transfer experiments.[4,5,6] In general, we envisage acyl group transfer reactions in solution possessing mechanisms with pathways that could take any direction in the energy contour diagram of Figure 1. We propose that there is smooth transition between the mechanisms resultant upon the changing energetics of the discrete intermediates.

It requires care to extrapolate the results of this study to enzyme mechanisms. Almost certainly the hydrolysis of peptides by serine proteinases will involve a stepwise path because of the stabilizing effect of the zwitterionic species ($R^+NH_2C(R)O^-$–O–enzyme) in the reaction. The oxyanion cavity[38] could stabilize the negative charge on the intermediate tetrahedral adduct in the serine protease catalyzed acyl transfer from esters.

(38) (a) Fersht, A. R.; Blow, D. M.; Fastrez, J. *Biochemistry* **1973**, *12*, 2035. (b) Robertus, J. D.; Kraut, J.; Alden, R. A.; Birktoft, J. J. *Ibid.* **1972**, *11*, 4293.

Acknowledgment. We are grateful to the Science and Engineering Research Council and to the Government of Saudi Arabia (S.B.-S.) for partial support of this work.

Registry No. 2,6-$Me_2C_6H_3O^-$, 25117-01-5; 4-$MeC_6H_4O^-$, 22113-51-5; 4-$FC_6H_4O^-$, 32376-34-4; $C_6H_5O^-$, 3229-70-7; 4-$ClC_6H_4O^-$, 24573-38-4; 3-$ClC_6H_4O^-$, 18938-14-2; 3,4-$Cl_2C_6H_3O^-$, 45670-76-6; 2-$ClC_6H_4O^-$, 29650-97-3; 4-$AcC_6H_4O^-$, 18983-84-1; 4-$NCC_6H_4O^-$, 14609-76-8; 2,3-$Cl_2C_6H_3O^-$, 96541-70-7; 3,4,5-$Cl_3C_6H_2O^-$, 60154-34-9; 4-$OHCC_6H_4O^-$, 18938-17-5; 2,6-$Cl_2C_6H_3O^-$, 53330-27-1; 2,4,5-$Cl_3C_6H_2O^-$, 45773-92-0; 2,3,5-$Cl_3C_6H_2O^-$, 100414-67-3; $C_6F_5O^-$, 26910-95-2; 2,3,5,6-$F_4C_6HO^-$, 91178-72-2; 2,3,4,5-$Cl_4C_6HO^-$, 68743-35-1; 4-$O_2NC_6H_4OAc$, 830-03-5; C_6F_5OAc, 19220-93-0; 2,3,5,6-C_6HF_4OAc, 110079-43-1; 4-$OHCC_6H_4OAc$, 878-00-2; 4-$O_2NC_6H_4O^-$, 14609-74-6; AcOAc, 108-24-7.

Supplementary Material Available: Table of physical properties of substituted phenyl acetates (1 page). Ordering information is given on any current masthead page.

Chiral Intermediates in Thiamin Catalysis. The Stereochemical Course of the Decarboxylation Step in the Conversion of Pyruvate to Acetaldehyde

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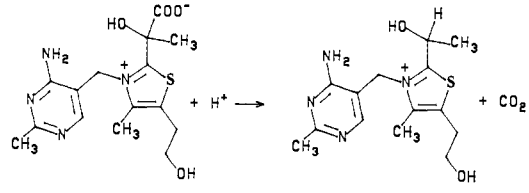
Abstract: The stereochemical course of the nonenzymic decarboxylation of the adduct of pyruvate and thiamin was determined. Racemic ethyl 2-(lact-2-yl)thiamin was resolved as the 2,3-dibenzoyltartrate salt under slightly acidic conditions. The specific rotations of the free enantiomers of ethyl 2-(lact-2-yl)thiamin are +8.4 ± 0.1° and −8.4 ± 0.1°. These were converted to the parent acid, 2-(lact-2-yl)thiamin, by acid hydrolysis. The (+) ethyl ester gave the (−) acid ($[\alpha]^{25}_D$ −4.7 ± 0.1°) while the (−) ethyl ester gave the (+) acid ($[\alpha]^{25}_D$ = +4.7 ± 0.1°). The acids were allowed to undergo spontaneous decarboxylation under a variety of conditions: aqueous solution, ethanol solution, dimethylformamide solution, and dimethylformamide solution containing brucine. Under all conditions the product was racemic 2-(1-hydroxyethyl)thiamin. These results require that the reaction proceed via an achiral intermediate or rapidly equilibrating achirally solvated enantiomeric intermediates. Since it is known that enzymic reactions produce a single enantiomer of 2-(1-hydroxyethyl)thiamin, the stereospecificity of the enzymic reaction is due to the chirality of the protein and not the intrinsic mechanism of the decarboxylation reaction.

The catalytic pathway of pyruvate decarboxylases involves two chiral intermediates derived from the combination of the substrate with thiamin diphosphate. The chiral adduct of thiamin diphosphate and pyruvate, 2-(lact-2-yl)thiamin diphosphate, exchanges carbon dioxide for a proton to produce a second chiral intermediate, 2-(1-hydroxyethyl)thiamin diphosphate.[1-4] The reaction of thiamin with pyruvate in the absence of enzyme produces similar adducts, 2-(lact-2-yl)thiamin ("lactylthiamin") and 2-(1-hydroxyethyl)thiamin ("hydroxyethylthiamin").



The decarboxylation of lactylthiamin formally involves substitution of a proton for carbon dioxide, with the potential intermediacy of a species which is the conjugate base of the product. Although thiamin is achiral, lactylthiamin and hydroxyethylthiamin are both chiral with the stereocenter located at the carbon atom at which the substitution occurs. Hydroxyethylthiamin diphosphate isolated from a subunit of pyruvate dehydrogenase (lacking subunits necessary for formation of acetyl-coenzyme A) has been shown to be a single enantiomer[5] and this is likely to result from the reaction of a single enantiomer of enzyme-bound lactylthiamin diphosphate. However, it is not clear if the stereochemical purity of the hydroxyethylthiamin diphosphate is a consequence of enzymic catalysis or an inherent property of the substitution process.

In order to study the stereochemical course of the nonenzymic reaction, it is necessary to have available the separated enantiomers of lactylthiamin and to relate the outcome of the reaction to the stereochemistry of the product. We have recently reported the resolution of the enantiomers of hydroxyethylthiamin and the determination of the absolute stereochemistry of the (+) and (−) enantiomers.[6,7] In this paper we report the isolation of the

(1) Breslow, R. *J. Am. Chem. Soc.* **1958**, *80*, 3719.
(2) Krampitz, L. O. *Thiamin Diphosphate and Its Catalytic Functions*; Marcel Dekker: New York, 1970.
(3) Kluger, R. *Ann. N.Y. Acad. Sci.* **1982**, *378*, 63.
(4) Kluger, R. *Chem. Rev.*, in press.
(5) Ullrich, J.; Mannschreck, A. *Eur. J. Biochem.* **1967**, *1*, 110.
(6) Kluger, R.; Karimian, K.; Gish, G.; Pangborn, W. A.; DeTitta, G. T. *J. Am. Chem. Soc.* **1987**, *109*, 618. The title and caption to Figure 1 contain a typographical error. The enantiomer of HETI shown has a *negative* (but very weak) specific rotation (circular dichroism is preferred for the assignment), as stated in the rest of the paper. This material results from (+)-hydroxyethylthiamin and the configuration at the stereocenter is *R* as indicated. We thank Professor W. Shin for noting the error.

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enantiomeric forms of the ethyl esters of lactylthiamin and their conversion to optically active lactylthiamin. The stereochemical course of the nonenzymic decarboxylation reaction was determined for a variety of conditions. The results provide information about the structure and solvation of a reactive intermediate.

Experimental Section

Methods. Instruments. Proton NMR spectra were recorded on a Varian T-60 spectrometer. Chemical shifts are reported relative to an external tetramethylsilane reference. UV spectra were obtained with a Varian Cary 210 spectrometer. Circular dichroism spectra were recorded on a Jasco spectrometer. Optical rotations were measured at 25 °C with a Perkin-Elmer digital polarimeter and are the average of 20 readings over a period of several minutes.

Materials. Thiamin chloride hydrochloride (USP) was purchased from Novopharm Limited (Scarborough, Ontario). Sodium pyruvate and ethyl pyruvate were obtained from the Sigma Chemical Co. and deuterium oxide (99%) was from MSD Isotopes Limited. Reagents and solvents were obtained from Fisher Scientific and BDH Canada Limited.

Synthesis. Ethyl 2-(lact-2-yl)thiamin (**1**) was prepared by the addition of the conjugate base of thiamin (prepared by the reaction of thiamin in absolute ethanol with 1 equiv of sodium ethoxide) with ethyl pyruvate as reported earlier.[8,9]

Resolution of Ethyl 2-(Lact-2-yl)thiamin. Preparation of 2-(Lact-2-yl)thiamin 2,3-Dibenzoyltartrate. A solution containing sodium hydroxide (1.143 g, 35.2 mmol) in 20 mL of water was added slowly to 40 mL of methanol containing 19.4 mmol of (+)-2,3-dibenzoyltartaric acid. This was added to a solution of 2-(lact-2-yl)thiamin (8.0 g, 17.6 mmol) in 20 mL of water. The sodium hydroxide was sufficient to neutralize the equivalent of lactylthiamin (which is in the form of chloride hydrochloride) and 1 equiv of 2,3-dibenzoyltartaric acid. The slight excess (1.8 mmol) of 2,3-dibenzoyltartaric acid is necessary in order to keep the solution acidic. We found that under neutral conditions, ethyl lactylthiamin decomposed to thiamin and ethyl pyruvate. The solution was concentrated on a rotary evaporator to the point of cloudiness and then allowed to stand for 48 h at room temperature at which time a fluffy white precipitate was observed. The precipitate was collected by filtration (sintered glass funnel) and washed with 10 mL of water. The solid was dissolved in 30 mL of warm methanol and water was added until the solution became slightly cloudy. After the solution stood at room temperature for 48 h, a precipitate resulted which was collected and recrystallized two more times. A similar procedure with (−)-2,3-dibenzoyltartaric acid produced the enantiomeric salt of ethyl lactylthiamin.

Isolation of (+)-Ethyl Lactylthiamin. The insoluble (+)-2,3-dibenzoyltartrate salt of ethyl lactylthiamin was dissolved in cold (4 °C) absolute ethanol and acidified with anhydrous HCl (from addition of concentrated aqueous HCl to anhydrous sulfuric acid) to pH 3 (measured with moistened indicator paper). Solvent was removed under aspirator vacuum on a rotary evaporator and the resulting oil was triturated with acetone to give a white solid. This was collected and washed twice with acetone and then with ether to yield 0.41 g of (+)-ethyl lactylthiamin, $[\alpha]^{25}_D$ +8.4 ± 0.1°. (Circular dichroism spectrum: θ_{271} = −3272, θ_{232} = (+)2154. UV λ_{max} = 271 nm, ϵ = 13400.) The analogous procedure with the (−)-2,3-dibenzoyltartrate salt of (−)-ethyl lactylthiamin gave a material with $[\alpha]^{25}_D$ −8.4 ± 0.1°.

(−) Lactylthiamin. The hydrolysis of the ethyl ester was conducted as reported previously for the racemic material.[8,9] (+)-Ethyl lactylthiamin (100 mg) was dissolved in 0.75 mL of concentrated aqueous HCl. After 24 h at 25 °C, the solution was concentrated under vacuum (without heating) until a glassy solid remained. This was dissolved in 10 mL of distilled water. The resulting material is (−)-lactylthiamin, $[\alpha]^{25}_D$ −4.7 ± 0.1° (Circular dichroism spectrum: θ_{277} = −2441, θ_{237} = (+)5826. UV λ_{max} = 263 nm, ϵ = 11423.) With use of (−)-ethyl lactylthiamin, (+)-lactylthiamin was obtained, $[\alpha]^{25}_D$ +4.7 ± 0.1°.

Decarboxylation Reactions. Aqueous Solution. The sample of (−)-lactylthiamin prepared as described above was dissolved in distilled water and left at room temperature for 24 h. The resulting solution was concentrated by rotary evaporation. Solutions of the material showed no optical activity and gave no circular dichroism spectrum. Addition of acetone to the residual oil afforded a white precipitate which was collected and washed twice with acetone and then with ether. ^{1}H NMR spectroscopy identified the material as hydroxyethylthiamin.[8]

Ethanol Solution. (+)-Ethyl lactylthiamin (50 mg) was dissolved in 0.4 mL of concentrated HCl and allowed to stand at room temperature overnight. The solution was cooled in an ice bath and 10 mL of absolute ethanol was added. Solvent was removed at room temperature under vacuum. The addition and removal of ethanol was repeated two more times to remove traces of water. Ethanol (2 mL) was then added to the residual material and the solution was left at room temperature for 2 days. Solvent was removed under vacuum and the resulting glassy material was treated with 5 mL of absolute ethanol. White crystals formed within a few hours. The crystals were isolated by centrifugation and washed in two portions of 2 mL of absolute ethanol and two portions of acetone (4 mL). The resulting material was dried (10 mg). It was identified by ^{1}H NMR and UV spectra as (hydroxyethyl)thiamin.[8] The material showed no optical activity.

Dimethylformamide (DMF) Solution. (+)-Ethyl lactylthiamin (50 mg) was dissolved in 0.4 mL of concentrated HCl and allowed to stand at room temperature overnight. The solution as cooled in an ice bath and 10 mL of dimethylformamide was added. The DMF had been dried over calcium oxide and distilled onto dry molecular sieves. Solvent was removed at room temperature under vacuum. The addition and removal of DMF was repeated two more times to remove traces of water. Dry DMF (2 mL) was then added to the residual material and the solution was left at room temperature for 2 days. Solvent was removed under vacuum and the resulting glassy material was treated with 5 mL of absolute ethanol. White crystals formed within a few hours. The crystals were isolated by centrifugation and washed in two portions of 2 mL of absolute ethanol and two portions of acetone (4 mL). The resulting material was dried (10 mg). It was identified by ^{1}H NMR and UV spectra as hydroxyethylthiamin.[8] The material showed no optical activity.

Effect of a Chiral Proton Source. Racemic ethyl lactylthiamin (1.0 g) was dissolved in 10 mL of concentrated HCl and allowed to stand at room temperature overnight. DMF (15 mL) was added to the solution at 0 °C and the solvent removed under vacuum without heating. Brucine (1.74 g, 2 equiv) in 15 mL of dry DMF was added and then the solvent was removed. Then 15 mL of dry DMF was added and the mixture was allowed to stand at room temperature for 4 days. The solvent was removed under vacuum without heat, and the resulting glassy material was treated with 20 mL of acetonitrile. The material was stirred for 1 h and the resulting precipitate was collected by filtration and washed with absolute ethanol. Solvent was removed by rotary evaporation and the resulting gum was treated with 20 mL of absolute ethanol. The solution was concentrated to 5 mL. The resulting precipitate was collected and dissolved in a minimal volume of 80% ethanol. Acetone was added to produce a precipitate which was collected and shown to be (hydroxyethyl)thiamin by its ^{1}H NMR and UV spectra. A solution of the material showed no optical activity.

Reaction in the Presence of Ethyl Pyruvate. The decarboxylation of (−)-lactylthiamin (0.05 g) in DMF was conducted as described above. In the last addition of DMF (2 mL), 1 mL (1.04 g) of ethyl pyruvate was also added. Isolation of the product revealed only the presence of racemic hydroxyethylthiamin (^{1}H NMR analysis). A very large molar excess of ethyl pyruvate was used in order to assure optimal conditions for potential trapping of the intermediate.

Effect of Solvent and Incubation on (+)-Hydroxyethylthiamin. The addition of (+)-hydroxyethylthiamin to acidic water followed by standing for 2 days and isolation as described above produced material with the same specific rotation as the starting material. Similarly, standing in DMF had no effect on optical activity of the sample.

Results

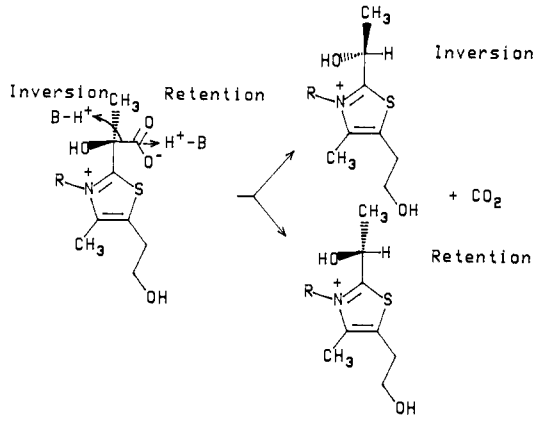
Resolution of Ethyl Lactylthiamin and Conversion to (+)- and (−)-Lactylthiamin. Ethyl lactylthiamin is readily resolved as the 2,3-dibenzoyltartrate salts under acidic conditions. Our initial attempts at this resolution were unsuccessful since we used the same conditions as for the resolution of hydroxyethylthiamin which involve neutral solutions.[7,10] We found that ethyl pyruvate and thiamin are formed from ethyl lactylthiamin in neutral solutions. The simple expedient of moderate acidification by the addition of a slight excess of dibenzoyltartaric acid permits the isolation of a single diastereomeric salt of ethyl lactylthiamin 2,3-dibenzoyltartrate. The properties of this material are listed in the Experimental Section.

Decarboxylation of the Enantiomeric Forms of Lactylthiamin. The progress of the decarboxylation of (−)-lactylthiamin in deuterium oxide was followed by ^{1}H NMR spectroscopy and by the optical rotation of the sample at 25 °C which was monitored for

(7) Kluger, R.; Stergiopoulos, V.; Gish, G.; Karimian, K. *Bioorg. Chem.* **1985**, *13*, 227.
(8) Kluger, R.; Chin, J.; Smyth, T. *J. Am. Chem. Soc.* **1981**, *103*, 884.
(9) Kluger, R.; Brandl, M. *J. Am. Chem. Soc.* **1986**, *108*, 7828.
(10) Shiobara, Y.; Sato, N.; Yogi, K.; Murakami, M. *J. Vitaminol.* **1966**, *12*, 286.

24 h. At the end of this time the sample showed no optical activity and no CD spectrum. The product of the decarboxylation of lactylthiamin was isolated and shown to be (hydroxyethyl)thiamin by its ^1H NMR spectrum. As a control, a solution of (+)-(hydroxyethyl)thiamin was kept under the same conditions for the same time. It did not undergo any change in its optical rotation and therefore the change in optical rotation of lactylthiamin upon decarboxylation is not due to racemization of the product.

The decarboxylation of lactylthiamin and related compounds is accelerated by solvents less polar than water. If the acceleration is partially due to stabilization of the transition state by concerted protonation or formation of an asymmetrically solvated intimate ion pair, then one would expect to see production of one enantiomer in excess.[11]



Decarboxylation of lactylthiamin in ethanol (24 h, room temperature) produced hydroxyethylthiamin with no optical activity. Therefore protonation is not concerted with decarboxylation nor is an asymmetrically solvated ion pair the immediate precursor of the product.

Since hydroxyethylthiamin is chiral, protonation of a prochiral intermediate by a chiral reagent should lead to two diastereomeric transition states. In an aprotic solvent, the only proton source is lactylthiamin itself or hydroxyethylthiamin Therefore we tested to see if this would produce optically active materials. The reaction was carried out in anhydrous DMF. The reaction produced racemic material as did the decarboxylation of racemic material in the presence of 2 equiv of an external, chiral proton source, (+)-brucine. When the reaction was conducted in DMF in the presence of excess ethyl pyruvate, only racemic hydroxyethylthiamin was produced; neither a condensation product (ethyl acetolactate) nor its adduct with thiamin was formed.

Discussion

The observation of formation of racemic products under all circumstances is consistent with the formation of a free achiral intermediate whose protonation is not subject to stereochemical preferences and whose formation is not assisted by the solvent or by the internal counterion. The presence of a chiral proton source which is capable of hydrogen bonding (the conjugate acid of brucine, with a stereocenter at the proton donor site) does not promote the preferred formation of a single enantiomer of hydroxyethylthiamin. The intermediate thus implicated is the conjugate base of hydroxyethylthiamin which is likely to have significant enamine character. The failure of the intermediate to react with a carbonyl compound (ethyl pyruvate) indicates that the reaction with protons is the favored process.

Doering and Pasternak reported the stereochemistry of decarboxylation of optically active 2-methyl-2-pyridylbutanoic acid in water.[12] That reaction produces racemic 2-(2-butyl)pyridine and thus implicates an achiral intermediate or transition state. Those workers reasoned that the reaction proceeds via an intermediate which has considerable enamine character. Cram and Haberfield examined the stereochemical outcome of the decarboxylation reaction of chiral 2-cyano-2-phenylbutanoic acid in a variety of solvents with ammonium ions and alkali metal ions as counterions.[11] Depending on the conditions of the reaction, substitution of the carbon dioxide moiety by a proton occurred with net retention, net inversion, or racemization. It is proposed that the reaction proceeds via a planar carbanion intermediate which may be subject to asymmetric solvation concerted with departure of carbon dioxide. Proton donation from the solvent thus facilitates the reaction and leads to substitution with inversion. In the presence of ammonium ions (which hydrogen bond to the carboxylate group), proton donation from the counterion leads to retention of relative configuration. In the absence of either of these factors, racemization occurs, as in our case.

Sable and co-workers showed that hydroxyethylthiamin undergoes base-catalyzed proton exchange at the C-2α position and thus provided the first direct evidence for the existence of the free conjugate base.[13,14] The rate of the proton exchange reaction is very slow (half-life 5 h at pD 8.5, 50 °C, $k = 4 \times 10^{-4}$ S^{-1}). This is consistent with the intermediate being a high-energy species due its very high basicity (estimated $pK = 17$[15]). Kuo and Jordan have shown that pyruvate decarboxylase can catalyze reactions of conjugated analogues of pyruvate to produce spectra which are consistent with the formation of an enamine structure[16,17] and have prepared ethers which are analogues of the proposed alcohol intermediate which would be derived from hydroxyethylthiazolium compounds.[18]

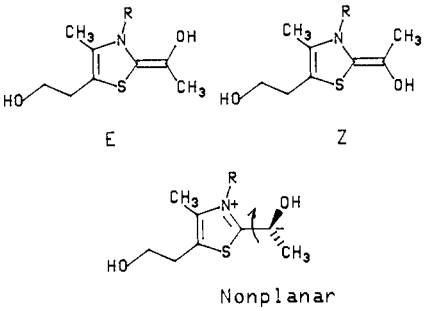
Bednar and Jencks have shown that hydrogen cyanide undergoes deprotonation by a mechanism which involves direct transfer of the proton from the carbon acid to a base.[19] This is in contrast to oxygen and nitrogen acids in which solvent molecules bridge the transfer and preassociate through hydrogen bonding. For carbon acids, preassociation with solvent or base through hydrogen bonding does not occur. For the case of hydroxyethylthiamin the situation is not necessarily the same as in hydrogen cyanide. The most acidic site on the side chain of hydroxyethylthiamin is the hydroxyl group. This may ionize and in turn accept a proton from carbon. Whatever the path, the outcome is that the carbanion is the ultimate product of proton removal and will also be the preferred protonation site.

The production of a single enantiomer of hydroxyethylthiamin diphosphate by the E1 subunit of pyruvate dehydrogenase[5] is thus not a consequence of the mechanism of the decarboxylation reaction but the result of the chiral environment. The protonation does not result from reaction of the intermediate with a catalytic group performing its normal function since the holoenzyme promotes oxidation rather than protonation of the enamine intermediate. One also would not expect protonation to be catalytically significant in the reactions of pyruvate decarboxylases. The nonenzymic conversion of lactylthiamin to hydroxyethylthiamin is dramatically accelerated in nonpolar solvents[8,15] and the active sites of pyruvate decarboxylases are known to be hydrophobic.[20] This is consistent with the observation that enzymic decarboxylations do not follow a common stereochemical pattern and therefore substitution of a proton for carbon dioxide does not follow a mechanistically determined pattern.[21,22]

In the proposed reaction scheme, the intermediate enamine can form as either the *E* or *Z* enantiomer.[1] In the enzymic reaction

(11) Cram, D. J.; Haberfield, P. *J. Am. Chem. Soc.* **1961**, *83*, 2354.
(12) Doering, W. E.; Pasternak, V. Z., *J. Am. Chem. Soc.* **1950**, *72*, 143.
(13) Mieyal, J. J.; Bantle, G.; Votaw, R. W.; Rosner, I. A.; Sable, H. Z. *J. Biol. Chem.* **1971**, *246*, 5213.
(14) Mieyal, J. J.; Votaw, R. G.; Krampitz, L. O.; Sable, H. Z. *Biochim. Biophys. Acta* **1967**, *141*, 205.
(15) Crosby, J.; Stone, R.; Lienhard, G. E. *J. Am. Chem. Soc.* **1970**, *92*, 2891.
(16) Kuo, D. J.; Jordan, F. *J. Biol. Chem.* **1983**, *258*, 13415.
(17) Kuo, D. J.; Jordan, F. *Biochemistry* **1983**, *22*, 3735.
(18) Jordan, F.; Kudzin, Z. H.; Rios, C. B. *Abstracts of Papers*, 191st National Meeting of the American Chemical Society; American Chemical Society: Washington, DC, 1986; Division of Organic Chemistry, Abstract No. 53.
(19) Bednar, R. A.; Jencks, W. P. *J. Am. Chem. Soc.* **1985**, *107*, 7126.
(20) Wittorf, J. H.; Gubler, C. J. *Eur. J. Biochem.* **1970**, *14*, 53.
(21) Hanson, K. R.; Rose, I. A. *CRC Crit. Rev. Biochem.* **1972**, *1*, 33.
(22) Benner, S. A.; Rozzell, J. D., Jr.; Morton, T. H. *J. Am. Chem. Soc.* **1981**, *103*, 993.

it is also possible that the enzyme could hold the material in a conformation that prevents formation of the enamine double bond and thus retains chirality. However, this would offer no particular mechanistic advantage and so is unlikely in terms of evolution of the catalytic function.



These *E* and *Z* forms should be produced proportionately from either of the two enantiomers of lactylthiamin. That is, either the *E* or the *Z* form will predominate, but the relative quantities will be independent of the absolute stereochemistry of lactylthiamin. Our results are consistent with the production of one or both of these and further work is necessary to determine the preferred stereochemistry of these systems.[18]

Conclusion

The stereochemical course of the nonenzymic decarboxylation of lactylthiamin under a variety of conditions indicates the preferential formation of a symmetrically solvated achiral intermediate from a chiral reactant to yield a chiral racemic product. The ability of an enzyme to generate a single enantiomer of hydroxyethylthiamin diphosphate from the decarboxylation of lactylthiamin diphosphate is consistent with a mechanism involving stereospecific protonation due to the effects of the enzymic medium. Knowledge of the stereochemistry of the decarboxylation reaction catalyzed by TDP-dependent enzymes as well as studies of the stereospecificity of binding processes will provide detailed information about catalysis in these systems.

Acknowledgment. This work was supported by the Natural Sciences and Engineering Research Council of Canada through an operating grant (R.K.) and a summer undergraduate research fellowship (K. Kitamura).

Molecular Design of Calixarene-Based Uranophiles Which Exhibit Remarkably High Stability and Selectivity

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Abstract: For the selective binding of the uranyl ion (UO_2^{2+}) a new class of uranophiles has been designed from calixarenes: they are para-sulfonated calix[*n*]arenes (*n* = 4, 5, 6: $\mathbf{2}_4$H, $\mathbf{2}_5$H, and $\mathbf{2}_6$H, respectively) and their carboxylated derivatives ($\mathbf{2}_4CH_2COOH$, $\mathbf{2}_5CH_2COOH$, and $\mathbf{2}_6CH_2COOH$, respectively). We have found that the cyclic pentamers ($\mathbf{2}_5$H and $\mathbf{2}_5CH_2COOH$) and the cyclic hexamers ($\mathbf{2}_6$H and $\mathbf{2}_6CH_2COOH$) have remarkably large stability constants ($K_{uranyl} = 10^{18.4-19.2}$ M^{-1}), whereas the cyclic tetramers ($\mathbf{2}_4$H and $\mathbf{2}_4CH_2COOH$) have very small stability constants ($K_{uranyl} = 10^{3.1-3.2}$ M^{-1}). This trend is very compatible with the X-ray data which show that UO_2^{2+} complexes invariably adopt the coplanar penta- or hexacoordination geometry. Hence, the high stability is better explained by "coordination-geometry selectivity" than by "hole-size selectivity". The selectivity factors ($K_{uranyl}/K_{M^{n+}}$) for $\mathbf{2}_6$H and $\mathbf{2}_6CH_2COOH$ were evaluated by comparing the K_{uranyl} with the stability constants for competing metal cations ($K_{M^{n+}}$). It was found that *the selectivity factors for these calixarenes are surprisingly large, 10^{12-17} as compared with competing Ni^{2+}, Zn^{2+}, and Cu^{2+} ions*! The remarkably high selectivity is attributed to the moderately rigid skeleton of calix[6]arene which can provide the preorganized hexacoordination geometry for the binding of UO_2^{2+} but cannot accommodate to the square-planar or tetrahedral coordination geometry for other metal cations in an "induced-fit" manner. Thus, calix[5]arene and calix[6]arene, which are easily synthesized from cheap starting materials, serve as excellent basic skeletons for the design of superior uranophiles.

The selective extraction of uranium from sea water has attracted extensive attention from chemists because of its importance in relation to energy problems. In order to design a ligand that can selectivity extract uranyl ion (UO_2^{2+}), one has to overcome a difficult problem: that is, the ligand must strictly discriminate between UO_2^{2+} and other metal ions present in great excess in sea water. A possibly unique solution to this difficult problem is provided by the unusual coordination structure of UO_2^{2+} complexes. X-ray crystallographic studies have established that UO_2^{2+} complexes adopt either a pseudoplanar pentacoordinate or hexacoordinate structure, which is quite different from the coordination structures of other metal ions.[1-6] This suggests that a macrocyclic host molecule having a nearly coplanar arrangement of either five or six ligand groups would serve as a specific ligand for UO_2^{2+} (i.e., as a uranophile). This approach has been investigated by Cram et al.,[7] Tabushi et al.,[8-10] and others.[11,12] For example, Tabushi et al.[9] synthesized a macrocyclic host molecule (**1**) having six carboxylate groups in the ring. Although the stability constant for UO_2^{2+} and **1** is quite large (log K_{uranyl} = 16.4 at pH 10.4 and 25 °C), the selectivity for UO_2^{2+} is not very high (e.g., $K_{uranyl}/K_{M^{n+}}$ = 80–210 for Ni^{2+} and Zn^{2+}) and the synthesis is not easy.[9]

(1) Graziani, R.; Forsellini, E. *J. Chem. Soc., Dalton Trans.* **1972**, 2059.
(2) Zachariasen, W. H. *Acta Crystallogr.* **1948**, *1*, 277.
(3) Zachariasen, W. H.; Ptettinger, H. A. *Acta Crystallogr.* **1959**, *12*, 526.
(4) Jayadevan, N. C.; Chackraburtty, D. M. *Acta Crystallogr.* **1959**, *12*, 526.
(5) Hall, D.; Rae, A. C.; Water, T. N. *Acta Crystallogr.* **1967**, *22*, 258.
(6) For a comprehensive review for the X-ray structures of UO_2^{2+} complexes see: Tabushi, I.; Kobuke, Y. *Nippon Kaisui Gakkaishi* **1982**, *36*, 205.
(7) (a) Alberts, A. H.; Cram, D. J. *J. Am. Chem. Soc.* **1977**, *99*, 3880. (b) Alberts, A. H.; Cram, D. J. *Ibid.* **1979**, *101*, 3545. (c) Alberts, A. H.; Cram, D. J. *J. Chem. Soc., Chem. Commun.* **1976**, 958.
(8) Tabushi, I.; Kobuke, Y.; Nishiya, T. *Tetrahedron Lett.* **1979**, 3515.
(9) Tabushi, I.; Kobuke, Y.; Ando, K.; Kishimoto, M.; Ohara, E. *J. Am. Chem. Soc.* **1980**, *102*, 5948.
(10) Tabushi, I.; Kobuke, Y.; Yoshizawa, A. *J. Am. Chem. Soc.* **1984**, *106*, 2481.
(11) Shinkai, S.; Koreishi, H.; Ueda, K.; Manabe, O. *J. Chem. Soc., Chem. Commun.* **1986**, 233.
(12) Fux, P.; Lagrange, J.; Lagrange, P. *J. Am. Chem. Soc.* **1985**, *107*, 5927.

0002-7863/87/1509-6371$01.50/0