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On the Mechanism of Isomerization of α-Hydroxypropionaldehyde to Hydroxyacetone

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Dimeric lactaldehyde and lactaldehyde-2-d in anhydrous or aqueous pyridine isomerize to hydroxyacetone. The isomerization is catalyzed by protic species that includes self-catalysis by the starting material and product, and catalysis by water and other Brönsted acids. The deuterium isotope effect which has values between 4 and 7, the isotopic exchange data, and the proton nmr assignment of the open dimer are consonant with the enediol mechanism with a rate-determining step involving the enolization of the open dimer.

INTRODUCTION

The aldo-keto isomerization and epimerization of aldoses and ketoses catalyzed by enzymes have attracted considerable attention in recent years because of their vital biological role in aldose synthesis, catabolism, and interconversion (*1*). Examples of nonenzymatic aldo-keto isomerization of carbohydrates and noncarbohydrate α-hydroxyaldehydes, catalyzed by base or acid, have been reported as early as 1900 (*2*). Fischer *et al.* (*3*) reported the isomerization of dimeric glyceraldehyde to dihydroxyacetone in anhydrous pyridine and Meyerhof and Lohmann (*4*) the conversion of D-glyceraldehyde-3-phosphate to dihydroxyacetone phosphate by triose phosphate isomerase. Most of the experimental evidence involving deuterium and tritium exchange for enzymic transformations suggests that these reactions are initiated by direct removal of an hydrogen alpha to the carbonyl with the formation of an enediol intermediate (*5*).

However, experiments with tritium labeling at the α-carbon of aldoses show an 80% tritium content in the ketose when the reaction is carried at low temperature (*6*). This result was explained either in terms of enolization and enzyme-bound tritium intermediate or by a combined enolization-hydride transfer mechanism. Recently the nonenzymic isomerization of glyceraldehyde 3-phosphate to dihydroxyacetone phosphate was studied extensively (*7*) and the rate constants for the individual steps were compared with those for the enzyme-catalyzed triosephosphate isomerase reaction. Both reactions were found to be compatible with the enediol mechanism but the enzyme increases the enolization rate by a factor of more than 10^9.

A hydride shift mechanism is supported by the data from the acid-catalyzed

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isomerization of DL-glyceraldehyde to dihydroxyacetone and their subsequent dehydration to pyruvaldehyde, conducted in tritiated water. Tritium was incorporated in the methyl but not the aldehydic carbon of pyruvaldehyde (*8*).

The experimental data available for the aldo-keto isomerization of simple α-hydroxyaldehydes are rather few. Although the favored mechanism is the enolization pathway (*9*) the kinetic data obtained from the isomerization of DL-glyceraldehyde to dihydroxyacetone (*5*) and the value of 1.3 for the kinetic isotope effect in the isomerization of mandelaldehyde dimer to 2-hydroxyacetophenone (*10*) do not exclude the hydride shift mechanism. Lactaldehyde (α-hydroxypropionaldehyde) provides a simple model for the non-enzymic study of aldo-keto isomerization. In the present work we report the kinetics and isotopic exchange of the isomerization of proteo- and deuterolactaldehyde to hydroxyacetone and we propose a mechanism in support of the enolization pathway.

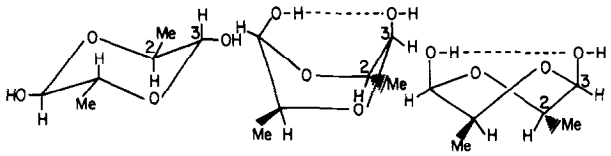
RESULTS AND DISCUSSION

The Structure of α-Hydroxypropionaldehyde

Freshly distilled monomeric α-hydroxypropionaldehyde (lactaldehyde) is a mobile liquid and dimerizes rapidly at room temperature. The dimerization is accompanied by evolution of heat and increase in viscosity. The liquid crystallizes slowly when kept in a desiccator or at low temperatures. A fresh solution of the crystalline lactaldehyde in DMSO-d_6 or pyridine does not show aldehydic protons. The spectrum of lactaldehyde and deuterolactaldehyde immediately after distillation is that of the monomer (*11*). The nmr spectra of solutions of crystalline lactaldehyde and lactaldehyde-2-d in DMSO-d_6 (Fig. 1 and Table 1) are consonant with the presence of two predominant dimeric forms in solution, one (23%) of which has a large vicinal spin–spin coupling constant ($J_{H,H}$ = 7 Hz) and another (77%) whose vicinal coupling constant is small ($J_{H,H}$ = 2.5 Hz). Out of the 10 possible dimeric structures, structure **1a**, i.e., the one where all large groups are equatorial, has all protons axial and J_{HH} *vic* is expected (*12*) as found to be large. Structures **1b** and **1b′**, i.e., the intramolecularly hydrogen-bonded structures, either in the boat or twist-boat form, have the hydrogens in a pseudoaxial, pseudoequatorial relationship and J_{HH} *vic* is expected to be, as observed, small (*12*). The assignments of the protons were obtained from decoupling experiments and the coupling constants and chemical shifts are shown in Table 1.

The 1,4-dioxane ring structure of dimeric lactaldehyde is commensurated with the infrared spectrum of the solid or of a fresh solution in pyridine which shows no absorption for an aldehydic group. When the pyridine solution is heated at 90°C for 1 hr a weak carbonyl band appears at 1730 cm^{-1}, presumably due to the open dimer.

The rate of dimerization of monomeric lactaldehyde in anhydrous pyridine was followed by measuring the decay of the aldehydic proton at 40°C. A 3.0 *M* solution (monomer) in pyridine dimerized with a second-order rate constant k = 0.006 min^{-1} mol^{-1} liter. We found that the rate of dimerization of monomeric lactalde-



1a 1b 1b′

hyde depends on the solvent. For example, the rate of dimerization in dimethylsulfoxide-d_6 is two to three times slower than that in pyridine.

Kinetic Measurements and Isotope Effects

Dimeric crystalline α-hydroxypropionaldehyde in anhydrous pyridine at 90°C isomerizes to hydroxyacetone. By using the nmr method we were able to follow the isomerization reaction and determine the formation of product, intermediates,

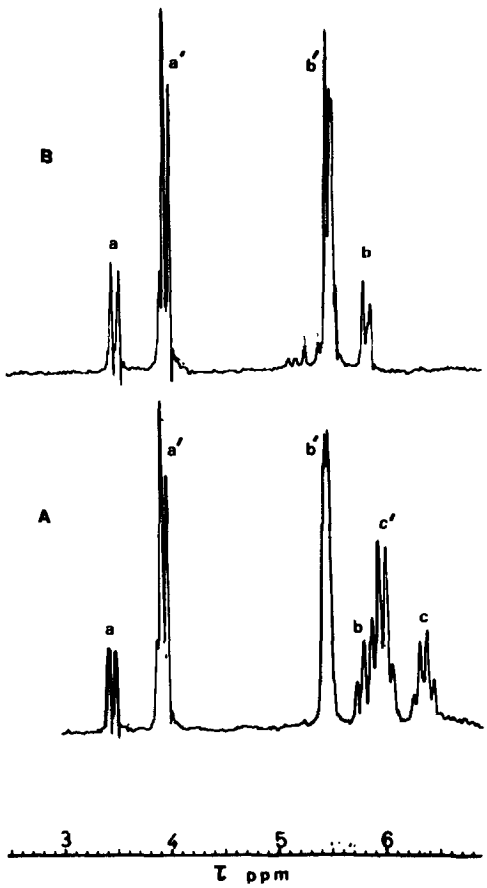


FIG. 1. (A) Partial nmr spectrum of dimeric lactaldehyde in DMSO-d_6. a, Hydroxyl; b, H of C-2; c, H of C-3 of structure **1a**; a′, hydroxyl; b′, H of C-2; c′, H of C-3 of structure **1b**. (B) Partial nmr spectrum of dimeric α-deuterolactaldehyde in DMSO-d_6; a, hydroxyl; b, H of C-2 of structure **1a**; a′, hydroxyl; b′, H of C-2 of structure **1b**.

TABLE 1

CHEMICAL SHIFTS AND COUPLING CONSTANTS OF DIMERIC LACTALDEHYDE AND DEUTEROLACTALDEHYDE IN DMSO-d_6 SOLUTIONS

Compound	Proton	τ_{ppm}	Intensity (%)	Coupling constants (Hz)		
				$J_{H_2-CH_3}$	$J_{H_2-H_3}$	J_{H_2-OH}
1a	OH	3.94	77	6.2	2.5	5.0
	$H-C_2$	5.45	78			
	$H-C_3$	5.97				
	CH_3	9.05				
1b	OH	3.40	23	6.3	7.0	7.0
	$H-C_2$	5.80	22			
	$H-C_3$	6.35				
	CH_3	9.03				
Deutero-**1a**	OH	3.96	76	—	—	5.0
	$H-C_2$	5.46	78			
	CH_3	9.07				
Deutero-**1b**	OH	3.48	24	—	—	7.0
	$H-C_2$	5.82	22			
	CH_3	9.02				

and the consumption of starting material. The formation of hydroxyacetone was determined by measuring the increase of the resonances at $\tau = 8.04$ ppm and $\tau = 5.62$ ppm corresponding to the methyl and methylene protons. The stability of hydroxyacetone under the experimental conditions was checked by heating at 90°C a solution of hydroxyacetone 1 *M* in anhydrous pyridine in a sealed tube for

TABLE 2

RATE OF ISOMERIZATION OF LACTALDEHYDE AND α-DEUTEROLACTALDEHYDE IN PYRIDINE AT 90°C: CONCENTRATION DEPENDENCE

	($k \times 10^{-4}$ min^{-1})
Lactaldehyde [*M*] (dimer)	
0.70	4.0
0.87	3.6
1.60	8.0
2.44	9.1
Lactaldehyde monomer [*M*][a]	
1.39	5.1
α-Deuterolactaldehyde [*M*] (dimer)	
0.7	1.0
0.87	1.0
1.60	1.6
	Isotope effects (k_h/k_d)
Concentration [*M*]	
0.7	4.0
1.6	5.0

[a] Molarity calculated as a dimer.

TABLE 3

RATE OF ISOMERIZATION OF LACTALDEHYDE AND α-DEUTEROLACTALDEHYDE IN PYRIDINE AT 90°C: CATALYSIS BY WATER AND DEUTERIUM OXIDE

	H_2O [*M*]	$k \times 10^{-4}$ min^{-1}
Lactaldehyde[a]		
1.6 [*M*]	0.00	8
	0.52	10.5
	0.69	11.2
	1.04	11.7
	1.37	11.8
	2.03	13.7
	2.67	17.8
α-Deuterolactaldehyde		
0.7 [*M*]	1.75	1.9
Lactaldehyde	D_2O [*M*]	
0.7 [*M*]	1.7	6.3
α-Deuterolactaldehyde		
0.7 [*M*]	1.7	1.4

[a] Molarity calculated as a dimer.

72 hr; there was no change in the nmr spectrum. The rate of the overall reaction was determined from the decrease of the intensities of the methyl protons at $\tau = 8.86$ ppm (middle of multiplet). *Pseudo*-first-order rate constants were obtained to greater than 85% reaction when the logarithm of the intensities of the methyl protons was plotted versus time.

The rate constants for the lactaldehyde isomerization to hydroxyacetone in anhydrous pyridine shown in Table 2 and Fig. 3 vary linearly with the concentration of the dimer from 0.7 to 2.4 *M*. This suggests that the substrate itself acts as a catalyst. The addition of hydroxyacetone (0.4 to 1.3 *M*) to solutions of lactaldehyde 1.5 *M* in pyridine enhanced slightly the rate of isomerization showing that the product is also acting as a catalyst. Similar self-catalysis has been reported for the mutarotation of tetramethyl and tetraacetylglucose in anhydrous pyridine or benzene (*13*) and the isomerization of mandelaldehyde dimer to 2-hydroxyacetophenone in pyridine (*10*).

The isomerization of α-deuterolactaldehyde (Table 2) in pyridine at 90°C is very slow and the kinetic isotope effect for the *pseudo*-first-order rate varies between 4 and 5. These values suggest that the deuterium on the carbon *alpha* to the carbonyl of the deuterolactaldehyde is directly involved in the rate-determining step (*14, 15*).

The isomerization reaction was also carried out in aqueous pyridine and the rates are shown in Table 3 and Fig. 3. The contribution of water catalysis to the overall rate for a 1.6 *M* lactaldehyde solution in pyridine is 3.6×10^{-4} min^{-1} mol^{-1} liter. Deuterolactaldehyde in pyridine in the presence of water or D_2O isomerizes with a rate four to five times slower than proteolactaldehyde under the same

TABLE 4

RATE OF ISOMERIZATION OF LACTALDEHYDE IN PYRIDINE AT 90°C AND D_2O AT 76°C: GENERAL ACID CATALYSIS

Nr	Lactaldehyde dimer [*M*]	Catalyst [*M*]	$k \times 10^{-4}$ min^{-1}
		2-Hydroxypyridine	
1	1.50	0.78	11.5
		Phenol	
2	1.50	0.69	11.8
		3-Hydroxypyridine	
3	1.50	0.86	18.8
		Benzoic acid	
4	1.50	0.70	21.3
		Salicylic acid	
5a	1.40	0.70	23.3
5b	1.30	1.20	59.0
		4-Hydroxypyridine	
6a	1.52	0.77	40.6
6b	1.55	0.26	11.0
		Trichloroacetic acid	
7	1.40	0.68	7.9
		Tyrosine ethylester	
8a	1.33	0.66	100.0
8b	1.15	0.17	7.0
		Hydroxyacetone	
9	1.42	0.69	8.0
		Phenol[b]	
10	0.8[a]	0.8	1.2
		2-Hydroxypyridine[b]	
11	0.8[a]	0.8	1.2
		4-Hydroxypyridine[b]	
12	0.8[a]	0.8	5.0

[a] Solvent, D_2O; temperature, 76°C.

[b] 0.8 *M* solutions of phenol, 2-hydroxypyridine, and 4-hydroxypyridine have pD at 25° 6.1, 6.5, and 6.8, respectively.

conditions. Deuterium oxide is found to be a less effective catalyst than water. The value of k_{H_2O}/k_{D_2O} for deuterolactaldehyde (0.7 *M*) is 1.36, which is a normal solvent isotope effect. The effect of concentration of the substrate and of water on the rates of isomerization suggests that the process is catalyzed by protic species.

Tables 4 and 5 show the effect of Brönsted acids on the rates of isomerization of lactaldehyde and deuterolactaldehyde in pyridine, respectively. The results from the various catalysts indicate that there is no simple correlation between catalytic efficiency and p*K* or nucleophilicity. However, the kinetic isotope effect for the same ratio of substrate/catalyst concentrations has values from 4 to 7. With salicylic acid and 4-hydroxypyridine (entries 5 and 6, Tables 4, 5) it can be seen that the rate of isomerization depends on the concentration of the catalyst. Trichloroacetic acid in pyridine proved to be a poorer catalyst than benzoic acid

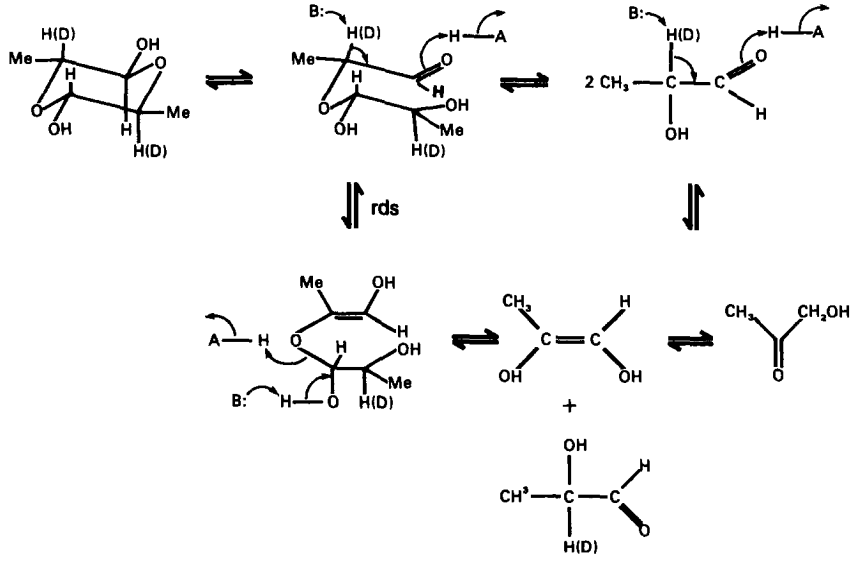
TABLE 5

RATE OF ISOMERIZATION OF α-DEUTEROLACTALDEHYDE IN PYRIDINE AT 90°C

Nr	α-Deuterolactaldehyde dimer [M]	Catalyst [M]	$k \times 10^{-4}$ min^{-1}
		2-Hydroxypyridine	
1	1.47	0.72	2.7
		Phenol	
2	1.52	0.70	2.6
		3-Hydroxypyridine	
3	1.45	0.70	3.5
		Benzoic acid	
4	1.43	0.68	4.0
		Salicylic acid	
5a	1.35	0.70	5.0
5b	1.23	1.27	9.7
		4-Hydroxypyridine	
6	1.52	0.74	6.8

(Table 4). This is expected since trichloroacetic acid is totally ionized and the acidic species, the pyridinium ion, is a weaker acid than the carboxylic acids. On the contrary tyrosine ethylester in pyridine is more effective than phenol under comparable conditions.

In all reactions the appearance and disappearance of intermediate species were detected by measuring the intensities of aldehydic protons at $\tau = 0.32$ ppm. A single aldehydic resonance was observed throughout the kinetic measurements which could belong either to the open dimer, or to the monomer, or to both (see Scheme 1). The rates reported at 90°C were obtained by starting with a crystalline



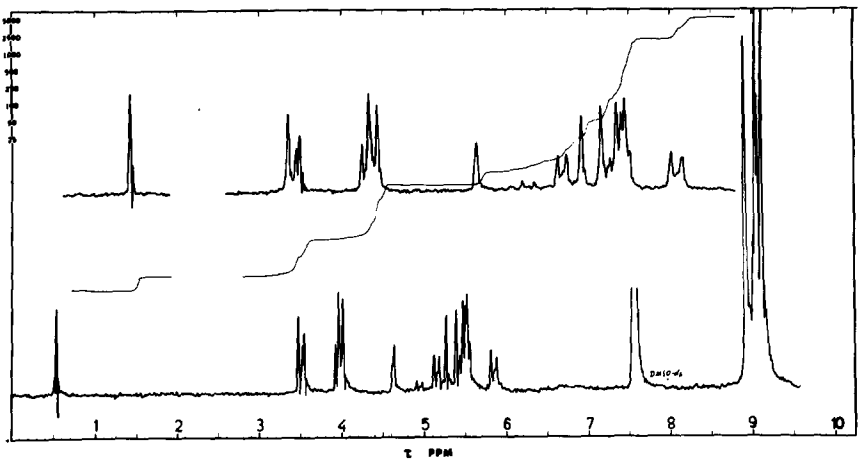


FIG. 2. Proton nmr spectrum of dimeric α-deuterolactaldehyde in DMSO-d_6 after heating at 90°C for 60 min. Inset: partial expanded spectrum (sweep width 500 Hz).

dimer where no aldehydic proton was detected from the nmr spectra at 40°C or by ir in a KBr pellet. When a solution of freshly distilled monomer in pyridine was used as the starting material the initial intensity of the aldehydic proton decayed rapidly at 90°C (half-life of decay was 60 min) and the isomerization proceeded with a *pseudo*-first-order rate 5×10^4 min^{-1}, which is almost equal to that of the dimer of comparable concentration (Table 2).

The nmr spectra of deuterated lactaldehyde in anhydrous DMSO-d_6 supply evidence which is consonant with the open dimer as the intermediate of the isomerization reaction. Lactaldehyde-2-d in DMSO-d_6 at room temperature exists in the dimeric form (no aldehydic proton is detectable). After heating at 90°C for 1 hr, aldehydic proton is detected at $\tau = 0.37$ ppm and new lines appear in the nmr spectrum at $\tau = 5.08$, $\tau = 3.43$ with $J_{H-HO} = 5$ Hz, and at $\tau = 4.57$, which belong respectively to the proton and hydroxyl of carbon-*3* and the hydroxyl of carbon-*4* of the open dimeric structure $\underset{4}{Me}\underset{3}{CH(OH)}-\underset{2}{CH(OH)}-O-\underset{1}{CD(Me)}-CHO$ (Fig. 2). From the intensities of these absorptions it was calculated that 25% of lactaldehyde-2-d exists as the open dimer in the DMSO-d_6 solution at 90°C. These data imply that the aldehydic proton which appears and disappears throughout the isomerization most likely belongs to the open dimer and not to the monomer. The open dimer is certainly in equilibrium with the monomer but apparently under the reaction conditions the monomer is less stable. For solutions in pyridine of the same concentration of proteo- or deuterolactaldehyde the percentage of the aldehydic species at 90°C, after temperature equilibration of the sample (approx. 5–10 min), appears to be the same, i.e., there is no observable isotope effect for the dimer to open dimer equilibrium. For example in a 2.1 *M* concentration proteo- or deuterolactaldehyde in pyridine at 90°C, 25% ± 3 of open dimer was present, as estimated from the nmr intensities. An equilibrium constant *K* of 0.3 ± 0.025 for the dimer to open dimer reaction in pyridine may be calculated.

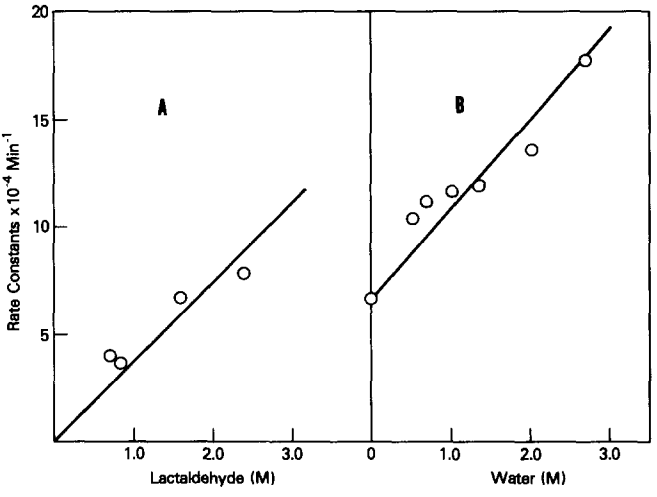


FIG. 3. (A) Concentration dependence of the *pseudo*-first-order rate constant for the isomerization of lactaldehyde in pyridine at 90°C. (B) Effect of water concentration on the rate of isomerization of a 1.6 *M* solution of lactaldehyde in pyridine at 90°C.

In the catalyzed reactions the appearance and disappearance of the aldehydic proton could also be observed at 0.32 τ, except in the case where 4-hydroxypyridine was used as a catalyst. The line width at half height of the resonance at τ = 0.32 ppm of the aldehydic proton (open dimer) at 90°C in pyridine is 1.5 Hz. In the presence of 4-hydroxypyridine (ratio of substrate/catalyst = 6) the resonance at 0.32 ppm is broadened to 16 Hz (half-height) indicating that the life time of the aldehydic proton is shortened. This broadening from the interaction of substrate-catalyst was also observed in DMSO-d_6. For example, gradual addition of 4-hydroxypyridine in a preheated solution of lactaldehyde in DMSO-d_6 or pyridine-d_5 for 1 hr at 90°C resulted in pronounced broadening of the aldehydic resonance without changing its chemical shift. When the ratio of lactaldehyde to 4-hydroxypyridine was 2, the aldehydic proton was broadened beyond detection. The broadening of the aldehydic resonance at τ = 0.32 ppm in the presence of 4-hydroxypyridine is probably due to the fast and reversible addition of the nitrogen of 4-hydroxypyridine to the carbonyl of the aldehydic species forming a tetrahedral intermediate (*16*). In addition the strong carbonyl absorption at 1620–1630 cm^{-1} (*17*) of 4-hydroxypyridine in pyridine shifts to lower frequencies (1600 cm^{-1}) in the presence of equimolar concentrations of lactaldehyde. The carbonyl absorption of 2-pyridone in pyridine at 1650 cm^{-1} is not affected by the presence of lactaldehyde.

Hydroxypyridines are tautomeric molecules and in aqueous solutions exist in the amide and the enol form (*18*). The 2- and 4-hydroxypyridines exist mainly in the amide form and are weaker acids than the 3-isomer. In solvents like dioxane and benzene dipole moment measurements and nmr data (*19*) show that 2- and 4-hydroxypyridines exist mainly as dipolar resonance hybrids of the amide form; the 3-isomer exists mainly in the enol form. The ratio of amide to enol tautomer of 4-hydroxypyridine is six times higher than the one of the 2-isomer in water. Also

the dipole moment of the 4-isomer in benzene exceeds the one of the 2-isomer by a factor of three. From the data in Tables 4 and 5 it can be seen that 4-hydroxypyridine is four times more efficient as a catalyst than 2-hydroxypyridine and the same relative catalytic efficiency is maintained when D_2O is used as solvent (entries 11 and 12, Table 4).[2]

A Brönsted plot was constructed from the data of Table 4 (entries 1–5a), by plotting the logarithms of the catalytic rate constants in liter per mole per minute versus the pK's of the corresponding catalysts.[3] The Brönsted coefficient had the value 0.09 ± 0.03. Griffith and Gutsche (*10*) reported a Brönsted coefficient of 0.11 for the isomerization of mandelaldehyde dimer to 2-hydroxyacetophenone under similar conditions (in pyridine at 90°C). The low value of the Brönsted coefficient suggests that the isomerization process is insensitive to the strength of the acid, or that a cooperative proton transfer of concerted acid–base catalyzed mechanism is involved. Since tautomeric molecules such as carboxylic acids and 2- and 4-hydroxypyridines exist in two tautomeric states which could both act as catalysts it is difficult to determine the mode of transfer of the proton between the substrate and the catalyst (*17*). The comparison of the catalytic behavior of 2- and 4-hydroxypyridine in pyridine and D_2O suggests that the acidity or basicity of these molecules plays only a secondary role in the isomerization process and that the strength of hydrogen bond between catalyst and substrate and the relative polarity of the resonance hybrids in the particular solvent may be of considerable importance (*25*). It is also conceivable that 4-hydroxypyridine, due to its *linear* tautomeric form, could fit in a Grotthuss mechanism of concerted proton transfer (*26*).

Isotopic Exchange

The following experiments were performed in order to examine the isotopic

[2] The pK's of 2- and 4-hydroxypyridines in water are (*18*): pK_{a_1} 0.75 and pK_{a_2} 11.62 for the 2-isomer; pK_{a_1} 3.27 and pK_{a_2} 11.09 for the 4-isomer. The pH of 0.8 M solution of dimeric lactaldehyde in water at 25°C is 4.4. Equimolar concentrations (0.8 M each) in water of lactaldehyde/catalyst have the following pH's at 25°C: (a) lactaldehyde and *2*-hydroxypyridine pH 5.1; (b) lactaldehyde and *4*-hydroxypyridine pH 6.4; (c) lactaldehyde and *3*-hydroxypyridine pH 6.7; and (d) lactaldehyde and phenol, pH 4.5.

[3] Brönsted acids in pyridine, in addition to existing in their ionized form, are present as hydrogen-bonded pyridine-acid adducts (pyr . . . HA) and as undissociated ion pairs ($pyrH^+A^-$) (*20*). The pK values of carboxylic acids maintain the relative relationships of their ionization constants in both pyridine and water and phenolic compound and purines show a relative enhancement of their acid strength in pyridine by 2.5 and 3.9 pK units, respectively, compared to that in water. This enhancement of the pK's in pyridine has been attributed to differences in solvation of the ions in pyridine (*21*) and to solvent–acid ion interactions controlled by dispersion forces (*20, 22*), which are stronger for delocalized ions such as phenols and purines as compared with carboxylic acids. The pK's of catalyst in pyridine estimated from Refs. (*20*) and (*23*) are: phenol, 7.48 (pK_{aq} 9.98–2.5); 2-pyridone, 9.2 (pK_{aq} 11.7–2.5); 3-hydroxypyridine, 6.55 (pK_{aq} 8.75–2.5); 4-hydroxypyridine 8.62 (pK_{aq} 11.12–2.5); benzoic acid 4.15 (pK_{aq} 4.20–0.05); and salicylic acid, 1.50 (pK_{aq} 2.98–1.48). Salicylic acid is a stronger acid in pyridine than water due to internal hydrogen bonding (*23*). Also acids in zwitterionic form, in low dielectric constant solvents, are stronger acids. However, any correlation of the pK's of Brönsted acids in water with acid–base equilibria in nonaqueous solvent are frequently unreliable because of serious errors arising from differences in the hydrogen-bonding properties of the anions and differences in their solvation (*24*).

exchange of starting materials and product during the isomerization reaction.

(a) Hydroxyacetone 1 *M* in pyridine in the presence of 1.7 *M* D_2O heated for 72 hr at 90°C did not show deuterium substitution of its methyl and methylene proton, as determined from the intensities of the proton nmr absorptions.

(b) Deuterolactaldehyde 0.7 *M* in anhydrous pyridine at 90°C throughout the isomerization to hydroxyacetone gave a ratio of 3 ± 0.2 for the methyl and methylene protons of the hydroxyacetone.

(c) When the same experiment was performed in the presence of D_2O 1.7 *M* the ratio CH_3/CH_2 was 2 ± 0.3 instead of 3.

(d) In the presence of H_2O 1.7 *M* the ratio was 1.5.

(e) In proteolactaldehyde in anhydrous pyridine, the ratio CH_3/CH_2 of hydroxyacetone was found to be 1.5.

(f) The same reaction in the presence of D_2O gave a ratio 2 ± 0.3.

Since the product hydroxyacetone does not exchange during the course of the reaction, the hydroxyacetone formed from experiment (b) gave the right value. In experiment (c) a ratio of 2 instead of 3 is justified because D_2O exchanges with the hydroxyl protons of the starting material. Experiment (d) shows complete exchange of the α-deuterium of lactaldehyde 2d with water. Finally from experiment (f) it is concluded that isotopic exchange with the solvent occurs during the isomerization reaction.

Mechanism of Isomerization

The results from the isotopic exchange and the values of the kinetic isotope effects between 4 and 7 reported in Tables 2–5 support the hypothesis that during the isomerization reaction the rate-determining step involves the abstraction of proton from the α-position to the carbonyl. In contrast to our values Griffith and Gutsche (*10*) report an overall isotope effect of 1.3 for the self-catalyzed isomerization of mandelaldehyde dimer and mandelaldehyde-2-d dimer to 2-hydroxyacetophenone. They favored as the rate-determining step the enolization pathway by involving an inverse equilibrium isotope effect equal to 3 for the opening of dimeric proteo- and deuteromandelaldehyde. In our system we did not observe an inverse equilibrium isotope effect. After temperature equilibration at 90°C, the amount of the aldehydic species (i.e., open dimer) measured for the proteo- and deuterolactaldehyde was about the same within the experimental error accepted for nmr measurements. The mechanism given in Scheme 1, with the rate-determining step involving the enolization of the open dimer, is consonant with all the experimental data that we have reported. Self-catalysis by the substrate, catalysis by water or Brönsted acids in pyridine are compatible with the mechanism where the isomerization is assisted by a base and an acid.

EXPERIMENTAL

Methylglyoxal dimethyl or diethylacetals (Fluka) were purified by fractional

distillation, 2-hydroxypyridine, mp 104–105°C, 3-hydroxypyridine mp 124–125°C, and 4-hydroxypyridine mp 140–144°C (Aldrich) were recrystallized twice from chloroform/petroleum ether. Phenol was purified by distillation. Benzoic and salicylic acid (Fluka) were recrystallized. All deuterated solvents were purchased from Merck Sharp Dohme and D_2O 100% and pyridine-d_5 from Stohler. Anhydrous pyridine was prepared by repeated distillations over barium oxide and kept in a desiccator.

A Varian A-60-A nmr spectrometer equipped with a variable temperature control unit V-4341/V-6057 was used for the kinetic measurements. The decoupling experiments were performed with a Varian XL-100 spectrometer. A Perkin-Elmer infrared spectrometer was used to record the ir spectra in pyridine.

Preparation of α-hydroxypropionaldehyde. Methylglyoxaldimethylacetal or diethylacetal (0.3 mol) in 200 ml anhydrous ether was added dropwise to a suspension of $LiAlH_4$ (0.11 mol) in 200 ml ether. The addition product was decomposed with water and ammonium chloride solution and the dimethylacetal of 2-hydroxypropionaldehyde was isolated as an oily material. NMR absorption in DMSO-d_6 at 8.87 τ (doublet), 6.67 τ (singlet), 6.30 τ (multiplet), and 5.87 τ (doublet) were assigned to the CH_3, OCH_3, H of C-2 and H of C-1, respectively. The coupling constants were $J_{CH_3-H_2} = 7.0$ Hz and $J_{H_1-H_2} = 6.5$ Hz. The acetal was hydrolyzed with 0.1 *N* H_2SO_4 as reported by Wohl (*27*). The crude product crystallized by standing for a few days in the refrigerator. The crystals (dimeric lactaldehyde) had a melting point of 101–104°C. The material was purified by distillation and monomeric lactaldehyde (bp 38–40°C at 0.1 mm Hg) was allowed to dimerize. The nmr spectrum of the fresh distillate in DMSO-D_6 corresponds to the lactaldehyde monomer (CH_3: 8.86 τ, H_2 of C-2: 5.99 τ, OH: 4.45 τ and CHO: 0.20 τ; $J_{CH_3-H_2} = 7.25$, $J_{H_2-OH} = 5.5$ Hz, and $J_{H_2-CHO} = 1$ Hz). The nmr parameters of the dimeric lactaldehyde are given in Table 1.

α-Hydroxypropionaldehyde-2-d was prepared by reduction of the methylglyoxaldimethylacetal with $LiAlD_4$ as described above. The nmr parameters of monomeric lactaldehyde-2-d (DMSO-d_6) are: CH_3: 8.85 τ, OH: 4.20 τ and CHO: 0.22 τ. The nmr parameters of dimeric lactaldehyde-2-d are given in Table 1. The assignments on Table 1 were verified by double resonance experiments. In proteolactaldehyde irradiation at 5.45 τ caused the collapse of the doublet at 3.94 τ into a singlet and irradiation at 5.97 τ converted the doublet at 9.05 τ to a singlet. Similarly, irradiation at 5.80 τ caused the collapsing of the doublet at 3.40 τ and irradiation at 6.35 τ the collapsing of the doublet at 9.03 τ. In deuterolactaldehyde irradiation at 3.48 τ brought the collapse of the doublet at 5.86 $\tau$and irradiation at 3.96 τ the collapse of the doublet at 5.46 τ.

Kinetic measurements. The samples for the kinetic measurements were degassed by freezing and thawing and sealed under vacuum. Samples closed by plastic caps wrapped with parafilm gave the same results as the sealed samples. A 90 ± 2°C constant temperature bath was used to keep the samples at this temperature for long intervals of time. All pseudo-first-order rate constants are the average of three or five experiments.

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REFERENCES

1. I. A. ROSE, "The Enzymes" (P. Boyer, ed.), Vol. 2, 3rd ed., p. 281. Academic Press, New York, 1970. I. A. ROSE, *Adv. Enzymol.* **43,** 491 (1975).
2. J. C. SPECK, JR., *Adv. Carbohydr. Chem.* **13,** 63 (1958) and references therein.
3. H. O. L. FISHER, C. TAUBE, AND E. BAER, *Chem. Ber.* **60,** 479 (1927).
4. O. MEYERHOF AND K. LOHMANN, *Biochem. Z.* **271,** 89 (1934).
5. (a) I. A. ROSE AND S. V. RIEDER, *J. Amer. Chem. Soc.* **77,** 5764 (1955); (b) E. A. NOLTMANN, "The Enzymes" (P. Boyer ed.), Vol. 6, 3rd ed., p. 271. Academic Press, New York, 1972; (c) J. R. KNOWLES, P. F. LEADLAY, AND S. G. MAISTER, *Cold Spring Harbor Symp. Biol.* **36,** 157 (1971); (d) C. D. GUTSCHE, D. REDMORE, R. S. BURIKS, I. NOWOTNY, H. GRASSNER, AND C. W. ARMBUSTER, *J. Amer. Chem. Soc.* **89,** 1235 (1967).
6. I. A. ROSE, *Brookhaven Symp. Biol.* **15,** 293 (1962).
7. A. HALL AND J. R. KNOWLES, *Biochemistry* **14,** 4348 (1975).
8. G. L. LOOKHART AND M. S. FEATHER, *Carbohydr. Res.* **60,** 259 (1978).
9. L. MICHAELIS AND P. RONA, *Biochem. Z.* **47,** 447 (1912).
10. D. W. GRIFFITHS AND C. D. GUTSCHE, *J. Amer. Chem. Soc.* **93,** 4788 (1971).
11. C. ZIOUDROU AND P. CHRYSOCHOU, *Tetrahedron* **33,** 2103 (1977).
12. A. S. PERLIN, *Canad. J. Chem.* **44,** 539 (1966); G. R. GRAY AND R. BARKER, *Biochemistry* **9,** 2455 (1970); D. W. GRIFFITHS AND C. D. GUTSCHE, *J. Org. Chem.* **36,** 2184 (1971).
13. (a) C. G. SWAIN AND J. F. BROWN, *J. Amer. Chem. Soc.* **74,** 2534 (1952); (b) A. S. HILL AND R. S. SHALLENBERGER, *Carbohydr. Res.* **11,** 541 (1969); (c) H. S. ISBELL AND W. PIGMAN, *Adv. Carbohydr. Chem. Biochem.* **24,** 13 (1969).
14. (a) L. MELANDER, "Isotope Effects on Reaction Rates," Chaps. 4 and 5. The Roland Press, 1960. (b) R. P. BELL, "The Proton in Chemistry," p. 201. Cornell University Press, Ithaca, 1959.
15. G. LAMATY, "Isotopes in Organic Chemistry" (E. Buncel and C. C. Lee, eds.), Vol. 2, Chap. 2, p. 46, 1976.
16. P. R. RONY, *J. Amer. Chem. Soc.* **91,** 6090 (1969).
17. S. F. MASON, *J. Chem. Soc.* 4874 and 5010 (1957).
18. (a) A. ALBERT AND Y. N. PHILLIPS, *J. Chem. Soc.* 1294 (1956); (b) S. F. MASON, *J. Chem. Soc.* 674 (1958); (c) P. BEAK, *Acc. Chem. Reas.* **10,** 186 (1977).
19. (a) U. VOGELI AND W. VON PHILIPSBORN, *Org. Mag. Reson.* **5,** 551 (1973); (b) A. R. KATRITZKY AND J. M. LAGOWSKI, *Adv. Heterocycl. Chem.* **1,** 339 (1963).
20. K. TSUJI AND P. J. ELVING, *Anal. Chem.* **41,** 286 (1969).
21. B. W. CLARE, D. COOK, E. C. F. KO, Y. C. MAE, AND A. J. PARKER, *J. Amer. Chem. Soc.* **88,** 1911 (1966).
22. E. GRUNWALD AND E. PRICE, *J. Amer. Chem. Soc.* **86,** 4517 (1964).
23. C. A. STREULI AND R. R. MIRON, *Anal. Chem.* **30,** 1978 (1958); C. A. STREULI, *Anal. Chem.* **32,** 407 (1969); R. R. MIRON AND D. M. HERCULES, *Anal. Chem.* **33,** 1770 (1961).
24. A. J. PARKER, *Quart. Rev. (London)* 163 (1962).
25. W. P. JENCKS, "Catalysis in Chemistry and Enzymology," Chap. 3, p. 239. McGraw-Hill, New York, 1969.
26. W. J. ALBERY, *Progr. Reaction Kinet.* **4,** 353 (1967).
27. A. WOHL, *Ber. Chem. Ges.* **41,** 3599 (1908).