Table I. Computed Energetics and Isotope Effects for the Substitution (SUB) and Electron Transfer (ET) Pathways in Equations 1 and 2[a]

reaction	$-\Delta E_{rxn}$	$\Delta E^{\ddagger}$(c,c)[c]	$\Delta G^{\ddagger}$(c,c)[c]	$\Delta S^{\ddagger}$[e]	α-D[e,f]	β-D[e,f]
SUB	26.3; 33 ± 3[b]	0.53; 0.071[d]	0.53	-27.9	0.582	1.080
					(0.679)	(1.062)
ET[g]	22.5; 24 ± 2[b]	0.05; 0.104[d]	0.72	-18.7	0.634	0.837
					(0.730)	(0.893)

[a] Energies in kcal/mol and entropies in eu. All thermodynamic quantities and isotope effects refer to a standard state of an ideal gas at 1 atm and T = 298.15 K. [b] Experimental ΔH° datum, based on data in ref 10. [c] Central barriers between precursor cluster and product cluster. [d] ZPE (scaled by 0.9) is included. [e] These quantities refer to the overall step from reactants to the TS. [f] α-D refers to the CH_3/CD_3 group in the α position to the nucleophile. β-D refers to the terminal CH_3/CD_3 group. Values in parentheses are obtained with *frequencies* scaled by 0.8. [g] ET without change in the original structures is endothermic by 28.25 kcal/mol at the cluster geometry and 22.5 kcal/mol at the geometry of the separated reactants.

deuterium isotope effects for the two pathways.

An important feature in Figure 1 is the clear stereochemical identities[11] of the two transition states, both belonging to the backside variety. Frontside type structures were found to be significantly higher in energy. For the ET pathway this stereospecificity is inconsistent with the idealized outersphere ET mechanism[1a] and points to a *significant innersphere character* as predicted recently on the basis of a curve-crossing analysis.[2]

Indeed, all the features of the ET-TS in the figure and table project the innersphere character of this TS. First, the spin density is delocalized and indicates significant orbital interaction between the nucleophilic and electrophilic portions of the TS. This is verified by the delocalized MO's of the species as well as by Hoffmann's fragment orbital analysis,[12] which shows a significant and dominant interaction between the HOMO (H_2S) and the SOMO ($C_2H_6^{\bullet+}$). Second, the *unusually inverse* α-D isotope effect, Table I, is dominated by bending-type modes (wagging and twisting) of the α-CH_3/CD_3 group, as expected from a "tight" TS, in the area of S_N2 TS's.[8b,13]

The entropy of activation $\Delta S^{\neq}$ serves normally as a potent mechanistic probe of polar vs ET pathways.[14] Surprisingly, $\Delta S^{\neq}$(ET) is seen to be more negative than the corresponding quantity for the SUB (polar) mechanism, contrary to what is observed in some cases and generally expected. A component analysis of $\Delta S^{\neq}$ reveals that it is the vibrational contribution which dominates the trend, $\Delta S^{\neq}$(ET) < $\Delta S^{\neq}$(SUB). The *molecular origin of this effect is the shorter bond in the ET-TS* which possesses thereby fewer accessible vibrational degrees of freedom. It seems worthwhile therefore to explore whether the opposite trend observed in ET-polar reactions of radical anions and alkyl halides does not have other origins than the postulated[14] outersphere character of the ET-TS.

Comparing the isotope effects in Table I, the largest qualitative difference is observed in the β-D isotope effect which is inverse for the ET pathway but normal for the SUB pathway. Consistent with Streitwieser's analysis,[15] this difference was found to originate in the bending-type modes of the terminal β-CH_3/CD_3 group whose frequencies increase in the ET-TS but decrease in the SUB-TS. These trends in the frequencies reflect the short C-C bond of the ET-TS as opposed to the long C-C bond in the SUB-TS (Figure 1). Indeed if it were possible to measure isotope effect, the total (α and β) effect for the ET reaction would still be inverse, while for the SUB reaction a resolution of the isotope effect would have revealed the inverse α effect as opposed to the normal β effect. Thus, the β-D isotope effect is a possible *probe of TS structure for the ET and SUB mechanisms* in our example. The applicability of this probe may carry over to other cases as well.[16]

Thus, our modeling of the polar-ET dichotomy projects an important conclusion, that the ET-TS, in organic reactions of radical cations,[2] may well possess a definite structure and robust stereochemistry which may be probed. This conclusion joins similar evidence in the literature.[2,17] and while many efforts have focused on the ET-polar dichotomy in reactions with an even number of electrons in the nucleophile and electrophile,[1a-c,g-j,18] it is in the area of odd-electron reactions[2] where possibilities lie in eventually basing the structure of the ET-TS on principles of bonding in common with the "conventional" TS's of polar mechanisms.

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Registry No. H_2S, 7783-06-4; $C_2H_6^{\bullet+}$, 34488-65-8; D_2, 7782-39-0.

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(18) In reactions with an even number of electrons the ET-TS is enforced to be outersphere. See: Shaik, S. S. *Acta Chem. Scand.* **1990**, *44*, 205.

Timing and Mechanistic Implications of Regiospecific Carbonyl Oxygen Isotope Exchange during Vitamin B_{12} Biosynthesis

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It has recently been shown[1] by ^{18}O labeling that during the biosynthesis of vitamin B_{12} (**1**; cyanocobalamin) almost complete $^{18}O/^{16}O$ exchange of the carbonyl oxygen of one of the three acetamido groups takes place, a result which has profound bearing on the mechanism of corrin biosynthesis. Thus in one hypothesis[2] δ-lactone formation from the ring-A acetate group (C-27) to C-20 has been proposed to rationalize the ring contraction of the

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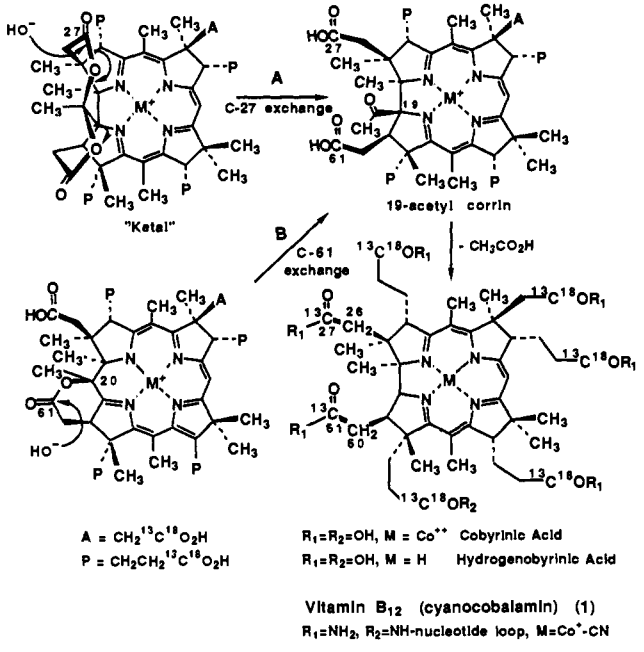
(2) Eschenmoser, A. *Angew. Chem., Int Ed. Engl.* **1988**, *27*, 6-39 and references cited therein. The mechanism suggested for the ring-contraction step is a stereoelectronically dictated dyotropic 1,2-migration of antiperiplanar groups within the dilactonic ensemble.

0002-7863/91/1513-9891$02.50/0

Scheme I



porphyrinoid to the corrinoid template (mechanism A, Scheme I) leading to incorporation of solvent label via hydrolysis. A second postulate[3] involves the sole intervention of the ring-D acetate carbonyl (C-61) in δ-lactone formation to C-20, followed by a subsequent hydrolysis step in which exchange of ^{18}O label (≥50%) exclusively at C-61 would be observed (mechanism B).

The unambiguous assignment of the ^{13}C signals for these two acetate carbonyls now becomes crucial, since the analysis for $^{18}O/^{16}O$ exchange is based on the isotopic shifts of ^{18}O on the $^{13}C{=}O$ resonances. Unfortunately, in the NMR spectra of cobalamins[4-7] the two carbonyls in question (C-27 and C-61) usually differ by less than 0.3 ppm in chemical shift and in several instances[6,7] are superimposable.

The problem was solved by enhancing the resolution of the ^1H-detected heteronuclear multiple bond (HMBC) 2D experiment via specific ^{13}C enrichment. Cyanocobalamin (**1**) was biosynthetically enriched in all seven carbonyl carbons from [1-^{13}C]ALA (**2**) using cells of *Propionibacterium shermanii*. The resulting high-resolution (<4 Hz/point in F_1) HMBC spectrum of **1** (Figure 1) clearly resolves all seven carbonyl carbons. More importantly, C-27 and C-61, separated by only 0.2 ppm in the 1D spectrum, can now be assigned unambiguously via correlation to their adjacent protons, C26H (δ 2.35) and C60H (δ 2.70) (Figure 1A,B), whose chemical shifts have been previously assigned.[5,7] This establishes that the correct assignment for C-27 (δ 178.40) had indeed been chosen in the earlier work, i.e., the acetate of ring A, rather than that of ring D, uniquely participates at least once (and possibly more than once) in reactions whose mechanisms require almost complete exchange of ^{16}O from the medium with the ^{18}O-labeled carbonyl at C-27.

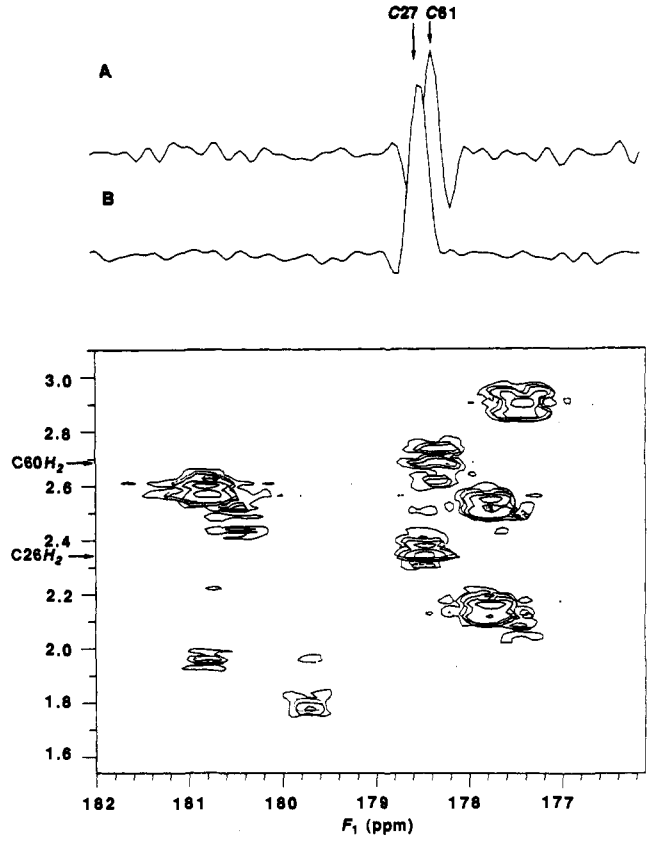


Figure 1. 2D HMBC absorptive-mode spectrum (300-MHz ^1H, 75-MHz ^{13}C) of cyanocobalamin (**1**) isolated from *P. shermanii* grown on [1-^{13}C]ALA. Spectra A and B represent slices through (→) δ 2.70 and 2.35, respectively, confirming the two bond correlations between C-61 (δ 178.28) and H-60 in A and C-27 (δ 178.40) and H-26 in B. Spectral conditions: 1.25-s repetition delay, τ_1 = 3.5 ms, τ_2 = 65 ms, SW (^1H) = 1200 Hz, SW (^{13}C) = 1000 Hz; 640 scans were accumulated for each 128t_1 increment, and the F_1 dimension was zero-filled to generate the final 512 × 512 matrix with a digital resolution of 3.9 Hz in F_1.

The next question concerns the timing of the loss of ^{18}O, i.e., before or after the formation of uro'gen III (**3**), the first tetrapyrrolic macrocycle of the B_{12} pathway. This question can, in principle, be answered by observation of the ^{18}O-induced chemical shift changes in the ^{13}C NMR spectrum of a sample of uro'gen III derived from [1-^{13}C,1-$^{18}O_2$]-5-aminolevulinic acid (ALA; **2a**), Scheme II. Although distinction can be made between the two sets of ^{13}C signals assigned to four acetate and four propionate carbonyls of uro'gen III, specific assignments for the individual acetate and propionate carbonyls cannot be made due to the symmetrical nature of the molecule. Accordingly a method for removing this pseudo symmetry was devised in order to provide unequivocal assignment for the rings-A and -D acetate carbonyls and information about possible ^{18}O exchange beyond uro'gen III, i.e., after the insertion of the first two methyl groups in the biosynthetic intermediate precorrin 2 (**4**) at C-2 and C-7. Uro'gen III (**3**) can be efficiently C-methylated in the presence of *S*-adenosyl-L-methionine (SAM) by uro'gen III methyltransferase (M-1)[8] to produce the unsymmetrical trimethylpyrrocorphin **5**

(3) Uzar, H. C.; Battersby, A. R. *J. Chem. Soc., Chem. Commun.* **1985**, 585. Leeper, F. J. *Nat. Prod. Rep.* **1985**, *2*, 561. In both mechanisms proposed in refs 2 and 3 it has been assumed that all five C-methylations and decarboxylation in ring C have taken place *before* ring contraction. The actual sequence may be modulated by the presence or absence of cobalt in the substrates. Thus in aerobic genetically engineered *P. denitrificans* the isolable intermediate precorrin 6x has already undergone ring contraction and deacylation,[13] but still has to receive C-5 and C-15 methyl groups, to suffer C-11 to C-12 methyl shift with decarboxylation, and to undergo NADPH-mediated reduction to form hydrogenobyrinic acid (Scheme I), which is not metalated enzymatically in this system. In contrast, recent studies (Müller, G.; Zipfel, F.; Hlineny, K.; Savvidis, E.; Hertle, R.; Traub-Eberhard, U.; Scott, A. I.; Williams, H. J.; Stolowich, N. J.; Santander, P. J.; Warren, M. J.; Blanche, F.; Thibaut, D. *J. Am. Chem. Soc.*, following paper in this issue) with *P. shermanii* show that while precorrins 2 and 3 accumulate in cobalt-deficient extracts, the true substrates for subsequent C-methylations (and ring contraction) are cobalt complexes. For clarity the mechanistic proposals for ring contraction are given in their original form. It will be of great interest to ascertain if the cobalt and cobalt-free sequences run in parallel or are different (i.e., in the timing of C-methylations and the ring-contraction step) due to the presence of the coordinated metal.

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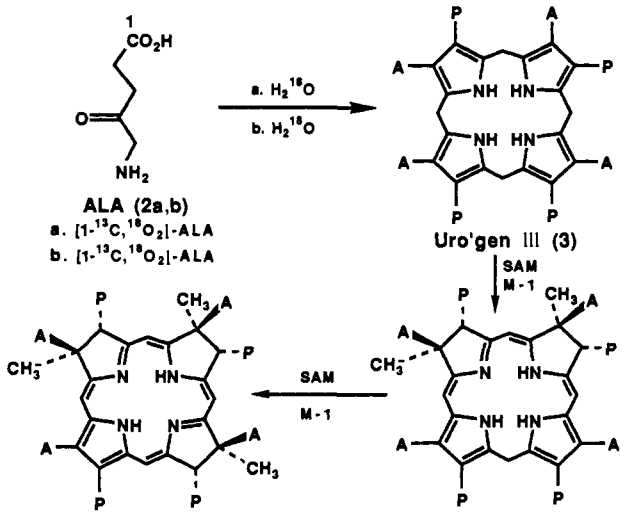
Scheme II



(via **4**, Scheme II) in which all four acetates of **5** are cleanly resolved in the ^{13}C NMR spectrum. Accordingly, **5a** was prepared from [1-^{13}C,1-$^{18}O_2$]ALA (**2a**) using the required set of enzymes[9] in $H_2{}^{16}O$. Analysis of the ^{18}O-perturbed ^{13}C chemical shifts for the acetate and propionate carbonyls of **5a** revealed that *no exchange* of ^{18}O by solvent ^{16}O had taken place. The complementary experiment using singly labeled ALA (**2b**) and $H_2{}^{18}O$ to give **5b** confirmed these results.

Thus the major exchange of the oxygen of the ring-A acetate carbonyl of cyanocobalamin with water from the medium must occur in an intermediate *after* precorrin 2 (**4**). The result is important not only in defining the timing of the exchange but also in providing a protocol for analyzing the fate of ^{18}O in the carbonyls of uro'gen III, one of which might be induced to undergo trapping of ^{16}O under conditions of high pH, as a test for possible lactonic intermediates[10] during uro'gen III formation.

Returning to the mechanism of corrin biosynthesis, it is now clear that the ring-A acetate (C-27) serves *at least once* in a scaffolding role before and/or during the ring-contraction step and is released by *hydrolysis* at C-27, whereas the ring-D acetate, which may still be involved in ketal formation of the 19-acetyl system (Scheme I), neither suffers direct hydrolysis nor participates in the hydrolytic step envisaged in a previous hypothesis.[3] The recent acquisition of genes for B_{12} biosynthesis in *Pseudomonas*[11] and in *Salmonella*[12] species may eventually allow the isolation of ring-A-lactonic intermediates whose suggested[2] intervention in the biosynthetic mechanism is reinforced by these experiments.

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Registry No. 1, 68-19-9.

(9) In each experiment (repeated in triplicate), 3 mg of the appropriately labeled ALA was incubated in the presence of ALA dehydratase, PBG deaminase, uro'gen III synthase, and uro'gen III methyltransferase in 100 mL of degassed buffer as described in ref 8.

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Timing of Cobalt Insertion in Vitamin B_{12} Biosynthesis

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The sequence of insertion of the seven *S*-adenosylmethionine-derived (SAM-derived) methyl groups of cobyrinic acid (**5a**) during its biosynthesis from uro'gen III (**2**) has been established using pulse labeling.[1,2] In *Propionibacterium shermanii*[1] it was observed that hourly pulsing with cobalt ion and [$^{13}CH_3$]SAM led to cobyrinic acid specimens whose ^{13}C methyl signal intensity ratios (in cobester **5b**) showed no evidence of deviation from those at natural abundance, indicating that cobalt is inserted either early (route a) into precorrin 2 (**3**) *or* precorrin 3 (**4**)[3,4,5] or subsequent to the five sequential C-methylation steps (at C-17, -12, -1, -5, and -15 in that order) on the way to corrin (route b), as depicted in Scheme I.

To distinguish between these two possibilities, we compared the synthetic capabilities of cell-free extracts from *P. shermanii* grown (A) in the absence and (B) in the presence of Co^{2+}. Whereas the oxidized cobalt complex of precorrin 2 (cobalt factor II; **8**)[6] could be isolated only after incubation of extract A with 5-aminolevulinic acid (ALA; **1**), SAM, and Co^{2+}, the cobalt complex of factor III[7] (**9**) was isolated directly from extract B. Extracts A and B were then partially purified (gel filtration) to remove endogenous small molecules. While the purified system A synthesizes cobyrinic acid only with the added substrates SAM/Co^{2+}/ALA or SAM/Co^{2+}/factor III, purified system B is capable of corrin synthesis in the presence of SAM but *in the absence of Co^{2+} or any other substrate.* We conclude that system

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(3) In discussing the post uro'gen III intermediates of corrin biosynthesis, it is assumed throughout that the biochemical pathway utilizes precorrins 1-3 which are at the hexahydro porphinoid oxidation level. The substances usually isolated are the corresponding (oxidized) factors I, II, and III, respectively. Both factors II and III (but not factor I) can be reduced by the cell-free system and reenter the pathway as precorrins 2 and 3, which are dipyrrocorphins.[4,5] The cobalt complexes of factors II and III discussed in this communication were used directly as substrates, their reduction to cobalt precorrins 2 and 3 in the cell-free extract being assumed.

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(6) Cobalt factor II octamethyl ester: UV/vis λ_{max} (rel ϵ) 285 (0.63), 382 (0.65 sh), 411 (1.00), 545 (0.29), 590 (0.63); FAB-MS, *m/e* 1058 (M^+ + H, 45), 1032 (M^+ + H - CN, 100).

(7) Cobalt factor III octamethyl ester: UV/vis λ_{max} (rel ϵ) 289 (0.58), 390 (0.76 sh), 420 (1.00), 551 (0.28), 594 (0.64); FAB-MS, *m/e* 1072 (M^+ + H, 50), 1046 (M^+ + H - CN, 50).

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