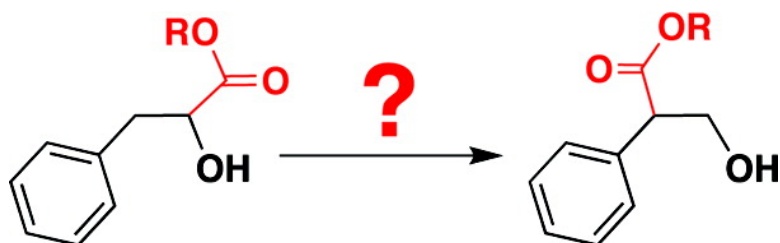Article

The Carbon-Skeleton Rearrangement in Tropane Alkaloid Biosynthesis

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The Carbon-Skeleton Rearrangement in Tropane Alkaloid Biosynthesis

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Abstract: High-level quantum chemistry calculations have been performed to examine the carbon-skeleton rearrangement of the tropane alkaloid littorine to hyoscyamine. Two pathways involving radical and carbocation intermediates have been investigated in this regard, namely, stepwise (or fragmentation−recombination) and concerted. The fragmentation products are calculated to be of high energy for both the radical- and carbocation-based mechanisms (136.3 and 170.9 kJ mol^{-1}, respectively). Similarly, the rearrangement barrier for the radical-based concerted pathway is calculated to be quite high (135.6 kJ mol^{-1}). In contrast, the carbocation-based concerted pathway is found to be associated with a relatively low barrier (47.4 kJ mol^{-1}). The ionization energy of the substrate-derived radical **3a** is calculated to be 7.01 eV, suggesting that its oxidation to generate the substrate-derived carbocation **3b** ought to be facile. In an attempt to investigate how an enzyme might modulate the rearrangement barriers, the separate and combined influences of partially protonating the migrating group and partially deprotonating the spectator OH group of the substrate were investigated. Such interactions can lead to significant reductions in the rearrangement barrier for both the radical- and carbocation-based concerted pathways, although the carbocation pathway continues to have significantly lower energy requirements. Also, the relatively high (gas-phase) acidity of the OH group of the product-related carbocation **4b** indicates that the direct formation of hyoscyamine aldehyde (**6**) is a highly exothermic process. Although we would not wish to rule out alternative possibilities, our calculations suggest that a concerted rearrangement mechanism involving carbocations constitutes a viable low-energy pathway for the carbon-skeleton rearrangement in tropane alkaloid biosynthesis.

1. Introduction

Tropane alkaloids comprise a group of medicinally useful, potentially toxic, and highly addictive substances whose members include scopolamine, a drug often used to help alleviate motion sickness, and cocaine, the infamous and tightly controlled medicinal and recreational drug.[1] One of the final steps in the biosynthesis of the tropane alkaloid hyoscyamine (**2**) from its precursor littorine[2] (**1**) involves a novel carbon-skeleton rearrangement:
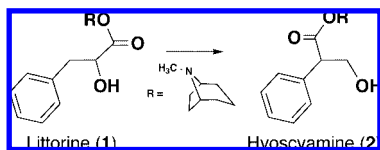
In many respects this transformation shows a striking similarity to the reactions catalyzed by the adenosylcobalamin (AdoCbl)-dependent enzyme methylmalonyl-CoA mutase (MCM) in that an sp^2-hybridized carbonyl moiety is transferred between two adjacent carbon atoms.[3] MCM effects its rearrangement via radical intermediates whereby 5′-deoxyadenosyl radical (Ado•), derived from the homolytic cleavage of the Co−C bond of AdoCbl, initiates catalysis with an H-atom abstraction from methylmalonyl-CoA to form a substrate-derived radical. This radical then rearranges via a so-called addition−elimination pathway,[4] assisted by partial protonation of the migrating group,[5,6] to generate the product-related radical directly. The reaction concludes with another hydrogen transfer between the product-related radical and 5′-deoxyadenosine to regenerate Ado• and form the final product.

Although AdoCbl is not known to be used by plants, investigations[7] of cell-free extracts from *Datura stramonium* and (*S*)-adenosylmethionine (AdoMet or SAM)[8] have revealed some interesting results. Specifically, it was found that the production of hyoscyamine (**2**) is stimulated 10−20 fold in the presence of AdoMet,[7] leading to the proposal that AdoMet

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(1) See, for example: (a) Lounasmaa, M.; Tamminen, T. In *The Alkaloids*; Cordell, G. A., Ed.; Academic Press: New York, 1993; Vol. 44, pp 1−114. (b) Robins, R. J.; Walton, N. J. In *The Alkaloids*; Cordell, G. A., Ed.; Academic Press: New York, 1993; Vol. 44, pp 115−187.
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(3) See, for example: Banerjee, R. *Chem. Rev.* **2003**, *103*, 2083−2094.
(4) Beatrix, B.; Zelder, O.; Kroll, F. K.; Örlygsson, G.; Golding, B. T.; Buckel, W. *Angew. Chem., Int. Ed. Engl.* **1995**, *34*, 2398−2401.
(5) See, for example: Smith, D. M.; Golding, B. T.; Radom, L. *J. Am. Chem. Soc.* **1999**, *121*, 9388−9399.
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Scheme 1. Possible Pathways for the Conversion of Littorine (**1**) to Hyoscyamine (**2**) with Radical (**a**)- or Carbocation (**b**)-Based Intermediates[15,16]
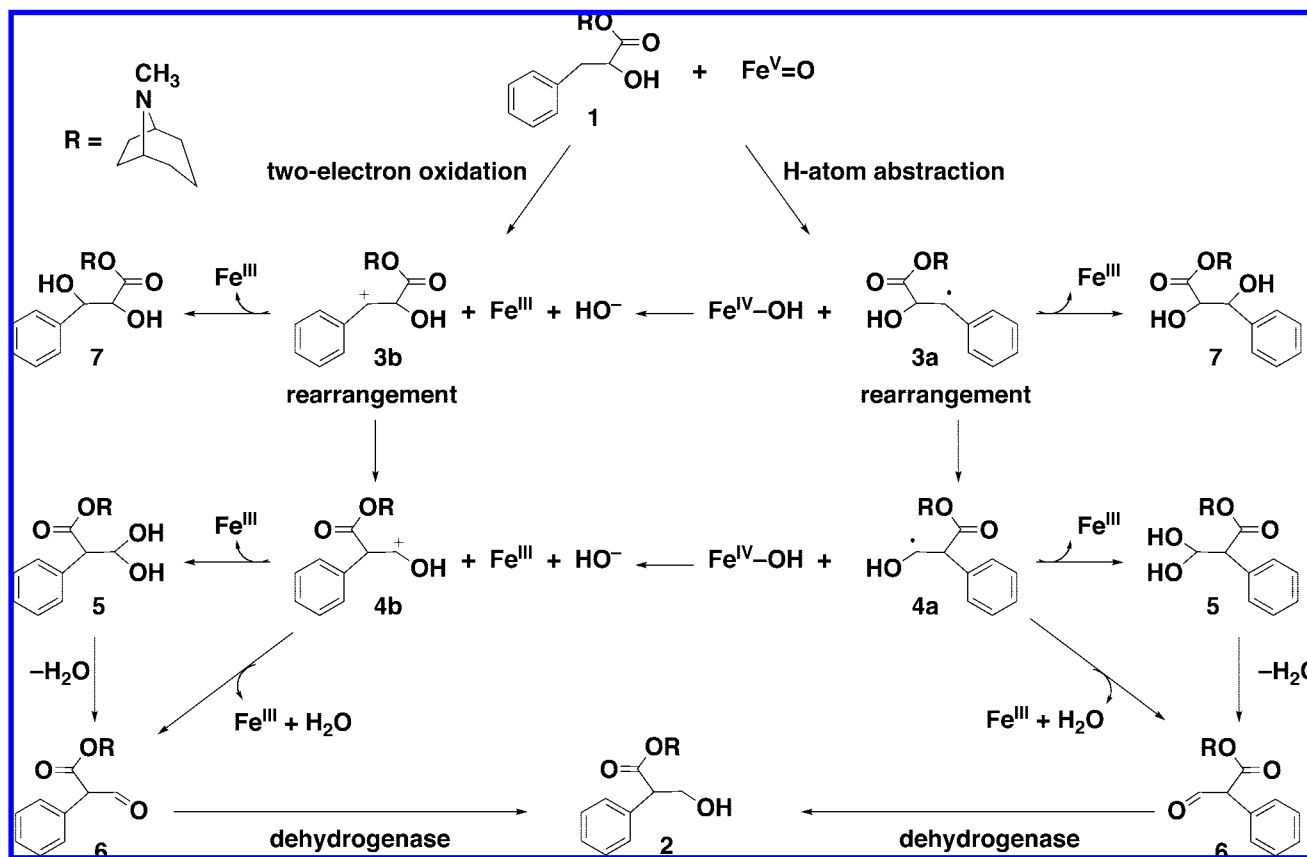
serves as a source of Ado• in the rearrangement of **1** to **2**. Indeed, such a role for AdoMet has recently been established for lysine 2,3-aminomutase, a pyridoxal 5′-phosphate-dependent enzyme involved in the reversible rearrangement of α- and β-lysine.[9] In the case of hyoscyamine production, however, tritium incorporation was not observed when the enzymatic reaction was carried out in the presence of [2,8,5′-³H]AdoMet,[7] which would have been expected for a mechanism involving Ado• as a hydrogen carrier. In addition, other stereochemical and isotopic labeling investigations concluded that a vicinal exchange mechanism was unlikely to be operational.[10] Overall, these results suggest that Ado• does not participate directly in the rearrangement of **1** to **2**.

An alternative proposal that has gained attention in recent years involves the use of a cytochrome P450 (P450) enzyme in the conversion of **1** to **2**. The fact that the P450 inhibitor chlotrimazole has been found to diminish the formation of **2** from **1** is certainly suggestive in this regard.[11] Further, suppression of the CYP80F1 gene in *Hyoscyamus niger* has been shown to attenuate hyoscyamine levels with concomitant accumulation of littorine.[12] Moreover, expression of CYP80F1

in yeast incubated with (*R*)-littorine and NADPH demonstrated hyoscyamine aldehyde (**6**) and (2′*R*,3′*R*)-3′-hydroxylittorine (**7**) to be the major products of the reaction.[12] The presence of the latter compound suggests that an oxygen-rebound process may be operative in the biosynthesis of **2**. In addition, observations of **6** remain consistent with previous studies[13] involving [2-¹⁸O,²H]littorine where it was found that up to 30% of the oxygen label was lost relative to deuterium in the product. An intermediate aldehyde (i.e., **6**) that partially exchanges oxygen with water prior to final reduction provides an attractive explanation for these observations.[13]

From these data, an iron-oxo P450 isomerase and a dehydrogenase have been proposed to be involved in the conversion of **1** to **2**.[12,14] Although the involvement of hyoscyamine aldehyde (**6**) and 3′-hydroxylittorine (**7**) in this reaction has been established, what remains unknown is whether the reaction proceeds via radical or carbocation intermediates, or some combination of the two. We highlight in Scheme 1 various pathways linking **1** and **2**, with the aim of providing insights to the reader regarding the diversity of ways in which this conversion *might* proceed.

In one possible pathway, a substrate-derived radical (**3a**) is generated from **1** via an initial H-atom abstraction by Fe^V=O[16]

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(15) For littorine (**1**) and its derivatives, the substituent R is tropane. However, for computational tractability, most models throughout this study use R = CH₃. In Section 3.2.3, these models are further truncated with R = H. A single nomenclature for the various reaction intermediates (i.e., **3a**, **4a**, **3b**, **4b**) has nevertheless been adopted for the sake of simplicity.

of the isomerase. Oxygen rebound at this stage would explain the formation of 3′-hydroxylittorine (**7**).[12] At the same time, a competing radical rearrangement can be envisaged that generates the product-related radical **4a**, which might then disproportionate with Fe^{IV}−OH to form hyoscyamine aldehyde **6** and Fe^{III} plus H$_2$O. Alternatively, a distinct oxygen-rebound step involving **4a** may occur to produce an Fe^{III} species plus **5**, with the latter able to lose H$_2$O to form **6**. Final product formation has been proposed to involve an alcohol dehydrogenase that reduces **6** to yield hyoscyamine (**2**).[12,14]

An alternative to the radical pathway is one involving cationic intermediates.[14] In this scenario, a formal two-electron oxidation combined with proton transfer produces **3b** plus Fe^{III} and OH$^-$. The recent observation of 3′-hydroxylittorine (**7**)[12] suggests that this may take place via an initial H-atom transfer to give **3a** and Fe^{IV}−OH, followed by one-electron oxidation of **3a** to form **3b**. If this were the case, it would be the oxidation of **3a** that competes with the 3′-hydroxylittorine (**7**)-forming rebound. Other sequences, or even other oxidants,[17] are possible but would probably require nucleophilic attack of OH$^-$ to account for the observed formation of **7** (Scheme 1). Irrespective of its origin, the cationic intermediate **3b** might then rearrange to produce the product-related carbocation (**4b**) in a similar fashion to the radical-based mechanism. Once formed, this species (**4b**) can lose a proton (formally to OH$^-$) to yield H$_2$O plus **6** directly. As with the radical pathway, the final step is thought to involve reduction of **6** by an alcohol dehydrogenase to generate hyoscyamine **2**.

Naturally, all such possibilities should be considered in the context of the existing P450 literature. The conventional view of these enzymes, particularly those performing hydroxylation reactions, favors a pathway involving hydrogen-abstraction and oxygen-rebound.[18,19] In the present situation, such a model would correspond to an initial H-atom abstraction from **1** to give **3a** plus Fe^{IV}−OH, followed by oxygen-rebound, either before or after rearrangement (Scheme 1). A challenge to this conventional view is the discovery of many complex, unusual, and diverse features of P450 systems.[20] In particular, a coherent picture in which the P450 oxidant may behave as a "chameleon", effecting different pathways for different purposes, is emerging.[21] Specific evidence for the involvement of one-electron reduction steps and carbocationic intermediates has arisen for the P450-catalyzed oxidation of aromatic compounds,[22] strained cycloalkanes,[23,24] and amines,[25] as well as for the biosynthesis of prostacylin and thromboxane.[26] Clearly, these studies indicate that a pathway involving cation participation should not be ruled out *a priori*. Indeed, the conversion of **1** to **2** may even proceed via the intermediacy of *both* radicals and carbocations, if one-electron oxidation of the radical[19] **3a** (or **4a**) were to occur (Scheme 1). Such a pathway would be an intriguing blend of traditionally understood P450 functionality (i.e., initial H-atom abstraction) with chameleonic overtones (i.e., electron transfer) to effect a specific outcome.

It is not currently possible to definitively evaluate the various mechanisms of Scheme 1 in their entirety because of uncertainties regarding the enzymes involved in the conversion of littorine to hyoscyamine. A more achievable goal is to attempt to distinguish between rearrangement mechanisms involving radical and carbocation intermediates (**3** → **4**, Scheme 1). Given that this is one of the principal roles of the hitherto unidentified P450 enzyme, a detailed examination of this step is thus valuable. To this end, the present investigation applies computational quantum chemistry techniques to examine the energetic demands of the two alternative rearrangements (**3a** → **4a** vs **3b** → **4b**, Scheme 1) with an emphasis on stepwise (or fragmentation−recombination) vs concerted rearrangement mechanisms. In addition, on the basis of the finding that partial protonation and deprotonation can reduce the rearrangement barriers for the related AdoCbl-dependent enzymes,[5,27] we have performed analogous investigations in the present situation to determine whether similar energetic savings might be realized.
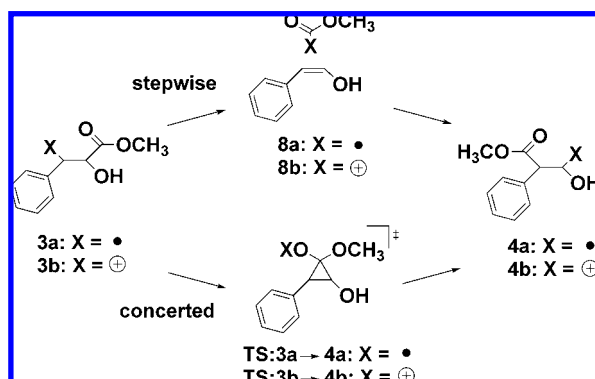
2. Theoretical Methodology

Standard ab initio[28] and density functional theory[29] calculations have been performed with the MOLPRO 2002.6[30] and Gaussian 03[31] programs. Geometries and scaled[32] vibrational frequencies were obtained at the B3-LYP/6−31G(d,p) level of theory. All relative energies have been obtained with the high-level composite method G3(MP2)-RAD. This method uses an additivity scheme involving RMP2/6−31G(d), RMP2/G3MP2Large, and URCCSD(T)/6−31G(d) single-point energy calculations on the B3-LYP/6−31G(d,p) geometries to approximate the URCCSD(T)/G3MP2Large level of theory. The suitability of closely related methods has been demonstrated in previous investigations on the mechanism for rearrangement of many AdoCbl-dependent enzymes.[5,27] All energies refer to isolated species in the gas phase at 0 K. The

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Scheme 2. Stepwise and Concerted Mechanisms for the Carbon-Skeleton Rearrangement (3 → 4) in Tropane Alkaloid Biosynthesis Involving Radical- (**a**) or Carbocation- (**b**) Based Intermediates

Table 1. Relative Energies (kJ mol^{-1}) for the Species Involved in the Carbon-Skeleton Rearrangement of **3** to **4**[15]

	B3-LYP/[a]	B3-LYP/[b]	RMP2/[c]	G3(MP2)-RAD
3a	0.0	0.0	0.0	0.0
8a	116.0	100.1	131.8	136.3
TS:3a→4a	126.0	129.3	107.0	135.6
4a	36.5	33.1	20.7	29.4
3b	0.0	0.0	0.0	0.0
8b	196.0	162.5	157.1	170.9
TS:3b→4b	52.8	46.5	26.4	47.4
4b	−12.7	−12.0	−40.7	−26.8

[a] 6−31G(d,p). [b] 6−311+G(3df,2p). [c] G3MP2Large.

effects of solvation have not been considered in detail in the present work. Preliminary results indicate that the use of a continuum model does not change the conclusions presented. On the other hand, we have investigated specific environmental effects within the active site of the enzyme through the interaction of appropriate Brønsted acids and bases with the substrate, and find that these can be very significant.

Because no structural information is available for the enzyme responsible for the carbon-skeleton rearrangement in tropane alkaloid biosynthesis, we have used model systems to assess the energy demands for this step. Such an approach has successfully been used in other enzyme-catalyzed processes[5,27] and should therefore provide useful insights in the present case. In order to provide a system small enough for high-level calculations, the tropane moiety in littorine and hyoscyamine was replaced in most calculations with a methyl group. In Section 3.2.3 where the combined effects of partial protonation and deprotonation are examined, this model is further truncated by replacement of CH$_3$ by H.[15] Essentially all of the species within each defined system represent the lowest-energy conformers. In a very small number of cases, conformations slightly lower in energy (by ca. 5−10 kJ mol^{-1}) than those employed herein were identified, corresponding, for example, to a rotation about the C−C bond of the migrating moiety, or the C−OH bond within this moiety. However, such species are found to correspond to higher-energy reaction paths. For these reasons, selected higher-energy intermediates were occasionally used.

3. Results and Discussion

3.1. Stepwise vs Concerted Rearrangement Pathways. Two distinct pathways for the 1,2-carbon-skeleton rearrangement in tropane alkaloid biosynthesis are explored in this study. The first, termed a stepwise or fragmentation−recombination pathway, involves cleavage of the C−C bond to the migrating moiety in the substrate-derived species **3** to produce two distinct fragments, collectively referred to as **8** (Scheme 2). Intermolecular recombination of **8** by way of C−C bond formation with the adjacent carbon atom then produces the rearranged product-related species **4**. Such a rearrangement mechanism is understood to be operative for the AdoCbl-dependent enzyme glutamate mutase.[33,34]

The second rearrangement pathway we have explored is characterized by the absence of discrete intermediates like **8**.

Instead, rearrangement proceeds through a transiently formed cyclopropyloxy species (**TS:3→4**) to generate the product-related species (**4**) directly. This concerted mechanism can be described (in the case involving radicals) as an addition of the unpaired electron to the π system of the migrating moiety to form the cyclopropyloxy species, which can then produce the rearranged product via homolytic cleavage of the appropriate adjacent bond. Comparable ring-closing/ring-opening descriptions can be put forward for the analogous situation involving carbocations. While the cyclopropyloxy species could in principle be a metastable intermediate, in the present systems it is found to correspond to a transition structure for the rearrangement of **3** to **4**.

In order to determine the inherent viability of the stepwise and concerted pathways for the carbon-skeleton rearrangement in tropane alkaloids, we have carried out appropriate quantum chemistry calculations.[15] Table 1 displays results of four different levels of theory for these processes, though only the most sophisticated, i.e., G3(MP2)-RAD, will be discussed in the text. The other results serve to demonstrate their performance relative to that of the benchmark G3(MP2)-RAD procedure with future applications on larger systems in mind.

Beginning with the radical-based stepwise pathway, we see that the combined relative energy of the fragmentation products **8a** lies 136.3 kJ mol^{-1} higher than that of the substrate-derived radical **3a**. We note that any transition structure for bond breakage (**TS:3a→8a**, not shown) would be associated with an even higher energy. Recombination of **8a** to form **4a** is found to be exothermic by 106.9 kJ mol^{-1}, though the overall reaction to form **4a** from **3a** is endothermic by 29.4 kJ mol^{-1}. The magnitude of this endothermicity reflects the ability of the phenyl substituent in **3a** to better stabilize the adjacent radical center than can the OH substituent in **4a**. Such electronic effects have recently been discussed in detail.[35] Curiously, the *barrier* for the concerted rearrangement from **3a** to **4a** (via **TS:3a→4a**, Scheme 2) is calculated to be 135.6 kJ mol^{-1}, which is essentially the same as the energy (136.3 kJ mol^{-1}) required to form the radical intermediates **8a**.

The results for the radical-based pathway differ significantly from those calculated for the pathways involving carbocation intermediates. In the latter case, we observe striking differences in energy requirements between the stepwise and concerted pathways (Table 1). The former is characterized by a relatively large 170.9 kJ mol^{-1} reaction energy required to form separated fragments **8b**. In contrast, the *barrier* associated with the concerted pathway is predicted to be just 47.4 kJ mol^{-1}, and the overall reaction is predicted to be exothermic by 26.8 kJ mol^{-1}. This latter result is

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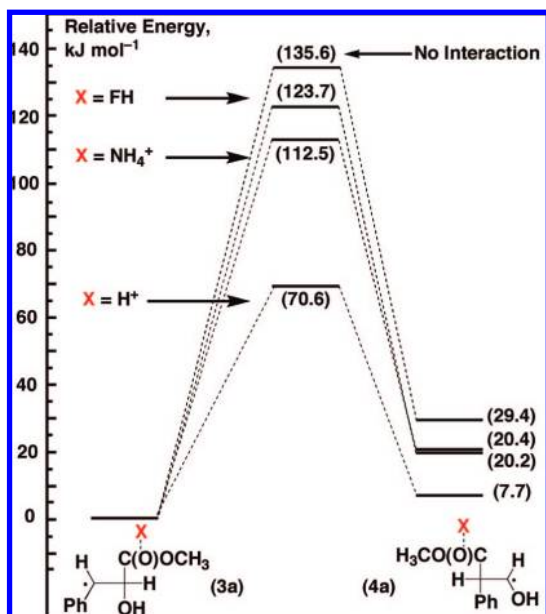
Figure 1. Schematic energy profiles showing the effect on the radical rearrangement (**3a** → **4a**) of partial protonation at the migrating moiety resulting from the interaction of various acids (**X**). Relative energies (G3(MP2)-RAD, kJ mol^{-1}) are given in parentheses.

Figure 2. Schematic energy profiles showing the effect on the radical rearrangement (**3a** → **4a**) of partial deprotonation at the spectator OH group resulting from the interaction of various bases (**Y**). Relative energies (G3(MP2)-RAD, kJ mol^{-1}) are given in parentheses.

consistent with the idea that, while a radical center is stabilized more strongly by a phenyl group than by an adjacent oxygen atom,[35] the reverse is true in carbocation chemistry, where the oxonium ion is expected to be more stable than the α-phenyl-substituted alternative.[36] In the present situation, it appears that this effect is reinforced by the presence of a β-OH substituent in **3b**, which inductively destabilizes the carbocation center,[37] tipping the balance further in favor of **4b**.

Clearly, there is a significant benefit to be gained from the concerted cationic mechanism that is not present in the radical-based pathway. Indeed, the very high barrier for rearrangement in the concerted radical-based pathway (135.6 kJ mol^{-1}) might well be outside the catalytic range for an enzyme. Thus, if radical intermediates are involved in the tropane alkaloid rearrangement, then how might the enzyme make this transformation possible? Previous investigations of the rearrangement mechanisms for various AdoCbl-dependent enzymes provide useful clues in this regard. In particular, it was found that the barriers to rearrangement are significantly facilitated by partial protonation of the migrating moiety and partial deprotonation of a spectator moiety.[5,27] Given the success of these previous studies, we chose to investigate whether similar effects might prove beneficial for the present systems.

3.2. The Concerted Radical Pathway. 3.2.1. The Effect of Partial Protonation of the Migrating Moiety. Figure 1 displays the barriers and reaction energies for the rearrangement of **3a** to **4a** in isolation and in the presence of various acids acting to partially protonate the C=O group of the migrating moiety.[15] As first noted in Table 1, in the absence of acid catalysis the rearrangement barrier is predicted to be 135.6 kJ mol^{-1} and the reaction is endothermic by 29.4 kJ mol^{-1}. A reduction of 11.9 kJ mol^{-1} in the rearrangement barrier is observed when the relatively weak gas-phase acid hydrogen fluoride is used to effect partial protonation of the migrating carbonyl oxygen. When the relatively strong gas-phase acid NH$_4^+$ is used instead, a reduction of 23.1
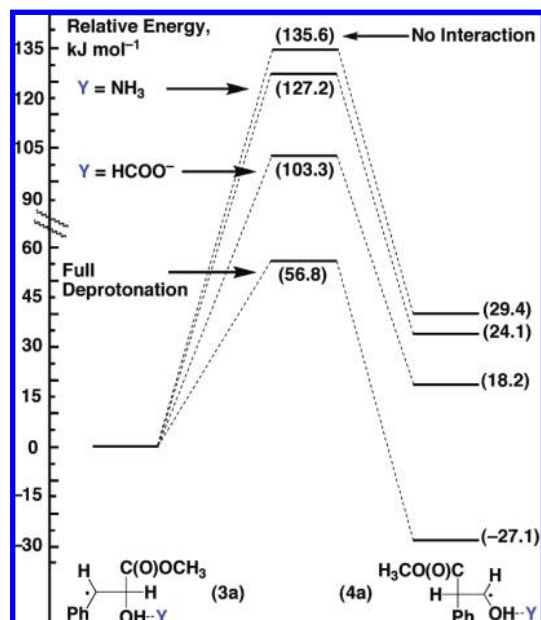
kJ mol^{-1} is observed, bringing the rearrangement barrier down to 112.5 kJ mol^{-1}. In the limit of full protonation (**X** = H$^+$), the rearrangement barrier is reduced to 70.6 kJ mol^{-1}.

The origin of the barrier reduction in this system can be understood in terms of the hydrogen bond strength between the acid and the migrating carbonyl oxygen. Because this interaction is stronger in the transition structure than in the reactant, a lowering of the barrier is observed. A measure of the degree of stabilization is provided by the distance between the acidic proton and the carbonyl oxygen of the substrate. For instance, we observe the distance between these entities to decrease across the series HF (1.584 Å), NH$_4^+$ (1.458 Å), and H$^+$ (0.975 Å). Correspondingly, we observe a lengthening of the acidic HF and NH bonds (by 0.039 and 0.082 Å, respectively) relative to their equilibrium values.

In summary, partial protonation of the migrating carbonyl oxygen does indeed reduce the barrier for rearrangement on the radical potential energy surface. However, even at the extreme of full protonation, which would not be expected to occur in the active site of an enzyme, the barrier remains relatively high. In order to assess how an enzyme might further reduce the barrier to rearrangement, we next explore the partial deprotonation of the spectator OH group.

3.2.2. The Effect of Partial Deprotonation of the Spectator OH Moiety. Previous investigations of the rearrangement step in the reactions catalyzed by the AdoCbl-dependent enzyme diol dehydratase (DDH) found that partially deprotonating the OH group adjacent to the migrating moiety had the effect of reducing the barrier for rearrangement.[27] Because the present system also includes such a 'spectator' OH group, we examined whether similar energy savings might be observed through partial deprotonation within **3** and **4**.

Figure 2 displays the barriers and reaction enthalpies for the radical-based concerted rearrangement in the absence and presence of various bases.[15] For convenience, we have again included the results for rearrangement in the absence of any external catalysis (i.e., a barrier and reaction enthalpy of 135.6 and 29.4 kJ mol^{-1}, respectively). Interaction of the relatively weak gas-phase base

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Table 2. Barriers and Reaction Enthalpies (G3(MP2)-RAD, kJ mol^{-1}) for the Carbon-Skeleton Rearrangement of **3a** to **4a** in the Presence of Acid (**X**) and Base (**Y**) Catalysts[15]

X	Y	$\Delta H^{\ddagger}$	ΔH
-	-	130.2	28.7
HF	HCOO$^-$	99.8	27.3
NH$_4^+$	NH$_3$	96.9	14.3

ammonia at the spectator OH group is seen to reduce the barrier for rearrangement by 8.4 kJ mol^{-1} to 127.2 kJ mol^{-1}. A larger reduction (of 32.3 kJ mol^{-1}) in the barrier height is observed when formate, HCOO$^-$, is used to effect partial deprotonation of the spectator OH group. In the limit of full deprotonation, we observe a reduction in the barrier to rearrangement of 78.8 kJ mol^{-1}, bringing the overall rearrangement barrier to 56.8 kJ mol^{-1}. In addition to this dramatic reduction in barrier, we also observe a marked change in reaction enthalpy. That is, when the spectator OH group is fully deprotonated, the reaction becomes exothermic by 27.1 kJ mol^{-1}.

It is interesting to observe that partial deprotonation (at the spectator OH) appears to provide a stronger influence in reducing the barrier and reaction enthalpy for rearrangement than does partial protonation (of the migrating group). Similar results have recently been observed in the rearrangement mechanism involving DDH.[38] In that case, a glutamate residue is well-poised in its active site to perform this role.[39]

3.2.3. The Combined Effects of Partial Protonation of the Migrating Moiety and Partial Deprotonation of the OH Spectator Moiety. A significant finding in previous studies of the effect of partial protonation and deprotonation on the reactions catalyzed by AdoCbl-dependent enzymes was that these effects worked synergistically.[27] That is, the combination of both of these interactions reduced the barrier to rearrangement significantly more than would be expected if their individual effects were simply added. Given the similarity between the AdoCbl-mediated reactions and the radical pathway in the present system, we next briefly examine such combined interactions.

The first row of Table 2 shows the barrier and reaction enthalpy for the uncatalyzed rearrangement. We have truncated our model system slightly in this section as a result of an increase in computational cost required to describe the simultaneous effects of partial protonation and deprotonation.[15] However, the barrier and reaction enthalpy obtained with this smaller model (130.2 and 28.7 kJ mol^{-1}, Table 2) agree nicely with those determined using the larger model (135.6 and 29.4 kJ mol^{-1}, Table 1), thus lending confidence to the reliability of the other results within this section.

The second row of Table 2 shows the effect of interaction of a weak acid (HF) at the migrating moiety with a simultaneous interaction of a strong base (HCOO$^-$) at the spectator OH group of **3a**. As can be seen, the barrier to rearrangement drops to 99.8 kJ mol^{-1} relative to the uncatalyzed case, while the reaction enthalpy is left essentially unchanged.

When a strong acid (NH$_4^+$) is used in combination with a weak base (NH$_3$), the rearrangement barrier is found to be reduced by 33.3 kJ mol^{-1} to 96.9 kJ mol^{-1}, while the reaction energy is reduced to +14.3 kJ mol^{-1}. On the whole, it appears as though there is not a significant synergistic effect for this rearrangement, at least within the constraints of these models.
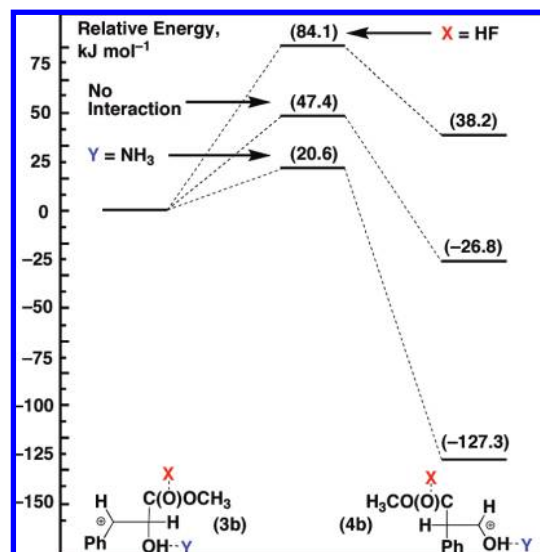
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Figure 3. Schematic energy profiles showing the effect on the cationic rearrangement (**3b** → **4b**) of partial protonation of the migrating moiety with HF (**X**) and partial deprotonation of the OH group with NH$_3$ (**Y**). Relative energies (G3(MP2)-RAD, kJ mol^{-1}) are given in parentheses.

The barrier remains relatively high at ca. 100 kJ mol^{-1}, and it is not clear how an enzyme would cope with such a situation.

3.3. The Concerted Carbocation Pathway. Having examined various aspects of a concerted rearrangement involving radical intermediates, we now examine the concerted carbocation-based rearrangement pathway in a similar fashion. It will be recalled that carbocationic species have been postulated previously to participate in the carbon-skeleton rearrangement of tropane alkaloids.[14] As shown in Scheme 1, a P450 enzyme may, for example, generate such species via a net two-electron oxidation with proton loss from **1**, or via a one-electron oxidation of the radical-derived species **3a** following an initial H-atom abstraction. Because the P450-dependent enzyme thought to be responsible for the carbon-skeleton rearrangement has yet to be isolated, it is not possible for us to directly assess these possibilities. However, some insight as to the feasibility of the latter scenario can be gained via an examination of the ionization energy (IE) of **3a** and comparing it with those for known compounds. We calculate the adiabatic IE of **3a** (to give **3b**) to be 7.01 eV, which is slightly higher than the corresponding IE of *tert*-butyl radical (6.58–6.70 eV),[40] and slightly lower than the first IE of the Mg atom (7.65 eV).[41] Overall, this suggests that the oxidation of **3a** to form **3b** can take place quite easily.

Moving on, Figure 3 displays the barrier and reaction enthalpy for the rearrangement of the substrate-derived carbocation **3b** to the product-related carbocation **4b** in the absence and presence of selected catalytic agents.[15] In the uncomplexed system, we observe that the barrier for rearrangement is 47.4 kJ mol^{-1} with an associated exothermicity of 26.8 kJ mol^{-1} (Table 1, Figure 3). Upon complexation of HF at the migrating carbonyl oxygen, we observe an *increase* of 36.7 kJ mol^{-1} in the rearrangement barrier, bringing it to 84.1 kJ mol^{-1}. Interestingly, we also observe an increase of 65.0 kJ mol^{-1} in the reaction enthalpy, making the overall rearrangement endothermic by 38.2 kJ

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mol^{-1}. Clearly, partial protonation does not impact favorably upon the **3b** → **4b** rearrangement.

These results contrast sharply with those obtained when NH$_3$ is used to partially deprotonate the spectator OH group; in this case, the barrier for rearrangement is just 20.6 kJ mol^{-1}. More impressive, however, is the effect that partial deprotonation has on the reaction enthalpy. Specifically, we find the exothermicity of the rearrangement of **3b** to **4b** in the presence of NH$_3$ to increase to 127.3 kJ mol^{-1}. This equates to a change in reaction exothermicity of 100.5 kJ mol^{-1}!

During the rearrangement of **3b**···NH$_3$ to **4b**···NH$_3$, we note a progressive transfer of the proton associated with the spectator hydroxyl group to the acceptor N atom of NH$_3$. This can be readily explained by recognizing that, while the gas-phase acidity of NH$_4^+$ (848 kJ mol^{-1}) is less than the relevant gas-phase acidity of **3b** (862 kJ mol^{-1}), it is greater than the gas-phase acidity of the product cation **4b** (803 kJ mol^{-1}); thus, the propensity for proton transfer from **4b** to NH$_3$ is strong. Further, these energy differences also explain the marked increase in exothermicity of the rearrangement, which now includes the ca. 60 kJ mol^{-1} difference in proton binding energies mentioned above.

Apart from the lowering of the rearrangement barrier and reaction enthalpy, the relatively high gas-phase acidity of the spectator hydroxyl group in the carbocation pathway has an important consequence on the overall conversion of **1** to **2**. Recalling Scheme 1, a step that follows the cationic rearrangement (**3b** → **4b**) is deprotonation of the spectator hydroxyl group to form the hyoscyamine aldehyde (**6**). Our results indicate that, in the presence of even a weak base, this proton loss occurs spontaneously with the rearrangement to yield **6** directly.

In summary, even in the absence of catalytic agents to partially deprotonate the substrate-derived carbocation **3b**, its rearrangement barrier to give **4b** is calculated to be a modest 47.4 kJ mol^{-1} and the reaction is exothermic by 26.8 kJ mol^{-1}. Partial protonation of the migrating group by HF is found to retard the concerted cationic rearrangement. In contrast, partial deprotonation of the spectator OH group by NH$_3$ offers significant energy savings, resulting in values for the barrier and reaction enthalpy of 20.6 and −127.3 kJ mol^{-1}, respectively. The relatively high gas-phase acidity of **4b** suggests that the subsequent and direct generation of the experimentally observed hyoscyamine aldehyde (**6**, Scheme 1) is a facile and highly favorable reaction.

4. Concluding Remarks

The present study has examined the carbon-skeleton rearrangement of littorine (**1**) to hyoscyamine (**2**) in tropane alkaloid biosynthesis. Although the precise enzymes involved in the overall transformation of **1** to **2** have not yet been identified, the participation of a cytochrome P450 enzyme and a dehydrogenase appears likely.[12,14] Of the various steps in the transformation of **1** to **2**, the carbon-skeleton rearrangement step involving either radical or carbocation intermediates has formed the focus of our investigations.

Our calculations of a rearrangement pathway involving radical species indicate this process to be energetically demanding. The fragmentation products derived from a stepwise rearrangement mechanism lie ca. 135 kJ mol^{-1} higher in energy than the substrate-derived radical. We find that the concerted radical pathway does not offer any improvement over this, with a calculated barrier for rearrangement of the same order (Table 1). Although both partial protonation of the migrating group and partial deprotonation of

the spectator hydroxyl group can serve to lower the radical rearrangement barrier, the strength of the acid or base required to reduce the barrier to a physiologically relevant level may be inaccessible in an enzyme environment.

As an alternative to the radical pathway, a cationic mechanism for the rearrangement was also investigated. Even though such pathways are not normally considered prominent in P450-catalyzed reactions, there is evidence that such pathways may be operational under certain circumstances. In the present situation, the calculated low ionization energy of the substrate-derived radical **3a** suggests that oxidation to the substrate-derived carbocation **3b** will be facile, opening up the possibility of a cationic mechanism. In a manner similar to that found for the radical pathway, the fragmentation−recombination involving carbocationic species is found to be associated with a high energy (ca. 170 kJ mol^{-1}). On the other hand, the concerted pathway is characterized by a relatively low barrier (47.4 kJ mol^{-1}).

Partially protonating the migrating moiety in the concerted carbocation-based pathway by HF has the effect of increasing the rearrangement barrier by 36.7 kJ mol^{-1} to 84.1 kJ mol^{-1}, and the reaction becomes endothermic by 38.2 kJ mol^{-1}. In contrast, partially deprotonating the spectator OH group with NH$_3$ has a dramatic beneficial effect on both the rearrangement barrier and the reaction enthalpy. More specifically, the barrier is reduced to just 20.6 kJ mol^{-1} and the reaction exothermicity increases to 127.3 kJ mol^{-1}. The enhanced acidity of the OH group in the product-related carbocation **4b** is reflected in the observed proton transfer to the relatively weak (gas-phase) base NH$_3$. This points to a direct and facile formation of the experimentally observed hyoscyamine aldehyde (**6**), perhaps even in concert with the carbocation rearrangement.

In closing, it is interesting to consider the littorine-to-hyoscyamine conversion in the light of a previously expressed opinion about P450 mechanisms. To wit, Akhtar et al. concluded that "The P-450 dependent hydroxylation and C−C bond cleavage reactions occur via a radical mechanism and the enzymes participating in these processes have evolved to deal with situations where the ionic processes are deemed energetically unfavourable."[42] Given the results obtained in the present study, the potential P450-dependent rearrangement of littorine may turn out to be an interesting counter-example to such a paradigm. As previously stated, however, there is much work to be done before any definitive mechanistic conclusion can be made along these lines.

Supporting Information Available: GAUSSIAN archive entries of the B3-LYP/6−31G(d,p) geometries, corresponding G3(MP2)-RAD total energies, and full citations for refs 30 and 31. This material is available free of charge via the Internet at http://pubs.acs.org.

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