Synthesis and Characterization of Cobalamines with Anionic and Neutral Phosphorous Donor Ligands

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Abstract

In dimethyl formamide as solvent aquacobalamine reacts with the triorganyl phosphites **3**–**7** to give the corresponding (diorganylphosphito-*P*)cobalamines, their new β-axial ligands $[P(O)(OR)_2]^-$ (**3a**–**7a**) being formed by partial hydrolysis. In methanol, however, additional methanolysis normally leads to (dimethylphosphito-*P*)cobalamine with the axial ligand $[P(O)(OMe)_2]^-$ (**2a**). Exceptions are $P(OCH_2CH_2NMe_2)_3$ (**4**) giving a complex with the only partially methanolized chiral ligand $[P(O)(OCH_2CH_2NMe_2)(OMe)]^-$ (**4b**), too, and the bicyclic phosphite **5** which is also coordinated in the unchanged, non-hydrolyzed form. All complexes are characterized by elementary analysis, electrophoresis, UV–Vis and ^1H, ^{31}P NMR spectra. The chirality of the cobalamine moiety causes diatropism of the two organyl groups in the prochiral ligands $[P(O)(OR)_2]^-$ which is well seen in the NMR spectra of the complexes with the methyl and phenyl derivatives **2a** and **6a**, whereas the spectra with ligands **3a** and (in part) **4a** are not resolved well enough to distinguish the two forms. With the chiral ligand **4b** two diastereomers are obtained in different yields; this asymmetric induction is indicated by the intensities of the respective signals in the NMR spectra.

Introduction

The biochemical significance of vitamin B12 is well known. Steric interactions between the β-axial ligand and the amide side chains of the corrin ring are believed to play an important role in all B12-dependent enzyme reactions [1–3], and therefore, the influence of different axial ligands on the corrin system is of great interest.

First examples of cobalamines with P-bounded β-axial ligands were obtained from aquacobalamine and trifluorophosphane (**1**) in methanol as solvent [4]. Hydrolysis as well as methanolysis gave (fluoromethylphosphito-*P*)- and (dimethylphosphito-*P*)-cobalamine; the latter was also isolated starting from trimethyl phosphite (**2**). The new anionic ligands $[P(O)(F)(OMe)]^-$ (**1a**) and $[P(O)(OMe)_2]^-$ (**2a**) are so tightly bounded to the cobalt ion of the cobalamine moiety that they cannot be replaced even by the strong ligand cyanide; this is in agreement with the results of studies on the *trans* effect of **2a** in cobaloximes [5, 6].

Further efforts to synthesize cobalamine derivatives with other (diorganylphosphito-*P*)- ligands $[P(O)(OR)_2]^-$ have not been reported in the literature, only the coordination behavior of the bicyclic phosphite 4-ethyl-2,6,7-trioxa-1-phospha-bicyclo-[2,2,2]-octane (**5**) was studied by means of UV–Vis spectroscopy [7].

In this paper we present some new cobalamine derivatives obtained by reacting aquacobalamine with different phosphites $P(OR)_3$ (**3**–**7**, Table 1). As the coordination place of the β-axial ligand is to some extent shielded by the amide side chains of the corrin ring, the phosphites were selected with the following criteria in mind: their organyl groups should have different steric requirements and/or contain some functional groups that might interact with the side chains, e.g. by hydrogen bonding. On the other hand, chelating organyl groups should influence the stability of the phosphites against hydrolysis because organometalloidal compounds, for instance, are much more stable having a 2,2′-biphenylylene ligand instead of two phenyl ligands [8–13].

Attempts to receive (diphenylphosphito-*P*)cobalamine from aquacobalamine and triphenyl phosphite (**6**) in methanol as solvent failed; the only product isolated was (dimethylphosphito-*P*)cobalamine [14]. In order to prevent methanolysis during the reaction another polar and aprotic solvent had to be found.

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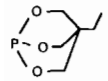
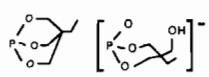
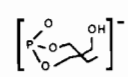
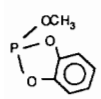
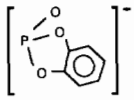
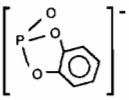
TABLE 1. Reactions Products $[\{Co\}\text{-}L]^{1\pm n}$ of Aquacobalamine and Triorganylphosphites[a]

Phosphite	Axial ligand of product	
	methanol as solvent	DMF as solvent
$P(OMe)_3$ (2)	$[P(O)(OMe)_2]^-$ (2a)	$[P(O)(OMe)_2]^-$ (2a)
$P(OCH_2CH_2Cl)_3$ (3)	$[P(O)(OCH_2CH_2Cl)_2]^-$ (3a)	$[P(O)(OCH_2CH_2Cl)_2]^-$ (3a)
$P(OCH_2CH_2NMe_2)_3$ (4)	$[P(O)(OMe)_2]^-$ (2a) $[P(O)(OCH_2CH_2NHMe_2)(OMe)]$ (4b)[b]	$[P(O)(OCH_2CH_2NHMe_2)]^+$ (4a)[b]
		
5	5 5a	5a
$P(OPh)_3$ (6)	$[P(O)(OMe)_2]^-$ (2a) $[P(O)(OMe)_2]^-$ (2a)	$[P(O)(OPh)_2]^-$ (6a)
		
7	7a	7a

[a]$\{Co\}^+$: abbreviation for the cobalamine moiety; $L^{\pm n}$: β-axial ligand; $\pm n$: charge of L, deduced from the charge of the complex at pH 2.5 as determined by paper electrophoresis in 0.5 M acetic acid. [b]Protonation of the dimethylamino group only in acidic solution (pH < 4).

Dimethyl formamide (DMF) proved to be the appropriate one.

Experimental

Aquacobalamine (as acetate), trimethyl phosphite (2), tris(2-chloroethyl) phosphite (3), tris(2-dimethylaminoethyl) phosphite (4), 4-ethyl-2,6,7-trioxa-1-phospha-bicyclo-[2,2,2]-octane (5) and triphenyl phosphite (6) were used as commercially received. 2-Methoxy-1,3,2-benzodioxaphosphole (7) was prepared according to ref. 15. All solvents were analytical grade and all reactions and manipulations were carried out under minimal exposure to light.

General Procedure

To a stirred solution of aquacobalamine in the solvent used (methanol or DMF) an excess of the phosphite, dissolved in the same solvent, was slowly added. After 10–18 h the volume was reduced and water was added, followed by extraction with ether. The aqueous solution was run on to an amberlite XAD-2 column [16], where the cobalamines were washed with water and then eluted with methanol. Chromatography on carboxymethylcellulose (CMC) in the H form was required to separate the new complexes from unreacted aquacobalamine. Recrystallization from water/acetone yielded the compounds as red needles.

Instruments

^1H and $^{31}P\{^1H\}$ NMR spectra were obtained from D_2O solutions at 298 K on an AM-360 Bruker spectrometer. In addition to the internal D_2O lock the chemical shifts of the ^1H NMR spectra are relative to TMS and the $^{31}P\{^1H\}$ NMR spectra relative to 85% H_3PO_4 as external standards. The UV–Vis spectra were recorded using 1 cm cells at 298 K on a Perkin-Elmer 554 spectrometer. Paper electrophoresis at pH 2.5 gave the charge of the cobalamines from which the charge of the β-axial ligand can be deduced. The purity and homogeneity of the products were also proven by paper chromatography.

Analyses

As a consequence of varying contents of crystal water combustion analyses never gave satisfactory results with respect to the absolute values, C:N and Co:P values found, however, were in excellent agreement with the expected values (see 'Supplementary Material').

Results and Discussion

As already pointed out, the solvent used has a great influence on the products formed in the reaction of aquacobalamine with triorganylphosphites (Table 1). The normally observed methanolysis using methanol as solvent is repressed to some extent in the

case of the phosphites **3** and **4** with functional groups capable of hydrogen bonding and of the cyclic phosphites **5** and **7** with dioxaphosphacyclohexane and dioxaphosphole rings as structural units. The bicyclic phosphite **5** even gives a complex of the unchanged ligand; the compound represents one of the first well characterized cobalamine derivatives with a neutral P-donor ligand. In DMF as solvent, however, the course of the reaction unambiguously leads to (diorganylphosphito-*P*)cobalamines without any by-products.

Important information on the new complexes can be received from their NMR spectra. Although the ^{1}H NMR spectra of cobalamine derivatives are quite complex an assignment of the signals has already been made to a large extent [17, 18 and refs. therein]. Thus, by comparing the spectra of the complexes with the published data the ligand signals can easily be recognized and assigned (Table 2). Only in a few cases in which the ligand and cobalamine signals overlap to some degree, a definite assignment could not be accomplished. Furthermore, the chemical shifts of the signals assigned to the protons of the different methyl groups and to the high field protons of the cobalamine moiety are given in Table 3.

The ^{31}P{^{1}H} spectra of the complexes show two signals, one of the ligand, the other of the phosphodiester. The signal of the phosphodiester was assigned to the base-on form, which was confirmed by recording the UV–Vis spectra of the solutions used for the NMR measurements. In contrast to the sharp signals due to the P atoms of the phosphodiesters those due to the P atoms of the ligands have linewidths of *ca.* 150 ppm. This effect is caused by the quadrupole moment of the Co atom and proves the P-coordination of the ligands. The ^{31}P{^{1}H} NMR data are given in Table 4.

The native cobalamine moiety $\{Co\}^+$ is chiral and therefore the two chemically equivalent organyl groups of a prochiral $[P(O)(OR)_2]^-$ β-axial ligand should show diastereotropism in the NMR experiment. Indeed, in all cases in which the ^{1}H NMR spectra of the ligands are not too complex the anisochronic signals of the two organyl groups can be distinguished. This is true for the complex of the methyl derivative **2a** and in part also for that of the dimethylaminoethyl derivative **4a** (protons of the NMe_2 group). This effect can be observed for the complex of the phenyl derivative **6a**. Since the *o*-, *m*- and *p*-protons of the phenyl rings are placed at

TABLE 2. ^{1}H NMR Data of the Ligands in the Cobalamine Complexes $[\{Co\}\text{-}L]^{1\pm n}$

Ligand $L^{\pm n}$	Assignment δ (ppm)	Chemical shifts	Coupling constant *J* (Hz)
$[P(O)(OMe)_2]^-$ (**2a**)	–OMe	3.282 (d, 3H)	^3J(P–H) 10.7
	–OMe	3.346 (d, 3H)	^3J(P–H) 10.7
$[P(O)(OCH_2CH_2Cl)_2]^-$ (**3a**)	$-CH_2-$	3.48–3.56 (m, 8H)	
$[P(O)(POCH_2CH_2NMe_2)_2]^-$ (**4a**)	$-OCH_2-$	3.53–3.68 (m, 4H)	
	$-CH_2-$	[a]	
	$-NMe_2$	2.119 (s, 6H)	
	$-NMe_2$	2.128 (s, 6H)	
$[P(O)(PCH_2CH_2NMe_2)(OMe)]^-$ (**4b**)	–OMe	3.285 (d)[b]	^3J(P–H) 10.7
	–OMe	3.361 (d)[b]	^3J(P–H) 10.7
	$-OCH_2-$	3.51–3.69 (m, 4H)	
	$-OCH_2N<$	[a]	
	$-NMe_2$	2.15 (s, 6H)	
$[P(OCH_2)_3CCH_2CH_3]$ (**5**)	$-OCH_2-$	4.364 (6H)	^3J(P–H) 4.7
	$C-CH_2-C$	[a]	
	$-CH_3$	0.685 (t, 3H)	^3J(H–H) 7.64
$[P(O)(OCH_2)_2C(CH_2CH_2OH)(CH_2CH_3)]^-$ (**5a**)	$-OCH_2-$	4.24[c]	
	$-CH_2-$	[a]	
	$-CH_3$	0.828 (t, 3H)	^3J(H–H) 8.0
$[P(O)(OPh)_2]^-$ (**6a**)	–OPh	6.493 (d, 2H, *ortho*)	N = 8.1 Hz
		6.937 (t, 1H, *para*)	N = 7.4 Hz
		7.050 (t, 2H, *meta*)	N = 7.2 Hz
	–OPh	6.757 (d, 2H, *ortho*)	N = 7.8 Hz
		7.029 (t, 1H, *para*)	N = 7.7 Hz
		7.176 (t, 2H, *meta*)	N = 7.7 Hz
$[P(O)(O_2C_6H_4)]^-$ (**7a**)	$>C_6H_4$	6.89 – 6.95 (m, 4H)	

Solvent: D_2O, external standard: TMS; $\{Co\}^+$: abbreviation for the cobalamine moiety. [a]Covered by signals of $\{Co\}^+$. [b]Correspond to three H atoms together. [c]Tentative assignment.

TABLE 3. ^{1}H NMR Data of the Cobalamine Moiety $\{Co\}^+$ in the New Complexes $[\{Co\}\text{-}L]^{1\pm n}$

Assignment[a]	Complex with ligand							
	2a	3a	4a	4b	5	5a	6a	7a
C1-CH_3	0.381	0.412	0.383	0.392	0.425	0.373	0.478	0.408 d
Pr3-CH_3	1.189	1.213 d	1.179 d	1.196 d	1.181 d	[b]	1.210 d	0.959
12β-CH_3	1.189	1.359	1.227	1.219	1.249	1.229	1.159	0.856
17β-CH_3	1.385	1.301	1.340	1.387	1.331	1.332	1.221	1.080
C2-CH_3	1.385	1.301	1.409	1.400	1.385	1.354	1.358	1.288
12α-CH_3	1.301	1.451	1.281	1.299	1.458	1.382	1.419	1.529
7α-CH_3	1.786	1.799	1.773	1.787	1.805	1.780	1.716	1.968
B6-CH_3	2.202	2.219	2.189	2.198	2.224	2.211	2.225	2.217
B5-CH_3	2.204	2.231	2.204	2.206	2.227	2.221	2.247	2.252
C5-CH_3	2.478	2.517	2.500	2.501	2.531	2.471	2.497	2.503
C15-CH_3	2.492	2.551	2.514	2.511	2.548	2.511	2.519	2.528
R1	6.270 d	6.306 d	6.265 d	6.312 d	6.368 d	6.317 d	6.321 d	6.318 d
C10	6.001	6.036	6.017	6.028 6.014	6.139	5.918	6.115	5.922
B4	6.319	6.347	6.288	6.279	6.429	6.366	6.425	6.388
B2	7.001	7.026	6.979	6.994	7.132	6.989	7.114	7.077
B7	7.183	7.218	7.177	7.190	7.300	7.315	7.231	7.238

Solvent: D_2O; external standard: TMS. [a]Numbering of atoms according to ref. 19. [b]Covered by signals of the ligand $L^{\pm n}$.

TABLE 4. $^{31}P\{^1H\}$ NMR Data of the New Complexes $[\{Co\}\text{-}L]^{1\pm n}$

Assignment	Complex with ligand							
	2a	3a	4a	4b	5	5a	6a	7a
Ligand $L^{\pm n}$	42.190	39.326	38.035	39.513 40.011	86.315	51.454	31.520	72.980
Phosphodiester	−0.316	−1.016	−0.316	−1.059	−0.776	−0.188	−0.178	−0.115

Solvent: D_2O; external standard: 85% H_3PO_4.

different positions in the cone of magnetic anisotropy of the corrin system their chemical shifts differ enough to produce deceptively simple spectra, *i.e.* a doublet (2H) and two triplets (1H and 2H) for each ring. The assignment of three of the signal groups to one ring or the other was achieved by decoupling experiments. In Fig. 1 the high field part of ^{1}H NMR spectrum of the complex with the phenyl ligand **6a** is reproduced together with that of the dimethylaminoethyl ligand **4a** for reasons of comparison.

When a chiral ligand like the dimethylaminoethylmethyl phosphite (**4b**) is coordinated to the chiral cobalamine moiety two diastereomers are formed which normally should differ in their physical properties. However, the product of the reaction between aquacobalamine and tris(dimethylaminoethyl) phosphite (**4**) in methanol could not be separated by means of paper chromatography or electrophoresis, giving only one spot in each case. But the ^{1}H NMR spectrum of this product unambiguously shows that the product consists of two compounds as is indicated by two different signals for the methoxy groups of **4b** and by two signals for the protons at C10 of the corrin ring. The ^{31}P NMR spectrum, too, shows two different signals for the phosphito ligand, but only one signal for the phosphodiester of the nucleotide loop. This fact excludes the product to be a mixture of the base-on and base-off forms since they are characterized by different chemical shifts of the phosphodiester signals [20]. Apparently, only regions in the near neighborhood of the ligands are effected by its chirality which is no more significant at the periphery of the complex, the axial ligand being shielded by the propionic amide groups of the cobalamine moiety. One of the two diastereomers is predominantly yielded so that the reaction is stereoselective.

The UV–Vis spectra of the complexes with the anionic diorganyl phosphito ligands differ only slightly and can easily be distinguished from the spectrum of the complex with the neutral bicyclic phosphite **5** which is identical to that obtained by Chemaly *et al.* [7] (Table 5). All spectra strongly depend on the pH value of the solution and show a

TABLE 5. Wave Lengths (pm) and Corresponding Extinction Coefficients (10^4 dm^3 mol^{-1} cm^{-1}, in parentheses) of Characteristic Bands in the UV–Vis Spectra of the New Complexes $[\{Co\}\text{-}L]^{1\pm n}$ in H_2O as Solvent

Complex with ligand	Notation of bands [21]						
				γ		β	α
2a	278	312	333	364 (2.1)	402	518 (0.77)	540 (0.81)
3a	278	312	332	363 (2.2)	402	517 (0.77)	542 (0.8)
4a	278	312	332	363 (2.1)	402	518 (0.78)	542 (0.8)
4b	278	312	332	364 (2.1)	402	518 (0.78)	542 (0.8)
5	280	306	324	364 (2.8)	413	522 (0.77)	553 (0.74)
5a	280	312	332	366 (2.1)	404	522 (0.7)	545 (0.8)
6a	278	310	330	364 (2.3)	404	520 (0.74)	544 (0.8)
7a	276	314	330	367 (2.3)	404	522 (0.8)	548 (0.8)

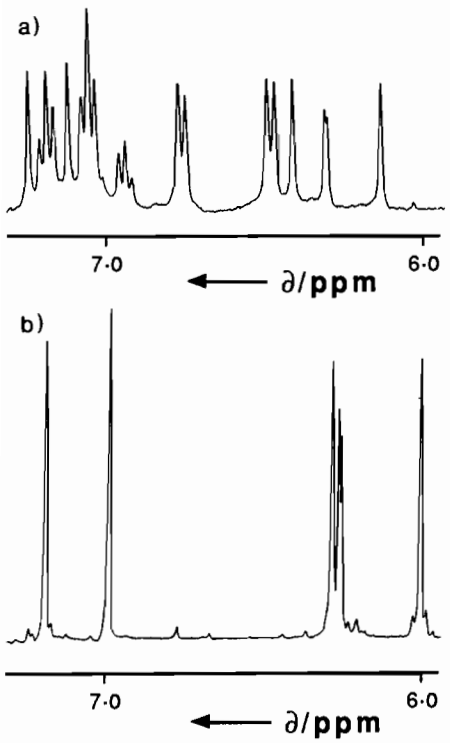


Fig. 1. The high field parts of the ^1H NMR spectra of the cobalamine complexes with (a) ligand **6a** and (b) ligand **4a**.

pH-dependent equilibrium giving an isosbestic point at approximately 482 nm. The protonation of the nucleotide loop is always accompanied by an absorption at 284 nm, assigned to the protonated nucleotide base [4].

Hydrolysis of the triorganylphosphites leading to the (diorganylphosphito-*P*)cobalamines is possible since aquacobalamine additionally contains 10–13% of crystal water, *i.e.* water is always present in the reaction mixture. The only phosphite of which a complex prior to hydrolysis could be isolated, probably due to its cage form, is the bicyclic compound **5**. But from these experimental results received upto now it cannot yet be decided when hydrolysis takes place during the course of the reaction. Reactions of coordinated ligands have been observed, e.g. the methanolysis of (fluoro-methylphosphito-*P*)cobalamine to (dimethylphosphito-*P*)-cobalamine [4]. On the other hand it is also known that dimethyl phosphite in which the electron pair at the phosphorous atom is blocked by a strongly bonded hydrogen (phosphonate form) slowly reacts with aquacobalamine to form the (phosphito-*P*) complex [7].

Supplementary Material

Analysis results are available from the author on request.

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