Carbon-13 Nuclear Magnetic Resonance Spectroscopy of $[CH_2CO_2H\text{-}^{13}C_2]$Carboxymethylcobalamin; an Acetic Acid Derivative with an Unusually High pK Value

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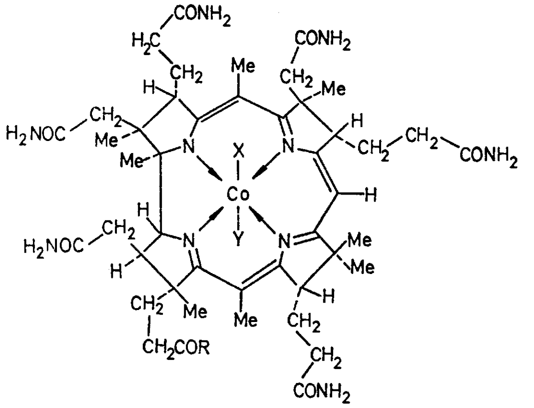
Summary The pH dependence of the ^{13}C chemical shifts of the 90% enriched carboxy, and methylene carbon atoms of $[CH_2CO_2H\text{-}^{13}C_2]$carboxymethyl cobalamin shows that the carboxymethyl unit has an unusually high pK value, 7·2.

THE ^{13}C chemical shifts of carbon atoms bonded to cobalt(III) in alkylcobalamins and alkylcobinamides (**1**; X = alkyl) are remarkably sensitive to the nature of the ligand (Y) *trans* to the alkyl group.[1] The wide range of ^{13}C shifts exhibited by these corrinoids reflect the profound influence of the axial ligand (Y) on the reactivity of the cobalt atom and of the organometallic bond.[2,3] Brodie[4] has demonstrated that axial ligation by a strong ligand is also accompanied by major conformational changes in the corrin ring. In investigations of these *trans*-effects, we have studied the pH dependence of the ^{13}C n.m.r. spectra of D_2O solutions of $[CH_2CO_2\text{-}^{13}C]$carboxymethylcobalamin (**1**; X = $^{13}CH_2\text{-}^{13}CO_2H$; Y = dimethylbenzimidazole) and have found that the carboxymethyl fragment has an unusually high pK value, 7·2. This very high pK value for an acetic acid derivative suggests that there is strong hydrogen bonding between the carboxy-group and the acetamide side chains of the corrin ring. Analagous interactions between co-ordinated water and the acetamide side chains have been found in aquocyanocobyric acid (**1**; X = H_2O, Y = CN^-), while the 6-amino-group of the adenine unit of adenosylcobalamin is hydrogen bonded to the carbonyl oxygen of the acetamide side chain of ring B.[6]



The pH dependence of the ^{13}C shifts of each component of the ^{13}C–^{13}C spin–spin doublets for the methylene and carboxy ^{13}C atoms of $[CH_2CO_2H\text{-}^{13}C_2$ (90%)]carboxymethylcobalamin is summarized in Figure 1. It is apparent from the shape of the curves that the pH dependence of the chemical shifts is associated with the titration of two groups (pH = 2·4 and 7·2), the equilibria among the protonated and unprotonated forms of the cobalamin being rapid on the ^{13}C n.m.r. time scale and resulting in exchange averaged ^{13}C chemical shifts. The most acidic centre (pH = 2·4) undoubtedly is the benzimidazole group whose protonation is accompanied by its displacement from the co-ordination sphere of cobalt(III) by a water molecule.[7] In this regard we note that the magnitude and the sign of the ^{13}C chemical shift measured for this protonation are similar to those obtained for the substitution of oxygen by a nitrogen donor in the structurally similar [^{13}C]methylcobinamides.[1]

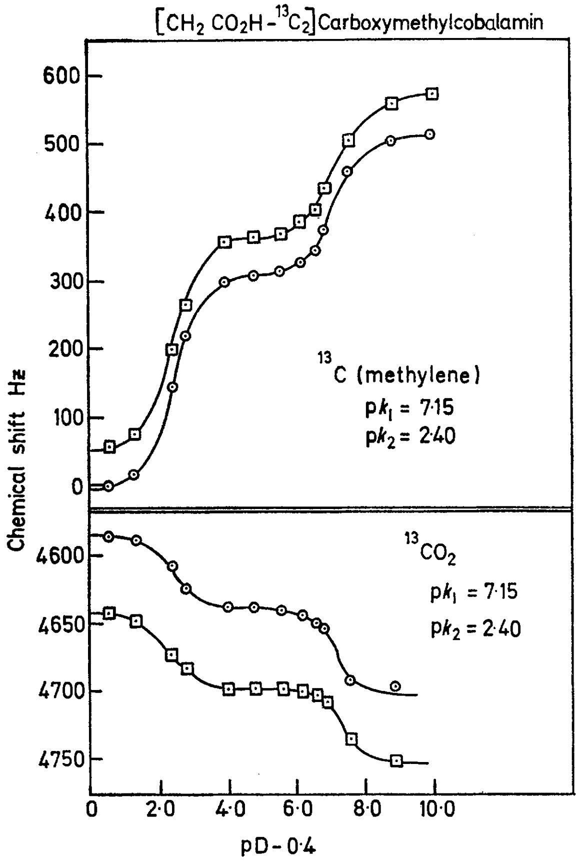


FIGURE 1. pH Dependence of the ^{13}C shifts (in Hz with respect to external Me_4Si) of each component of the ^{13}C–^{13}C spin–spin doublets for the methylene (upper) and carboxyl (lower) ^{13}C atoms of $[CH_2CO_2H\text{–}^{13}C_2$ (90%)]carboxymethyl cobalamin.

The ^{13}C chemical shift–pH titration curve of the ethyl ester, ethoxycarbonylmethylcobalamin (Figure 2), is similar in the pH region 0—6. Above pH6, however, the ^{13}C chemical shifts of the carboxy and methylene carbons of the esters are independent of pH. Thus the second group ($pK = 7{\cdot}2$) of carboxymethylcobalamin being titrated above pH 6 must be the carboxy-group. The large value of this pK relative to those for other acetic acid derivatives indicates either an unusual substituent effect for cobalt(III) or an unusual environment for the carboxymethyl unit. As discussed above, we believe the latter to be the case because Co^{III} in these compounds and related derivatives behaves as a highly electronegative substituent.[1]

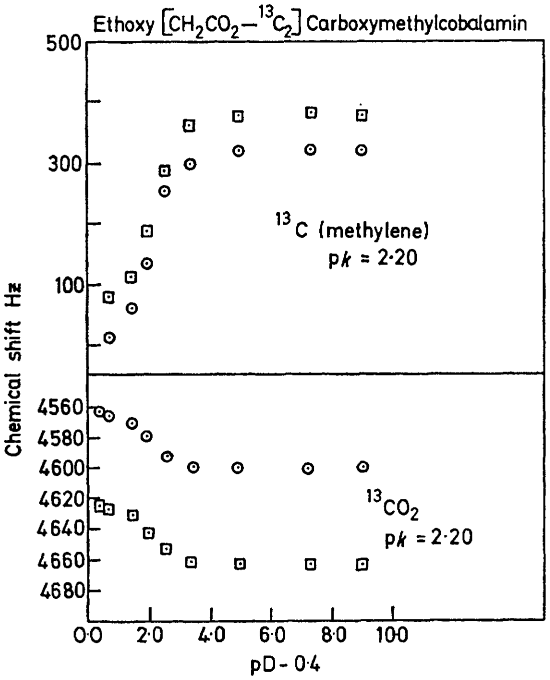


Figure 2. pH Dependence of the ^{13}C shifts (in Hz with respect to external Me_4Si) of each component of the ^{13}C–^{13}C spin–spin doublets for the methylene (upper) and carboxy (lower) ^{13}C atoms of ethoxy[CH_2CO_2–$^{13}C_2$]carboxymethylcobalamin.

Proton decoupled ^{13}C FT-n.m.r. spectra were obtained at 25·2 MHz with a Varian XL-100-15 spectrometer interfaced to a Data General Supernova Computer using the deuterium resonance (15·4 MHz) of internal D_2O as a lock. Since the measurements were made with D_2O solutions, the following relationship[8] was used in calculating the pK values: pD = pH (meter reading) + 0·4. [CH_2CO_2-$^{13}C_2$]carboxymethylcobalamin and ethoxy[CH_2CO_2-$^{13}C_2$]carbonylmethylcobalamin were prepared from 2-bromo-[1,2-$^{13}C_2$]acetic acid and ethyl 2-bromo[1,2-^{13}C]acetate respectively as described before;[7] 2-bromoacetic acid and ethyl 2-bromoacetate were prepared from 90% enriched [1,2-$^{13}C_2$]acetic acid according to the method of Ropp.[9] The purity of the cobalamins was established by spectral analysis and by paper chromatography in three solvent systems.

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