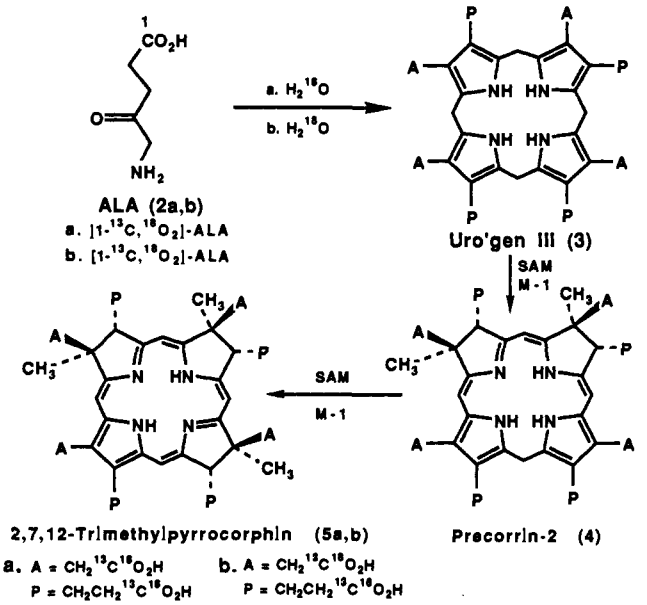
Scheme II



(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.

Acknowledgment. We thank the National Institutes of Health for their generous support of this work.

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

Gerhard Müller,* Franz Zipfel, Kersten Hlineny, Elephterios Savvidis, Ralph Hertle, and Ute Traub-Eberhard

Institut für Organische Chemie und Isotopenforschung Universität Stuttgart, D-7000 Stuttgart 1 Federal Republic of Germany

A. Ian Scott,* Howard J. Williams, Neal J. Stolowich, Patricio J. Santander, and Martin J. Warren

Center for Biological NMR Department of Chemistry, Texas A&M University College Station, Texas 77843-3255

Francis Blanche* and Denis Thibaut

Department de Chimie Analytique Centre de Recherche de Vitry Rhône-Poulenc Rorer S.A., BP14, F-94403 Vitry-sur-Seine Cedex, France

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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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Scheme I

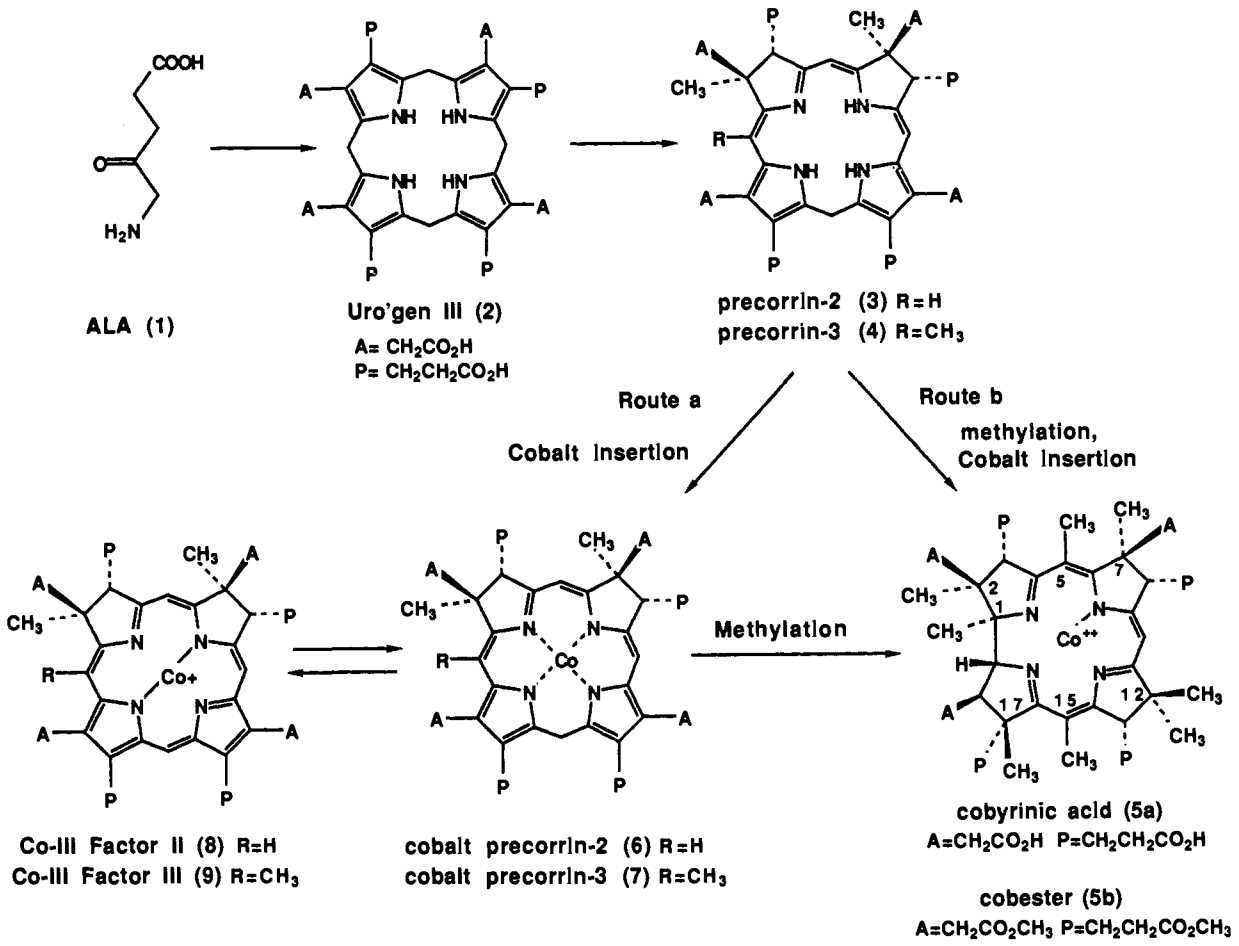

Table I. Conversion of Radioactively Labeled Isobacteriochlorins[a] to Cobyrinic Acid by Substrate-Free Enzyme Preparations of *P. shermanii*[b,c]

	substrates				isolated cobester			
expt		nmol	dpm/nmol	ratio ($^3H/^{14}C$)	nmol[d]	dpm	nmol[e]	ratio ($^3H/^{14}C$)
1	[methyl-$^3H_9$-$^{14}C_8$]	500	^3H: 5460	4.55	20	80 080		3.3
	Co–factor III		^{14}C: 1200			24 010		
2	[methyl-$^3H_9$]Co–factor III	430	3175		40	90 000	42.5	
	+ [$^{14}C_8$]factor II	430	2563			330	0.13	
3	[methyl-$^3H_9$]Co–factor III	500	6633		38	90 600	20.5	
	+ [$^{14}C_8$]Co–factor II	500	2740			59 340	21.6	
4	[$^{14}C_8$]Co–factor II	300	1735		12	22 610	13	
	+ [methyl-$^3H_6$]factor II	300	1430			<40	-	
5[f]	[$^{14}C_8$]Co–factor II	430	1306		27.5	36 822	28	
	+ [methyl-$^3H_9$]factor III	430	2094			4702	3.3	

[a] Radioactive substrates were synthesized with cell suspensions of *P. shermanii* from [4-^{14}C]ALA and/or [methyl-^{3}H]Met; cobalt insertion was carried out chemically. [b] Prepared from 80 g of wet cells in each case. [c] Time of incubation, 16 h with SAM (40 μmol) and EDTA (0.1 mM). [d] Estimated spectrophotometrically, after purification to constant specific radioactivities. [e] Estimated from specific radioactivities of substrates. [f] EDTA omitted.

B contains the cobalt complexes of precorrin 2 (**6**) and/or precorrin 3 (**7**) possibly as protein-bound species. When separate samples of extract A were preincubated (i) with factor III and (ii) with factor III + Co^{2+}, the *small molecules removed by gel filtration*, and the preparation (system C) incubated (i) with and (ii) without Co^{2+} in the presence of SAM, only procedure ii synthesized cobyrinic acid, indicating than an enzyme-bound cobalt complex of factor III (**9**) and hence cobalt precorrin 3 (**7**) had been formed during preincubation. When [$^{13}CH_3$]SAM was added to systems B or C as in procedure ii, the spectrum of isolated cobester showed no isotopic dilution.

Direct confirmation of the role of cobalt precorrin 3 (**7**) as a true substrate came from the incorporation of doubly labeled ($C^3H_3/^{14}C$) cobalt factor III with retention of two-thirds of the original $^3H/^{14}C$ ratio (Table I, experiment 1) as required for the loss of the C^3H_3 group and C-20 as acetic acid. The competition experiments (Table I, experiments 2–4) clearly demonstrate intact bioconversion, without interchange of coordinated metal, of cobalt factors II and III to cobyrinic acid, although in the absence of EDTA (experiment 5) some exchange of cobalt between the complexes can be detected. By careful experimentation it is possible to demonstrate the cell-free conversion of ^{14}C-labeled cobalt factor II in the presence of [C^3H_3]SAM to cobalt factor III which exhibits a $^3H/^{14}C$ ratio of 1.15, and to cobyrinic acid with the $^3H/^{14}C$ ratio (5.65) expected for the five introduced methyl groups.

It has not yet proved possible to determine if cobalt insertion in *P. shermanii* is an enzymatic or spontaneous process, but it is clear that previous ideas concerning the mechanism of biological C-methylation of precorrin intermediates must be revised to account for the "early" introduction of cobalt, whose modulation of the electronic configuration of the precorrins could provide the

biochemical counterpart of the regiospecificity of Eschenmoser's biomimetic C-methylation of dipyrrocorphins[8] which is controlled by the presence (or absence) of a coordinating metal. The recent isolation[9] of a cobalt-free intermediate, precorrin 6x, in which six methylations, an oxidation, and ring contraction have already occurred, from genetically engineered *Pseudomonas denitrificans*, and its bioconversion to hydrogenobyrinic acid **5a** (Co^{++} = H) strongly suggest that, in this aerobic organism, the B_{12} pathway is different from that of *P. shermanii* in that cobalt insertion is postponed in *P. denitrificans*, whereas hydrogenobyrinic acid **5a** (Co^{++} = H) is *not* a precursor of cobyrinic acid in *P. shermanii*.[10]

Further implications of these new findings are under investigation.[11]

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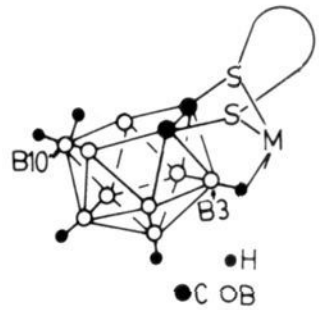

Figure 1. Schematic representation of the B(3)-H-M interaction in *exo*-dithiocarbaborane compounds.

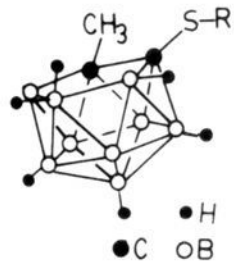

Figure 2. Molecular representation of the series of anions {7-SR-8-Me-7,8-$C_2B_9H_{10}$}. Only relevant hydrogen atoms have been indicated.

Simultaneous Conversion of Pd-PPh_3 and B-H to B-PPh_2 under Exceedingly Mild Conditions. Crystal and Molecular Structure of $PdPPh_3Cl\{7\text{-}SMe\text{-}8\text{-}Me\text{-}11\text{-}PPh_2\text{-}7,8\text{-}C_2B_9H_{10}\}$

Francesc Teixidor,*[†] Jaume Casabó,*[‡]
Antonio M. Romerosa,[†] Clara Viñas,[†] Jordi Rius,[†] and Carles Miravitlles[†]

Institut de Ciència de Materials de Barcelona and Departament de Química, Universitat Autònoma de Barcelona, Campus de Bellaterra, Cerdanyola 08193 Barcelona, Spain

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We report an unexpected reaction that has implications for organoborane/organocarborane chemistry, the consequences of which are currently being tested in our program in asymmetric catalysis. Our research on the coordination chemistry of macrocyclic derivatives of *exo*-dithiocarbaborane compounds[1-6] has shown the singularity of their chemistry: great reactivity resemblance with diphosphines and, remarkably, the ability to form the unprecedented B(3)-H-M bond (Figure 1) which is modulated by the length of the exo-cluster macrocyclic chain.[7] This observation led us to the synthesis of $[N(CH_3)_4][RhCl\{7,8\text{-}\mu\text{-}S(CH_2CH_2)S\text{-}C_2B_9H_{10}\}\{\sigma\text{-}7,8\text{-}\mu\text{-}S(CH_2CH_2)S\text{-}C_2B_9H_9\}]$,[8] the first reported example of a B(3)-M interaction. All metal complex derivatives of these *exo*-dithiocarbaborane ligands contain the five-membered ring $\overline{S_a\text{-}M\text{-}S_b\text{-}C_c\text{-}C_c}$ as a common feature. The geometrical requirements of this ring together with the sulfur-carbaborane interaction have to be, in great part, responsible for this novel chemistry. To get further insight in the sulfur-carbaborane mutual influence, we have studied the reactivity of *exo*-monothiocarbaborane compounds. Even though there is only one sulfur atom on the molecule, the system evolves to the formation of a new five-membered $\overline{S_a\text{-}M\text{-}P\text{-}B\text{-}C_c}$ ring.

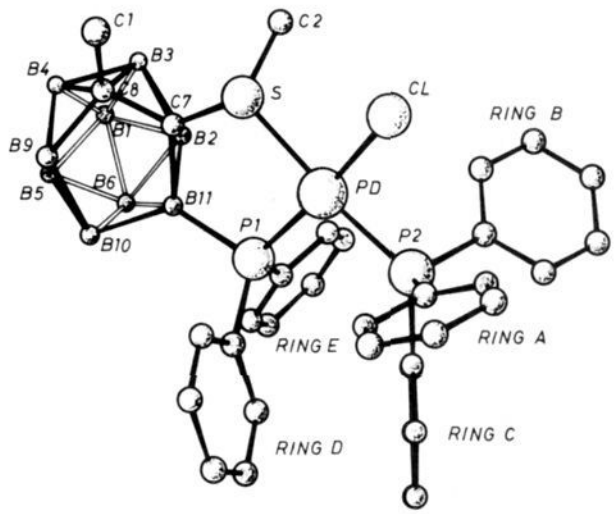

Figure 3. Molecular structure of **3** (hydrogens omitted). Selected intramolecular distances (Å) angles (deg) are as follows: Pd-S 2.355 (1); Pd-P1, 2.266 (1); Pd-P2, 2.309 (1); Pd-Cl, 2.342 (1); P1-B11, 1.922 (5); C7-B11, 1.609 (7); C7-S, 1.803 (5); C7-C8, 1.573 (6); C8-B9, 1.651 (8); B9-B10, 1.853 (9); B10-B11, 1.783 (7); S-Pd-P1, 90.2 (0); P2-Pd-P1, 100.2 (0); Cl-Pd-P2, 85.0 (0); Cl-Pd-S, 84.4.

The reaction of $[NMe_4]\{7\text{-}SMe\text{-}8\text{-}Me\text{-}7,8\text{-}C_2B_9H_{10}\}$ (**1**) (Figure 2) with $Pd(PPh_3)_2Cl_2$ (**2**) in degassed ethanol resulted in the formation of a yellow solid (**3**). Absorptions (ppm) at -4.5, -6.3, -8.9, -12.9, -14.1, -20.15, -23.1, -27.2, and -33.8, all of intensity 1, are observed in the ^{11}B NMR {H} spectrum. The absorption at -8.9 was split in two equal absorptions in the ^{11}B NMR and ^{11}B NMR {H} spectra (J = 200 Hz), suggesting B-P bond formation. It was previously known that thermolysis of the *closo*-3,3-(triarylphosphine)$_2$-3,1,2-$NiC_2B_9H_{11}$ series in benzene solution leads to the formation of the corresponding *closo*-3,8-(triarylphosphine)$_2$-3-H-3,1,2-$NiC_2B_9H_{11}$.[9] Similar ligand-interchange

[†] Institut de Ciència de Materials de Barcelona.
[‡] Departament de Química, Universitat Autònoma de Barcelona.

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