

Conformational Variability of Corrins: Some Methods of Analysis

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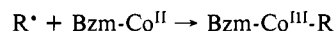
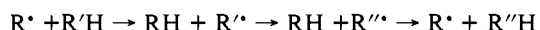
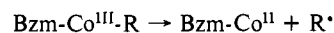
Abstract: The detailed three-dimensional structures of several corrins that have been studied by X-ray crystallographic techniques are analyzed to reveal the variations in structure. Methods used include matrix partitioning, factor analysis, and surface accessibility calculations. The results indicate a flexing of the corrin about the Co...C(10) line; when C(5) and C(15) move up, C(10) moves down and vice versa. Most of the flexing occurs on the C(5) side of the molecule. Details of the results of the various methods of analysis are presented. The amount of folding, which is related to the bulkiness of the upper and lower axial groups bound to the cobalt atom, affects the distances between axially oriented groups projecting from the β -positions of the five-membered rings. These atoms, C(26), C(37), C(46), and C(54), protect the cobalt atom. In adenosylcobalamin there is steric pressure on the adenosyl group from a group of atoms on the corrin, principally the hydrogen atom on C(19), but also C(46) and a hydrogen atom on C(54); this pressure may aid Co-C bond cleavage. When the Co-C bond in the adenosyl coenzyme is broken, the adenosyl group must move either to make room for substrate or to carry the adenosyl to a position where it can abstract a hydrogen atom from substrate. Possible pathways for such movement of the free radical within the enzyme-coenzyme-substrate complex are analyzed by computer graphics techniques, and the consequences of such movements are described. It seems reasonable that the adenine group may remain somewhat fixed and that rotation about its glycosidic bond may occur, swinging the free radical to a site where it can interact with substrate; this type of movement can move the free radical to a site nearly 8 Å from the cobalt atom.

Many of the B₁₂-dependent enzymes require the cofactor adenosylcobalamin for activity. Most of these enzymes catalyze the interchange of a hydrogen atom with a substituent on an adjacent carbon atom.¹⁻³ Adenosylcobalamin has been shown by Hodgkin and co-workers⁴ to be a derivative of vitamin B₁₂, with an adenosyl replacing the cyanide of the vitamin (Figure 1 and deposited Figure A). The most significant result of this X-ray analysis was the finding that adenosylcobalamin contains a Co-C bond and is therefore an organometallic compound, the first to be found occurring naturally. The other cofactor of B₁₂-dependent enzymes, methylcobalamin, also contains a Co-C bond. Vitamin B₁₂ and its two coenzymes, adenosylcobalamin and methylcobalamin, contain a corrin ring system; this is similar to a porphyrin ring system with one bridge atom missing and with saturated β -positions on the five-membered rings (see Figure 1). The corrin ring structure is much more saturated than that of a porphyrin, and it also has a slightly helical sense as a result of the C(1)-C(19) saturated direct bond between five-membered rings. The side chains attached to the β -positions of the rings are acetamido or propionamido groups. These side chains, which project axially from the rings, are bulky and sterically limit the flexibility in the system. They also, interestingly, serve to protect the sensitive Co-C bond from external attack. One axial substituent on the cobalt atom is cyanide, adenosyl, or methyl and the other is 5,6-dimethylbenzimidazole (Bzm).

The mode of action of the cofactor adenosylcobalamin is believed, at least in the case of the reaction of enzymes such as methylmalonyl CoA mutase, to involve homolytic cleavage of the Co-C bond⁵ to give a deoxyadenosyl free radical (R^{*}) and a lowered valence state for the cobalt atom (Scheme I). A hydrogen atom is then abstracted from the substrate (R'H) by the 5'-deoxyadenosyl radical. Subsequent rearrangement of the substrate radical results in the product (R''H) and, finally, regeneration of the adenosylcobalamin (when the 5'-deoxyadenosyl radical reacts with the Co(II) corrin). The energy of the Co-C bond,

measured by Halpern,¹ is of the order of 26 kcal mol⁻¹ in aqueous solution. While cleavage of the bond in free coenzyme is extremely slow (rate constant 10⁻⁷ s⁻¹, half-life ~ 0.5 years), on interaction of adenosylcobalamin with a B₁₂-dependent enzyme this rate is increased by a factor of 10³-10⁵; it is further increased when substrate binds ($k_{cat} \sim 10^2$ s⁻¹) to give an overall enhancement of lability of the Co-C bond by a factor of 10⁹-10¹¹.⁶ Thus, in some manner, the interaction of the enzyme with coenzyme and, additionally, the interaction of substrate with the enzyme-coenzyme complex result in increased lability of the Co-C bond.

Scheme I



Two effects could account for an increased lability of the Co-C bond. One is an electronic effect, such as the trans effect. This effect, which involves an electronic effect of a group on the group trans to it, has been examined in detail by Elder and co-workers⁷ and appears to be particularly significant in octahedral cobalt complexes. The cobalt-bound 5,6-dimethylbenzimidazole group is trans to the adenosyl group in the coenzyme, and if it were replaced by an enzymic side chain, the trans effect might help to labilize the Co-C bond. However, Lipscomb⁸ and others provide evidence from theoretical calculations to suggest that steric, rather than electronic effects modulate the lability of the Co-C bond in B₁₂ coenzyme. This is particularly evident in the experimental

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work of Randaccio and Marzilli^{9,10} in which structural studies of model compounds (cobaloximes) indicate a lengthening of an axial Co–C bond (and, hence, a decrease in the dissociation energy of this bond) when there is steric overcrowding between the groups attached to the C atom and the roughly planar equatorial ligand of cobalt; for example, the half-life of Co–C cleavage varies from more than a year when the carbon atom belongs to a methyl group to three seconds with a 3-pentyl group.

The structures of both vitamin B₁₂ and its coenzymes, particularly adenosylcobalamin, indicate that they have strained ground states. The distortions in the immediate surroundings of the cobalt atom reveal this;¹¹ the 5,6-dimethylbenzimidazole is too bulky to coordinate to a cobalt atom that is complexed with a large, rigid, planar equatorial ligand. What happens in vitamin B₁₂ is that the corrin ring is flexed at one side (near C(5)) when the bulky axial benzimidazole ligand is bound. When two axial substituents (e.g., adenosyl above and 5,6-dimethylbenzimidazole below) bind, the distortion of the corrin ring is less, presumably because of opposing effects. No amino acid side chain normally found in proteins can provide distortions in the corrin, due to bulk, in the way that 5,6-dimethylbenzimidazole can.

The manner by which substrate is affected by coenzyme has been the subject of much research.¹⁻³ It appears probable, as shown by Finke¹² from model studies, that the substrate does not bind directly to the cobalt atom but is held by the varying B₁₂-dependent enzymes in an area of the active site that allows interaction of substrate with 5'-deoxyadenosyl free radical. However, the alternate model, in which substrate binds directly onto cobalt, cannot be definitely ruled out. Obviously the enzyme needs great control over its potentially dangerous free-radical-forming coenzyme; thus the role of B₁₂ coenzyme is to provide, in a manner that can be carefully modulated by an enzyme, a free radical that can be used in the enzymatic reaction.

We address here the problem of how to analyze the amount of flexibility in corrin structures and how to relate this to the biochemical reaction that occurs when a specific B₁₂-utilizing enzyme and its substrate bind. This type of analysis allows for a preliminary probe into the mode of action of the coenzyme. The data to be used consist of the X-ray crystallographic results from the determinations of structures of various corrins.¹³⁻²¹

Experimental Methods

The structures of a large number of corrins have been determined since the first X-ray diffraction studies of vitamin B₁₂ and adenosylcobalamin. These comprise a metal-free corrin,¹³ a hexacarboxylic acid derivative with a lactam ring formed as the *c* side chain interacts with C(8),¹⁴ a monocarboxylic acid derivative with a side chain (either *b* or *e*) hydrolyzed,¹⁵ cobyrinic acid with the *f* side chain hydrolyzed,¹⁶ neovitamin B₁₂ which is epimerized at C(13),¹⁷ vitamin B₁₂-5'-phosphate,¹⁸ Factor A which has adenine replacing the benzimidazole,¹⁹ a synthetic

dicyanocorrin and its rhodium analogue,²⁰ and a synthetic Co(II) dimer.²¹ The computer-based studies aimed at analyzing the variability in these corrin structures will now be described.

1. Direct Superposition of Corrin Structures. The simplest method for comparing corrins is to superpose each directly on the other and then to compare the results. This we have done either by computing various least-squares planes through groups in the molecule or by using the computer program DOCK²² to superimpose the molecules. The common grouping chosen for such a comparison was the four central equatorial nitrogen atoms of each structure (via a computation of their least-squares planes); unlike the case for simple porphyrins, there is no ambiguity or symmetry to such a superposition, since asymmetry is caused by the facts that the C(1)–C(19) bond directly links two five-membered rings and since there is a methyl group on C(1) but only a hydrogen atom on C(19). Other ways of analyzing differences, for example, by comparing torsion angles and so obtaining a numerical evaluation of differences or by analyzing the motion of the atoms (via their temperature factors), are not as satisfactory. The study of torsion angles suffers from difficulties in visualizing results.²³ Studies of experimentally determined temperature factors are not very satisfactory for an analysis of corrin vibration because most corrins crystallize with many molecules of solvent of crystallization, some of it highly disordered; therefore temperature factors, while higher for the side chains than the ring system, are not very precisely determined. In addition, refinements of the structures of corrins generally have involved isotropic, rather than anisotropic, temperature factors so that measures of the amount of motion perpendicular to and in the general plane of the corrin ring system are usually not available.

2. Partitioned Distance Matrix Analysis. The comparisons of corrins made by superposing molecules with respect to an arbitrarily chosen least-squares plane (as described above) indicate that there are probably many better ways of aligning the molecules, particularly if a detailed analysis of the comparison is required. A distance matrix analysis does not necessitate a superposition of molecules in order to compare them. The various molecules are compared by computing distances between analogous atoms or groups in each molecule, and a matrix of these distances is prepared for each molecule for comparison. Differences are then noted.

Distance matrix analysis²⁴⁻²⁶ has proven useful for structure analysis and comparisons for both small molecules and macromolecules; originally, it was suggested for use in analysis of protein folding and was applied to the distances between α -carbon atoms in proteins.²⁴ Comparison of distance matrices of several proteins (for which the *j*th atom is the *j*th α -carbon atom in the amino acid sequence number) has allowed comparisons to be made of the folding of different proteins and has revealed changes in through-space orientations between substructures.²⁷ The distance matrix gives information analogous to that obtained from two-dimensional NMR studies, but it does not preserve the directional information that three-dimensional X-ray crystallographic results provide.

To obtain such a matrix it is first necessary to prepare an ordered list of the atoms of the molecule under consideration. The distance matrix contains all of the distances from each atom in the structure to every other atom; the *i*–*j*th element of the matrix is the distance (r_{ij}) between the *i*th and *j*th atom of the structure. Thus, in comparing two structures, it is essential that atoms labeled *i* or *j* represent analogous atoms. The distance matrix is symmetrical about the diagonal $r_{ij} = r_{ji}$; the diagonal elements (where *i* = *j*) are all zero. The important feature of the distance matrix for use in structural pattern matching is that it is invariant to translations and rotations of the whole molecule. As a result molecules can be compared and analyzed regardless of relative orientation and without requiring direct superposition.

However, a distance matrix for the B₁₂ coenzyme, with ~110 non-hydrogen atoms in the molecule, is unwieldy and therefore it was considered necessary to "partition" the matrix. This means that various groupings such as pyrrole-like rings, bridging atoms, and the benzimidazole were chosen and represented each as one unit by summing the

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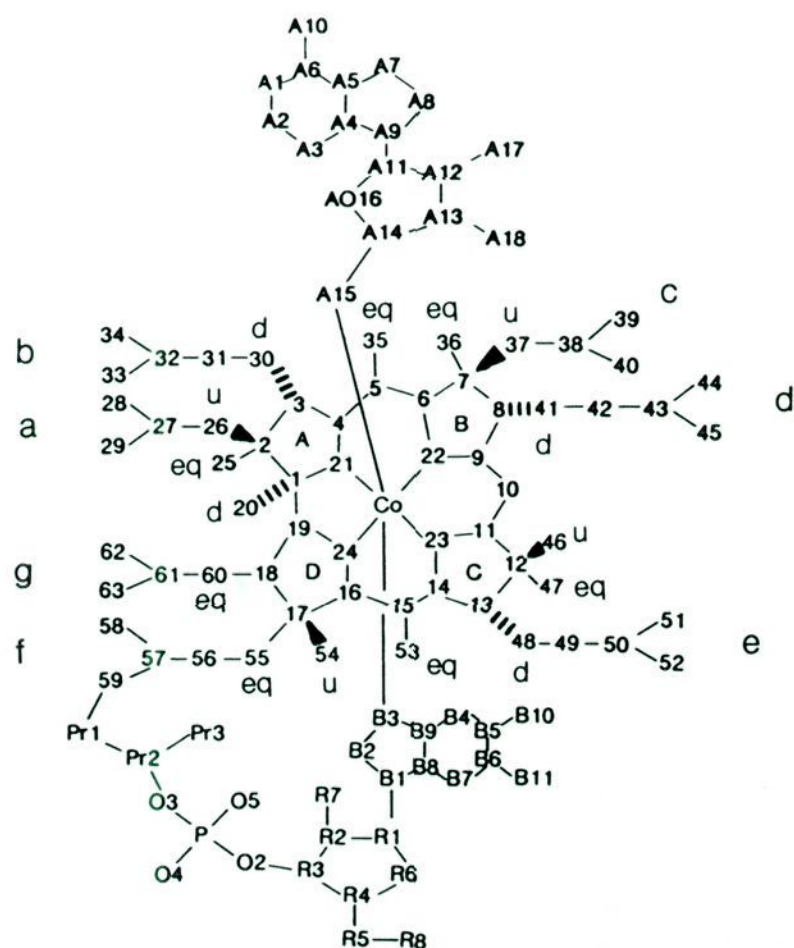
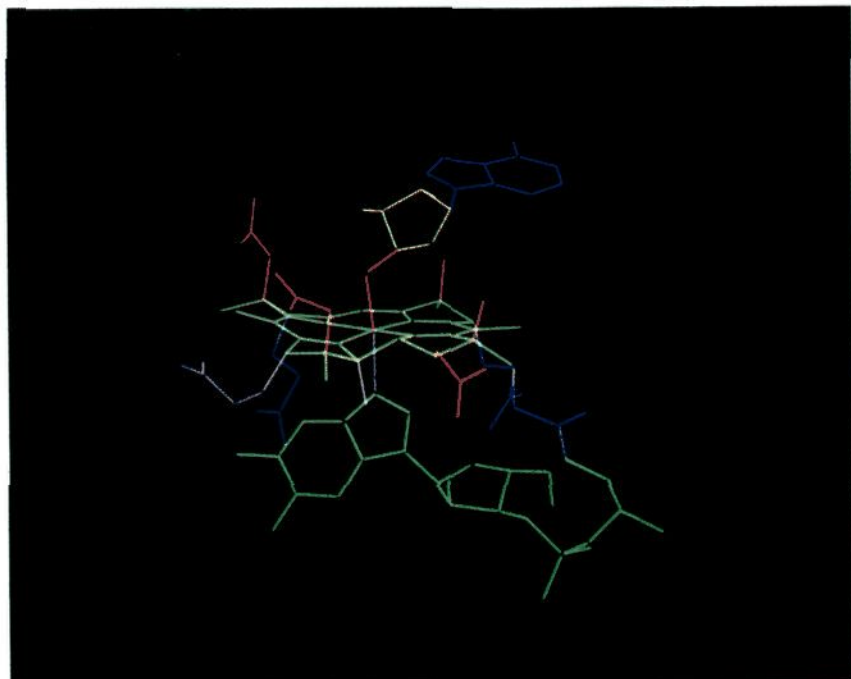


Figure 1. (a, top) Diagram of adenosylcobalamin: Acetamido side chains (red), propionamido side chains (purple), adenosyl ribose (yellow), and adenine (blue). (b, bottom) Numbering system for corrins. The rings are lettered A to D and the side chains *a* to *g*. Conformations of the side chains at their points of origination on the five-membered rings are indicated (*u* = up, *d* = down, *eq* = equatorial). The adenosyl group on A15 is above the corrin plane and the benzimidazole group, starting at B3, is below this plane.

distances in that group. For example, in a pyrrole ring, the 5×5 interatomic distances would be summed as one entry in the partitioned matrix. The distances between each atom in the pyrrole and each atom in another group would be summed as another entry in the matrix. In this way we were able to simplify the matrix and make it more amenable to analysis.

In order to simplify the analysis further, difference matrices were computed, that is, the differences in partitioned matrix entries for two molecules at any locus in the matrix. The partitioned distance matrix for one molecule may be compared with that for a second by subtracting one from the other. The difference partitioned distance matrix is the difference in absolute or actual values $[(\sum |r_{ij}|_a - \sum |r_{ij}|_b)$ or $(\sum r_{ija} - \sum r_{ijb})]$, and differences in these two types of entries may indicate concerted movements of groups. It is convenient to correct for the number of entries in each partition to give an average value per entry as each matrix element. The differences found between matrices must be compared with those expected from measured estimated standard deviations (esds). These values, expected from reported esd values for atomic

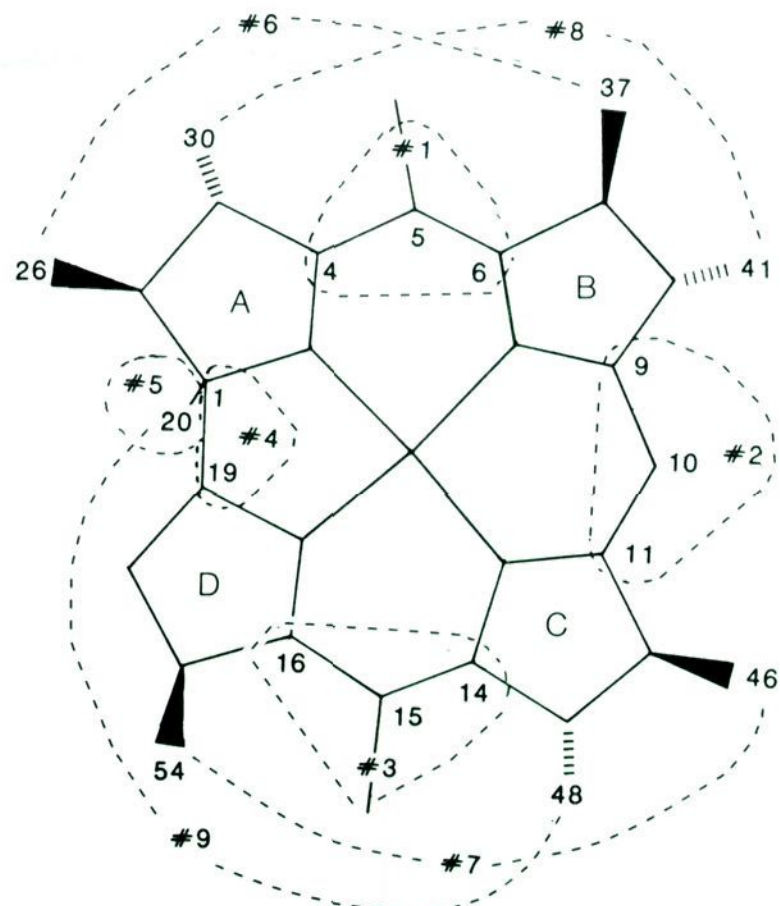


Figure 2. Diagram of the selection of partitions for a comparison of cobyric acid with the monocarboxylic acid. The nine partitions selected for this example are indicated by broken lines (#1 to #9). Partitions #1, #2, and #3 involve the bridges at C(5), C(10), and C(15), respectively. Partition #4 involves the C(1)–C(19) bond and partition #5 involves the attached methyl group C(20). Partitions #6 to #9 involve the axial groups on β -carbon atoms. Large entries in both difference matrices are #1–#5, #1–#7, #4–#4, #6–#7, and #8–#8.

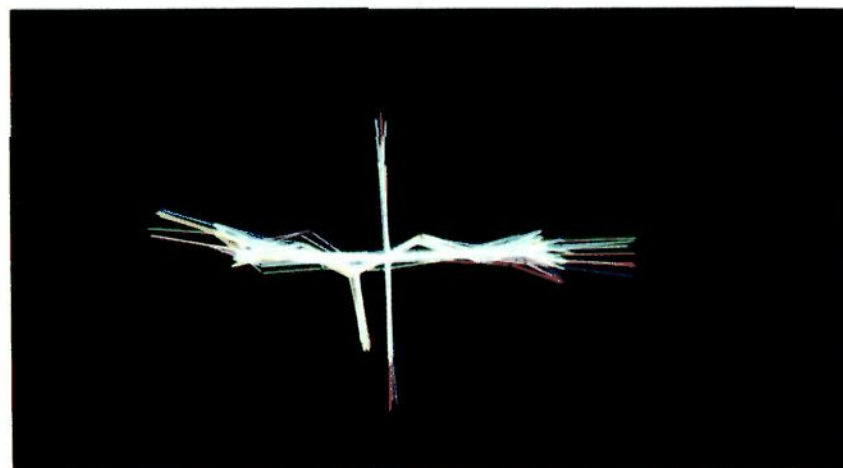


Figure 3. A side view of the result of superimposing 9 corrins each onto the best plane through the four nitrogen atoms N(21) to N(24). The molecules are the coenzyme (yellow), wet and air-dried vitamin B₁₂ (green), the monocarboxylic acid (blue), neovitamin B₁₂ (blue), cobyric acid (purple), the hexacarboxylic acid (purple), and synthetic cobalt and rhodium corrins (red). The view is approximately along the Co–C(10) direction.

coordinates and for unit cell dimensions, are computed.

If two corrin molecules differ by the amount of flexing in the ring system, this variation should be indicated by a large entry in the difference matrix for the distances between the atoms of the C(5) bridge [C(4), C(5), C(6), C(35)] and the C(15) bridge [C(14), C(15), C(16), C(53)]. Unfortunately because the corrin ring is a rather flat system, differences in distances computed in this way are small and therefore the method is not very sensitive to these flexing types of structural changes. However, the positions of C(20) or the axial substituents such as C(26), C(37), C(46), and C(54) constitute more satisfactory probes of information on the types of variability in corrin ring conformation (folding of the corrin ring system or some readjustment of the ring pucker).

A portion of the comparison of cobyric acid with the monocarboxylic acid by distance matrix analysis may serve as an example of the steps used in this method (Figure 2 and deposited Figure B). These two corrins occur naturally. Their structures have been determined with fair accuracy, since most of their respective hydrogen atoms have been located during the X-ray and neutron structure determinations. Although 23 partitions were used in the full analysis, only 9 were used here (deposited

Table A). Analysis of the partitioned matrices is done relative to the estimated errors that may be derived from the errors in atomic coordinates and unit cell parameters. The diagonal partitions ($i = j$) contain information on changes in the relative arrangements of the atoms within a partition, whereas off-diagonal partitions ($i \neq j$) contain information on the changes in the through-space orientations of partitions (conformation). In the present example entries 1-1, 1-5, 1-7, 1-8, 3-3, 4-4, 6-7, 8-8, and 8-9 in the absolute error partitioned matrix are all $\geq 10\sigma$. By comparison, in the standard error partitioned matrix, entries 1-5 and 4-4 are $\geq +10\sigma$ and 1-7, 6, and 8 are $\leq -10\sigma$. Thus for these partitions, which are significant at the 10σ level in both difference matrices, the changes in positions are somewhat concerted, i.e., all pairwise atom distances in these partitions are in the same direction with respect to the intramolecular comparison; entries in both the absolute and standard matrices have similar absolute values. The matrix entries 1-1 and 1-8 are, by this criterion, not concerted.

As shown in Figure 2, the 1-5 entry represents a movement of the bridge 1 group away from the methyl 20 group in the monocarboxylic acid as opposed to cobyrinic acid (i.e., the distances are greater in the monocarboxylic acid and hence the entry is negative in the standard matrix). This may be the result of the corrin flexing caused by the benzimidazole group in the monocarboxylic acid. There is not an analogous large entry for bridge 3; this implies that the folding only involves the C(5) side of the B₁₂ coenzyme molecule. The 6-7 entry (positive in the standard matrix) implies that C(26) and C(37) move toward C(46) and C(54) in the monocarboxylic acid. Entry 8-8 implies some change in the C(30)-C(41) distance (larger in cobyrinic acid). A similar type of distance matrix analysis was also applied to the amide groups at the ends of the seven side chains of the coenzyme.

3. Factor Analysis. However, when we have to analyze molecular geometry from a set of similar structures of which some are of limited accuracy, we need a technique that allows us to see a significant effect above the noise created by experimental error. The technique of factor analysis (particularly the principal components variant) has been found to be a powerful method of eliminating random (normally distributed) experimental error.²⁸⁻³¹ In particular, the technique has been used to analyze the out-of-plane deformations of the porphyrin skeleton.³² This study showed that out-of-plane distortions could be described by low-energy processes similar to the lowest normal modes of the porphyrin. More recently we have shown the technique to be valuable in understanding the crystal packing induced deformations in the steroid skeleton (Δ^4 -en-3-ones) in terms of its lowest normal modes.³³ This showed that a very significant signal could be obtained from a small number of structures (15) with very small deformations from a mean geometry (~ 0.05 Å) which were not much greater than the mean experimental error (~ 0.01 Å). In the present case the experimental errors are higher (~ 0.03 Å) but the deviations from a mean geometry are larger and gave us hope that this technique might show one or more significant factors.

In this technique we take a set of n observations for m parameters P_{ij} and form the correlation matrix, \mathbf{R} . There are great advantages in choosing Cartesian coordinates as the P_{ij} since these are the experimentally determined quantities and are formally independent (unlike the set of all internal coordinates which involve complex ring closure conditions, etc.). We fit, by iterative least squares, all molecules (in this case the corrin fragment already defined of N atoms) into a common mean geometry, so that if \mathbf{x}_i represents the $3N$ -dimensional vector of (rotated and translated) Cartesians of the i th molecule and $\bar{\mathbf{x}}$ is the mean geometry

$$\bar{\mathbf{x}} = \frac{1}{n} \sum_i^n \mathbf{x}_i$$

$$\text{and } \Delta_i = (\mathbf{x}_i - \bar{\mathbf{x}})^T (\mathbf{x}_i - \bar{\mathbf{x}})$$

is a minimum for each molecule. The covariance between the structures is then

$$\mathbf{R} = \frac{1}{n} \sum_i^n (\mathbf{x}_i - \bar{\mathbf{x}})(\mathbf{x}_i - \bar{\mathbf{x}})^T$$

This matrix is Gramian (i.e., symmetric with no negative eigenvalues) of order $3N$ but is singular with maximum rank (r) = $3N - 6$. If there are fewer data (n) than $3N - 6$ the rank of the matrix is lower, viz (n)

- 1). (If there are linear relationships between the observations the rank may drop further.) Diagonalization gives

$$\mathbf{R} = \mathbf{E}^T \boldsymbol{\lambda} \mathbf{E} = \mathbf{E}^T \boldsymbol{\lambda}^{1/2} \boldsymbol{\lambda}^{1/2} \mathbf{E}$$

(where $\boldsymbol{\lambda}$ is a diagonal matrix of r non-zero eigenvalues) and may be rewritten

$$\mathbf{R} = \mathbf{F}^T \mathbf{F}$$

where \mathbf{F} is a matrix of scaled eigenvectors (factors) representing the direction and magnitude of the principal components of the distribution of \mathbf{x} . The factors will result from a mixture of real effects combined with experimental error. If the latter is distributed approximately isotropically in $3N$ -space (a reasonable approximation for most X-ray analyses), the major axes reveal significant variation from other effects. If we are fortunate, we can reliably interpret these as chemical or crystallographic effects (or sometimes as uncorrected systematic errors).

In the case of B₁₂ derivatives the input to the factor analysis consisted of the 26 atoms of the corrin ring system, but it excluded the cobalt atom. However, not all data were included; wet and dried B₁₂ (old data), B₁₂ phosphate, the monocarboxylic acid (neutron data), and a cobalt(II) dimer were eliminated because they were outliers in bond length tests. The hexacarboxylic acid was removed because the corrin ring system was perturbed by the formation of a lactam ring attached to ring B. This left 12 structures (listed in deposited Table B). In the factor analysis, coordinates of each of the 12 corrins were referred to their inertial axes, and their mean Cartesian geometries were found. This mean corrin structure was used as a reference for analysis of each experimentally studied corrin; each structure was rotated (by a least-squares method) to give a minimum root-mean-square deviation from this mean geometry. The fit was indicated by the total squared displacement of the atoms from the mean geometry (Table B). There were 26 atoms and hence the problem had a dimensionality of 78; since only 12 cases were studied the problem was underdetermined so that only the first 11 factors would be meaningful.

4. Surface Accessibility. The steric accessibility of key functional groups in a molecule will determine, in part, the availability of that part of the molecule to attack by other molecules.^{34,35} In order to evaluate the total surface area surrounding a particular atom and quantitate its accessibility, a computer program, SURVOL,³⁶ was used. This program is based on a Monte Carlo simulation of space filling within a box of enclosure. A box is constructed so that it will have a clearance of twice the radius of boundary atoms. The box is then randomly filled with points at a fixed density of 50 points per cubic angstrom (a value found experimentally to give reasonable results). The ratio of the number of points within the van der Waals radius of an atom to the total number of points leads to a ready computation of the van der Waals volume. Accessible surface area is computed in an analogous manner to that for molecular volume. Random points are generated at uniform density around each atom at the fixed van der Waals radius. If the points are within the van der Waals radius of any other atom they are considered buried, otherwise they are exposed. The ratio of exposed points to total points is then computed. In molecules as complex as the B₁₂ coenzymes certain atoms must necessarily be buried; such an analysis will indicate which atoms these are.

Results

1. Direct Superposition of Corrin Structures. The results of superposing all molecules via the least-squares plane through the four central nitrogen atoms are shown in Figure 3 and deposited Table C. This shows that the mean difference in corrin structure is a flexing of the ring system, best illustrated by the view along the plane of the corrin system. However, this diagram also indicates that some further small adjustments could be made so that the alignment of corrins on each other would be improved. Most of the flexing of corrin occurs on the side C(4) to C(10) (rather than C(10) to C(16)), i.e., the angle A:C is much larger than C:B (where A involves N(21) to C(10), B involves N(24) to C(10), and C involves the four equatorial nitrogen atoms N(21)-N(24) attached to the cobalt). This difference in flexing is the result

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Table I. Distance Matrix Analysis

(a) The Partition Groups Involve the Following						
# 1-4	rings A-D	# 12, # 13, # 14	C(25), C(26), C(30)			
# 5-8	bridges C(5), C(10), C(15), C(1)-C(19)	# 15, # 16, # 17	C(36), C(37), C(41)			
# 9	C(24)	# 18, # 19, # 20	C(46), C(47), C(48)			
# 10, # 11	bridge methyl groups C(35), C(53)	# 21, # 22, # 23	C(54), C(55), C(60)			

(b) Highest Entries Are Listed Below. Those Occurring in More than One Corrin Are Indicated by an Asterisk						
neo B ₁₂	monocarboxylic acid	air-dried B ₁₂	wet B ₁₂	synthetic corrin	cobyric acid	hexa acid
# 15-17(-)	# 18-19(+)*	# 3-18(+)*	# 18-19(-)*	# 9-22(+)	# 14-17(-)*	# 14-17(-)*
# 15-18(+)	# 3-18(+)*	# 10-16(-)*	# 13-23(+)	# 10-16(-)*	# 16-18(-)*	# 11-20(+)
# 18-19(-)*	# 7-18(+)	# 16-18(+)*	# 12-13(+)	# 14-15(-)	# 17-20(+)	# 10-14(+)
# 17-19(+)*	# 8-18(+)	# 12-14(-)	# 18-21(+)*	# 13-21(-)	# 16-21(-)	# 13-18(+)
# 2-18(+)*	# 18-21(+)*	# 4-21(+)	# 7-21(+)	# 17-19(+)*	# 17-19(+)*	# 16-18(-)*
# 3-17(+)	# 9-20(-)*	# 8-9(-)	# 10-16(-)*	# 22-23(-)*	# 3-16(-)	# 17-19(+)*
# 2-17(-)	# 3-19(+)	# 6-18(+)	# 4-8(-)	# 9-23(-)	# 6-16(-)	# 6-17(+)
# 18-20(-)	# 16-18(+)*	# 2-18(+)	# 22-23(-)*	# 8-21(-)*	# 5-17(-)	# 9-20(-)*
		# 18-19(+)*	# 16-18(+)*	# 20-22(+)		

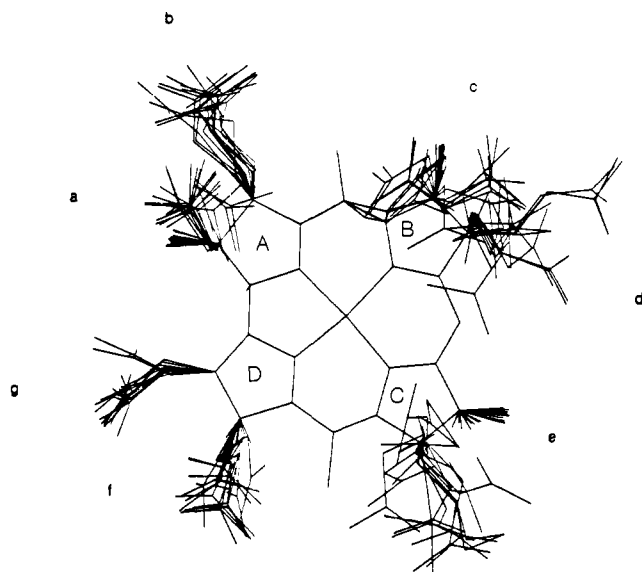


Figure 4. Variations in the side chain conformations for 13 corrins (the coenzyme, wet and air-dried vitamin B₁₂, the monocarboxylic acid, neovitamin B₁₂, cobyric acid, the hexacarboxylic acid, synthetic cobalt and rhodium corrins, a Co(II) dimer, and three stable yellow corrinoids). This diagram is drawn with respect to the best plane through the four central nitrogen atoms of the corrin ring. Note that the *c*, *d*, and *e* side chains vary more than do others.

of the axial binding of the bulky dimethylbenzimidazole with its major effect near C(5).¹¹

In a similar way a comparison of entire molecules is shown in Figure 4 and illustrates deviations in side chain orientations. This figure was drawn from the same superposition used for Figure 3. The side chains *a*, *b*, *f*, and *g* do not vary much but the side chains *c*, *d*, and *e* are extremely variable.

2. Partitioned Distance Matrix Analysis. The distance matrix analysis gave detailed information on the nature of the folding of the corrin ring system. All structures were compared to that of the coenzyme, adenosylcobalamin; this was chosen because of its biological activity. The molecule was partitioned into pyrrole rings (# 1 to # 4), bridges (# 5 to # 8) and C(20) (# 9), the bridging methyl groups (# 10 and # 11), and side chains (# 12 to # 23). The four major upper groups are C(26) (# 13), C(37) (# 16), C(46) (# 18), and C(54) (# 21).

Table I contains a listing of the highest entries in the various distance matrices; they are also diagrammed in deposited Figures C-I. Those entries that occur more than once are indicated with an asterisk. The entries for neovitamin B₁₂ are dominated by the changes in # 18 and # 19 that result from the epimerization. Note the following frequent entries for the corrins listed in Table I: # 16-18, which represents folding about the Co...C(10) line; # 3-18 and # 18-19, which indicate some conformational var-

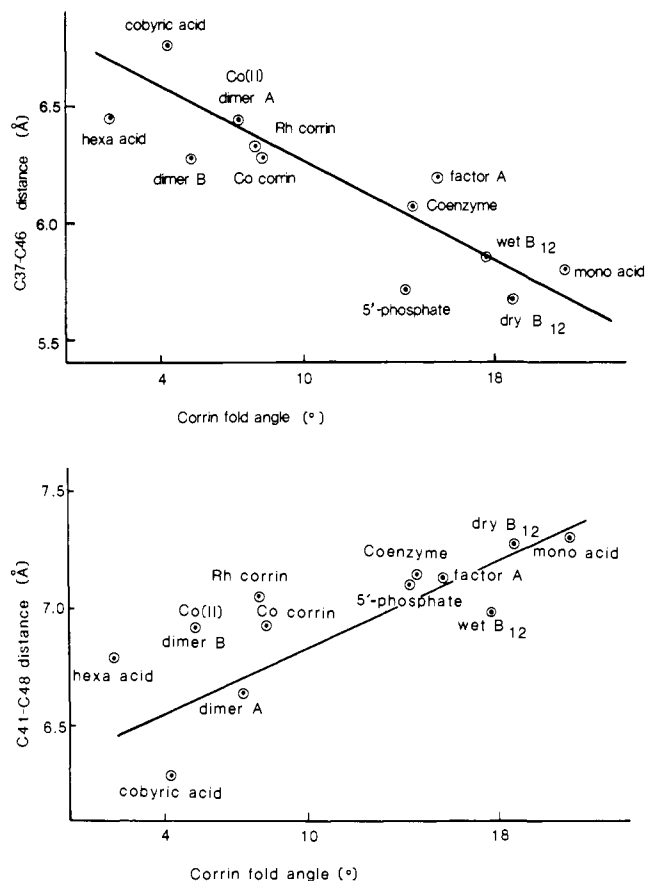


Figure 5. Correlation of the corrin fold angle with the distances between axial carbon atoms on β -positions of five-membered rings. (a) Most variable upper axial carbon distances, C(37)-C(46); (b) most variable lower axial carbon distances, C(41)-C(48). The corrin fold angle is defined as the angle between planes A = (N(21), C(4), C(5), C(6), N(22), C(9) and C(10)) and B = (N(24), C(16), C(15), C(14), N(23), C(11), and C(10)). See deposited Table C for numerical values.

iability in the C ring; # 18-21, which represents some folding over C(15); and # 14-17, which indicates some folding over C(5).

The highest values in the matrix for the air-dried vitamin imply that there is some change (between vitamin and coenzyme) in the C ring and between C(37) and C(35) (distance longer for the vitamin). High values also indicate that a fold occurs from the D ring to C(10). The data for the "wet vitamin" also imply a change in the C ring and a longer C(35)...C(37) distance. The fold about C...C(10) is also indicated by changes in distances between C(37) and C(46) but further complicated by increases in C(25)-C(60) (lower side) and C(46)-C(54) (upper side). Thus more distortions occur in this molecule.

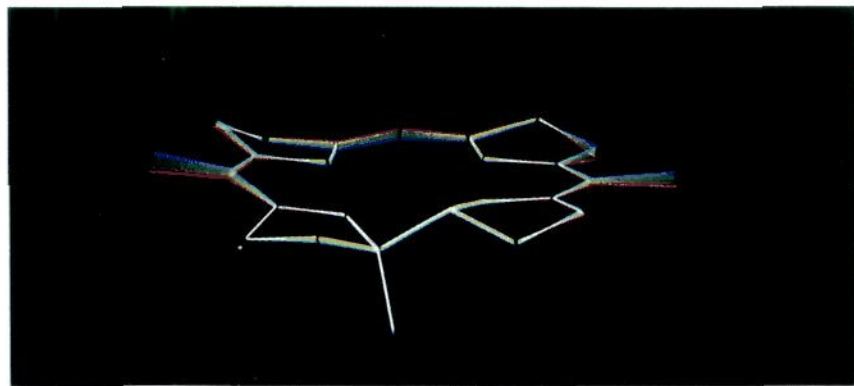


Figure 6. Results of a factor analysis of 12 corrins. The first factor (probably the only significant one) is illustrated. For clarity, the limits in variation are indicated by different colors, red through blue. This diagram may be compared with the experimental result in Figure 3.

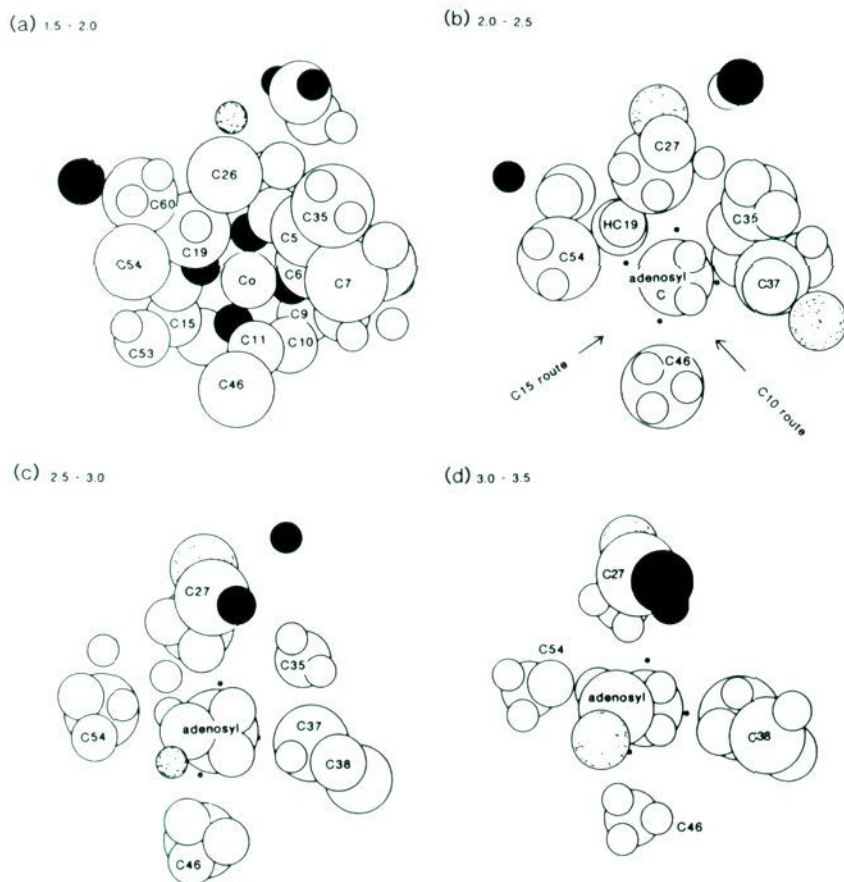


Figure 7. Sections parallel to the plane N(21), N(22), N(23), N(24) of adenosylcobalamin at various heights above the cobalt atom showing possible routes of access to the cobalt atom. The routes over C(10) and C(15) are clear but the route over C(5) is hindered by the flexing of the corrin. Each atom is drawn with a radius of half the van der Waals radius. Heights above the cobalt atom are (a) 1.5–2.0 Å, (b) 2.0–2.5 Å, (c) 2.5–3.0 Å, and (d) 3.0–3.5 Å. The locations of the four nitrogen atoms are indicated by small black circles.

In the monocarboxylic acid the major matrix differences are #18–19 (C(46) to C(47)) and #3–18 (ring C to C(46), its substituent). These imply some change in the area of the C ring, a possible region in which amide hydrolysis to give this acid has occurred. Other large entries include C(46) (#7–18, #8–18, #16–18, and #18–21), C(47) (#3–19), and C(48) (#9–20). Thus all the large differences involving ring C which is pushed on so that negative values (larger distances in the monocarboxylic acid) are found for interactions with C(24) and C(41), all to C(48). Positive values are found for the interactions of C(46) and C(47) with C(37) (a flexing about the C(10)–C(15) bridge), C(54), and the C(1)–C(19) bond. The overall impression is that the major fold difference occurs across the C(10)–C(15) (or the B–D ring) direction.

Finally, the three largest entries for cobyrinic acid represent a longer distance from C(30) to C(41) and from C(37) to C(46) and a shorter distance from C(41) to C(48). Thus the fold appears to occur across the Co...C(10) line.

We then examined distances between axial carbon atoms of the corrin ring system. This analysis showed how the most variable distances, those measured across the fold line, vary as the fold angle (the angle between the planes N(21)–C(4)–C(5)–C(6)–N(22)–C(9)–C(10) and N(24)–C(16)–C(15)–C(14)–N(23)–C(

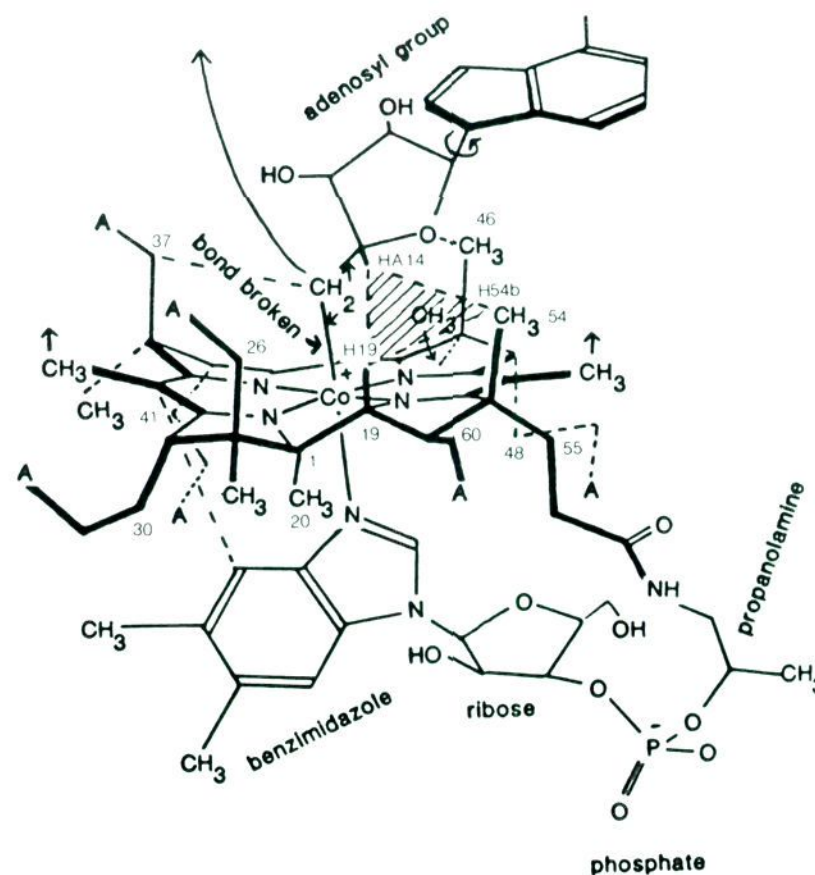


Figure 8. Diagram of adenosylcobalamin (A = side chain amide, Co–NH₂). The axial groups attached to β -carbon atoms are numbered. Short C...O and N...H distances, including C(46)...adenosyl ribose O and the triangle involving H(19), H(54b), and HA(14), are indicated.

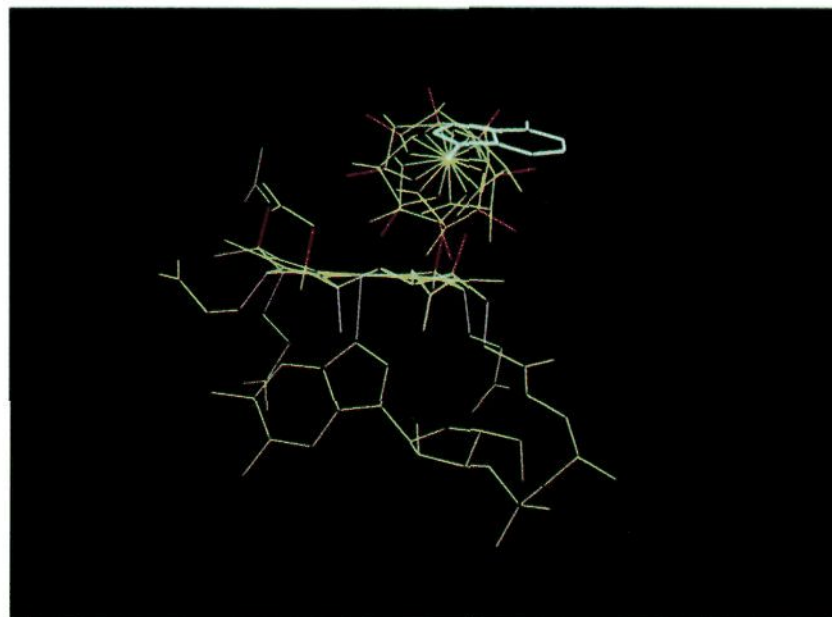


Figure 9. Computer-graphics-generated diagram of rotation about the glycosidic bond. Eight positions are illustrated for the C–C bond (in red). The route over C(15) (counterclockwise rotation, right-hand side of diagram) is hindered but the vertical pathway (clockwise rotation) is clear. See deposited Figures S and T for further details. In the uppermost position the adenosyl free radical can abstract hydrogen from enzyme-bound substrate.

(11)–C(10)) increases. This is diagrammed in Figure 5. Data are listed in deposited Table D. Deposited Table E contains distances between the cobalt atom and amide termini (average of Co...O and Co...N distances).

3. Factor Analysis. The study of 12 corrin structures by factor analysis gave the results illustrated in Figure 6 (and deposited Figures J–L). The first four factors had eigenvalues of 21.5%, 11.8%, 9.8%, and 8.5%. The approximate estimated standard deviation of a (C–C) bond is 0.04 Å; the root-mean-square deviation per atom in the factor analysis is 0.07 Å. Thus, approximately 50% of the latter must be due to experimental error, and the rest is (possibly) the variation from structure to structure being investigated. The first factor was found to have a good χ^2 distribution, and it appeared to be the only factor that made good sense. The others were rejected, as they were considered to be lost in the noise of the measurements. In Figure 6, it can be seen that the variation is mostly perpendicular to the ring and that it

is presented by flexing, with directions of this variation for C(1), C(19), and C(10) opposite to those for C(5), C(35), C(15), and C(53). Thus, while there is not much motion near the A and D rings (apart from a slight tilt change) there is considerable motion of C(35) and C(53), the methyl groups on bridging atoms C(5) and C(15). As a result there is considerable conformational change in the B and C rings, as C(5) and C(15) move up, C(10) moves down (or vice versa).

The precision of the data prevented us from using any other factors, but it is clear, from an examination of Figure 6, that the nature of the conformational change in corrins, shown by the raw data in Figure 3, is best represented by this method of principal factor analysis.

4. Surface Accessibility. The major result of the analysis of surface accessibility, shown in deposited Table F, is to show that the area around C(10) is less hindered and more accessible than other parts of the corrin ring, while the area near C(1) and C(19) is the least accessible. When cobalt (octahedrally coordinated) is replaced by nickel (square-planar coordination) some of the metal becomes accessible. The area around C(5) to C(8) is the most readily oxidized area in the coenzyme molecule. Data are listed in deposited Table F for those molecules for which hydrogen atom coordinates are available; included are the two coenzymes, adenosylcobalamin,³⁷ and methylcobalamin³⁸ (calculated hydrogen atom positions for methylcobalamin). In Figure 7 is shown the accessibility of the cobalt atom from the top (adenosyl side) of adenosylcobalamin. Three main routes are available if the adenosyl is removed: one directly onto the cobalt, one over C(10), and one over C(15).

Discussion

The major conformational variability in corrin nuclei, at the present level of precision of measurement of corrin structure and from our study by partitioned distance matrix analyses and factor analyses, appears to be the amount of folding about the Co...C(10) line. This variability has symmetry such that C(5) and C(15) move up while C(10) moves down and vice versa; this is similar to that of one of the largest factors for porphyrins.³² The distance matrix analysis has shown that most of the flexing occurs on the C(5) side of the molecule; presumably a merging of these two views more nearly approximates the true picture. This implies that C(5) moves up much more than does C(15). The area of the molecule around the C(1)–C(19) bond is sterically crowded by the many groups there, but the area around C(10) is free from such constraints and therefore quite flexible; there is not even a methyl group on it such as is found on C(5) or C(15).

What is the effect of such flexing of the corrin ring? There are four β -carbon atoms projecting axially from the five-membered rings above the corrin on the adenosyl side; these are C(26), C(37), methyl C(46), and methyl C(54) and they stand like gateposts above each corner of the corrin ring (see Figure 8 and deposited Figure M). We compared the distances between these four gatepost carbon atoms in 13 crystal structures with fold angles that varied from 2 to 22° (Figure 5 and deposited Table D). The C(37) methyl C(46) distance, which spans the Co...C(10) fold line, was most sensitive to changes in fold angle and varied by 1.1 Å depending upon fold angle. The positions of the four upper axial carbon atoms for various molecules were plotted and were compared, in two ways, with use of the adenosylcobalamin structure as the standard. In the first method, the cobalt atom and four nitrogen atoms were superimposed (deposited Figure N), while in the second method the Co...C(10) vectors were aligned. By either method, it was found that, of the four carbon atoms listed, C(37) moved the most. Its position varied by 0.9 Å in a direction perpendicular to the Co...C(10) vector. The range of motion of the other axial carbon atoms was on the order of 0.6 Å. In deposited Figure N, the axial carbon atom positions are compared in two corrin structures, cobyrinic acid and B₁₂ mono-

carboxylic acid. These structures are well-defined (hydrogen atoms were located) and differ by 17° in fold angle (4° for cobyrinic acid, 21° for the monocarboxylic acid).

The effect of flexing on the underside of corrins was also considered. The axial carbon atoms below the corrin ring [C(30), C(41), C(48), C(55)] can affect the binding of the lower axial ligand; the variation in the C(30)–C(41) and C(41)–C(48) distances (1.0 Å) was twice as great as the variation in the other two distances (see deposited Table D). C(41) and C(48) moved as much as 0.8 Å; C(48) moved in a direction perpendicular to the Co–C(10) fold line, but C(41) moved in a direction approximately 45° to the fold line (deposited Figure N). The oblique motion of C(41) is responsible for the large variation in the C(30)–C(41) distance, even though the position of C(30) changes very little. The positions of methyl C(20), methyl C(25), and methyl C(47) were also plotted, but they remained approximately constant.

A distance analysis indicated that a primary effect of flexing of the corrin ring system is the close proximity of the hydrogen atom on C(19), which points directly up, onto the hydrogen atom on C(4') of the ribose ring (2.02 Å) (deposited Figure O). In the coenzyme methylcobalamin this same hydrogen atom pushes, in an analogous manner, against the methyl group coordinated axially to the cobalt atom (deposited Figure P). Some short H...H distances in the adenosyl coenzyme are listed in deposited Table G. They show that there is a triangle involving H(19), H(54b), and HA(14), illustrated in Figure 8, together with a short distance between C(46) and the adenosyl ribose oxygen atom that could aid Co–C cleavage as the corrin ring flexes. In addition, the hydrogen atoms on C(46) lie under the adenine ring (H...C 3.08 Å) and may, if folding about Co...C(10) occurs, push the adenine up. In the crystal structures of (*R*)- and (*S*)-2,3-dihydroxypropylcobalamin³⁹ the Co–C–C bond angle is 113.6° in the (*S*) conformer which has no strong interaction with H(19). In the (*R*) conformer, there is an interaction with H(19) and the Co–C–C angle is increased to 119.6°.

Such flexing of the corrin ring may cause it to move to a conformation resembling the transition state (deposited Figure Q), which involves Co(II) which is known to lie out of the plane of the corrin (0.13 Å in the case of a Co(II) corrin dimer²¹). If the 5,6-dimethylbenzimidazole remains attached then, after Co–C cleavage, the cobalt will move down toward the benzimidazole; this will decrease the steric accessibility of the cobalt from above the corrin plane. In this arrangement there is an analogy to the flexed corrin in which the cobalt lies below the average plane of corrin atoms due to the increased height of C(5) and C(15) and their surrounding atoms.

It is tempting to suggest that side chain variability, illustrated in Figure 4, may also give information on the mode of action of the coenzyme. The *a*, *b*, *f*, and *g* side chains are fairly rigidly constrained by all the methyl and methylene groups in their area. However, the *c*, *d*, and *e* side chains are free to show some conformational changes. Flexing of these side chains plus steric pressure on C(35) and C(53) might aid the flexing of the corrin ring. The approximate constancy of unit cell dimensions of vitamin B₁₂ variants (not the coenzyme) had intrigued us; variations in molecular shape are accommodated by differing water structure. An analysis of side-chain-to-side-chain packing is shown in deposited Table H. It is clear that in all cases there is involvement of each side chain except *c*. It is also interesting that this *c* side chain swings round in "wet-B₁₂", "B₁₂'", and in one of two dihydroxypropylcobalamins³⁹ and forms a hydrogen bond to a group coordinated axially to the cobalt (above the plane of the corrin ring). In cases where bonding of an intermediate to the cobalt occurs, this type of interaction may be significant in the mechanism.

Accessibility of the Upper Surface of a Corrin. The variation in the types of enzymes for which adenosyl cobalamin is a cofactor intuitively suggests that the enzyme, rather than cofactor, must

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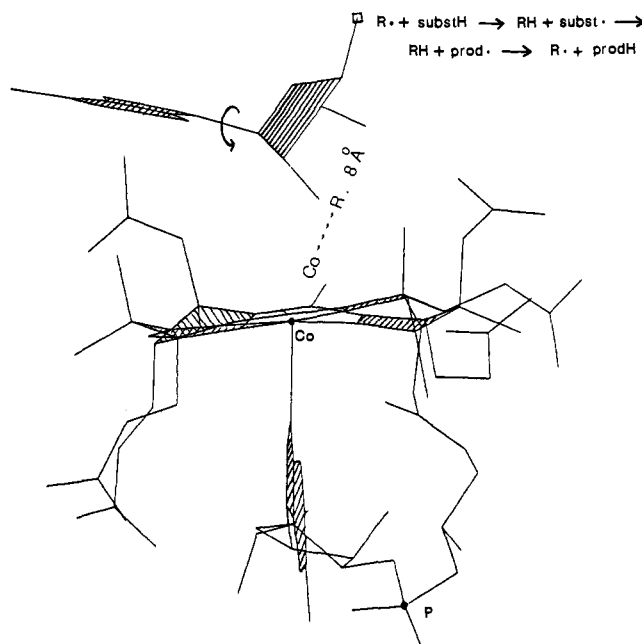


Figure 10. Diagram of results of rotation of adenosyl ribose after C---C bond cleavage.

have a large proportion of the control of the stereochemistry of the catalyzed reaction and that the coenzyme mainly serves as a source of free radicals. We considered what pathways are available for approach to the cobalt atoms and pathways that the adenosyl free radical might take as it leaves the cobalt after Co-C cleavage. Methyl groups and amide side chains that extend above and below the plane of the corrin ring may protect the deoxyadenosyl free radical generated by Co-C bond homolysis from unwanted side reactions, but they may also restrict the access to the cobalt. This problem of cavity size was studied by computing the van der Waals radii⁴⁰ of atoms in planes at 0.5-Å intervals above the plane of the four nitrogen atoms of the corrin ring, as illustrated in Figure 7. Circles representing half the size of the van der Waals sphere of the atom at the level were plotted⁴¹ on the same scale as CPK models.

It has not been shown conclusively whether the substrate moves onto the cobalt atom after the Co-C bond is broken or whether the free radical on the C is moved to another place where substrate is bound. We consider access in both cases. Between the gatepost carbon atoms on the upper surface of the corrin there are several pathways to the cobalt, illustrated in Figure 7. One route is over C(10) and a second is over C(53). The proposed route over C(10) would be hindered in the 10-chloro derivative of B₁₂ coenzyme. In glutamate mutase the halogenated derivative of the coenzyme is inactive;⁴² in diol dehydrase the catalytic activity of the 10-Cl analogue of the coenzyme is much lower.⁴³ It is interesting that in a recent X-ray crystallographic study of (*R*)- and (*S*)-2,3-dihydroxypropylcobalamins the bulkiest part of the dihydroxypropyl group lies toward C(15) (see deposited Figure R). This seems, then, a likely type of arrangement for groups that interact with the cobalt atom. Similarly in adenosylcobalamin this is where the ribose of the adenosyl group is positioned.

The alternative situation, presently preferred, is that the free radical at C(A15) moves to another site. If this is the case, after Co-C cleavage during catalysis, how does the adenosyl move toward the substrate in a protected manner so that only substrate may accept the free radical? One possibility is that if the adenine group is fixed by the enzyme by hydrogen bonding,⁴⁴ for example,

then the deoxyribose group can move by rotation about the glycosidic linkage (rotation about C(8)-N(9)-C(1')-O(1')). In Figure 9 and deposited Figures S and T the possible pathway of C(A15) (the site of the free radical) is illustrated. It appears that if C(A15) moves toward C(53) as the glycosidic torsion angle increases from 65 to 180° there are steric problems; torsion angles 120-180° are not allowed because contacts between C(A15) and C(53) are too close. However, if the glycosidic torsion angle decreases, C(A15) swings up almost vertically, a more likely situation; the Co...C distance may then extend to 7.9 Å, a value near to the value (~10-12 Å) deduced from ESR studies.⁴⁵ In addition the sugar pucker may further change this distance. Presumably, once it has swung out of place, the adenosyl free radical can interact with substrate as shown in Figure 10. By facilitating this process the enzyme could enhance the reaction rate. The free radical from C(A15) must lie near the substrate so that the resulting C(A15) methyl group can interact with product radical before it interacts with anything else. In addition there must be some mechanism for bringing C(A15) back to the cobalt atom. In this context it is interesting that, in a Co(II) corrin, a methyl group (from an esterified side chain) lies over the cobalt, implying some attraction between these, perhaps analogous to Co(II) interacting with an adenosyl group.

Conclusions

Our computer-based analyses of corrin flexibility have indicated that the major variation is a flexing about the Co...C(10) line. This flexing is aided by bulky axial substituents on the lower side of the corrin. In Co(III) adenosylcobalamin the corrin ring is slightly flexed up toward the adenosyl group. If the Co-C bond is broken homolytically the Co(II), now pentacoordinate, will lie below the plane of the corrin,²¹ displaced toward the base. If the base also swings away from the lower side the corrin will be planar (possibly with Co(I) in the plane⁴⁶) and hence the highly reactive Co(I) will be accessible from above for interaction with an alkyl group or radical.

In the case of the adenosylcobalamin, steric pressure from the hydrogen atom on C(19) may assist in Co-C cleavage. If the substrate moves onto the cobalt then, once the adenosyl group has moved, the substrate may enter from directly above or over (C10) or C(15). If the substrate interacts with the adenosyl group at an alternative site, the movement of the adenosyl group must be controlled, since coenzyme can be formed again. If the adenine group is held by the enzyme then the ribose group is free to swing up, once the Co...C bond is broken, and carry the free radical on R-CH₂•, in a protected environment, to substrate and remain there until the need arises to regenerate the coenzyme.

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Supplementary Material Available: Table A, Results of Partitioned Matrix Analysis; Table B, Output from Factor Analysis; Table C, Fold Angles of Corrins and Deviations from Plane N(21) to N(24); Table D, Distances between Axial Groups; Table E, Co...P and Co...amide distances; Table F, Accessible Areas in Corrins; Table G, Short H...H and H...C, N, O Distances; Table H, Side Chain-Side Chain Interactions; Figure A, Formula; Figure

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B, Comparison of Corrins of Cobaltic and Monocarboxylic Acid; Figures C-I, Results of Partitioned Matrix Analysis; Figures J-L, Results of Factor Analysis; Figure M, "Gate Post" atoms; Figure N, Movement of "Gate Post" atoms Unfolding; Figure O, Short H...H and H...O Distances; Figure P, Steric Factors Affecting

Methyl Groups in Methylcobalamin; Figure Q, Co(III) and Co(II) Corrins; Figure R, Orientations of Upper Ligands in Some B₁₂ Derivatives; Figures S and T, Rotation about the Glycosidic Bond (37 pages). Ordering information is given on any current masthead page.

Collisional Relaxation of Photoexcited Bromobenzene Ions by Various Neutral Partners

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Abstract: With use of the technique of competitive collisional quenching of two-photon dissociation, the collisional quenching of photoexcited bromobenzene ions was measured for 35 neutral partners. The number of collisions required for quenching ranged from 3.5 to 180, generally increasing with molecular complexity. For quench gases not containing unsaturation or heavy atoms (except H₂O), the quenching efficiency gives an excellent plot against $NM_r^{0.5}$, where N is the number of atoms in the neutral, and M_r is the reduced mass. Neutrals containing double or triple bonds, or heavier-than-third-row atoms, show markedly enhanced quenching efficiency. The results are compared with other quenching efficiency studies of polyatomic ions and neutrals and show similarities to several other studies which have been interpreted in terms of a collision-duration or energy-flow limitation on the rate of energy removal. The present results are not in accord with models assuming energy equilibration in the collision complex on each collision.

Intermolecular energy transfer in collisions, a common activation and deactivation process of excited molecules, plays an important role in many gas-phase reactions. The thermalization of vibrationally excited molecules is of particular interest in many aspects of gas-phase chemistry.

Study of the collisional quenching of vibrationally excited neutral molecules has a long history, but only more recently have techniques and interest developed in studying gas-phase ions. Because of the longer range and greater strength of ion-neutral interactions, compared with neutral-neutral interactions, one might expect that energy transfer in the ion case might tend to be more efficient, but not enough is known to give a general conclusion on this fundamental question. Several studies have looked at quenching of small ions, yielding absolute efficiencies¹⁻³. Among polyatomic ions, the relative-efficiency study of Miasek and Harrison⁴ and some dimer-ion stabilization studies in Bowers' laboratory^{5,6} have looked at a variety of quenching neutrals, while several other studies using one or a few quench gases have been reported.⁷⁻⁹

We have reported some results¹⁰⁻¹² using the technique of competitive collisional quenching of two-photon photodissociation as a method having the capability of measuring the absolute quenching efficiency for some polyatomic ions. We describe here the results of a more extensive study with bromobenzene ion using enough different neutrals to make various trends clear. The vibrationally excited bromobenzene ions are created by 514.5-nm photon absorption, with the collisional quenching experiment giving the number of collisions required to reduce the ions from 20000 to 2850 cm⁻¹ of internal energy.

Experimental Section

Instrumentation and experimental techniques for the determination of the two-photon pressure dependence in bromobenzene ion with ICR mass spectrometry have been previously described in detail.¹¹ Bromobenzene ions were formed by a 200-ms pulse of a nominally 11 eV electron beam. After irradiation by the Coherent Radiation I-12 argon ion laser at 514.5 nm for 1 s, the ions were sampled during a detect pulse of 4-ms duration. The pressure of bromobenzene was maintained at 2 × 10⁻⁸ Torr (ion gauge reading), while the pressure of the quench gas

Table I. Quenching Efficiencies

neutrals	ORC ^a	Z	neutrals	ORC ^a	Z
Simple Molecules					
1. ³ He	6.1	181	10. CH ₃ Cl	13.8	27
2. ⁴ He	5.3	150	11. C ₂ H ₆	9.83	24
3. Ne	3.5	100	12. C ₂ H ₅ F	13.9	19
4. Ar	5.34	77	13. C ₂ H ₅ Cl	13.2	19
5. H ₂	14.75	160	14. <i>c</i> -C ₃ H ₆	9.1	17
6. D ₂	10.5	93	15. <i>c</i> -C ₂ H ₄ O	14.7	21
7. H ₂ O	6.86	8	16. <i>n</i> -C ₃ H ₈	10.03	18
8. CH ₄	9.91	40	17. <i>n</i> -C ₄ H ₁₀	10.27	13
9. CH ₃ F	18.5	29	18. <i>c</i> -C ₆ H ₁₂	8.05	8
Unsaturated Molecules					
19. O ₂	5.75	30	25. C ₂ H ₂	9.05	12
20. N ₂	6.69	70	26. C ₂ H ₄	9.91	12.5
21. NO	6.08	28	27. C ₃ H ₆	9.98	7
22. CO	6.36	40	28. <i>i</i> -C ₄ H ₈	10.2	10
23. CO ₂	6.5	18	29. <i>c</i> -C ₆ H ₁₀	8.29	5.5
24. COS	9.8	8			
Heavy-Atom Molecules					
30. Kr	5.05	30	32. CH ₂ Br	11.5	19
31. Xe	5.64	24	33. C ₆ F ₅ I	11.5	3.5

^aORC = orbiting rate constant (10⁻¹⁰ cm³ molecule⁻¹ s⁻¹).

varied up to about 10⁻⁵ Torr (corrected). The ionization gauge pressure readings were converted into absolute pressure with use of published

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