to the simplification of proton spectra in a manner similar to that of spin-echo difference spectroscopy.[20]

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Received March 18, 1980

Electrochemistry of Vitamin B_{12}. 6. Diaquocobinamide

Sir:

The chemistry of cobinamides has been widely investigated since it may provide a source of information for a better understanding of the properties of cobalamins.[1,2] The comparison between the two classes of compounds may indeed help in the evaluation of the role of the nucleotide side chain Bzm ligand. There have been, in this connection, few studies on electrochemistry of cobinamides.[3-6] The polarographic investigation of an extended series of cobinamides[3] has led to a rather puzzling observation concerning diaquocobinamide(III): its reduction wave is located at −0.74 V vs. SCE, i.e., considerably negative (700 mV) to the standard potential of the Co(III)/Co(II) couple in aquocobalamin.[7,8] This observation has been later taken as evidence that diaquocobinamide(III) has a standard potential more negative than that of aquocobalamin(III) in a discussion of the electrochemical reduction mechanism of the later compound.[9] One would have, on the contrary, anticipated that due to the electron-donating ability of Bzm being clearly higher than that for water the reduction of Co(III) to Co(II) would be much more difficult for aquocobalamin than for diaquocobinamide. It is noted that the pH (6.2) where the polarographic determination was carried out is slightly higher than the pK_a (6) of the ionization of a first axial water molecule[10] and that the half-wave potential may be affected by electron-transfer kinetics and/or adsorption phenomena. It seems unlikely, however, that these effects could reverse the expected behavior to such a large extent. On the other hand, one could invoke stereochemical differences between cobinamides and cobalamins such as bent-up conformation of the corrin ring in the latter case as compared to the former,[11] leading to different electron-donating properties of the ring which might counterbalance the influence of the axial ligands. Again, however, the large magnitude of the inversion in reducibility appears difficult to rationalize within this context.

Previous determinations of the Co(III)/Co(II) thermodynamics of cobalamins have suggested, through extrapolation of the data obtained in strongly acidic media, that the standard potential would be +0.270 V vs. SCE for the base-off forms.[7] It is expected that the behavior of diaquocobinamide is similar to that of the base-off aquocobalamin, leading to an $E°$ value in a more reasonable agreement with the anticipated effect of the axial ligands. It was the purpose of the work reported hereafter to determine directly the main redox characteristics of the Co(III)/Co(II) and Co(II)/Co(I) couples in diaquocobinamide and to see whether or not the expectations derived from the extrapolation of the aquocobalamin behavior are legitimate.

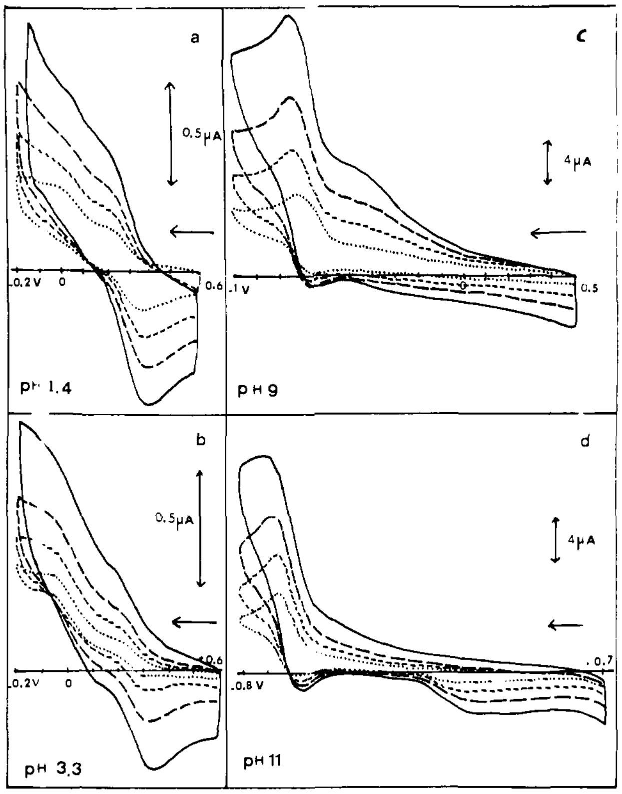

Figure 1. Cyclic voltammetry of diaquocobinamide as a function of pH. Electrodes: (a and b) glassy carbon, (c and d) platinum. Buffer: (a) $HClO_4$; (b–d) Britton–Robinson. Concentration: (a and b) 1, (c) 0.9, (d) 0.8 mM. Sweep rate (from bottom upward): 0.02, 0.05, 0.1, and 0.2 V s^{-1}.

Instrumentation and procedures for cyclic voltammetry were the same as already described.[7,8,12] Diaquocobinamide was prepared from aquocobalamin and purified according to previously described procedures.[13]

The general features of the cyclic voltammetry of the Co(III)/Co(II) couples as a function of pH (Figure 1) are similar to what was observed with aquocobalamin:[7,8] while in acidic media, the Co(III)/Co(II) wave is clearly separated from the Co(II)/Co(I) wave, there is a negative shift of the former upon increasing the pH over the pK_a (6) of the aquo–hydroxo interconversion, finally leading to an almost complete merging of the two waves. For pH < 6, it is noted, however, that the Co(III)/Co(II) cathodic pattern is split into two waves, which was not the case for aquocobalamin. Determination of $E°$ on the first of these waves and on the anodic Co(II)/Co(III) wave leads to an approximate value of 0.265 V, i.e., close to the figure found for the base-off aquocobalamin in strongly acidic medium.

A spectroelectrochemical investigation of the oxidoreduction of diaquocobinamide was carried out at pHs 1.6 and 3.8 on a platinum-grid electrode in order to reach a more reliable determination of the Co(III)/Co(II) $E°$. The results obtained upon reduction at pH 3.8 are summarized in Figure 2. The logarithms of the analyses (Figure 2c) have a slope close to 60 mV, as expected for a reversible process. The reversibility has actually been reached and confirmed by a reoxidation experiment in which the

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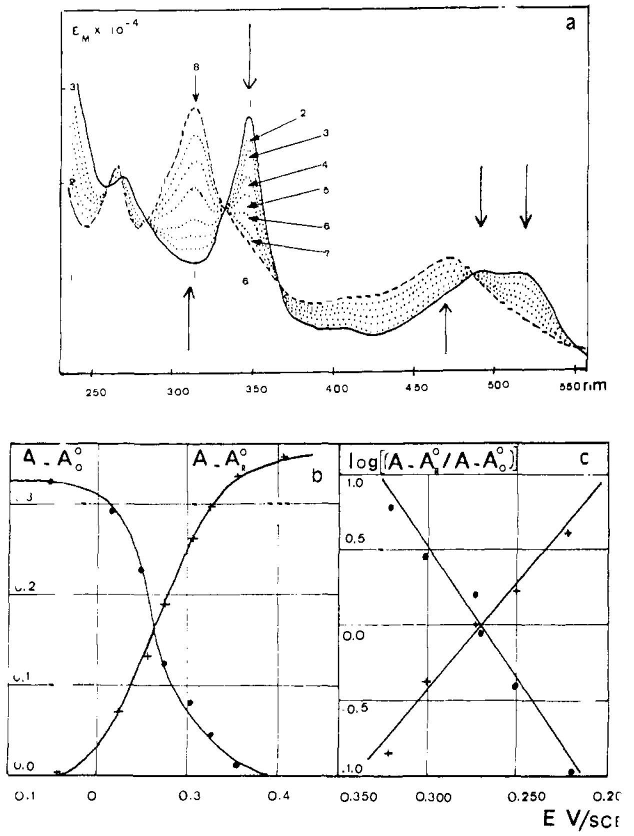

Figure 2. Spectroelectrochemistry of cobinamide(III)/cobinamide(II) (0.5 mM) on a platinum-grid electrode in Britton–Robinson buffer, pH 3.8. (a) Variation of the spectrum upon reduction; electrode potential (V vs. SCE) 0.4 (1), 0.35 (2), 0.32 (3), 0.30 (4), 0.27 (5), 0.25 (6), 0.22 (7), and 0.15 (8). (b) Spectrovoltammograms at 346 (●) and 310 nm (+); (A) observed absorbance and ($A_0°$, $A_R°$) absorbances of base Co(III) and Co(II), respectively. (c) Logarithmic analyses of the spectrovoltammograms.

same behavior as seen for reduction is observed. $E°$ is found equal to +0.270 V vs. SCE. The same value is found for pH 1.4. We failed in obtaining reversible behavior and meaningful $E°$ values above the pK_a of the aquo–hydroxo interconversion. At, e.g., pH 8, the slopes of the logarithmic analyses were on the order of 100 mV. This is probabily due to the slowness of the electron transfer to the hydroxo form and to its interaction with the electrode material. Around pH 10, a partial spontaneous reduction of diaquocobinamide(III) into Co(II) was observed, involving probably the anion of the buffer system as the electron source. The splitting of the Co(III)/Co(II) wave in acidic medium may also be related to the adsorption of the cobinamide on the electrode surface. It is indeed noted that the cobalt in diaquocobinamide is readily accessible to axial ligandation, clearly more than in aquocobalamin, and that the surface of the platinum electrode may well, in the rather positive potential range corresponding to the Co(III)/Co(II) reduction, be covered by superficial oxide layers that may interact with the cobalt atom.

The cyclic voltammetry behavior of the Co(II)/Co(I) couple is shown in Figure 3. In acidic medium, the Co(II)/Co(I) wave is not completely reversible and is followed by an irreversible S-shaped wave. Both phenomena are probably related to catalytic hydrogen discharge. A more satisfactorily reversible wave is obtained when starting from Co(I) and sweeping in the reverse direction at slightly higher scan rates (Figure 3a), thus allowing the determination of $E°$. Reversibility is easily obtained at higher pH (Figure 3b, d), even starting from Co(II). The $E°$ values are very close at the three pHs (−0.73 V vs. SCE in average).

The above results show that the $E°$–pH diagram for diaquocobinamide as predicted from the extrapolation of the results obtained for aquocobalamin (Figure 10 in ref 7) is essentially correct: diaquocobinamide does behave as base-off aquocobalamin as far as the redox equilibrium properties of the Co(III)/Co(II) and Co(II)/Co(I) couples are concerned. The diagram should, however, be improved as follows. The 60-mV slope oblique line separating the monohydroxocobalt(III) from the monohydroxocobalt(II) regions should be stopped at pH 10.5 and then replaced by a 120-mV slope line featuring the separation of the dihydroxycobalt(III) from the dihydroxocobalt(II) regions as corresponds to the pK_a of the monohydroxo/dihydroxo equilibrium.[10] An important point is that the $E°$ of the Co(III)/Co(II) couple is found to be much more positive than in the case of base-on aquocobalamin, in agreement with the expected electron-donating effect of Bzm as compared to that of water. The previous value of −0.74 V vs. SCE appears as corresponding actually to the Co(II)/Co(I) couple. The Cobinamide(II) could have indeed been formed through reduction of cobinamide(III) by the mercury of the dropping electrode in a chloride-containing medium. This is confirmed by an experiment in which the addition of mercury to a 0.02 M NaCl aqueous solution of diaquocobinamide was found to result in a rapid and total conversion into cobinamide(II) as checked spectroscopically.

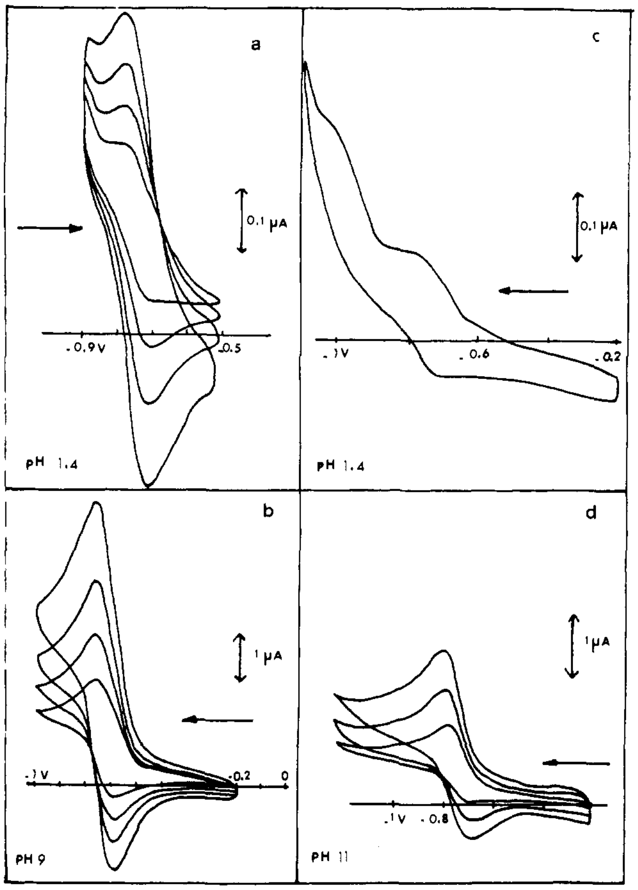

Figure 3. Cyclic voltammetry of cobinamide(II)/cobinamide(I) on a mercury drop electrode. Sweep rate (from bottom upwards): (a and b) 0.02, 0.05, 0.1, 0.02 V s^{-1}, (c) 0.2 V s^{-1}, (d) 0.02, 0.05, and 0.1 V s^{-1}. Concentration: (a and c) 0.9, (b) 0.8, (d) 0.7 mM.

Acknowledgments. The work was supported in part by the CNRS (Equipe de Recherche Associée 309 "Electrochimie Moléculaire"). The Roussel Uclaf Co. is gratefully thanked for the gift of aquocobalamin samples.

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Received October 10, 1979