Understanding the Mechanism of B_{12}-Dependent Diol Dehydratase: A Synergistic Retro-Push−Pull Proposal

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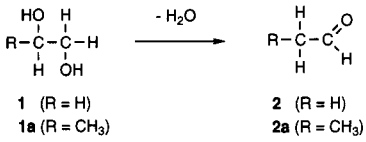
Abstract: Ab initio molecular orbital theory is used to investigate the coenzyme B_{12}-dependent reactions catalyzed by diol dehydratase. The key step in such reactions is believed to be a 1,2-hydroxyl migration, which occurs within free-radical intermediates. The barrier for this migration, if unassisted, is calculated to be too high to be consistent with the observed reaction rate. However, we find that "pushing" the migrating hydroxyl, through interaction with a suitable acid, is able to provide significant catalysis. This is denoted retro-push catalysis, the retro prefix signifying that the motion of the migrating group is in the direction opposite to the electron motion. Similarly, the "pulling" of the migrating group, through interaction of the spectator hydroxyl with an appropriate base, is found to substantially reduce the rearrangement barrier. Importantly, the combination of these two effects results in a barrier reduction that is notably greater than additive. This synergistic interplay of the push and the pull provides an attractive means of catalysis. Our proposed retro-push−pull mechanism leads to results that are consistent with isotope-labeling experiments, with experimental rate data, and with the crystal structure of the enzyme.

Introduction

Among the enzyme-catalyzed transformations reliant on coenzyme B_{12} (adenosylcobalamin) is a group of reactions in which water is eliminated from 1,2-diols.[1] A representative example is the diol dehydratase-catalyzed conversion of ethane-1,2-diol (**1**) into acetaldehyde **2** plus water.[2] This same protein



(1)

1 (R = H)
1a (R = CH_3)

2 (R = H)
2a (R = CH_3)

also acts on propane-1,2-diol (**1a**) giving propionaldehyde (**2a**) plus water at similar rates for both enantiomers. The detailed mechanism of the reactions has been the subject of much experimental study,[3] but no complete mechanistic picture has yet emerged.

Experiments with tritium-labeled substrate and cofactor,[4] as well as EPR studies,[5] give strong support for the involvement of free-radical intermediates in the diol dehydratase-catalyzed reactions. These experiments, combined with the generally accepted mode of action of coenzyme B_{12}, make it highly likely that a substrate hydrogen atom is abstracted by the 5′-deoxyadenosyl radical (Ado•, supplied by the coenzyme; see Scheme 1) to form 5′-deoxyadenosine and a substrate-derived radical **3**.[6] This radical could conceivably eliminate water to yield an allyloxy radical, which abstracts a hydrogen atom from 5′-deoxyadenosine with formation of the product aldehyde. Indeed, recent studies have proposed just this type of mechanism for the diol dehydratase-catalyzed processes.[7,8] However, mechanisms of this kind are seemingly at odds with experimental isotope-labeling studies.

Experiments carried out with ^{18}O-labeled propane-1,2-diols have shown that, depending on the chirality at C2 of the substrate, either the 1-hydroxyl or 2-hydroxyl group can be eliminated in the formation of propionaldehyde. The generally accepted interpretation of this result is that the hydroxyl group at C2 is transferred to C1 and that propane-1,1-diol (**5a**) is an obligatory intermediate.[9−11] By analogy, it would seem likely that the dehydration of ethane-1,2-diol should proceed via

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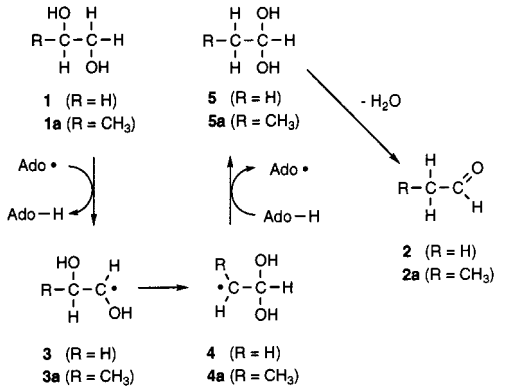
Scheme 1. Proposed Mechanism for the Diol Dehydratase-Catalyzed Reactions[a]



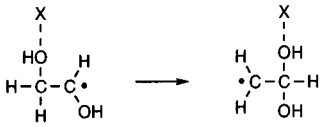
[a] Ado• denotes the 5′-deoxyadenosyl radical supplied by the coenzyme. See ref 6.

ethane-1,1-diol. In contrast, the proposal of George et al.[7] would require that the dehydration mechanisms of ethane-1,2-diol and propane-1,2-diol proceed via different pathways.

The combination of the evidence for radical intermediates with the isotope-labeling studies has thus led the bulk of researchers in this field to accept the general mechanistic picture shown in Scheme 1, valid for both ethane- and propane-1,2-diols.[3d] Here, hydrogen atom abstraction is followed by a 1,2-shift of a hydroxyl group, resulting in a *gem*-diol-related radical. This radical intermediate (**4**, Scheme 1) can then reabstract a hydrogen atom from 5′-deoxyadenosine (Ado−H, Scheme 1), forming the closed-shell 1,1-diol **5**. Enzyme-catalyzed elimination of water from this prochiral species affords the product aldehyde **2**, in a manner consistent with the isotope substitution experiments.

The main difficulty with the mechanism shown in Scheme 1 is that the radical rearrangement step (**3** → **4**) is known to be associated with a high barrier. For example, a recent examination of the 1,2-hydroxyl shift in the 1,2-dihydroxyethyl radical **3** puts the activation energy at 113.1 kJ mol^{-1}.[12] In contrast, the likely barrier for the rate-limiting step in the enzymatic reaction has been estimated, from the experimental rate constant, to lie between 55 and 70 kJ mol^{-1}.[8b] It is therefore of interest to investigate how it is that the enzyme might be able to bring about the barrier lowering required to drive the dehydration at a biologically useful reaction rate. We shall address elsewhere the question as to why such an intrinsically difficult step should be involved in the mechanism at all.

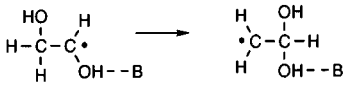
We have examined two primary types of catalytic assistance for the radical rearrangement step. The first of these we term "retro-push" catalysis to encompass the various means by which the migrating hydroxyl group can be *pushed* through interaction with a suitable acid (X):



(2)

We use the retro prefix to signify that the motion of the migrating group is in the direction opposite to the electron flow, although the prefix is occasionally dropped in the interests of simplicity. The initial suggestion of this type of catalysis (for diol dehydratase) came from early model studies which showed that protonation of the migrating hydroxyl (i.e., X = H^+ in reaction 2) results in a substantial reduction in the rearrangement barrier.[13] This kind of interaction is able to withdraw electron density from the migrating hydroxyl, thus weakening the associated C−O bond and enabling more facile rearrangement. Subsequent work, on a larger model system, demonstrated some problems associated with full protonation and offered an alternative ("predissociation") mechanism.[8] This predissociation mechanism is one possibility that struggles to be consistent with the experimental isotope-labeling studies.[9,12] Very recently, however, we demonstrated that partial proton transfer to the migrating oxygen from a range of Brønsted acids (e.g., X = $CH_3NH_3^+$, NH_4^+, etc.) is able to overcome the difficulties associated with full protonation and thus provide a viable alternative.[12]

The second type of assistance considered in the current work centers on what we term "retro-pull" catalysis. In this case, the migrating group is *pulled* across as a result of an interaction between the spectator hydroxyl and a suitable base (B):



(3)

Again we use the retro prefix to signify that the motion of the migrating group is in the direction opposite to the electron flow. Numerous studies have shown that α-hydroxyalkyl radicals (such as **3**) are ∼10^5 times more acidic than the corresponding alcohols. Those with a leaving group in the β position are seen to rapidly undergo a base-catalyzed fragmentation to $^{\bullet}CH_2$−CHO in solution.[3d,13b,14] Recognition of these properties has recently led to the suggestion of mechanisms, for the diol dehydratase-catalyzed reactions, involving "ketyl" radical anion intermediates.[1,6] These mechanisms postulate that, following hydrogen atom abstraction (**1** → **3**, Scheme 1), the proton attached to the spectator oxygen is lost (Scheme 2) to give the radical anion **6**. In keeping with its solution properties, this species is thought to eliminate the adjacent hydroxyl function to form an allyloxy radical **7** plus the hydroxide ion. To enable stereochemical consistency, it is proposed that the eliminated hydroxide readds to the carbon framework (to form **8**), whence recapture of a proton results in the *gem*-diol-related radical **4**. This species can then reabstract a hydrogen atom and lose water, as before, yielding the product aldehyde. Our present investigations reveal that pulling the migrating group, via abstraction of a proton (or by partial deprotonation) from the spectator hydroxyl, can indeed assist the radical rearrangement. We find, however, that it is not necessary to invoke a fully dissociative

(9) Experiments carried out with enantiomeric propane-(1-^{18}O)-1,2-diols show that most of the label is retained in product propanal starting from the *S* isomer while most of the label is lost starting from the *R* isomer. Experiments carried out with racemic propane-(2-^{18}O)-1,2-diol show that approximately half of the label is retained in the product aldehyde. The simplest explanation for these results involves the intermediacy of a 1,1-diol.[3f,10,11]

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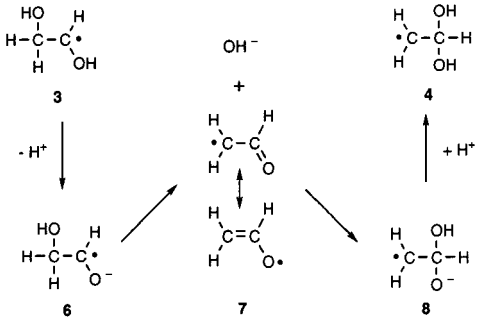
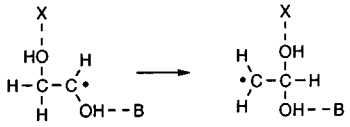
Scheme 2. The Ketyl (or Radical Anion) Mechanistic Proposal[a]



[a] See refs 1 and 6.

process that would involve the elimination and readdition of OH^- to make use of this type of assistance.

Finally, we have examined the effect of retro-push and retro-pull catalysis acting in concert:



(4)

We want to determine whether such a combination shows a reduced catalytic effect, whether the effect is roughly additive, or whether there is synergism between the push and the pull.

The X-ray crystal structure of diol dehydratase from *Klebsiella oxytoca* was recently reported,[15] and associated theoretical calculations were carried out.[16] It has been suggested that an alkali metal cation (in particular K^+) might be directly involved in the catalysis. It is of interest to examine this conclusion and other aspects of the crystal structure in the light of our calculations.

Theoretical Procedures

Standard ab initio molecular orbital calculations[17] were performed with GAUSSIAN 94,[18a] GAUSSIAN 98,[18b] and MOLPRO 97.[19] Geometries and zero-point vibrational energies were obtained using the B3-LYP/6-31G(d,p) procedure, the ZPVEs being scaled by 0.9806.[20] Polarization functions on hydrogen atoms have been included here and in our other related studies[12,21] to provide a better description of species involving hydrogen bonds. For species without hydrogen bonds, the exclusion of these light-atom polarization functions (for geometries and frequencies) is expected to have a minimal effect.[22] Improved relative energies (ΔE), for the bulk of the species reported herein, were obtained using a previously outlined modification[12] of the G2(MP2,SVP)-RAD[23] procedure:

$$\Delta E(\text{G2(MP2,SVP)-RAD(p)}) = \Delta E(\text{URCCSD(T)/6-31G(d)}) + \Delta E(\text{RMP2/6-311+G(3df,2p)}) - \Delta E(\text{RMP2/6-31G(d)}) + \Delta E(\text{ZPVE}) \quad (5)$$

It is well known that the inclusion of diffuse functions is necessary to obtain a proper description of anionic species. In the context of G2-type theories, it has recently been found that including the effect of diffuse functions directly, rather than relying on the additivity approximation, leads to improved results.[24] In an effort to incorporate this approach into our current treatment, we have replaced the 6-31G(d) basis set in eq 5 with the slightly larger 6-31+G(d) for use with anionic species. The resultant method (which we have termed G2(MP2,SVP)(+)-RAD(p)) is computationally more expensive than its parent, but should provide more reliable energetics for the negatively charged systems. We found that including diffuse functions during the geometry and frequency calculations had no significant effect and so continued to use B3-LYP/6-31G(d,p) for obtaining these properties.

Unless otherwise specified, the relative energies presented in this paper refer to G2(MP2,SVP)-RAD(p) values for surfaces involving neutral species and G2(MP2,SVP)(+)-RAD(p) values for surfaces involving anionic species, all at 0 K. The corresponding total energies and GAUSSIAN 94 and GAUSSIAN 98 archive entries for the RMP2/6-31G(d)//B3-LYP/6-31G(d,p) calculations for all relevant structures are presented in Tables S1−S4 of the Supporting Information, while selected thermochemical parameters are displayed in Table S5−S7.

We note that our calculations refer to isolated molecules in the gas phase. The extent to which they are applicable to the enzyme system will depend in part on the extent to which the active site of the enzyme is sequestered from water.

Results and Discussion

A. The Retro-Push Mechanism. (1) Protonation. As mentioned above, it was suggested some time ago that the diol dehydratase enzyme might facilitate the radical rearrangement through protonation of the migrating hydroxyl.[13] This proposal was supported by molecular orbital calculations on the 2-hydroxyethyl radical which showed that protonation lowered the rearrangement barrier from more than 100 kJ mol^{-1} to only 18 kJ mol^{-1}.[8,13] More recent higher-level calculations have confirmed this finding, with protonation reducing the barrier to 26.8 kJ mol^{-1} at the G2(MP2,SVP)-RAD(p) level of theory.[12]

Enlargement of the model system to include a second hydroxyl substituent, however, exposed some problems with the concept of assistance to hydroxyl migration by full protonation.[8] It was found that protonation of the "migrating" hydroxyl of the 1,2-dihydroxyethyl radical does not result in either a stable reactant complex or product complex. Instead,

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(19) MOLPRO 97 is a package of ab initio programs written by Werner, H.-J. and Knowles, P. J. with contributions from Almlof, J., Amos, R. D., Berning, A., Cooper, D. L., Deegan, M. J. O., Dobbyn, A. J., Eckert, F., Elbert, S. T., Hampel, C., Lindh, R., Lloyd, W. A., Meyer, W., Nickless, A., Peterson, K., Pitzer, R., Stone, A. J., Taylor, P. R., Mura, M. E., Pulay, P., Schütz, M., Stoll, H., Thorsteinsson, T.

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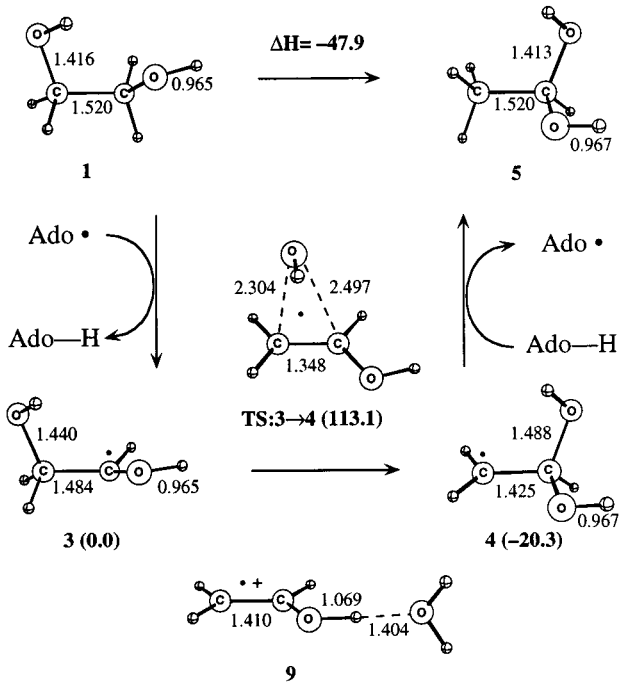

Figure 1. B3-LYP/6-31G(d,p) structures and selected bond lengths (Å) for the species involved in the transformation of ethane-1,2-diol (**1**) into ethane-1,1-diol (**5**), via free-radical intermediates. Included is the structure of the "protonated 1,2-dihydroxyethyl radical" **9**. Relative energies (G2(MP2,SVP)-RAD(p) values in kJ mol^{-1}) are given in parentheses.

the protonated 1,2-dihydroxyethyl radical spontaneously rearranges to a hydrogen-bonded hydrate of the vinyl alcohol radical cation (**9**, Figure 1). On the basis of this observation, George et al. proposed a "predissociation" mechanism whereby, after various proton- and hydrogen atom-transfer steps, the resulting complex **9** dissociates to give acetaldehyde plus water.[8] However, their postulated mechanism does not appear to be consistent with the ^{18}O-labeling results,[12] since the "migrating" oxygen will always end up in the eliminated water.[9] Furthermore, the hydrogen-bonding site postulated to be taken up by water in **9** is almost certainly used by the enzyme to bind the substrate (see later) and would therefore be unlikely to be available for participation in such complexes.

(2) Partial Proton Transfer. Following our calculations on the methylmalonyl-CoA mutase-catalyzed reaction,[21] we became interested in whether partial transfer of a proton to the migrating hydroxyl of the 1,2-dihydroxyethyl radical might be sufficient to catalyze the rearrangement. Indeed, we found that a range of Brønsted acids were remarkably effective in this regard as a result of interaction with the migrating hydroxyl group.[12]

Perhaps the simplest demonstration of the partial proton-transfer concept, in the context of diol dehydratase, comes from an examination of the catalysis of the rearrangement by the ammonium ion (NH_4^+). In contrast to the fully protonated analogue **9**, the partially protonated radical (**10**, Figure 2) is found to be a stable entity. The C−O bond adjacent to the migrating hydroxyl can be seen to be significantly lengthened (1.510 Å) with respect to the isolated radical (1.440 Å in **3**, Figure 1). This bond lengthening can reasonably be taken to imply a more weakly bound, and hence more labile, hydroxyl group. In accordance with this observation, we find a transition structure (**TS:10→11**) for the 1,2-shift of the partially protonated hydroxyl group, lying just 49.6 kJ mol^{-1} higher in energy than the reactant complex. The product of this rearrangement, the partially protonated 1,1-dihydroxyethyl radical **11**, is also found

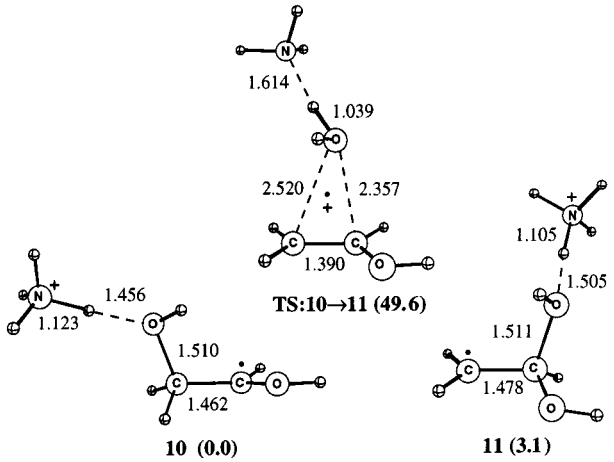

Figure 2. B3-LYP/6-31G(d,p) structures and selected bond lengths (Å) for the species involved in the catalysis by NH_4^+ of the 1,2-shift of the hydroxyl group in the 1,2-dihydroxyethyl radical. Relative energies (G2(MP2,SVP)-RAD(p) values in kJ mol^{-1}) are given in parentheses.

to be a stable entity. This latter species is found to lie only slightly higher in energy than the reactant, making the radical rearrangement endothermic by 3.1 kJ mol^{-1} (Figure 2).

Even from the early results with the 2-hydroxyethyl radical, it was clear that protonation of the migrating hydroxyl is able to impart a stabilizing influence on the transition structure for the 1,2-shift. The problems associated with the protonation of the 1,2-dihydroxyethyl radical arise because the extreme of full protonation is too harsh for the reactant and product radicals to remain stable. Interaction of a Brønsted acid rather than an isolated proton with the migrating hydroxyl, however, exerts a moderating influence and allows for the best of both worlds. That is, the conjugate base of the interacting acid is able to hold back the acidic proton to the extent that the reactant and product radicals become stable entities. At the same time, however, the partially transferred proton preferentially stabilizes the transition structure, causing the migration barrier to be reduced from more than 100 to ∼50 kJ mol^{-1} (in the case of NH_4^+). In addition, we find that, by choosing acids of differing strength, we can manipulate the barrier toward either extreme. Figure 3 shows a schematic energy profile depicting the effects of these various retro-push catalysts on the radical-rearrangement step. Additional details are given in Table S5 of the Supporting Information.

(3) Lewis Acid Catalysis. The recent resolution of the X-ray crystal structure of diol dehydratase from *K. oxytoca* has raised the additional possibility that an alkali metal cation (in particular K^+) might be directly involved in catalysis[15] (see section D for further discussion of this and other aspects of the reaction). To this end, we have investigated the effect of Li^+ and Na^+ on the reaction. Figure 4 shows the radical rearrangement in the presence of Li^+, as a representative example. The similarities between the rearrangement shown in Figure 4a and the system involving NH_4^+ (Figure 2) are immediately obvious. There is, again, a lengthening of the C−O bond in the substrate-derived radical **12** and the Li^+ is drawn closer to the more electron-rich oxygen in the transition structure (**TS:12→13**), in much the same way as the proton of NH_4^+. It is evident, however, from the calculated barrier of 70.4 kJ mol^{-1}, that the Lewis acid is not able to impart quite as much benefit to the transition structure as is the mobile proton from ammonium. As might be expected on the basis of a simple nuclear shielding argument, Li^+ is a more efficient catalyst than Na^+ (the latter being associated with a calculated barrier of 93.2 kJ mol^{-1}).

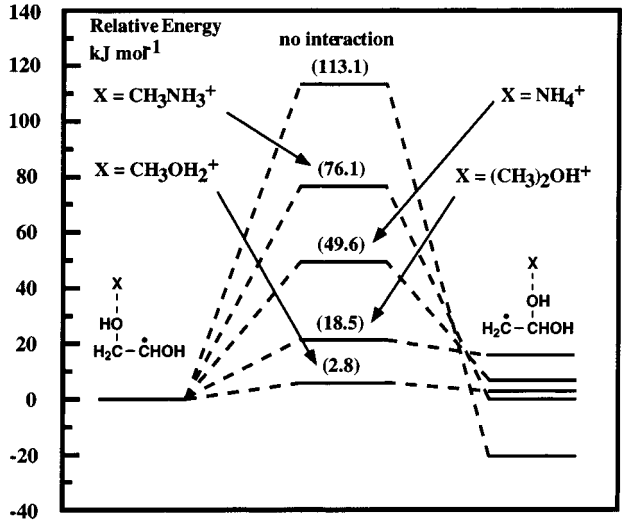

Figure 3. Schematic energy profile for the retro-push catalysis of the 1,2-shift of the hydroxyl group in the 1,2-dihydroxyethyl radical through interaction of the migrating hydroxyl group with various acids (X). Relative energies (G2(MP2,SVP)-RAD(p) values in kJ mol^{-1}) are given in parentheses.

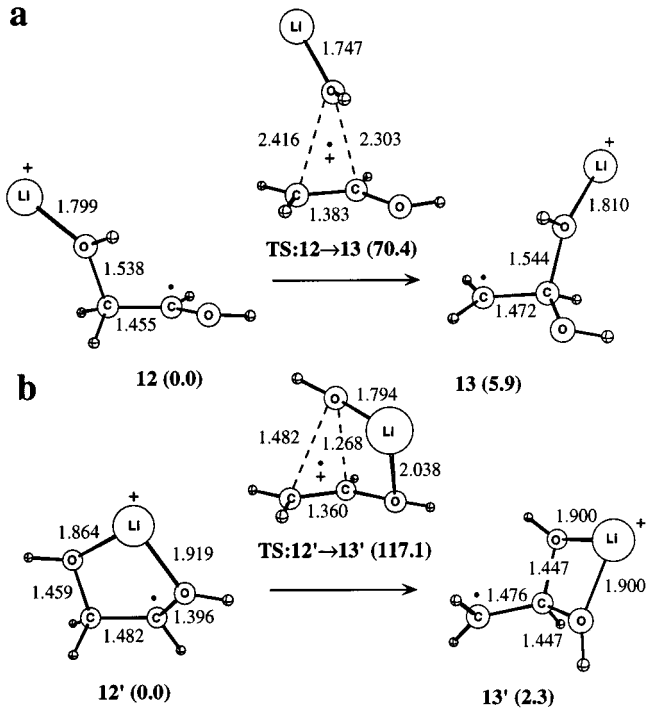

Figure 4. B3-LYP/6-31G(d,p) structures and selected bond lengths (Å) for species involved in the catalysis by Li^+ of the 1,2-shift of the hydroxyl group in the 1,2-dihydroxyethyl radical, in (a) the single-contact arrangement and (b) the bridging arrangement. Relative energies (G2(MP2,SVP)-RAD(p) values in kJ mol^{-1}) are given in parentheses.

The preferred arrangement for Li^+ interacting with the 1,2-dihydroxyethyl radical is actually one in which the metal ion bridges the two oxygens (**12′**).[25] This structure is 49.7 kJ mol^{-1} lower in energy than the single-contact arrangement (**12**). The bridged transition structure for the 1,2-shift (**TS:12′→13′**), however, is only 2.9 kJ mol^{-1} lower in energy than its single-contact counterpart. This differential stabilization is so extreme that the barrier for the bridged system in Figure 4b (117.1 kJ mol^{-1}) is actually higher than that for the unassisted rearrangement (113.1 kJ mol^{-1}, Figure 1). The same is true for Na^+ (with a calculated barrier in the bridging arrangement of 113.7 kJ mol^{-1}).

Interestingly, if the above energy comparisons are made using only the lower-level (and therefore less reliable) B3-LYP/6-31G(d,p) results, a Lewis acid in the bridging arrangement is predicted to offer a small amount of catalysis. The B3-LYP method predicts that the unassisted rearrangement requires 97.4 kJ mol^{-1}. The introduction of Li^+ in the bridging position (as in Figure 4b) reduces the barrier (with B3-LYP/6-31G(d,p)) to 90.4 kJ mol^{-1}, whereas Na^+ in this same position is associated with an activation energy of 91.7 kJ mol^{-1}.

On the basis of results similar to the above B3-LYP/6-31G(d,p) predictions, it has been suggested[16] that the 1,2-shift is actually catalyzed by a Lewis acid in the bridging arrangement. Our higher-level G2(MP2,SVP)-RAD(p) results, however, suggest that this conclusion is an artifact of the level of theory used and that in reality such a situation is anticatalytic and unlikely *in its own right* to be responsible for facilitating the 1,2-shift. On the other hand, we shall see below that the Lewis acid *does* facilitate the rearrangement in the presence of a retro-pull catalyst.

B. The Retro-Pull Mechanism. (1) Deprotonation. The second catalytic mechanism that we have considered involves various degrees of deprotonation of the spectator hydroxyl. As mentioned before, consideration of this effect has arisen from the known acidity of the hydroxyl proton in related radicals,[13b,14] and the proposal of mechanisms involving ketyl (or radical anion) intermediates (Scheme 2) is based upon the loss of this proton.[1]

Ab initio investigations of the ketyl mechanism are complicated by the fact that 2-hydroxyacetaldehyde is calculated to have a negative electron affinity in the gas phase. That is, although calculations can be (and have been, see below) performed upon the ketyl intermediate (**6**, Scheme 2), the electron is artificially bound by the limitations of the finite one-particle basis set. Given the chance, the system would benefit from losing an electron to give 2-hydroxyacetaldehyde. This is not to say that the radical anion species **6** is not stable in solution or at the active site of an enzyme, where the surrounding medium may provide some stabilizing influence. Before consideration of these effects, however, it is instructive to examine the isolated ketyl species in the gas phase.

Figure 5a shows the calculated structure of the proposed ketyl intermediate, the radical anion of 2-hydroxyacetaldehyde (**6**). Our calculations indicate that the energy of this species would be lowered by 63.0 kJ mol^{-1} by the loss of a single electron (Table S6). Nevertheless, we find a transition structure on this somewhat unstable surface that describes the intramolecular 1,2-shift of a hydroxyl group (**TS:6→8**). The barrier associated with this shift is 47.5 kJ mol^{-1} (Figure 5a); i.e., deprotonation has a substantial catalytic effect. The product of hydroxyl transfer (**8**) is found to lie 30.1 kJ mol^{-1} above the reactant 2-hydroxyacetaldehyde radical anion (**6**).

(2) Partial Proton Transfer. In an effort to stabilize the ketyl species as well as to extend the partial proton-transfer concept to partial deprotonation, we have investigated the effect of allowing various bases to interact with the spectator hydroxyl group. The presence of OH^- at this position results in a partial proton transfer from the spectator hydroxyl to the base to produce a complex which can be described as the interaction of H_2O with the deprotonated substrate-derived radical at the spectator oxygen (**14**, Figure 5b). The binding energy of the

(25) For the Brønsted acids with two or more protons (NH_4^+, $CH_3NH_3^+$, etc.), this bridging arrangement is also the one of lowest energy. It does not occur when the Brønsted acid is monoprotic such as $(CH_3)_2OH^+$ or for many of the acidic groups that might appear in the enzyme.

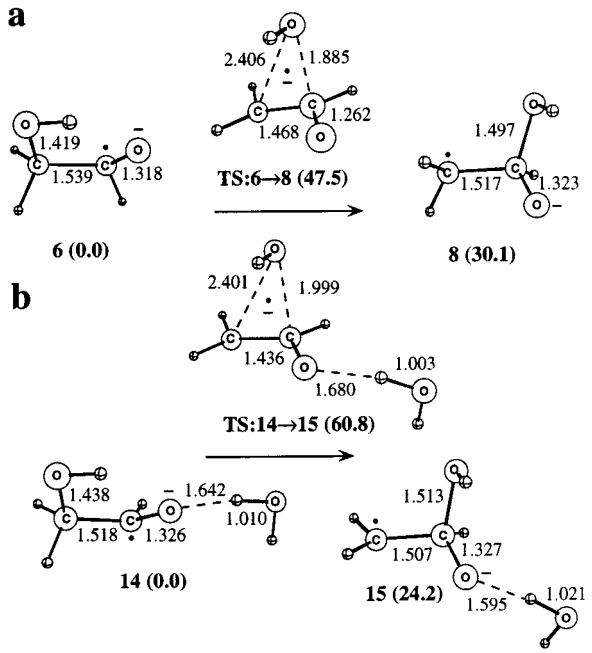

Figure 5. B3-LYP/6-31G(d,p) structures and selected bond lengths (Å) for species involved in the 1,2-shift of the hydroxyl group in the 1,2-dihydroxyethyl radical, involving catalysis by (a) deprotonation (**6** → **8**) and (b) the hydroxide ion (**14** → **15**). Relative energies (G2(MP2,SVP)(+)-RAD(p) values in kJ mol^{-1}) are given in parentheses.

additional electron in this complex (−8.5 kJ mol^{-1}) is considerably less negative than in the isolated case. We find that the presence of OH^- causes the barrier for the 1,2-hydroxyl shift to decrease from 113.1 to 60.8 kJ mol^{-1}, with an associated endothermicity of 24.2 kJ mol^{-1} (Figure 5b).

The replacement of OH^- in Figure 5b by the slightly less basic CH_3O^- results in a structurally similar complex, with the proton again localized on the base. The presence of the methyl group is almost sufficient for the system to bind the electron in an energetically beneficial manner, with a calculated electron affinity for the corresponding neutral species of −1.1 kJ mol^{-1}. The barrier for the 1,2-shift in this case is calculated to be 63.4 kJ mol^{-1}, while the product radical anion lies 24.1 kJ mol^{-1} above the reactant (Table S6).

Interaction of a still weaker base such as CN^-, not surprisingly, causes the complex formed with the 1,2-dihydroxyethyl radical (**16**, Figure 6) to exhibit a positive electron binding energy (37.7 kJ mol^{-1}, Table S6). However, the partial proton transfer effected by CN^- is less than the above cases and the hydroxyl proton is now localized on the spectator oxygen (see Figure 6). The *partial deprotonation* mechanism that ensues bears many similarities to the *partial protonation* examples discussed earlier. For example, the degree of proton transfer to the base is greater for the transition structure for the 1,2-shift (**TS:16→17**) than in the reactant complex **16**, as might have been expected for a reaction facilitated by deprotonation. Indeed, the acidic proton is transferred to the interacting base at the transition structure, again reminiscent of the catalysis by ammonium. The barrier associated with this transition structure is 78.7 kJ mol^{-1}, substantially less than in the uncatalyzed reaction. Following the rearrangement, the proton is transferred back to the spectator oxygen in the complex between CN^- and the 2,2-dihydroxyethyl radical **17**. Interestingly, the CN^- prefers to accept hydrogen bonds from both hydroxyl groups in this complex, causing the radical rearrangement to be exothermic

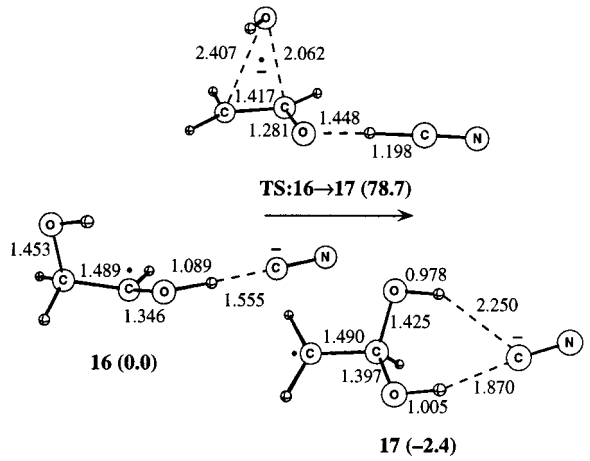

Figure 6. B3-LYP/6-31G(d,p) structures and selected bond lengths (Å) for the species involved in the catalysis by CN^- of the transformation of ethane-1,2-diol into ethane-1,1-diol, via free-radical intermediates. Relative energies (G2(MP2,SVP)(+)-RAD(p) values in kJ mol^{-1}) are given in parentheses.

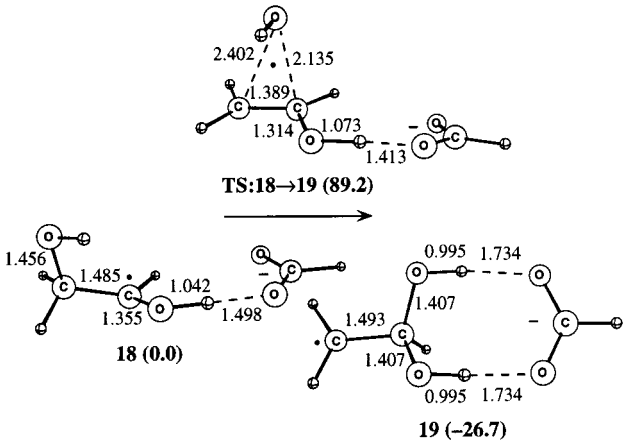

Figure 7. B3-LYP/6-31G(d,p) structures and selected bond lengths (Å) for species involved in the catalysis by $HCOO^-$ of the 1,2-shift of the hydroxyl group in the 1,2-dihydroxyethyl radical. Relative energies (G2(MP2,SVP)(+)-RAD(p) values in kJ mol^{-1}) are given in parentheses.

by 2.4 kJ mol^{-1}, in stark contrast to the situation with the stronger bases.

The complex between the 1,2-dihydroxyethyl radical and the formate anion (**18**, Figure 7) is calculated to bind an electron by 52.3 kJ mol^{-1} (Table S6). This weaker base ($HCOO^-$) is seen not to abstract the acidic proton from the spectator hydroxyl to the same extent as in the previous examples. Nevertheless, the extra lengthening of the O−H bond evident in the transition structure (**TS:18→19**) is still able to impart significant catalysis, with a calculated rearrangement barrier of 89.2 kJ mol^{-1}. The structure of the formate anion is such that it can interact with both hydroxyls extremely well in the product-related radical **19**. Consequently, the rearrangement is even more exothermic (26.7 kJ mol^{-1}) than in the case of CN^- (Figure 7).

We have also investigated the effect on the rearrangement of the interaction of the neutral bases ammonia and methylamine with the spectator hydroxyl group. As expected, the effect is not large, with calculated barrier reductions of just 2.3 and 3.6 kJ mol^{-1}, respectively (Table S6). On this basis, one could expect still weaker bases (such as water or hydrogen fluoride) to have an even smaller catalytic effect.

A schematic energy profile summarizing the results from this part of our investigation is displayed in Figure 8. We conclude

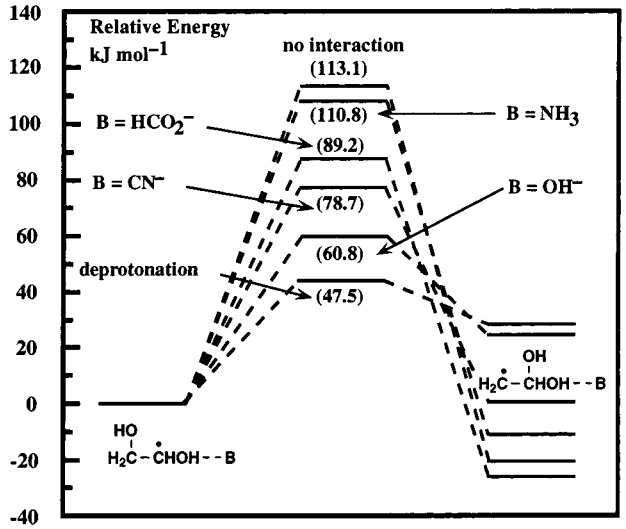

Figure 8. Schematic energy profile for the catalysis of the 1,2-shift of the hydroxyl group in the 1,2-dihydroxyethyl radical through interaction of the spectator hydroxyl group with various bases (B). Relative energies (in kJ mol^{-1}) are given in parentheses. Values correspond to G2(MP2,SVP)-RAD(p) for no interaction and B = NH_3, and G2(MP2,SVP)(+)-RAD(p) otherwise.

that abstraction of the proton attached to the spectator oxygen substantially lowers the barrier associated with the 1,2-hydroxyl shift. We find that it is not necessary to invoke a process involving complete dissociation of OH^- (Scheme 2) to make use of this kind of assistance. We also find that a significant barrier lowering can be achieved without complete removal of the acidic proton. As we have observed in the case of partial protonation of the migrating hydroxyl above, the partial deprotonation of the spectator hydroxyl is an attractive and perhaps more realistic alternative than complete deprotonation. This is particularly relevant in the case of enzymatic deprotonation since the protein environment is unlikely to provide a base significantly stronger than formate.

We previously proposed that any reaction that is facilitated by protonation will also be facilitated by the partial proton transfer[21] that interaction with a Brønsted acid provides. We now propose that *any reaction that is facilitated by deprotonation will be facilitated by the partial deprotonation that interaction of a base at the deprotonation site provides*.

C. The "Retro-Push−Pull" Mechanism. (1) Effect of HF on the NH_4^+-Catalyzed Rearrangement. In the previous sections, we have seen how the rearrangement barrier can be lowered, either by the pushing of the migrating hydroxyl group through interaction of an appropriate acid (section A) or the pulling of the migrating group resulting from the interaction of an appropriate base at the spectator hydroxyl group (section B). In this section, we see how these two mechanistic alternatives are able to work together in such a way that the combined catalytic effect is greater than additive. This synergistic interplay between the push and the pull of the migrating group provides what we believe to be the key ingredient in explaining the catalytic mechanism of the diol dehydratase-catalyzed reactions.

The push−pull synergy is probably most easily understood as a perturbation on the previously discussed (retro-push) system involving the interaction of ammonium with the migrating hydroxyl (Figure 2). The introduction of a relatively weak base (HF) at the spectator hydroxyl (Figure 9) perturbs this system to a surprisingly large extent and so serves as a useful illustration of the concept.

The structural features and relative energies of the now doubly complexed stationary points on the radical surface are shown in Figure 9. At the B3-LYP/6-31G(d) level, the potential surface is complicated by double-well potentials for the hydrogen-bonded complexes (Figure 9, italics). However, with the G2(MP2,SVP)-RAD(p) higher-level theoretical treatment (Figure 9, bold text), the hydrogen bonds on both sides of Figure 9 are likely to be described by single wells corresponding to NH_4^+ complexes. The bulk of the energy required to effect the 1,2-shift is associated with the initial proton transfer from the acid to the migrating hydroxyl. Once the proton is localized on the oxygen, the potential surface is predicted to be quite flat, with the highest point corresponding to the more conventional transition structure for the 1,2-shift (**TS:21→22**). Significantly, the barrier associated with this transition structure is only 28.9 kJ mol^{-1} (Figure 9), some 20.7 kJ mol^{-1} lower than was calculated in the absence of HF (e.g., Figure 2). This result should be considered in the knowledge that HF is a substantially weaker base than NH_3 (see later for quantitative confirmation) and the latter on its own is able to convey only 2.3 kJ mol^{-1} of catalysis to the rearrangement (Figure 8). In this example, we thus get our first glimpse of the synergistic interplay between the push and the pull of the migrating group. A very weak base interacting with the spectator hydroxyl, which on its own would be expected to impart only a very small degree of catalysis, is able to have a substantial impact upon the rearrangement barrier when allowed to cooperate with an acidic group interacting with the migrating hydroxyl. Another important point that arises from the data in Figure 9 is that one must treat small basis set B3-LYP results for this type of problem with some caution.

(2) The Effects of H_2O, NH_3 and HCOOH on the NH_4^+-Catalyzed Rearrangement. Interaction of H_2O with the spectator hydroxyl of the system in Figure 2 exemplifies the situation for a base slightly stronger than HF,[26] and we find that the barrier to rearrangement is further reduced. That is, we calculate a barrier for the 1,2-hydroxyl migration of only 18.3 kJ mol^{-1}, some 31.3 kJ mol^{-1} lower than in the absence of a base (Table S7).

A still more striking illustration of the push−pull synergy comes from the perturbation of the system shown in Figure 2 by the introduction of NH_3 at the spectator hydroxyl. We have seen (Figure 2) that the "push" of the migrating group supplied by NH_4^+ is able to reduce the migration barrier by 63.5 kJ mol^{-1} from 113.1 to 49.6 kJ mol^{-1}. On the other hand, we have seen (Figure 8) that the "pulling" of the migrating group provided by NH_3 is able to impart a barrier reduction (from 113.1 kJ mol^{-1}) of only 2.3 kJ mol^{-1}. If these two effects were to combine in an additive manner, then we would expect the barrier to be lowered by 65.8 kJ mol^{-1}, to 47.3 kJ mol^{-1}. In a powerful demonstration of the positive reinforcement associated with cooperation, we calculate a barrier[26] of just 7.5 kJ mol^{-1}, when NH_4^+ and NH_3 are allowed to work together. The barrier reduction associated with this catalytic duo is an enormous 105.6 kJ mol^{-1}, to a value that is less than 10% of the energy required for the unassisted rearrangement.

(26) For bases slightly stronger than HF, the previously shallow B3-LYP/6-31G(d,p) potential well corresponding to the product-related NH_3 complex **22** disappears. Thus, B3-LYP/6-31G(d,p) predicts the hydrogen bond (involving the migrating hydroxyl) in the product-related radical to be described by only a single well corresponding to an NH_4^+ complex. Consequently, the more conventional transition structure for the 1,2-shift (i.e., one resembling **TS:21→22**) no longer exists. This is not surprising given the B3-LYP energies shown in Figure 9. In all cases, however, we are able to verify that the proton-transfer transition structure (analogous to **TS:22→23**) connects the NH_4^+ complex of the product-related radical (analogous to **23**) to the NH_3 complex of the substrate-derived radical (analogous to **21**).

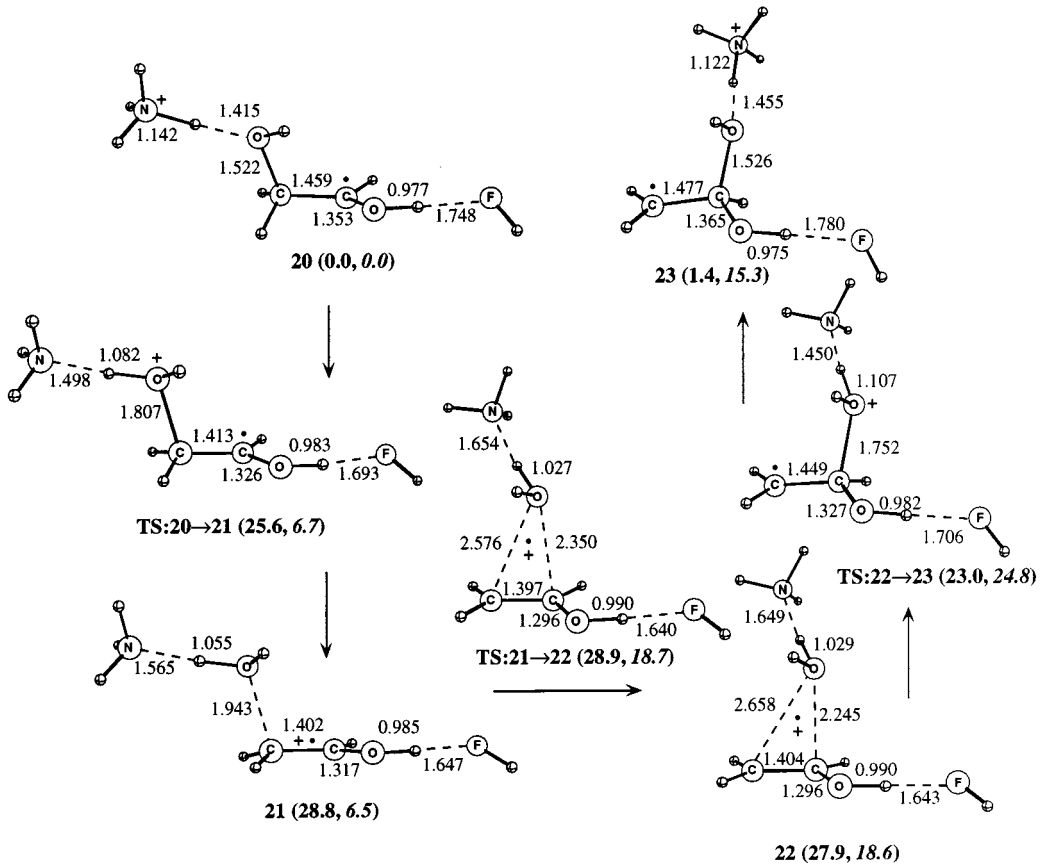

Figure 9. B3-LYP/6-31G(d,p) structures and selected bond lengths (Å) for species involved in the cooperative catalysis by NH_4^+ and HF of the 1,2-shift of the hydroxyl group in the 1,2-dihydroxyethyl radical. G2(MP2,SVP)-RAD(p) relative energies (in kJ mol^{-1}) are boldface text, while B3-LYP/6-31G(d,p) relative energies (in kJ mol^{-1}) are italic text.

We have also included the results pertaining to the retro-push−pull catalysis by NH_4^+ and neutral formic acid (HCOOH) (Table S7). This pair, which give rise to a rearrangement barrier of just 17.6 kJ mol^{-1}, can be seen to offer a catalytic advantage similar to that obtained with NH_4^+ and H_2O. These results have been included here for two main reasons. First, it is known, for the related ribonucleotide reductase-catalyzed reaction, that an amino acid with a carboxylic acid side group (glutamate) is in a position to accept a hydrogen bond from the equivalent of the spectator hydroxyl group.[27] This result has more recently been confirmed (by the X-ray crystal structure) also to be the case for diol dehydratase.[15] Second, the results for this pair serve to demonstrate the ability of the G2(MP2,SVP)-RAD(p) method to treat relatively large systems.

A schematic energy profile summarizing the effect of retro-push−pull catalysis is displayed in Figure 10.

(3) Retro-Push−Pull Catalysis by Formic Acid. During the course of our calculations with neutral formic acid, we found that this species was able to provide both retro-push and retro-pull catalysis, on its own. A similar proposal has very recently been published,[7] but we believe that the published results stop short of a satisfactory mechanism.

Figure 11 shows our calculated results[28] for the relevant stationary points and energetics of this mechanism. The acidic proton of formic acid is shown to be "pushing" the migrating hydroxyl of the substrate-derived radical, while the carbonyl oxygen, acting at the site of the spectator hydroxyl, is "pulling" the migrating hydroxyl (see **24**). We find a transition structure (**TS:24→25**) that describes the transfer of a proton to the migrating hydroxyl at the same time that a proton is being transferred from the spectator oxygen. This process requires 65.2 kJ mol^{-1}. The stable structure formed after these proton transfers have occurred (**25**) resembles a complex of formic acid with water and the allyloxy radical. A similar complex was found by George et al., and they proposed that it dissociates and abstracts a hydrogen atom to form the product aldehyde.[7] We believe this suggestion does not constitute a viable mechanism for the diol dehydratase-catalyzed reactions since, if this course

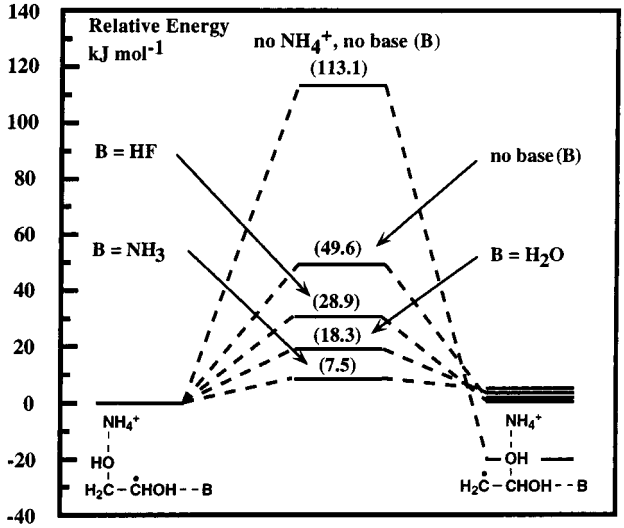

Figure 10. Schematic energy profile for the catalysis of the 1,2-shift of the hydroxyl group in the 1,2-dihydroxyethyl radical by NH_4^+ together with various bases (B). Relative energies (G2(MP2,SVP)-RAD(p) values in parentheses) are given in kJ mol^{-1}.

(27) Siegbahn, P. E. M. *J. Am. Chem. Soc.* **1998**, *120*, 8417−8429.
(28) Smith, D. M. PhD Thesis, Australian National University, 1999.

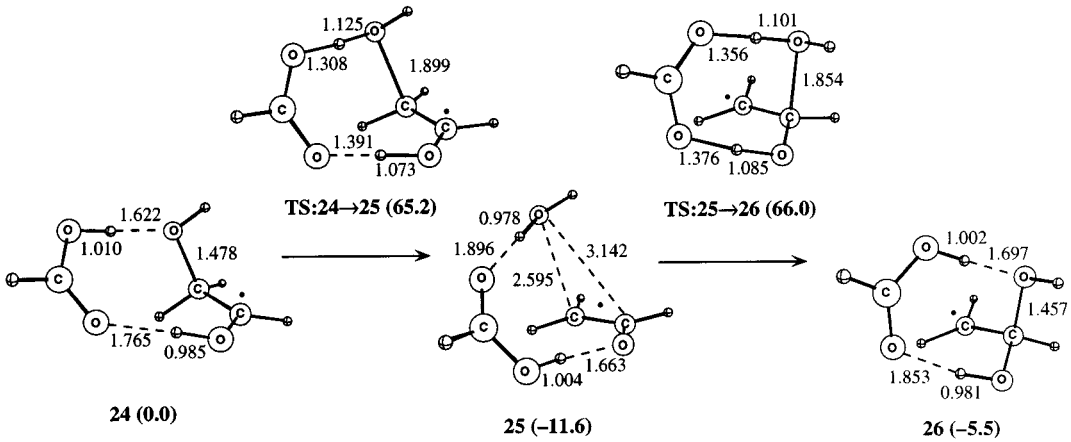

Figure 11. B3-LYP/6-31G(d,p) structures and selected bond lengths (Å) for the species involved in the catalysis by HCOOH of the 1,2-shift of the hydroxyl group in the 1,2-dihydroxyethyl radical. Relative energies (G2(MP2,SVP)-RAD(p) values in kJ mol^{-1}) are given in parentheses.

of events were to occur, the migrating oxygen would *always* end up in the leaving water. As discussed earlier, this is an outcome that is known to be inconsistent with labeling experiments for propane-1,2-diol.[9] While it possible that the diol dehydratase enzyme acts upon ethane-1,2-diol in a manner completely different from the way it interacts with propane-1,2-diol, a unified mechanism seems far more attractive.

Our calculations indicate that the complex **25** can rearrange, relatively easily, to incorporate a 1,1-diol intermediate **26** on the pathway. We find a transition structure (**TS:25→26**) for this process at an energy (66.0 kJ mol^{-1}) only marginally higher (0.8 kJ mol^{-1}) than the initial transition structure (**TS:24→25**). Intrinsic reaction coordinate calculations confirm the connectivity of the potential surface as indicated in Figure 11. We submit, therefore, that if a carboxylic acid were to be involved in the single-handed assistance of the diol dehydratase-catalyzed dehydration of ethane-1,2-diol, the most likely course of events is one which incorporates a 1,2-OH shift such as that shown in Figure 11.

(4) Retro-Push−Pull Catalysis by Li$^+$ and HCOO$^-$. Up to this point, we have considered only the action of neutral bases in the context of the retro-push−pull mechanism. This is because the separation of oppositely charged species in the gas phase is generally an energetically expensive exercise. Nevertheless, we find that the combination of Li$^+$ and HCOO$^-$ is able to catalyze the radical rearrangement in an interesting manner.[28] Figure 12 shows Li$^+$ in the bridging arrangement with the base positioned to enable interaction with both the spectator hydroxyl and the Lewis acid. Such a situation is able to provide both retro-push and retro-pull catalysis (as evidenced by the reduction of the barrier to 55.7 kJ mol^{-1}) while avoiding any large separation of charge. Recall that, in this (bridging) arrangement, Li$^+$ on its own actually increases the barrier for the 1,2-shift (section A3). Figure 7 shows that HCOO$^-$, acting alone, is able to reduce the barrier by 23.9 kJ mol^{-1} to 89.2 kJ mol^{-1}. From these data, we can again see that the barrier lowering in the cooperative system is considerably greater than the sum of the two parts. Thus, the Lewis acid does have the potential to catalyze the rearrangement (as suggested on the basis of its observation in the crystal structure[15] and lower-level calculations[16]), but only when combined with a suitable retro-pull catalyst which allows the push−pull synergy to be realized.

D. Biological Relevance and the Dehydration Step. (1) The X-ray Crystal Structure. As noted earlier, the X-ray crystal structure of diol dehydratase from *K. oxytoca* has recently been solved and interpreted.[15] It is useful to reexamine the structure in the light of our mechanistic predictions.

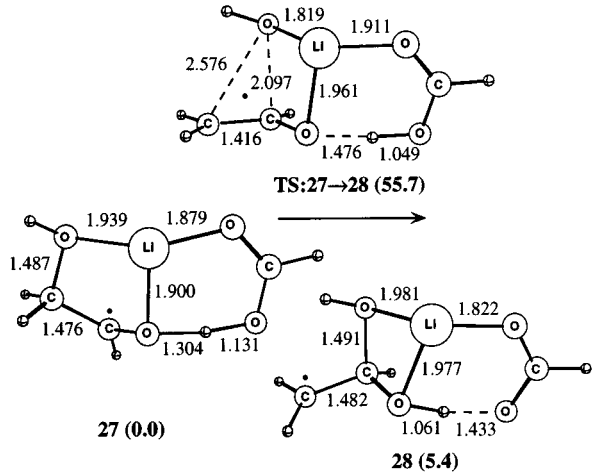

Figure 12. B3-LYP/6-31G(d,p) structures and selected bond lengths (Å) for species involved in the cooperative catalysis by Li$^+$ and HCOO$^-$ of the 1,2-shift of the hydroxyl group in the 1,2-dihydroxyethyl radical, in the bridging arrangement. Relative energies (G2(MP2,SVP)-RAD(p) values in kJ mol^{-1}) are given in parentheses.

Figure 13 shows a closeup view of the substrate bound at the active site.[29,30] A potassium ion can be seen to interact with both oxygens of the substrate as well as with the carboxyl group of a glutamate residue (Glu 170). Indeed, the potassium ion is found to have a total of seven coordinating oxygens, the remaining four (not shown) being derived from the side chains of Ser 362, Gln 141, Gln 296, and Glu 221. It is an interesting facet of the diol dehydratase-catalyzed reactions that they actually require a monovalent cation such as K$^+$ for their normal function.[2c] Other cations that have been found to fulfill this role are NH_4^+ and $CH_3NH_3^+$.

The position of the potassium ion in a bridging arrangement with respect to the substrate may suggest a mechanism for the

(29) The pdb code for the structure shown in Figure 13 is 1dio. For information on the protein data bank, see: (a) Abola, E. E.; Sussman, J. L.; Prilusky, J.; Manning, N. O. In *Methods in Enzymology*; Carter, C. W., Jr., Sweet, R. M., Eds.; Academic Press: San Diego, 1997; Vol. 277; pp 556−571. (b) Sussman, J. L.; Lin, D.; Jiang, J.; Manning, N. O.; Prilusky, J.; Ritter, O.; Abola, E. E. *Acta Crystallogr.* **1998**, *D54*, 1078−1084.

(30) (a) On the basis of the fact that the *S* isomer is thought to bind more tightly to the enzyme,[30b] the substrate in the crystal structure was previously assigned *S* stereochemistry.[11] However, the substrate in the pdb file[29] has *R* stereochemistry (Figure 13). Given this, and the fact that the difference in binding affinities is small,[30c] we discuss the bound substrate as having *R* stereochemistry. (b) Bachovchin, W. W.; Eagar, R. G.; Moore, K. W.; Richards, J. H. *Biochemistry* **1977**, *16*, 1082. (c) Golding, B. T. In *Comprehensive Organic Chemistry*; Barton, D., Ollis, W. D., Eds.; Pergamon Press: Oxford, 1979; Vol. 5; p 580.

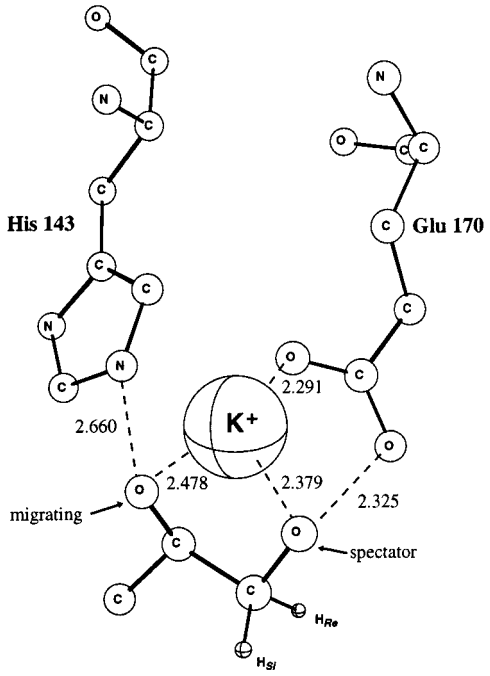

Figure 13. Closeup view of the active site of diol dehydratase showing the substrate, potassium ion, and two potentially important amino acids (bond lengths given in Å). See refs 15 and 29.

1,2-shift such as that shown in Figure 4b. However, we find that the barrier in this arrangement is actually higher than that calculated in the absence of any interacting species. On the other hand, our results from Figure 12 indicate that, if this bridging arrangement is supplemented by an appropriate retro-pull catalyst, significant and synergistic catalysis can be realized.

The pictured glutamate residue (Glu 170) is found to be in an ideal position, with respect to the spectator hydroxyl,[31] to act as a pull catalyst through partial deprotonation of the substrate. This residue also interacts with the potassium ion at the active site, further strengthening the resemblance to the structures in Figure 12. A potential problem in proposing a retro-push−pull mechanism in which a potassium pushes the migrating group from the bridging position and a glutamate residue pulls from the spectator hydroxyl (Figure 12) is that the effectiveness of the potassium is likely to be strongly diminished by the seven oxygens surrounding the cation in the active site. Such an environment would be expected to reduce the available charge and hence to dampen the catalytic ability of the K^+ (or indeed of alternative monovalent cations such as NH_4^+ or $CH_3NH_3^+$ if they were playing this role).

We therefore propose that the retro-push catalysis is assisted by a nearby histidine residue (His 143, Figure 13). The $N^{\epsilon 2}$ nitrogen of His 143 can be seen to be quite close to the migrating hydroxyl of the substrate.[31] Indeed, the N−O distance in the crystal structure (2.660 Å) is only marginally longer than that calculated in our model complexes (2.579 Å in Figures 2 and 2.557 Å in Figure 9). These results indicate that the histidine residue is certainly close enough to partially protonate the migrating hydroxyl and act as an effective retro-push catalyst. It would seem that an extra interaction such as this is required because the "solvation" of the potassium ion by its coordination sphere reduces its ability to "push" the migrating hydroxyl. In this scenario, the potassium could play more of a binding and directing role in the migration, rather than being strongly involved in the catalysis. Interestingly, a histidine residue can also be found in the ideal position to partially protonate the migrating group in methylmalonyl-CoA mutase,[21b] a closely related B_{12}-dependent enzyme.

(2) The Stereochemistry of the Elimination. Our proposed mechanism is potentially consistent with the results of studies with ^{18}O-labeled substrates, which indicate the presence of a 1,1-diol on the reaction pathway.[9] However, in addition to explaining how the 1,1-diol is formed, a viable mechanism must also account for the mode of dehydration of this diol. It has previously been suggested that the hydroxyl that migrates from C2 of the 1,2-diol substrate will be preferentially eliminated due to its coordination to K^+.[16] Similarly, the mechanisms of George et al. would also require that the hydroxyl at C2 is eliminated.[7,8] However, none of these mechanisms would be consistent with the experimental labeling results which indicate that while the hydroxyl group that migrates is eliminated for (*S*)-propane-1,2-diol, it is the *spectator* hydroxyl that is predominantly eliminated if the substrate has *R* stereochemistry.[9] A new explanation is clearly required.

On the basis of our calculations and in the context of our mechanistic proposals, we are indeed able to rationalize all the observed stereochemistry of the diol dehydratase-catalyzed reactions (see Appendix for a detailed analysis). For the first time, we make a link between which hydrogen atom is removed from C1 and which oxygen atom is ultimately incorporated into the solvent water.

Concluding Remarks

In the present study, we have investigated the mechanism for the diol dehydratase-catalyzed reactions. It is generally accepted that the first step in the dehydration involves abstraction of a substrate hydrogen atom by the coenzyme, resulting in a substrate-derived radical. Recent theoretical studies have proposed that this substrate-derived radical loses water and abstracts a hydrogen atom to form the required product. Experimental oxygen-labeling studies, however, strongly suggest a more complicated pathway involving the intermediacy of a 1,1-diol, which requires the 1,2-shift of a hydroxyl group to an adjacent radical center. The calculated barrier for this hydroxyl migration, if unassisted, is too large to be consistent with the observed reaction rate. The main thrust of our study has thus been to examine means by which this rearrangement might be facilitated.

Previous work has shown that protonation of the migrating group facilitates 1,2-shifts in radicals. However, in the case of the 1,2-dihydroxyethyl radical, full protonation has been found to be too extreme and results in a pseudodecomposition pathway. The softening of the extreme, through the use of either a Brønsted or Lewis acid, is sufficient to prevent such decomposition. That is, in both these cases, the reactant and product radicals are found to be stable entities. At the same time, however, preferential stabilization of the transition structure, leading to a significantly lowered rearrangement barrier, is maintained. We have termed this effect retro-push catalysis to describe the pushing of the migrating hydroxyl group through interaction with an appropriate acid. The retro prefix signifies that the motion of the migrating group is in the direction opposite to the electron motion.

The recent suggestions of mechanisms involving "ketyl" (or radical anion) intermediates led us to investigate the effect of deprotonation of the spectator hydroxyl. We find that this type of perturbation is indeed able to facilitate the radical rearrange-

(31) Recall that propane-1,2-diol is dehydrated to give propionaldehyde so that the migrating hydroxyl must move from C2 to C1 (Figure 13).

ment and can do so without requiring a fully dissociative process. As is the case with protonation, the extreme of full deprotonation is not ideal. However, the softening of the extreme again provides a satisfactory solution. The use of appropriate bases to partially deprotonate the spectator hydroxyl results in anionic systems with a bound electron. These bases are still able to impart significant catalysis to the rearrangement. Consideration of these results also allows us to extend our previous conclusions regarding partial protonation to the case of partial deprotonation. We have termed this effect retro-pull catalysis to describe the pulling of the migrating group that is effected by a suitable base interacting with the spectator hydroxyl. Again, the retro prefix signifies that the motion of the migrating group is in the direction opposite to the electron motion.

While the retro-push and retro-pull effects are both capable of providing viable mechanisms in isolation, it is their cooperative nature that is truly remarkable. That is, the combined effect of a push and a pull is calculated to be considerably greater than the sum of the two parts. This synergistic interplay, in the most favorable case, is shown to effect a barrier reduction in excess of 100 kJ mol^{-1}.

Consideration of our predicted mechanism in light of the experimental X-ray crystal structure reveals several interesting points. We find both a potassium ion and a histidine residue to be well-positioned for provision of retro-push catalysis. A glutamate residue is found to be in an optimum position to be involved with retro-pull catalysis. In addition, we are able to propose a feasible explanation for the stereochemistry of the reactions based upon the investigation of the structure and the interpretation of our calculations. All of the results presented herein are applicable to the enzyme-catalyzed dehydrations of both ethane-1,2-diol and propane-1,2-diol.

On the basis of our calculations, we are able to assign likely roles to the active site participants in the X-ray crystal structure. First, while we concur with Toraya[15,16] that the potassium ion (or alternative monovalent cation) imparts some degree of retro-push catalysis to the radical rearrangement, we propose that its predominant purpose is to bind the substrate and direct the reaction. The histidine residue is also likely to provide retro-push catalysis for the radical rearrangement, as well as supplying the proton for the leaving water. Finally, the glutamate residue should be able to provide retro-pull catalysis at the spectator hydroxyl, as well as to act as an acceptor for the proton lost by the departing aldehyde.

Appendix

The Stereochemistry of the Elimination. As noted earlier, there are problems in reconciling some of the previous mechanistic proposals for diol dehydratase.[7,8,15] with the observed isotope-labeling results.[9] Our proposed mechanism is potentially consistent because of the involvement of a 1,1-diol on the pathway, but we need to elaborate further to see if we can account for the observed stereochemistry.[9] It is helpful initially to examine the available stereochemical data in more detail. For the dehydration of (*R*)-propane-1,2-diol, it has been established first that H_{re} (Figure 13) is specifically removed from C1 by the 5′-deoxyadenosyl radical.[11] Second, the ^{18}O-labeling results described above are consistent with the notion that the migration of the hydroxyl group from C2 to C1 occurs with inversion of configuration at the newly formed stereocenter (C1).[32] Finally, it is the oxygen originally attached to C1 (the spectator oxygen) that is predominantly eliminated, leaving the hydroxyl originally bonded to C2 (the migrating oxygen) to be incorporated into the product aldehyde.[3f,10,11]

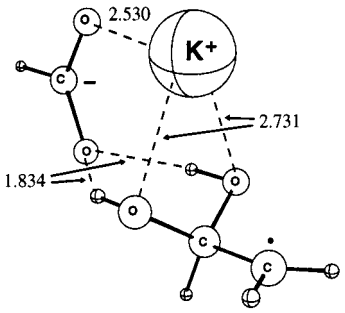

Figure 14. B3-LYP/6-31G(d,p) structure and selected bond lengths (Å) of a potentially important structure involving the product-related radical.

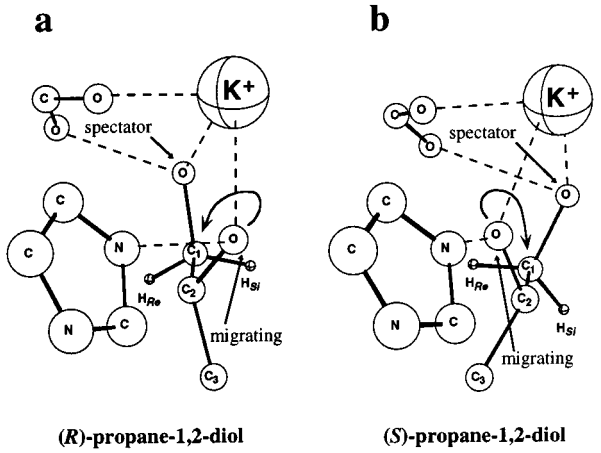

Figure 15. An alternative perspective of the active site of diol dehydratase. See refs 15 and 29.

In the dehydration of (*S*)-propane-1,2-diol, it is H_{si} that is removed from C1. Inversion of configuration at this center following the migration is also consistent with the experimental results,[32] and it is the oxygen originally attached to C2 (the migrating oxygen) that is lost in the subsequent elimination step.[10,11]

A useful observation at this point is that one of the low-energy structures of the product-related radical in the presence of K^+ and $HCOO^-$ (such as the structure shown in Figure 14) has C_s symmetry, as does the product 1,1-diol. In the absence of a chiral influence, e.g., the enzyme, there is no intrinsic preference as to which hydroxyl group is eliminated from such a 1,1-diol. However, in the presence of the enzyme, one of the hydroxyl groups of the product 1,1-diol could preferentially receive a proton and leave as water. In an arrangement such as that in Figure 14, the elimination preference is likely to be governed by the location of the additional proton source. It appears that the pictured histidine residue (His 143, Figure 13) is well-placed to perform this task. Using this information, and that obtained by examination of the three-dimensional structure of the active site, we are able to postulate a feasible mechanism to account for the stereochemistry. While this mechanism is somewhat speculative, it provides, in our opinion, the best explanation of the available data at the present time.

For the *R* isomer[30] (shown from a slightly different perspective in Figure 15 to that in Figure 13), it is H_{re} that is removed from C1 by the coenzyme. This is followed by the 1,2-shift of

(32) Strictly, the ^{18}O-labeling data are consistent with inversion at C1 plus loss of the *re* hydroxyl group or retention at C1 and loss of the *si* hydroxyl group. The former pathway is more attractive because it separates in space the hydrogen abstraction by the adenosyl radical from the migration of the hydroxyl group.[21b]

the migrating hydroxyl (presumably facilitated by retro-push−pull catalysis) from C2 to C1. To occur with inversion of configuration at C1, this migration must proceed on the opposite face of the molecule to H_{re}, or from the *right* of the potassium ion from the perspective of Figure 15 (i.e., away from the histidine residue in a manner represented approximately by the path of the curly arrow). If this migration were to result in an arrangement like Figure 14, the histidine residue would end up adjacent to the original spectator hydroxyl group. Acceptance of a proton (from His 143) by this group, and donation of a proton by the original migrating hydroxyl to Glu 170 (presumably both proton transfers are preceded by hydrogen atom transfer from the coenzyme), would result in the release of water and the product aldehyde in a manner consistent with all of the isotopic-labeling data.

It has been previously suggested that the *R* and *S* isomers could bind to the protein in slightly different ways.[10] Indeed, given that different hydrogens are abstracted from C1 for the two isomers, this is quite likely. Figure 15 shows the *S* isomer bound in the active site so that H_{si} is on the lower face of the substrate (as was H_{re} for the *R* isomer) and is thus likely to be abstracted by the coenzyme. If this abstraction were to occur, the migration of the hydroxyl group would need to occur from the *left* of the potassium ion in Figure 15 (roughly following the path of the curly arrow) in order for the stereochemistry at C1 to be inverted. That is, for inversion to occur, the hydroxyl group would need to migrate on the opposite face of the molecule to H_{si}, i.e the reverse of that discussed for the *R* isomer. If this migration were to result in an arrangement like that in Figure 14, it would be the newly migrated hydroxyl that ends up adjacent to the histidine residue. In this scenario, the migrating hydroxyl group would accept a proton from the histidine and leave as water. The original spectator hydroxyl would be the one to donate a proton to Glu 170 and become incorporated into the product aldehyde. Once again, this sequence of events is consistent with all the available isotopic-labeling data.

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Supporting Information Available: G2(MP2,SVP)-RAD(p) or G2(MP2,SVP)(+)-RAD(p) total energies (Tables S1−S3), GAUSSIAN 94 and GAUSSIAN 98 archive entries for the RMP2/6-31G(d)//B3-LYP(6-31G(d,p) calculations for all relevant structures (Table S4), and thermochemical parameters (Tables S5−S7) (pdf). This material is available free of charge via the Internet at http://pubs.acs.org.

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