On the Mechanism of Action of Vitamin B_{12}: Theoretical Studies of the 2-Methyleneglutarate Mutase Catalyzed Rearrangement

David M. Smith,[†] Bernard T. Golding,*[,‡] and Leo Radom*[,†]

Contribution from the Research School of Chemistry, Australian National University, Canberra, ACT 0200, Australia, and Department of Chemistry, University of Newcastle upon Tyne, Newcastle upon Tyne NE1 7RU, UK

Received July 31, 1998. Revised Manuscript Received November 2, 1998

Abstract: Ab initio molecular orbital theory is used to investigate the coenzyme-B_{12}-dependent rearrangement of 2-methyleneglutarate to (*R*)-3-methylitaconate catalyzed by 2-methyleneglutarate mutase. We use a 'model system' approach whereby substituents such as carboxylate groups are replaced by computationally less expensive hydrogen atoms. The validity of this approach is tested and supported by investigations which compare the results obtained with and without this simplification. In both rearranging systems, we find a recently suggested mechanism, involving a transient fragmentation of the substrate followed by recombination of the fragments, to be associated with a high activation energy. A cyclization/ring-opening (addition/elimination) mechanism is found to require substantially less energy than the fragmentation/recombination mechanism. Even lower in energy requirements are mechanisms involving protonation/deprotonation of the substrates.

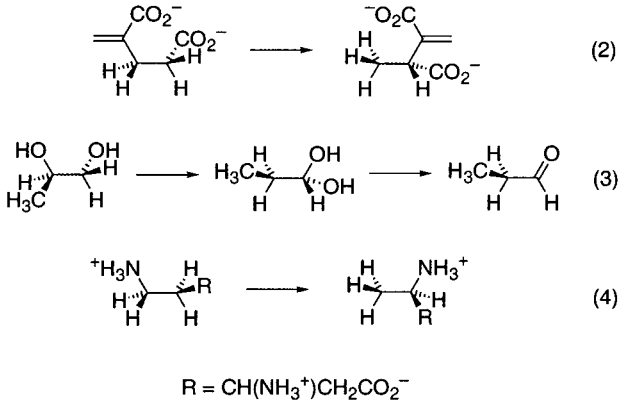
Introduction

Enzyme-catalyzed reactions dependent on the B_{12} coenzyme called adenosylcobalamin[1] have baffled many investigators since the discovery of the first example of this class 40 years ago.[2] Each enzyme−coenzyme partnership facilitates a rearrangement, in which a substrate hydrogen atom and functional group (X) apparently change places on adjacent carbon atoms:

(1)

These reactions and the associated enzymes have been divided into three classes: the carbon skeleton mutases (exemplified by the 2-methyleneglutarate mutase catalyzed reaction shown in Scheme 1, reaction 2), the eliminases (e.g., diol dehydratase in Scheme 1, reaction 3), and the aminomutases (e.g., β-lysine 5,6-aminomutase in Scheme 1, reaction 4).[3] Such reactions initially had no precedent in known chemistry, and attempts to replicate them in experimental model systems have had varying success.[4] In addition, it has not been established whether any of the model reactions proceed by the same type of mechanism as the corresponding enzymatic process.

Scheme 1. Representative Examples of the Three Classes of Coenzyme B_{12}-Dependent Reactions



In previous work, we explored possible mechanisms for the coenzyme B_{12}-dependent mutases using ab initio molecular orbital methods for the determination of the energetics of selected reaction pathways.[5] It had already been proposed at that time that the mutases operated via intermediate protein-bound free radicals,[5,6] and subsequently, this idea has been developed.[7] These intermediate radical species are thought to arise in an initiation step in which the 5′-deoxyadenosyl radical (Ado−CH_2• in Scheme 2), derived by homolysis of the cobalt−

[†] Australian National University.

[‡] University of Newcastle upon Tyne.

(1) For recent developments and leading references see, *Vitamin B_{12} and B_{12}-Proteins;* Kräutler, B., Arigoni, D., Golding, B. T., Eds.; Wiley-VCH: Weinheim, 1998.

(2) Barker, H. A.; Smyth, R. D.; Wilson, R. M.; Weissbach, H. *J. Biol. Chem.* **1959**, *234*, 320−328.

(3) Golding, B. T.; Buckel, W. Corrin-dependent Reactions. In *Comprehensive Biological Catalysis*; Sinnott, M. L., Ed.; Academic Press: London, 1997; Vol. III, pp 239−259. The stereochemistry for the β-lysine 5,6-aminomutase catalyzed reaction has not yet been established.

(4) (a) Dowd, P.; Shapiro, M.; Kang, D. *J. Am. Chem. Soc.* **1975**, *97*, 4754. (b) Fountoulakis, M.; Retey, J. *Chem. Ber.* **1980**, *113*, 650−668. (c) Golding, B. T. In *B_{12}*; Dolphin, D., Ed.; Wiley: New York, 1982; Vol. 1; pp 543−582. (d) Wollowitz, S.; Halpern, J. *J. Am. Chem. Soc.* **1988**, *110*, 3112−3120. (e) Ashwell, S.; Davies, A. G.; Golding, B. T.; Hay-Motherwell, R.; Mwesigye-Kibende, S. *J. Chem. Soc., Chem. Commun.* **1989**, 1483−1485. (f) Anderson, R. J.; Ashwell, S.; Dixon, R. M.; Golding, B. T. *J. Chem. Soc., Chem. Commun.* **1990**, 70−72. (g) Finke, R. G. In *Molecular Mechanisms in Bioorganic Processes*; Bleasdale C., Golding B. T., Eds.; The Royal Society of Chemistry: Cambridge, 1990; pp 244−280. (h) Darbre, T.; Keese, R.; Siljegovic, V.; Wollebgygi, A. *Helv. Chim. Acta* **1996**, *79*, 2100. (i) Hisaeda, Y.; Ogawa, A.; Ohno, T.; Murakami, Y. *Inorg. Chim. Acta* **1998**, *273*, 299−309.

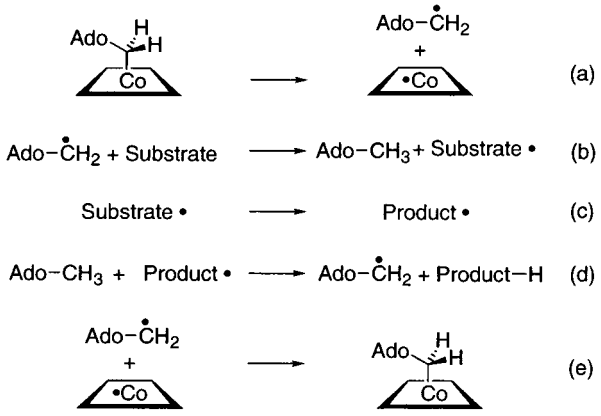
(5) (a) Golding, B. T.; Radom, L. *J. Chem. Soc., Chem. Commun.* **1973**, 939−941. (b) Golding, B. T.; Radom, L. *J. Am. Chem. Soc.* **1976**, *98*, 6331−6338.

(6) (a) Eggerer, H.; Stadtman, E. R.; Overath, P.; Lynen, F. *Biochem. Z.* **1960**, *333*, 1−9. (b) Halpern, J. *Ann. N. Y. Acad. Sci.* **1974**, *239*, 2−21.

(7) (a) Finke, R. G.; Schiraldi, D. A.; Mayer, B. J. *Coord. Chem. Rev.* **1984**, *54*, 1−22. (b) Halpern, J. *Science* **1985**, *227*, 869−875.

10.1021/ja9827245 CCC: $18.00
Published on Web 01/26/1999

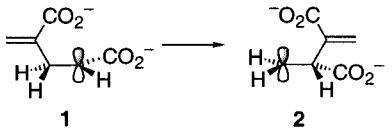
Scheme 2. Schematic Representation of the Bound-Free-Radical Hypothesis for B_{12}-Dependent Reactions



carbon σ-bond of 5′-deoxyadenosylcobalamin (Scheme 2, step a), abstracts a hydrogen atom from a substrate molecule giving 5′-deoxyadenosine and a substrate-derived radical (Scheme 2, step b). This is transformed into a product-related radical (Scheme 2, step c), which is converted to product by abstraction of a hydrogen atom from the methyl group of 5′-deoxyadenosine (Scheme 2, step d). The aim of our earlier studies, which were focused on the eliminases, was to probe the conversion of the substrate-derived radical into the product-related radical (Scheme 2, step c). We concluded that this conversion is greatly facilitated by protonation of the migrating group.

In recent years, the genes coding for several B_{12}-dependent enzymes have been cloned,[8] and the crystal structures of two B_{12}-binding proteins have been elucidated.[9] Dowd's preference for mechanisms of rearrangement involving cobalt participation has recently been reiterated through model studies.[10] In contrast, EPR spectroscopic work has provided evidence for the intermediacy of a substrate-derived radical with cob(II)alamin some 6 Å away.[11] Advances in computer technology and new molecular orbital methods provide theoretical data on molecular systems more reliably than ever before. Hence, the time is now right for a fresh theoretical attack on the mechanistic problem of the coenzyme B_{12}-dependent reactions. Indeed, recent experimental results[12] have led to the resurgence of an old mechanistic proposal[5] involving fragmentation/recombination, the validity of which needs to be probed both by experimental and theoretical approaches. An important feature of the calculations is that they provide a means of evaluating the feasibility of the key rearrangement step in the absence of cobalt.

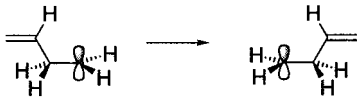
In this paper, we present the results of calculations applied to the B_{12}-dependent rearrangement catalyzed by 2-methyleneglutarate mutase in which 2-methyleneglutarate is converted to (*R*)-3-methylitaconate (Scheme 1, reaction 2).[13] If the bound-free-radical hypothesis is accepted, then the crucial radical rearrangement step can be represented by reaction 5, in which a 2-methyleneglutarate-derived radical (**1**) is transformed into an (*R*)-3-methylitaconate-related radical (**2**)



1 **2** (5)

The migrating group (X) in this reaction has been shown by labeling experiments, both for the enzyme[14] and for a model,[15] to be the acrylate moiety.

The system shown in reaction 5 is relatively large and conformationally flexible, making its detailed theoretical investigation computationally demanding although not impossible. In the present work, we use a model system approach whereby substituents such as carboxylate groups are replaced by computationally less expensive and simpler hydrogen atoms. Applying this simplification to the 2-methyleneglutarate mutase catalyzed rearrangement results in the system shown in reaction 6



3 **3'** (6)

We have effectively replaced the transformation of 2-methyleneglutarate to (*R*)-3-methylitaconate with the degenerate rearrangement of the but-3-enyl radical. This approach greatly simplifies the calculations, while keeping the rearranging carbon skeleton intact. The merits of such a model system approach must be tested, however, and this is done in the present work by comparing the results obtained using the full system (reaction 5) with those from the model in which both carboxylate groups have been removed (reaction 6).

Theoretical Procedures

Standard ab initio molecular orbital calculations[16] were performed with GAUSSIAN 94.[17] Geometries and zero-point vibrational energies were obtained using the B3-LYP/6-31G(d) procedure. For the model system (reaction 6), improved relative energies were obtained using the B3-LYP/6-311+G(3df,2p) and CBS-RAD techniques. The latter procedure, in conjunction with the aforementioned geometries and zero-point energies, corresponds to a model chemistry which has been previously referred to as CBS-RAD(B3-LYP,B3-LYP),[18] but in the interests of simplicity will be referred to as CBS-RAD in the present paper. The improved relative energies for the larger species involved in reaction 5 were obtained by using only the B3-LYP/6-311+G(3df, 2p) theoretical method. Unless otherwise noted, relative energies in the text refer to CBS-RAD values for the species involved in reaction

(8) Zelder, O.; Beatrix, B.; Leutbecher, U.; Buckel, W. *Eur. J. Biochem.* **1994**, *226*, 577−585.

(9) (a) Luschinsky-Drennan, C.; Huang, S.; Drummond, J. T.; Matthews, R. G.; Ludwig, M. L. *Science* **1994**, *266*, 1669−1674. (b) Mancia, F.; Keep, N. H.; Nakagawa, A.; Leadlay, P. F.; McSweeney, S.; Rasmussen, B.; Bosecke, P.; Diat, O.; Evans, P. R. *Structure* **1996**, *4*, 339−350.

(10) He, M.; Dowd, P. *J. Am. Chem. Soc.* **1998**, *120*, 1133−1137.

(11) Bothe, H.; Darley, D. J.; Albracht, S. P. J.; Gerfen, G. J.; Golding, B. T.; Buckel, W. *Biochemistry* **1998**, *37*, 4105−4113.

(12) Beatrix, B.; Zelder, O.; Kroll, F. K.; Orlygsson, G.; Golding, B. T.; Buckel, W. *Angew. Chem., Int. Ed. Engl.* **1995**, *34*, 2398−2401. During the inhibition of 2-methyleneglutarate mutase by acrylate, it was found that a plot of $1/v$ versus the square of the acrylate concentration gave a straight line (Dixon plot, v = initial velocity). This linear correlation suggests that two acrylate molecules are involved in the inhibition of the enzyme.

(13) (a) Kung, H. F.; Cederbaum, S.; Tsai, L.; Stadtman, T. C. *Proc. Natl. Acad. Sci. U.S.A.* **1970**, *65*, 978. (b) Buckel, W.; Golding, B. T. *Chem. Soc. Rev.* **1996**, *26*, 329−337.

(14) (a) Kung, H. F.; Tsai, L. *J. Biol. Chem.* **1971**, *246*, 6436−6443. (b) Hartrampf, G.; Buckel, W. *Eur. J. Biochem.* **1986**, *156*, 301−304.

(15) Dowd, P.; Hershline, R. *J. Chem. Soc., Perkin. Trans. 2* **1988**, 61−70.

(16) Hehre, W. J.; Radom, L.; Schleyer, P. v. R.; Pople, J. A. *Ab Initio Molecular Orbital Theory*; Wiley: New York, 1986.

(17) Frisch, M. J.; Trucks, G. W.; Schlegel, H. B.; Gill, P. M. W.; Johnson, B. G.; Robb, M. A.; Cheeseman, J. R.; Keith, T.; Petersson, G. A.; Montgomery, J. A.; Raghavachari, K.; Al-Laham, M. A.; Zakrzewski, V. G.; Ortiz, J. V.; Foresman, J. B.; Cioslowski, J.; Stefanov, B. B.; Nanayakkara, A.; Challacombe, M.; Peng, C. Y.; Ayala, P. Y.; Chen, W.; Wong, M. W.; Andres, J. L.; Replogle, E. S.; Gomperts, R.; Martin, R. L.; Fox, D. J.; Binkley, J. S.; Defrees, D. J.; Baker, J.; Stewart, J. P.; Head-Gordon, M.; Gonzalez, C.; Pople, J. A. GAUSSIAN 94 (Rev. E.2) (Gaussian, Inc., Pittsburgh, 1995).

(18) Mayer, P. M.; Parkinson, C. J.; Smith, D. M.; Radom, L. *J. Chem. Phys.* **1998**, *108*, 604−615.

6 and B3-LYP/6-311+G(3df,2p) values for the species involved in reaction 5, in both cases at 0 K. We have also included, within the tables of results, the B3-LYP/6-31G(d) relative energies for both systems to allow an examination of the suitability of the smaller basis set, with potential application to still larger systems in mind.

One possible mechanism for the degenerate rearrangement of the but-3-enyl radical (reaction 6) involves the formation of the cyclopropylcarbinyl radical as an intermediate (see below).[7b] The process by which the cyclopropylcarbinyl radical ring-opens to form the but-3-enyl radical has received considerable attention.[19] In particular, the CBS-RAD and B3-LYP methods have been found to perform well in describing this ring-opening reaction.[19e] The alternative fragmentation/recombination mechanism for the rearrangement of **3** involves the addition of a radical to ethylene as the recombination step. Such radical additions to double bonds have also been shown to be well-described by both the CBS-RAD and B3-LYP methodologies.[20] Due to the similarities between the systems contained in the current work and those in the previous assessment studies,[19e,20b] we are confident that the selected theoretical methods are well-suited to our investigation of the 2-methyleneglutarate mutase catalyzed rearrangement. Calculated CBS-RAD and B3-LYP/6-311+G(3df,2p) total energies and GAUSSIAN 94 archive entries for the B3-LYP/6-311+G(3df,2p) calculations for all relevant structures are presented in Tables S1−S3 of the Supporting Information.

Results and Discussion

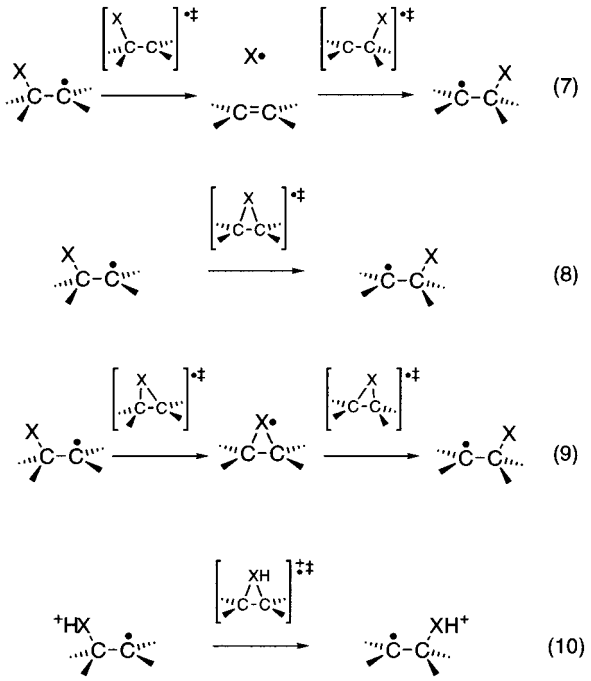
A. Possible Mechanisms for 1,2-Shifts in Isolated Free Radicals. We have considered four mechanistic possibilities for the migration of a functional group X (bonded to carbon) to an adjacent carbon atom bearing an unpaired electron. These are shown in Scheme 3 and have all been discussed, to a varying extent, in the literature.[5,21]

Reaction 7 depicts a transient fragmentation to form ethylene and an X-derived radical (X•). Re-addition of X• to the adjacent ethylenic carbon atom yields the rearranged target. This mechanism, referred to as fragmentation/recombination, has recently received renewed attention in the B_{12} field, in the light of experimental evidence for inhibition of 2-methyleneglutarate mutase by acrylate.[12]

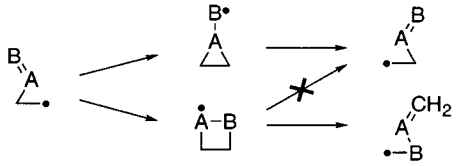
Reactions 8−10 all involve migration of the group X without detachment from the two-carbon unit. The first possibility of this type (reaction 8) will be referred to as the concerted mechanism and has the symmetrical bridged species as a transition structure. Such rearrangements are usually associated with large energy barriers, often requiring one-electron occupancy of a high-energy orbital.[5,21b] Exceptions are observed with migrating groups involving low-lying d orbitals[22] or containing π-electron systems.[23]

An unsaturated migrating group allows a mechanism slightly different from the one shown in reaction 8. In this case, the migrating group X contains a pair of doubly bonded atoms (A═B, with A bonded to the framework carbon, see Scheme 4).

Scheme 3. Possible Mechanisms for the Free-Radical-Based 1,2-migration of a Group X



Scheme 4. Possible Ring-closing/Ring-opening Modes in the Case of an Unsaturated Migrating Group X (Scheme 3, reaction 9, X equals A═B)



Such a mechanism is shown in reaction 9, which is best described as an intramolecular addition of the unpaired electron to atom A of the π system to form a stable intermediate in which the free electron resides on atom B. The three-membered ring thus formed may then eliminate, by homolytic fission of the appropriate adjacent bond, to give the rearranged product. This pathway is referred to as the addition/elimination mechanism. The distinction between the concerted (reaction 8) and the addition/elimination (reaction 9) mechanisms depends formally on whether the symmetric species is a transition structure or a stable intermediate. This distinction is not always clear-cut since there are cases in which the depth of the well containing the intermediate becomes very small, making it difficult to classify the rearrangement one way or the other. There do exist, however, definitive examples of both classes of behavior.[24]

An alternative possibility to the formation of a three-membered ring via reaction 9 is the addition of the unpaired electron to atom B of the migrating group, resulting in the formation of a four-membered ring with the free electron residing on atom A (cf. Scheme 4). Ring opening can lead to an isomeric radical but not that corresponding to migration of the A═B (X) fragment.

A further variation to the concerted mechanism arises from protonation of the migrating group (reaction 10). Previous studies have shown that protonation is able to facilitate the 1,2-

(19) See, for example: (a) Newcombe, M.; Glenn, A. G. *J. Am. Chem. Soc.* **1989**, *111*, 275−277. (b) Nonhebel, D. C. *Chem. Soc. Rev.* **1993**, 347−359. (c) Beckwith, A. L. J.; Bowry, V. *J. Am. Chem. Soc.* **1994**, *116*, 2710−2716. (d) Martinez, F. N.; Schlegel, H. B.; Newcombe, M. *J. Org. Chem.* **1996**, *61*, 8547−8550. (e) Smith, D. M.; Nicolaides, A.; Golding, B. T.; Radom, L. *J. Am. Chem. Soc.* **1998**, *120*, 10223−10233.

(20) See, for example: (a) Wong, M. W.; Radom, L. *J. Phys. Chem.* **1995**, *99*, 8582−8588. (b) Wong, M. W.; Radom, L. *J. Phys. Chem.* **1998**, *102*, 2237−2245.

(21) See, for example: (a) Russell, J. J.; Rzepa, H. S.; Widdowson, D. A. *J. Chem. Soc., Chem. Commun.* **1983**, 625−627. (b) Greenberg, A.; Liebman, J. F. In *Energetics of Organic Free Radicals*, 1st ed.; Simoes, J. A. M., Greenberg, A., Liebman, J. F., Eds.; Blackie Academic and Professional: London, 1996; Vol. 4; pp 224−294.

(22) Hoz, T.; Sprecher, M.; Basch, H. *J. Phys. Chem.* **1985**, *89*, 1664−1670.

(23) Lindsay, D. A.; Lusztyk, J.; Ingold, K. U. *J. Am. Chem. Soc.* **1984**, *106*, 7087−7093.

(24) Smith, D. M.; Golding, B. T.; Radom, L., to be published.

(25) Hoz, T.; Sprecher, M.; Basch, H. *THEOCHEM* **1987**, *150*, 51−65.

(26) For further applications of protonation, see: George, P.; Glusker, J. P.; Bock, C. W. *J. Am. Chem. Soc.* **1997**, *119*, 7065−7074.

Table 1. Relative Energies (kJ mol^{-1})[a] of the Species Involved in the Degenerate Rearrangement of the But-3-enyl Radical (Reactions 11−13) at 0 K

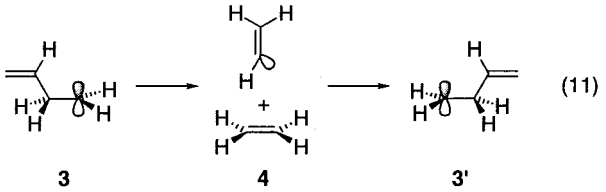
	CBS-RAD	B3-LYP/ 6-311+G(3df,2p)	B3-LYP/ 6-31G(d)
but-3-enyl radical (**3**)	0.0	0.0	0.0
TS:3→4	147.5	141.6	153.3
vinyl radical + ethylene (**4**)	137.2	122.5	141.0
TS:3→5	42.4	45.1	44.8
cyclopropylcarbinyl radical (**5**)	12.4	17.3	12.7
methylcyclopropane radical cation (**6**)	0.0	0.0	0.0
TS:6→6′	8.8	10.2	10.5

[a] Energies relative to either **3** or **6**.

shift in cases where it may otherwise seem unfavorable.[5,25,26] A possible explanation for these findings in acyclic systems is that protonation of X weakens the C−X bond,[27] thus making migration of the XH moiety easier. An additional factor that should be considered is that protonation introduces a stabilizing charge−dipole interaction in the transition structure, at least in the gas phase.

B. The Degenerate Rearrangement of the But-3-enyl Radical. (1) Fragmentation/Recombination. The simplest way of assessing the feasibility of the recently suggested fragmentation/recombination mechanism in the context of the 2-methyleneglutarate catalyzed rearrangement[12] is to investigate the energetics of this pathway in the model system (reaction 6). One advantage of this approach is that the smaller but-3-enyl radical may be treated with theoretical techniques more accurate than possible for the species involved in reaction 5.

The fragmentation/recombination mechanism for the rearrangement of the but-3-enyl radical (**3**) is shown in reaction 11



(11)

This reaction proceeds via a bond fission to give the vinyl radical plus ethylene (collectively referred to as **4**) followed by an intermolecular radical addition to form the rearranged product. Table 1 shows the relative energies for the species involved in this pathway. The B3-LYP/6-31G(d) structures and selected geometric parameters may be found in Figure 1.

The energy of **TS:3→4** is found to be quite high, nearly 150 kJ mol^{-1} above the but-3-enyl radical (**3**), demonstrating that the fragmentation step of reaction 11 is energetically unfavorable. We find that the energy increases relatively steeply as the two fragments separate, rising to more than 50 kJ mol^{-1} above that of **3** at a separation of just 1.8 Å. This finding may be relevant in considerations of the reaction within the confines of the cavity of the active site of the enzyme. The two fragments (**4**) lie in a relatively shallow energy well (10−20 kJ mol^{-1} deep), indicating that if fragmentation were to be effected, then the recombination could occur relatively easily. Indeed, recent experiments[28] have given an approximate activation energy for this process of 30 kJ mol^{-1}, slightly higher than our calculated values.

(27) See, for example: Mayer, P. M.; Glukhovtsev, M. N.; Gauld, J. W.; Radom, L. *J. Am. Chem. Soc.* **1997**, *119*, 12889.

(28) Roscoe, J. M.; Jayaweera, I. S.; Mackenzie, A. L.; Pacey, P. D. *Int. J. Chem. Kinet.* **1996**, *28*, 181−193.

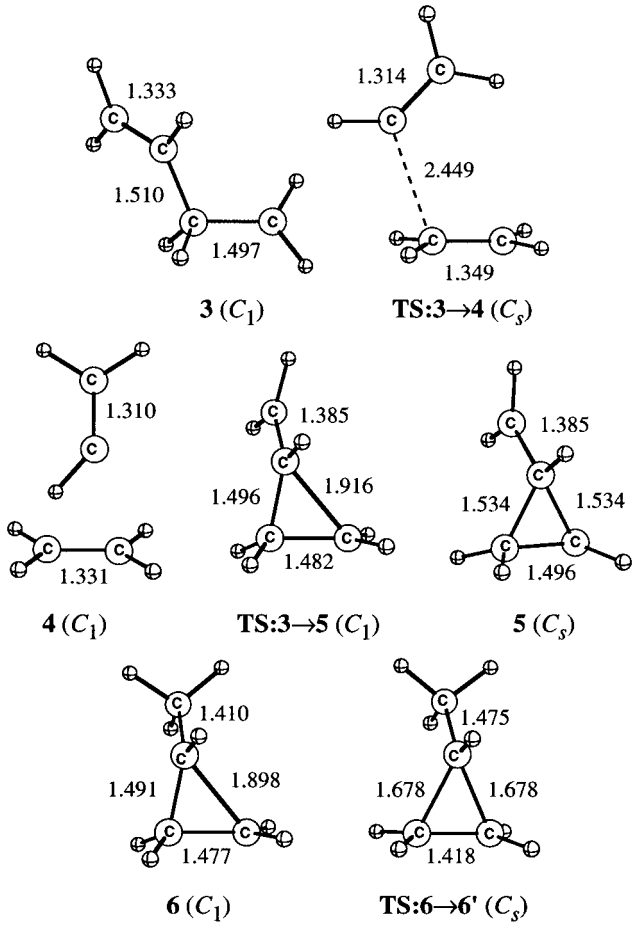
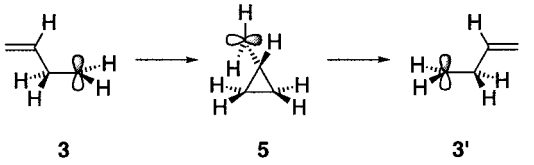


Figure 1. B3-LYP/6-31G(d) structures and selected bond lengths (Å) for the species involved in the degenerate rearrangement of the but-3-enyl radical.

(2) Addition/Elimination. The presence of a C=C double bond in the migrating group of the but-3-enyl radical introduces the possibility of the addition/elimination mechanism



(12)

The appropriate intermediate, as shown in reaction 12, is the cyclopropylcarbinyl radical (**5**).

The relative energies of the species involved in reaction 12 are included in Table 1. B3-LYP/6-31G(d) structures and selected geometrical parameters are given in Figure 1, while Figure 2 shows a schematic energy profile, comparing the addition/elimination mechanism with the fragmentation/recombination alternative. We find a significant preference (ca. 100 kJ mol^{-1}) for the addition/elimination pathway shown in reaction 12 compared with the fragmentation/recombination pathway of reaction 11. Thus it is more favorable, in the gas phase at least, for the migrating HC=CH$_2$ group to stay bonded to the remaining framework rather than to become detached from it.

The cyclopropylcarbinyl radical intermediate involved in the addition/elimination mechanism is predicted to lie in a well of depth 30 kJ mol^{-1}. The characteristics of this radical have been discussed in detail in recent work,[19e] where it was found that the currently employed theoretical techniques give good agreement with experiment for the ring-opening reaction.

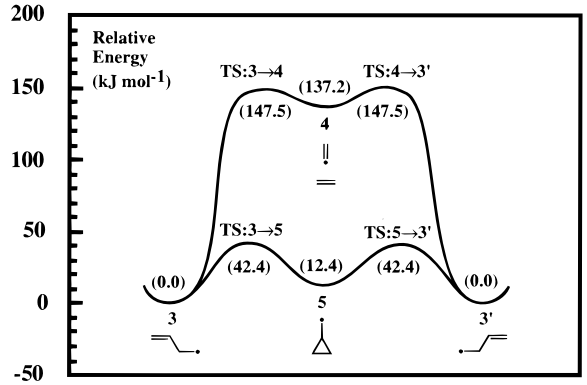
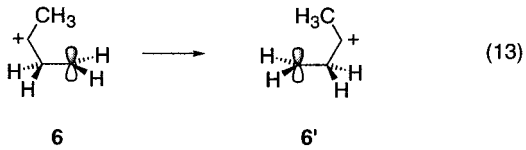


Figure 2. Schematic CBS-RAD energy profile for the degenerate rearrangement of the but-3-enyl radical via the fragmentation/recombination and addition/elimination mechanisms. Relative energies (kJ mol^{-1}) shown in parentheses.

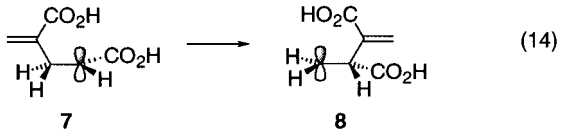
(3) Facilitation by Protonation. Guided by a previous study,[5] we were encouraged to investigate the facilitation of the concerted 1,2-shift in the but-3-enyl radical by protonation of the migrating group. Of the two possible protonation sites on the migrating group, we have chosen the terminal carbon for our current investigation. The resulting reaction is equivalent to the degenerate rearrangement of a partially ring-opened methyl cyclopropane radical cation (**6**)



The unsubstituted cyclopropane radical cation has received considerable experimental[29] and theoretical[30] attention and is thought to exist as three equivalent $^2A_1$ partially ring-opened structures. These three equivalent structures are able to interconvert relatively easily, via three equivalent $^2B_2$ structures. Although the symmetry is reduced in the methyl-substituted system, we are able to observe the appropriate 1,2-shift operating by a mechanism analogous to that of the unsubstituted case (see Figure 1 and ref 30b). The barrier to interconversion for the two methylcyclopropane radical cations (ca. 10 kJ mol^{-1}, Table 1) is found to be significantly lower than the barrier for addition/elimination.

The general agreement between the three theoretical techniques presented in Table 1 is quite good. Perhaps fortuitously, the B3-LYP results with the smaller basis set (6-31G(d)) are closer to the CBS-RAD predictions than are the values obtained with the more complete basis set (6-311+G(3df,2p)). With this and the possible application to larger systems in mind, we continue to present the B3-LYP results using both basis sets.

C. The 2-Methyleneglutarate to (*R*)-3-Methylitaconate Rearrangement. (1) The Gas-Phase Model. In the context of the biological medium, the carboxylic acid substituents of both substrate and product would be expected to exist formally in their anionic form. However, the process of binding the substrate to the protein presumably utilizes positively charged groups such as could be provided by arginine residues as, for example, in chorismate mutase.[31] Interactions of this type would result in only a small net charge in the vicinity of the carboxylate groups. In the gas phase, a molecule with an excess negative charge at either end is destabilized as a result of simple electrostatics. Consideration of these two factors has led us to choose a neutral carboxylic acid group as our gas-phase model for a protein-bound carboxylate group. Following this approach, reaction 14 depicts the rearrangement of a 2-methyleneglutaric-acid-derived radical (**7**) to form an (*R*)-3-methylitaconic-acid-based radical (**8**)



Investigation of this system allows us to ascertain the importance of the carboxylic acid groups in the gas-phase rearrangement and thus test the validity of our model system approach.

(2) Mechanistic Comparisons. As with the degenerate rearrangement of the but-3-enyl radical (the parent system), we have assessed three different mechanistic possibilities for the rearrangement shown in reaction 14. Reaction 15 shows fragmentation of the 2-methyleneglutaric-acid-derived radical (**7**) to form acrylic acid and the 2-acrylic acid radical (collectively referred to as **9**)[32] before recombination to form the (*R*)-3-methylitaconic-acid-related radical (**8**)

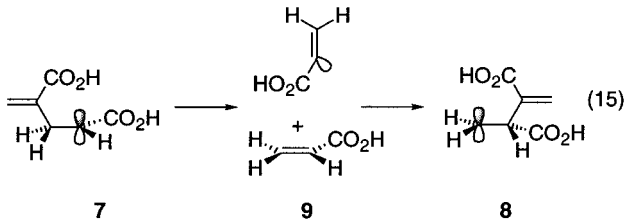


Table 2. Relative Energies (kJ mol^{-1})[a] of the Species Involved in the Rearrangement of the 2-Methyleneglutaric Acid Derived Radical (**7**) to the (*R*)-3-Methylitaconic Acid Related Radical (**8**) (Reactions 15−17) at 0 K

	B3-LYP/6-311+G(3df,2p)	B3-LYP/6-31G(d)
7	0.0	0.0
TS:7→9	153.8	163.7
9	140.5	161.0
TS:9→8	166.9	175.3
8	50.5	50.2
TS:7→10	55.2	52.2
10	49.9	44.2
TS:10→8	86.6	85.1
11	0.0	0.0
TS:11→12	11.2	11.1
12	11.5	11.3

[a] Energies relative to either **7** or **11**.

(29) (a) Sieck, L. W.; Gordon, R.; Ausloos, P. *J. Am. Chem. Soc.* **1972**, *94*, 7157−7159. (b) Qin, X. Z.; Williams, F. *Tetrahedron* **1986**, *42*, 6301−6314. (c) Lunell, S.; Yin, I.; Huang, M. B. *Chem. Phys.* **1989**, *139*, 293−299.

(30) (a) Du, P.; Hrovat, D. A.; Borden, W. T. *J. Am. Chem. Soc.* **1988**, *110*, 3405−3412. (b) Krogh-Jespersen, K.; Roth, H. D. *J. Am. Chem. Soc.* **1992**, *114*, 8388−8394. (c) Skancke, A. *J. Phys. Chem.* **1995**, *99*, 13886−13889.

(31) Lee, A. Y.; Stewart, J. D.; Clardy, J.; Ganem, B. *Chem. Biol.* **1995**, *2*, 195−203.

(32) In this paper, all relative energies involving **9** correspond to the separated species. We note that in the course of our investigations we found hydrogen-bonded complexes between the 2-acrylic acid radical and acrylic acid that were more stable than the separated species but these are not discussed further in the present work.

(33) The stereochemistry indicated for **10** corresponds to the lowest energy B3-LYP/6-31G(d) conformation (see Figure 3). This stereochemistry has not yet been established experimentally. However, if a cycylopropylcarbinyl radical such as **10** is an intermediate, it must have *R* stereochemistry at the carbon atom corresponding to the chiral center in (*R*)-3-methylitaconate.

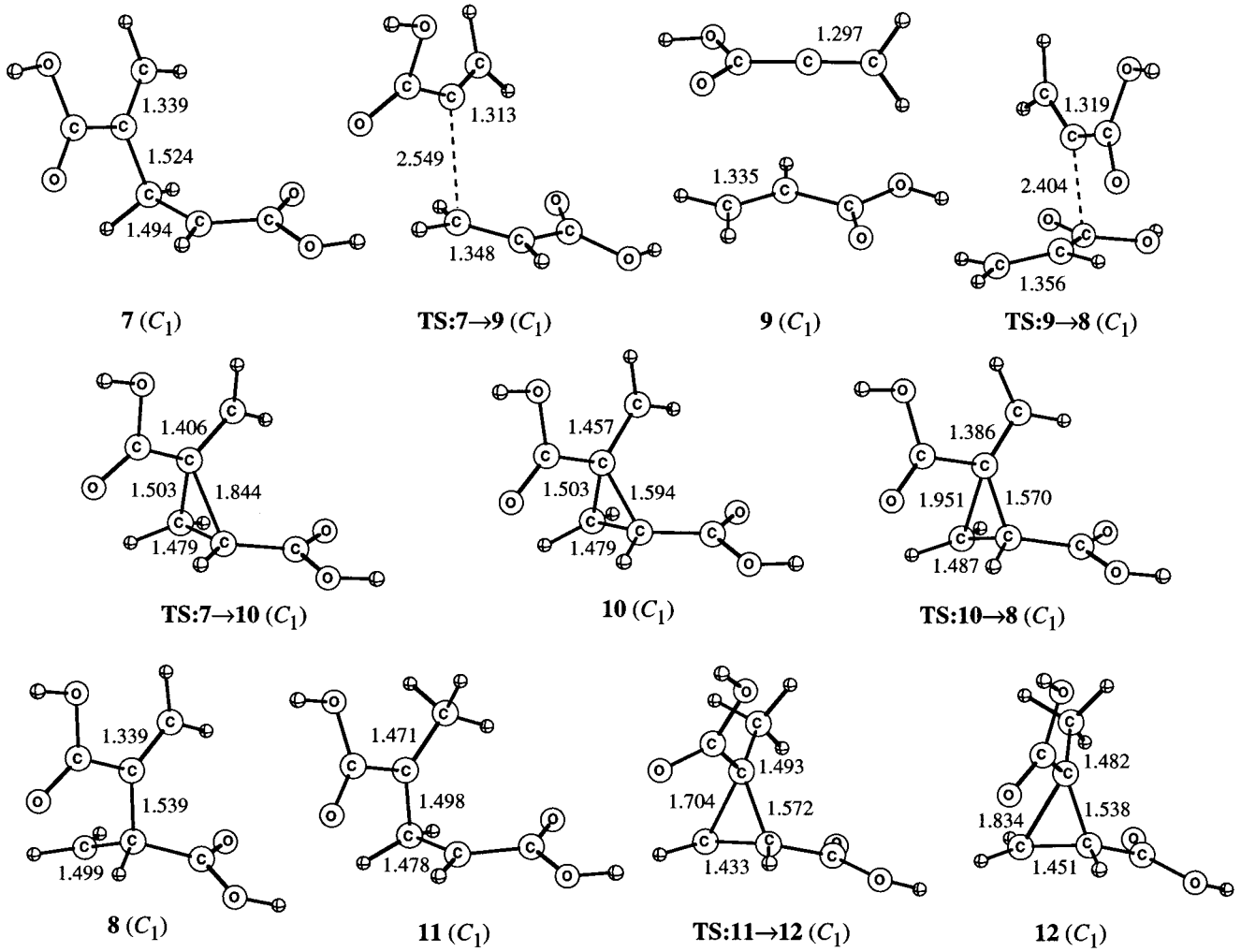
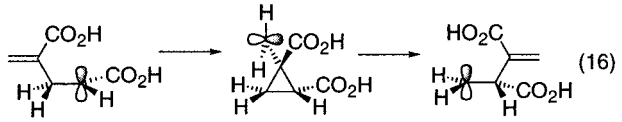
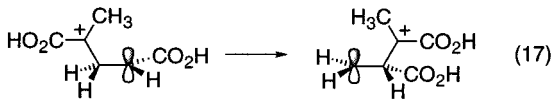


Figure 3. B3-LYP/6-31G(d) structures and selected bond lengths (Å) for the species involved in the rearrangement of the 2-methyleneglutaric-acid-derived radical (**7**) to the (*R*)-3-methylitaconic-acid-related radical (**8**).[40]

Reaction 16[33] involves cyclization of **7** to form the 1-methyl-enecyclopropane-1,2-dicarboxylic acid radical (**10**) followed by ring opening to give the product-related radical (**8**)



Reaction 17 (**11** → **12**) is the carboxylic-acid-substituted analogue of the degenerate rearrangement of the methylcyclopropane radical cation (reaction 13)



The results of the investigation of these three possibilities are presented in Table 2 (relative energies), Figure 3 (B3-LYP/6-31G(d) structures and geometrical parameters), and Figure 4 (schematic energy profile).

Although the degeneracy between reactant and product is lost in reaction 14, which is endothermic by 51 kJ mol^{-1}, the general

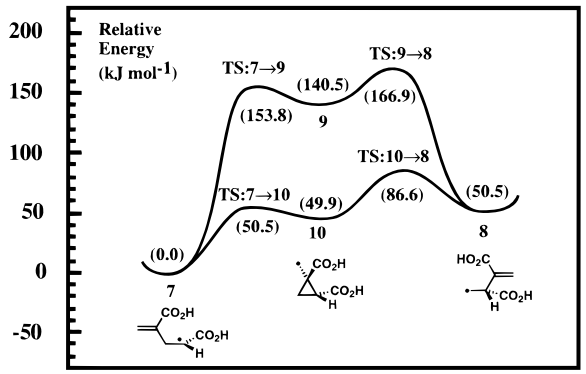


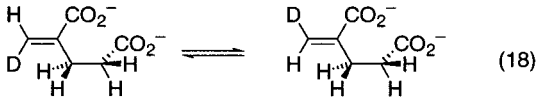
Figure 4. Schematic B3-LYP/6-311+G(3df,2p) energy profile for the rearrangement of the 2-methyleneglutaric acid derived radical (**7**) to the (*R*)-3-methylitaconic-acid-related radical (**8**). Relative energies (kJ mol^{-1}) shown in parentheses.

trends in Table 2 are not dissimilar to those shown by the parent system (Table 1). The initial barrier to fragmentation for reaction 15 (corresponding to **TS:7→9**) is predicted to be 154 kJ mol^{-1}, approximately 10 kJ mol^{-1} higher than that for the parent system. The transition structure corresponding to recombination of the two fragments (**TS:9→8**) is about a further 10 kJ mol^{-1} higher in energy. The barrier for the cyclization of **7** (**TS:7→10**) is 55 kJ mol^{-1}, also approximately 10 kJ mol^{-1} higher than the analogous process in the parent system. The ring opening of **10** to form **8** is predicted to require 37 kJ mol^{-1}, only marginally

more energy than is required for the ring opening of the cyclopropylcarbinyl radical (Table 1 and ref 19e). Thus, as with the model system, the addition/elimination mechanism is predicted to be energetically more favorable (by roughly 100 kJ mol^{-1}) than the fragmentation/recombination mechanism. We also find good general agreement between the two systems (reactions 6 and 14) with respect to the predicted bond lengths within the rearranging carbon skeleton (Figures 1 and 3).

Protonation of the migrating group (**11**) is found to facilitate the concerted rearrangement in much the same way as was observed for reaction 13. Table 2 shows **12** to be marginally higher in energy than **TS:11→12** but this is not the case in the absence of zero-point energy. The lack of a reverse barrier in this rearrangement is indicative of the lateness of the transition structure as well as the flatness of the potential energy surface in the region of the product. A striking feature of the results is the significant reduction in the energy difference between reactant and product accompanying protonation. B3-LYP/6-311+G(3df,2p) predicts the reaction to be endothermic by only 11 kJ mol^{-1}.

(3) Equilibration of Deuterium-Labeled Isomers of 2-Methyleneglutarate. In previous work aimed at providing support for the addition/elimination mechanism, a sample of deuterated 2-methyleneglutarate (**13** in reaction 18) was incubated with 2-methyleneglutarate mutase in the presence of coenzyme B_{12}.[34] It was found that an equilibrium between **13** and **14** was established



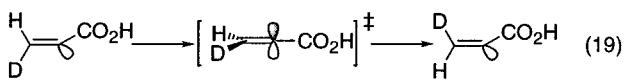
(18)

13 **14**

Given that rotation about the C=C double bond in either **13** or **14** is likely to involve a significant barrier, there must be some other species along the reaction pathway within which facile methylene rotation is possible.

In the context of the addition/elimination mechanism, the equilibration of reaction 18 can be rationalized if the •CH_2 group in the intermediate cyclopropylcarbinyl-type radical (**10**) can rotate easily. The calculated barrier for this rotation is indeed quite small at 15.2 kJ mol^{-1} (B3-LYP/6-311+G(3df,2p)), only slightly larger than the analogous barrier in the parent cyclopropylcarbinyl radical (14.5 kJ mol^{-1}).[19e]

The fragmentation/recombination mechanism can also provide an explanation of the above experimental observations. In the proposed intermediate species (**9**), the relevant CH_2 group is part of the 2-acrylic acid radical and the process that allows the two appropriate hydrogens to interconvert is described by reaction 19



(19)

It appears that the B3-LYP technique does not adequately describe this motion in vinyl-type radicals.[35] MP2, on the other hand, does seem to provide a satisfactory description of the above inversion/rotation process. Using MP2(full)/6-31G(d) geometries and zero-point energies, MP2/6-311+G(3df,2p) predicts that only 5.2 kJ mol^{-1} is required to interconvert the two methylene hydrogens. In the parent system, the relevant barrier is that of the inversion of the vinyl radical. This process is predicted by MP2(full)/6-311+G(3df,2p) to require 16.3 kJ mol^{-1}.[36]

Finally, the proton-assisted mechanism also leads to a low barrier for interconversion of the methylene hydrogens. If the methylene group of 2-methyleneglutarate were to become fully protonated, as in **11**, rotation about the C−C bond connecting the resulting methyl group to the carbocation center would be very easy (7.7 kJ mol^{-1} at B3-LYP/6-311+G(3df,2p)). Similarly, the methyl rotations in the protonated (*R*)-3-methylitaconate-radical (**12**) and the methylcyclopropane radical cation (**6**) are low-energy processes (7.5 and 8.0 kJ mol^{-1}, respectively). Thus protonation/deprotonation would also lead to facile equilibration of the methylene hydrogens. In the enzymatic system, such a mechanism might also result in isotopic exchange between substrate methylene and solvent water. This exchange has not been observed but cannot be entirely discounted.[14a] Alternatively, it is possible that the protein may allow for partial protonation of the substrate by utilizing an acidic amino acid residue of appropriate acidity. This pathway could enable the equilibration between isomers **13** and **14** to take place without incorporation of isotopic label from the group effecting partial protonation.[37]

The barriers to methylene rotation in the cyclopropylcarbinyl-type radical **10**,[38] methylene rotation in the 2-acrylic acid radical part of **9**, and methyl rotation in **11** and **12** are all small. These results indicate that it is not possible to discriminate between the different mechanisms for the rearrangement of **7** to **8** simply on the basis of the experimental observation of rapid equilibration of **13** and **14** in the presence of enzyme.

Concluding Remarks

The calculations in the present study show that the energetics of the full 2-methyleneglutarate rearrangement (reaction 14) do not differ significantly from those predicted for the parent but-3-enyl system (reaction 6). Although there are differences in detail between the two, it seems that the smaller model system gives a reasonably adequate description of the fundamental electronic structure changes required to effect the gas-phase rearrangements. Therefore, if ab initio gas-phase calculations can provide useful information relating to the biological system,

(34) Edwards, C. H.; Golding, B. T.; Kroll, F.; Beatrix, B.; Broker, G.; Buckel, W. *J. Am. Chem. Soc.* **1996**, *118*, 4192−4193.

(35) B3-LYP/6-31G(d) predicts the 2-acrylic acid radical to have a near-linear CCC arrangement, with the methylene group lying in a plane perpendicular to the carboxylic acid group (see Figure 3). UHF, UMP2, and UQCISD, on the other hand, all predict that such a geometry is a first-order saddle point on the surface and that the minimum has all of the atoms coplanar with a bent CCC angle (as shown in reaction 19). This latter finding is consistent with previous work on the closely related cyanovinyl radical[18] where the B3-LYP/6-31G(d) structure has C_{2v} symmetry with a linear CCC arrangement, in contrast to the results obtained with UHF, UMP2, and UQCISD, and with results at the highest level examined (CCSD(T)/cc-pVTZ). These all predict that the coplanar, bent C_s structure is the global minimum. Using MP2(full)/6-31G(d) energies, geometries, and zero-point energies gives an energy difference of 11.6 kJ mol^{-1} in favor of the bent-coplanar structure for the 2-acrylic acid radical.

(36) With MP2(full)/6-31G(d) energies, geometries, and zero-point energies, the inversion barrier in the vinyl radical is predicted to be 20.6 kJ mol^{-1}. B3-LYP/6-311+G(3df,2p), in combination with B3-LYP/6-31G(d) structures and zero-point energies, gives a barrier for the same process of 12.1 kJ mol^{-1}.

(37) For a discussion of the partial-proton-transfer concept in the context of enzyme-catalyzed reactions, see: Smith, D. M.; Golding, B. T.; Radom, L. *J. Am. Chem. Soc.* **1999**, *121*, in press.

(38) The MP2(full)/6-31G(d) barrier to methylene rotation in **10** is 10.7 kJ mol^{-1}. This value includes a zero-point energy contribution calculated using B3-LYP/6-31G(d) (because the MP2/6-31G(d) force constants are currently computationally too demanding). For the parent cyclopropylcarbinyl radical, the MP2 barriers to methylene rotation are 8.4 kJ mol^{-1} (6-31G(d)) and 12.3 kJ mol^{-1} (6-311+G(3df,2p)). Both values were obtained with the inclusion of MP2(full)/6-31G(d) zero-point vibrational energies.

the results presented here seem to justify the simplification of the enzyme-based rearrangement by the removal of the CO_2H groups. This is particularly true for studies aimed at discriminating between the three proposed mechanisms.

The rearrangement mechanism involving complete detachment of the migrating group from the rest of the molecule (i.e., fragmentation/recombination) is found to require significantly more energy than mechanisms in which the migrating group stays attached (i.e., addition/elimination and protonation/deprotonation). Given our finding that the intrinsic activation energy for the fragmentation/recombination mechanism is well in excess of 100 kJ mol^{-1}, the enzyme would need to reduce this barrier substantially for it to represent a viable route. It is not clear how this could be achieved.

The addition/elimination mechanism is found to offer a substantial energetic improvement to the fragmentation/recombination mechanism.[39] Accompanying this by protonation provides an even lower energy pathway. Although direct protonation of the substrate at carbon is unlikely because of the low basicity of the methylene group and the lack of isotopic exchange between the protons of this methylene group and those of water,[14a] it may be possible for the protein to effect a partial protonation of the substrate. The latter situation could be regarded as intermediate between addition/elimination (no protonation) and full protonation. Work is currently in progress to investigate this possibility more thoroughly.[37]

Acknowledgment. We gratefully acknowledge generous allocations of time on the Fujitsu VPP300 and SGI Power Challenge computers of the Australian National University Supercomputing Facility. We thank Professor Wolfgang Buckel for helpful discussions and Dr Alistair Rendell for making available code that facilitated efficient execution of the large B3-LYP energy calculations. We would also like to thank the British Council for the award to D.M.S. of a Partnerships for Excellence Postgraduate Bursary to study at the University of Newcastle upon Tyne and the Research School of Chemistry at the Australian National University for the provision of a Visiting Fellowship for B.T.G.

Supporting Information Available: Total CBS-RAD (Table S1) and B3-LYP/6-311+G(3df,2p) (Table S2) energies and GAUSSIAN 94 archive entries for the B3-LYP/6-311+G(3df, 2p) calculations for all of the relevant structures (Table S3) (10 pages, print/PDF). See any current masthead page for ordering information and Web access instructions.

JA9827245

(39) (a) Interestingly, the calculated barriers for hydrogen-atom transfer (Scheme 2, steps b and d) for model systems lie between those for fragmentation/recombination and addition/elimination. That is, using the ethyl radical as a model for the Ado−CH_2• (Scheme 2) and 1-butene as a model for the substrate, the barrier at 0 K for step b is calculated to be 64.3 kJ mol^{-1} (B3-LYP/6-311+G(3df,2p)). Using an analogous model gives a barrier for step d of 59.1 kJ mol^{-1}. Experimental determinations of primary isotope effects for the related glutamate mutase catalyzed reaction[39b] suggest that H-atom abstraction is substantially rate-limiting in the overall transformation. Our calculations are consistent with the same conclusions for 2-methyleneglutarate mutase *if* the addition/elimination mechanism is in operation. However, in the case of the fragmentation/recombination mechanism, its higher barrier should cause it to become the rate-limiting step. (b) Marsh, E. N. G. *Biochemistry* **1995**, *34*, 7542−7547.

(40) The 2-acrylic-acid-radical (a component of **9**) is shown in its lowest energy B3-LYP/6-31G(d) conformation. The conformational details of this species differ among the various theoretical procedures used. For a discussion, see text and note 35.