Isomerization of 59 to Precapnelladiene. A magnetically stirred solution of **59** (60 mg, 0.294 mmol) and rhodium(III) chloride trihydrate (107 mg, 0.410 mmol) in absolute ethanol (20 mL) was heated at reflux for 30 h. At this point, an additional 105 mg of catalyst was introduced and heating was resumed for 42 h. The cooled mixture was filtered, a fresh 95 mg of $RhCl_3$ was added to the filtrate, and heating was resumed for a final 48 h (total reaction time of 5 days). Capillary GC analysis indicated the **59**:**1** ratio to be 1:10. The reaction mixture was poured in 15% potassium cyanide solution and extracted with purified petroleum ether (4 × 10 mL). The combined organic extracts were dried and carefully evaporated. Chromatography in the aforementioned manner provided 27 mg (45%) of pure precapnelladiene.

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Transition-State Structures for Ester Aminolysis with and without Rate-Limiting Proton Transfer[1]

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Abstract: The reaction of phenyl acetate ($CH_3CO_2C_6H_5$ and $CD_3CO_2C_6H_5$) with methoxyamine at 25 °C in water exhibits β-secondary deuterium isotope effects k_{3H}/k_{3D} of 0.857 ± 0.024 (general-acid catalysis by $CH_3O_2CCH_2CH(CO_2CH_3)NH_3^+$, k_{HOH}/k_{DOD} = 3.9 ± 0.8) and 0.867 ± 0.009 (general-acid catalysis by $CH_3ONH_3^+$, k_{HOH}/k_{DOD} = 1.75 ± 0.06). These values are consistent with rate-limiting proton transfer from general acid to a zwitterionic tetrahedral adduct and rate-limiting solvent reorganization in a complex of general acid and zwitterionic adduct (Cox, M. M.; Jencks, W. P. *J. Am. Chem. Soc.* **1981**, *103*, 572-580). The reaction of hydrazine with phenyl acetate, with general-base catalysis by hydrazine, exhibits k_{3H}/k_{3D} = 0.972 ± 0.002 (H_2O solvent) and 0.970 ± 0.009 (D_2O solvent) and k_{HOH}/k_{DOD} = 1.44 ± 0.02. This is not consistent with proton transfer from a tetrahedral adduct but rather suggests a near-trigonal structure at carbonyl in the transition state. Possibly leaving-group expulsion in a cyclic process with external stabilization by a base catalyst is rate-determining. The uncatalyzed reaction of semicarbazide with 2,4-dinitrophenyl acetate has k_{3H}/k_{3D} = 0.975 ± 0.009, also consistent with a near-trigonal transition state.

The general acid–base catalyzed aminolysis of esters (and by implication its reverse, the general catalyzed alcoholysis of amides, the reaction catalyzed by serine–hydrolase enzymes) is thought, on the basis of an analysis of structure–reactivity relationships and kinetic isotope effects by Jencks and his co-workers,[2] to involve for certain structures and conditions rate-limiting proton transfers between general catalyst and the zwitterionic tetrahedral intermediates (**1** and **2**). Such processes follow special mechanisms in order for the actual proton-switch itself to become kinetically significant, as opposed to the slower diffusional steps which precede and succeed proton transfer. Examples of these special mechanisms include generation of tetrahedral adducts within a solvent cage already containing the general acid or base, perhaps hydrogen bonded to one of the reaction partners (pre-association), or the occurrence of a further chemical reaction, after proton transfer, more rapidly than diffusion apart of the proton-transfer products (spectator catalysis). Other general-catalyzed reactions which do not meet such criteria may have the diffusional steps of the proton-transfer sequence rate-limiting, or there may be, in the rate-limiting transition state, one form or another of coupling between the proton transfer and the formation or fission of heavy-atom bonds.

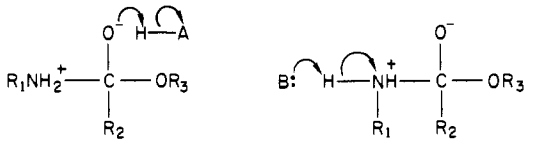

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Table I. Observed First-Order Rate Constants k_0^{3L} for Reaction of Phenyl Acetate ($C_6H_5OCOCL_3$, L = H, D) with Methoxyamine (0.055 M) and Methoxyammonium Ion (0.045 M) in the Presence of Aspartic Acid Dimethyl Ester Conjugate Acid (HA,DA) in H_2O and D_2O Solvents, μ = 1.0 (KCl), 25.00 ± 0.05 °C

[HA] or [DA], M	$10^6k_0^{3H}$, s^{-1}	$10^6k_0^{3D}$, s^{-1}	k_0^{3H}/k_0^{3D}
	Solvent H_2O, pH 4.80[a]		
0.08	2.95, 2.97	3.22, 3.38	0.895
0.16	3.28, 3.22	3.76, 3.78	0.861
0.32	3.57, 3.66	4.34, 4.23	0.849
0.48	3.92, 4.07	4.74, 4.63	0.853
0.64	4.44, 4.45	5.11, 5.21	0.862
0.80	4.89, 4.94	5.60, 5.64	0.874
	Solvent D_2O, pD 5.30[b]		
0.08	1.49 ± 0.10		
0.16	1.76 ± 0.03		
0.32	1.84 ± 0.06		
0.48	1.86 ± 0.01		
0.64	1.87[c]		
0.80	2.09 ± 0.08		

[a] Reactions of protiated and deuterated substrates measured in alternation with the same solutions. [b] Except as indicated, rate constants are the mean of four measurements. [c] Single measurement.

(1) The research was supported by the National Institute of General Medical Sciences through research Grant No. GM-20198.

(2) (a) Kirsch, J. F.; Jencks, W. P. *J. Am. Chem. Soc.* **1964**, *86*, 843. (b) Jencks, W. P.; Gilchrist, M. *J. Am. Chem. Soc.* **1968**, *90*, 2622. (c) Blackburn, G. M.; Jencks, W. P. *J. Am. Chem. Soc.* **1968**, *90*, 2638. (d) Fersht, A. R.; Jencks, W. P. *J. Am. Chem. Soc.* **1970**, *92*, 5442. (e) Jencks, W. P. *J. Am. Chem. Soc.* **1972**, *94*, 4731. (f) Jencks, W. P. *Chem. Rev.* **1972**, *72*, 705. (g) Gravitz, N.; Jencks, W. P. *J. Am. Chem. Soc.* **1974**, *96*, 499. (h) Fox, J. P.; Jencks, W. P. *J. Am. Chem. Soc.* **1974**, *96*, 1436. (i) Satterthwait, A. G.; Jencks, W. P. *J. Am. Chem. Soc.* **1974**, *96*, 7018. (j) Jencks, W. P. *Acc. Chem. Res.* **1976**, *9*, 425. (k) Hupe, D. J.; Jencks, W. P. *J. Am. Chem. Soc.* **1977**, *99*, 451. (l) Gresser, M. J.; Jencks, W. P. *J. Am. Chem. Soc.* **1977**, *99*, 6963 and 6970. (m) Fox, J. D.; Jencks, W. P. *J. Am. Chem. Soc.* **1978**, *100*, 2077. (n) Cox, M. M.; Jencks, W. P. *J. Am. Chem. Soc.* **1978**, *100*, 5956. (o) Cox, M. M.; Jencks *J. Am. Chem. Soc.* **1981**, *103*, 572.

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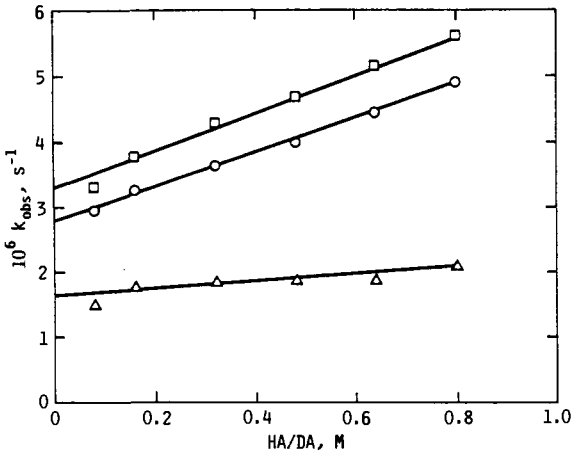


Figure 1. Dependence of the first-order rate constants of methoxyaminolysis of phenyl acetate ($C_6H_5OCOCL_3$; L = H, D) on the concentration of the conjugate acid of aspartic acid dimethyl ester in H_2O: HA, L = H (○), L = D (□) and in D_2O; DA, L = H (△), at 25.00 ± 0.05 °C, μ = 1.0 (KCl). The solutions contained methoxyamine (0.055 M) and methoxyammonium ion (0.045 M) in all cases. The data are from Table I. The lines are calculated from the parameters in Table II.

A test of this model is provided by isotope effects that measure the degree of tetrahedral character in carbonyl addition–elimination transition states, principally α-deuterium[3] and β-deuterium[4] secondary isotope effects. For example, the hydrazinolysis of methyl formate[3a] has $k_{\alpha D}$ = 0.725 ± 0.005, equal to the limiting effect for tetrahedral adduct formation[5] and thus fully consistent with rate-limiting proton transfer to hydrazine from the zwitterionic adduct of methyl formate and hydrazine.[6]

In this paper we report β-deuterium effects for several aminolysis reactions. In some cases, the expected tetrahedral character in the transition state is confirmed, in others it is not.

Results

Methoxyaminolysis of Phenyl Acetate. This reaction (eq 1, L = H, D) exhibits the first-order rate constants shown in Table I, when studied at pH 4.80 at varying levels of aspartic acid dimethyl ester conjugates acid (which we denote HA in H_2O or DA in D_2O). The corresponding forms of methoxyammonium ion we designate HS and DS. Data for D_2O solvent at pD 5.30 are also given in Table I. Figure 1 shows the data to be adequately linear in HA or DA. The rate constants of Cox and Jencks,[2o] who made a thorough study of this reaction, show that both the slopes

$$CH_3ONH_2 + C_6H_5OCOCL_3 \rightarrow CH_3ONHCOCL_3 + C_6H_5OH \quad (1)$$

and intercepts of the plots in Figure 1 are determined by general-acid catalyzed terms.[7] The slopes are equal to k_{HA} or k_{DA} for general-acid catalysis of methoxyaminolysis by aspartic acid dimethyl ester conjugate acid. The intercepts are determined to the extent of 94% by k_{HS} or k_{DS} for general-acid catalysis of the methoxyaminolysis by methoxyammonium ion. Values of the rate constants k_{HA}^{3H} and k_{DA}^{3H} are about 30% higher than those determined by Cox and Jencks under quite similar conditions, while the intercept values are about 30–35% lower. The isotope effects are the same as theirs:

$$k_{HA}^{3H}/k_{DA}^{3H} = 3.9 \pm 0.6 \text{ (Cox and Jencks: 3.8)}$$

$$k_{HS}^{3H}/k_{DS}^{3H} = 1.75 \pm 0.04 \text{ (Cox and Jencks: 1.75)}$$

Table II. Catalytic Constants for General-Acid Catalyzed Methoxyamminolysis of Phenyl Acetate ($C_6H_5OCOCL_3$, L = H, D) by Aspartic Acid Dimethyl Ester Conjugate Acid ($k_{L'A}^{3L}$) and Methoxyammonium Ion ($k_{L'S}^{3L}$) at 25 °C in L'$_2$O Solvent (L' = H, D), μ = 1.0 (KCl)

catalytic constant	magnitude,[e] M^{-2} s^{-1}
$(k_{HA}^{3H})^a$	4.74 ± 0.11 × 10^{-5}
k_{HA}^{3D}	5.55 ± 0.22 × 10^{-5}
$(k_{HS}^{3H})^b$	1.12 ± 0.01 × 10^{-3}
(k_{HS}^{3D})	1.29 ± 0.02 × 10^{-3}
$(k_{DA}^{3H})^c$	1.21 ± 0.19 × 10^{-5}
$(k_{DS}^{3H})^d$	0.64 ± 0.01 × 10^{-3}

[a–d] Reference 2o: [a] 3.52 × 10^{-5}. [b] 1.45 × 10^{-3}. [c] 0.92 × 10^{-5}. [d] 0.83 × 10^{-3}. [e] Obtained by dividing the slopes of Figure 1 by the concentration of methoxyamine as free base (0.055 M in both H_2O and D_2O) and the intercepts by the product $[CH_3ONH_2][CH_3ONH_3^+]$ = (0.055)(0.045) M^2 in both H_2O and D_2O.

Table III. Observed First-Order Rate Constants and Isotope Effects for Hydrazinolysis of Phenyl Acetate ($C_6H_5OCOCL_3$, L = H, D) at pH 8.87 in H_2O Solvent, 25.00 ± 0.05 °C, μ = 1.0 (KCl)

$[N_2H_4]_{total}$,[a] M	$10^6 k_0^{3H}$, s^{-1}	$10^6 k_0^{3D}$, s^{-1}	k_0^{3H}/k_0^{3D}
0.116	2913, 2847, 2883, 2914, 2815 av: 2874 ± 43	2952, 2944, 2939, 2927, 2892 av: 2931 ± 23	0.981 ± 0.016
0.155	4774, 4763, 4735, 4778, 4769 av: 4764 ± 17	4920, 4828, 4922, 4948, 4898 av: 4906 ± 46	0.972 ± 0.010
0.194	7272, 7189, 7135, 7120, 7065 av: 7156 ± 78	7454, 7424, 7473, 7295, 7180 av: 7340 ± 109	0.975 ± 0.018
0.232	10257, 10062, 10268, 10203, 10133 av: 10184 ± 87	10785, 10142, 10253, 10486, 10506 av: 10434 ± 249	0.976 ± 0.03
0.318	18198, 17896, 17885, 18174, 18216 av: 18074 ± 168	18270, 18375, 18665, 18619, 18985 av: 18583 ± 278	0.973 ± 0.017
0.404	29478, 28793, 28865, 29029, 28104 av: 28854 ± 497	29954, 29897, 29258, 29530, 28840 av: 29496 ± 463	0.978 ± 0.023

[a] Fraction of free N_2H_4, 0.83.

(3) (a) Bilkadi, Z.; de Lorimier, R.; Kirsch, J. F. *J. Am. Chem. Soc.* **1975**, *97*, 4317. (b) Kirsch, J. F. In "Isotope Effects on Enzyme Catalyzed Reactions"; Cleland, W. W., O'Leary, M., Northrop, D. B., Eds.; University Park Press, 1977; Chapter 4, p 100. (c) Ortiz, J. J.; Cordes, E. H. *J. Am. Chem. Soc.* **1978**, *100*, 2080. (d) do Amaral, L.; Bastos, M. P.; Bull, H. C.; Ortiz, J. J.; Cordes, E. H. *J. Am. Chem. Soc.* **1979**, *101*, 169. (e) Pohl, E. R.; Hupe, D. J. *J. Am. Chem. Soc.* **1980**, *102*, 2763.

(4) (a) Hogg, J. L. In "Transition States in Biochemical Processes"; Gandeour, R. D., Schowen, R. L., Eds.; Plenum Press: New York, 1978, Chapter 5. (b) Hogg, J. L.; Rodgers, J.; Kovach, I. M.; Schowen, R. L. *J. Am. Chem. Soc.* **1980**, *102*, 79. (c) Kovach, I. M.; Hogg, J. L.; Raben, T.; Halbert, K.; Rodgers, J.; Schowen, R. L. *J. Am. Chem. Soc.* **1980**, *102*, 1991. (d) Kovach, I. M.; Elrod, J. P.; Schowen, R. L. *J. Am. Chem. Soc.* **1980**, *102*, 7530. (e) Kovach, I. M. *J. Org. Chem.* **1982**, *47*, 2235. (f) Kovach, I. M.; Quinn, D. M. *J. Am. Chem. Soc.* **1983**, *105*, 1947. (g) Mata-Segreda, J. F. *Rev. Latinoam. Quim.* **1979**, *10*, 105. (h) Stein, R. L.; Fujihara, H.; Quinn, D. M.; Fischer, G.; Kuellertz, G.; Barth, A.; Schowen, R. L. *J. Am. Chem. Soc.* **1984**, *106*, 1457.

(5) The equilibrium isotope effect for hydration of acetaldehyde is $k_{\alpha H}/k_{\alpha D}$ = 0.73 ± 0.03: Lewis, C. A.; Wolfenden, R. V. *Biochemistry* **1977**, *16*, 4886.

(6) As discussed in ref 3a, there may be a small secondary isotope effect on the proton-transfer reaction itself, arising from electrostatic sources and perhaps as far from unity as $k_{\alpha H}/k_{\alpha D}$ = 0.93. This could lead to an equilibrium effect for addition as close to unity as 0.725/0.93 = 0.78. Under the conditions of the work in ref 3a, pH 10 and 0.2 M free hydrazine, the proton transfer is mainly to hydrazine.

(7) The slopes of Figure 1 are the k_{cat} values of ref 2o. For HA, which has a pK_a of 6.71 and is thus 98.8% present as ammonium ion at pH 4.80, $k_{cat} = k_{HA}$. The pD in DOD is shifted to 5.30, the same shift expected for the pK_a of an ammonium ion (Schowen, K. B.; Schowen, R. L. *Methods Enzymol.* **1982**, *87c*, 551), so the fraction of DA ionized will remain unchanged. The intercepts of Figure 1 should have, according to the rate constants of ref 2o, a contribution of 3.6 × 10^{-6} s^{-1} from the term (1.45 × 10^{-3} M^{-2} s^{-1})$[CH_3ONH_2][CH_3ONH_3^+]$, 0.1 × 10^{-6} s^{-1} from the term (1.9 × 10^{-6} M^{-1} s^{-1})$[CH_3ONH_2]$. The intercept is thus 94% determined by the general-acid catalysis term.

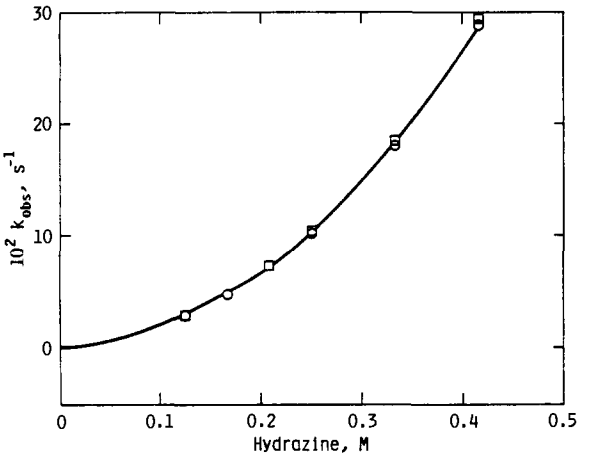


Figure 2. Dependence of the first-order rate constants of hydrazinolysis of phenyl acetate ($C_6H_5OCOCL_3$; L = H, D) on total hydrazine concentration (fraction of free base, 0.83) in H_2O, at 25.00 ± 0.05 °C, μ = 1.0 (KCl); L = H (○) and D (□). The data are taken from Table III and the line is calculated from parameters given in Table V.

This close agreement in the isotope effects suggests that the discrepancies between our slopes and intercepts and estimates from the kinetic parameters tabulated by Cox and Jencks result only from failure to reproduce conditions exactly and from cumulative errors in (a) reducing observed data to kinetic constants and (b) predicting observed rate constants from calculated constants.

To obtain good values of the secondary isotope effects on k_{HA} and k_{HS}, we used Stein's procedure.[8] At any value of [HA], the first-order rate constant k_0^{3D} is given by

$$k_0^{3D}/[CH_3ONH_2] = k_{HS}^{3D}[CH_3ONH_3^+] + k_{HA}^{3D}[HA] \quad (2)$$

and

$$k_0^{3D}/k_0^{3H} = (k_{HS}^{3H}[CH_3ONH_3^+]/k_0^{3H})(k_{HS}^{3D}/k_{HS}^{3H}) + (k_{HA}^{3H}[HA]/k_0^{3H})(k_{HA}^{3D}/k_{HA}^{3H}) \quad (3)$$

$$k_0^{3D}/k_0^{3H} = w_{HS}(k_{HS}^{3D}/k_{HS}^{3H}) + (1 - w_{HS})(k_{HA}^{3D}/k_{HA}^{3H}) \quad (4)$$

Thus with the weighting factor w_{HS} obtained only from data for the H substrate and the isotope effects k_0^{3D}/k_0^{3H} obtained in paired experiments under identical conditions, values of the two isotope effects are found by a least-squares fit to eq 4. The result is

$$k_{HA}^{3H}/k_{HA}^{3D} = 0.857 \pm 0.024$$

$$k_{HS}^{3H}/k_{HS}^{3D} = 0.867 \pm 0.009$$

Hydrazinolysis of Phenyl Acetate. The reaction of eq 5 (L = H, O) was studied in hydrazine–hydrazinium ion buffers as a function of their concentration in H_2O and D_2O (Tables III and IV). As Figure 2 illustrates, the observed first-order rate constants

$$NH_2NH_2 + C_6H_5OCOCL_3 \rightarrow NH_2NHCOCL_3 + C_6H_5OH \quad (5)$$

are a quadratic function of total hydrazine. A similar profile is obtained in D_2O. Since the fraction of free hydrazine was 0.83 in H_2O and 0.80 in D_2O, eq 6 is obeyed to an extent of 96%.

$$k_0^{3L} = k_1^{3L}(N_2H_4) + k_2^{3L}(N_2H_4)^2 \quad (6)$$

Least-squares fitting to eq 6 yields the rate constants of Table V. Our values of k_1^{3H} and k_2^{3H} are about 20% smaller than those obtained by Satterthwait and Jencks[2i] (9.33×10^{-3} M^{-1} s^{-1} and 0.27 M^{-2} s^{-1}, respectively), which may again reflect chiefly difficulties in reproducing conditions accurately and errors in estimation of the parameters of complex rate laws. The solvent isotope

(8) Stein, R. L. *J. Org. Chem.* **1981**, *46*, 3328. This procedure was applied and illustrated in ref 4h, where it is shown to be particularly powerful in defining isotope effects in complex systems more precisely than the rate constants to which they refer.

Table IV. Observed First-Order Rate Constants and Isotope Effects for Hydrazinolysis of Phenyl Acetate ($C_6H_5OCOCL_3$, L = H, D) at pD 9.48 in D_2O Solvent, 25.00 ± 0.05 °C, μ = 1.0 (KCl)

$[N_2D_4]_{total}$,[a] M	$10k_0^{3H}$, s^{-1}	$10^0k_0^{3D}$, s^{-1}	k_0^{3H}/k_0^{3D}
0.100	1753, 1701, 1634, 1622, 1614 av: 1665 ± 60	1773, 1687, 1728, 1744, 1693 av: 1725 ± 36	0.965 ± 0.040
0.167	3949, 3973, 3906, 3916, 3909 av: 3930 ± 30	2936, 4010, 4018, 4044, 3966 av: 3993 ± 47	0.984 ± 0.014
0.200	5593, 5443, 5451, 5556 av: 5511 ± 75	5629, 5623, 5649, 5665, 5824 av: 5678 ± 83	0.971 ± 0.019
0.240	7439, 7372, 7302, 7410, 7293 av: 7363 ± 64	6714, 7589, 7621, 7766, 7709 av: 7660 ± 74	0.962 ± 0.013
0.300	10949, 11161, 11175, 11064, 11089 av: 11088 ± 91	11540, 11211, 11460, 11449, 11629 av: 11458 ± 156	0.968 ± 0.015
0.400	20166, 19857, 19698, 19746, 19729 av: 19839 ± 192	20175, 20379, 20260, 20365 av: 20295 ± 96	0.978 ± 0.011

[a] Fraction of free N_2D_4, 0.80.

Table V. Catalytic Constants[a] for Hydrazinolysis of Phenyl Acetate ($C_6H_5OCOCL_3$, L = H, D) in L'$_2$O Solvent (L' = H, D), pH 8.87, pD = 9.48, 25.00 ± 0.05 °C, μ = 1.0 (KCl)

catalytic constant	magnitude
k_1^{3H}(HOH), M^{-1} s^{-1}	$6.77 \pm 0.68 \times 10^{-3}$
k_1^{3D}(HOH), M^{-1} s^{-1}	$7.24 \pm 0.73 \times 10^{-3}$
k_2^{3H}(HOH), M^{-2} s^{-1}	0.236 ± 0.002[b]
k_2^{3D}(HOH), M^{-2} s^{-1}	0.241 ± 0.003[c]
k_1^{3H}(DOD), M^{-1} s^{-1}	$6.74 \pm 0.37 \times 10^{-3}$
k_1^{3D}(DOD), M^{-1} s^{-1}	$7.25 \pm 0.40 \times 10^{-3}$
k_2^{3H}(DOD), M^{-2} s^{-1}	0.169 ± 0.002[d]
k_2^{3D}(DOD), M^{-2} s^{-1}	0.173 ± 0.002

[a] Calculated by least-squares fitting of eq 6. [b] If a correction for the hydrazinium ion catalyzed hydrazinolysis was applied from ref 21 ($0.005 \times 0.17 = 0.0085$) to the calculation of k_2^{3H}(HOH) its value would change to 0.227, i.e., ~4% reduced. [c] Even if extreme values of β-deuterium isotope effects are assumed for the neglected term, the corresponding decrease in the value of k_2^{3D}(HOH) is 4 ± 0.5%. [d] The solvent isotope effect for the neglected term is 2.3[9] thus the corrected value of k_2^{3H}(DOD) is 0.164, ~3% less.

effects can be calculated from the least-squares parameters in Table V:

$$k_1^{3H}(HOH)/k_1^{3H}(DOD) = 1.0 \pm 0.1 \text{ (Bruice and Benkovic: 1.1)}[9]$$

$$k_1^{3D}(HOH)/k_1^{3D}(DOD) = 1.0 \pm 0.1$$

$$k_2^{3H}(HOH)/k_2^{3H}(DOD) = 1.44 \pm 0.02 \text{ (Bruice and Benkovic: 1.2)}[9]$$

$$k_2^{3D}(HOH)/k_2^{3D}(DOD) = 1.45 \pm 0.02$$

The secondary isotope effects were calculated with greater precision from the weighting factor approach (eq 7).

$$k_0^{3D}/k_0^{3H} = w_1(k_1^{3D}/k_1^{3H}) + (1 - w_1)(k_2^{3D}/k_2^{3H}) \quad (7a)$$

$$w_1 = k_1^{3H}[N_2H_4]/k_0^{3H} \quad (7b)$$

(9) At 18 °C; Bruice, T. C.; Benkovic, S. J. *J. Am. Chem. Soc.* **1963**, *86*, 418.

The results are

$$k_1^{3H}(HOH)/k_1^{3D}(HOH) = 0.99 \pm 0.03$$

$$k_1^{3H}(DOD)/k_1^{3D}(DOD) = 0.99 \pm 0.07$$

$$k_2^{3H}(HOH)/k_2^{3D}(HOH) = 0.972 \pm 0.002$$

$$k_2^{3H}(DOD)/k_2^{3D}(DOD) = 0.970 \pm 0.009$$

Reaction of Semicarbazide with 2,4-Dinitrophenyl Acetate. This reaction (eq 8, L = H, D) was studied in H_2O (data in Table VI).

$$NH_2CONHNH_2 + ArOCOCL_3 \rightarrow NH_2CONHNHCOCL_3 + ArOH \quad (8)$$

Here k_0^{3L} is linear in $[NH_2CONHNH_2]$ with zero intercept as Figure 3 shows. The β-secondary isotope effect from the slopes of the lines ($k^{3H} = 9.20 \pm 0.05 \times 10^{-2}$ M^{-1} s^{-1}; $k^{3D} = 9.44 \pm 0.07 \times 10^{-2}$ M^{-1} s^{-1}) is

$$k^{3H}/k^{3D} = 0.975 \pm 0.009$$

Discussion

The results are gathered in Table VII along with $\hat{I}$ values, which we suppose to vary from zero to unity as the carbonyl structure in the transition state varies from trigonal to tetrahedral.[4d] They are calculated from eq 9, as discussed previously by us.[4d] Here K_{3H}/k_{3D} is the limiting effect for complete conversion to a tetrahedral adduct, taken

$$k_{3H}/k_{3D} = (K_{3H}/K_{3D})^{\hat{I}} \quad (9)$$

before[4d] as 0.87, the observed effect (corrected to three deuteriums) for equilibrium hydration of 1,3-dichloroacetone. Although it was previously uncertain how exactly this limit, derived from a ketone, could be applied to esters,[10] the work we describe here confirms its correctness, as we shall explain.

Methoxyaminolysis of Phenyl Acetate. Cox and Jencks[2o] suggested that the methoxyaminolysis of phenyl acetate with HA catalysis had a proton-transfer process like that shown in **1** as an important contributor to limitation of the rate, accounting for the solvent isotope effect of 3.9. When the acidity of the catalyst was either increased or decreased from 6.71 for HA, Cox and Jencks found k_{HOH}/k_{DOD} to drop sharply. They were able to fit their observations to a model in which $k_{HOH}/k_{DOD} = 12$ for the proton switch itself, (1), and in which solvent reorganization processes (more acidic catalysts) and diffusional separation of the products of the proton switch (less acidic catalysts) also participate in limiting the rate. Following Stein,[8] it is easy to show that the weighting factor for the proton switch, w_s, for HA catalysis must be

$$w_s = (3.9 - 1)/(12 - 1) = 0.24$$

so that the proton switch is 24% rate-limiting[11] and other processes 76%.

The agreement of k_{3H}/k_{3D} (0.86 ± 0.02) with K_{3H}/k_{3D} (0.87 ± 0.02) for 1,3-dichloroacetone hydration[4b] suggests (a) that the ketone equilibrium–addition effect can also be extended to esters[10] and (b) that *all* participating activated complexes in the (virtual) transition state for HA catalysis are fully tetrahedral. The latter point adds strong substantiation to the ideas of Cox and Jencks,[2o] according to which the processes that "dilute" the proton-switch isotope effect all involve tetrahedral carbonyl adducts.

Methoxyammonium ion, with a pK_a of 4.7, is a thousandfold more acidic than HA; according to Cox and Jencks, solvent reorganization before the proton switch should now dominate in limiting the rate. From the solvent isotope effect, the weighting factor for the proton switch is now

$$w_s = (1.8 - 1)/(12 - 1) = 0.07$$

Presumably solvent reorganization is around 93% rate limiting for HS catalysis. This, or an analogous hypothesis, is supported by the secondary isotope effect which yields $\hat{I} = 1.00$. All contributing activated complexes are tetrahedral or nearly so.

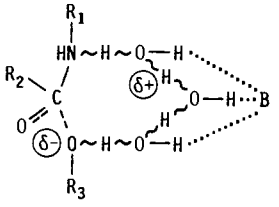
Hydrazinolysis of Phenyl Acetate. Here the secondary isotope effect for the k_1 term is so subject to error as to render any attempted interpretation highly doubtful. Our concern will be limited to the k_2 term.

The isotope effect of 0.97 and the $\hat{I}$ value of 0.22 indicate that all activated complexes contributing to the virtual transition state for the k_2 term are close to trigonal in character. This means that, contrary to what has been believed, proton transfer from the zwitterionic adduct to hydrazine (as in **2**) *cannot* limit the rate. Such a process would have an activated complex with a fully tetrahedral activated complex ($\hat{I} = 1.0$) and this is excluded by the β-deuterium secondary isotope effect. The small solvent isotope effect, $k_{HOH}/k_{DOD} = 1.45$, is also consistent with the finding that proton transfer does not limit the rate.

This result is surprising. Excellent isotope-effect evidence of Kirsch and co-workers shows that the carbonyl carbon of methyl formate is fully tetrahedral in the transition state for the corresponding reaction of this ester ($k_{\alpha H}/k_{\alpha D} = 0.725 \pm 0.005$, cited above; $\hat{I} = 1.02 \pm 0.18$). Further, the *methoxy*-^{18}O effect ($k_{16}/k_{18} = 1.0048 \pm 0.0006$[12]) shows the bond to this atom not to be breaking in the transition state. Thus proton transfer as in **2** is an extremely probable mechanism for hydrazine-catalyzed hydrolysis of methyl formate.

Satterthwait and Jencks[2i] argued for a similarity in mechanism between alkyl and phenyl esters because both have $\beta_{lg} = 0.6$. It is true that the correlation line for phenyl esters lies below that for alkyl esters by an amount corresponding to 10–15-fold in rate, but this was attributed to an unfavorable, constant steric effect of aryl vs. alkyl in the formation of a tetrahedral adduct.

The new findings show that in spite of having β_{nuc} the same as the alkyl substrates which do undergo rate-limiting proton transfer, the phenyl acetate transition state has a nearly planar carbonyl function. We suggest **3** as a possible structure for the activated complex. In **3**, the general base B interacts in a stabilizing manner with one or more of the acidic protons on a water



3

bridge uniting the nucleophilic atom with the leaving group (the number of participating waters is of course unknown and the number shown is arbitrarily chosen for the purpose of illustration only). The charge on entering and leaving groups could remain the same as in **1** or **2**, satisfying observations on β_{nuc} and β_{lg}. Tertiary amines should function as well as primary and secondary amines. The interaction of B could be weak, yielding the observed small value of β (≤ 0.25). A near-trigonal structure at carbonyl would produce $\hat{I} \sim 0.2$.

The change in mechanism on going from alkyl to aryl esters can be understood in terms of steric effects. Transition states like **3** may be inherently less stable than those like **2** for alkyl esters (perhaps for entropic reasons, among others). The changeover to phenyl esters should produce a strong rise in steric energy for **2**, where R_3O is in a tetrahedral environment, but a much smaller rise in steric energy for **3**, where R_3O is in an unconstricted environment. If the actual steric effect in **2** is larger than the 10–15-fold rate reduction experimentally seen for phenyl vs. alkyl esters—say, 100-fold—and if the inherent instability of **3** relative to **2**, aside from steric effects, corresponds to 10–15-fold, then the

(10) Cross conjugation of the ester–alkoxy electron pair into the carbonyl group, decreasing delocalization of the β-CH electrons, might have made the equilibrium isotope effect for addition to an ester smaller than that for a ketone. Apparently this phenomenon is of negligible influence, as are electrostatic effects from the positive and negative charges of the zwitterion.

(11) The expression "Step Y X% rate-limiting" means that the weighting factor w_Y for step Y is X/100. For a two-step serial process where $k^{-1} = k_Y^{-1} + k_Z^{-1}$, $w_Y = k_Y^{-1}/k^{-1}$.

(12) Sayer, C. B.; Kirsch, J. F. *J. Am. Chem. Soc.* **1973**, *95*, 7375.

Table VI. Observed First-Order Rate Constants and Isotope Effects for the Reaction of Semicarbazide Buffers[a] with 2,4-Dinitrophenyl Acetate ($2,4\text{-}(NO_2)_2C_6H_3OCOCL_3$, L = H, D) at pH 4.46, 25.00 ± 0.05 °C, μ = 1.0 (KCl)

$[NH_2CONHNH_2]_{free}$, M	$10^6k_0^H$, s^{-1}	$10^6k_0^D$, s^{-1}	k^{3H}/k^{3H}
0.020	1954, 2008, 2012 av: 1988 ± 38	2031, 2039, 2050 av: 2040 ± 10	0.975 ± 0.109
0.040	3694, 3729, 3735, 3806, 3863, 3878 av: 3784 ± 76	3812, 3812, 3815, 3857, 3956, 3922 av: 3822 ± 63	0.980 ± 0.025
0.080	7249, 7331, 7356, 7382 av: 7330 ± 108	7508, 7536, 7693, 7765 av: 7623 ± 120	0.962 ± 0.020
0.160	14143, 14331, 14456, 14675, 14699, 14826 av: 14520 ± 250	14812, 14814, 14818, 14915, 14942, 14980 av: 14880 ± 75	0.976 ± 0.107
0.240	21835, 21671, 21422, 21404, 21445, 21539 av: 21553 ± 170	22496, 22589, 22930, 21910, 21949, 22747 av: 22390 ± 3.93	0.963 ± 0.018
0.320	28216, 28379, 28471, 28886, 28940, 28942 av: 28639 ± 322	28803, 28828, 29437, 29907, 29940, 29982 av: 29483 ± 553	0.971 ± 0.021
0.400	35786, 36298, 36613, 37040, 47041	36096, 37092, 37657, 38140, 38260	0.971 ± 0.027
0.480	45658, 44898, 44694, 44585, 43960 av: 44760 ± 610	45939, 45943, 45945, 46955 av: 46056 ± 506	0.972 ± 0.017

[a] Fraction of free semicarbazide, 0.80.

Table VII. Secondary Isotope Effects, Measures of Tetrahedral Structure in the Transition State, and Solvent Isotope Effects for Ester Aminolysis

reaction	k_{3H}/k_{3D}	$\hat{I}^a$	k_{HOH}/k_{DOD}
methoxyaminolysis of phenyl acetate with general-acid catalysis by			
$CH_3O_2CCH_2CH(CO_2CH_3)NH_3^+$(HA)	0.86 ± 0.02	1.08 ± 0.15	3.9 ± 0.8
$CH_3ONH_3^+$(HS)	0.87 ± 0.01	1.00 ± 0.12	1.75 ± 0.06
hydrazinolysis of phenyl acetate with general-base catalysis by			
NH_2NH_2	0.97 ± 0.01	0.22 ± 0.08	1.45 ± 0.02
reaction of semicarbazide with 2,4-dinitrophenyl acetate, uncatalyzed	0.975 ± 0.009	0.18 ± 0.07	

[a] Calculated from eq 9 with K_{3H}/K_{3D} = 0.87 ± 0.01.

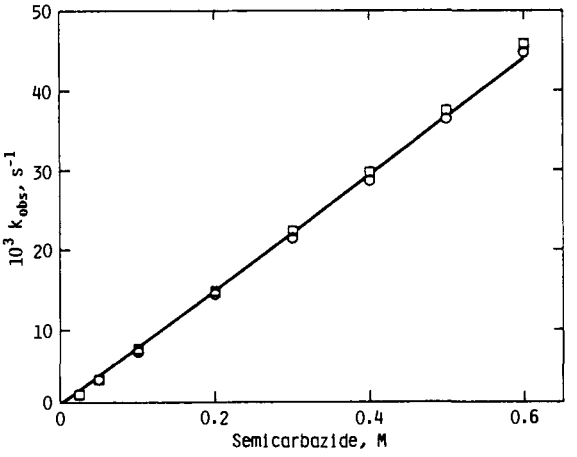


Figure 3. Dependence of the first-order rate constant for the reaction of semicarbazide with 2,4-dinitrophenyl acetate ($ArOCOCL_3$; L = H, D) on total semicarbazide concentration (fraction of free base, 0.80) in H_2O, at 25.00 ± 0.05 °C; μ = 1.0 (KCl); L = H (○) and D (□). The data are from Table VI.

bilinear correlation is accounted for. Introduction of the large phenyl group makes **2** so unstable that it no longer participates; **3**, which suffers a smaller steric effect, not takes over at a 10–15-fold reduced rate. Since the charge on the leaving group is similar in **2** and **3**, both have the same value of β_{lg}.

Reaction of Semicarbazide with 2,4-Dinitrophenyl Acetate. In this reaction, the pK_a of the protonated nucleophile[2b] (3.9) and that of the protonated leaving group[2b] (4.1) are nearly equal, so we expect the rate-limiting transition state to be that for leaving-group expulsion from the zwitterionic adduct (oxyanion leaving groups having an intrinsic disadvantage of around 3.5 pK units vs. ammonium leaving groups[28]). This is entirely consistent with a quasi-trigonal structure at carbonyl as indicated by $\hat{I}$ = 0.18.

Conclusions. Previous views[20] of transition-state structure for the methoxyaminolysis of phenyl acetate with catalysis by an acid of pK_a = 6.7 (proton transfer to oxygen of the tetrahedral zwitterionic adduct) and by an acid of pK_a = 4.7 (solvent reorganization preparatory to protonation of the tetrahedral zwitterionic adduct) are confirmed in both cases by $\hat{I}$ values of unity. The expectation of rate-limiting leaving group expulsion for the "class II" reaction of semicarbazide with 2,4-dinitrophenyl acetate is consistent with $\hat{I} \sim 0.2$ for this reaction.[2i] In the hydrazine-catalyzed hydrazinolysis of phenyl acetate, the previously held[2i] view of rate-limiting deprotonation of a tetrahedral, zwitterionic adduct is *not* consistent with $\hat{I} \sim 0.2$, which indicates a nearly trigonal, rather than a tetrahedral, transition state. A cyclic structure with solvent bridging between nucleophile and departing leaving group is suggested. General-base stabilization of the solvent bridge by interaction at protruding protons rationalize the high catalytic activity of tertiary amines.

Experimental Section

Materials and Solutions. Reagent grade inorganic salts were recrystallized and dried before use. Hydrazine monohydrochloride was prepared by dropwise addition of cold, concentrated hydrochloric acid to cold liquid hydrazine (Chemical Procurement Lab. Inc.) in an equimolar amount. The crude crystals harvested from water were recrystallized twice from absolute methanol and dried (mp 91–92.5 °C; lit.[13] mp 91–93 °C). Titration showed >99% monohydrochloride. Semicarbazide hydrochloride (Fisher Scientific Co.) was recrystallized from a mixture of 3.6 mol % methanol and 6.4 mol % water. Methoxamine hydrochloride (Chemical procurement Lab. Inc.) was recrystallized from absolute ethanol (mp 149–151 °C; lit.[14] mp 149–151 °C). DL-Aspartic acid

(13) Sisler, H. H.; Neith, F. T.; Boatman, C. B.; Shellman, R. W. *J. Am. Chem. Soc.* **1954**, *76*, 3914.

dimethyl ester hydrochloride (Sigma Chemical Co.) was recrystallized from absolute methanol. Water was distilled from a copper-bottom still, passed through a Barnstead mixed-bed ion-exchange column, boiled for 20 min, and cooled suddenly. Heavy water (Stohler 99.8 atom % deuterium) was distilled and the 100-120 °C fraction was used. Ester substrates used in this study in protiated and deuterated forms (>99.5% labeled) were prepared from the appropriate purified phenol and labeled acetyl chloride as previously described.[4c]

Solutions of 1.0 M hydrazine monohydrochloride and semicarbazide hydrochloride were made and titrated with standard KOH solutions to give 80% free base. The pH of such a semicarbazide buffer is 4.46 and that of the hydrazine buffer is 8.90 or pD 9.5 in DOD. Serial dilutions of these buffers were made with a 1.0 M KCl solution and a deviation of pH ±0.03 was tolerated on dilution. Methoxyamine was used at 0.2 M concentration, ionic strength 0.4 M with KCl, and was 50% neutralized with a KOH solution to pH 4.80 (pD 5.30) shortly before use. A 1.6 M solution of DL-aspartic acid dimethyl ester was prepared, KOH was added to pH 4.80 (pD 5.30), and the solution was diluted with 1.6 M KCl to give concentrations 0.1-1.0 M at ionic strength 1.00 M after a 1:1 dilution with the methoxyamine stock solution. The reaction solutions contained 0.1 M methoxyamine at a fraction of 0.555 free base; the fraction of the DL-aspartic acid dimethyl ester in the acidic form was 0.99. The pH of reaction mixture was determined at 25 °C before and after each experiment. A Radiometer 26 pH meter was employed for pH measurements.

(14) Goldfarb, A. R. *J. Am. Chem. Soc.* **1945**, *67*, 1852.

Kinetics. Rate measurements were made with a Cary 16 spectrophotometer interfaced to a Hewlett Packard 2100-A minicomputer or to a DEC-Heathkit H-11 microcomputer. First-order rate constants for reactions of hydrazine and semicarbazide were obtained by a weighted, nonlinear, least-squares fit of the data to an exponential function. Methoxyaminolysis was slow and was studied under zero-order conditions. Duplicate samples at 0.01 M initial concentration of phenyl acetate were followed for 2-3 h, corresponding to 1-5% reaction. A linear least-squares fit of absorbance A vs. time t gave dA/dt. The difference in extinction coefficients of phenol and phenyl acetate at 275 nm is 1340 M^{-1} cm^{-1}. Exact values of initial substrate concentrations, E_0, were obtained by hydrolysis of an aliquot of the phenyl acetate stock solution in methanol with 0.1 M NaOH. The absorbance of the liberated phenoxide ion was measured at 286.9 nm (ϵ 2544 M^{-1} cm^{-1}). The first-order rate constant was calculated from

$$k_0 = (dA/dt)/E_0(1340)$$

In all kinetic studies, rates for the protiated and deuterated substrates were measured in alteration with as little intervening time as possible. Identical solutions were employed with the two isotopic substrates in these paired experiments. Reaction temperatures were controlled by a Lauda K4/RD circulating water bath connected to the cell compartment and cuvet holder. Temperatures were monitored with a digital thermistor.

Registry No. D_2O, 7789-20-0; CH_3ONH_2, 67-62-9; NH_2NH_2, 302-01-2; $NH_2CONHNH_2$, 57-56-7; $CH_3O_2CCH_2CH(CO_2CH_3)NH_3^+$, 98858-59-4; phenyl acetate, 122-79-2; 2,4-dinitrophenyl acetate, 4232-27-3; deuterium, 7782-39-0; methoxyamine hydrochloride, 593-56-6.

Spectroscopy and Photochemistry of Thiouracils: Implications for the Mechanism of Photocrosslinking in tRNA

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Abstract: Nanosecond laser photolysis studies of 4-thiouridine (4t-Urd), 1,3-dimethyl-4-thiouracil (DMTU), and uracil (U) are described. Absorption spectra of the triplet states of these molecules are presented along with INDO/S calculations of ground-state and excited-state spectra. The excited triplet-state spectra of 4t-Urd and DMTU are similar, indicating that the T_1 state of 4t-Urd must be primarily in the thione tautomeric form. Intersystem crossing yields for 4t-Urd and DMTU are 0.9 ± 0.1 and 1.0 ± 0.1, respectively. Quenching of the lowest triplet state of 4t-Urd and DMTU by a number of quenchers is shown to proceed primarily by an electron-transfer mechanism. Separated electron-transfer products are observed on quenching of 4t-Urd and DMTU by the two amines diethylaniline (DEA) and triethylamine (TEA). This suggests that photocrosslinking of 4t-Urd with cytidine in tRNA may occur by a mechanism which involves an initial electron-transfer quenching reaction.

Near-ultraviolet light has been shown to inhibit the growth of *E. Coli*[1-3] with an action spectrum having a maximum at 330nm. The action spectrum is similar to the absorption spectrum of 4-thiouridine (4t-Urd)[1], a somewhat uncommon tRNA nucleoside base. It has been shown that near-UV photolysis of tRNAs leads to a decrease in their activity due to crosslinking of the 4t-Urd that is in the 8 position of tRNA with a cytidine that is in the tRNA 13 position.[4-7] Given that the action spectra of photocrosslinking and growth inhibition are similar, it is believed that the photocrosslinking reaction is responsible for the near-UV inhibition of *E. coli*. Furthermore, in both solution and in vitro intact tRNA, 4t-Urd exhibits a weak phosphorescence.[8,9] Quenching of the phosphorescence of 4t-Urd in tRNA leads to a concomitant decrease in crosslinking, implying that the 4t-Urd triplet is the photoactive state.[9]

Despite the central importance of the 4t-Urd triplet state in the photoreaction of tRNA, no comprehensive spectroscopic study of this excited state has appeared. Thus, we have explored the ground- and excited-state spectroscopy of 4t-Urd and related compounds. Among other properties of this state, we have investigated the extent of tautomerization in the triplet state and whether tautomerization is important in its photoreactions. While 4t-Urd only exists in the thione form in its ground state, the ground state of the closely related 2-thiouracil exists in an equilibrium

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