On the Mechanism of Action of Adenosylcobalamin

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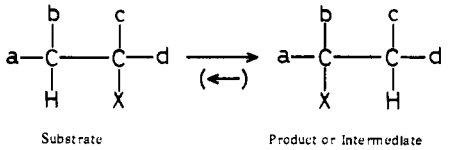
Received December 8, 1975

Abstract: Ab initio molecular orbital theory is used to demonstrate that intramolecular 1,2 shifts in radicals may be facilitated by protonation of the migrating group. On the basis of this observation, a mechanistic scheme is proposed for some reactions catalyzed by adenosylcobalamin. This scheme is consistent with all experimental data to date. Previously postulated mechanistic schemes are briefly reviewed.

Adenosylcobalamin (**1**), a derivative of vitamin B_{12}, acts as a coenzyme for several biological reactions.[2] With one exception (ribonucleotide reductase),[3] these are considered to be molecular rearrangements, the nature of which can be described by eq 1. A group X and a hydrogen atom interchange their positions.



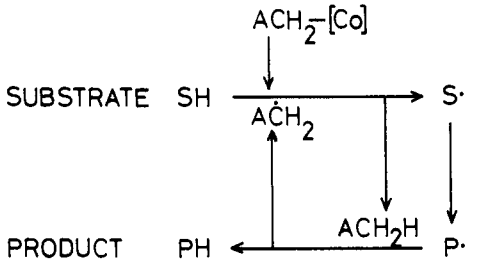
(1)

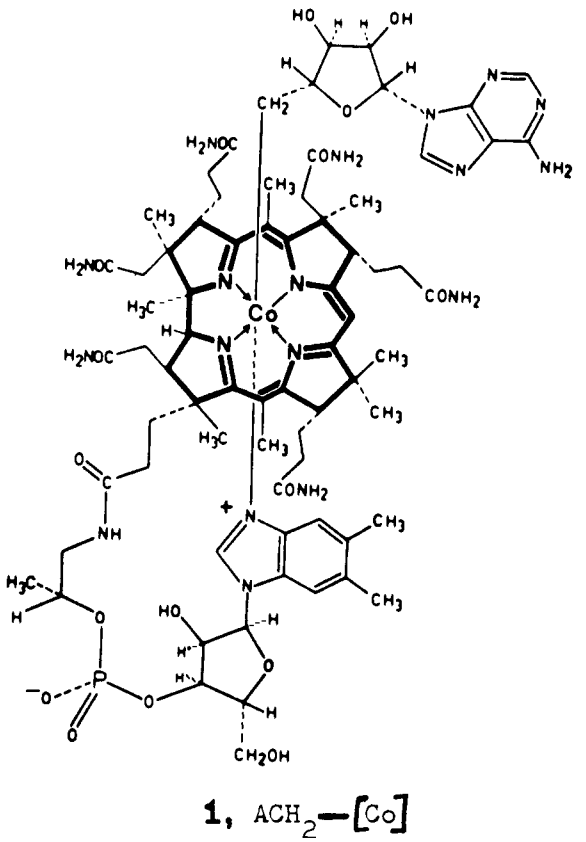
The principal known examples[4-12] of the substituents a, b, c, d, and X involved in these reactions are given in Table I. It has been shown that the 1,2 migration of H does not occur by a direct intramolecular 1,2 shift and so the reactions are unlike the well-known 1,2-hydride shifts which may occur in carbocations. Furthermore, there is no exchange with solvent H.[13] It is found that a specific hydrogen is transferred from the substrate to a reactive intermediate derived from adenosylcobalamin, whence it mixes with two C(5′)-methylene hydrogens in the intermediate 5′-deoxyadenosine. One of the resulting three hydrogens at C(5′) is then returned to become a specific hydrogen atom of the product. On the other hand, the migration of X does occur intramolecularly, either by a direct 1,2 shift or by some other mechanism in which X becomes, say, transiently bound to cobalt or to an acceptor on the enzyme.

The transformations summarized by eq 1 and Table I are of unusual interest since, unlike many enzymatic reactions, they cannot be looked upon as familiar organic reactions subject to special catalysis. It may well be that these systems are revealing new chemistry, which awaits definition in nonenzymatic situations. We and others have undertaken model studies designed to elucidate such chemistry. In this paper these studies are reviewed. Our main purpose, however, is to examine the possibility that the transformations of Table I proceed by rearrangements of intermediate organic radicals. This will be discussed in the light of available experimental evidence and the results of our ab initio molecular orbital calculations on model systems.[14] We hope thereby to provoke informative new experiments.

Possible Overall Pathways for the Reactions Catalyzed by Adenosylcobalamin. Following intensive studies of the conversion of (*R*)- and (*S*)-propane-1,2-diol to propanal catalyzed by adenosylcobalamin and the enzyme diol dehydrase (cf. Table I), Abeles[15] has proposed a mechanism which, because it may apply to all reactions of Table I, is given in a generalized form in Scheme I. While a universal mechanistic scheme for the reactions of Table I may be appealing, the diverse chemistry of alkylcobalt compounds[16,17] like adenosylcobalamin may presage a greater complexity. However, persuading evidence has been gathered at least for the operation of parts of Scheme I, in the case of reactions A-D of Table I. There is evidence (ultraviolet-visible[18] and electron spin resonance[19-21] spectroscopy) for the reversible formation of B_{12r} and organic radicals in these reactions and for the generation of 5′-deoxyadenosine.[22] The easy formation of B_{12r} and the adenosyl radical **2** is implicit from studies of the behavior of adenosylcobalamin under (nonenzymatic) anaerobic photolysis or thermolysis.[23] Although the adenosyl radical **2** traps itself under these conditions with the formation of the cyclonu-

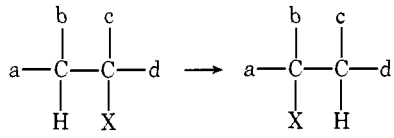
Scheme I. Mechanism (Based on Ref 15) for Reactions Catalyzed by Adenosylcobalamin





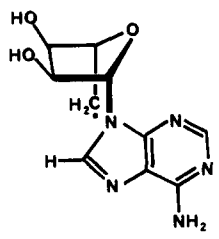
1, ACH_2—[Co]

Table I. Principal Enzymatic Reactions Catalyzed by Adenosylcobalamin



Rxn	Enzyme	a	b	c	d	X	Ref
A(i)	Diol dehydrase[a]	OH	H	H	H or CH_3	OH	4
A(ii)	Glycerol dehydrase[a]	OH	H	H	CH_2OH	OH	2d
B	Ethanolamine ammonia-lyase	OH	H	H	H or CH_3	NH_2	5
C	(*R*)-Methylmalonyl-CoA mutase	H or CH_3	H	CO_2H	H	COSCoA	6
D	(*S*)-Glutamate mutase	H	H	H	CO_2H	$CHNH_2$ CO_2H	7
E	α-Methyleneglutarate mutase	CO_2H	H	H	H	$C{=}CH_2$ CO_2H	8
F	Aminomutase utilizing either (i) (*S*)-3,6-Diaminohexanoate	$CH_2CHCH_2CO_2H$ NH_2	H	H	H	NH_2	9
	(ii) (*R*)-2,6-Diaminohexanoate	$(CH_2)_2CHCO_2H$ NH_2	H	H	H	NH_2	10
	(iii) (*R*)-2,5-Diaminopentanoate	CH_2CHCO_2H NH_2	H	H	H	NH_2	11

[a] In the reactions catalyzed by these enzymes 1,1-diols are intermediates which undergo enzyme-catalyzed dehydration to the observed product aldehyde (see ref 12).



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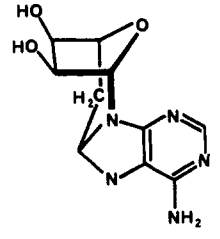
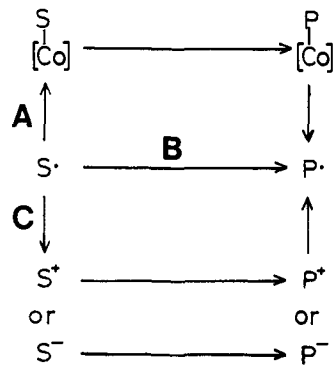


3, Double bond between N-9 and C-8
4, • at C-8, • at N-9

cleoside **3**,[24] this cyclization can only proceed via a specific conformation (as drawn for **2**). In the enzymatic reactions attainment of this conformation may be impeded or the formation of intermediate radical **4** might be reversible, leaving a sufficient concentration of radical **2** at equilibrium to act on a substrate. Alternatively, fission of the Co–C bond of adenosylcobalamin may be concerted with approach of a substrate C–H bond toward the C(5′)-methylene group, such that a free adenosyl radical never materializes.

Possible modes of conversion of the substrate-derived radical S· to the product-related radical P· (cf. Scheme I) are shown in Scheme II. Pathway A involves organocobalt intermediates

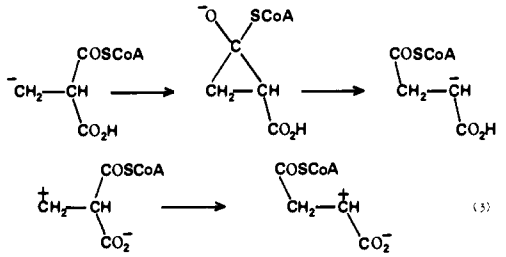
Scheme II. Possible Modes of Conversion for S· to P· (cf. Scheme I)



and has been much favored recently.[25] The possible operation of pathway B (direct rearrangement of S· to P·) has been given scant attention.[26] Pathway C was the basis of some early proposals (involving cationic intermediates with diol dehydrase;[4a] carbanion intermediates with methylmalonyl-CoA mutase[27]) and has recently been revived for the reaction catalyzed by ethanolamine ammonia-lyase.[28]

Studies of the chemistry of alkylcobaloximes[29] as models for alkylcobalamins led to the discovery of the solvolytic chemistry of β-acetoxyalkyl(pyridine)cobaloximes,[30] shown to involve π complexes.[30-33] Babior et al.[32] proposed this system as a chemical model for the conversion of S· to P· via organocobalt intermediates, applicable to the reactions catalyzed by diol dehydrase, ethanolamine ammonia-lyase, and the other aminomutases (cf. Table I). For methylmalonyl-CoA mutase, a mechanism involving organocobalt intermediates has been proposed and it was claimed to be supported by a chemical model.[34] However, the extreme conditions of this model and the very low yield obtained of rearranged product makes it barely convincing. In the case of glutamate mutase, a fragmentation–recombination via organocobalt intermediates has been put forward[35] but as yet has no foundation within the chemistry of alkylcobalamins.

The purpose of forming an organocobalt intermediate (pathway A, Scheme II) is to eventually impart ionic character to the system as in the mechanisms of ref 32, 34, and 35, permitting rearrangements to occur within te framework of conventional mechanistic experience. An ionic intermediate could alternatively arise from an initially formed radical by one-electron transfer leading to a Co^{I} intermediate (B_{12s}) + a substrate-derived carbocation or a Co^{III} intermediate + a substrate-derived carbanion, or electron transfer to the protein could occur. The conversion of propane-1,2-diols to propanal, and glutamate to 3-methylaspartate, could then be represented by pathways involving intermediate carbocations and acceptable intramolecular 1,2 shifts, while the conversion of methylmalonyl-CoA to succinyl-CoA could proceed[27] via intermediate carbanions (cf. eq 2). Another possibility (eq 3) is suggested by the stabilizing effect of the carboxylate anion in carbocations[36] and could explain why the COSCoA group migrates in preference to CO_2H since the former, like all neutral carbonyl functions, will destabilize an adjacent car-



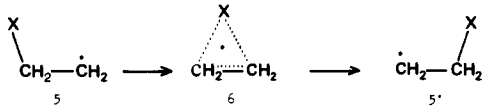
(3)

bocation center. The cation on the left-hand side of eq 3 could be derived from the corresponding radical, by a one-electron transfer in the manner indicated above.

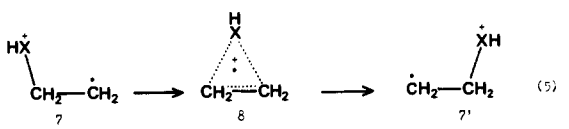
The possibility of a direct conversion of S· to P· (cf. Scheme II) via an intramolecular or intermolecular 1,2 shift has been given little regard due to the belief that such shifts are unlikely with organic radicals.[37] We examine this subject in detail below.

In concluding this section, we note other mechanistic proposals which fall outside the framework of Schemes I and II. Following the discovery of certain base-catalyzed reactions of 2-hydroxyalkyl- and 2-alkoxyalkylcobaloximes and cobalamins, Schrauzer[38] has proposed mechanisms for propane-1,2-diols → propanal involving B_{12s} as a reactive intermediate. Certain features of these mechanisms and their supporting experiments have been criticized.[39,40] A proposal for methylmalonyl-CoA mutase,[41] based on a supposed model reaction proceeding by undefined mechanism at 300 °C in 20% yield also suffers from the defect ascribed above to another model[34] for this enzymatic reaction. That is, the severe conditions required to operate the model, and then only in low yield, tend to discredit it.

An Approach Based on Molecular Orbital Calculations. We have applied ab initio molecular orbital theory to small systems which might serve as models for various possible mechanistic pathways for the reactions of Table I. Initially we considered the simple intramolecular radical shift shown as eq 4.



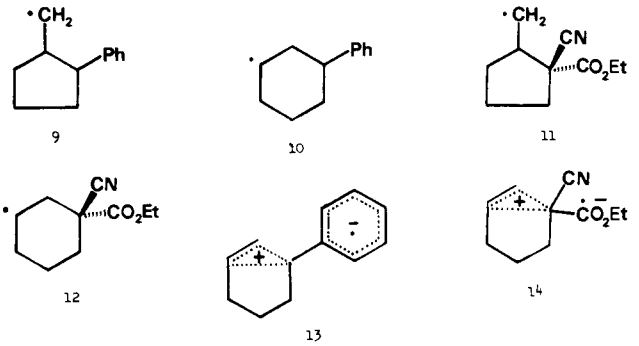
Calculations confirmed the expected result that the intramolecular shift requires high activation energy (see below for details). We then considered possible ways by which an enzyme catalyzing the transformations of Table I could facilitate a 1,2 shift. Our strategy in this regard was based on the well-established result that whereas 1,2-intramolecular shifts in carbocations occur with ease, those in radicals occur quite rarely.[37] Our aim, therefore, was to devise a means of increasing the cationic character of species such as **5.** In an attempt to accomplish this end, we have considered the effect of protonation of the migrating group leading to the intramolecular shift shown in eq 5.



(5)

We have studied reaction 5 with substituents X = OH, NH_2, and CH.

Earlier suggestions of facilitation of 1,2 shifts in radicals through increased carbocation character at the radical center have been advanced by Walling.[42] He has recently reported[43] an intramolecular 1,2 shift described as "the first clear-cut case of an intramolecular 1,2-alkyl migration in a monoradical rearrangement (**9** → **10**). He also drew attention to reactions discovered by Julia[44] (e.g., **11** → **12**) which may also have proceeded via an intramolecular 1,2 shift of a C group. It was



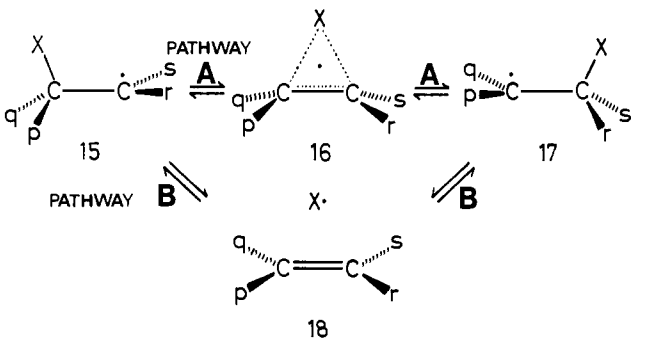
proposed that these shifts may be facilitated by charge separation in the transition state (e.g., **13** for **9** → **10**). This leads to increased cationic character at the radical center and 1,2 shift occurs (Wagner-Meerwein rearrangement). For the rearrangement of radical **11** to **12,** presumably prior electron transfer could occur (**11** → **14**), followed by 1,2 shift.

Computational Procedure. In order to investigate the energetics of reactions such as in eq 4 and 5, we have used standard linear combination of atomic orbitals, unrestricted, self-consistent field molecular orbital theory with the Gaussian 70 system of programs.[45] Calculations were carried out with two basis sets, the minimal STO-3G set[46] and the split-valence 4-31G set.[47] The 4-31G basis set is the more reliable but is considerably more expensive in terms of computation time. As a compromise, optimum geometries for the systems under consideration were obtained with the STO-3G set and corresponding relative energies with the 4-31G basis. Such a procedure for determining heats of reaction has already been used extensively.[48] Theoretical geometries derived with the STO-3G basis set have generally been found in reasonable agreement with experiment although results for cations (with standard exponents) and radicals (with the unrestricted SCF procedure) are somewhat worse than for neutral molecules.[49] Relative energies with the 4-31G basis set are least reliable in comparisons of bridged and open structures. In such cases, the relative stabilities of the bridged structures are underestimated. The error decreases as the ring strain decreases and is ~16 kcal mol^{-1} for the bridged vs. open ethyl cation and ~5 kcal mol^{-1} for the propene-cyclopropane pair.[50] Finally, we should note that our computations refer to isolated molecules in the gas phase at 0 K. The behavior in experimental systems in solution may be affected by interaction with solvent molecules.

Results and Discussion

1,2 Shifts in Radicals. Two possible pathways for 1,2 shifts **15** → **17** in radicals are shown in Scheme III. In the *intra*molecular pathway A, X never detaches itself completely from the rest of the molecule and passes through the bridged transition state or intermediate **16.** In the *inter*molecular pathway

Scheme III. Possible Pathways (A and B) for 1,2 Shifts in Radicals



15a-18a, X = OH; p = q = r = s = H

15b-18b, s = X = OH; p = Me; q = r = H

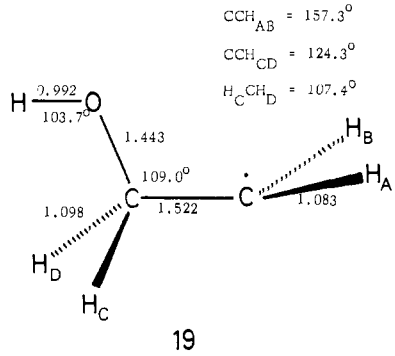


Figure 1. Calculated geometrical parameters for the hydroxyethyl radical. The notation H_{AB} denotes a point on the bisector of the angle H_ACH_B.

B, the bond between X and C-1 is broken, giving the radical Ẋ and alkene **18**. Ẋ then attacks C-2 to produce the isomeric radical **17**.

Examples of pathway A are well documented[37,51,52] for cases in which X contains a π system (e.g., X = phenyl) or low-lying vacant d levels (e.g., X = chlorine). However, if X is a hydrogen atom or the hydride of any first row element, the bridged structures involve one-electron occupancy of an antibonding molecular orbital and, with the possible exception of Walling's work,[43] the bona fide observation of intramolecular 1,2 shifts in such cases has yet to be reported.[37]

We have examined the situation for the 2-hydroxyethyl radical **15a** where pathway A might serve as a model for a possible mechanism of action of diol dehydrase. In this mechanism, the radical **15b** derived from propane-1,2-diol according to Scheme I rearranges via **16b** by pathway A of Scheme III to radical **17b**. This radical abstracts a hydrogen from deoxyadenosine giving 1,1-dihydroxypropane, which finally loses one of its enantiotopic hydroxyl groups (in an enzymatically catalyzed step) to produce propanal.

The calculated structure of the 2-hydroxyethyl radical **19** [E (STO-3G) = −151.48821 hartrees, E (4-31G) = −153.21723 hartrees)] is shown in Figure 1. We could find no structure for the hypothetical bridged radical **16a** lower in energy than that of the separated species ethylene and hydroxyl radical. Hence, if the rearrangement of radical **15a** to **17a** does occur, it should proceed by a dissociation-recombination mechanism corresponding to pathway B of Scheme III. The energy difference between the hydroxyethyl radical **15a** and separated hydroxyl radical and ethylene is 6 kcal mol^{-1} (4-31G). Note that this mechanism, if applied to the reaction catalyzed by diol dehydrase (A, Table I), temporarily generates from the 1,2-dihydroxyprop-1-yl radical an enol of propanal and a hydroxyl radical which subsequently recombine to give the 1,1-dihydroxyprop-2-yl radical.

1,2 Shifts in Protonated Radicals. We next carried out calculations on radicals **7** and **8** derived by protonation of X in **5** and **6**. This choice was provoked (but not necessitated; see below) by the following experimental evidence. Using ^{18}O-labeled substrates with diol dehydrase, Retey et al.[4a] demonstrated that ^{18}O at C-2 of substrate is transferred to C-1 of an intermediate (possibly propane-1,1-diol) and is then either largely lost [propanal from (*R*)-propane-1,2-diol] or largely retained [propanal from (*S*)-propane-1,2-diol]. For this result to be compatible with a dissociation-recombination mechanism, it is necessary for water, if that is what dissociates, not to mix with solvent water. (If it is a hydroxyl radical which dissociates, it must recombine with the enol of propanal without damaging the protein at or away from the active site.) While neither of these requirements is impossible (e.g., the active site of this enzyme may well be shielded from water[53]), the postulate of a direct 1,2 shift provides an alluringly simple explanation for the results of Arigoni and Retey. We therefore

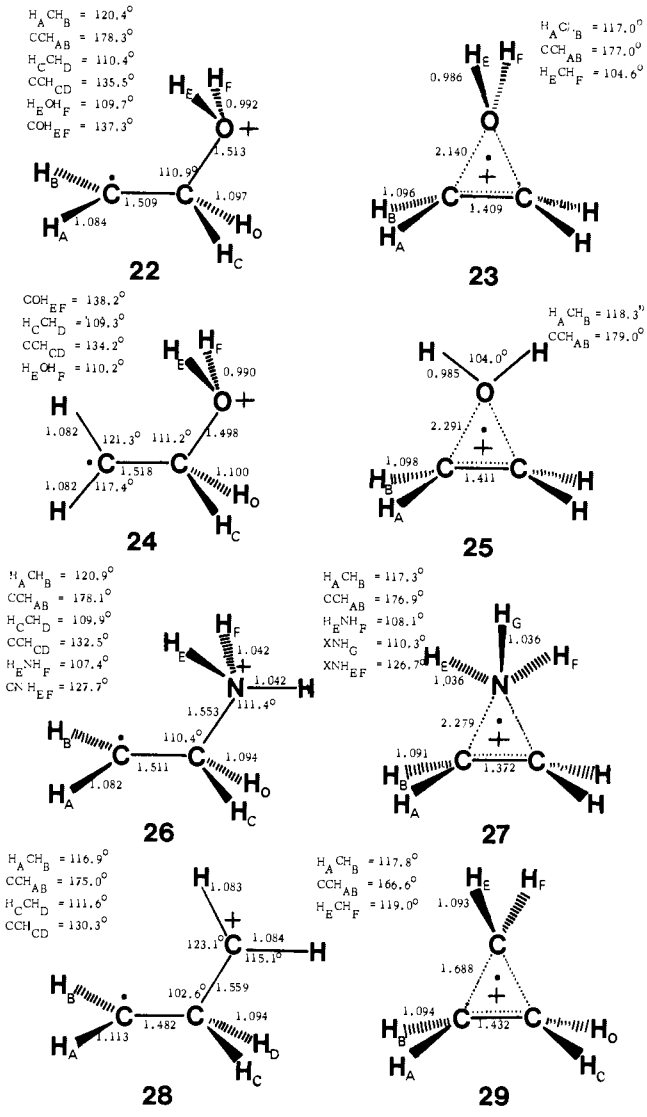
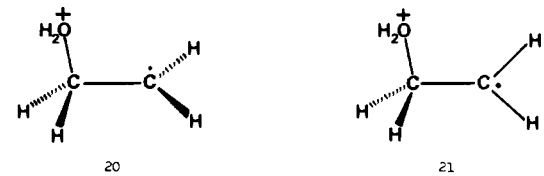


Figure 2. STO-3G optimized structures for protonated radicals. In **22**, **26**, and **28** H atoms at the radical center are bent slightly upwards.

performed calculations on protonated radicals **7** and **8** (X = OH, NH_2 and CH) for which we believed that such a direct shift is more likely to occur.

Calculations were initially carried out with assumed standard bond lengths and bond angles on perpendicular (**20**) and bisected (**21**) conformations of the protonated hydroxyethyl



radical. These calculations showed a preference for the perpendicular conformation by an amount (~2-3 kcal mol^{-1}) significantly greater than in the hydroxyethyl radical or other simple radicals where there is no strong conformational preference.[54,55] In addition, there was a slight preference for the bonds at oxygen to be tetrahedral and symmetrically staggered rather than trigonal.

Calculations with optimization of all bond lengths and bond angles were then carried out on appropriate conformations of open and bridged structures of $HX^+CH_2CH_2$ with X = OH, NH_2, and CH. The resulting optimum geometries (**22**–**29**) are shown in Figure 2 and calculated total energies in Table II. The calculated geometries of the open ions show few unexpected

Table II. Calculated Total Energies for Protonated Radicals

Substituent (XH)	Structure	Calcd total energy, hartrees[a] STO-3G	4-31G
OH_2 (open)	22	−151.87781	−153.54003
OH_2 (bridged)	23	−151.79942	−153.52676
OH_2 (open)	24	−151.87422	−153.53540
OH_2 (bridged)	25	−151.79093	−153.52730
NH_3 (open)	26	−132.40422	−133.79148
NH_3 (bridged)	27	−132.30378	−133.73839
CH_2 (open)	28	−115.34724	−116.54198
CH_2 (bridged)	29	−115.37460	−116.56038

[a] 1 hartree = 627.5 kcal mol^{-1}

features and require little comment. The bridged ions **23, 25,** and **27** have structures which lie between those expected for weak complexes of $HX\cdot^+$ and CH_2CH_2 and of HX and $CH_2CH_2\cdot^+$. The published[49] STO-3G geometries for the component species HX, $HX\cdot^+$, CH_2CH_2, and $CH_2CH_2\cdot^+$ are reproduced in Figure 3 to demonstrate this feature more clearly.

The geometrical consequences of such complex formation may be readily predicted with the aid of perturbation theory. For example, the complex $H_2O \rightarrow C_2H_4\cdot^+$ corresponds to electron donation from a lone pair orbital on water into the partly filled π-bonding orbital on $C_2H_4\cdot^+$. The bonding character in the C-C bond of the $C_2H_4\cdot^+$ fragment of the complex is thus increased and the C-C bond length decreased from its value in $C_2H_4\cdot^+$ itself. The alternative picture of the complex, $C_2H_4 \rightarrow H_2O\cdot^+$, involves electron donation from the filled π-bonding orbital of C_2H_4 into a partly filled orbital on H_2O^+. This would lead to decreased π bonding and a longer C-C bond compared with that in C_2H_4. Our calculated structures (**23, 25**) for $[H_2O\cdots C_2H_4]\cdot^+$ show C-C bond lengths only slightly reduced from the $C_2H_4\cdot^+$ value, suggesting that these systems are well described as $H_2O \rightarrow C_2H_4\cdot^+$ complexes. For the bridged structure $[NH_3\cdots C_2H_4]\cdot^+$, the calculated C-C length is further reduced, indicating an increased contribution from the $C_2H_4 \rightarrow NH_3\cdot^+$ description. The binding energy for $[CH_2\cdots C_2H_4]\cdot^+$ is too large for the structure to be usefully discussed using perturbation arguments.

The structural predictions are consistent with energetic results for the component pairs HX and $CH_2CH_2\cdot^+$ or $HX\cdot^+$ and CH_2CH_2 as summarized in Table III. Both theory[49] and experiment indicate that the combination $H_2O + C_2H_4\cdot^+$ is substantially lower in energy than $H_2O\cdot^+$ and C_2H_4. Accordingly, the bridged structures $[H_2OCH_2CH_2]\cdot^+$ resemble complexes between H_2O and $C_2H_4\cdot^+$. For X = NH_2, the more stable pair is $NH_3\cdot^+$ and C_2H_4, and it is therefore not surprising that in this case the bridged structure looks more like a weak complex between these species.

Binding energies for the most stable forms of the open and bridged structures relative to the more stable of the possible pairs of separated species are given in Table IV. These range from 12 kcal mol^{-1} in bridged $NH_3{}^+CH_2\dot{C}H_2$ to 81 kcal mol^{-1} in bridged $\dot{C}H_2CH_2CH_2{}^+$. If we assume the bridged structures represent transition states for intramolecular 1,2 shifts, the activation energies fo these shifts are 8 kcal mol^{-1} for $H_2O^+CH_2\dot{C}H_2$ and 33 kcal mol^{-1} for $^+NH_3CH_2\dot{C}H_2$. For $\dot{C}H_2CH_2CH_2{}^+$, the bridged structure (which is the radical cation of cyclopropane) is 12 kcal mol^{-1} more stable than the open form.

We noted above that the hypothetical bridged structure of the hydroxyethyl radical is unbound, i.e., there is no structure with energy lower than that of separated hydroxyl radical + ethylene. Protonation leads to a relative stabilization of the bridged structure to the extent that is bound by 17.4 kcal mol^{-1} relative to H_2O and $C_2H_4\cdot^+$. An approximate calculation

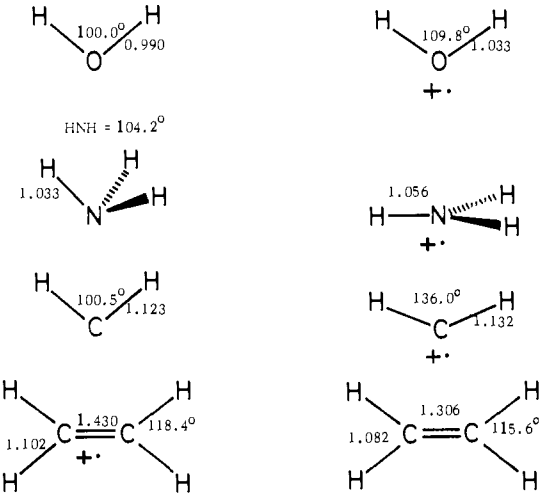


Figure 3. STO-3G optimized structures for component species (from ref 49).

Table III. Theoretical (4-31G) and Experimental Estimates (kcal mol^{-1}) of $E(HX\cdot^+ + CH_2CH_2) - E(HX + CH_2CH_2\cdot^+)$

HX	Theor	Exptl
H_2O	+43	+50
NH_3	--15	−7
CH_2[a]	−16	−10

[a] Calculations based on singlet methylene.

Table IV. Binding Energies (4-31G, kcal mol^{-1}) of $H\dot{X}CH_2\dot{C}H_2$ Relative to More Stable $(HX\cdot^+ + CH_2CH_2)$ or $(HX + CH_2CH_2\cdot^+)$ Pairs

HX	Open	Bridged
H_2O	−25.7	−17.4
NH_3	−45.7	−12.4
CH_2[a]	−69.9	−81.4

[a] Binding energies relative to $CH_2\cdot^+ + CH_2CH_2$.

shows that charge-dipole interaction is likely to contribute significantly to this binding energy.

Because reaction A (Table I) catalyzed by adenosylcobalamin involves OH and CH_3 substituents, we have also carried out calculations on $H_2O^+CH_2\dot{C}H_2$ substituted by these groups. Calculations were carried out for α- and β-substituted open forms (taking the radical center as the reference carbon) and for the substituted bridged structures. The optimized geometries of the $H_2O^+CH_2\dot{C}H_2$ skeleton were assumed and the bond lengths to the substituent and orientation of the substituent separately optimized in each case. The substituents were themselves taken to have standard geometries. Geometries and total energies so obtained are listed in Table V together with data derived for smaller species in order to calculate binding energies. Corresponding binding energies are listed in Table VI.

Both OH and CH_3 substituents lead to a lowering in calculated binding energies, i.e., they have a greater stabilizing effect in $CH_2CH_2\cdot^+$ than in $H_2O^+CH_2\dot{C}H_2$. In addition, the bridged forms are stabilized relative to the open structures to the extent that within the accuracy of the theoretical method used, it is difficult to predict whether the bridged or open structures have lower energies. Our conclusion is then that the 1,2-intramolecular shifts in these species should occur quite readily.

Relevance to the Mechanism of Action of Adenosylcobalamin. Our calculations suggest that whereas 1,2 shifts in simple radicals are likely to occur via a dissociation-recombination

Table V. Optimum Geometric Parameters and Calculated Total Energies for Substituted $H_2\overset{+}{O}CH_2\dot{C}H_2$ Radical Ions and for Substituted and $CH_2CH_2CH_2\cdot^+$

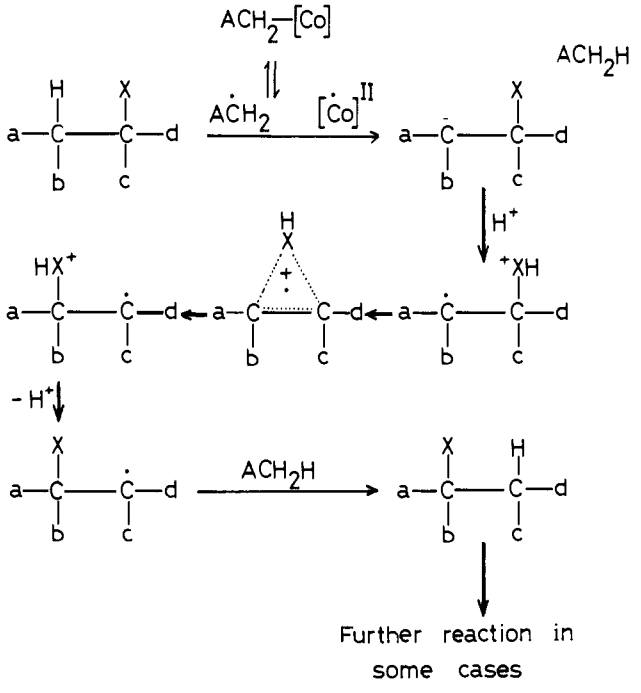
Species	Substituent	Geometric parameters	Total energy, hartrees STO-3G	4-31G
α-Substituted open $H_2\overset{+}{O}CH_2\dot{C}H_2$	CH_3	C–C = 1.523, HCCC = 76.0°	−190.46670	−192.52539
	OH	C–O = 1.369, HOCC = 171.5°	−225.72216	−228.28547
β-Substituted open $H_2\overset{+}{O}CH_2\dot{C}H_2$	CH_3	C–C = 1.548, HCCC = 64.9°	−190.46876	−192.52557
	OH	C–O = 1.394, HOCC = 154.5°	−225.71894	−228.28501
Substituted bridged $H_2\overset{+}{O}CH_2\dot{C}H_2$	CH_3	C–C = 1.522, HCCC = 14.7°	−190.39971	−192.52203
	OH	C–O = 1.318, HOCC = 180.3°	−225.67439	−228.29048
Substituted CH_2CH_2	CH_3	C–C = 1.527, HCCC = 0°	−115.65956	−116.90353
	OH	C–O = 1.389, HOCC = 0°	−150.91396	−152.66653
Substituted $CH_2CH_2\cdot^+$	CH_3	C–C = 1.513, HCCC = 0°	−115.40619	−116.59962
	OH	C–O = 1.309, HOCC = 180°	−150.69552	−152.37220

Table VI. Binding Energies (4-31G, kcal mol^{-1}) of Substituted $H_2\overset{+}{O}CH_2\dot{C}H_2$ Radical Ions Relative to H_2O Substituted $CH_2CH_2\cdot^+$

Substituent	α-Substituted open $H_2\overset{+}{O}CH_2\dot{C}H_2$	β-Substituted open $H_2\overset{+}{O}CH_2\dot{C}H_2$	Substituted bridged $H_2\overset{+}{O}CH_2\dot{C}H_2$
H	−25.7	−25.7	−17.4
CH_3	−14.1	−14.3	−12.0
OH	−6.3	−6.0	−9.4

mechanism, an intramolecular rearrangement is favored when the radicals are protonated. On the basis of our calculations, we suggest the general mechanism displayed as Scheme IV for

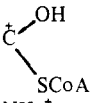
Scheme IV. General Mechanism for Reactions (A–C and F, cf. Table I) Catalyzed by Adenosylcobalamin



the reactions A–C and F listed in Table I. The protonated bridging groups XH^+ are summarized in Table VII. Our mechanisms require that the enzymes participating in the reactions of Table I have an acidic functional group capable of effectively protonating an O or N of the migrating group in the substrate-derived radical (of course, the amino substrates are likely to be appreciably protonated at nitrogen anyway). This enables the rearrangement to take place in an intramolecular fashion.

There are certain nonenzymatic reactions of radicals which bear a suggestive resemblance to the reactions catalyzed by diol dehydrase and ethanolamine ammonia-lyase for which our

Table VII. Protonated Bridging Groups (XH) According to the Mechanism of Scheme IV for Reactions of Table I

Rxn	Enzyme	XH
A	Diol dehydrase and glycerol dehydrase	H_2O^+
B	Ethanolamine ammonia-lyase	NH_3^+
C	(*R*)-Methylmalonyl-CoA mutase	
F	Aminomutase	NH_3^+

mechanism may be equally applicable. When vicinal diols[56] and amino alcohols[57] in aqueous solution are allowed to react with HO· radicals, then the corresponding radicals (e.g., $HOCH_2\dot{C}HOH$ from ethane-1,2-diol, $H_3N^+CH_2\dot{C}HOH$ from ethanolamine) are formed as shown by their ESR spectra. These radicals are readily converted to α-carbonyl radicals (e.g., $\dot{C}H_2CHO$ from $HOCH_2\dot{C}HOH$ and $H_3N^+CH_2\dot{C}HOH$). Depending on the pH of the system, two mechanisms have been proposed[56c,56e] for the derivation of $\dot{C}H_2CHO$ from $HOCH_2\dot{C}HOH$. At low pH[56d]

$$HOCH_2\dot{C}HOH \overset{H^+}{\rightleftharpoons} H_2\overset{+}{O}CH_2\dot{C}HOH \longrightarrow H_2O + \dot{C}H_2CHO + H^+ \quad (6)$$

At neutral and higher pH

$$HOCH_2\dot{C}HOH \rightleftharpoons H^+ + HOCH_2\dot{C}HO^- \longrightarrow HO^- + \dot{C}H_2CHO \quad (7)$$

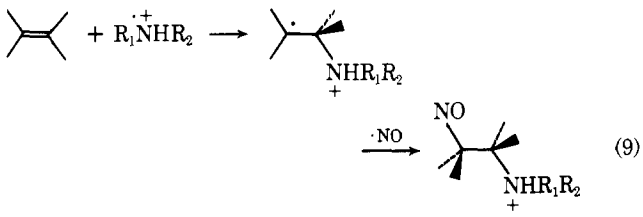
The hydroxy group of α-hydroxyalkyl radicals is appreciably more acidic (ca. 5 pH units lower)[58] than the hydroxy groups in alcohols and the reactions of eq 7 proceed with extreme ease even at physiological pH.[56e] with With ethanolamine, the following process is supposed to occur at neutral pH.

$$H_3\overset{+}{N}CH_2\dot{C}HOH \rightleftharpoons H_3\overset{+}{N}CH_2\dot{C}HO^- + H^+ \longrightarrow H_3N + \dot{C}H_2CHO \quad (8)$$

In the reaction of ethane-1,2-diol above, $CH_2CH(OH)_2$ was thought to have been formed from $HOCH_2\dot{C}HOH$ which is consistent with the mechanism of Scheme IV, but it has not been established whether it arises from the latter radical *before* $\dot{C}H_2CHO$ (see also ref 57a, footnote 4).

The mechanism in Scheme IV ($^+\dot{X}H = {}^+\dot{N}H_3$) for the reactions catalyzed by ethanolamine ammonia-lyase (Table I, B) and aminomutases (Table I, F) recall reactions between aminium radical cations and olefins,[59] e.g.

Although the possibility has been considered,[60] so far no evidence has been obtained for the intermediacy of a π complex like **27** in these reactions.



(9)

For the reaction catalyzed by methylmalonyl-CoA mutase (Table I, C), our mechanism [Scheme IV, $^{+}XH = {}^{+}C(OH)SCoA$] involves the formation of a cyclopropane radical cation. This intermediate can ring-open either to substrate-derived radical or to product-related radical.[61]

The reactions catalyzed by glutamate mutase (Table I, D) and α-methyleneglutamate mutase (Table I, E) cannot be convincingly explained by mechanisms which invoke proton-catalyzed radical rearrangements. The failure to incorporate solvent H into carbon-bound H during the reaction catalyzed by α-methyleneglutarate mutase[8] precludes a mechanism for this reaction (involving protonation of the methylene group) similar to that proposed for methylmalonyl-CoA mutase (see above). In this case, rearrangement via a cyclopropylcarbinyl radical is an attractive possibility and more so following the recent model studies of Dowd et al.[62] For glutamate mutase, protonation of the CH of the migrating group ($CHNH_2CO_2H$) is very unlikely, thus excluding a mechanism involving a derived bridged intermediate (cf. Scheme IV). A direct 1,2 shift of the group $CHNH_2CO_2H$ is also unlikely (cf. discussion above concerning intramolecular shift of HO· in $\cdot CH_2CH_2OH$). We are evaluating certain other possibilities and have initiated model studies in this area.

Concluding Remarks. In this paper, we have used ab initio molecular orbital theory to demonstrate that intramolecular 1,2 shifts in radicals may be facilitated by protonation of the migrating group. On the basis of this observation, we have suggested a mechanistic scheme for some of the reactions catalyzed by adenosylcobalamin, a scheme which is consistent with all experimental data to date.

Our mechanism does not require the intervention of σ-bonded organocobalt intermediates. However, we do not wish to explicitly exclude the possibility of such involvement. The very nature of enzyme-active sites means that intermediate organic radicals in these reactions can never be far from $Co^{II}(B_{12r})$.[63] It is therefore important in future research on reactions catalyzed by adeosylcobalamin to establish whether the Co atom of B_{12r} is a conductor or spectator.

Acknowledgment. B.T.G. thanks the Australian National University for a Visiting Fellowship in 1973, when part of the work described was completed.

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Tautomerism in Cytosine and 3-Methylcytosine. A Thermodynamic and Kinetic Study

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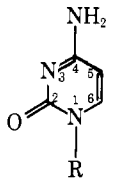
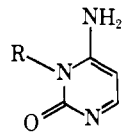
Received December 26, 1975

Abstract: Aqueous cytosine, which exists mainly as the 1(*H*)-aminooxo form (Ia), is shown by temperature-jump spectroscopy to tautomerize slightly to the 3(*H*)-aminooxo form (IIa); the equilibrium constant K = (IIa)/(Ia) is estimated to be (2.5 ± 0.5) × 10^{-3} at 25 °C and the tautomerization enthalpy is 3.1 ± 0.1 kcal M^{-1}. At 10 °C, The interconversion process is catalyzed by water [k_{H_2O} = (1.8 ± 0.3) × 10^3 s^{-1}], by H^+ [k_{H^+} = (1.6 ± 0.3) × 10^{10} M^{-1} s^{-1}], by OH^- [k_{OH^-} = (1.2 ± 0.05) × 10^{10} M^{-1} s^{-1}], and by the cytosinium cation [k_{cation} = (4.4 ± 0.2) × 10^8 M^{-1} s^{-1}]. On the other hand, 3-methylcytosine is shown by uv and ir spectroscopy to exist as a mixture of two tautomeric forms, the aminooxo form IIb and the iminooxo form IIIb; while IIIb is the major form in nonpolar solvents, the amino form IIb largely predominates in water, the equilibrium constant K' = (IIIb)/(IIb) being estimated as ca. 3 × 10^{-2} at 25 °C. The IIb ⇌ IIIb interconversion mechanism was investigated in D_2O solution at 10 °C by temperature-jump spectroscopy. The interconversion is catalyzed by D_2O [k_{D_2O} = (2.9 ± 0.3) × 10^4 s^{-1}], by OD^- [k_{OD^-} = (4.3 ± 0.2) × 10^9 M^{-1} s^{-1}], and by cationic 3-methylcytosine [k_{cation} = (2.2 ± 0.1) × 10^8 M^{-1} s^{-1}]. In both tautomeric equilibria the solvent-catalyzed term in the rate law is shown to be closely related to the basicity of the minor tautomer. The thermodynamics and kinetics of tautomerization of cytosine and cytidine to their rare imino forms are finally tentatively discussed in the light of this work.

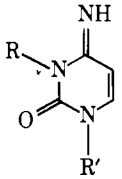
It is now firmly established, by a variety of physical methods,[1] that cytosine exists in solution essentially in the 1(*H*)-aminooxo tautomeric form, Ia.



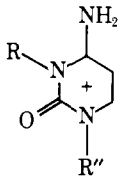
Ia, R = H
b, R = CH_3



IIa, R = H
b, R = CH_3



IIIa, R = R′ = H
b, R = CH_3; R′ = H
c, R = R′ = CH_3



IV

However, as many as six other structures can formally be written for the cytosine molecules, so that small amounts of rare tautomeric forms may exist along with Ia. Much theoretical work has been concerned with the relative stabilities of the various tautomers,[2] but so far, none of the rare forms has been unambiguously observed experimentally. Our present knowledge on the possible occurrence of some of them is based on indirect arguments, such as comparison of the pK's of various nontautomeric methylated derivatives.[3] It has been concluded this way that Ia should predominate over IIa by a factor of about 800 in aqueous solution, while the iminooxo form IIIa would be even less favored, the equilibrium constant K = (IIIa)/(Ia) being about 2 × 10^{-5}. These estimates relied heavily on the assumption that the cations resulting from protonation of cytosine and its methylated derivatives 3-methylcytosine and 1,3-dimethylcytosine all have the common structure IV; another, less severe restriction arises from the hypothesis that the substitution of the labile proton by a methyl group does not modify significantly the pK of the various tautomers.

Despite increasing evidence showing that cytosine and its methylated analogues do form cations of similar structure,[3a,4] there has been a trend in the literature to disregard these conclusions, possibly because they are based on rather indirect arguments. Thus, several claims for a much higher proportion of the iminooxo tautomer IIIa have been published[5-7] and criticized.[8,9] Therefore, there clearly exists a need for a direct characterization of the rare tautomeric forms of cytosine, especially in water. Unfortunately, no experimental technique will, at present, allow the detection of a tautomeric form present in a proportion as low as 2 × 10^{-5}. However, this difficulty could be overcome indirectly by studying separately the two equilibria