hexane–ethyl acetate mixtures and recrystallized from petroleum spirit, bp 80–100 °C.

The methoxy dimethylamino derivatives **5** and **6** were prepared according to the procedure suggested by Suschitzky[16] for **5**. Thus, refluxing 0.3 g of *N,N*-dimethyl-4-nitrosoaniline and 0.3 g of 4-methoxyphenyl azide in 3 mL of bromobenzene for 1 h under nitrogen, evaporation of the solvent, chromatography of the residue on alumina, and double recrystallization from cyclohexane yielded 0.325 g (60%) of compound **5**, yellow needles, mp 133–134 °C. Compound **6** (0.2 g, 35%) was similarly obtained from 10 min of refluxing of 0.3 g of methoxynitrosobenzene and 0.36 g of 4-(dimethylamino)phenyl azide, chromatography, and several recrystallyzations from methanol, mp 135–136 °C.

Photochemical Reactions. A solution of 100 mg of (4-methoxyphenyl)phenyldiazene 1-oxide (**1**) in 100 mL of ethanol was irradiated for 6 h at 17 °C by means of a Helios Italquartz 125-W medium-pressure arc through a Pyrex filter. Evaporation of the solvent and chromatography on silica gel eluting with a cyclohexane–benzene mixture yielded 45 mg of unreacted **1**, 25 mg (39% of reacted **1**) of 2-hydroxy-4-methoxyazobenzene (**7**), and 22 mg (34%) of 4-hydroxy-4′-methoxyazobenzene (**8**). Similar irradiation of compounds **1–6** in the conditions stated in Tables I and II yielded the reported products.

Physical Data. UV spectra were recorded with a Cary 19 spectrophotometer and luminescence spectra with a Aminco Bowman MPF spectrophotofluorometer. IR spectra were recorded in KBr pellets by means of a Perkin-Elmer 197 spectrophotometer, ^{1}H NMR spectra (in C_6D_6 or $CDCl_3$) by means of a Bruker 80 instrument with tetramethylsilane as internal standard.

Structure Assignment. In most cases, photochemical products were recognized by direct comparison with authentic samples. The hydroxy azo dyes **7**,[17] **9**,[18] **10**,[19] **14**,[20] and **15**[21] were prepared according to published methods, as was the biphenyl **18**.[22] The formyl derivatives **16** and **19** were prepared by formylation of the corresponding *N*-methyl derivatives.[14] The hydroxyazo derivative **8** corresponds to the product described by Bunce[6] in its physical and spectroscopic properties.

The structure of 2,4′-dimethoxy-4-hydroxy-5-phenylazoazobenzene was assigned to product **11**, orange crystals, mp 63–66 °C, on the basis of the elemental analysis (C, 65.9; H, 5.2; H, 15.3. Calcd for $C_{20}H_{18}N_4O_3$: C, 66.3; H, 5; N 15.5) and spectroscopic properties: NMR (C_6D_6) δ 3.15 (3 H); 3.2 (3 H), Aromatic signals between δ 6.5 and 8.18 include an AA′BB′ and an AB systems but no proton with only para or meta coupling. Mass spectrum is in accord with the structure.

Reaction Quantum Yield. The photochemical reaction was effected with 313- or 366-nm radiation (intensity ca. 10^{-7} einstein min^{-1} cm^{-2}) obtained from a focalized high-pressure mercury arc by means of an interference filter ($\Delta\lambda_{1/2}$ 5 nm).

Solutions of 10^{-4} M **1–6** were irradiated to a ca. 15% conversion. Light intensity was measured by ferrioxalate actinometry. Formation of the hydroxy azo derivative was measured by either of two methods, viz., either making the irradiated solution 0.5 M in KOH and measuring the absorbance of the corresponding anion or by HPLC chromatography (Waters apparatus, Corasyl 18 column). Disappearance of the starting material was determined by chromatography and UV absorbance measurements.

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Notes

Proton Inventory Investigation of the Specific Acid Catalyzed Hydrolysis of γ-Ethoxy-γ-butyrolactone

Allan D. Bokser, Kathryn A. York, and John L. Hogg*

Department of Chemistry, Texas A&M University, College Station, Texas 77843

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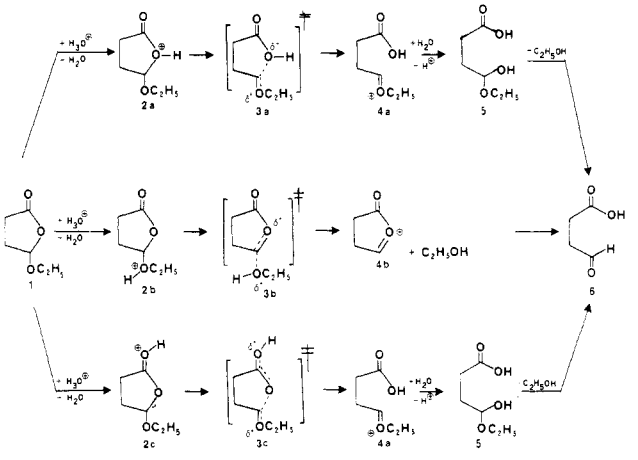
It has been proposed that some enzyme-catalyzed hydrolyses of glycosidic bonds or enzyme-catalyzed transglycosylations may involve a mixed acetal–acylal intermediate that would then rapidly hydrolyze.[1] We report a study of the hydrolysis of such a compound, γ-ethoxy-γ-butyrolactone (**1**), as a model for such a process.[1] Fife has previously explored the hydrolysis of the compound and has proposed an A-1 mechanism for the acid-catalyzed hydrolysis of **1** as shown in path **a** of Scheme I.[2] Protonation of the lactone gives intermediate **2a** that breaks down in the rate-determining step to yield **4a**. The intermediate **4a** then suffers attack by water to give the hemiacetal **5** that rapidly hydrolyzes to the aldehyde **6**. The aldehyde chromophore allows the reaction to be monitored spectrophotometrically.

Scheme I



Fife's mechanism was based on the following pieces of information, among other things. A specific acid-catalyzed reaction that exhibits a solvent deuterium isotope effect of $k(D_2O)/k(H_2O) = 2.37$ is observed at low pH. The

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relatively small negative entropy of activaion of −6.9 eu indicates that there is minimal involvement of water in the rate-determining transition state.

We have extended the mechanistic study by using the proton inventory technique to examine the origin of the solvent deuterium isotope effect on the acid-catalyzed reaction.

Results and Discussion

Scheme I represents three of the possible mechanisms for the acid-catalyzed hydrolysis of 1. Path **a** shows a preequilibrium protonation on the *endo*-acetal oxygen to form **2a**. The rate-determining transition state, **3a**, would have bond breaking between the ring oxygen and the acetal carbon. This bond breaking would afford the acyclic intermediate, **4a**, which then hydrolyzes rapidly through **5** to form the product **6**. Path **b** shows the preequilibrium protonation on the *exo*-acetal oxygen to form **2b**. The rate-determining transition state **3b** would have bond breaking between the *exo*-oxygen and the acetal carbon. This bond breaking would afford ethanol and the cyclic intermediate, **4b**, which then hydrolyzes rapidly to the product, **6**. Path **c** shows a preequilibrium protonation of the carbonyl oxygen to form **2c**. The rate-determining transitions state, **3c**, would have bond breaking between the ring oxygen and the acetal carbon. This bond breaking would afford the same intermediates, **4a** and **5**, as found in path **a**.

Each of the possible specific acid-catalyzed mechanisms in Scheme I is reasonable based upon studies done on **1** and similar compounds. Fife has proposed path a for the acid-catalyzed hydrolysis of **1** for reasons mentioned above.[2] Kankaanpera has studied the acid-catalyzed hydrolysis of several cyclic acetals including 2-ethoxytetrahydrofuran[4] and 2,5-dimethoxytetrahydrofuran[5] and has proposed a mechanism similar to path **b** in which exo fission of the carbon–oxygen bond from the ring occurs after protonation. He based this mechanism on the fact that the rates of the acid-catalyzed hydrolysis of 2-alkoxytetrahydrofuran and 2-alkoxytetrahydropyran increased with increasing polarity of the *exo*-alkoxy group. He concluded that the polar influences of the alkoxy group on the basicity of the endocyclic oxygen should be very slight and, therefore, the rate of the hydrolysis involving endocyclic oxygen cleavage should have been almost independent of the polarity of the alkoxy group.[4] Kankaanpera has also studied the acid-catalyzed hydrolysis of 2,5-dimethoxytetrahydrofuran in an anhydrous acidic ethanol solution and found that 2,5-diethoxytetrahydrofuran, the expected product of a cyclic intermediate, and not 1,1,4,4-tetraethoxybutane, the expected product of an acyclic intermediate, forms.[5]

Weeks and co-workers studied the acid-catalyzed hydrolyses of substituted phthalides and 3-methoxy-3-arylperinaphthalides.[6-9] They proposed that the acid-catalyzed hydrolysis of 3-methoxyphthalide occurs by a mechanism similar to either path **b** or path **c**. This conclusion was based on the slope of about 1 obtained from the Zucker–Hammett plot of log k_w vs. H_0 and on the Bunnett ϕ value of 0.66 in 1 M H_2SO_4.[6] Both of these values indicate an A1 type mechanism. Weeks later studied the acid-catalyzed hydrolysis of substituted 3-methoxy-3-arylphthlalides and found a Hammett ρ value of −1.2 and a decrease in reaction rate with an increase in steric bulk at the C-3 position. He concluded from this that the mechanism of the acid-catalyzed hydrolysis of 3-methoxyphthalide occurs by a mechanism similar to path **b**.[7,8]

Table I. Second-Order Rate Constants for the Specific Acid Catalyzed Hydrolysis of γ-Ethoxy-γ-butyrolactone in H_2O–D_2O Mixtures of Atom Fraction (n) of Deuterium at 25.0 ± 0.1 °C[a]

	10^2k_n, M^{-1} s^{-1}	
n	obsd[b]	calcd[c]
0.00	39.1 ± 0.3[d]	39.1
0.20	43.6 ± 0.5	44.9
0.25	46.1 ± 0.5	46.5
0.40	51.3 ± 0.9	51.9
0.50	55.6 ± 1.6	56.1
0.60	60.5 ± 0.6	60.8
0.75	68.2 ± 1.2	69.0
0.80	68.5 ± 2.4	72.2
0.988[e]	86.9 ± 1.1	86.8

[a] The ionic strength was maintained at 0.25 M with sodium chloride. [b] Determined as the slope of a plot of the pseudo-first-order rate constants for hydrolysis vs. the concentration of acid at each atom fraction of deuterium. [c] Calculated on the basis of eq 3. [d] Error limits are standard deviations. [e] Atom fraction of deuterium in "pure" DCl in D_2O as determined by Josef Nemeth.[11]

We felt that proton inventory studies of the specific acid-catalyzed hydrolysis of **1** might allow a distinction of the three paths of Scheme I. The proton inventory technique involves the measurement of reaction rates in mixtures of protium oxide and deuterium oxide of known isotopic composition. These rate constants (k_n) are related to the rate constant in pure protium oxide (k_0) by the atom fraction (n) of deuterium in the solvent and the isotopic fractionation factors (ϕ) of the protons responsible for the isotope effect. The equation expressing this relationship is the Gross–Butler equation (1). Each isotopically ex-

$$k_n = k_0 \prod_i^{TS}(1 - n + n\phi_i)/\prod_j^{RS}(1 - n + n\phi_j) \qquad (1)$$

changeable reactant state proton contributing to the isotope effect will be characterized by a fractionation factor (ϕ_j) while the contributing transition state protons will have fractionation factors (ϕ_i). A plot of the rate constant vs. the atom fraction of deuterium constitutes the proton inventory. It is the shape of this plot that reveals details about the origin of the solvent deuterium isotope effect. The theory is treated in several reviews.[10]

Table I lists the second-order rate constants for the acid-catalyzed hydrolysis of **1** as a function of the atom fraction (n) of deuterium in the solvent. These data yield a solvent deuterium isotope effect of $k(D_2O)/k(H_2O) = 2.22$ that is slightly different from the literature value of 2.37.[2] A plot (not shown) of these second-order rate constants vs. the atom fraction of deuterium is bowl shaped. The bowl-shaped curvature of the proton inventory plot indicates that more than a single proton is contributing to the solvent deuterium isotope effect.

Recalling eq 1, we see that the reactant-state hydronium ion contains three isotopically exchangeable protons with

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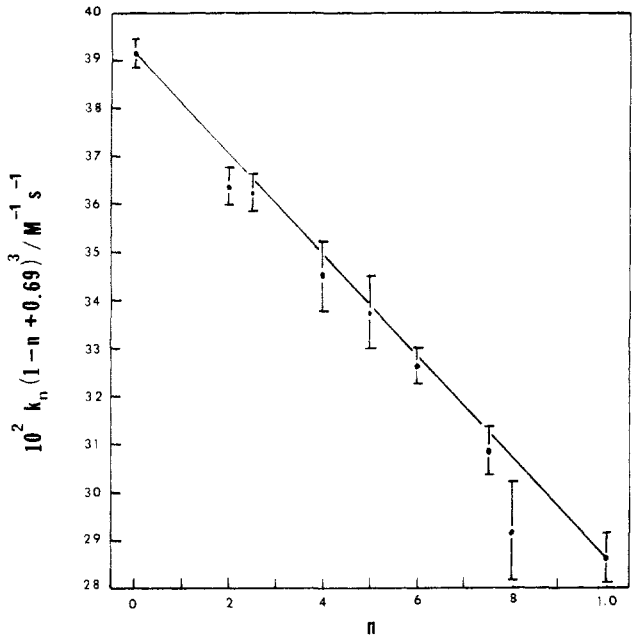

Figure 1. Proton inventory plot for the specific acid-catalyzed hydrolysis of γ-ethoxy-γ-butyrolactone in which the reactant-state fractionation factors shown in eq 2 have been factored out. The slope (−0.104) of this linear replot of the data of Table I is $-k_0 + \phi k_0$, which allows a value of $\phi = 0.73$ to be determined for the transition-state proton.[12]

nonunit fractionation factors of 0.69.[10a,b] Thus, eq 1 becomes

$$k_n = k_0 \prod_i^{TS} (1 - n + n\phi_i)/(1 - n + 0.69n)^3 \qquad (2)$$

Rearrangement of eq 2 allows us to plot $(1 - n + 0.69n)^3 k_n$ vs. n to gain some information about the transition-state contributions to the isotope effect. This plot (Figure 1) is linear, indicating a single transition-state proton contributes to the observed isotope effect.[11] The least-squares slope (−0.104) of this line is $-k_0 + \phi k_0$, which gives a value of the fractionation factor for the transition-state proton of 0.73 when the equation is solved with the value of k_0. This means that the single transition-state proton contributing to the solvent isotope effect is responsible for a *normal* isotope effect of 1/0.73 = 1.37. This normal isotope effect is in contrast to the inverse isotope effect contribution of the three reactant-state protons. Thus, the equation describing the proton inventory for the hydrolysis of **1** is shown in eq 3. The calculated numbers are presented in Table I.

$$k_n = k_0(1 - n + 0.73n)/(1 - n + 0.69n)^3 \qquad (3)$$

The expected value of the fractionation factor for the transition-state proton of each possible path in Scheme I is different. The fractionation factor for the proton on the *endo*-acetal oxygen, in **2a**, should have a value between 1.0 and 1.2 due to the large barriers to rotation about the C–O bonds of the positively charged ring oxygen.[13] The fractionation factor for the carboxylic acid proton of **4a**, which is formed from the transition state, **3a**, is 1.0. Therefore, the fractionation factor of the transition-state proton should be between 1.0 and 1.2 if the reaction occurs via path **a**. The fractionation factor for the proton on the *exo*-acetal oxygen, **2b**, should be near 0.69 due to the low barrier to rotation about the C–O bond of the positively charged oxygen.[10a,b] The hydroxyl proton on the ethanol molecule that forms with **4b** from the transition state, **3b**, should be unity.[10a,b] Therefore, the fractionation factor for the proton of transition state **3b** should be between 0.69 and 1.0 if the reaction occurs via path **b**. The fractionation factor for the proton on the carbonyl oxygen, **2c**, should have a value between 1.0 and 1.2 due to the large barrier to rotation about the C–O bond of the positively charged oxygen. The hydroxyl proton on the carboxylic acid group of **4a**, which is formed from the transition state, **3c**, should be unity.[10a,b] Therefore, the fractionation factor for the proton of transition state **3c** should have a value between 1.0 and 1.2.

The fractionation factor for the proton involved in the transition state is 0.73. Therefore, any proposed mechanism must have a transition-state proton that could account for a fractionation factor of 0.73. We propose that the proton inventory study supports path **b** as the mechanism for the acid-catalyzed hydrolysis of **1**. The value of the fractionation factor for the proton suggests it is in a bonding situation very nearly like that in intermediate **2b**. On the basis of this result, the rate-limiting transition state for the specific acid-catalyzed hydrolysis of **1** can best be described by the structure shown in **3b** in which the dotted lines represent a very minimal amount of bonding change (i.e., an early transition state).

It remains to be seen whether or not such an intermediate is involved in other acetal–acylal reactions and whether or not such an intermediate is actually involved in an enzymatic reaction. At the present time, the most that can be said is that it is not possible to entirely rule out the formation of such a covalent glycosyl enzyme.

Experimental Section

γ-Ethoxy-γ-butyrolactone (**1**) was prepared according to the literature procedure.[3]

Pseudo-first-order constants for the hydrolysis of **1** were measured at five different concentrations of HCl (DCl) or a mixture of the two over the range 5×10^{-3} to 6×10^{-2} M at 25.0 ± 0.1 °C at a constant ionic strength of 0.25 M (NaCl). Measurements were made at nine different atom fractions of deuterium oxide. These mixtures were prepared gravimetrically from concentrated stock solutions of HCl or DCl.

Reactions were initiated by injection of 100 μL of a 0.76 M solution of **1** in acetonitrile into 3 mL of the appropriate acid solution in the cuvette. The concentration of **1** in the cuvette was 0.024 M. Each reaction at a given concentration of acid and given atom fraction of deuterium oxide was run in triplicate. Reactions were monitored by following the absorbance increase at 280 nm by using a Cary 118C ultraviolet–visible spectrophotometer equipped with a constant-temperature cell compartment and linked to a Micromation computer for data acquisition. Reactions were followed for at least 3 half-lives and exhibited good first-order kinetics. Plots of the pseudo-first-order rate constants at each atom fraction of deuterium oxide vs. the concentration of acid produced the second-order rate constants as the slopes of the linear plots.

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Registry No. **1**, 932-85-4; D_2O, 7789-20-0; deuterium, 7782-39-0.

(11) Josef Nemeth, Urbana, IL 61801.

(12) A polynomial regression "best fit" program obtained from Phil Huskey from the University of Kansas, Lawrence, KS, was employed to determine whether the proton inventory data best fits a linear, quadratic, or cubic equation. The best fit is a linear equation in which $k_n \approx -0.1047n + 0.3873$.

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