole, "Introduction to Statistical Thermodynamics," Prentice-Hall, Inc., New York, N. Y., 1954, pp. 114, 164.

$$\left(\frac{k_{\mathrm{H}}}{k_{\mathrm{T}}}\right) = \alpha \left(\frac{k_{\mathrm{H}}}{k_{\mathrm{D}}}\right)^{1.442}$$

$$\alpha = \frac{\prod_{j=1}^{2} \gamma(\beta_j{}^*)}{\prod_{j=1}^{3} \gamma(\beta_j)}$$

$$\gamma(\beta) = \frac{(1-\beta)(1-\beta^{\sqrt{m_3/m_1}})^{0.442}}{(1-\beta^{\sqrt{m_3/m_2}})^{1.442}}$$

$$\beta = e^{-h\nu_3/kT}$$

The function $\gamma(\beta)$ ranges only from 1.00 (at $T = 0$, $\beta = 0$) to 0.952 (at $T = \infty, \beta = 1$). Therefore if only two vibrations in the transition state and three in the ground state are involved, α cannot exceed $(1.00)^2/(0.952)^3 = 1.157$ nor be less than $(0.952)^2/(1.00)^3 = 0.907$. These are shown in Fig. 2 along with the low temperature relation.

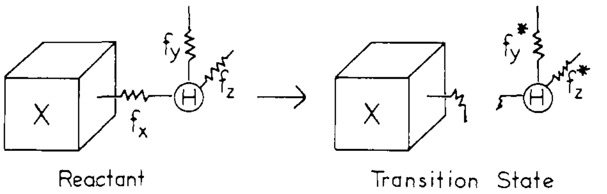

Fig. 1.—Model for comparison of isotope effects with different hydrogen isotopes. The cleavage of the $X-H^i$ bond is pictured for the protium isotope.

At extremely high temperatures where $h\nu/kT < 1$ (where the vibrations are classical), the isotope effect should be simply the inverse ratio of square roots of the effective masses for motion along the reaction coördinate, *i.e.* close to $\sqrt{3}$ for $k_{\mathrm{H}}/k_{\mathrm{T}}$ and $\sqrt{2}$ for $k_{\mathrm{H}}/k_{\mathrm{D}}$.

Experimental Comparison of Tritium and Deuterium Isotope Effects.—It seemed desirable to test equation 7 experimentally for a system where isotope effects are large and accurately measurable. Accordingly we compared the rate of racemization of α-phenylisocaprophenone (I), the rate of deuterium exchange between α-phenylisocaprophenone-α-*d* (II) and water, and the rate of tritium exchange between α-phenylisocaprophenone-α-*t* (III) and water, all under the same conditions in aqueous solution with 8.4 *M* dioxane, 1:1 acetic acid–sodium acetate buffers and 0.1 *M* ionic strength (sodium chloride) at 97.8°. These are conditions under which the assumptions seem to be justified. Furthermore, only one species, acetate ion, catalyzes the reaction significantly in this system. The deuterium isotope effect was 5.02, from which the calculated tritium isotope effect is 10.2. The observed tritium isotope effect was also 10.2.

There is one other accurate comparison of tritium and deuterium isotope effects in the literature, in a different kind of reaction where the isotope effects are numerically much smaller. Kaplan and Wilzbach have measured both the deuterium isotope effect and the tritium isotope effect in the hydrolysis of triphenylsilane at 25°.[17] From the observed deuterium isotope effect of 1.152, the calculated tritium isotope effect is 1.23. The observed tritium isotope effect was 1.26, again in good agreement.

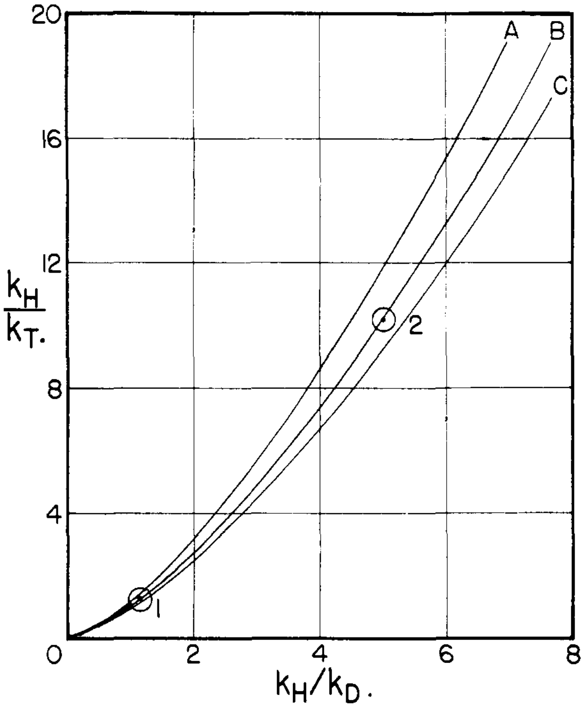

Fig. 2.—Tritium isotope effect *vs.* deuterium isotope effect: A, maximum value of $k_{\mathrm{H}}/k\mathrm{T}$; B, low temperature relation; C, minimum value based on model in Fig. 1; 1, experimental point for hydrolysis of triphenylsilane; 2, experimental point for enolization of α-phenylisocaprophenone catalyzed by acetate ion.

Experimental[3]

D(+)-α-Phenylisocaprophenone (I).—This compound is easily purified by recrystallization from absolute ethanol. A 0.1 *M* solution has an observed rotation of over 8° in a 2-dm. tube. It is completely soluble in 72% dioxane even with strong electrolytes present up to 0.2 *M* ionic strength. It can be prepared in 52% over-all yield from natural leucine by the procedure below.

L(−)-leucine (100 g., 0.76 mole, U.S.P., Nutritional Biochemicals Corp.) was placed in a 1-liter flask with 600 ml. of absolute ethanol. Anhydrous hydrogen chloride was bubbled into the reaction solution, which was constantly stirred with a Teflon-covered magnetic stirring bar. The leucine went into solution immediately, but the hydrogen chloride gas was allowed to bubble through the solution until it was no longer absorbed, or about two hours. The reaction solution was then evaporated nearly to dryness on a steam-bath, 500 ml. of reagent ethyl ether was added and the mixture refluxed. The ether-alcohol was decanted from the remaining solid residue and filtered into a 1-liter erlenmeyer flask. The flask was placed in a rock salt–ice-bath and the ester hydrochloride crystallized out and filtered. The mother liquor was returned to the residue in the reaction flask, 10 ml. of ethanol and 200 ml. of ether were added, and this mixture was refluxed. This time no solid residue was left. The solution was filtered, chilled in a rock salt-ice-bath, and the crystalline L(+)-leucine ethyl ester hydrochloride filtered, m.p. 134.6–135.0°, 124.3 g., 83%, $[\alpha]^{26}\mathrm{D}$ +19.8° (2% solution in absolute ethanol).

Bromobenzene (454 g., 2.9 moles, Merck reagent) was added slowly to a three-necked, 3-liter flask fitted with dropping funnel, Hershberg stirrer and reflux condenser which contained 70 g. (2.9 moles) of oven-dried magnesium turnings and 2 liters of anhydrous reagent ethyl ether. After addition was complete, stirring was continued for 3 hours more. The leucine ester hydrochloride (102.7 g.,

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[17] L. Kaplan and K. E. Wilzbach, THIS JOURNAL, **77**, 1297 (1955).

0.526 mole) was added slowly from an erlenmeyer flask through a wide rubber (Gooch) tube with stirring at a rate which caused gentle refluxing. After addition was complete, the reaction mixture was allowed to stand overnight. It was hydrolyzed by pouring it slowly over 600 g. of ammonium chloride and 2 kg. of ice in a 10-liter beaker. The combined ether extracts (6 liters) were evaporated and the residue crystallized from ligroin (b.p. 90–100°), with final cooling to 0°. The fluffy crystals were placed in a vacuum desiccator overnight. The yield of L(−)-α-(I-aminoisoamyl)-benzohydrol was 110.7 g., 78.2%, based on the ester hydrochloride used, m.p. 134.6–135.3°; Kanao and Yaguchi[10] report m.p. 133°.

Anal. Calcd. for $C_{18}H_{23}ON$: C, 80.25; H, 8.61; N, 5.20. Found: C, 80.40; H, 8.57; N, 5.19.

L-(−)-α-(1-Aminoisoamyl)-benzohydrol (103 g., 0.383 mole) was dissolved in 2 liters of 25% acetic acid solution with stirring in a 5-liter, three-necked, round-bottomed flask, fitted with a Hershberg stirrer and dropping funnel and cooled by an ice–water bath. A solution of sodium nitrite (33.3 g. in 150 ml. of distilled water) was added dropwise with continued stirring and cooling. After a further half hour of stirring and cooling, the reaction mixture was allowed to stand overnight at room temperature. The ketone was extracted with three 300-ml. portions of ether. The ether extracts were evaporated to dryness on the steam-bath. The isocaprophenone was recrystallized three times from absolute ethanol. The yield of D(+)-α-phenylisocaprophenone melting at 50.4–50.6° was 76.7 g. or 80% based on amino alcohol used, $[\alpha]^{25}\text{D}$ +160.5° (absolute ethanol); Bell[10] reports m.p. 53° and $[\alpha]^{20}\text{D}$ 150° (absolute ethanol), 138° (nitrobenzene), 167° (benzene), 212° (amyl acetate); Kanao and Yaguchi[10] report m.p. 33–36° and $[\alpha]^{20}\text{D}$ 109.18° (acetone).

Anal. Calcd. for $C_{18}H_{20}O$: C, 85.67; H, 7.99. Found: C, 85.80; H, 7.85.

DL-α-Phenylisocaprophenone-α-*d* (II).—For comparison of deuterium and tritium isotope effects, deuterated ketone was prepared by refluxing 25 g. of I with 0.1 *M* sodium monoxide in a mixture of 300 ml. of dioxane and 40 ml. of 99.5% deuterium oxide for 4 days. The ketone was isolated by the same procedure used in the preparation of I, and recrystallized four times from absolute ethanol. The deuterium content was 4.56 atom % determined by combustion (see below).

DL-α-Phenylisocaprophenone-α-*t*-(III).—A portion of I (50 g.) was placed in a 2-liter round-bottomed flask containing 30 ml. of concentrated hydrochloric acid and one liter of dioxane and fitted with a reflux condenser. Tritiated water (25 ml. containing 75 millicuries of tritium) was then added and the mixture refluxed for 7 days. The ketone was isolated as before and recrystallized from absolute ethanol three times. The yield of III melting at 77.6–77.8° was 38.0 g. or 76%. It has a specific activity of 7.70 microcuries per millimole.

For comparison of deuterium and tritium isotope effects, tritiated ketone was prepared by refluxing 25 g. of I with 12 ml. of 0.1 *M* sodium monoxide in tritiated water containing 25 millicuries of tritium for 4 days. The ketone was isolated and purified as before. The yield was 16.9 g. (67.6%), m.p. 77.5–77.7°, 8.01 microcuries per millimole.

Other Reactants.—Du Pont sodium monoxide was used in the preparation of deuterated and tritiated ketone. Other chemicals were analytical reagent grade. Sodium chloride was dried for 12 hours at 380°. Sodium hydroxide solutions were carbonate-free (50% solutions filtered and diluted with freshly boiled distilled water). The acetate buffer solutions were made from analytical reagent acetic acid and carbonate-free sodium hydroxide solution.

Dioxane ("pure" commercial grade) was purified by refluxing 5 liters with 100 g. of sodium for 160 hr., then distilling through a 100-cm. column packed with glass helices, b.p. 103–104°. It was stored in frozen condition in glass-stoppered bottles sealed with paraffin at 3°.

Infrared Spectra of I and II.—The light and deuterated ketones were examined separately in carbon tetrachloride solution in a Baird model 100 infrared spectrophotometer by Messrs. H. Rao and M. Evans using rock salt cells and lithium fluoride optics. The only apparent differences were a peak at 3003 cm.$^{-1}$ in I only and a peak at 2140 cm.$^{-1}$ in II only. These peaks are probably due to C–H and C–D stretching. Their ratio is 1.403, close to the theoretical ratio of 1.414 for a classical harmonic oscillator.

Evidence for No Exchange with Acetic Acid.—After four months at 98°, the contents of a reaction vial from run 61 (with 0.50 *M* acetic acid) was extracted with two 20-ml. portions of 1% sodium hydroxide solution. The caustic extract was evaporated to dryness on a steam-bath. The solid residue was mixed with 10 ml. of water and filtered. The filtrate was acidified until just acid to litmus and to it was added 5 ml. of a 10% solution of *p*-phenylphenacyl bromide in ethanol. The resulting mixture was heated under reflux for one hour. Upon cooling overnight at 0°, *p*-phenylphenacyl acetate separated. It was recrystallized from ethanol, m.p. 110–112°, reported[18] 111°. The specific activity of this sample was less than 10^{-5} microcurie per millimole.

Analysis of Products.—After 6 months at 98° (100 half-lives) the contents of a reaction vial from run 40 (with 0.04 *M* hydrochloric acid) was extracted with two 20-ml. portions of anhydrous ether. The ether extracts were evaporated to dryness. The residue was taken up with hot ethanol and filtered. The filtrate was left overnight at 0° after which the DL-ketone separated out as crystals. It was recrystallized twice from absolute ethanol and assayed for tritium. It had a molar activity of 4.90×10^{-2} microcurie per millimole. The water portion of the ether extraction was treated with helium as described below and assayed for tritium. The water had a molar activity of 9.75×10^{-2} microcurie per millimole. Since the original ketone assayed at 15.40 microcuries per millimole and the concentration of ketone was 0.1000 *M*, the concentration of water in this reaction mixture was 15.7 *M*. Because the molar activity of the water was 1.99 times that of the ketone (there are two exchangeable hydrogens on water compared to one on the ketone), there is no isotope fractionation in this reaction.

Analysis for Tritium.—The procedure of Wilzbach, Kaplan and Brown was used.[19] This involves conversion of the tritium in an organic compound to a mixture of methane and hydrogen for counting in an ionization chamber by heating with zinc, nickelic oxide and water in a sealed tube of special Pyrex glass no. 1720 at 640 ± 10° for three hours. They demonstrated that results by this method agree satisfactorily with results from combustion and subsequent reduction of water to hydrogen. We used bombs prepared from 17 mm. o.d. tubing, because it was more readily available than the 11 mm. recommended. It was found necessary to use zinc dust rather than granulated zinc since, in initial calibration using samples of water of 1.36 microcuries per millimole, reduction was found incomplete with granulated zinc. Tank hydrogen was used to fill the ionization chamber to atmospheric pressure rather than methane. The mole % of methane was calculated from the measured volume of gas from the sample and the millimoles of hydrogen that it was known to contain. The increase in counting rate due to the methane was calculated from data obtained in hydrogen and in methane containing 7 mole % hydrogen,[20] and the specific activity corrected accordingly. This correction was always less than 2%.

Kinetic Procedure.—All reaction solutions were initially 0.100 *M* ketone and 8.4 *M* dioxane. The ionic strength in all runs was initially 0.10 *M* as a result of adding the required amount of sodium chloride. The ketone and dioxane were introduced as a dioxane stock solution of 34.10 g. of ketone per liter of solution. The other components (about 26% by volume before mixing) were added as aqueous solutions. A typical acetate buffer run was made up as follows. To 37.00 ml. of the stock solution of ketone in a 50-ml. volumetric flask was added 8.00 ml. of 1.5 *M* acetic acid–0.5 *M* sodium acetate solution, 2.00 ml. of 0.5 *M* sodium chloride solution and distilled water up to 50 ml. at 25°. The dioxane solution introduced actually contained 35.74 ml. of dioxane. The solution was shaken thoroughly and pipetted into drawn-out Pyrex test-tubes. The top parts of the test-tubes were drawn off with a gas–oxygen flame and the bulbs sealed. The volumes of solution in the bulbs did not have to be measured exactly, but were about 4–5 ml. for racemization, 10 ml. for deuterium exchange and 5 ml. for

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[18] N. L. Drake and J. Bronitsky, This Journal, **52**, 3715 (1930).

[19] K. E. Wilzbach, L. Kaplan and W. G. Brown, *Science*, **118**, 522 (1953).

[20] W. A. Sheppard, Ph.D. Thesis in Organic Chemistry, M.I.T., November, 1954, p. 136.

tritium exchange. It was shown[2] that it made no difference whether Pyrex or soft glass tubes were used. The bulbs were placed in an oil-bath at 97.8 ± 0.2°. At various times, they were removed from the thermostat, cooled immediately to room temperature, and the solution was investigated polarimetrically or the water isolated and assayed for deuterium or tritium increase.

For racemization kinetics, a 5-ml. hypodermic syringe fitted with a 9-inch 20-gage needle was used to fill a 2-dm., 2.3-ml. polarimeter tube with the solution. The polarimetric readings were taken with a Schmidt and Haensch polarimeter, using a Cenco sodium arc lamp with no filter. It was estimated that the polarimetric readings were accurate to 0.02°. Readings were taken in a room where temperature fluctuated between 23 and 26°.

For racemization studies using sodium hydroxide as a catalyst, Teflon bombs were used. Runs made in Pyrex glass bulbs were erratic at best and titration with hydrochloric acid indicated that no base was left in aliquots removed after one hour in the constant-temperature bath. The Teflon bombs were thoroughly flushed with purified nitrogen before filling with 5 ml. of the reaction solution. The solution was placed in the tubes using a pipet preflushed with nitrogen. Before the Teflon caps were put in place, nitrogen gas was flushed over the solutions. The bomb with cap was then placed in an aluminum jacket which was machined so that the bomb and cap would fit snugly inside. A brass disk was placed over the cap and the apparatus was clamped in a brass vise-like device to seal the cap by compression against the bomb tube. The assembled bombs with clamps were placed in the bath. After 10 minutes the first bomb was removed and a stop watch started.

The first-order rate constant was calculated by plotting log $(\alpha - \alpha_\infty)$ *vs.* t, where α is observed rotation, α_∞ is rotation at infinite time, and t is time. In most runs the rotation at the start (α_0) was about 8° and at infinite time (α_∞) was 0.15 ± 0.02°. The plot was inspected for the best straight line by use of a straight edge. The rate constant was calculated using the equation $k_1t = 2.303 \log [(\alpha_0 - \alpha_\infty)/(\alpha - \alpha_\infty)]$.

The deuterium and tritium exchange kinetics were carried out exactly as the racemization studies except that DL-α-phenylisocaprophenone (II or III) was used instead of the optically active ketone I and the rate of exchange of heavy hydrogen from ketone to solvent was measured. After each bulb or bomb was opened, its contents were placed in a 125-ml. separatory funnel which contained the 20-ml. of anhydrous ethyl ether for the 5-ml. aliquots or 40 ml. for the 10-ml. aliquots. The contents of the funnel were shaken vigorously four times and the water layer then drawn off into another 125-ml. separatory funnel which again contained 20 ml. (or 40 ml.) of ether. The contents were shaken four times and the water layer was transferred by means of a tuberculin syringe fitted with a 6-inch, 20-gage needle, to a small glass vial. The vial was placed in a silicone oil-bath at 80° and specially purified helium gas was passed over the surface of the water in the vial for 2 min. to ensure complete removal of any ether from the water layer. The vials were then sealed and labeled. The deuterium or tritium content of the water at infinite time was determined by making up a reaction mixture with 0.1 M hydrochloric acid as the catalyst, letting it exchange for 6 weeks at 97.8° and then analyzing with water.

For deuterium analysis, the water samples were purified by what was essentially Fetcher's modification[21] of the procedure described by Keston, Rittenberg and Schoenheimer.[22] The distillation train used for purification is shown in Fetcher's Fig. A few mg. of calcium oxide and a few mg. of potassium permanganate were placed in trap B, and the system was assembled using Apiezon M stopcock grease on all the ground-glass joints. The system was evacuated to approximately 0.01 mm., and trap B was heated with a microburner in order to dry the potassium permanganate and the calcium oxide. Air was admitted through the drying tower, and approximately 0.5 ml. of the water to be purified was placed in trap A. The system was again evacuated, and the water was allowed to distil into trap B which had been immersed in an acetone–solid carbon dioxide mixture. To accelerate the distillation, the trap from which the water was being distilled was always immersed in a beaker of water at room temperature. After all the water was collected in B, air was admitted. The chilling bath was removed from B, and trap A was replaced by a ground-glass stopper. The water in B was boiled gently by heating with a microburner. Trap C was now chilled, the system evacuated, and the water allowed to distil into C. After the distillation was complete, air was admitted, and B was replaced by a glass plug. The system was again evacuated and the water allowed to distil successively into chilled traps D and E.

After use, the trap containing potassium permanganate and calcium oxide was rinsed with water, then with a sodium sulfite–sulfuric acid solution, and finally seven times with distilled water. Trap E was rinsed with an approximately 5% hydrofluoric acid solution and then ten times with distilled water. All the other traps were rinsed seven times with distilled water after every use and were cleaned occasionally with hydrofluoric acid solution.

The purified water samples were analyzed for deuterium by the falling-drop method.[23] Briefly, the falling-drop apparatus consists of a graduated dropping tube containing *o*-fluorotoluene, a micropipet to deliver drops of uniform size into the dropping tube and a thermostat to maintain the temperature of the dropping tube to ±0.001°. Six drops of each sample to be analyzed were allowed to fall through the dropping tube, and each drop time between two lines 15 cm. apart was recorded with stopwatches. A standard of distilled water, purified in the same manner as the samples, was dropped with each series of samples. The temperature of the thermostat was regulated near 27° so that the drop time of the standard was approximately 140 seconds.

The falling-drop apparatus was calibrated by measuring the deuterium content of solutions of known concentrations. These solutions were made by diluting 99.5% deuterium oxide with distilled water which had been purified in the same manner as the unknown water samples. A calibration chart was made by plotting the difference in the reciprocals of the times of sample and standard *vs.* % deuterium.

A sample of II was also burned to water to determine its deuterium directly. A combustion train similar to that described by Keston, Rittenberg and Schoenheimer[22] was used. Tank oxygen, purified by passing it successively through a drying tower, sulfuric acid, and an acetone–solid carbon dioxide trap, was allowed to flow through a combustion tube packed with cupric oxide at 780°. The sample was placed in the combustion tube just before the heated cupric oxide. The sample was burned by means of a movable furnace heated to 650°. The water collected at the end of the train in a trap immersed in an acetone–solid carbon dioxide-bath. The water was weighed on a microbalance and diluted with a weighed amount of purified distilled water. The resulting sample was purified and analyzed for deuterium as described above.

The procedure for tritium assay used in this work has been described.[24] Essentially the technique was to reduce about 18 mg. of water with magnesium amalgam and then to measure monometrically the yield of tritiated hydrogen gas formed. A portion of this gas was then placed in an ionization chamber and the activity of the gas determined by measuring the rate of change of voltage with time using a vibrating reed electrometer. The factors for converting to molar activities were derived from assay of analyzed tritiated water from Tracerlab, Inc., Boston, Mass. The first-order rate constant was calculated by using the equation $k_1t = 2.303 \log [(1 - F_0)/(1 - F)]$ where F_0 is the fraction exchanged at zero time and F is the fraction exchanged at time t. The activity of the water at infinite time was generally 3.28×10^{-2} microcurie per millimole. Since the half-time for the β-decay of tritium is in the order

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[21] E. S. Fetcher, Jr., *Anal. Chem.*, **16**, 412 (1944).

[22] A. S. Keston, D. Rittenberg and R. Schoenheimer, *J. Biol. Chem.*, **122**, 227 (1937).

[23] C. G. Swain and M. M. Labes, THIS JOURNAL, **79**, 1087 (1957); for further details *cf.* reference there cited and J. T. McKnight, Ph.D. Thesis in Organic Chemistry, M.I.T., September, 1953, pp. 70–85; M. M. Labes, Ph.D. Thesis in Organic Chemistry, M.I.T., February, 1954, pp. 32–41; and J. F. Reuwer, Jr., M.S. Thesis in Organic Chemistry, M.I.T., October, 1956, pp. 18–20.

[24] C. G. Swain, V. P. Kreiter and W. A. Sheppard, *Anal. Chem.*, **27**, 1157 (1955); for further details on tritium assay *cf.* W. A. Sheppard, Ph.D. Thesis in Organic Chemistry, M.I.T., November, 1954, pp. 125–156; E. C. Stivers, Ph.D. Thesis in Organic Chemistry, M.I.T., May, 1956, pp. 66–68, 87–92, 96–97, 100–101.

of 13.2 years and since for some runs the duration between zero time and the last point was 0.3 year, the radioactive assays on the water samples for any given run were done within a period of one week to avoid corrections for tritium decay.

The kinetics of isotope exchange was considered in a previous paper.[25] In the present study the ratio of concentrations of water to ketone is large, *viz.*, 157. The unlabeled hydrogens (protiums) are present in enormous concentrations relative to the labeled hydrogens (tritiums). There is no significant isotope fractionation at equilibrium (*cf.* "Analysis of Products" section above). In addition, all of the species reacting with the ketone are true catalysts, *i.e.*, regenerated by the reaction. Thus four conditions are present in any one of which by itself is sufficient for first-order kinetics. Therefore it is not surprising that first-order kinetics was found.

Kinetic Data.—Table VI summarizes the kinetic data.[3]

TABLE VI

KINETIC DATA FOR ENOLIZATION OF 0.1 M α-PHENYLISOCAPROPHENONE IN AQUEOUS DIOXANE[a] AT 97.8°

Catalyst	M	k_r[b] × 10⁶, sec.⁻¹	k_e[c] × 10⁶, sec.⁻¹
H^+	0.0100	2.31 ± 0.14	....
H^+	.0200	4.92 ± .27	0.520 ± 0.008
H^+	.0400	10.1 ± .25	1.053 ± .042
H^+	.0600	15.4 ± .3	1.609 ± .036
H^+	.0800	20.8 ± .9	....
H^+	.100	26.5 ± .9	....
HO^-	.0025	108 ± 3	....
HO^-	.0037	230 ± 1	....
HO^-	.0050	354 ± 11	25.7 ± 2.5
HO^-	.0075	605 ± 23	43.0 ± 3.6
HO^-	.0100	852 ± 17	64.1 ± 4.1
AcO^-[d]	.0100	0.083 ± 0.011	0.0112 ± 0.0007[e]
AcO^-[d]	.0200	.192 ± .009	.0201 ± .0011[f]
AcO^-[d]	.0400	.406 ± .031	....
AcO^-[d]	.0600	.634 ± .018	.0687 ± .0016[g]
AcO^-[d]	.0800	.780 ± .025	.0912 ± .0055[h]
AcO^-[d]	.100	1.04 ± .057	.1145 ± .0077[i]
HOAc[j]	.500	.0453 ± .0029	.00394 ± .00010
HOAc[j]	.750	.0668 ± .0028	.00556 ± .00034
HOAc[j]	1.00	.0890 ± .0048	.00778 ± .00028

[a] See section on "Kinetic Procedure" for exact composition of solvent. [b] First-order rate constant for racemization of I at the stated catalyst concentration. [c] First-order rate constant for exchange of III at the stated catalyst concentration. [d] A concentration of acetic acid equal to that of the acetate ion also was added. [e] 0.0112 M in both acetic acid and acetate ion (instead of 0.100 M). [f] 0.0224 M in both. [g] 0.0672 M in both. [h] 0.0896 M in both. [i] 0.112 M in both; ionic strength 0.112 M in this run only. [j] No acetate ion was added.

In all cases, plots of the first-order rate constants (k_1) in Table VI *vs.* concentration of catalyst are linear. Accordingly, the catalytic coefficients (k) recorded in Table IV were obtained by application of the method of least squares to the data given in Table VI.

$$\Sigma[1k_1 - a - k\ (\text{catalyst})]^2 = \text{minimum}$$

The intercepts (a) were very small and usually negative: -3.7×10^{-7} and -2.8×10^{-8} for H^+, -1.4×10^{-4} and -1.2×10^{-5} for HO^-, -2.0×10^{-9} and -2.4×10^{-10} for AcO^-, and $+1.5 \times 10^{-9}$ and $<1 \times 10^{-11}$ for HOAc for k_H and k_T, respectively. Therefore the catalysis due to solvent alone cannot be estimated.

An attempt was made to measure directly the rate of racemization in 0.10 M sodium chloride. The rotation of the solution changed only from 6.90 ± 0.02° initially to 6.91 ± 0.01° after five weeks at 98°, hence the first-order rate constant is less than 10^{-10} sec.⁻¹.

The intercepts were most negative for the reactions with hydroxide ion. We believe that this was due to absorption of small amounts of carbon dioxide by the very dilute sodium hydroxide solutions used in spite of precautions to exclude carbon dioxide at all stages. This is unlikely to seriously affect the isotope effect calculated for hydroxide ion for the following reason. The plots of first-order rate constant *vs.* concentration of hydroxide ion are linear and have the same intercept on the concentration axis (0.0015 M) for both k_H and k_T. This appears to require the assumption that the same amount of carbon dioxide entered each solution (perhaps with the dioxane or from the Teflon bombs) and that carbonate has negligible catalytic activity compared to hydroxide.

To prove that the rates with only acetic acid added are valid measures of the term k_{HOAc}(HOAc) and do not include significant contributions from $k_{H^+}(H^+)$ or $k_{AcO^-}(AcO^-)$, we did four runs with constant acetic acid (1 M) but varying low concentrations of added acetate ion (0.00000, 0.00047, 0.00065 and 0.00147 M). The rate constant k_1 had a trend which *increased* with increasing acetate ion (8.90×10^{-8}, 9.33×10^{-8}, 9.31×10^{-8}, 10.0×10^{-8} sec.⁻¹). The increase is within a factor of two in each case of that expected for catalysis by the acetate ion added, using the catalytic coefficient for acetate ion determined with high concentrations of acetate ion. Precise agreement is not expected because small differences are involved and these magnify the experimental error. However, if the ionization constant for acetic acid in this medium had been large enough to give significant catalysis by H^+, the first effect of adding acetate ion would have been to *decrease* the rate, because k_{H^+} is 25 times larger than k_{AcO^-}. For example, if K_{HOAc} were as high as 10^{-8}, k_1 would have reached a minimum at 0.00050 M acetate with a k_1 1.7×10^{-8} sec.⁻¹ below k_1 with no added acetate, and with 0.00257 M acetate it would only just equal the value with no added acetate. We conclude that K_{HOAc} in this medium is of the order of 10^{-9} or less and that the catalysis observed with no added acetate is very predominantly due to acetic acid in the relatively concentrated

TABLE VII

RACEMIZATION OF 0.1 M D(+)-α-PHENYLISOCAPROPHENONE IN AQUEOUS DIOXANE WITH ACETIC ACID–ACETATE BUFFERS AT 97.8°

(AcO^-), M	(HOAc)/(AcO^-)	k_1 × 10⁶, sec.⁻¹
0.067	1	0.692
.112[a]	1	1.17
.01	2	0.092
.02	2	.193
.04	2	.411
.06	2	.652
.08	2	.827
.10	2	1.07
.01	3	0.096
.02	3	.197
.04	3	.418
.06	3	.657
.08	3	.856
.10	3	1.09
.003	10	0.030
.006	10	.062
.012	10	.118
.018	10	.179
.024	10	.242
.030	10	.321

[a] Ionic strength 0.112 M in this run only.

TABLE VIII

KINETIC DATA FOR ENOLIZATION OF 0.1 M α-PHENYLISOCAPROPHENONE IN AQUEOUS SOLUTION WITH 8.4 M DIOXANE, 1:1 ACETIC ACID-ACETATE ION BUFFERS AND 0.1 M IONIC STRENGTH AT 97.8°

(AcO^-), M	DL-Ketone, k_r × 10⁶, sec.⁻¹	Ketone-α-d, k_d × 10⁶, sec.⁻¹	Ketone-α-t, k_e × 10⁶, sec.⁻¹
0.030	...	0.0573	....
.050	...	.0985	....
.060	0.616	....	0.0614
.070	...	.1400	....
.080	.835	....	.0811
.100	1.41	.2047	.1029

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[25] C. G. Swain and M. M. Labes, THIS JOURNAL, **79**, 1084 (1957).

solutions of acetic acid which were used, with contributions from hydrogen ion very minor and contributions from acetate ion still less important by a factor of 25.

Catalysis by acetic acid (or by hydrogen ion) is negligible in the 1:1 acetic acid–acetate ion buffers used for evaluating the catalytic coefficient for acetic ion, because doubling the acetic acid concentration on going to a 2:1 buffer ratio does not significantly change the rate. Table VII shows this. The data of Table VII were not used in evaluating the catalytic coefficients.

Only in the plots of first-order rate constant for 10:1 acetic acid–acetate ion buffers *vs.* buffer concentration could any curvature be observed, and it was too slight to permit the coefficient of the product term $k_p(\text{HOAc})(\text{AcO}^-)$ to be calculated to even one significant figure. No curvature was observed in the plots for 1:1, 2:1 or 3:1 buffers nor in the plots *vs.* H_3O^+, HO^- or HOAc. However, these results are compatible with a coefficient for the product term of the order of 10^{-8} M^{-2} sec.$^{-1}$ or less.

Table VIII gives the first-order rate constants for racemization, deuterium exchange and tritium exchange. The catalytic coefficients are 10.59×10^{-6}, 2.111×10^{-6} and 1.037×10^{-6} M^{-1} sec.$^{-1}$, respectively.

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COMMUNICATIONS TO THE EDITOR

AN ACTIVE ACETALDEHYDE-THIAMINE INTERMEDIATE

Sir:

Recently, Breslow has proposed a mechanism of action of thiamine and thiamine pyrophosphate (TPP) in which an α-hydroxyethyl group at the 2 position of the thiazole moiety is considered to be the active 2-carbon component ("active acetaldehyde") in various enzymatic reactions catalyzed by TPP as well as non-enzymatic reactions catalyzed by thiamine.[1]

In continuing earlier work[2] on the mechanism of thiamine action, we have synthesized DL-3-[(2-methyl-4-amino-5-pyrimidyl)methyl]-2-(1-hydroxyethyl)-4-methyl-5-(2-hydroxyethyl)-thiazolium chloride hydrochloride (I) and determined some of its growth-promoting and enzymatic activities.

2-(1-Benzoyloxyethyl)-4-methyl-5-(2-hydroxyethyl)-thiazole was prepared by the reaction of α-(benzoyloxy)-thiopropionamide with 3-acetyl-3-chloro-1-propanol in pyridine, b.p. 200–208° (0.7 mm.), n^{23}_D 1.5672. *Anal.* Calcd. for $C_{15}H_{17}NO_3S$: C, 61.83; H, 5.88; N, 4.81. Found: C, 61.66; H, 5.96; N, 5.04. Removal of the benzoyl group by potassium hydroxide in methanol at 50° yielded 2-(1-hydroxyethyl)-4-methyl-5-(2-hydroxyethyl)-thiazole, m.p. 80–81.5° cor. (from ethyl acetate). *Anal.* Calcd. for $C_8H_{13}NO_2S$: C, 51.31; H, 7.00; N, 7.48; S, 17.12. Found: C, 51.12; H, 7.02; N, 7.58; S, 17.30. Reaction of this thiazole with 2-methyl-4-amino-5-bromomethylpyrimidine hydrobromide in dimethyl formamide at 50–55° gave I as the bromide hydrobromide. Purification and conversion to the chloride hydrochloride (I) were accomplished by fractional crystallization from a methanol–acetone solution, by paper chromatography (R_f 0.54 in an isopropyl alcohol–hydrochloric acid–water system (170 ml. isopropyl alcohol, 41 ml. concentrated hydrochloric acid, water to 250 ml.) and by picrate formation, m.p. 225° dec. (uncor.) (from methanol–ether). *Anal.* Calcd. for $C_{14}H_{22}Cl_2N_4O_2S$: C, 44.09; H, 5.82; N, 14.69; Cl, 18.60; S, 8.41. Found: (dried at 78°, 0.2 mm.); C, 44.21; H, 6.06; N, 14.77; Cl, 18.46; S, 8.60. The ultraviolet spectra of I were similar to thiamine: in 0.005 *N* HCl, λ_{max} 245–257 mμ (*E* 13,901); in phosphate buffer, *p*H 7.0, λ_{max} 228–230 mμ (*E* 13,353) and 267.5–269.5 mμ (*E* 11,747); in phosphate buffer, *p*H 8.0 λ_{max} 234–236 mμ (*E* 17,347).

The activity of I in the reactivation of carboxylase was demonstrated. Alkaline-washed yeast deficient in TPP[3] contains thiaminokinase but apparently the cells are impermeable to adenosine triphosphate (ATP) inasmuch as the carboxylase activity is not restored by thiamine and ATP. However, if phosphoenolpyruvate (PEP), adenosine monophosphate (AMP), and thiamine are added to the preparation,[4] ATP is apparently generated from PEP and the resulting pyruvate is decarboxylated to acetaldehyde and carbon dioxide. The DL-hydroxyethylthiamine (I) can be substituted for thiamine in this system with equal activity.[5] With a soluble yeast carboxylase preparation possessing thiaminokinase, preliminary data show that acetaldehyde is liberated from hydroxyethylthiamine. The growth-promoting activity[5] of I was found to be approximately 80% of the activ-

TABLE I

REACTIVATION OF CARBOXYLASE

Alkaline washed yeast 1.0 ml.; potassium phosphate buffer 0.05*M* *p*H 6.2; $MgCl_2$ 10 μmoles; total volume 2.0 ml.; time 45 minutes; temperature 30°

Additions	μmoles CO_2 evolved
PEP 10 μmoles	0
PEP 10 μmoles + AMP 3 mg.	0
PEP 10 μmoles + AMP 3 mg. + thiamine 30 μg.	8
PEP 10 μmoles + AMP 3 mg. + hydroxyethyl thiamine 30 μg.	8
Pyruvate 10 μmoles + TPP 4 μg.	9
Pyruvate 10 μmoles + thiamine 30 μg.	0
Pyruvate 10 μmoles + hydroxyethylthiamine 30 μg.	0

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[1] R. Breslow, *Chemistry and Industry*, R28 (1956); 893 (1957); THIS JOURNAL, **80**, 3719 (1958).

[2] H. Koffler and L. O. Krampitz, *J. Bacteriol.—Proceedings*, 113 (1955).

[3] K. Lohman and P. Schuster, *Biochem. Z.*, **294**, 188 (1937).

[4] H. Weil-Malherbe, *Biochem. J.*, **33**, 1997 (1939).

[5] It must be emphasized that the hydroxyethylthiamine (I) employed in these tests is the DL form and very probably only one antipode is biologically active.