EPR Study of the Photolysis of Methyl- and Adenosylcobinamides in the Presence of Phosphine and Pyridine Bases. Evidence for the Need of a Judicious Choice of Irradiation Temperature and Solvent to Assess Ligand Binding

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The products of anaerobic photolysis of methylcobinamide ($MeCbi^+$) and adenosylcobinamide ($AdoCbi^+$) in the presence of phosphine and pyridine bases have been studied in methanol, at two irradiation temperatures (77 and 300 K) by EPR spectroscopy. The presence of cobalt(II) species and organic radicals in solution is indicative that homolytic cleavage of the Co−C bond is induced upon irradiation with visible light. The EPR results obtained upon photolysis of cobinamide solutions in the presence of several cobinamide:phosphorus base molar ratios show that only for a large excess of base is the 1:1 adduct quantitatively formed, and is, in those experimental conditions, the single Co(II) species in solution. For molar ratios below 1:20 the EPR spectra allow identification of a mixture of the 1:1 adduct and a species with no phosphorus ligand bound to the cobalt(II) center. For pyridine derivatives, (a) in equimolar solutions of cobinamide:nitrogen base, EPR spectra exhibit one signal characteristic of the five-coordinate species, and (b) in the presence of a large excess of base a less intense EPR signal characteristic of a 1:2 adduct is observed together with that of the 1:1 adduct. Photolysis in frozen matrix allowed us to confirm that phosphines do not show a propensity to bind to the organocobalt(III)(corrin)$^+$ compounds but do bind to the $[Co^{II}(corrin)]^+$ species formed upon photolysis, thus evidencing the reactivity of the latter. Another aspect concerning ligand binding is the fact that the choice of the solvent may be determinant in the assignment of coordination numbers. EPR parameters and spin densities calculated for the Co(II) species are in agreement with those described for a $^2A_1$ ground state and vary according to basicity of pyridine bases and with Tolman cone angle for the phosphines, confirming that both electronic and steric effects are important to determine the type of adducts formed and the usefulness of EPR parameters to get insight into structural changes regarding the coordination of axial ligands. The possibility of establishing such correlations is recently becoming more important, as in many of the processes catalyzed by B_{12} cofactors displacement of axial ligands is observed and proved to be determinant to the enzymatic mechanism.

Introduction

B_{12} system compounds have been the object of intense interdisciplinary research since the identification of the presence of the cofactors in enzymatic processes regarding the transfer of hydrogen atoms, methyl groups, and other molecular rearrangements.[1–8]

The physiologically relevant B_{12} derivatives are light sensitive and chemically labile organometallic cofactors, and the cleavage of the cobalt−carbon bond is known to be the key step for all the enzymatic mechanisms in which they are involved. For coenzyme B_{12} the mechanism of the holoenzyme-catalyzed reaction is attributed to homolytic cleavage of the Co−C bond, while for methylcobalamin mechanistic studies point to heterolytic bond fission.[9,10]

Investigations performed in different enzymatic reactions allowed identification of *base-on* (coordination of 5,6-dimethylbenzimidazole) and *base-off* (absence of coordination or ligand replacement) active forms of the cofactor. Replacement of the axial ligand 5,6-dimethylbenzimidazole by histidine has been reported for *methionine synthase, methylmalonyl-CoA mutase*, and *glutamate mutase*. In crystals of Co(II) corrins, external axial ligands have been found to coordinate at the less hindered

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β-face of the Co(II) center,[11–13] whereas in B_{12r} the nucleotide base is intramolecularly directed to the α-face of the Co(II) center.[14]

The relationship between the catalytic activity and the nature of the axial ligand and its involvement in the enzymatic reactions is undoubtedly one of the most important aspects to be clarified in this field, particularly concerning bond homolysis.[8]

Considering that axial base effects are greater on cob(II)alamin and on the transition state than in the ground state,[15–17] the characterization of the cobalt(II) complexes formed upon homolytic cleavage of the Co−C carbon can provide valuable information on the elucidation of B_{12} mechanisms.

Homolytic Co−C bond fission can be promoted by photolysis, and this method has been used in many studies performed with organocobalamins and B_{12} model compounds to characterize the homolysis products and to get insight into the factors that may influence the mechanisms of cobalt−carbon bond cleavage.[18–35]

On the basis of evidence provided mainly from EPR data Giannotti et al. concluded that following light absorption there are two chemical processes, one being the loss of a hydrogen atom from the equatorial ligand and the other the homolytic fission of the cobalt−carbon bond, with subsequent formation of organic radicals and cobalt(II) species. For alkylcobalamins and coenzyme B_{12} Symons et al.[19] proposed that the latter mechanism must be operative following light absorption. Irradiation causes electron transfer from the corrin ring to the Co(d_{z^2}) σ^* orbital, and the antibonding character of this orbital is relieved by homolytic fission of the Co−C bond.

More recently, Sension et al.[36–39] have been using time-resolved absorption spectroscopy to investigate the photolysis of the B_{12} coenzymes as a function of solvent environment, particularly in what concerns its influence on intermediate states. Their very interesting studies corroborate the formation of cobalt(II) species and organic radicals as photolysis primary products and show that recombination rate is solvent-dependent, reflecting the competition between recombination, cage escape, and formation of caged radical pairs.

Our group has been interested in studying photolysis of B_{12} model compounds, particularly the influence of electronic and steric contributions of axial bases on the labilization of the cobalt−carbon bond. For that purpose we selected a set of axial nitrogen and phosphorus ligands and synthesized alkyl derivatives of cobaloximes, imino-oxime, and Schiff base compounds and characterized the Co(II) species formed upon photolysis by EPR spectroscopy. Parallel studies have been performed on solutions of the aquoalkyl derivatives, which are particularly useful as ligands for which isolation of the organocobalt(III) compound could not be achieved. Photolysis experiments performed at three temperatures allowed us to identify photolysis primary products and to study its reactivity by characterizing new species arising from further reaction in solution.

The results obtained for the set of compounds studied show that homolysis of the Co−C bond is generally induced by visible light with the exception of some methyl derivatives in which stronger bonds are observed.[30] Photolysis primary products have been characterized as Co(II) species and organic radicals, and we found that the type of Co(II) species formed as secondary products strongly depends on the nature of the axial Lewis base.

For all the Co(II) species formed we determined the spin-Hamiltonian parameters as well as spin densities on the cobalt atom and the nitrogen and phosphorus ligand donors. The values of the obtained parameters correlate with proton affinities of the N-donor bases and values of the Tolman cone angle (TCA) for the phosphorus bases, thus implying that both basicity and steric hindrance are involved in stabilizing a five-coordinate species or favoring the coordination of a second axial ligand.

Regarding the influence of the equatorial ligand, we may infer that its type is not crucial in terms of the nature of the photolysis primary products, but its flexibility is of major importance in determining the number of ligands that the Co(II) center is able to accommodate and consequently the reactivity of photolysis primary products toward available substrates present in solution media.

Taking into account that the model systems most closely related to cobalamins are cobinamides and the important results reported by Marzilli et al.[8] on the photolysis of methylcobinamide, we felt that to complete our work it was imperative to study the anaerobic photolysis of cobinamides in the presence of the set of P- and N-donor axial ligands we had used previously.

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Table 1. EPR Spin-Hamiltonian Parameters and Spin Densities (values of the A tensor components are expressed as × 10^{-4} cm^{-1})

	interaction with Co										interaction with axial base				
axial base	PA	TCA	g_x	g_y	g_z	A_x	A_y	A_z	ρ_{3d}	ρ_{4s}	A_x	A_y	A_z	A_{iso}	ρ_L
py	924		2.281	2.221	2.003	15	5	105	0.72	0.06	14	13	18.9	15	0.15
4-CNpy	882		2.288	2.224	2.003	20	20	108	0.69	0.06	14	13	18.5	15	0.14
4-Etpy	940		2.28	2.22	2.003	12	5	104	0.73	0.06	14	13	19	15	0.15
4-Mepy	942		2.279	2.22	2.003	12	5	104	0.73	0.06	14	13	19	15	0.15
4-N(Me)$_2$py	970		2.277	2.21	2.003	7	18	101	0.67	0.06	14	13	20	16	0.17
3-CNpy	882		2.289	2.223	2.003	20	23	109	0.69	0.06	14	13	18.4	15	0.13
3-Clpy	899		2.287	2.223	2.003	19	18	108	0.69	0.06	14	13	18.6	15	0.14
3-Brpy	900		2.287	2.223	2.003	19	18	108	0.69	0.06	14	13	18.6	15	0.14
3-Etpy	937		2.28	2.22	2.003	12	5	105	0.73	0.06	14	13	18.9	15	0.15
3-Mepy	938		2.28	2.22	2.003	12	5	105	0.73	0.06	14	13	18.9	15	0.15
PMe$_2$Ph		125	2.177	2.157	2.003	−44	−20	73	0.68	0.06	200	220	246	222	0.15
PEt$_3$		130	2.195	2.165	2.003	−35	−20	74	0.68	0.06	190	206	232	209	0.15
PBu$_3$		132	2.195	2.165	2.003	−35	−20	74	0.69	0.06	190	206	232	209	0.15
PMePh$_2$		137	2.19	2.171	2.003	−43	−20	79	0.69	0.06	185	205	230	205	0.14
PBuPh$_2$		145	2.192	2.174	2.003	−43	−20	79	0.7	0.06	180	200	224	201	0.14

In the present work we report the EPR characterization of the photolysis products of methylcobinamide ($MeCbi^+$) and adenosylcobinamide ($AdoCbi^+$) in the presence of phosphine and pyridine bases in methanol. The behavior of the cobalt(II) fragments formed upon photolysis toward excess base present in solution is also analyzed.

Experimental Section

Reagents and Synthesis. The solvents were from Merck, and other chemicals from Aldrich. All solvents used in EPR experiments were purified by standard methods[40] and kept under an atmosphere of nitrogen. The alkylcobinamides were prepared from the corresponding alkylcobalamins by hydrolysis in aqueous solution at 80 °C with cerium(III) hydroxide, as described in the literature.[41,42] The compounds were purified by passing sequentially through two chromatographic columns, one with a SP Sephadex resin and the other with a DEAE resin, both from Sigma. The authenticity and purity of the compounds prepared were monitored by ^1H NMR spectroscopy on D_2O solutions, in a Bruker AM200 (200 MHz) spectrometer. The ^1H NMR spectra obtained for the prepared compounds are identical to those described for $AdoCbi^+$ and $MeCbi^+$ and clearly show that the organic radical is bound to the cobalt center, while the benzimidazole nucleotide has been removed.[8]

Sample Preparation. All manipulations were carried out under nitrogen; the solvents were deoxygenated with nitrogen, and all solids were degassed under vacuum. The samples to be irradiated were prepared by adding the corresponding N- or P-donor bases to solutions of the alkylcobinamide complexes in methanol. Photolyses of resulting solutions were performed with a Hg lamp (250 W Philips HP/T; $\lambda = 560-580$ nm) in anaerobic conditions at two different temperatures (77 and 300 K). Samples were irradiated up to 1 h, and the results were independent of irradiation time.

Visible Spectroscopy. Optical spectra of samples of methanolic solutions of the alkylcobinamides titrated with the appropriate Lewis base were recorded on a Shimadzu UV-3101 PC spectrometer in order to establish the molar ratios at which displacement of the axial water molecule is achieved.

EPR Spectroscopy. The X-band EPR spectra were obtained on a Bruker ESP E-300 instrument with a Bruker variable-temperature unit. Spectra were calibrated with diphenylpicrylhydrazyl (dpph), and the magnetic field was calibrated by using Mn^{2+} in MgO.

Computer Simulation. EPR spectra of cobalt(II) compounds were simulated using a program based on Pilbrow's formalism,[43] which uses a spin-Hamiltonian of the type $H = H(\text{Zeeman}) + H(\text{hyperfine}) + H(\text{ligand})$. The hyperfine term was deduced assuming a C_{2v} point-group symmetry at the cobalt center and the noncoincidence of the g and A(Co) tensor axes in the xy plane. As no interaction of the unpaired electron with the nitrogen atoms of the equatorial plane was observed, the ligand term includes only the nitrogen axial interaction, and it was assumed that the nitrogen tensor has the same principal axis as that of the g tensor. For the simulation of the spectra of the compounds reported, the best fit was obtained for collinear g and A tensors in the x and y plane (rhombic symmetry) with neglect of any quadrupolar contribution to the spin-Hamiltonian. The results obtained are registered in Table 1.

Cobalt Spin Density Calculation. Analysis of the g and cobalt hyperfine tensors allows deduction of the unpaired spin density in the metal 3d and 4s orbitals. The procedures for this have been fully described by us[27] and are based on those of McGarvey.[44] The results obtained are shown in Table 1.

Ligand Spin Density Calculation. From the observed hyperfine coupling to the axial donor nitrogen and phosphorus atoms, spin density on base nitrogen 2s and 2p orbitals and on base phosphorus 3s and 3p orbitals could be determined. The procedures for these calculations have already been described,[29,31] and the results are shown in Table 1. No hyperfine coupling with the equatorial nitrogen atoms was observed in the experimental conditions used.

Results

Adenosylcobinamide and methylcobinamide can be described as derivatives of the B_{12} cofactors that have the axial benzimidazole nucleotide chemically removed, thus bearing as ligands the corrin and the organic radicals. In solution, the presence of a solvent molecule is uncertain, and high-pressure spectroscopic techniques evidenced an equilibrium between five-coordinate species and six-coordinate aqua(solvato) forms.[45,46] Solutions of such compounds are EPR silent and remain so for a few days while kept in the dark. Upon exposure to light, observation of EPR signals, namely, spectra characteristic of cobalt(II) species, is indicative of homolytic cleavage of the Co−C bond.

In the present work, solutions of methylcobinamide and adenosylcobinamide were irradiated with visible light, in fluid

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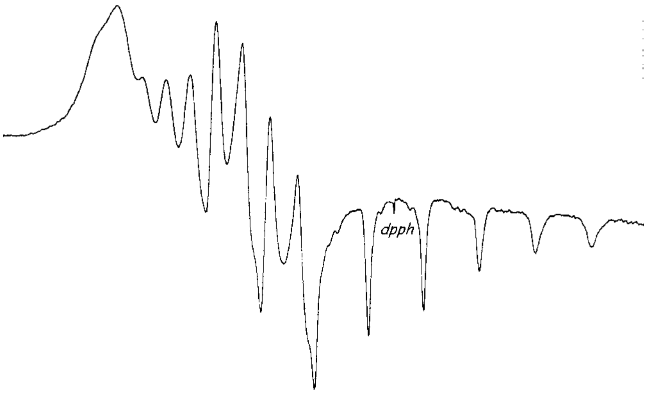

Figure 1. Frozen solution, 100 K, EPR spectrum of adenosylcobinamide irradiated at RT.

and frozen solution, in the absence and in the presence of Lewis bases. Monitoring of Co−C bond cleavage and characterization of the species formed were achieved using EPR. The extension of Lewis base coordination in the sixth position was checked prior to photolysis experiments by electronic spectroscopy since it has been described in the literature that a red shift is observed in the spectrum as a ligand molecule coordinates.[8,47] For B_{12} model compounds, such as alkylaquacobaloximes, the displacement of an axial water molecule is quantitative at a 1:1 molar ratio, but that is not the case for all model compounds. For alkylcobinamides it has been reported that in methanol and in the presence of pyridine or other N-donor bases the compounds undergo fast replacement of water molecules, and in the presence of excess base only the base-bound alkylcobinamide is observed.[9] For P-donor bases the situation is different, and it has been reported that for none of the phosphine bases used was binding to the cobalt(II) center detected, thus implying that water replacement did not occur even in the presence of a large excess of phosphorus base.[10] In order to confirm ligand displacement/base coordination in our samples, methanol solutions of $AdoCbi^+$ and $MeCbi^+$ were titrated with the Lewis bases used in this work. For N-donor bases it was observed that optical spectra significantly change with the addition of base, and quantitative displacement is achieved with a large excess of base. For P-donor bases no significant changes were detected as the amount of base is raised.

Irradiation of Methylcobinamide and Adenosylcobinamide in Fluid Solution. Solutions of $AdoCbi^+$ and $MeCbi^+$ irradiated at room temperature yield EPR spectra that are indistinguishable from those reported for base-off cob(II)alamin (B_{12r})[18,26,37] and that exhibit at 100 K large g-tensor anisotropy. The spectra observed (Figure 1) are characteristic of low-spin d^7 ($S = ½$) cobalt(II) species, clearly evidencing eight lines representative of the interaction of the unpaired electron with the cobalt nucleus (^{59}Co, $I = 7/2$).

Irradiation of Fluid Solutions of Alkykcobinamides in the Presence of P-Donor Bases. EPR spectra obtained upon irradiation of $MeCbi^+$ or $AdoCbi^+$ in the presence of variable amounts of phosphorus base show evidence of significant changes as the cobinamide:phosphorus base ratio is raised. The spectra obtained for PBu_3 at molar ratios (1:1, 1:5, 1:20) are depicted in Figure 2.

The three spectra are clearly different, and a comparative analysis, taking into account the spectrum obtained for photolysis of cobinamides in the absence of base (Figure 1), is indicative of the formation of a new species as the amount of L base increases. Spectra a and b are undoubtedly the superposition of two signals that have different intensities according to the amount of base in solution. Spectrum a is very close to that of the species $[Co^{II}(corrin)]^+$, just showing additional lines in the high-field region indicating the binding of an axial ligand and that are more noticeable in spectrum b. The spectra depicted in a and b are thus assigned to a mixture of the species $[Co^{II}(corrin)]^+$ and $[Co^{II}(corrin)L]^+$ in different percentages according to the molar ratio used.

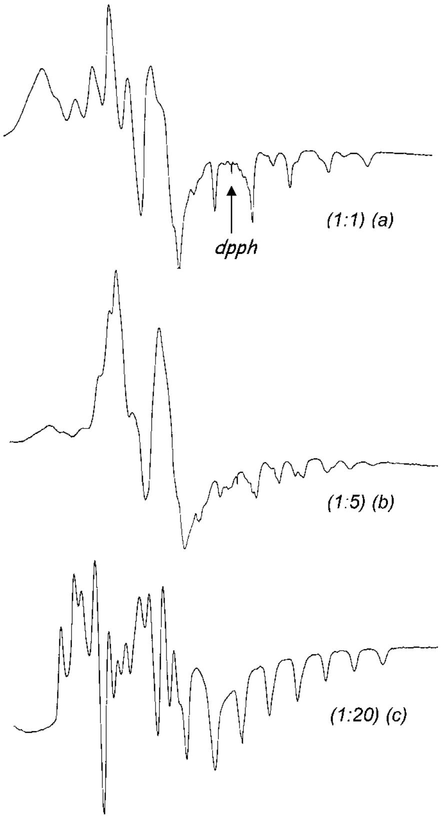

Figure 2. Frozen solution, 100 K, EPR spectra of adenosylcobinamide irradiated at RT in the presence of PBu_3.

Spectrum c is clearly due to a single species and resembles those obtained for 1:1 adducts of cobaloximes[28] and iminooxime[32] compounds with phosphines. The spectrum exhibits in the high-field region hyperfine coupling to a cobalt nucleus (^{59}Co, $I = 7/2$) and to one phosphorus nucleus (^{31}P, $I = ½$), is characteristic of a five-coordinate low-spin d^7 ($S = ½$) cobalt(II) compound, and so is assigned to the species $[Co^{II}(corrin)L]^+$. The single spectrum of the five-coordinate compound could not be observed for PPh_3, for which the EPR spectra are always a mixture of the two signals, suggesting that coordination of PPh_3 to $[Co^{II}(corrin)]^+$ is not quantitatively achieved.

The formation of a six-coordinate species, with two bound phosphine molecules, was never achieved even in the presence of very large excess of base.

Irradiation of Fluid Solutions of Alkykcobinamides in the Presence of N-Donor Bases. Solutions of $AdoCbi^+$ or $MeCbi^+$ in the presence of N-donor bases in a 1:1 molar ratio and irradiated at room temperature exhibit frozen solution EPR spectra, which resemble those of cob(II)alamin. The spectra are characteristic of low-spin d^7 ($S = ½$) cobalt(II) species and show in the high-field region hyperfine coupling to a cobalt(II) nucleus and superhyperfine coupling to an axial nitrogen atom. Each cobalt hyperfine line is split into three with relative intensities

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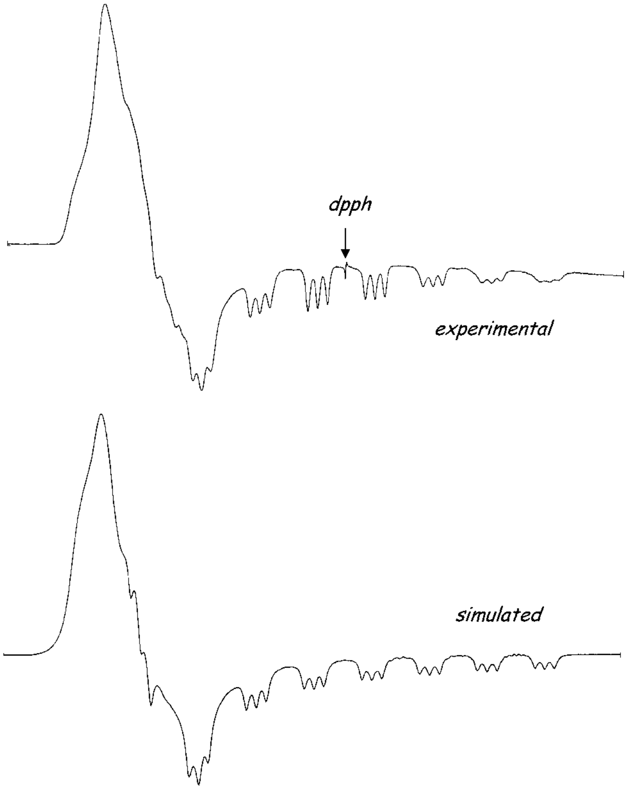

Figure 3. Frozen solution, 100 K, EPR spectra of adenosylcobinamide irradiated at RT in the presence of pyridine.

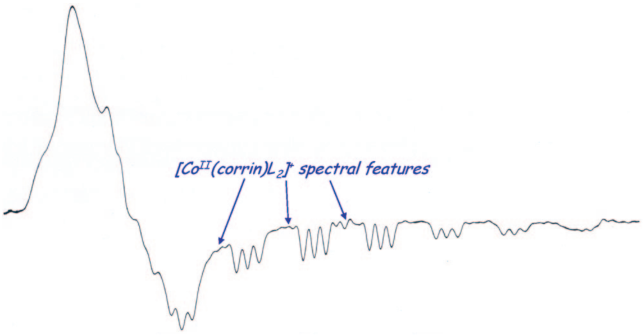

Figure 4. Frozen solution, 100 K, EPR spectra of adenosylcobinamide irradiated at RT in the presence of a large excess of pyridine.

1:1:1, a pattern characteristic of interaction of the unpaired electron with the cobalt atom with one nitrogen atom (^{14}N, $I = 1$) (Figure 3), thus indicating the formation of a cobalt(II) species with one N-donor base coordinated to the metal center, [Co^{II}(corrin)L]$^+$.

Photolysis in the presence of a very large excess of N-donor base yields solutions that exhibit EPR spectra that show two superimposed signals, one of which is the signal of the [Co^{II}(corrin)L]$^+$ species (Figure 4).

The new features correspond to a new set of cobalt hyperfine lines, which exhibit a smaller spacing and consequently a smaller A_z component of the cobalt hyperfine tensor and which are split into five, thus indicating the axial coordination of two nitrogen bases and providing evidence for the formation of the six-coordinate species, [Co^{II}(corrin)L$_2$]$^+$. The intensity of the new signal increases slightly as the amount of N-donor base is raised, but is always observed together with the signal characteristic of the five-coordinate species [Co^{II}(corrin)L]$^+$, even in the presence of a 50-fold excess of base. The much lower intensity of the EPR signal of the 1:2 species may be due to the fact that the corrin ligand is not flexible enough to accommodate the sixth ligand, thus favoring the five-coordinate species.

Results obtained for B$_{12}$ model compounds, namely, cobaloximes,[31] imino-oxime compounds,[34] and some Schiff bases,[35] show that the formation of 1:2 adducts with cobalt(II) species and N-donor bases is favored in compounds with more flexible equatorial ligands that can adjust according to the characteristic of the incoming ligand. For cobaloximes and imino-oxime compounds signals characteristic of six-coordinate species are observed for 3- and 4-substituted pyridines even for a 1:1 molar ratio,[31] while for a bulky Schiff base coordination of a second pyridine molecule was never observed.[35]

Irradiation of Methylcobinamide and Adenosylcobinamide in Frozen Solution. Irradiation, at 77 K, of frozen solutions of AdoCbi$^+$ or MeCbi$^+$ produced samples that are EPR silent and whose NMR spectra in CD$_3$OD are indistinguishable from those obtained before irradiation, thus demonstrating that no light-induced process has occurred.

Irradiation in the Presence of a Large Excess of P-Donor Bases. Irradiation at 77 K of MeCbi$^+$ and AdoCbi$^+$ in the presence of phosphine bases yields EPR-silent solutions. Furthermore, no EPR signals are detected after a thermal cycle in which the sample is brought up to RT and recooled again, thus implying that no overall reaction occurred upon photolysis.

Irradiation in the Presence of a Large Excess of N-Donor Bases. Contrasting with the previous results, the behavior observed for MeCbi$^+$ and Ado Cbi$^+$ when samples were irradiated at 77 K is different.

Solutions of AdoCbi$^+$ irradiated at 77 K in the presence of the N-donor bases are EPR silent, indicating that the cobalt−carbon bond was not cleaved in these conditions.

Frozen solution EPR spectra, obtained after irradiation of MeCbi$^+$, at 77 K, in the presence of N-donor bases are similar to those described in the literature for methylcobalamin.[19] Each spectrum (Figure 3) shows one signal identical to that ascribed to the five-coordinate cobalt(II) species, [Co^{II}(corrin)L]$^+$, and an intense and much narrower signal centered at $g \approx 2.00$, which is assigned to an alkyl radical or to a reaction product of the "hot" alkyl radicals with solvent molecules The spectra also exhibit additional features, which are identical to those observed after photolysis of methylcobalamin and that have been proposed to arise from formation of radical pairs.[19,48] The EPR spectra of these solutions change as the temperature is raised with the observation of broadening of the cobalt bands and loss of intensity of the radical signal. After relaxation of the matrix to room temperature, recooling the solutions to 77 K results in EPR spectra with well-resolved cobalt(II) features that are identical to those obtained for the photolysis in fluid matrix.

Spin-Hamiltonian Parameters and Spin Densities. The EPR spectra of the Co(II) five-coordinate species were simulated and the spin densities on the cobalt and axial ligands donor atoms were determined as described in the Experimental Section; the results obtained are shown in Table 1.

Discussion

Photolysis Mechanism. Irradiation in fluid solution of methyl- and adenosylcobinamides in the absence and presence of Lewis bases resulted always in homolysis of the cobalt−carbon

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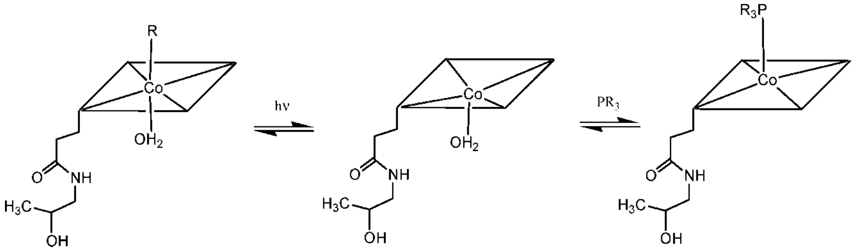
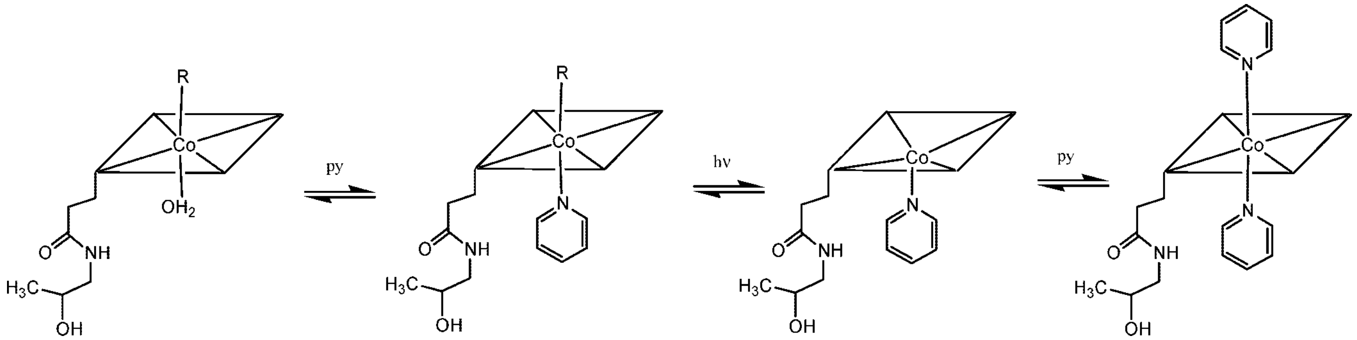
(48) Pezeshk, A.; Coffman, R. E. *J. Chem. Soc., Dalton Trans.* **1985**, 891–893.

Scheme 1. Coordination of a Phosphine Base



Scheme 2. Coordination of a Pyridine Base



bond, as confirmed by the detection in solution of EPR signals characteristic of Co(II) species.

The results obtained for irradiation of the cobinamides in frozen matrix at 77 K were somehow different, as homolysis of the cobalt–carbon bond was induced only in the presence of N-donor bases. Photolysis at 77 K of AdoCbi$^+$ or MeCbi$^+$ in the absence of base did not produce paramagnetic species, a fact that can be rationalized by the lack of the *trans* effect of the axial base, which weakens the Co–C bond. A difference of ca. 5 kcal mol^{-1} has been reported for the BDE values of the Co–C bond in the *base-on* and *base-off* forms of adenosylcobalamin,[49] and we observed in a previous work regarding the photolysis of methylcobaloximes[32] that such a difference was sufficient to prevent bond cleavage, which could be achieved using an UV light source as an alternative to visible light.

Irradiation at 77 K of AdoCbi$^+$ or MeCbi$^+$ in the presence of P-donor bases also did not result in bond homolysis. The result contrasts with those reported for several organocobalt(III) B_{12} models in which the homolytic cleavage of the Co–C bond was more easily achieved than for the corresponding N-donor base adducts, a fact explained by the decrease in the strength of the Co–C bond resulting from structural modifications imposed by the bulkier groups of the axially bounded P-donor base.[30] In the present case, we think that as the extent of coordination upon addition of the P-donor base to the alkylcobinamide is very low, the photolyzed solution contains, initially, a very small amount of alkylcobalt(III) phosphine complex and an appreciable amount of the base-off forms.

The EPR spectra of photolyzed solutions of MeCbi$^+$ in the presence of N-donor bases exhibit at 77 K bands assigned to a cobalt(II) five-coordinated adduct and a strong and narrow signal at $g \approx 2.00$ attributed to an alkyl radical. After relaxing the irradiated solutions at 77 K to room temperature and recooling, the EPR spectra exhibit the same cobalt(II) fragments produced when photolysis is performed in fluid solutions.

Solutions of AdoCbi$^+$ prepared in the same conditions did not yield detectable paramagnetic species, a result that is identical to that reported by Symons et al. for coenzyme B_{12} and that was explained as occurrence of homolysis followed by back-reaction of the large adenosyl radical with B_{12r} since the radical cannot escape and diffuse away to react with proximal solvent molecules.[19,30]

Photolyses of frozen and fluid solutions of alkylcobalamins,[19–21] alkylcobaloximes,[29–34] and alkyl-Schiff base-cobalt(III) compound[35] in the presence of pyridine or phosphine bases and of fluid solutions of methylcobinamide (MeCbi$^+$) in the presence of pyridine and quinoline bases[8] have been reported in literature. The results have been interpreted assuming that on light absorption an electron from the equatorial ligand moves into the antibonding Co(d_{z^2}) σ* orbital, thus leading to the homolytic fission of the Co–C bond, with formation of an organic radical and a five-coordinate cobalt(II) complex as photolysis primary products.

Considering the results described, we propose that the same mechanism must be operative for the photolysis of the AdoCbi$^+$ and MeCbi$^+$ studied in this work.

Comparing the results obtained here for cobinamides and those of the aquoorganocobalt(III) model compounds, it seems evident that with respect to induction of homolysis of the Co–C bond by visible light the differences reside essentially in the extent of the reaction of displacement/coordination of axial ligands that occurs prior to photolysis and that is quantitatively achieved for N-donor bases and not for P-donor bases. However, when formation of the cobinamide Co(II) fragments occurs in fluid solution, these react with the added phosphines, giving the corresponding five-coordinate species.

Photolysis experiments in frozen matrix were crucial to confirm that phosphines do not bind to the organocobalt(III)(corrin)$^+$ compounds but do bind to the Co(II)(corrin)$^+$ species formed upon photolysis, thus evidencing the reactivity of the latter.

The EPR spectra after irradiation of solutions with a large excess of P-donor base exhibit only the signal of the [Co^{II}(corrin)L]$^+$, indicating that the [Co^{II}(corrin)H_2O]$^+$ species does coordinate one phosphine molecule. We think that coordination must occurr in the less hindered β-face of the cobalt(II) center, leading to a β-phosphine-Co(II)-corrin, as has been suggested

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(49) Hay, B. P.; Finke, R. G. *J. Am. Chem. Soc.* **1987**, *109*, 8012–8018.

for the coordination of other external ligands. EPR parameters are characteristic of nonsolvated five-coordinate cobalt(II) adducts, and consequently the water molecule must have been displaced (Scheme 1).

For N-donor bases, the EPR experiments of photolysis at 77 K provide strong evidence that organocobalt(III) pyridine derivatives are formed prior to photolysis, as the five-coordinate Co(II) species is observed in frozen matrix before any temperature relaxation. In this work we observed that coordination of a second pyridine molecule occurred leading to formation of six-coordinate species (Scheme 2), although the extension of the reactions is small, suggesting that the coordination at the sixth position of the cobalt atom is hindered.

The detection of an EPR signal with features characteristic of the coordination of a second pyridine molecule contrasts with the result reported by Marzilli et al.,[8] who could not observe such a signal even in neat pyridine and at 120 K. We would like to stress that the signal we observed is much less intense than the one due to the five-coordinate species, indicating that a small percentage of species coordinates a second pyridine molecule. Also, we believe that the reason for this discrepancy may arise from the fact that we are using methanol as a solvent instead of ethylene glycol or pyridine and registering the spectra at a lower temperature (100 K). The extreme importance of the solvent and temperature with respect to spectra resolution is well known, and in particular, in the case of the B_{12} system compounds this fact has been reported by Bayston et al.,[18] who also detected the coordination of a second pyridine molecule in EPR experiments performed in methanol, and more recently by Van Doorslaer et al.,[7] who reported the same result and demonstrated that axial binding is temperature dependent and correlates with the phase transition of the solvent.

The much lower intensity of the 1:2 signal seems to be an indication that the coordination at the β-face of Co(II)-corrin species must be hindered. Previous studies on adducts formed by cob(II)alamin (B_{12r}) with N-donor bases have revealed an identical behavior, since it as been observed that in the presence of excess axial base the EPR spectra exhibit a signal attributable to B_{12r} superimposed onto a much weaker signal assigned to a six-coordinate species.[28] These results indicate that the five-coordinate cobalt(II) species derived from the natural systems can also coordinate an N-donor base molecule in the β-face of the cobalt(II) center, but that coordination of a sixth ligand is hindered. This explanation is consistent with the crystallographic data available for Co(II)-corrin systems, as the known structures have a five-coordinate metal center, while for Co(III)-corrins six ligands are bound to the cobalt atom.

Structure of the Five-Coordinated Species. The aptitude of the five-coordinate cobalt(II) species for a second axial ligand is rationalized using indirect evidence obtained from analysis of the spin-Hamiltonian parameters;[10,11] the results are interpreted assuming that the interaction of the axial ligand with the metal center will pull the cobalt atom well above the equatorial plane and will fold the equatorial moiety from the axial ligand, thus rendering the other axial position less likely to be involved in bonding.

The X-ray data described for cobalt corrinoids[2] can be used to derive a similar explanation for our cob(II)inamide adducts with N- and P-donor bases. The corrin ring has been shown to be nonplanar, and a widely used parameter to measure this effect is the folding around the metal C-10 of cobalt corrins. The values for fold angles in cob(III)alamins range from 1.9° to 23.8°, and it has been shown that these values correlate with the Co$-$N$_{ax}$ bond lengths, a short bond implying a higher folding. On the other hand, upward folding is much more severe in cob(III)alamins with Co$-$N$_{ax}$ bonds shorter than 2.0 Å. Thus as in coenzyme B_{12} and methylcobalamin, the Co$-$N$_{ax}$ bond is close to 2.2 Å; the steric interaction of the DMB nucleotide base with the corrin ring is considerably reduced. In contrast the fold angle in the Co-β-cyanoimidazolylcobamide (the vitamin B_{12} analogue in which the cobalt DMB base is replaced by imidazole) is considerably less than in vitamin B_{12}, despite a shorter Co$-$N$_{ax}$ bond (1.97 Å vs 2.01 Å). This result clearly points to an effect of the bulk of the DMB base, which sterically interacts more strongly with the corrin ligand than in the case of imidazole. These observations suggest that electronic and steric effects of the axial base are important in controlling the structure of the cob(III)alamin.

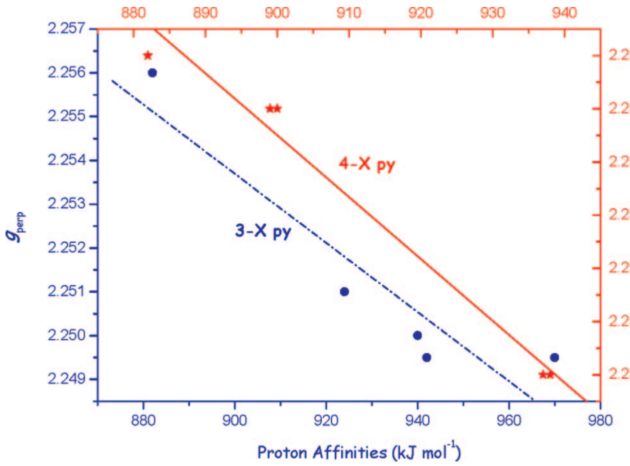

Figure 5. Variation of $g_{perpendicular}$ with proton affinities of the N-donor bases.

The X-ray structure of cob(II)alamin resolved by Kräutler[14] shows that the folding in the corrin ligand falls within the range indicated by the correlation between the length of the Co$-$N$_{ax}$ bond and the fold angle in cob(III)alamins. Thus the corrin system presents structural features identical to those observed for methylcobalamin and coenzyme B_{12}, with the axial bond slightly shorter (2.13 Å) and the fold angle slightly larger (16.3°) in cob(II)alamin. On the other hand the distance between the coordinating DMB base and the corrin ring is almost the same in B_{12r} and in coenzyme B_{12}, as a result of a downward displacement of the cobalt from the plane of the equatorial ligand. This type of structure for cob(II)alamin must hinder the coordination at the sixth position of the metal center, which explains the difficulty of these species to coordinate a second molecule of base.

EPR data can be used to provide some details on this type of structure for the complexes studied in this work by analyzing the dependence of the values of the g and A tensor components.

Influence of the Nitrogen Bases on the Structure of the Five-Coordinated Cobalt(II) Species. The g tensor components determined for the complexes studied in this work show a small dependence on N-donor base strengths. A plot of $g_{\perp}$ [$g_{\perp}= 1/2(g_x + g_y)$] against base strength (as measured by their proton affinity) of the axial N-donor base (Figure 5) shows that an increase in nitrogen base strength decreases slightly the value of $g_{\perp}$, while the g_z values are invariant. This dependence on the base strength can be interpreted as implying a smaller Co$-$N bond length for the stronger bases, as a decrease in axial bond length must imply an increase of the energy of the antibonding d_{z^2} orbital, leading to lower values of g_x and g_y.

The A_z component of the hyperfine interaction of the cobalt nucleus correlates with base strength, showing that the hyperfine

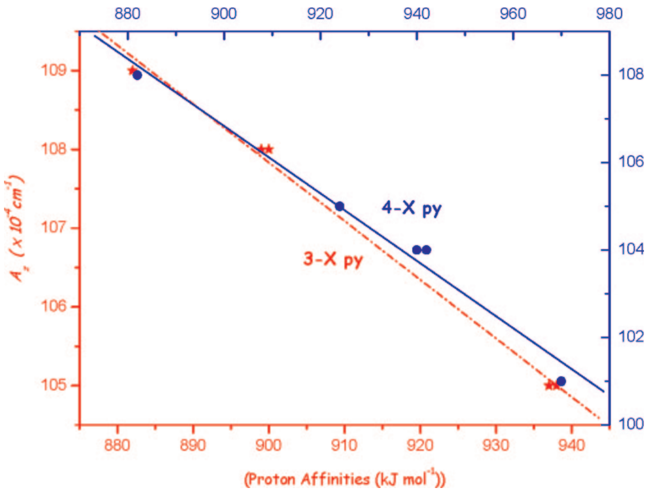

Figure 6. Variation of *Az* with proton affinities of the N-donor bases.

splitting increases as the donor ability of the base decreases (Figure 6). The same result has been observed for complexes with other equatorial ligands such as cobaloximes and is interpreted in that case considering that the size of the as N lone pair orbital decreases as ligand basicity increases, making the overlap between that pair and the 3d_{z^2} Co orbital less effective and thus explaining the decrease in the hyperfine spliting values.[31]

The dependence on electronic factors is identical for 3- and 4-pyridines, and in both cases a weak coordination at the sixth position has been observed, thus implying that, although the Co−N bond lengths may differ, the structural modifications induced by sterically hindered pyridines seem to be more important in rendering the sixth position less accessible for coordination of a second molecule of base.

Influence of the Phosphorus Bases on the Structure of the Five-Coordinated Cobalt(II) Species. The spin-Hamiltonian parameters show dependence with Tolman's cone angle of P-donor bases. Phosphines with large TCA values show large g_x and g_y values and consequently must have smaller energy differences, $d_{z^2} - d_{xz}$ and $d_{z^2} - d_{yz}$. This result suggests a longer Co−P bond length for the bulkier axial ligands, as an increase in axial bond length must imply a decrease in the energy of the antibonding d_{z^2} orbital. This interpretation is supported by the observation that the bulkier phosphines exhibit lower values of the phosphorus isotropic superhyperfine coupling constant (A_{iso}). As A_{iso} decreases, so does the unpaired spin density on the phosphorus s orbital, suggesting larger Co−P bond lengths for phosphines with higher TCA values.

All these data are indicative of longer axial Co−P bonds for bulkier phosphines, supporting higher steric repulsions between this axial ligands and the [Co^{II}(corrin)] moiety that deviate the cobalt atom from the equatorial plane toward the axial base.

On the other hand, this interpretation can explain the observation that coordination to cobalt(II) of the axial bulkier phosphines is more unfavorable than for N-donor bases since coordination of phosphines was completed only when excess axial base was used. The steric interactions between these bulkier phosphines and corrin moiety must be higher, leading to a lower axial interaction between phosphorus and cobalt, thus destabilizing these five-coordinated adducts.

Conclusions

Photolysis of MeCbi$^+$ and AdoCbi$^+$ in the presence of N-donor bases or P-donor bases yields, as products, complexes of the added bases with [Co^{II}(corrin)]$^+$, thus implying homolytic cleavage of the cobalt−carbon bond; additional support for this mechanism is obtained from irradiation at 77 K of MeCbi$^+$ in the presence of pyridines since we detect the presence of two superimposed EPR signals characteristic of a radical and a cobalt(II) complex, respectively. These results are identical to those described for methylcobalamin and coenzyme B$_{12}$, suggesting that removal of the benzimidazole nucleotide in the cobalamins and substitution by the pyridine bases studied leads to similar photolysis paths. For organocobalt B$_{12}$ model compounds with P-donor bases it has been observed that the same mechanism is operative, and as a consequence of longer and weaker Co−P bonds, photolysis is achieved with smaller irradiation times than those observed for analogous compounds with N-donor bases.[30–32] Unfortunately in the present study we cannot derive such a conclusion since the results evince that the P-donor bases were unable to coordinate to the cobalt(III) metal center in the initial MeCbi$^+$ or AdoCbi$^+$ systems, although they could react with the photolysis products, giving rise to five-coordinate species in solution in the presence of an excess of base.

For the N-donor bases used in this work we observed EPR signals characteristic of the presence of cobalt(II) five-coordinated adducts for molar ratios up to 1:20 but also detected the formation of 1:2 adducts when a large excess of base is used. These results are similar to those found for Schiff base models,[35] but must be contrasted with those obtained for cobaloximes and imino-oxime compounds, since in the five-coordinated cobalt(II) species the sixth coordination position is more accessible and these adducts are highly reactive through the coordination of a second molecule of base.[34]

The results regarding the type of adducts that N-donor bases, namely, pyridine and 4-substituted pyridines, may form with the corrin moiety [Co^{II}(corrin)]$^+$ contrast with those previously reported in the literature since only the 1:1 adduct was detected,[8] and we believe that this fact is due to the use of methanol and a different temperature of spectra acquisition.

We would like to stress that the use of various solvents and temperatures of observation chosen according to its physical characteristics may be of value to assess ligand binding.

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